Addendum 10

to the Draft Assessment Report

of 30 December 2005

(relating to confirmatory information according to Commission Implementing Regulation (EU) No 485/2013)

Confirmatory Information

Imidacloprid
B.9 Ecotoxicology

~~27 November 2015~~
19 July 2016

Rapporteur Member State: Germany

Version history

When	What
27.11.2015	final version by RMS
19.07.2016	revision by RMS (comments from reporting table and discussion in Pesticides Peer Review Meeting 145)

Table of contents

B Summary, evaluation and assessment of the data and information

1. Introduction

The active substance imidacloprid was included in Annex I to Directive 91/414/EEC on 1 August 2009 by Commission Directive 2008/116/EC, and has been deemed to be approved under Regulation (EC) No 1107/2009, in accordance with Commission Implementing Regulation (EU) No 540/2011, as amended by Commission Implementing Regulation (EU) No 541/2011.

The specific provisions of the approval were later amended by Commission Directive 2010/21/EU, to permit use as a seed treatment only if

- the seed coating is performed in professional seed treatment facilities, which must apply the best available techniques to ensure that the release of dust during application to the seed, storage and transport can be minimised, and
- if adequate drilling equipment is used to ensure a high degree of incorporation in soil, minimisation of spillage and minimisation of dust emission.

Following new scientific information in spring 2012 on the sub-lethal effects of neonicotinoids on bees and the respective EFSA conclusion of January 2013 (EFSA Journal 2013; 11(1):3068), The Commission considered that there are indications that the authorised uses of imidacloprid (and clothianidin and thiamethoxam) no longer satisfy the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009 with respect to their impact on bees. To exclude the high risk for bees further restrictions were imposed by Commission Implementing Regulation (EU) No 485/2013. These restrictions comprised

- the limitation to professional uses
- the prohibition of uses as seed treatment and soil treatment for crops attractive to bees and for cereals, excepts for uses in greenhouses and for winter cereals
- the prohibition of uses as foliar treatments for crops attractive to bees and for cereals with the exception of uses in greenhouses and uses after flowering.

In addition, Commission Implementing Regulation (EU) No 485/2013 also requested the submission of confirmatory information as regards

a) the risk to pollinators other than honey bees
b) the risk to honey bees foraging nectar or pollen in succeeding crops
c) the potential uptake via roots to flowering weeds
d) the risk to honey bees foraging on insect honey dew
e) the potential guttation exposure and the acute and the long-term risk to colony survival and development, and the risk to bee brood resulting from such exposure
f) the potential exposure to dust drift following drill and the acute and the long-term risk to colony survival and development, and the risk to bee brood resulting from such exposure
g) the acute and long term risk to colony survival and development and the risk to bee brood for honey bees from ingestion of contaminated nectar and pollen.

On 19 December 2014, the company Bayer CropScience AG submitted a dossier to address these confirmatory data requirements for imidacloprid.

This Addendum presents the evaluation performed by the RMS Germany on these confirmatory data and is focused and structured along the questions posed in the Commission Implementing Regulation (EU) No 485/2013.

The risk assessment was performed following the risk assessment scheme for honey bees as proposed in the EFSA Guidance Document on bees. This was due to the special situation for these confirmatory data on imidacloprid and the relating mandate for EFSA by Commission. Due to the potential risk to honey bees from imidacloprid, the screening steps were not performed, and the risk assessment started at the first tier.
With regard to contact toxicity following dust drift the Guidance Document "Draft Authorisation of Plant Protection Products for Seed Treatment" (SANCO/10553/2012, January 2014) was used for the risk assessment, as it was agreed at the corresponding Pesticides Peer Review Meeting 145.

~~Please take note that this assessment was performed according to the following guidelines:~~

- ~~In accordance with SANCO/10329/2002 rev 2 Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC and with ANNEX to SANCO/11803/2010 Rev. 4 for~~
  - ~~the risk to honey bees~~
  - ~~the risk to commercially used pollinators other than honey bees~~

- ~~In accordance with the 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) for~~
  - ~~the risk to wildlife pollinators other than honey bees~~

  ~~This is because there is currently no formally valid guidance document for the assessment of wildlife pollinators (in contrast to honey bees). In the opinion of the German Federal Environment Agency (the authority responsible for the assessment of wildlife pollinators in Germany), the EFSA Guidance Document therefore represents for certain parts the current state of the scientific and technical knowledge (see also „Implementation plan for the EFSA Guidance Document on the Risk Assessment of Plant Protection Products on Bees") and thus is the only basis for the risk assessment on the submitted data for wildlife pollinators which is available for all parties involved in the EU.~~

2. Conclusions

a) The risk to pollinators other than honey bees

- *Commercially used pollinators other than honey bees*
  There is no unacceptable risk for commercially used pollinators due to exposure via residues in guttation fluids or nectar and pollen. As no adverse effects on honey bees are expected following sowing of sugar beet of good seed treatment quality, there are yet no data that indicate that other pollinators are likely to be at risk, however it can also not be fully excluded. In conclusion, the risk is considered acceptable for the intended use in sugar beets.

  For dust drift during sowing of cereals, a risk cannot be excluded for commercial pollinators such as *Bombus* and *Osmia*. However the argumentation that the likelihood of exposure of individual bumble bees is low in autumn and that no exposure takes place for solitary bees like *Osmia* is shared by the RMS.

- *Wildlife pollinators other than honey bees*
  An unacceptable risk for wild bumble bees and solitary wild bees due to different routes of exposure following the uses under consideration has been identified in accordance with the 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). A risk for wild bumble-bees and solitary wild bees cannot be excluded for exposure resulting from residues of imidacloprid in drifted dust, ~~from foraging on weeds in the treated crop,~~ from foraging on plants in the field margin, from foraging on succeeding crops and from foraging on the treated crop (uses in potato and leafy vegetables, which come to flowering stages). A risk for wild bumble bees and solitary wild bees resulting from foraging on the treated crop is not considered relevant for the uses in winter cereals, beets and leafy vegetables harvested before flowering. Regarding the risk for wild bumble bees and solitary wild bees resulting from foraging on weeds in the treated crop, this scenario can be considered of low relevance as exposure route for potato, cereals and sugar beet pending on further clarifications on the data submitted by the applicant.

b) The risk to honey bees foraging nectar or pollen in succeeding crops

The risk of imidacloprid to honey bees from consumption of contaminated pollen and nectar in succeeding crops can be considered acceptable; as the level of residues in nectar and pollen detected in the investigated flowering crops were in the range or below levels of primary crops, for which in former assessments (DAR 2005, EFSA 2008 and EFSA 2013) no clear effects on acute mortality and honey bee colony development were observed.

c) The potential uptake via roots to flowering weeds

The assessment of the potential uptake via roots to flowering weeds could not be finalized as no data on residues in nectar and pollen of flowering weeds were provided.

d) The risk to honey bees foraging on insect honey dew

The exposure of honey bees to imidacloprid through honey dew present in the treated field can be considered negligibly low, provided that there is no resistance of aphids.

e) The potential guttation exposure and the acute and the long-term risk to colony survival and development, and the risk to bee brood resulting from such exposure

Although the concentrations of insecticides such as imidacloprid in guttation fluids arising from the use as a seed treatments do present levels potentially harmful to bees, acute and chronic colony level effects were not observed in the studies presented here. Furthermore, honey bee behaviour as well as other factors relating to colony wellbeing (colony strength, health status such as presence and level of *Varroa*, viruses and other pathogens) were unaffected by exposure to guttating winter cereals, potatoes or sugar beets treated with imidacloprid (and clothianidin) as a seed treatment. Therefore, it can be concluded that residues of imidacloprid in guttation fluid produced by winter cereals, sugar beet and potato plants at the maximum seed dressing rates do not pose an unacceptable acute or chronic risk to honey bee colony development or survival.

f) The potential exposure to dust drift following drill and the acute and the long-term risk to colony survival and development, and the risk to bee brood resulting from such exposure

As an overall conclusion, a risk to bees following dust drift from treated cereal seeds cannot be excluded, both for imidacloprid seed treated wheat and barley.
The risk to bees following dust drift from treated sugar beet seeds is considered acceptable. No further data were available for granules (use of the product “Merit” in turf) and no data for the abrasiveness of the granules provided, thus the assessment could not be finalized for machine assisted spreading, whereas the risk of dust drift is considered low for hand spread granules.

g) The acute and long term risk to colony survival and development and the risk to bee brood for honey bees from ingestion of contaminated nectar and pollen

As no exposure is expected to nectar and pollen from sugar beet, potatoes and winter cereals as a result of the treatment of seeds the risk can be considered acceptable.

3. Overall summary conclusion

The risk to honey bees

- Unacceptable risks to bees cannot be excluded for dust drift of imidacloprid treated seed of wheat and barley as well as for the application of granules, whereas the risk of dust drift is considered low for hand spread granules. ~~The treatment of seed potatoes or seeds of sugar beets with imidacloprid did not pose an unacceptable acute or chronic risk to honey bee colony development or survival in accordance with SANCO/10329/2002 rev 2 Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC and with ANNEX to SANCO/11803/2010 Rev. 4~~. However, the assessment of the potential systemic uptake via flowering weeds could not be finalised.

The risk to pollinators other than honey bees

- *Commercially used pollinators other than honey bees*
  For dust drift during sowing of cereals, a risk cannot be excluded for commercial pollinators such as *Bombus* and *Osmia* ~~in accordance with SANCO/10329/2002 rev 2 and with ANNEX to SANCO/11803/2010 Rev. 4~~.

- *Wildlife pollinators other than honey bees*
  Unacceptable risks for wild bumble bees and solitary wild bees due to the exposure to residues of imidacloprid via several exposure routes have been identified or cannot be excluded ~~in accordance with the 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).

B.9 Summary of new studies on the active substance imidacloprid considering the risk assessment of seed treatments uses

B.9.1 Introduction

In the Implementing Regulation No. 485/2013 published on 25th May 2013 the EU Commission amended the conditions of inclusion of the active substances clothianidin, thiamethoxam and imidacloprid. According to this regulation the following questions have to be addressed:

a) the risk to pollinators other than honey bees
b) the risk to honey bees foraging nectar or pollen in succeeding crops
c) the potential uptake via roots to flowering weeds
d) the risk to honey bees foraging on insect honey dew
e) the potential guttation exposure and the acute and the long-term risk to colony survival and development, and the risk to bee brood resulting from such exposure
f) the potential exposure to dust drift following drill and the acute and the long-term risk to colony survival and development, and the risk to bee brood resulting from such exposure
g) the acute and long term risk to colony survival and development and the risk to bee brood for honey bees from ingestion of contaminated nectar and pollen.

As the new data submitted by the applicant (Bayer CropScience) are intended to address these questions for existing, currently permitted, registrations a summary of these registrations is given here (refer to table 9.1-1). Full details of the currently registered uses of imidacloprid are given in the documents M-505297-01-1 and M-505302-01-1 submitted by the applicant.

Table 9.1-1: Summary of seed treatment uses of imidacloprid currently registered in Europe

Crop	Use rate of IMD (range) dose/unit	Use rate of IMD (range) dose/grain	Use rate of IMD (range) dose g a.s./ha	Countries where registered
Winter cereals	27 – 70 g a.s./ 100 kg	0.006 – 0.043 mg a.s./grain**	48 – 126	FRA, POL, HUN, ROM, CZE
Beet	15 – 90 g a.s./U	0.15 – 0.9 mg a.s./grain	15 – 117 (135, 162)[a]	BEL, CZE, DNK, FIN, FRA, GRE, POL, SVK, DEU, SWE, ESP, ITA
Potato	No data#	No data#	120 – 180	AUS, BUL, CZE, DEU, DNK, ESP, EST, FIN, HUN, LAT, LIT, POL, ROM, SWE, SVK
Leafy vegetables[1] (outdoor)	114 g a.s./U	1.14 mg a.s./grain	80 – 104	BEL
Leafy vegetable[2] (greenhouse)	80 – 150 g a.s./U	0.8 – 1.50 mg a.s./grain	90 – 120	BEL, NLD
Turf*	No data#	No data#	150	DNK, DE, IRE, ESP, SWE, UK

* granulate
** calculated with a thousand seed weight of 21 - 61 g
U (1 unit) = 100 000 seeds
due to the application technique it is not possible to determine the amount of imidacloprid applied
[1] lettuce, endive; [2] brassica, lettuce, endive, radicchio
[a] This values are not considered as representative, these are extreme values relevant for an individual country (135 g a.s./ha= ITA; 162 g a.s./ha= ESP).

B.9.2 Effects on bees (Laboratory, Exposure, Tier I, Tier II) ~~Acute toxicity~~

B.9.2.1 Acute toxicity (Laboratory)

Honey bees

In the first EU evaluation of imidacloprid (2008) it was concluded that technical and formulated imidacloprid is highly toxic to honey bees. Since then, a number of new acute toxicity studies with other formulations have been conducted. This studies showing in some cases higher toxicity than studies with the technical substance. As the difference between the technical substance and the formulation is within a factor of five it was decided at the Pesticides Peer Review 145 Ecotoxicology meeting to still use the data of the technical substance for further

calculations. It was also considered that the data on formulations provide some indications of a complete different toxicity profile of products compared to the technical substance.

Table 9.2-1 presents a summary of all new submitted honey bee acute toxicity studies. Further details regarding these tests are provided in section B.9.5.1. For further calculations the table also presents the agreed endpoints of the first EU evaluation of imidacloprid (2008).

Table 9.2-1: Summary of the acute oral and contact toxicity of imidacloprid to honey bees

Test substance	Test organism	Exposure route	LD_{50}	EU agreed endpoint	Reference
Imidacloprid (active substance)	Honey bee	oral 48 h	0.0037 µg IMD/bee	Yes	Conclusion on the peer review of imidacloprid EFSA Scientific Report (2008) 148, 1-120
		contact 48 h	0.081 µg IMD/bee		
Confidor SC 200 (200 g a.s./L)		oral 48 h	0.0056 µg IMD/bee	Yes	
		contact 48 h	0.042 µg IMD/bee		
Imidacloprid FS 350 (350 g a.s./L)		oral 96 h	0.0244 µg IMD/bee	New data	Sekine, T. 2014 Report no.: 89281035
		contact 96 h	0.0476 µg IMD/bee		
Clothianidin + Imidacloprid FS 275 (100 + 175 g a.s./L)		oral 48 h	0.058 µg prod./bee (0.005 µg CTD + 0.009 µg IMD/bee)	New data	Schmitzer, S. 2014a Report no.: 89691035
		contact 48 h	0.29 µg prod./bee (0.026 µg CTD + 0.046 µg IMD/bee)		
Imidacloprid + Pencycuron FS 370 (120 + 250 g a.s./L)		oral 96 h	0.96 µg prod./bee (0.10 µg IMD/bee)	New data	Schmitzer, S. 2014b Report no.: 89661035
		contact 96 h	0.38 µg prod./bee (0.040 µg IMD/bee)		

CTD Clothianidin; IMD Imidacloprid

No new laboratory tests on chronic toxicity, effects on bee brood and sub-lethal effects of imidacloprid to honey bees were submitted as these issues are not the subject of the present document. However, more information on these topics are available in the first EU review of imidacloprid (DAR on Imidacloprid (2005); EFSA Scientific Report (2008) 148, 1-120), in the more recent evaluation of EFSA (EFSA Journal 2013;11(1):3068) and listed in table 9.2-3 and table 9.4-2.
Furthermore, additional information regarding effects of imidacloprid on honey bee brood taken from population assessments and sub-lethal effects as foraging and flight behaviour, food storage or colony development to honey bees have been submitted as part of the higher tier studies (refer to section B.9.5.2.).

Bumble bees

At the moment, there are no agreed guidelines for testing the toxicity of pesticides to bumble bees. However, acute effects of imidacloprid to bumble bees have been addressed in the first EU review on imidacloprid (2008) by laboratory studies with a number of imidacloprid containing formulations. Here the RMS concluded that based on NOED bumble bees show a somewhat lower species susceptibility to imidacloprid compared to honey bees.
Since then, a number of new acute contact toxicity studies have been conducted. The results of this study are listed in the table below (refer to table 9.2-2). Further details regarding the tests are provided in section B.9.5.1.

Table 9.2-2: Summary of the acute contact toxicity of imidacloprid to bumble bees

Test substance	Test organism	Exposure route	LD_{50}	EU agreed endpoint	Reference
Imidacloprid (active substance)	Bumble bee	oral 96 h	0.038 µg IMD/bee	Yes	Conclusion on the peer review of the pesticide risk assessment for bees for the active substance imidacloprid considering all uses other than seed treatments and granules, EFSA Journal 2015; 13(8):4211
		contact 96 h	0.218 µg IMD/bee		

		contact 72 h	> 0.05 < 0.1 µg IMD/bee*	Yes	Conclusion on the peer review of imidacloprid EFSA Scientific Report (2008) 148, 1-120
Imidacloprid FS 350 (350 g a.s./L)		contact 96 h	85.3 µg IMD/bee	New data	Pfeiffer, S. 2014a Report no.: S13-05153
Clothianidin + Imidacloprid FS 275 (100 + 175 g a.s./L)		contact 72 h	54.9 µg prod./bee (19.9 µg CTD + 35.0 µg IMD/bee)	New data	Pfeiffer, S. 2014b Report no.: S13-05151
Imidacloprid + Pencycuron FS 370 (120 + 250 g a.s./L)		contact 96 h	270 µg prod./bee (28.1µg IMD/bee)	New data	Pfeiffer, S. 2014c Report no.: S13-05154

CTD Clothianidin; IMD Imidacloprid
* could not be accurately determined

No new laboratory tests on chronic toxicity, effects on bumble bee brood and sub-lethal effects of imidacloprid to bumble bees were submitted as confirmatory data.
However, more information on these topics are available in the first EU review of imidacloprid (EFSA Scientific Report (2008) 148, 1-120), in the recent EFSA evaluation (EFSA Journal 2013;11(1):3068) and listed in table 9.2-3 and table 9.4-2.
Furthermore, additional information regarding effects on bumble bee brood and sub-lethal effects of imidacloprid to bumble bees have been submitted as part of the new higher tier studies (refer to section B.9.5.2).

Solitary bees
No laboratory test on solitary bees has been submitted by the applicant. Thus, the RMS follows the instruction of the EFSA Guidance Document on bees to extrapolated from the endpoint for honey bee by using a factor of 10. These calculated endpoints can be found in table 9.2-3 and table 9.4-2, respectively.

B.9.2.2 Exposure

The recommended use pattern for imidacloprid includes application as a seed treatment in winter cereals, sugar beet, potato and leafy vegetables at a maximum application rate of up to 180 g a.s./ha and as a granulate application in turf with a maximum application rate of 150 g a.s./ha (please refer to table 9.1-1).

Bees may be exposed orally to residues from systemic compounds present in pollen, nectar honey dew, guttation fluid or to product dust drift in the field margin or adjacent crops during sowing resulting to oral and contact exposure.

Information on the specific route of exposure to honey bees to be checked are given in table 9.2-2b. Information regarding bumble bees and solitary bees on this issue can be found in section B.9.4.1 part "Dust drift and the risk to pollinators other than honey bees, commercially used".

Table 9.2-2b: Route of exposure to honey bees to be checked in relation to the recommended use pattern

Crop	Route of exposure to be checked					
	Pollen and nectar			Honey dew	Guttation	Dust***
	Crop	Succeeding crop	Weeds in the field			
Winter cereals	Yes-for pollen[1]	Yes	No* - Yes**	No	Yes	Yes
Sugar beet	No[2]	Yes	No* - Yes**	No	Yes	Yes[5]
Potato	Yes-for pollen[3]	Yes	No* - Yes**	Yes	Yes	No[6]
Leafy vegetables	No[4]	Yes	No* - Yes**	No	Yes	Yes
Turf	No	Yes	No* - Yes**	No	Yes	Yes[7]

* during sowing (because weeds will not be present in the field when the crop is sown)
** grown after sowing
*** dust drift in the field margin or adjacent crops

[1] PPR meeting 145: "The attractiveness of cereals was further analysed by van der Steen et al. in 2015. This analysis is based on a literature review and experts judgment. Here cereals were reported as not attractive. However, the paper is in Dutch and not available to other MSs e.g. not peer reviewed. Therefore EFSA identified an open point for the RMS to provide the Tier I risk assessment for pollen."

[2] The experts considered in the PPR meeting 145 that a specific treated crop scenario should be developed for bi-annual crop. However, for the use under evaluation, it was concluded that this scenario is not relevant if the beets are not grown for seed production.

[3] Potato are not considered attractive to honey bees for the consumption of pollen by the EFSA Guidance Document on bees. However, data were provided by Denmark during PPR Meeting 129 indicating that honey bees collect pollen from potatoes. Therefore, the risk from the consumption of pollen will be assessed.

[4] As currently no flowering vegetables are registered in the EU (only lettuce and endive) no assessment has to be performed in the scope of this addendum.

[5] Following the EFSA Guidance Document on bees, the risk from treated sugar beet seeds is acceptable. However, at the PPR Meeting 145, it was considered necessary to include the Tier 1 risk assessment.

[6] due to the type of application (in-planter or in-furrow)

[7] PPR meeting 145: "For the granular formulation in areas such as golf-tees and sport fields, the RMS considered as not attractive i.e. only grass and no considerable flowering weeds present. The risk for hand held applications was considered low for all the scenarios. For machinery application the field margin scenario is considered relevant. However, no data are available for granule dust drift."

B.9.2.3 First and second tier risk assessment

The risk assessment was performed following the risk assessment scheme for honey bees as proposed in the EFSA Guidance Document on bees. Due to the potential risk to honey bees from imidacloprid, the screening steps were not performed, and the risk assessment started at the first tier.

With regard to contact toxicity following dust drift the Guidance Document "Draft Authorisation of Plant Protection Products for Seed Treatment" (SANCO/10553/2012, January 2014) will be used for the risk assessment.

Table 9.2-3: Toxicity endpoints used for the following risk assessment

Risk assessment type	Endpoint	Honey bees	Bumble bees	Solitary bees
Acute contact	LD_{50} (µg a.s./bee)	0.081 (48h)	0.218 (96h)	0.0081***

Risk assessment type	Endpoint	Honey bees	Bumble bees	Solitary bees
Acute oral	LD_{50} (µg a.s./bee)	0.0037 (48h)	0.038 (96h)	0.00037***
Chronic (oral)	10-day LDD_{50} (µg a.s./bee/day)	> 0.00282*	> 0.000282***	> 0.000282***
Larval	NOEC (µg a.s./larva) 7days (=22days)	0.00528 as provisional**	No endpoint available or extrapolated	No endpoint available or extrapolated
Development of hypopharyngeal glands	NOEC hpg (µg a.s./bee/day)	No endpoint available	Not applicable	Not applicable

*: Endpoint set at the highest concentration tested
**: Endpoint determined at 7 days but only 3 day exposure during the study. Endpoint is the highest dose tested. Endpoint is based on nominal amount of food offered to the larvae.
***: Extrapolated from the endpoint for honey bee by using a factor of 10.

a) The risk to pollinators other than honey bees

The first and second tier risk assessment for bumble bees and solitary bees can be found in section B.9.4.1 (“Pollinators other than honey bees (wild pollinators)”).

b) The risk to honey bees foraging nectar or pollen in succeeding crops

According to the EFSA Guidance Document on bees, the following formulae should be used to determine the Exposure Toxicity Ratio (ETR) for acute adult oral exposure, chronic adult oral exposure and chronic exposure to larvae, for product applied as seed treatment in the first tier risk assessment:

The relevant shortcut values are presented in Table J6 of Appendix J of the EFSA Guidance Document. The shortcut values for crops attractive for both pollen and nectar are considered. The relevant exposure factor E_f is presented in Appendix X of the EFSA Guidance Document.

ETR for the **acute adult oral** exposure:

$$ETR_{acute\ adult\ oral} = \frac{AR * E_f * SV}{LD_{50oral}}$$

AR = application rate in kg a.s./ha
SV = shortcut value for acute exposure to forager honey bees (0.70= Appendix J, Table J6)
E_f = exposure factor (1= taken from Appendix X)

Note: If the ETR is > 0.2 a potential risk is identified and a higher tier risk assessment should be performed. If the ETR is below this trigger, the risk is acceptable.

ETR for the **chronic adult oral** exposure:

$$ETR_{chronic\ adult\ oral} = \frac{AR * E_f * SV * twa}{LDD_{50}}$$

AR = application rate in kg a.s./ha
SV = shortcut value for chronic exposure to forager honey bees (0.54= Appendix J, Table J6)
twa = 1
E_f = exposure factor (1= taken from Appendix X)

Note: If the ETR is > 0.03 a potential risk is identified and a higher tier risk assessment should be performed. If the ETR is below this trigger, the risk is acceptable.

ETR for **larvae**:

$$ETR_{larvae} = \frac{AR * E_f * SV * twa}{NOED}$$

AR = application rate in kg a.s./ha
SV = shortcut value for exposure to honey bee larvae (0.40= Appendix J, Table J6)
twa = 1
E_f = exposure factor (1= taken from Appendix X)

Note: If the ETR is > 0.2 a potential risk is identified and a higher tier risk assessment should be performed. If the ETR is below this trigger, the risk is acceptable.

ETR for **hypopharyngeal glands** (HPG):

As there is currently no validated methodology for the assessment of sub- lethal effects, no endpoint for the effects on the HPG of honey bees is available for imidacloprid. Therefore, the first tier risk assessment for honey bees based on HPG is not possible yet.

The first tier risk assessment has been performed using the highest and lowest authorized application rate for winter cereals, beets, potato, leafy vegetables and turf (see table 9.2-4). The relevant toxicity endpoints are taken from table 9.2-3. The calculated tier 1 ETR values are shown in table 9.2-5.

Table 9.2-4: Lowest and highest authorized application rate of imidacloprid

Crop	Application rate (kg IMD/ha)
Winter cereals	0.048 – 0.126
Sugar beet	0.015 – 0.117
Potato	0.12 – 0.18
Leafy vegetables[1]	0.08 – 0.104
Leafy vegetable[2]	0.09 – 0.12
Turf	0.15

[1] outdoor: lettuce, endive; [2] greenhouse: brassica, lettuce, endive, radicchio

Table 9.2-5: Tier 1 ETR calculations for acute adult oral, chronic adult oral and larval exposure for the lowest and highest authorized application rate of imidacloprid (consumption of pollen and nectar from succeeding crops)

Acute adult oral exposure								
Crop	Application rate (kg a.s./ha)		E_f	SV	twa	$LD_{50\ oral}$ (µg a.s./bee)	ETR	Trigger
Winter cereals	Lowest	0.048	1	0.70	-	0.0037	9.1	0.2
	Highest	0.126					23.8	
Sugar beet	Lowest	0.015					2.8	
	Highest	0.117					22.1	
Potato	Lowest	0.12					22.7	
	Highest	0.18					34.1	
Leafy vegetables[1]	Lowest	0.08					15.1	
	Highest	0.104					19.7	
Leafy vegetables[2]	Lowest	0.09					17.0	
	Highest	0.12					22.7	
Turf	0.15						28.4	
Chronic adult oral exposure								
Crop	Application rate (kg a.s./ha)		E_f	SV	twa	LDD_{50} (µg a.s./bee/day)	ETR	Trigger
Winter cereals	Lowest	0.048	1	0.54	1	> 0.00282	< 9.2	0.03
	Highest	0.126					< 24.1	
Sugar beet	Lowest	0.015					< 2.9	
	Highest	0.117					< 22.4	
Potato	Lowest	0.12					< 23.0	
	Highest	0.18					< 34.5	
Leafy vegetables[1]	Lowest	0.08					< 15.3	
	Highest	0.104					< 19.9	
Leafy vegetables[2]	Lowest	0.09					< 17.2	
	Highest	0.12					< 23.0	
Turf	0.15						< 28.7	
Larval exposure								
Crop	Application rate (kg a.s./ha)		E_f	SV	twa	NOED (µg a.s./larva /development period)	ETR	Trigger
Winter cereals	Lowest	0.048	1	0.40	1	0.00528	3.6	0.2
	Highest	0.126					9.5	
Sugar beet	Lowest	0.015					1.1	
	Highest	0.117					8.9	
Potato	Lowest	0.12					9.1	
	Highest	0.18					13.6	
Leafy vegetables[1]	Lowest	0.08					6.1	
	Highest	0.104					7.9	
Leafy vegetables[2]	Lowest	0.09					6.8	
	Highest	0.12					9.1	
Turf	0.15						11.36	

[1] outdoor: lettuce, endive; [2] greenhouse: brassica, lettuce, endive, radicchio

As all ETR values exceed the relevant trigger values, a potential risk is identified for all honey bee developmental stages and for all uses. Further consideration is thus necessary.

Tier 2 risk assessment

The EFSA Guidance Document on bees suggests a number of options to refine the tier 1 risk assessment. For these refinements further data are required. For example, the shortcut values (SV), which are used for the estimation of the oral exposure via nectar and pollen consumption at first tier, could be refined with valid compound or crop specific residue data.

The applicant submitted a number of studies considering both natural residues and forced studies with exposure with an artificially applied plateau. As discussed in the experts meeting (PPR 145), the natural aged residue studies are considered acceptable, and residue values suitable for use in the risk assessment were selected. It was noted that the number of trials and their representativeness was not sufficient to allow an assessment of the 90th percentile. Thus, it was agreed to use the highest residue values from these trials for the exposure assessments. These values are 2.5 µg a.s./kg for pollen and 3.5 µg a.s./kg for nectar (table 9.3-2b).

In the experts meeting (PPR 145) it was decided to use the SHVAL calculation which is a tailored made MC tool developed by EFSA to refined the SVs. First, two “test” calculations were made to check whether the tool, the PC and the user perform well. Later on a 3rd test run was done. In these tests the same input parameters were used than the ones that had been used for the tier 1 calculations for HB nurse, HB larva and HB forager chronic for the seed dressing use.

The SHVAL tool requires to insert the natural logarithm form of residue data expressed in mg/kg. Therefore, these were calculated before running the model, as:

Relevance	Residue level in mg/kg	Ln
Test	1	0
IMD pollen	0.0025	-5.99146
IMD nectar	0.0035	-5.65499

As a summary, the following input parameters were inserted in the SHVAL tool for the different calculations:

Bee type & category	Pollen consumption in mg/bee/day or mg/larvae	Sugar consumption in mg/bee/day or mg/larvae	Sugar content of nectar in mg/mg	Chemical concentration		Relevance
				Pollen	Nectar	
HB nurse	12	34-50	0.15	0	0	
HB forager chronic	0	32-128	0.15	0	0	Test
HB larva	2	59.4	0.15	0	0	
HB forager acute	0	80-128	0.15	-5.99146	-5.65499	
HB forager chronic	0	32-128	0.15	-5.99146	-5.65499	IMD
HB larva	2	59.4	0.15	-5.99146	-5.65499	

The calculated refined SVs were the following:

Bee type & category	Tier 2 SV (µg/bee or µg/bee/day or µg/larva)	Relevance	Comment
HB nurse	**0.293**		Expected value was 0.29
HB forager chronic	**0.540**	Test	Expected value was 0.54
HB larva	**0.398**		Expected value was 0.4
HB forager acute	**0.00244**		
HB forager chronic	**0.00189**	IMD	
HB larva	**0.00139**		Value was confirmed by 'hand' calculation (as no variability in input parameters)

Conclusion
The tier 2 SVs for imidacloprid are more than 2 orders of magnitude lower than the tier 1 SVs considering the residue levels of 2.5 µg a.s./kg and 3.5 µg a.s./kg in the pollen and nectar of the succeeding annual crop.

Since the used residue values are not RUD values, but they were considered as representative for the uses under evaluation, the refined SVs should be used in the refined RAs without considering the application rate of the primary crop (i.e. these SVs can be considered as representative for any GAP, provided that the crop rotation and the ageing processes leading to a certain $PEC_{plateau}$ is considered representative). Additionally, both the E_f and the twa values are supposed to be 1 in the RAs for these scenarios.

Therefore, the formula to be used can be simplified as:

$$ETR = \frac{SV}{\text{Toxicity endpoint}^{1}}$$

SV= shortcut value for acute exposure to forager honey bees (0.00244)
shortcut value for chronic exposure to forager honey bees (0.00189)
shortcut value for exposure to honey bee larvae (0.40)

[1]= $LD_{50,oral}$ (0.0037 µg a.s./bee)
LDD_{50} (> 0.00282 µg a.s./bee/day)
NOED (0.00528 µg a.s./larva /development period)

Using this formula the risk quotients for imidacloprid are the following:

Bee type & category	**Tier 2 SV** (µg/bee or µg/bee/day or µg/larva)	**Toxicity endpoint**	**ETR**	**Trigger**
HB forager acute	0.00244	0.0037	**0.7**	> 0.2
HB forager chronic	0.00189	> 0.00282	**< 0.7**	> 0.03
HB larva	0.00139	0.00528	**0.3**	> 0.2

As all ETR values exceed the relevant trigger values, a potential risk is identified for all honey bee developmental stages and for all uses. Therefore, higher tier test are required.

c) The potential uptake via roots to flowering weeds

Theoretically residues in weeds in the treated field could also be a route of exposure to honey bees. However, as describe in the EFSA Conclusion on the risk assessment for bees for imidacloprid (2013) the risk via this route of exposure was considered to be negligible as weeds will not be present in the field when the crop is sown and considerable uptake via the roots is unlikely as the substance is concentrated around the seed. Therefore no first and second tier risk assessment was performed. Nevertheless, a data gap was identified to further address the potential uptake of imidacloprid via roots of flowering weeds growing shortly after sowing till harvest. Therefore the applicant submitted a statement in which the occurrence of flowering weeds in agricultural crops was evaluated (Garside C. M. et al, 2014). Further information regarding this statement are summarized in section B.9.4 and section B.9.5.2 (the potential uptake via roots to flowering weeds), respectively.

d) The risk to honey bees foraging on insect honey dew

No assessment of the risk to bees from honey dew is proposed in the current EFSA Guidance Document on bees because the available information was not sufficient to produce a robust risk assessment scheme for this exposure route. Therefore no first and second tier risk assessment was performed. However, to estimate the potential risk for honey bees foraging on honey dew the applicant submitted two statements which were summarised in section B.9.4.4.

e) The potential guttation exposure and the acute and the long-term risk to colony survival and development, and the risk to bee brood resulting from such exposure

The the first and second tier risk assessment will be supplemented by EFSA.

f) The potential exposure to dust drift following drill and the acute and the long-term risk to colony survival and development, and the risk to bee brood resulting from such exposure

Note: Generally, the first and secound tier risk assessment in this document was performed following the EFSA Guidance Document on bees. However, at PPR Meeting 145, the draft SANCO guidance document for seed treatment (SANCO/10553/2012, January 2014) was considered to be the appropriate guidance document to calculate the dust drift exposure for the risk assessment instead the EFSA Guidance Document for bees. This descision based on more recent data that shows that the amount of active substance in the dust is strongly dependent on the seed quality (calculation basis of the SANCO document), more than on the application rate (calculation basis of the EFSA Guidance Document for bees).Therefore, the majority of the experts considered that SANCO document should be used in the exposure assessment, while the minority considered that the EFSA Guidance Document for bees, should be used as it is a final version and published.

For the present assessment it was decided in the PPR Meeting 145 to calculated the HQ/ETR by replacing f_{dep}*AR or AR*E_f by the PEC_{3D} values calculated on the basis of the SANCO guidance document for seed treatment (SANCO/10553/2012, January 2014). The deposition values presented in SANCO document were standardized for a certain amount of seeds/ha (see table 10-2, section 10.5.2, SANCO document). Therefore, in a first step, these values have to be corrected according to the seed units given in the GAP table. The correction factors to be used in the exposure calculations for the use in winter cereals, sugar beet and leafy vegetables are shown in table 9.2-6. Based on the corrected Heubach values and the content of a.s. in dust, the Heubach a.s. value was calculated for the lowest and highest application rate of imidacloprid (table **9.2-7**) and then extrapolated to the PEC_{2D} and PEC_{3D}, respectively (table **9.2-8**).

Table 9.2-6: Calculation of the correction factors to be used in the Heubach a.s. value calculation.

Crop		According to GAP		According to SANCO	Correction factor
		Application rate (g a.s./ha)	Seed units (kg seeds/ha)	Seed units (kg seeds/ha)	
Winter cereals	Lowest	48	178	180	**0.99**
	Highest	126	180	180	**1**
Beet	Lowest	15	100,000	100,000	**1**
	Highest	117	130,000	100,000	**1.3**
Leafy vegetables	Lowest	80	100,000	100,000*	**1**
	Highest	104	130,000	100,000*	**1.3**

* as no data were avaible for leafy vegetables the values generated for beet were used

Table 9.2-7: Calculation of the Heubach a.s. value to be used in the PEC_{2D} and PEC_{3D} calculation.

Crop	Application rate (g a.s./ha)		Regulatory scenario***	Heubach value (g dust/ha)		Content of a.s. in dust (%)*	Heubach a.s. value (g a.s. in dust/ha)
				SANCO*	Corrected**		
Winter cereals	Lowest	48	Reference value	2	1.98	10	**0.198**
			Worst case	3	2.97	25	**0.74**
	Highest	126	Reference value	2	2	10	**0.2**

			Worst case	3	3	25	**0.75**
Beet	Lowest	15	Reference value	0.05	0.05	2	**0.001**
			Worst case	0.1	0.1	10	**0.01**
	Highest	117	Reference value	0.05	0.065	2	**0.0013**
			Worst case	0.1	0.13	10	**0.013**
Leafy vegetables	Lowest	80	Reference value	0.05	0.05	2	**0.001**
			Worst case	0.1	0.1	10	**0.01**
	Highest	104	Reference value	0.05	0.065	2	**0.0013**
			Worst case	0.1	0.13	10	**0.013**

* SANCO/10553/2012, January 2014 (section 10.5.2, table 10-2)

using the correction factor from table 9.2-6 * quality parameters regarding the seed treatment

Table 9.2-8: Calculation of the PEC_{2D} and PEC_{3D}

Crop	**Application rate (g a.s./ha)**		**Regulatory secenario**	**Heubach a.s. value (g a.s. in dust/ha)**	PEC_{2D} dust deposition (g a.s./ha)	**PEC_{3D} dust deposition (g a.s./ha)**
Winter cereals	Lowest	48	Reference value	0.198	0.099	**1.29**
			Worst case	0.74	0.37	**4.81**
	Highest	126	Reference value	0.2	0.1	**1.3**
			Worst case	0.75	0.375	**4.88**
Beet	Lowest	15	Reference value	0.001	0.02	**0.26**
			Worst case	0.01	0.2	**2.6**
	Highest	117	Reference value	0.0013	0.026	**0.34**
			Worst case	0.013	0.26	**3.38**
Leafy vegetables	Lowest	80	Reference value	0.001	0.02	**0.26**
			Worst case	0.01	0.2	**2.6**
	Highest	104	Reference value	0.0013	0.026	**0.34**
			Worst case	0.013	0.26	**3.38**

PEC_{2D}= Heubach a.s. value * *crop-specific deposition factor*
PEC_{3D}= PEC_{2D} * *3D extrapolation factor*

Crop-specific deposition factor (from table 10-3; section 10.5.2, SANCO document): For **cereals**, this crop specific deposition factor was determined to be **0.5**. For **sugar beet**, the data available when the SANCO Guidance Document was drafted was not sufficient to determine a general deposition factor. A reference PEC_{2D} value of 0.02 g a.s./ha was derived from one study instead, which is a factor 20 higher than the Heubach a.s. value for this scenario. Therefore, as a conservative approach, the same factor of **20** was used to calculate a PEC_{2D} value for the worst-case scenario.

Extrapolation factor (from table 10-4; section 10.5.2, SANCO document): According to SANCO/10553/2012 (Version January 2014), it has been shown that species living or foraging in 3-dimensional structures like hedgerows, trees or other crops are exposed to higher deposition rates of contaminated dust than species living on the ground. To address this issue, an extrapolation factor between 2-D and 3-D deposition was derived. Based on the experimental results from several studies in different crops, a factor of **13** has been determined.

According to the requirements of the EFSA Guidance Document, the acute risk through contact exposure and the oral acute and chronic risk to adult bees as well as the larvae toxicity was assessed.

The relevant shortcut values are presented in Table J7 of Appendix J of the EFSA Guidance Document.
As stated in table 9.2-2b, only exposure to dust drift in the field margin and adjacent crops is considered relevant. As exposure in the latter will be lower than in field margins, the risk assessment was only performed for field margins.

HQ for the **acute adult contact** exposure:

$$HQ_{acute\ adult\ contact} = \frac{f_{dep} * AR}{LD_{50contact}} \qquad \frac{\mathbf{PEC_{3D}}}{\mathbf{LD_{50contact}}}$$

AR = application rate in g a.s./ha
f_{dep} = fraction of the dose deposited
PEC_{3D} = predicted environmental concentration; refer to table 9.2-8 above

Note: If the HQ is > 14 a potential risk is identified and a higher tier risk assessment should be performed. If the HQ is below this trigger, the risk is acceptable.

ETR for the **acute adult oral** exposure:

$$ETR_{acute\ adult\ oral} = \frac{AR * E_f * SV}{LD_{50oral}} \qquad \frac{\mathbf{PEC_{3D} * SV}}{\mathbf{LD_{50oral}}}$$

AR = application rate in kg a.s./ha
E_f = exposure factor
SV = shortcut value for the acute exposure to forager honey bees (3.7= Appendix J, Table J7)
PEC_{3D} = predicted environmental concentration; refer to table 9.2-8 above

Note: If the ETR is > 0.2 a potential risk is identified and a higher tier risk assessment should be performed. If the ETR is below this trigger, the risk is acceptable.

ETR for the **chronic adult oral** exposure:

$$ETR_{chronic\ adult\ oral} = \frac{AR * E_f * SV * twa}{LDD_{50}} \qquad \frac{\mathbf{PEC_{3D} * SV * twa}}{\mathbf{LDD_{50}}}$$

AR = application rate in kg a.s./ha
E_f = exposure factor
SV = shortcut value for chronic exposure to forager honey bees (2.9= Appendix J, Table J7)
twa = 1
PEC_{3D} = predicted environmental concentration; refer to table 9.2-8 above

Note: If the ETR is > 0.03 a potential risk is identified and a higher tier risk assessment should be performed. If the ETR is below this trigger, the risk is acceptable.

ETR for **larvae**:

$$ETR_{larvae} = \frac{AR * E_f * SV * twa}{NOED} \qquad \frac{\mathbf{PEC_{3D} * SV * twa}}{\mathbf{NOED}}$$

AR = application rate in kg a.s./ha
E_f = exposure factor
SV = shortcut value for exposure to honey bee larvae (2.2= Appendix J, Table J7)
twa = 1
PEC_{3D} = predicted environmental concentration; refer to table 9.2-8 above

Note: If the ETR is > 0.2 a potential risk is identified and a higher tier risk assessment should be performed. If the ETR is below this trigger, the risk is acceptable.

The first tier risk assessment was performed using the highest and lowest authorized “maximum application rate”. The relevant toxicity endpoints are taken from table 9.2-3. As the PEC_{3D} was calculated assuming that pneumatic sowing machines equipped with pertinent devices ensuring

dust deflection to soil are used, the risk assessment is only valid for situations where this equipment is used. The calculated Tier 1 HQ values are shown in table 9.2-9. The ETR values are shown in table 9.2-10a, table 9.2-10b and table 9.2-10c, respectively.

Table 9.2-9: Tier 1 HQ calculations for acute adult contact exposure through dust drift for the lowest and highest authorized "maximum application rate"

Acute adult contact exposure							
Crop	Application rate (g a.s./ha)		Regulatory secenario	PEC_{3D} (g a.s./ha)	$LD_{50\ contact}$ (µg a.s./bee)	HQ	Trigger
Winter cereals	Lowest	48	Reference value	1.29	0.081	15.89	14
			Worst case	4.81		59.38	
	Highest	126	Reference value	1.3		16.05	
			Worst case	4.88		60.19	
Sugar beet	Lowest	15	Reference value	0.26		**3.21**	
			Worst case	2.6		32.1	
	Highest	117	Reference value	0.34		**4.17**	
			Worst case	3.38		41.73	
Leafy vegetables[1]	Lowest	80	Reference value	0.26		**3.21**	
			Worst case	2.6		32.1	
	Highest	104	Reference value	0.34		**4.17**	
			Worst case	3.38		41.73	

[1] lettuce, endive

For the uses in beet and leafy vegetables, the HQ value is below the trigger for the lowest and highest application rate if the assessment is based on reference values, which indicates that the risk is acceptable. However, if worst case dust deposition values are considered, the HQ value exceeds the trigger. For the highest application rate, the HQ value exceeds the trigger for both regulatory scenarios. For the use in winter cereals, the HQ values for both the lowest and highest 'maximum application rate' exceed the trigger, regardless of the regulatory scenario considered. Further consideration is thus needed.

Table 9.2-10a: Tier 1 ETR calculations for acute adult oral exposure from plants in the field margin for the lowest and highest authorized "maximum application rate"

Acute adult oral exposure									
Crop	Application rate (kg a.s./ha)		Regulatory secenario	PEC_{3D} (kg a.s./ha)	SV	twa	$LD_{50\ oral}$ (µg a.s./bee)	ETR	Trigger
Winter cereals	Lowest	0.048	Reference value	0.00129	3.7	-	0.0037	1.29	0.2
			Worst case	0.00481				4.81	
	Highest	0.126	Reference value	0.0013				1.3	
			Worst case	0.00488				4.88	
Sugar beet	Lowest	0.015	Reference value	0.00026				0.26	
			Worst case	0.0026				2.6	
	Highest	0.117	Reference value	0.00034				0.34	
			Worst case	0.00338				3.38	
Leafy vegetables[1]	Lowest	0.08	Reference value	0.00026				0.26	
			Worst case	0.0026				2.6	
	Highest	0.104	Reference value	0.00034				0.34	
			Worst case	0.00338				3.38	

[1] lettuce, endive

Table 9.2-10b: Tier 1 ETR calculations for chronic adult oral exposure from plants in the field margin for the lowest and highest authorized "maximum application rate"

Chronic adult oral exposure									
Crop	Application rate (kg a.s./ha)		Regulatory secenario	PEC_{3D} (kg a.s./ha)	SV	twa	LDD_{50} (µg a.s./bee/day)	ETR	Trigger
Winter cereals	Lowest	0.048	Reference value	0.00129	2.9	1	> 0.00282	1.32	0.03
			Worst case	0.00481				4.95	
	Highest	0.126	Reference value	0.0013				1.34	
			Worst case	0.00488				5.01	
Sugar beet	Lowest	0.015	Reference value	0.00026				0.27	
			Worst case	0.0026				2.67	
	Highest	0.117	Reference value	0.00034				0.35	
			Worst case	0.00338				3.48	
Leafy vegetables[1]	Lowest	0.08	Reference value	0.00026				0.27	
			Worst case	0.0026				2.67	
	Highest	0.104	Reference value	0.00034				0.35	
			Worst case	0.00338				3.46	

[1] lettuce, endive

Table 9.2-10c: Tier 1 ETR calculations for larval exposure from plants in the field margin for the lowest and highest authorized "maximum application rate"

Larval exposure									
Crop	Application rate (kg a.s./ha)		Regulatory secenario	PEC_{3D} (kg a.s./ha)	SV	twa	NOED (µg a.s./larva /development period)	ETR	Trigger
Winter cereals	Lowest	0.048	Reference value	0.00129	2.2	1	0.00528	0.54	0.2
			Worst case	0.00481				2.00	
	Highest	0.126	Reference value	0.0013				0.54	
			Worst case	0.00488				2.03	
Sugar beet	Lowest	0.015	Reference value	0.00026				**0.11**	
			Worst case	0.0026				1.08	
	Highest	0.117	Reference value	0.00034				**0.14**	
			Worst case	0.00338				1.41	
Leafy vegetables[1]	Lowest	0.08	Reference value	0.00026				**0.11**	
			Worst case	0.0026				1.08	
	Highest	0.104	Reference value	0.00034				**0.14**	
			Worst case	0.00338				1.41	

[1] lettuce, endive

All ETR values exceed the relevant trigger values, indicating a potential risk. Only for the chronic risk assessment for larval exposure, some ETR values are below the trigger. Thus, further consideration are necessary.

Tier 2 risk assessment

The EFSA Guidance Document on bees suggests a number of options to refine the first tier risk assessment. For these refinements further data are required. For several studies these measurements (Heubach values) were available. However, in the PPR meeting 145 it was

argued that individual studies with few varieties might be not sufficiently representative (and not sufficient to overrule the default values in SANCO 2015, which based on a larger dataset) as the amount of dust drift is very much dependent on the quality of the seed dressing rather than the properties of the a.s.. Therefore it was agreed that the available data are not suitable for tier 2 calculations.

g) The acute and long term risk to colony survival and development and the risk to bee brood for honey bees from ingestion of contaminated nectar and pollen.

According to the EFSA Guidance Document on bees, the following formulae should be used to determine the Exposure Toxicity Ratio (ETR) for acute adult oral exposure, chronic adult oral exposure and chronic exposure to larvae, for product applied as seed treatment in the first tier risk assessment:

The relevant shortcut values are presented in Table J6 of Appendix J of the EFSA Guidance Document. As there is a potential exposure to honey bees through the consumption of pollen from winter cereals and potatoes (see table 9.2-2b), the risk assessment was performed for the uses in these two crops. As both corps does not produce nectar, the shortcut values for crops attractive for pollen only are considered. The relevant exposure factor E_f is presented in Appendix X of the EFSA Guidance Document.

ETR for the **acute adult oral** exposure:

$$ETR_{acute\ adult\ oral} = \frac{AR * E_f * SV}{LD_{50oral}}$$

AR = application rate in kg a.s./ha
SV = shortcut value for nurse honey bees as proposed in the EFSA Guidance Document (0.012= Appendix J, Table J6)
E_f = exposure factor (1= taken from Appendix X)

Note: If the ETR is > 0.2 a potential risk is identified and a higher tier risk assessment should be performed. If the ETR is below this trigger, the risk is acceptable.

ETR for the **chronic adult oral** exposure:

$$ETR_{chronic\ adult\ oral} = \frac{AR * E_f * SV * twa}{LDD_{50}}$$

AR = application rate in kg a.s./ha
SV = shortcut value for nurse honey bees as proposed in the EFSA Guidance Document (0.012= Appendix J, Table J6)
Twa = 1
E_f = exposure factor (1= taken from Appendix X)

Note: If the ETR is > 0.03 a potential risk is identified and a higher tier risk assessment should be performed. If the ETR is below this trigger, the risk is acceptable.

ETR for **larvae**:

$$ETR_{larvae} = \frac{AR * E_f * SV * twa}{NOED}$$

AR = application rate in kg a.s./ha
SV = shortcut value for exposure to honey bee larvae (0.002= Appendix J, Table J6)
Twa = 1
E_f = exposure factor (1= taken from Appendix X)

Note: If the ETR is > 0.2 a potential risk is identified and a higher tier risk assessment should be performed. If the ETR is below this trigger, the risk is acceptable.

ETR for **hypopharyngeal glands** (HPG):

As there is currently no validated methodology for the assessment of sub- lethal effects, no endpoint for the effects on the HPG of honey bees is available for imidacloprid. Therefore, the first tier risk assessment for honey bees based on HPG is not possible yet.

The first tier risk assessment has been performed using the highest and lowest authorized application rate for winter cereals and potatoes (see table 9.2-4). The relevant toxicity endpoints are taken from table 9.2-3. The calculated tier 1 ETR values are shown in table 9.2-11.

Table 9.2-11: Tier 1 ETR calculations for acute adult oral, chronic adult oral and larval exposure for the lowest and highest authorized application rate of imidacloprid (consumption of pollen from treated crops)

Acute adult oral exposure								
Crop	Application rate (kg a.s./ha)		E_f	SV	twa	$LD_{50\ oral}$ (µg a.s./bee)	ETR	Trigger
Winter cereals	Lowest	0.048	1	0.012	-	0.0037	**0.16**	0.2
	Highest	0.126					0.41	
Potato	Lowest	0.12					0.39	
	Highest	0.18					0.58	
Chronic adult oral exposure								
Crop	Application rate (kg a.s./ha)		E_f	SV	twa	LDD_{50} (µg a.s./bee/day)	ETR	Trigger
Winter cereals	Lowest	0.048	1	0.012	1	> 0.00282	< 0.20	0.03
	Highest	0.126					< 0.54	
Potato	Lowest	0.12					< 0.51	
	Highest	0.18					< 0.77	
Larval exposure								
Crop	Application rate (kg a.s./ha)		E_f	SV	twa	NOED (µg a.s./larva /development period)	ETR	Trigger
Winter cereals	Lowest	0.048	1	0.002	1	0.00528	**0.02**	0.2
	Highest	0.126					**0.05**	
Potato	Lowest	0.12					**0.05**	
	Highest	0.18					**0.07**	

The ETR values for acute adult oral exposure in the lowest application rate of winter cereals and larval exposure in both winter cereals and potato are below the relevant trigger, indicating an acceptable risk.
For all remaining scenarios a potential risk was identified and further consideration is necessary.

Tier 2 risk assessment

The EFSA Guidance Document on bees suggests a number of options to refine the first tier risk assessment. For these refinements further data are required. For example, the shortcut values, which are used for the estimation of the oral exposure via nectar and pollen consumption at first tier, could be refined with valid compound or crop specific residue data. However, no new or appropriate informations on imidacloprid residues in winter cereal or potato pollen are available. Therefore it is not possible to perform a secound tier risk assessment.

B.9.3 Higher tier studies

In the first EU review on imidacloprid (2008) a total of 24 tent/field studies were conducted to investigate potential adverse effects of imidacloprid containing seed treatments of sunflower crops and oilseed rape crops to honey bees. In these test no adverse effects were recorded. However, EFSA reassessed these studies in 2013 and confirmed that no clear effects were observed, but indicated some potential effects on bees (e.g. increased mortality). Thus, uncertainties were identified both on the methodologies and the results of those studies.

Since then, according to the questions posed in the Implementing Regulation No. 485/2013 several new field studies with honey bees and bumble bees have been conducted. The main results of these investigations are briefly presented in the following sentence. Further details regarding these studies are provided in section B.9.5.2

a) The risk to pollinators other than honey bees

Two new higher tier studies with bumble bees were submitted. These studies examined the effects of potential exposure of bumble bees to residues of imidacloprid following the use of the active substance as an in-furrow application on potatoes.
An overview of the studies is presented in table 9.3-1. Further details regarding the tests are provided in section B.9.5.2.

Table 9.3-1: Summary of all new submitted bumble bee higher tier studies

* further endpoints (e.g. sugar consumption, weight of the hives) are reported in section B.9.5.2

b) The risk to honey bees foraging nectar or pollen in succeeding crops

In the first EU review on imidacloprid (2008) it was concluded that succeeding crop plants do not exhibit residue levels of imidacloprid (including the monohydroxy- and olefine-metabolites) higher than 2 ppb in nectar or pollen. However these studies have usually been performed under "forced" conditions where imidacloprid was specifically applied to the soil surface to create an artificial plateau and an untreated crop then sown (following variable time intervals). This situation is, however, not representative of the exposure situation under field conditions whereby any "accumulated" residues arising from multi-year use will have been exposed to natural aging processes in the soil. Considering that imidacloprid has been registered and used extensively over several years a more realistic study was performed.
In this approach the untreated succeeding crops were sown in soils with a history of several years use of imidacloprid, and hence to "natural residues" in the soil. These residues have undergone natural degradation and ageing and are therefore representative of the residues that can be expected after agricultural use of imidacloprid as a seed treatment.

The applicant has performed new studies considering both natural residues and forced studies with exposure with an artificially applied plateau.
For the "forced" studies the appropriate theoretical concentration of imidacloprid which could occur in a succeeding crop situation the possible crops which could be treated with imidacloprid and the potential rotations of these crops were elaborated. Imidacloprid is currently used as a seed treatment on cereals, sugar beet and potatoes. As the crop rotations may vary from country to country the applicant has performed a survey in a number of European countries and based on this survey the potential rotations were elaborated. However, the most critical rotation (considering use of neonicotinoids) was considered to be potato, cereals, cereals as all three crops could potentially be treated with a neonicotinoid product. Furthermore as imidacloprid is used in different formulations in the same crop two plateau concentrations were calculated, the first using the maximum rate for all relevant seed treated crops (H= high loading) while the second accounts for a lower use rate of the seed treatment formulations (L= low loading).

A summary of all studies with natural aged residues and forced plateau concentrations is reported below. Further details regarding the studies are provided in section B.9.5.2.

Test-organism/ substance	Endpoints*	Crop / Application rate / Exposure	Result	Ref.	Guideline
Bumble bee / Monceren G (active ingredient: 120 g IMD/L + 250 g pencycuron /L)	• Flight activity • Mortality • Population assessment	Potatoe 1.5 L product/ha In-furrow application at planting	Flight activity Crop (mean number of the flight): C= 3.8 bees/4 m^2/10 minutes T= 1.7 bees/4 m^2/10 minutes Mortality Adults/Larvae (mean exposure phase): C= 1.6 bees; 0.5 larvae T= 1.5 bees; 0.9 larvae Adults/Larvae (mean post-exposure phase): C=3.3 bees; 14.4 larvae T= 2.9 bees; 12.9 larvae Population assessment The results of the final brood evaluation did not show any statistically significant differences between the control and the test item treatment.	Klein, O.; 2014a; Report No.: S14-03553	OEPP /EPPO Guideline No. 170 (4), 2010
Bumble bee / Monceren G (active ingredient: 120 g IMD/L + 250 g pencycuron /L)	• Flight activity • Mortality • Population assessment	Potatoe 1.5 L product/ha In-furrow application at planting	Flight activity Crop (mean number of the flight): C= 0.9 bees/4 m^2/10 minutes T= 2.0 bees/4 m^2/10 minutes Mortality Adults/Larvae (mean exposure phase): C= 1.0 bees; 0.7 larvae T= 0.6 bees; 0.8 larvae Adults/Larvae (mean post-exposure phase): C=2.6 bees; 9.5 larvae T= 2.7 bees; 5.6 larvae Population assessment The results of the final brood evaluation showed a statistically significant difference in one out of all parameters assessed, a lower number of live young queen larvae. However, the number of live young queens and live	Klein, O.; 2014b; Report No.: S14-03554	OEPP /EPPO Guideline No. 170 (4), 2010

			queen pupae were higher in the test item treatment resulting in a total queen reproduction that was well above the reproduction in the control.		

Table 9.3-2a: Summary of concentration of imidacloprid detected in succeeding crops

Crop		**Pollen** [µg IMD/kg]				**Nectar** [µg IMD/kg]			
		No. of values > LOQ /Total	Mean	Median	90th percentile	No. of values > LOQ /Total	Mean	Median	90th percentile
Natural aged residues									
Phacelia		2/18	0.47	0.4	0.4	9/18	0.36	0.2	0.4
Winter OSR		2/15	0.49	0.4	0.7	3/15	0.22	0.2	0.3
Maize		10/18	0.46	0.4	0.8	-	-	-	-
Model studies with artificially applied plateau									
Mustard	H	9/18	1.7	1.0	4.5	11/18	0.3	0.3	0.4
	L	14/18	1.8	1.4	3.8	15/18	1.8	3.8	0.6
Maize	H	6/18	0.3	0.2	0.9	-	-	-	-
	L	8/18	0.4	0.3	1.2	-	-	-	-
Phacelia	H	4/12	0.7	0.3	2.0	7/12	0.4	0.8	0.3
	L	1/12	0.3	0.3	0.4	5/12	0.3	0.2	0.4

For calculation of the mean, median and 90th percentile values, concentrations reported as <LOQ were assigned as 0.4 µg/kg for pollen and 0.2 µg/L for nectar (equal to mid-way between LOD and LOQ), all values reported as < LOD were assigned as 0 in the calculation).
H= high loading; L= low loading

Table 9.3-2b: Minimum and maximum concentration of imidacloprid detected in succeeding crops

Phacelia		**Maize**	**Winter OSR / Mustard***		**Reference**
Pollen	Nectar	Pollen	Pollen	Nectar	
[µg IMD/kg]					
Natural aged residues					
< LOQ	< LOQ - **3.5**	< LOD - < LOQ	-	-	Ythier, E. 2014a; M-504801-01-1
< LOQ - 1.5	< LOD - 0.4	< LOD - **2.5**	< LOQ	< LOQ - 0.3	Ythier, E. 2014b; M-504806-01-1
< LOQ - 1.2	< LOD - 0.4	0.64 - 0.91	-	-	Ythier, E. 2014c; M-504836-01-1
-	-	-	< LOQ - 1.3	< LOD - 0.7	Ythier, E. 2014d; M-504810-01-1
Model studies with artificially applied plateau					
1.9 - 2.0 (H) < LOQ - 0.6 (L)	0.8 - 1.0 (H) < LOD - < LOQ (L)	< LOQ - 0.93 (H) < LOQ - 1.2 (L)	1.6 - 4.7 (H) 1.8 - 5.1 (L)	< LOQ - 0.5 (H) 0.7 - 3.9 (L)	Ythier, E. 2014e; M-504842-01-1
< LOD- 0.62 (H) < LOD - < LOQ (L)	< LOD - 0.49 (H) < LOQ - 0.43 (L)	< LOD (H) < LOD (L)	< LOQ (H) < LOQ - 1.0 (L)	< LOD - 0.63 (H) < LOD - 0.57 (L)	Striffler, B., Ballhaus, F. 2014; M-504854-01-1

LOD; LOQ (pollen) = 0.2 µg IMD/kg ; 0.6 µg IMD/kg
LOD; LOQ (nectar) = 0.1 µg IMD/kg ; 0.3 µg IMD/kg
(H)= high loading; (L)= low loading
- = no data
*= model studies
Bold values: used for further calculations

c) The potential uptake via roots to flowering weeds

The potential uptake of neonicotinoid pesticides into flowering weeds, as route of exposure of bees, has been identified as a data gap in the first EU evaluation of imidacloprid (2008). Although the occurrence of weeds is not routinely assessed during trials performed with insecticides these data are available for efficacy trials of herbicides. During such trials the identity and occurrence of weeds in control and treated plots is assessed.

Data extracted from efficacy trials on herbicidal active ingredients were used to evaluate the potential occurrence (and relative importance) of flowering weeds in relevant seed treatment crops by the applicant. Therefore, only data from the control plots were analysed as this represented a worst case scenario and from this the potential relevance of flowering weeds for honey bees was determined considering uses in cereals, sugar beets, and potatoes. The condition where weeds are at BBCH stage ≥ 60 (flowering) and ≥ 10% ground cover was considered suitable to identify situations which have the potential to be attractive to foraging bees.

For cereals, flowering weeds exceeding 10% ground cover were only observed in 14 out of 2327 observations (i.e. 0.6 %) and out of these 14 only one was possibly relevant under certain circumstances, exposure via flowering weeds is confirmed not to be a relevant route of exposure for honey bees in this crop. In the trials with sugar beet and potatoes there were no flowering weeds present on the control plots, where no herbicide was used.

Further details regarding this evaluation are provided in section B.9.5.2.

d) The risk to honey bees foraging on insect honey dew

Instead of a study a statement paper written by Nauen et al. 2013 has been submitted by the applicant (refer to section B.9.5.2). It was concluded here that no resistance of aphids to neonicotinoids is known up to date. However, recently *Myzus persicae* was shown to have developed resistance to neonicotinoid insecticide sprays in peaches in southern Europe, based on a target-site mutation in the nicotinic acetylcholine receptor ß-subunit. No neonicotinoid resistance was detected from *M. persicae* on any secondary host species yet, including sugar beet and potatoes.

Additionally the applicant has submitted a statement to demonstrate that exposure to honeydew is negligible (see text below, in italic).

Honey dew

Honeydew is a sugar-rich sticky liquid, secreted by aphids and some scale insects which feed on phloem sap. This liquid is sugar-rich and has high water content, but is low in nitrogen. Consequently aphids must eat large quantities of phloem sap to get sufficient nitrogen. The aphid gut is therefore adapted so that sugar and water can quickly pass from foregut to hindgut then rectum avoiding passing through the midgut where amino acids are absorbed. The excreted liquid is commonly known as honeydew.

Need for sap feeding insect control

Deposits of honeydew on leaf surfaces can cause sooty mould growth which can be deleterious to plants in that they can indirectly damage the plant by coating the leaves to the point that it reduces or inhibits sunlight penetration affecting photosynthetic production.

In addition the presence of aphids (and other sap feeding pests) can be harmful to plants as heavy infestations can weaken plants due to feeding damage. However, the most important deleterious effect of aphid infestations is the transmission of disease causing viruses on the

aphid's stylets. Significant damage by virus transmission can be caused even by very light aphid infestations if virus transmission occurs. Hence aphid efficient control can be highly important to prevent the spread of many economically important virus diseases in winter cereal, beet and potato crops. Consequently it is economically important for the grower to ensure control of aphid pests on these crops.

Sap feeding insect control

Control is achieved by seed treatment by neonicotinoid insecticides and also by foliar sprays of various different effective classes of insecticides including neonicotinoids. Seed treatments can provide highly effective and timely control of insect pests especially during the crop establishment phase and due to the sensitivity of aphids to neonicotinoid insecticides and other strategies employed by growers aphid numbers are managed so as not to build up to large infestations which can provide a food source for honey bees. At later crop growth stages the concentrations of neonicotinoid may be much lower which may not be sufficient to control aphid pests or affect honey bees. However, at these later stages virus transmission is no longer the aim and it is the reduction of aphid numbers which could lead to reductions in crop yield.

Exposure of bees to residues of neonicotinoid in honey dew

There is a highly theoretical exposure scenario where aphids are able to feed from a seed treated plant and not be killed, but still to produce honeydew on which bees will forage. For this situation to happen, levels of neonicotinoid must be present in honey dew without killing the pest but also at levels which may harm honey bees at the colony level. This could only occur if the aphids were not killed by the insecticide treatment (i.e. resistant) which as described above would need to pass through gut of the pest and be present in honey dew at environmentally relevant concentrations. At present there are no documented cases of such resistance in aphids infesting crops which grown using neonicotinoid seed treatments.

Aphid resistance to Neonicotinoids

Neonicotinoid insecticides act on the insect nicotinic acetylcholine receptors (nAChR) via both contact and ingestion routes of administration the exposure route is ideal for targeted insect pest control. Imidacloprid and clothianidin are both neonicotinoid insecticides with the same mode of action (MOA) and belong to IRAC MOA class 4 A.

To date, the occurrence of resistance to this class of insecticide in aphid pests is rare. Moderate imidacloprid resistance in green peach aphid Myzus persicae collected in Greek tobacco has been reported but this is possibly an adaption to nicotine-containing tobacco plants. This metabolic mechanism also confers cross-resistance to other neonicotinoids such as clothianidin and thiamethoxam.

In 2011 target-site resistance in a M. persicae clone derived from a French field population collected in peach was first described (Slater et al 2011[1]). The R81T mutation provides resistance to all neonicotinoid insecticides tested. However similar mechanisms of resistance in M. persicae populations collected in any other crop such as for example cabbage and potatoes have not been described. No reports on neonicotinoid resistance mechanisms have been described in any of the other sucking, chewing and soil pests controlled by clothianidin and imidacloprid used as seed treatment, including thrips and all major aphid species occurring in cereals (e.g. Metapolophium dirhodum, Sitobion avenae and Rhopalosiphum padi)

[1] Slater, R, Paul, V.L, Andrews, M., Garbay, M., Camblin, P. (2011). Identifying the presence of neonicotinoid resistant peach-potato aphid (*Myzus persicae*) in the peach-growing regions of Southern France and northern Spain. *Pest Manag Sci*; **68**:634-638. DOI 10.1002/ps.2307

or in sugar beet such as Apis fabae. The peach-potato aphid is potentially of risk in potato cultivation but as already noted no such accounts have been documented for seed treatment uses or on potato crops for this species. In addition, anti-resistance strategies are in place which restrict the use of consecutive sprays with the same MOA and require the implementation of long-term rotation with insecticides with other MOAs. Furthermore, field performance is regularly monitored by growers and where performance is poor a repeat application with the same MOA is not permitted and an alternative class of insecticide must be used. Label instructions for anti-resistant management strategies can be crop and use specific and are hence on all product labels and adherence to them is mandatory.

The risk to honey bees foraging on insect honey dew – seed treatment uses
The risk of exposure of honey bees to neonicotinoid insecticides seed treatments via honey dew is considered to be low. The seed treatments themselves control the honey dew producing insects and hence no exposure can occur. At later stages of crop development when the levels of systemic insecticide have declined and no longer provide sap feeding insect control these levels are of low risk to bees. Also there is a large difference in size and body between aphids and honey bee foragers. Adult aphid body weights for cereal aphids and those found on beets such as Apis fabae are about 1 mg, with young aphids considerably smaller (Dixon and Kindlmann, 1994[2]). Honey bee foragers are approximately 100 – 120x larger and would be expected to be far less sensitive than aphid pests. Consequently when levels in the plant have fallen to those which do not affect aphids they would also not be expect to also impact honey bees. As there is no incidence of aphid resistance to a neonicotinoid insecticides seed treatment the risk of exposure to honey bees via honey dew produced by sap feeding insects is low. In addition, resistance management strategies are well known by growers and advisors and they are on labelled on all products. Furthermore, Bayer CropScience operates a product stewardship programme for its products.
Consequently, as sap feeding pests are controlled by neonicotinoid insecticides seed treatments, there are no current incidents of resistance to seed treatments (even after many years of use), and the implementation of anti-resistance strategies mean that the risk to bees foraging on honey dew is low.

e) The potential guttation exposure and the acute and the long-term risk to colony survival and development, and the risk to bee brood resulting from such exposure

This issue was not a subject of the first EU evaluation of imidacloprid (2008). At the moment, there are no agreed guidelines for testing the potential risk for honey bees from guttation drops of seed treated crops.

A total of seven field studies have been submitted. These studies cover the maximum use conditions for imidacloprid (IMD) seed treatment uses in winter cereals 70 g a.s./dt (126 g a.s./ha), beet crops 90 g/U (117 g a.s./ha) and potato 180 g a.s./ha, respectively.

The studies were conducted in Germany in different geographical locations (Northern, Central and Southern Germany) and over a period of years to ensure a wide range of natural and agricultural conditions. As winter cereals are sown in autumn there are potentially two guttation periods in which honey bees could be exposed; one in autumn, shortly after crop emergence and before overwintering and again in the spring after winter hibernation. Here the same

[2] Dixon, AFG, Kindlmann, P. (1994). Optimum body size in aphids. Ecological Entomology **19**, 121-126

colonies were exposed to both guttation periods. Sugar beets are drilled in the spring and hence have one guttation period during that time.
All studies were conducted under standard agricultural conditions with honey bee colonies sited at the edge of either fields sown with insecticide treated or untreated seed. The studies were set up to provide appropriate conditions so that there were no major flowering crops present within 3 km of the test locations and that there were no open water bodies within 300 m to the field to ensure that the colonies collected any water necessary for their needs from the immediate area as either guttation fluid, dew or rainfall.
Effects on bee colonies, with each five honey bee colonies per field, in a total of nine imidacloprid treated and nine untreated cereal fields. These colonies were located at the edge of each field during sowing.
For the sugar beet and potato studies, eight colonies were placed at the edge of each of the four fields (two treated and 2 untreated).

All studies investigated the following parameters:

- Occurrence and proportion of guttation of the (treated) crop and off-crop
- Observation of honey bees visiting the crop and off-crop areas
- Behaviour of the bees in the crop and around the hive
- Honey bee mortality (mean number of dead bees per colony per day)
- Condition of the colonies (e.g. colony strength, brood, food storage) and health status (e.g. presence and levels of *Varroa*, viruses and other pathogens)
- Overwintering performance of exposed colonies
- Levels of clothianidin residues and metabolites in guttation fluid.

An overview of the studies is presented in table 9.3-3. Further details regarding the tests are provided in section B.9.5.2.

Table 9.3-3: Overview of field studies to address the risk of residues in guttation fluid to honey bees

Test-organism/ crop	**Application rate/test item(s)**	**Colony exposure**	**Result**	**Ref.**
Honey bees/ winter wheat	Sowing rate: 200 kg seed/ha No. sites: IMD= 2 CTD= 2 Con= 2 1: 0.7 g IMD/kg seed 2: 0.5 g clothianidin /kg seed 3: control Colonies per site:	Placing of the colonies: Pre-sowing Duration: 2009 (autumn) - 2010 (spring)	Guttation freq.: 86.4 % autumn 87.9 % spring Coincides with bee flight: 72.7 % autumn 64.4 % spring Max. residues in guttation fluid mg/L: IMD $_{autumn}$ 6.9 IMD $_{spring}$ 0.2 CTD $_{autumn}$ 13.0 CTD $_{spring}$ 0.4 Overwintering success: IMD: 80 %	Hofmann, S.; Lueckmann, J.; 2014, Report No.: R09247-4

Test-organism/ crop	Application rate/test item(s)	Colony exposure	Result	Ref.
	IMD= 5 CTD= 2 Con= 5		CTD: 89 % Con: 86 % Behaviour: No effects Colony strength: No effects	
Honey bees/ winter barley	Sowing rate: 200 kg seed/ha No. sites: IMD= 2 CTD= 2 Con= 2 1: 0.7 g IMD/kg seed 2: 0.5 g clothianidin /kg seed 3: control Colonies per site: IMD= 5 CTD= 5 Con= 5	Placing of the colonies: Pre-sowing Duration: 2009 (autumn) - 2010 (spring)	Guttation freq.: 84.2 % autumn 80.7 % spring Coincides with bee flight: 46.6 % autumn 56.3 % spring Max. residues in guttation fluid mg/L: IMD $_{autumn}$ 15.0 IMD $_{spring}$ 0.1 CTD $_{autumn}$ 2.3 CTD $_{spring}$ 0.2 Overwintering success: IMD: 80% CTD: no data Con: 80 % Behaviour: No effects Colony strength: No effects	Hofmann, S.; Garrido, C.; Lueckmann, J.; 2012, Report No.: R09247-3
Honey bees/ winter barley	Sowing rate: 200 kg seed/ha No. sites: CTD+IMD = 5 Con= 5 1: CTD+IMD (175 + 100g a.s./L); 500 mL/dt 2: control Colonies per site: CTD+IMD = 5 Con= 5	Placing of the colonies: Pre-sowing Duration: 2011 (autumn) - 2012 (spring)	Guttation freq.: 100 % autumn 89.4 % spring Coincides with bee flight: 73.1 % autumn 69.1 % spring Max. residues in guttation fluid mg/L: IMD $_{autumn}$ 6.7 IMD $_{spring}$ 0.1 CTD $_{autumn}$ 8.5 CTD $_{spring}$ 0.2 Overwintering success: CTD+IMD: 67.9 % Con: 57.8 % Behaviour: No effects Colony strength: No effects	Hofmann, S.; Staffel, J.; Aumeier, P.; 2014; M-501261-01-1

Test-organism/ crop	Application rate/test item(s)	Colony exposure	Result	Ref.
Honey bees/ sugar beets	Sowing rate: 1.3 U/ha (3.55 kg seed/ha) No. sites: CTD+IMD= 1 Con= 1 1: CTD+IMD 0.6+0.3mg/pill 2: control Colonies per site: CTD+IMD= 8 Con= 8	Placing of the colonies: BBCH 12 Duration: 2013 (spring, 42 days)	Guttation freq.: 14.3 % spring Coincides with bee flight: Yes, but bees do not visit crop Max. residues in guttation fluid mg/L: IMD: 0.018 – 0.061 CTD: 0.035 – 0.057 Overwintering success: CTD+IMD: 100 % Con: 100 % Behaviour: No effects Colony strength: No effects	Rexer, H. U.; 2014; M-500724-01-1
Honey bees / sugar beets	Sowing rate: 1.3 U/ha (3.55 kg seed/ha) No. sites: CTD+IMD = 1 Con= 1 1: CTD+IMD 0.6+0.3mg/pill 2: control Colonies per site: CTD+IMD = 8 Con= 8	Placing of the colonies: BBCH 12 Duration: 2013 (spring, 40 days)	Guttation freq.: 35 % spring Coincides with bee flight: Yes, but bees do not visit crop Max. residues in guttation fluid mg/L: IMD: 0.003 – 0.01 CTD: 0.017 – 0.064 Overwintering success: CTD+IMD: 100 % Con: 100 % Behaviour: No effects Colony strength: No effects	Rexer, H. U.; 2014; M-500734-01-1
Honey bees /potato seed	Sowing rate: 1.5 L prod./ha= 180 g IMD No. sites: IMD= 1 Con= 1 1: 180 g IMD /L 2: control Colonies/site: IMD= 8 Con= 8	Placing of the colonies: BBCH 10 Duration: 2014 (spring, 57 days)	Guttation freq.: 60.3 % spring Coincides with bee flight: Yes, but bees do not visit crop Max. residues in guttation fluid mg/L: IMD: 0.032 – 0.791 Overwintering success: IMD = 100% Con = 100% Behaviour: No effects Colony strength: No effects	Rexer, H. U.; 2014; M-503349-03-1

Test-organism/ crop	Application rate/test item(s)	Colony exposure	Result	Ref.
Honey bees /potato seed	Sowing rate: 1.5 L prod./ha= 180 g IMD No. sites: IMD= 1 Con= 1 1: 180 g IMD /L 2: control Colonies/site: IMD= 8 Con= 8	Placing of the colonies: BBCH 10 Duration: 2014 (spring, 59 days)	Guttation freq.: 39.8 % spring Coincides with bee flight: Yes, but bees do not visit crop Max. residues in guttation fluid mg/L: IMD = 0.001 – 1.982 Overwintering success: IMD = 100 % Con = 100 % Behaviour: No effects Colony strength: No effects	Rexer, H. U.; 2014; M-503344-03-1

f) The potential exposure to dust drift following drill and the acute and the long-term risk to colony survival and development, and the risk to bee brood resulting from such exposure

This issue was not a subject of the first DAR in 2005 and the EU evaluation of imidacloprid (2008) and at the moment, there are no agreed guidelines for testing the potential risk from dust drift for honey bees.

A total of three field studies which investigate the drift of dusts during sowing of imidacloprid and clothianidin-treated seeds, have been submitted.

An overview of the studies is presented in table 9.3-4. Further details regarding the tests are provided in section B.9.5.2.

Table 9.3-4: Determinations of imidacloprid and clothianidin residues in dust drift deposits

Crop	Test-substance	Sampling method	Active substance content on filter paper	Ref.	Guideline
Winter barley	Sowing rate: 200 kg seed/ha 1: Manta Plus (0.7 g IMD /kg seed) 2: Smaragd forte (0.5 g CTD /kg seed)	Shortly after sowing: Petri-dishes (1, 3, 5 m)= 120 24h-following sowing: Petri-dishes (1m)= 159	**Shortly after sowing[1]:** **1m** max. : 0.045g a.s./ha 90th%tile: 0.037 g a.s./ha **3m** max. : 0.283g a.s./ha 90th%tile: 0.031 g a.s./ha **5m** max. : 0.272g a.s./ha 90th%tile: 0.027 g a.s./ha **24h- following sowing[1]:** max.: 0.026g a.s./ha 90th%tile: < LOD	Hofmann, S. 2010a, Report No.: R09247-1	91/414/EEC of July 15, 1991, SANCO/3029/99 Rev. 4, 2000-07-11
Winter barley	Sowing rate: 200 kg seed/ha 1: 0.2 g CTD + 0.35 g IMD/kg seed	Heubach analysis Gauze-netting-samplers (3 m)= 45 samples Petri-dishes (1, 3 m)= 180	**Heubach values:** Site1 0.097 g a.s./100 kg seed Site2 0.022 g a.s./100kg seed Site3 0.144 g a.s./100kg seed **Gauze-netting-samplers[2]:** **3m** max.: <LOQ 90th%tile: <LOQ **Petri-dishes[3]:** **1m** max.: 1.66g CTD/ha 90th%tile: 0.12g a.s./ha max.: 2.41g IMD/ha 90th%tile: 0.20g a.s./ha **3m** max.: 0.50 g CTD/ha 90th%tile: 0.07g a.s./ha max. 0.75g IMD/ha Residue level of all non-spiked control samples and soil samples were < LOD.	Lueckmann, J.; 2014a, Report No.: R11129	BBA Drift Guideline Part VII, 2-1.1

Crop	Test-substance	Sampling method	Active substance content on filter paper	Ref.	Guideline
Winter wheat	Sowing rate: 200 kg seed/ha 1: Manta Plus (0.7 g IMD /kg seed) 2: Smaragd forte (0.5 g CTD /kg seed)	Shortly after sowing: Petri-dishes (1, 3, 5 m)= 120 24h-following sowing: Petri-dishes (1m)= 160	**Shortly after sowing[1]:** **1m** max. : 0.034g a.s./ha 90th%tile: < LOQ **3m** max.: 0.030g a.s./ha 90th%tile: < LOQ **5m** max.: 0.258g a.s./ha 90th%tile: < LOQ **24h- following sowing[1]:** max.: 0.027g a.s./ha 90th%tile: < LOD Residue level of all non-spiked control samples and soil samples were < LOD.	Hofmann, S. Lueckmann, J.; 2010b, Report No.: R09247-2	91/414/EEC of July 15, 1991, SANCO/3029/99 Rev. 4, 2000-07-11

CTD Clothianidin; IMD Imidacloprid

[1] Petri-dishes: LOQ (Limit of quantification) = 0.014 g a.s./ha (imidacloprid, clothianidin)
LOD (Limit of detection) = 0.004 g a.s./ha (imidacloprid, clothianidin)

[2] Gauze samples: LOQ (Limit of quantification) = 0.04 g a.s./ha (imidacloprid, clothianidin)
LOD (Limit of detection) = 0.01 g a.s./ha (imidacloprid, clothianidin)

[3] Petri-dishes: LOQ (Limit of quantification) = 0.07 g a.s./ha (imidacloprid, clothianidin)
LOD (Limit of detection) = 0.02 g a.s./ha (imidacloprid, clothianidin)

In addition, two field studies which investigate on the risk of dust drift during and after sowing to honey bee colonies have been submitted.
An overview of the studies is presented in table 9.3-5. Further details regarding the tests are provided in section B.9.5.2.

Table 9.3-5: Overview of field studies to address the risk of residues in dust to honey bee colonies

Test-organism	Application rate	Observations	Result	Ref.
Honey bee/*Phacelia tanacetifolia* (full flowering)	Sowing rate (winter barley): 200 kg/ha 1: Imidacloprid FS 350A G (0.7 g IMD /kg seed) 2: Control	A: Honey bee mortality and behaviour B: Population development and health assessment C: Gauze-netting-samplers (3 m) D: Heubach analysis	A: no test item related effect B: no statistical differences C: Gauze-netting-samplers[1]: 1a = max. 0.125 ± 0.085 g a.s./ha 1b = max. 0.320 ± 0.019 g a.s./ha 2 = <LOD D: At the time of bagging = 0.22 g dust/100 kg seeds 0.032 g a.s./100 kg seeds At the time of sowing = 0.62 g dust/100 kg seeds	Lueckmann, J.; Staffel, J.; 2014, GLP200
Honey bee/*Phacelia tanacetifolia* (full flowering)	Sowing rate (sugar beet - treatment): 130,000 pills/ha Sowing rate (maize - control): 100,000 seeds/ha 1: Poncho Beta Plus (0.60 mg CTD/pill + 0.30 mg IMD/pill) 2: Control (maize)	A: Honey bee mortality and behaviour B: Population development and health assessment C: Dust drift sampling	A: no test item related effect B: no statistical differences C: Gauze-netting-samplers[1]: 1 = <LOD 2 = <LOD	Staffel, J.; Lueckmann, J.; 2014, Report No 195

CTD Clothianidin; IMD Imidacloprid

[1] Gauze samples: LOQ (Limit of quantification) = 0.04 g a.s./ha (imidacloprid, clothianidin)
LOD (Limit of detection) = 0.004 g a.s./ha (imidacloprid, clothianidin)

g) The acute and long term risk to colony survival and development and the risk to bee brood for honey bees from ingestion of contaminated nectar and pollen

No new studies focused on imidacloprid residues in nectar and pollen of seed treated crops were submitted as confirmatory data. However, in the first DAR of imidacloprid (2005) several field-residue trials with non-labelled imidacloprid on sunflower, maize and rape were carried out in various countries to examine the imidacloprid residue levels which honey bees may be exposed to under realistic field conditions. Based on the these data, it was concluded that at currently registered European seed dressing rates of Gaucho®, honey bees will not encounter imidacloprid residue levels higher than 5 ppb in nectar or pollen. More recent data are summarized in EFSA Journal 2013;11(1):3068.

B.9.4 Risk assessment

The high acute and contact toxicity as well as the chronic dietary toxicity to bees of the active substance imidacloprid and its main metabolites olefine-imidacloprid and hydroxy-imidacloprid have been already assessed in the DAR on imidacloprid (2005). Further assessments on newer data were performed by EFSA (2013). More information on these studies, substance properties, the different routes of exposure and the identified concerns as well as the breached trigger values are available in these documents.

This risk assessment performed here focused on the questions posed in the Implementing Regulation No. 485/2013 published on 25th May 2013. According to this regulation the following questions have to be addressed:

a) the risk to pollinators other than honey bees
b) the risk to honey bees foraging nectar or pollen in succeeding crops
c) the potential uptake via roots to flowering weeds
d) the risk to honey bees foraging on insect honey dew
e) the potential guttation exposure and the acute and the long-term risk to colony survival and development, and the risk to bee brood resulting from such exposure
f) the potential exposure to dust drift following drill and the acute and the long-term risk to colony survival and development, and the risk to bee brood resulting from such exposure
g) the acute and long term risk to colony survival and development and the risk to bee brood for honey bees from ingestion of contaminated nectar and pollen.

B.9.4.1 The risk to pollinators other than honey bees

Pollinators other than honey bees (commercial used)

The possible risk to bumble bees has been addressed by laboratory studies with a number of imidacloprid containing formulations as well as in field studies. No studies have been submitted for other pollinators.

Toxicity

The high acute and contact toxicity as well as the chronic dietary toxicity of the active substance imidacloprid and the metabolites to bumble bees has been assessed in the DAR on imidacloprid (2005). Further assessments were performed by EFSA in 2008 and in 2013, which in principle confirmed the conclusions of very high dietary toxicity made in the DAR (2005). Laboratory studies provided as confirmatory data indicate a lower contact toxicity of imidacloprid to bumble bees (*Bombus terrestris* L.) per individual bee. The contact toxicity in a formulation was lower compared to the active substance for all tested formulations. Consistently, for both the active substance and formulations a lower contact toxicity was found for bumble bees than for honey bees. Further information are provided in section B.9.2.1.

Pollinators other than honey bees (wild pollinators)

For the risk assessment of wild pollinators the RMS considers as current scientific knowledge the 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, 04. July 2014). With regard to oral and contact toxicity following dust drift the Guidance Document "Draft Authorisation of Plant Protection Products for Seed Treatment" (SANCO/10553/2012, January 2014) will be used to derive the exposure values for the risk assessment.

Toxicity to pollinators other than honey bees

The Notifier proposed to use the following endpoints for the risk assessment of wild pollinators:

Table 9.4-1: **Toxicity endpoints for imidacloprid**

Tested formulation	Contact toxicity to bumble bees LD_{50}	Contact toxicity to honey bees LD_{50}	Reference (bumble bee studies)	Reference (honey bee studies)
Imidacloprid (active substance)	LD_{50} >0.05 < 0.1 µg IMD/bee (could not be accurately determined)	0.042 – 0.081 µg IMD/bee	IMD DAR[3]	EFSA conclusion[4]
Clothianidin + Imidacloprid FS 275 (100 + 175 g/L)	54.9 µg total CNI/bee = (19.9 µg CTD + 35.0 µg IMD)/bee	0.072 µg total CNI/bee = (0.026 µg CTD + 0.046 µg IMD)/bee	M-494283-01-1	M-501653-01-1
Imidacloprid FS 350 (350 g/L)	85.3 µg IMD/bee	0.0476 µg IMD/bee	M-494307-01-1	M-500305-01-1
Imidacloprid + Pencycuron FS 370 (120 + 250 g/L)	(270 µg product/bee) = 28.1µg IMD/bee	0.38 µg product/bee = 0.040 µg IMD/bee	M-494321-01- 1	M-503109-01-1

According to the EFSA Guidance Document on bees, it can be assumed that the toxicity endpoints for bumble bees and solitary bees can be lower than for honey bees. Therefore, EFSA proposed to use an assessment factor of ten when extrapolating from honey bee endpoints to endpointsfor bumble bees and solitary bees. For bumble bees, the notifier presented acute contact toxicity studies. Information on the toxicity of solitary wild bees is not available.

Thus, the RMS follows the evaluation of the EFSA conclusion on the peer review of the pesticide risk assessment for bees for the active substance imidacloprid considering all uses

[3] Imidacloprid DAR Volume B.9, (Public version) 2008

[4] EFSA Scientific Report (2008) 148, 1-120, Conclusion on the peer review of imidacloprid

other than seed treatments and granules, EFSA Journal 2015; 13(8):421. The relevant endpoints for the risk assessment of bumble bees and solitary bees are presented in Table 9.4-2.

Table 9.4-2: EFSA conclusion 2015[5]: Toxicity endpoints for the active substance imidacloprid

Risk assessment type	Endpoint	Honey bees	Bumble bees	Solitary bees
Acute contact	LD50 (µg a.s./bee)	0.081 (48h)	0.218 (96h)	0.0081***
Acute oral	LD50 (µg a.s./bee)	0.0037 (48h)	0.038 (96h)	0.00037***
Chronic (oral)	10-day LDD50 (µg a.s./bee/day)	> 0.00282*	> 0.000282***	> 0.000282***
Larval	NOEC (µg a.s./larva) 7days (=22days)	0.00528 as provisional**	No endpoint available or extrapolated	No endpoint available or extrapolated
Development of hypopharyngeal glands	NOEChpg (µg a.s./bee/day)	No endpoint available	Not applicable	Not applicable

*: Endpoint set at the highest concentration tested
**: Endpoint determined at 7 days but only 3 day exposure during the study. Endpoint is the highest dose tested. Endpoint is based on nominal amount of food offered to the larvae
***: Extrapolated from the endpoint for honey bee by using a factor of 10.

Due to the lack of any reliable studies on larval toxicity and the development of the hypopharyngial glands, no risk assessment will be performed for these endpoints.

Exposure for pollinators other than honey bees

In the opinion of the notifier, only three major potential routs of exposure are relevant for assessing the risk to non-*Apis* pollinators; exposure to seed treatment dust for winter cereals and to pollen in potato and fruiting vegetable cultivation. The RMS disagrees with this assumption.

According to the EFSA Guidance Document on bees (EFSA, 2013), the risk assessment for products applied as seed treatment should consider both contact exposure and oral exposure via contaminated food items.

For the uses in winter cereals, beets, potatoes and leafy vegetables (field application) the following routes of exposure have to be assessed:

- exposure via contact from dust particles
- consumption of pollen and nectar from the treated crop, weeds in the field, plants in the field margin and succeeding crops in the following year.
- consumption of contaminated water from puddles (solitary wild bees). This could be relevant in the opinion of the RMS, because mason bees collect their muddy soil material for the purpose of constructing the walls of their brood cells. But this cannot be considered in the sope of this addendum.

The exposure via adjacent crop is covered by the assessment of plants in the field margin.

[5] EFSA Conclusion on the peer review of the pesticide risk assessment for bees for the active substance imidacloprid considering all uses other than seed treatments and granules, EFSA Journal 2015; 13(8):4211

Furthermore, the RMS agrees with the following assumption of the notifier: "*non Apis bees obtain their water requirements from nectar and do not use water to cool the colony or to dilute stored honey they do not collect guttation water*".

The crop definition "leafy vegetables" (field application) is considered not precise enough for a risk assessment. It is not possible to assess the risk for all kinds of vegetables in this addendum. Therefore, the RMS will distinguish between vegetables which will be harvested before flowering (e.g. carrots, mangolds etc.) and vegetables which will come to flowering (for seed production or where the fruits are harvested e.g. beans, tomatoes etc.). For both groups of leafy vegetables, the exposure routes dust drift, weeds in the field, plants in the field margin and succeeding crop scenario are relevant for the risk assessment. In general, ~~in case of group of~~ for leafy vegetables which might come to flowering the risk from the treated crop ~~has~~ should ~~to~~ be considered. But due to the fact that flowering vegetables are currently not registered in the EU (only lettuce and endive) no assessment has to be performed in the scope of this addendum.

For seed treatments of leafy vegetables sown or planted in greenhouses ~~only~~ the only route of exposure considered relevant by the RMS is consumption of pollen and nectar from the treated crop in the cases of flowering.

The application of granular imidacloprid products in amenity vegetation (golf courses, sports grounds, commercial and residential lawns) is not considered in this document since ~~the notifier has not provided any relevant data or an appropriate risk assessment~~ either for application by hand a risk can be considered negligible or for e.g. rotary/spin type broadcast spreaders application technique no specific dust drift data are available.

Therefore, the RMS could not finalise the risk assessment for this use. In general, the exposure via dust drift and contact from dust particles as well as consumption of pollen and nectar from plants in flowering the amenity vegetation and field margin are considered relevant by the RMS.

Risk assessment dust drift scenario

The risk assessment for the exposure route "contact toxicity following dust drift" follows the Guidance Document "Draft Authorization of Plant Protection Products for Seed Treatment" (SANCO/10553/2012, January 2014). For this exposure route the RMS did not follow the EFSA bee guidance document with regard to the exposition parameters, because in this special point it is in our opinion not reflecting current scientific knowledge. There are discrepancies between both guidance documents in the derivation of deposition values, the extrapolation factor between ground deposition and deposition on 3-D structures (e.g. hedges) and the finding that deposition of the amount of active substance is more related to the seed quality than to the application rate.

In a first step, the deposition values of the SANCO guidance document (see chapter 10.5.2 Table 10-2) have to be corrected according to the seed units given in the GAP table. According to the GAPs presented by the notifier the following units are relevant.

Table 9.4-3: Correction factor for the seed unit

Crop	Seed units in the GAP	Seed units in SANCO dust GD	Correction factor
Winter cereals	178-180 kg seeds/ha	180 kg seeds/ha	Not necessary
Beet	100,000 seeds	100,000 seeds	Not necessary

Beet (117 g a.s./ha)	130,000 seeds	100,000 seeds	1.3
Leafy vegetables	100,000 seeds	-	-

The GAP table presented by the notifier cannot be considered very precise. The RMS had the impression the highest application rate for beets given in the table does not correspond to the seed unit of 100,000 seeds and thus, has to be corrected accordingly. This leads to a max. drilling rate of 130,000 seeds/ha for the use in beet (Table 9.4-4). With this factor the Heubach value has to be adjusted accordingly.

Table 9.4-4: Amount of dust/ha

Crop	Drilling rate according to the GAP [unit seeds/ha]	Heubach value [g dust/ha]
Winter cereals		
Worst case		3 (with sticker)
Min drilling rate	178 kg seeds/ha	3
Max drilling rate	180 kg seeds/ha	3
Beet/leafy vegetables		
Worst case		0.1
Min drilling rate	100,000 seeds/ha	0.1
Max drilling rate	130,000 seeds/ha	0.13

These seed dressing rates are used to derive the standard deposition values [for details please refer to Appendix III of the SANCO Guidance Document (2014)] are summarised and adjusted in the following table.

Table 9.4-5: Content of a.s. in dustas for the seed dressing rate

Crop	seed dressing rate according to the GAP [g a.s./unit]	content of a.s. in dust according to SANCO dust GD [%a.s. in dust]
Winter cereals		
Worst case		25
Min dressing rate[1]	48	6.2
Max dressing rate[1]	126	16.4
Beet/leafy vegetables		
Worst case		
Min dressing rate[2]	15-60	10
Max dressing rate[2]	90	15

[1] 13 % of seed dressing rate (g/180 kg) please refer to Table 10-2 of the SANCO GD
[2] worst case value refers to appl. rate of 60 g a.s /ha (100,000 seeds)

The active substance in dust (Heubach a.i. value) will be calculated on the basis of the above mentioned Heubach value and the content (%) of a.s. in dust (Table 9.4-6) and transformed to the PEC 2D dust ground deposition (See also SANCO GD Table 10-3). Non-target arthropods outside the field sown

with treated seeds will be exposed to the active substance through the deposition of abraded dust. Foliar dwelling non-target arthropods like wild pollinators have to be considered particularly at risk. Thus, the realistic worst case exposure for terrestrial invertebrates – especially pollinators – is not on the ground but in 3 dimensional spatial structures (e.g. trees, hedges, adjacent crops). Thus, the predicted 3-D exposure data as in the SANCO Guidance Document are applied in the assessment of the risk for foliar-dwelling non-target arthropods exposed to contaminated dust. As long as no generic factors are available for every crop, a worst case extrapolation factor of 13 is used to derive 3-D exposure data from 2-D ground deposition data.

Table 9.4-6: Active substance in dust, Heubach a.s. (g a.s in dust/ha)

Crop	Heubach a.s. [g a.s./ha]	PEC 2D ground deposition in off-crop areas [g a.s./ha]	PEC 3D dust deposition in off crop areas [g a.s./ha]
Winter cereals			
Worst case	0.75	0.375	4.88
Min dressing rate	0.19	0.095	1.24
Max dressing rate	0.49	0.245	3.19
Beet/leafy vegetables			
Worst case[1]	0.01	0.2	2.6
Min dressing rate	0.01	0.2	2.6
Max dressing rate	0.02	0.4	5.2

[1] ~~Factor of 20 according to the SANCO GD~~ The underlying study in the SANCO GD resulted in a factor of 20 between 'Heubach a.s' value and the PEC 2D dust ground deposition from the calculation of the reference value (Table 10-3: 0.001'Heubach a.s.' correspond to 0.02 PEC2Ddust ground deposition). In the SANCO GD no worst case deposition could be derived from studies. Thus, the factor of 20 has been applied for the worst case scenario.

Deposition data for the use in leafy vegetables are not available. Therefore, the RMS proposes to use data from beet as a preliminary best estimate.

However, a data gap for deposition data of dressed seed of leafy crops has to be defined, since the risk assessment should principally be based on data mirroring the seed quality of the corresponding crops.

The granular application on turf could not be assessed because for rotary/spin type broadcast spreaders application technique no specific dust drift data are available.

The HQ-ratio can be calculated as follows:

$$HQ_{contact} = f_{dep} / 100 * \frac{AR}{LD_{50} contact}$$

$$ETR_{acuteadultoral} = \frac{AR * E_f * SV}{LD_{50}oral}$$

where

HQ	=	Hazard Quotient
ETR	=	Exposure Toxicity Ratio
a.s.	=	active substance
$f_{dep}/100$	=	Exposure. Predicted Environmental Concentration after deposition of abraded dust in adjacent 3-dimensional structures
E_f	=	Exposure factor
SV	=	Shortcut value

The risk assessment for wild pollinators exposed by imidacloprid residues via dust drift is summarised in Table 9.4-7 below.

Table 9.4-7: Risk assessment for wild pollinators exposed by imidaclopid residues via dust drift

Crop	Species	Toxicity LD50 (µg a.s./bee)	Exposure (see Table 9.4-6)		HQ[1]	Trigger
Contact exposure						
Winter cereals	BB	0.218	1.24-4.88		**5.69-22.39**	2.3
	SB	0.0081	1.24-4.88		**153-603**	2.6
Beet/leafy vegetables	BB	0.218	2.6-5.2		**11.9-23.9**	2.3
	SB	0.0081	2.6-5.2		**321-642**	2.6
Oral exposure						
Winter cereals	BB	0.038	1.24-4.88	11.2	**366-1438**	0.036
	SB	0.00037	1.24-4.88	5.7	**19103-75178**	0.04
Beet/leafy vegetables	BB	0.038	2.6-5.2	11.2	**766-1533**	0.036
	SB	0.00037	2.6-5.2	5.7	**40054-80108**	0.04

[1] Values in bold do~~es~~ not meet the trigger value.
BB: bumble bees
SB: solitary bees

Based on the field rates of imidacloprid calculated according to the GAP the acceptability criterion for wild non-*Apis* pollinators (HQ < 2.3/2.6 and ETR < 0.036/0.04) is not achieved ~~for wild pollinators~~. This indicates an unacceptable risk for wild non-Apis pollinators due to the intended use of imidacloprid in winter cereals, beet and leafy vegetables.

Conclusion:

An unacceptable risk for wild bumble bees and solitary wild bees due to the exposure with residues of imidacloprid in drifted dust has been identified.

Risk assessment foraging on treated crop

According to the EFSA Conclusion on the risk assessment for bees for imidacloprid (2013) and Appendix D of the EFSA Guidance Document on bees (2014), winter cereals and beets are not considered attractive to honey bees for the consumption of pollen and nectar.

It should be noted that the attractiveness of a crop to bumble bees and solitary bees is not necessarily the same as for honey bees. However, beets are harvested before flowering. Furthermore, winter cereals do not produce nectar and are generally considered to be of low attractiveness for pollen. Consequently, it is considered that non-Apis bees will not be exposed to nectar and pollen from these crops as well.

EFSA PPR 145 Ecotoxicology Meeting:

"The applicant provided some argumentations e.g. wind pollinated, not attractive. No data where provided to support this argumentation. EFSA (2013), due to diverging data from literature, considered that further data should be provided to exclude collection of pollen by honeybees, bumblebees and solitary bees.

The attractiveness of agricultural horticultural crops was further analysed by van der Steen, et. Al., 2015 report n. 606, Wageningen University. This analysis is based on a literature review and experts judgment. Cereals are reported as not attractive. However, the paper is in Dutch and not available to other MSs e.g. not peer reviewed. By quickly looking at the references of the report, it seems that only one paper, published after 2013, is cited.

Overall, the experts concluded that EFSA (2013) is still the reference point for attractiveness of cereals. Therefore an open point was identified for the RMS to provide the Tier I risk assessment."

For the calculation please refer to **Fehler! Verweisquelle konnte nicht gefunden werden.**

Potatoes are considered as not attractive to honey bees. However, in the EFSA conclusion (2013) it is noted that it some bumble bee species are known to collect pollen from potato flowers. Additionally, the notifier did not present data on the attractiveness of potato flowers for solitary wild bees. In general, there are plants from the family of *Solanaceae* known as food plants for solitary wild bees. Thus, it cannot be excluded that solitary bees might use potato flowers as food source. A data gap to address the attractiveness of potato flowers for solitary bees should be set.

A detailed description of the use in leafy vegetables is lacking. Therefore, the RMS will not conduct a risk assessment for the vegetables which will be harvested before flowering.

The risk for wild pollinators has generally to considered in leafy vegetables that will come to flowering, e.g. if they are grown to produce seeds.

For the use in potatoes:

The RMS considers the scenario "downward spraying" for the tier1 assessment as appropriate. The relevant shortcut values are presented in chapter 3.2.2 of the EFSA Guidance Document Table 5 for the first tier. The shortcut values or bumble bees and solitary bees in potatoes is selected according to table Jx (p.24) "Treated crop – application before emergence, crop attractive for pollen only".

For leafy vegetables:

According to the EFSA Guidance Document on bees, the following formulae should be used to determine the Exposure Toxicity Ratio (ETR) for acute adult oral exposure, chronic adult oral exposure and chronic exposure to larvae, for product applied as seed treatment. The relevant shortcut values (and the methodology used to determine these values) are presented in chapter 3.3.2 of the EFSA Guidance Document Table 9 and are refined according to table Jxx (p. 38).

The *ETR for the acute adult oral exposure* is calculated by the following equation:

$$ETR_{acute\ adult\ oral} = \frac{AR * E_f * SV}{LD_{50\ oral}}$$

Where: AR = application rate in kg a.s./ha

SV = shortcut value for acute exposure to bees

Ef = exposure factor

$LD_{50,\ oral}$ is expressed as µg a.s./bee

The *ETR for the chronic adult oral exposure* is calculated by the following equation:

$$ETR_{chronic\ adult\ oral} = \frac{AR * E_f * SV * twa}{LC_{50}}$$

Where: AR = application rate in kg a.s./ha

SV = shortcut value for acute exposure to bees

Ef = exposure factor

LC_{50} is expressed as µg a.s./bee per day

Due to the high toxicity of imidacloprid the RMS did not conduct a risk assessment on screening level.

Table 9.4-8: First tier step ETR calculation for oral exposure from downward spraying/solid formulations

Type of assessment	Type of bee	Endpoint	Application Rate AR [kg/ha]	Exposure factor Ef	twa	Shortcut value SV	ETR[2]	Trigger[1]
	Potoato							
Acute oral adult	BB	0.038	0.120-0.180 kg/ha lowest appl. Rate is considered sufficient for the calculation	-	-	0.03	**0.095**	0.036
	SB	0.0037		-	-	0.01	**0.32**	0.04
Chronic oral adult	BB	> 0.000282		-	0.72	0.03	**9.2**	0.0048
	SB	> 0.000282		-	0.72	0.01	**3.06**	0.0054
	Leafy vegetables for seed production or flowering							
Acute oral adult	BB	0.038	0.8-1.5 mg a.s./grain lowest appl. rate is considered sufficient for the calculation	-	-	0.9	**19.0**	0.036
	SB	0.0037		-	-	0.49	**106**	0.04
Chronic oral adult	BB	> 0.000282		-	1	0.78	**2213**	0.0048
	SB	> 0.000282		-	1	~~0.93~~ 0.49	~~**2638**~~ **1390**	0.0054
	cereals							
Acute oral adult	BB	0.038	0.006-0.043 mg a.s./grain lowest appl. rate is considered sufficient for the calculation	-	-	0.9	**0.14**	0.036
	SB	0.0037		-	-	0.49	**0.80**	0.04
Chronic oral adult	BB	> 0.000282		-	1	0.78	**16.6**	0.0048
	SB	> 0.000282		-	1	0.49	**10.4**	0.0054

[1] The protection goal is met if the calculated ETR value is smaller than the trigger.
[2] Values in bold does not meet the trigger value.
[3] The shortcut value was calculated for consumption over 1 day, therefore the shortcut value needs to be multiplied by 10 in order to account for exposure over the whole developmental period of bumble bee larvae.
BB: bumble bees
SB: solitary bees

As all ETR values exceed the relevant trigger values, a potential risk is identified for all wild bees by the use in potatoes and leafy vegetables coming to flowering, e.g when used for seed production. Thus, further consideration is thus necessary.

Higher Tier

The EFSA Guidance Document on bees (2014) suggests a number of options to refine the tier 1 risk assessment. For these refinements further data are required. For example, the shortcut values, which are used for the estimation of oral exposure via nectar and pollen consumption at first tier, could be refined by valid compound or crop specific residue data. Further refinements of the risk assessment could be based on field effect studies.

For bumble bees two potato field studies (Pfeiffer et al 2014) were submitted by the notifier to refine the risk. An extensive conclusion of the RMS on these 2 studies is given in chapter 9.5.2.

Relevance of the two potato bumble bee field studies (Pfeiffer et al 2014) for the risk assessment of wild non-*Apis* pollinators:

Both submitted field studies (S14-03553 and S14-03554) are considered not appropriate for addressing the risk to wild living bumble bee species exposed to pollen of treated potato plants.

Reasoning:

1. Sufficient exposure of the bumble bee colonies at the treatment site to pollen of treated potato plants is questionable.

   This is due to:

   - small field sides compared to the possible foraging home ranges of the test species *B. terrestris*

   - the lacking detailed description of the field sites surrounding in greater distances including lacking information about treated or non-treated potato field within the potential foraging home range of *B. terrestris.*

   - the bordering of treatment plots to wood sides with potentially flowering trees

   Evidence can be found in the results of pollen source analysis showing high portions of pollen from other plant species.

   Furthermore, the determined portions of potato pollen are not in line with the residue analysis in the pollen samples.

2. As observations were carried out at one control and one treatment plot only, it is not possible to distinct between effects caused by environmental site conditions and effects attributed to the exposure to pollen from imidacloprid treated potato plants. Furthermore, detailed environmental conditions as the amount of precipitation at each field side was not provided.

3. Approaches for determining effects on the parameters flight activity in crop and at the entrance of the hives are not considered appropriate. The time of observation was too short (10 or 15 minutes/day) and the area of observation for recording the flight activity in crop is regarded too small (2 x 4 m^2). Moreover, environmental influences on the measured parameters are not assessable since detailed plot and time specific information was not given.

4. The informative value of these studies regarding potential effects to bumble bee species other than *Bombus terrestris* is questionable. The effects to the bumble bee species Bombus terrestris in field may not cover other bumble bee species since:

   - *Bombus terrestris* is known to have a wide foraging home range compared to other bumble bee species.

   - it is not clear whether other bumble bee species will be more susceptible to the pesticide

5. Subsequent to the exposure period at potato field sites, colonies were further observed at special monitoring sites providing sufficient food sources (e.g. wild flowers) without intensive agriculture. Natural bumble bee hives are located at one site during the total season. Thus, wild bumble bee colonies in the agricultural landscape may be subject to food shortage as well as multiple pesticides, which may hinder their recovery from an initial stress.

6. Bumble bees were additionally fed with sugar solution. Although this approach was reasoned with the lacking or reduced nectar production in potato flowers, it is not appropriate for assessing effects to wild bumble bees.

No specific information on the level of residues in pollen and nectar of any kind of leafy vegetables is available in the submitted data set of confirmatory information. Thus, it is not possible to further refine the risk assessment for bumble bees and solitary wild bees by this means.

The reasoning from the RMS has been discussed in the EFSA PPR 145 Ecotoxicology Meeting without the attendance of the RMS but for reason of completeness it should be reported in the addendum:

"Two bumblebee effect studies were available. The following shortcomings were highlighted by the RMS in the addendum (assessment of wild pollinators).

1 - Studies were conducted with *B. terrestris*. However, its representativeness for other bumble bee species has to be questioned.

2 - Post exposure period at uncontaminated sites.

3 - Provision of sugar solution as additional food.

4 - Both studies were carried out with only one control and one treatment plot.

5 - The residue levels in pollen were rather low.

Not all the MSs at the meeting agreed that the shortcomings above would question the suitability of the studies for the RA (shortcomings 1, 2 and 3 were not considered as such by all the MSs). It was noted that the extrapolation to other *Bombus* species is a general risk assessment issue rather than a real shortcoming of the study design.

Anyway, it was noted that it would be necessary to rely on other lines of evidence for addressing the risk to wild pollinators.

Overall, the majority of experts agreed that, due to the uncertainties (i.e., low statistical **power, questionable exposure), the studies are not sufficient to draw any solid conclusion on** the effects of imidacloprid on wild bees."

Conclusion:

A risk for bumble bees and solitary wild bees resulting from foraging on the treated crop is not considered relevant for the use in winter cereals, beet and leafy vegetables harvested before flowering.

An unacceptable risk for solitary wild bees and wild bumble bees could not be excluded for the use in potato and leafy vegetables (which come to flowering stages).

Risk assessment foraging on weeds in the field

In addition to the exposure route "risk from foraging on the treated crop" the "risk from foraging on weeds in the field" should be discussed for non-Apis pollinators.

The EFSA Guidance Document on bees (2014) does not consider this oral exposure route from solid formulations relevant for seed treatments (p. 32, Table 8).

The RMS assumes that the risk from foraging on weeds in the field is covered by the risk assessment from foraging in the treated crop, because there will be greater levels of residues in the treated crop plants than in flowering weeds.

However, according to the risk assessment carried out above, an unacceptable risk from foraging in the treated potato crops and leafy vegetable crops that come to flowering was concluded.

As a comparative risk assessment for the application in beets, winter cereals and non-flowering leafy vegetables was not conducted, the risk from foraging on weeds in the field still has to be discussed.

Due to the high toxicity of imidacloprid a screening step assessment is not deemed to be necessary.

For the use in potatoes:

The RMS considers the scenario “downward spraying” for the tier1 assessment appropriate. The relevant shortcut values are presented in chapter 3.2.2 of the EFSA Guidance Document Table 5 for the first tier. For bumble bees and solitary bees the shortcut values for potatoes will be selected according to table Jx (p. 24) “Treated crop – application before emergence, crop attractive for pollen and nectar”.

For leafy vegetables, cereals and beets:

The relevant shortcut values (and the methodology used to determine these values) are presented in chapter 3.3.2 of the EFSA Guidance Document Table 9 and are refined according to table Jxx (p. 38) “weeds in the field (application before emergence of weeds)”.

The lowest application rate per grain will be considered here as surrogate for all seed treatment uses in the GAP.

Table 9.4-9: First tier step ETR calculation for oral exposure from downward spraying/solid formulations

Type of assessment	Type of bee	Endpoint	Application Rate AR [kg/ha]	Exposure factor Ef	twa	Shortcut value SV	ETR[2]	Trigger[1]
	Potato							
Acute oral adult	BB	0.038	0.120-0.180 kg/ha lowest appl. rate is considered sufficient for the calculation	-	-	0.90	**2.84**	0.036
	SB	0.0037		-	-	0.49	**15.9**	0.04
Chronic oral adult	BB	> 0.000282		-	0.72	0.78	**239**	0.0048
	SB	> 0.000282		-	0.72	0.49	**150**	> 0.0054
	Cereals*, Vegetables and Beet							
Acute oral adult	BB	0.038	0.012-0.032 mg a.s./grain lowest appl. rate is considered sufficient for the calculation*	-	-	0.46	**0.15**	0.036
	SB	0.0037		-	-	0.17	**0.55**	0.04
Chronic oral adult	BB	> 0.000282		-	1	0.40	**17**	0.0048
	SB	> 0.000282		-	1	0.17	**7.2**	0.0054

[1] The protection goal is met if the calculated ETR value is smaller than the trigger.
[2] Values in bold does not meet the trigger value.
[3] The shortcut value was calculated for consumption over 1 day, therefore the shortcut value needs to be multiplied by 10 in order to account for exposure over the whole developmental period of bumble bee larvae.
BB: bumble bees
SB: solitary bees

The first tier risk assessment for non-*Apis* pollinators from foraging on flowering weeds does not meet the trigger values for none of the intended uses.

Higher tier risk assessment

The notifier presented a study (Garside et al, 2014, see chapter B.9.5.2) that investigated the occurrence of flowering weeds in cereal, sugar beet and potato fields in the context of (herbicide) efficacy trials.

In addition to the deficiencies of this study mentioned in the comment by the RMS, it is not comprehensible that only flowering weeds with more than 10% ground cover should be relevant for wild pollinators. A large percentage of the solitary wild bees are specialized on certain plant families (some of them even on certain genera). Thus, ground cover cannot be considered as useful mean to assess the attractiveness of weeds. Furthermore, the RMS is of the opinion that in arable crops displaying little competition (like sugar beet or potatoes), (flowering) weed infestation cannot be excluded (at a later point of time. In DE this fact is reported regularly. Therefore, the results of this study are not appropriate to refine the risk for wild pollinators foraging on weeds in the field.

In the Pesticide Peer Review Meeting 145 the study from Garside 2014 had been discussed:

"The study was considered useful to address the relevance of the weeds scenario for the specific case. However, some clarification would be needed:

- no. of plots analysed (trials, replicates, observations)
- observation timing date and BBCH stage for the crop
- no. of species per plot
- clarification with regard to the ground cover % reported in the study (average or total ground cover)

Therefore an open point was identified for the RMS to provide these clarifications in a revised RAR. Addressing this point the RMS may request the applicant to provide the data in the study Garcide et al 2014 in a tabular format (xls). Pending on these clarifications a final conclusion can be drawn by EFSA.

Overall, pending on the clarification to be provided in the revised addendum, if all the available data will demonstrate that the flowering weed coverage is below the 10% trigger, the weed scenario for potato, cereals and sugar beet can be considered of low relevance as exposure route. Other uses were not covered by these data i.e. leafy vegetable and amenity vegetation."

Conclusion:

~~**A risk for bumble bees and solitary wild bees resulting from foraging on weeds in the treated crop cannot be excluded for none of the intended uses.**~~ **See outcome of the meeting above.**

Risk assessment foraging in the field margin

The exposure route "risk from foraging on plants in the field margin" is generally considered relevant for non-*Apis* pollinators.

For the use in potatoes:

Data on spray drift deposition of furrow spraying application in potatoes are not available.

Nonetheless, the RMS considers the scenario "downward spraying" for the tier1 assessment appropriate. The relevant shortcut values are presented in chapter 3.2.2 of the EFSA Guidance Document Table 5 for the first tier. Due to the high toxicity of imidacloprid the screening step is not deemed to be necessary. For bumble bees and solitary wild bees the exposure factor will be used according to table X1a (p. 22) and the shortcut values for potatoes will be selected according to table Jy (p. 25) "Plants in the field margin".

For leafy vegetables, cereals and beets:

The risk assessment will be conducted according to chapter 3.3.2 of the EFSA Guidance Document Table 9. The relevant exposure factor will be selected according to table X1b (p. 35) and the shortcut values are refined according to table Jxx (p. 38) "weeds in the field (application before emergence of weeds)".

The lowest application rate per grain will be considered here as surrogate for all seed treatment uses in the GAP.

Due to the high toxicity of imidacloprid the screening step is not deemed to be necessary.

Table 9.4-10: First tier step ETR calculation for oral exposure from downward spraying/solid formulations

Type of assessment	Type of bee	Endpoint	Application Rate AR [kg/ha]	Exposure factor Ef	twa	Short cut value SV	ETR[2]	Trigger [1]
	Potato							
Acute oral adult	BB	0.038	0.120-0.180 kg/ha lowest appl. Rate is considered sufficient for the calculation	0.0092	-	6.5	**0.19**	0.036
	SB	0.0037		0.0092	-	2.3	**0.69**	0.04
Chronic oral adult	BB	> 0.000282		0.0092	0.72	5.9	**16.6**	0.0048
	SB	> 0.000282		0.0092	0.72	2.3	**6.5**	0.0054
	Cereals							
Acute oral adult	BB	0.038	0.012-0.032 mg a.s./grain lowest appl. rate is considered sufficient for the calculation	0.099	-	6.5	**0.2**	0.036
	SB	0.0037		0.099	-	2.3	**0.74**	0.04
Chronic oral adult	BB	> 0.000282		0.099	1	5.9	**25**	0.0048
	SB	> 0.000282		0.099	1	2.3	**9.7**	0.0054
	Beets							
Acute oral adult	BB	0.038	0.15-0.9 mg a.s./grain	0.0003	-	6.5	0.008-**0.046**	0.036
	SB	0.0037		0.0003	-	2.3	0.028-**0.17**	0.04
Chronic oral adult	BB	> 0.000282		0.0003	1	5.9	**0.95-5.7**	0.0048
	SB	> 0.000282		0.0003	1	2.3	**0.37-2.2**	0.0054
	Application rate for leafy vegetables is above the maximum application rate for beet. Thus, risk is not acceptable							

[1] The protection goal is met if the calculated ETR value is smaller than the trigger.

[2] Values in bold does not meet the trigger value.

BB: bumble bees

SB: solitary bees

As stated above, the RMS is of the opinion that the exposure factor for dust drift in EFSA (2014) is not derived under the consideration of current knowledge. Therefore, the presented values have to be considered as not conservative enough.

As all other ETR values exceed the relevant trigger values, a potential risk is identified for all wild bees for the intended uses in potatoes, cereals, beets (chronic) and leafy vegetables which will come to flowering, e.g. when used for seed production.

Further consideration is thus necessary. However, information on further refinement of the risk from foraging in the field margin is not available in the set of confirmatory data.

The RMS does not agree with assumption that wild pollinators (bumble bees and solitary wild bees) are not at risk during an autumn application (e.g. in winter cereals) due to reduced activity and stopped reproduction. The number of worker bumble bees may decrease during autumn, but the exposure of newly emerged and mated queens may have consequences for next year

generations. This risk cannot excluded here. Additionally, there are wild solitary bee species known to be active until mid of October (in Germany e.g. http://www.wildbienen.de/wba-kale.htm). Therefore, the risk for solitary wild bees is relevant even for autumn applications.

Conclusion:

A risk for bumble bees and solitary wild bees resulting from foraging on plants in the field margin cannot be excluded.

Risk assessment foraging on succeeding crops

The exposure route "risk from foraging on succeeding crops" is considered relevant for non-*Apis* pollinators, because imidacloprid is a persistent (DT_{50} = 288 $_{max, europ. field studies}$) and systemic compound (see additionally EFSA Conclusion on the risk assessment for bees for imidacloprid, 2013).

Furthermore, the applicant submitted a number of studies determining the concentrations of imidacloprid in nectar and pollen of bee attractive crops (*phacelia*, maize or mustard) under conditions of "naturally" aged residues (succeeding crops grown on soils with a history of imidacloprid use) or "forced" plateau concentration" (succeeding crops grown on soils treated with imidacloprid to obtain a theoretical plateau concentration of imidacloprid in the soil).

Results from these studies show that there are low but measurable residues of imidacloprid in pollen and nectar of succeeding crops. Hence, exposure to bees is possible. The exposure to natural aged residues was generally lower than in the model studies with artificial soil residues.

This could be explained by the fact that in the naturally-aged-residue studies, imidacloprid had already undergone ageing processes, making them less available for plant uptake than in the "forced plateau concentration" studies.

However, independent of the study design residues in all samples were lower than those obtained in primary crops (refer to DAR 2005).

As an unacceptable risk on wild solitary bees and bumble bees was concluded for all intended uses and all relevant exposure scenarios (foraging on the treated crop, foraging on weeds in the field, foraging on weeds in field margin), an additional risk assessment will be performed.

For potatoes:

The relevant shortcut values are presented in chapter 3.2.2 of the EFSA Guidance Document Table 5 for the first tier. For bumble bees and solitary wild bees the exposure factor is not applicable according to table X4 (p. 20) and the shortcut values for potatoes will be selected according to table Jy (p. 25) "Succeeding crop".

For leafy vegetables, cereals and beets:

The risk assessment will be conducted according to chapter 3.3.2 of the EFSA Guidance Document Table 9. The exposure factor is not relevant according to table 8 (p. 32) and the shortcut values are refined according to table Jxx (p. 38) "Succeeding crop".

The lowest application rate per grain will be considered here as surrogate for all seed treatment uses in the GAP.

Due to the high toxicity of imidacloprid the screening step is not deemed to be necessary.

Table 9.4-11: First tier step ETR calculation for oral exposure from downward spraying/solid formulations

Type of assessment	Type of bee	Endpoint	Application Rate AR [kg/ha]	Exposure factor Ef	twa	Short cut value SV	ETR[2]	Trigger [1]
	Potato							
Acute oral adult	BB	0.038	0.120-0.180 kg/ha lowest appl. Rate is considered sufficient for the calculation	-	-	0.9	**2.8**	0.036
	SB	0.0037		-	-	0.49	**15.9**	0.04
Chronic oral adult	BB	> 0.000282		-	0.72	0.78	**239**	0.0048
	SB	> 0.000282		-	0.72	0.49	**150**	0.0054
	Cereals							
Acute oral adult	BB	0.038	0.012-0.032 mg a.s./grain lowest appl. rate is considered sufficient for the calculation	-	-	0.9	**0.28**	0.036
	SB	0.0037		-	-	~~0.2~~ 0.49	~~**0.65**~~ **1.59**	0.04
Chronic oral adult	BB	> 0.000282		-	1	0.78	**33.2**	0.0048
	SB	> 0.000282		-	1	~~0.2~~ 0.49	~~**8.5**~~ **20.9**	0.0054
	Application rate for leafy vegetables and beet is above the maximum application rate for cereals. Thus, risk is not acceptable							

[1] The protection goal is met if the calculated ETR value is smaller than the trigger.

[2] Values in bold does not meet the trigger value.

BB: bumble bees

SB: solitary bees

As all ETR values exceed the relevant trigger values, a potential risk is identified for adult bumble bees and solitary wild bees and for all uses. Further consideration is thus necessary.

Tier 2 risk assessment

The EFSA Guidance Document on bees suggests a number of options to refine the tier 1 risk assessment. Data needed for further refinements might be valid information on compound or crop specific residues in pollen and nectar. This could be used for the refinement of shortcut values, which are used for the estimation of oral exposure via nectar and pollen consumption at first tier.

The applicant submitted numerous studies providing imidacloprid residues in nectar and pollen in several succeeding crops

A summary of results derived from these studies is presented in Chapter B 9.3.

Table 9.4-12: The applicant provided the following summary of concentration of imidacloprid detected in succeeding crops

Natural residues									
		Pollen #				Nectar #			
Crop		IMD [µg/kg]				IMD [µg/kg]			
		No. of values > LOQ /Total	Mean	Median	90th percentile	No. of values > LOQ /Total	Mean#	Median	90th percentile
Phacelia		2/18	0.47	0.4	0.4	9/18	0.36	0.2	0.4
Winter OSR		2/15	0.49	0.4	0.7	3/15	0.22	0.2	0.3
Maize		10/18	0.46	0.4	0.8	-	-	-	-
Model studies with artificially applied plateau									
Mustard	H	9/18	1.7	1.0	4.5	11/18	0.3	0.3	0.4
	L	14/18	1.8	1.4	3.8	15/18	1.8	3.8	0.6
Maize	H	6/18	0.3	0.2	0.9	-	-	-	-
	L	8/18	0.4	0.3	1.2	-	-	-	-
Phacelia	H	4/12	0.7	0.3	2.0	7/12	0.4	0.8	0.3
	L	1/12	0.3	0.3	0.4	5/12	0.3	0.2	0.4
For calculation of the mean, median and 90th perecntile values, concentrations reported as <LOQ were assigned as 0.4 µg/kg for pollen and 0.2 µg/L for nectar (equal to mid-way between LOD and LOQ), all values reported as < LOD were assigned as 0 in the calculation).									

~~The highest 90th percentile residue values from the 'natural exposure' succeeding crop studies were considered suitable, and will be used in the risk assessment (0.8 µg/kg for pollen and 0.4 µg/kg for nectar).~~

~~As these values were obtained by exposing a number of succeeding crops to soils with a history of imicloprid application, the selected residue values are considered to cover the succeeding crop scenarios for all registered uses of imidacloprid as seed treatment.~~

~~In table J1 of appendix J of the EFSA Guidance Document on bees (2014), data on the consumption of nectar and pollen by bumble bee and solitary wild bees are reported. These values are shown in~~

~~Table 9.4-13. Since the energy demand of the bumble bees or larvae is available (sugar consumption) rather than the nectar consumption, the sugar content of the nectar needs to be considered. In the studies that measured the residue content of nectar and pollen in succeeding crops, the sugar content of the sampled nectar was not determined. According to the EFSA Guidance Document on bees (2014), some data from the literature is available. However, little is known about the distribution and frequency of the sugar content carried by bees. Thus, further research in this field is necessary.~~

~~For the time being worst case values (i.e. nectar with the lowest sugar content from the ranges which may be foraged by bees), namely 15% for bumble bees and 10 % solitary bees, are to be used for the risk assessment for the succeeding crop scenario.~~

~~Taking this sugar concentration into account, the nectar consumption was calculated and reported in~~

~~Table 9.4-13.~~

Table 9.4-13: Pollen, sugar and nectar consumption of non-*Apis* bees

Organism	Pollen consumption (mg/bee/day)	Sugar consumption (mg/bee/day)	Nectar consumption[1] (mg/bee/day)
Adult bumble bee	26.6-30.3	73-149	487-993
Adult solitary bee	10.2	18-77	180-770

[1]*Nectar consumption was calculated based on a worst case sugar concentration of 15 % in nectar for bumble bees and 10 % for solitary bees*

Based on the nectar and pollen consumption, the daily residue uptake for adult bees and the total residue uptake for larvae can be calculated using the following formula:

$$RI = \frac{(R_n \times C_n) + (R_p \times C_p)}{1000}$$

Where: RI is the residue intake by an adult bee of bee larva (expressed in µg/bee/day or µg/larva)
Rn is the residue level in nectar (in mg/kg)
Rp is the residue level in pollen (in mg/kg)
Cn is the consumption of nectar in mg (mg/bee/day or mg/larva)
Cp is the consumption of pollen in mg (mg/bee/day or mg/larva)

For the calculation of the residue intake (RI), the worst case values for nectar consumption will be used for the acute exposure for adult honey bees. For the chronic adult exposure, the mean from the minimum and maximum value will be used. The calculated RI values, taking into account the available measured residue values, are shown in Table 9.4-14 below.

Table 9.4-14: Calculation of residue intake (RI) values for the different scenarios and bumble bees and solitary bees, respectively

Scenario	Residue in pollen (mg/kg)	Pollen consumption (mg/bee/day or mg/larva)	Residue nectar (mg/kg)	Nectar consumption (mg/bee/day or mg/larva)	Residue intake (µg/bee/day or µg/larva)
BB acute	0.0008	30.3	0.0004	993	0.00042
SB acute	0.0008	10.2	0.0004	770	0.00032
BB chronic	0.0008	30.3	0.0004	740	0.00032
SB chronic	0.0008	10.2	0.0004	475	0.00020

Based on the calculated RI values, a refined ETR can be calculated with the following equation:

$$ETR = \frac{RI}{LD_{50\ oral}\ /\ LC_{50}\ /\ NOEC}$$

The calculated ETR values are shown in

~~Table 9.4-155.~~

~~For assessing the acceptability of the risk, the same trigger values as for the tier 1 risk assessment are applied.~~

Table 9.4-15: Tier 2 ETR calculations for acute adult oral and chronic adult oral exposure for the lowest and highest authorised 'maximum application rate' of imidaclprid in winter cereals and sugar beet

Scenario	Residue intake (µg/bee/day or µg/larva)	Toxicity endpoint (µg/bee or µg/larva)	ETR[2]	Trigger[1]
BB acute	0.00042	0.038	0.011	0.036
SB acute	0.00032	0.0037	**0.09**	0.04
BB chronic	0.00032	> 0.000282	**1.14**	0.0048
SB chronic	0.00020	> 0.000282	**0.71**	0.0054

[1] The protection goal is met if the calculated ETR value is smaller than the trigger.
[2] Values in bold does not meet the trigger value.

Experts at the PPR 145 have agreed that the following residue levels can be used for tier 2 risk assessments for the succeeding crop scenario:

Table 9.4-16: Single maximum residue values

	pollen	nectar
imidacloprid	2.5 µg/kg	3.5 µg/kg

The following is noted:

- these are single, maximum values without distributions
- these values are not RUD values as they are originating from 'naturally aged' residue studies where several years of crop rotation was studied. The application rates of the treated crops were not unique; therefore it would be difficult (and not necessary) to link these values to a certain application rate. Therefore, these values will be used in the MC calculations without any modification (i.e. not expressed as RUDs; see RAs proposals in Appendix 1).

Materials and Method:

The calculations were made by SHVAL, which is a tailored made MC tool developed by EFSA.

The calculations were made for imidacloprid for the different bees and risk categories with the chemical specific residue values. The SHVAL tool requires to insert the natural logarithm form of residue data expressed in mg/kg. Therefore, these were calculated before running the model, as:

Table 9.4-17: Residue value recalculated

Relevance	Residue level in mg/kg	Ln
Test	1	0
Imidacloprid pollen	0.0025	-5.99146
Imidacloprid nectar	0.0035	-5.65499

As a summary, the following input parameters were inserted in the SHVAL tool for the different calculations:

Table 9.4-18: Input parameters for the SV calculation

bee type & category	Pollen consumption in mg/bee/day or mg/larvae	Sugar consumption in mg/bee/day or mg/larvae	Sugar content of nectar in mg/mg	chemical concentration in pollen (see above)	chemical concentration in nectar (see above)	Relevance
BB acute	30.3	111-149	0.15	-5.99146	-5.65499	imidacloprid
BB chronic	30.3	73-149	0.15	-5.99146	-5.65499	imidacloprid
SB adult	10.2	18-77	0.10	-5.99146	-5.65499	imidacloprid

Results

The calculated refined SVs were the following:

Table 9.4-19: SV calculation

Relevance	bee type & category	Tier 2 SV (µg/bee or µg/bee/day or µg/larva)	Comment
imidacloprid	BB acute	0.00312	
imidacloprid	BB chronic	0.00269	
imidacloprid	SB adult	0.00171	

The tier 2 SVs for imidacloprid are more than 2 orders of magnitude lower than the tier 1 SVs considering the residue levels of 2.5 µg/kg and 3.5 µg/kg in the pollen and nectar of the succeeding annual crop.

Refined risk assessment

Since the used residue values are not RUD values, but they were considered as representative for the uses under evaluation, the refined SVs should be used in the refined RAs without considering the application rate of the primary crop (i.e. these SVs can be considered as representative for any GAP, provided that the crop rotation and the ageing processes leading to a certain PECplateau is considered representative). Additionally, both the Ef and the twa values are supposed to be 1 in the RAs for these scenarios. Therefore, the formula to be used can be simplified as:

ETR = SV/tox. endpoint

Using this formula the risk quotients are the following:

Table 9.4-20: Tier 2 ETR calculations for acute adult oral and chronic adult oral exposure of imidaclprid in winter cereals and sugar beet

Chemical	bee type & category	Tier 2 SV (µg/bee or µg/bee/day or µg/larva)	Toxicity endpoint	ETR	Trigger
imidacloprid	BB acute	0.00312	0.038	**0.082105**	> 0.036
imidacloprid	BB chronic	0.00269	> 0.000282	**< 9.539007**	> 0.0048
imidacloprid	SB adult acute	0.00171	0.00037	**4.621622**	> 0.04
imidacloprid	SB adult chronic	0.00171	> 0.000282	**< 6.06383**	> 0.0054

Taking into account these conservative measured residue values, an unacceptable risk to wild solitary bees and bumble bees has the be concluded for all intended uses according to the GAP table~~, except for the acute risk to bumble bees~~.

Further refinements to the risk assessment could be based on field effect studies. No higher tier effect studies specifically assessing the risk to bumble bees or solitary wild bees from the consumption of nectar and pollen in succeeding crops are available.

Conclusion:

A risk for bumble bees and solitary wild bees resulting from foraging on succeeding crops cannot be excluded.

Pollinators other than honey bees (commercially used)

Guttation

As it is known that honey bees need high amounts of water for brood rearing the RMS considers the issue of guttation, for other commercial pollinators than honey bees (e.g. *Osmia* and *Bombus terrestris*), risk assessment is covered by the higher tier assessment of honey bees. Furthermore for e.g. *Osmia* no relevant water uptake is known and no brood nest humidity control is necessary as for honey bees.

Residues in nectar and pollen

Sugar beets (if harvested before flowering) and cereals do not provide nectar or pollen for honey bees and other bees. On some potato varieties, bumble bees may intensively forage pollen. In two field studies conducted with bumble bee colonies situated at the edge of either fields grown with either imidacloprid or untreated potatoes flight and foraging activity was demonstrated. Only low residues were detectable, and in both studies no indication of any adverse effects on colony mortality, behaviour or colony development were observed following exposure of bumble bee colonies in field conditions. However in the PPR meeting 145 the majority of experts agreed that, due to the uncertainties (i.e., low statistical power, questionable exposure), the studies are not sufficient to draw any solid conclusion on the effects of imidacloprid on wild bees. Therefore, and in absence of other sufficiently data, an unacceptable risk could not be excluded for the use in potato.

Dust drift

The high acute and contact toxicity as well as the chronic dietary toxicity of the active substance Imidacloprid and the metabolites to bumble bees has been assessed in the DAR on Imidacloprid (2005). Further assessments were performed by EFSA in 2008 and in 2013, which in principle confirmed the conclusions of very high dietary toxicity made in the DAR (2005).

No semi-field and field studies have been submitted as confirmatory data to specifically assess the effect of the use of imidacloprid as seed treatment in cereals and sugar-beet on bumble bees or non-*Apis* bees which are used as commercial pollinators. For biological reasons, there is no likelihood of exposure of *Osmia rufa* to dust drift in autumn, also the likelihood of exposure of individuals of *Bombus terrestris* foraging is rather low in autumn.

Many pollinators, such as the commercial used solitary bee *Osmia rufa* are not active as adults at this time of year and are not nesting, nor brood-caring. At this time of year solitary bees are at larval and pupal stages within nests and are not exposed to dust drift, thus a risk can be excluded. Non-*Apis* bees such as bumble bees may be exposed to dust generated by machinery at the time of sowing winter cereals. However, by autumn, the annual colonies of bumble bees are no longer viable. The colony collapses and does not overwinter and new colonies are founded each spring by new queens. Consequently the only relevant caste of bee is the newly emerged and mated queens (or gynes). These larger and more robust individuals will overwinter and found new colonies in the spring. They are typically 2x larger in size compared to worker bumble bees and at least 5x larger than honey bees used in the laboratory studies. As no adverse effects are expected following sowing of sugar beet of good seed treatment quality, for honey bees, there are yet no data that indicate that other pollinators are likely to be at greater risk, however it can also not be fully excluded.

For dust drift during sowing of cereals, on the basis of honey bee data a risk for honey bees could not be excluded, and thus likewise a risk cannot be excluded also for commercial pollinators such as *Bombus* and *Osmia*. However the argumentation that the likelihood of exposure of individual bumble bees is low in autumn and no exposure takes places for solitary bees like *Osmia* is shared by the RMS.

B.9.4.2 The risk to honey bees foraging nectar or pollen in succeeding crops

The first tier risk assessment has been performed using the highest and lowest authorized application rate for winter cereals, beets, potato, leafy vegetables and turf. Here, a potential risk was identified for all honey bee developmental stages and for all uses, as all ETR values exceed the relevant trigger values. However, the applicant submitted a number of studies in which the concentrations of imidacloprid in nectar and pollen of bee attractive crops (*phacelia*, maize or mustard) were measured under conditions of "naturally" aged residues (succeeding crops grown on soils with a history of imidacloprid use) or "forced" plateau concentration (succeeding crops grown on soils treated with imidacloprid to obtain a theoretical plateau concentration of imidacloprid in the soil). The results from these studies show that there are low but measurable residues of imidacloprid in pollen and nectar of succeeding crops. The exposure to natural aged residues was generally lower than in the model studies with artificial soil residues. This could be explained by the fact that in the naturally-aged-residue studies, imidacloprid had already undergone ageing processes, making them less available for plant uptake as compared to the "forced plateau concentration" studies. For the second tier risk assessment the highest residue values from the "naturally" aged trials was used (2.5 µg a.s./kg for pollen and 3.5 µg a.s./kg for nectar). Here, again a potential risk was identified for all honey bee developmental stages and for all uses. Following the EFSA Guidance Document on bees higher tier test are required which were not submitted by the applicant. However, independent of the study design discussed here no residues in any samples was higher than those obtained in primary crops which have no effects on honey bee colony development (refer to DAR 2005). Therefore the risk was considered as acceptable.

Overall conclusion:
The risk of imidacloprid to honey bees from consumption of contaminated pollen and nectar in succeeding crops can be considered acceptable; as the level of residues in nectar and pollen detected in the investigated flowering crops were in the range or below levels of primary crops, for which in former assessments (DAR 2005, EFSA 2008 and EFSA 2013) no clear effects on acute mortality and honey bee colony development were observed.

B.9.4.3 The potential uptake via roots to flowering weeds

The applicant did not assess the potential uptake of imidacloprid via roots into flowering weeds. Instead, a collection of data from multiple years of efficacy trials was provided in which the occurrence of flowering weeds in agricultural crops was assessed. In principle, the methodology of this summary seems reasonable. However, since the experiments were conducted in order to investigate efficacy of herbicides, no data were collected right before harvest. Hence, there is no information on how many weeds reached flowering stages during the period after the last sampling and before harvest. However, in arable crops that display little competition like sugar beets there will be an extensive weed control until the plant is large enough. At this time the sugar beet covers the majority of the ground. Therefore it can be expected that only a few weeds occur, whereas the occurrence of flowering weeds in the later development stages of cereals and potatoes cannot be ruled out. For the methodologically correct determination of the probability and abundance of flowering weed in later development stages, a further monitoring is necessary.

Overall conclusion

Based on available data, a risk for honey bees reading imidacloprid residues in nectar and pollen of flowering weeds growing on treated fields can only be excluded for the first development stages of the arable crops observed. In addition, no data on residues in nectar and pollen of flowering weeds were provided. Thus, the risk assessment of the potential uptake via roots to flowering weeds could not be finalized.

B.9.4.4 The risk to honey bees foraging on insect honey dew produced by aphids sucking on seed treated plants

The applicant did not provide any data regarding the presence of honey dew in crops grown from imidacloprid treated seeds. Instead, two statements were submitted to demonstrate that exposure to honeydew is negligible and that the development of resistance against a plant protection product containing the active substances imidacloprid and clothianidin will be unlikely (Nauen R. 2013, M-453965-01-1).

From the RMS point of view, imidacloprid has a very high efficacy on aphids and therefore no aphid population build up and relevant honeydew production has to be expected. Aphids need to be able to feed on a treated plant without being killed by the imidacloprid present in the phloem sap to produce honey dew. At later stages of crop development, when the levels of systemic insecticide have declined and no longer provide sap feeding insect control, these levels may pose a low risk to bees. Moreover, due to the possible impacts of aphids and other sap feeding insects on crop yield, even at later stages of crop development, aphids will be chemically controlled by other insecticides. Consequently, it is unlikely that large aphid infestations (and thus high levels of honey dew) will occur in crops grown from imidacloprid treated seeds. Furthermore, no resistance of aphids to neonicotinoids is known yet. However, recently *Myzus persicae* was shown to have developed resistance to neonicotinoid insecticide sprays in peaches in southern Europe, based on a target-site mutation in the nicotinic acetylcholine receptor ß-subunit. No neonicotinoid resistance was detected from *M. persicae* on any secondary host species yet, including sugar beet and potatoes.
The statement paper by Nauen et al. 2013 was also discussed at the Pesticides Peer Review 145 Ecotoxicology meeting. Here, the argumentation provided was agreed since imidacloprid is intended to control sap sucking insects and at least during the first weeks of growth the exposure of honey bees is likely to be low. In relation to that, the paper by Foster 2008 was also considered. It was noted that the ED_{50} in the study by Foster 2008 was not consistent among the tested clones as there were some apparent variability (although this variability in the effects concentration on *M. persicae* was lower than the one for clothianidin). It was agreed that neonic resistence to aphids could not be excluded (there are several reported cases of neonics resistant strains of aphids in literature, including *M. persicae*, which is a highly polyphagous species). Moreover, it was noted that at later crop growth stages (i.e., after the 8th week) the efficacy of the aphids control will be lower, therefore a certain exposure of honey bees through honeydew might occur. Overall, the experts agreed on the basis of the available data that honeydew can be considered as a low relevance route of exposure for the treated crop scenario of the uses under evaluation.

Overall conclusion
The exposure of honey bees to imidacloprid through honey dew present in the treated field can be considered negligibly low, provided that there is no resistance of aphids.

B.9.4.5 The potential guttation exposure and the acute and the long-term risk to

colony survival and development, and the risk to bee brood resulting from such exposure

An initial theoretical calculation regarding the acute toxicity of guttation fluid from seed treated crops and an evaluation of studies concerned with several aspects of guttation was made by EFSA in 2013 (EFSA Journal 2013;11(1):3068). A data gap was concluded as no studies were available that specifically investigating effects on bees triggered by guttation fluid from plants seed treatment with imidacloprid. Therefore the applicant has submitted seven higher tier studies to address the exposure, and hence the risk (i.e. the acute and long-term risk to colony survival and development, and the risk to bee brood) to bees from exposure via guttation fluid for all crops for which imidacloprid is authorised as a seed treatment. These studies cover the maximum use conditions for imidacloprid (IMD) seed treatment uses in winter cereals, beet crops and potato i.e. 70 g a.s./dt (126 g a.s./ha), 90 g/U (117 g a.s./ha) and 180 g a.s./ha, respectively. In some studies seeds were treated with the maximum use rates of both clothianidin and imidacloprid in a single formulation (Hofmann S. et al 2014, M-501261-01-1; Rexer H.U. 2014a, M-500724-01-1; Rexer H.U. 2014b, M-500734-01-1). However, the levels of parent molecules present in guttation water of both substances together were similar to when they are used separately. Although formulations containing both imidacloprid and clothianidin are not currently registered in Europe, a combination of both active substances could be applied during seed treatment, and additionally the notifier has on-going registrations for formulations which contain a mixture of both clothianidin and imidacloprid. Thus, this situation represents a realistic exposure scenario. Based on the physiological properties which determine guttation, and on the observations in these studies, it can be demonstrated that the presence of one active substance does not influence the uptake and expression of the second active substance. Hence the study can be used for both substances and represents a worst case exposure scenario covering both substances and co-formulations.

For each crop the occurrence and proportion of guttation, behaviour observation on honey bee, mortality condition of the colonies overwintering performance and residue analyses have been performed.
As winter cereals are sown in autumn there are potentially two guttation periods to which honey bees could be exposed: one in autumn shortly after crop emergence and before overwintering and again in the spring after winter hibernation.
Sugar beets and potatoes are drilled in the spring and hence have one guttation period during that time.

Winter cereals
In winter cereals guttation was observed in both treated and untreated crops and was a fairly common occurrence in both the autumn and spring exposure periods. The frequency to which guttation occurred in cereals was similar between wheat and barley and was also generally independent of the year of study.
Residue levels of imidacloprid and its major plant metabolites (imidacloprid 5 hydroxy and imidacloprid olefin) in guttation fluid produced by winter cereals were similar with an indication that residues in the spring are far lower than those observed in autumn. This can be explained by the fact that in the spring the cereal plants are older, larger and in a phase of rapid growth in contrast to the plants in the autumn about to enter winter.
Bees were similarly likely to be active on days where guttation occurred in winter cereals in autumn as they were in spring. However; far fewer bees (as a proportion of those observed at the study sites) were observed to be collecting guttation water in the autumn compared to the spring. This can be explained by the fact that in autumn the colonies are declining in size and preparing to overwinter and in the spring colonies are active and increasing in size as egg laying

recommences after the overwintering period. Thus, the autumn colonies have a lower demand for resources compared to those in spring.
The daily mortality levels of colonies located at the edge of the winter cereal fields were generally observed to be at a low level. Occasional peaks of mortality were observed but these occurred at both treated and control sites and were of similar magnitude. There was a slight tendency for more frequent peaks at treated field sites than at control sites. However, these do not a follow a systematic pattern related to guttation events or exposure and are most probably due to local weather conditions; especially in the studies conducted in autumn 2009 where the weather was cold approaching winter.
Furthermore, no treatments related differences in the overwintering performance between the control and the imidacloprid groups were observed.

Sugar beet and potato
In contrast, guttation was far less common in sugar beet and potato than observed for winter cereals.
Residue levels of imidacloprid and its major plant metabolites (imidacloprid 5 hydroxy and imidacloprid olefin) in guttation fluid produced by sugar beet and potato plants in spring (i.e. shortly after emergence) were at least an order of magnitude lower than the residues found in guttation fluid produced by winter cereals in the autumn.
Bees were active on days when guttation occurred but were not observed to visit the fields sown with either treated or untreated seeds for sugar beet and treated seed tubers for potato. Furthermore, they were not observed collecting guttation water from sugar beet or potato plants at any time during these experiments.
In the studies where honey bee colonies were exposed to guttating sugar beet and potato mortality was generally low and consistent with no differences between the colonies located at treated and control site.
Furthermore the overwintering success for sugar beet and potatoes was 100% for all colonies.

Additionally, the applicant submitted a number of studies in which the concentrations of imidacloprid in guttation fluid excreted from succeeding crops were measured. For this, maize grow on field with “naturally” aged residues or “forced” plateau concentration (please refer to B.9.4.2) were observed. The measured values were mostly below or in rare cases equal to the concentration in guttation droplets of seed treated crops. As for seed treated crops the risk was considered acceptable, this conclusion also applies for guttation of succeeding crops.

Overall conclusion:
Although the concentrations of insecticides such as imidacloprid in guttation fluid arising from the use as a seed treatments can be present at levels theoretically capable of harming individual bees, acute and chronic colony level effects were not observed in the studies presented here. Furthermore, honey bee behaviour as well as other factors relating to colony wellbeing (colony strength, health status such as presence and level of *Varroa*, viruses and other pathogens) were unaffected by exposure to guttating winter cereals, potatoes or sugar beets treated with imidacloprid (and clothianidin) as a seed treatment. Therefore, it can be concluded that residues of imidacloprid in guttation fluid produced by winter cereals, sugar beet and potato plants at the maximum seed dressing rates do not pose an unacceptable acute or chronic risk to honey bee colony development or survival.

B.9.4.6 The potential exposure to dust drift following drill and the acute and the long-term risk to colony survival and development, and the risk to bee brood

resulting from such exposure

Higher tier studies were provided to address the potential side effects of insecticidal dust drift in realistic conditions.

As there is a limited published data set on the effects of insecticidal dust on bees, and there is no agreed risk assessment scheme for dusts and no clear guidance available, additional considerations are needed. Dust particles abraded from seed treatments containing imidacloprid or clothianidin are highly toxic to bees. Dust drift may result in exposure via dust drift during sowing and contact with particles while flying through the dust cloud, exposure during foraging activity on treated flowers and leaves and exposure to particle contaminated nectar and pollen. For the evaluation of side effects it is highly relevant how much dusts can be abraded and which residue content these dusts contain, while it is only of little relevance what the seed loading and the amount of a.s. sown per ha is. The calculation on the basis of the Heubach g a.s./ha is considered more appropriate than the application rate per ha.

In addition, the machinery used has a significant role in emitting dusts into the environment. When dusts are emitted, an exposure of bees may occur. In spite of the important role in determining the risk for bees, an assessment of the machinery and their potential emission could not be performed within this assessment. For the practical use this implies that the use of specific machinery used may be regulated within the risk management, as e.g. it is known that pneumatic sowing machines emit more dusts than mechanical machines, but also that there is variation within pneumatic machines.

Cereals

While first tier calculations both on basis of HQ calculations (draft EU SANCO/10553/2012) and likewise ETR-calculations in indicated that a risk could not be excluded at this level, two new higher tier studies with sowing of winter barley and one study with sowing of winter wheat were conducted with measurements of dust deposits (Lueckmann & Staffel, 2014b). Another study with investigation of dust deposits after sowing of barley and one further study after sowing of sugar beet were performed in combination with an assessment of potential side effects on bees.
In the bee effect studies, no acute and chronic effects on honey bee colonies (including mortality, behaviour, health status, colony strength and overwintering success) were found after sowing of cereals. However, the study design, using large flowering fields adjacent as exposure areas, does not necessarily cover a realistic worst case scenario in the specific dust risk assessment. Furthermore, for the issue of dust drift, additional aspects need to be considered to evaluate the risk in practical conditions: a higher variability for the quality of the seed treatments available on the market needs to be assumed for cereals compared to sugar beet. While the seed treatment of sugar beets is performed in specialized seed treatment facilities and it is known that the sugar beet pill has a high seed treatment quality and low abrasiveness of the seed treatment, treatment of barley and winter wheat is done in different manner. It is not possible to ensure for risk assessment on the basis of the available confirmatory data, that a worst-case seed batch has been investigated.
Dust drift values used as a basis for risk assessment should not be generated with seed batches having outstandingly good treatment quality (i.e. relatively low Heubach-values and/or low concentrations of a.i. in the dust), since these are not representative for the quality of seed on the market (DR SEED GD SANCO). Thus, for insecticide treated crops, crop-specific requirements should be set for a worst-case approach.

The registered rates for seed treatments in cereals and barley are in between 48 – 126 g a.s./ha. Therefore the available study is representative for the currently registered uses in winter cereals and barley considering only the application rate; however there is uncertainty to which extent the provided studies represent the market quality keeping in mind that max. Heubach values for cereals have been defined in France as 5 g /100kg and in Germany as 5 g / 150-250 kg of seeds. While it is agreed that for winter sown cereals the likelihood of exposure for bees is not always given, as there may be little flowering bee attractive crops downwind of the treated fields, the situation may arise in the agricultural practice, especially with the increase of flowering strips and flowering plants used for greening, e.g. Sinapis which may flower late in the year and weather circumstances may result in single days where bee flight activity takes place.
Regarding the worst-case exposure scenarios, it is discuss worthy if field studies may reflect scenarios in landscape with small-sized agricultural fields, in which a scenario of contaminated flowering weeds or adjacent crops may be higher, however it needs to be acknowledged that in other circumstances with larger fields this may be a more field-realistic situation for many agricultural areas.

Considering a field realistic-worst case scenario, considering the basis of available studies, for sowing of cereals, a risk for bees cannot be excluded. It is assumable that this conclusion can be extrapolated from winter cereals to summer cereals.

Sugarbeets

No dust drift deposits above the LOD were measured following sowing of sugar beet pills. In parallel, a field effect study in *Phacelia* with dust drift from sowing of sugar beet was performed at two different locations (Lueckmann & Staffel, 2014a). In the study, no acute and chronic effects on honey bee colonies (including mortality, behaviour, health status, colony strength and overwintering success) were found after sowing of sugar beet pills.

The confirmatory data presented here confirm the former conclusion of EFSA (2013) in which in first-tier on the basis of the draft SANCO/10553/2012 was considered risk to bees was considered to be low and also confirm the formerly submitted higher tier data. Overall it is concluded the risk to honey bees from dust drift of treated sugar beet seeds is acceptable.

The registered rates for sugar beet are 15 – 117 (the highest application rate of up to 135-162 is registered in a individual country and therefore not representative for the EU); for these rates CTD and IMD were added if combined in one seed treatment, which seems justified considering the similar mode of action and toxicity to bees. Therefore, the available study with the application rate of 78 g Clothianidin + 10.4 g ß-Cyfluthrin + 39 g Imidacloprid is representative for most of the registered uses in sugar beet, however the high application rates of 135- 162 g as/ha are not fully covered by submitted data.

However, it is noted that also for sugar beet seed treatment quality assurance is essential to guarantee the high level of resistance against abrasion of dusts during sowing.

Considering a field realistic-worst case scenario, the risk to honey bees following dust drift from treated sugar beet seeds is considered acceptable.

Granules

Product: Merit turf

In the conclusion of EFSA (2013), it was concluded for the product Merit Turf that on the basis of trigger values a risk to honey bees from exposure via dust drift cannot be excluded. The data were not considered sufficient to demonstrate a low risk.
For evaluation of confirmatory data presented here no further data on potential abrasion of dusts were provided by the applicant. A statement on the machinery used and way of distribution was provided.
While the notifier argued that due to the fact that the product is applied via spreading, that there is no mixing of the granules and the granules are irrigated straight after application the risk of dust drift and hence exposure of honey bees from this technique is low, concern needs to be pointed out because within the evaluation no data on the composition of the granules are available. If it is demonstrated that only very low amounts of dusts can be abraded from the formulated granules, in combination with certified machinery a low risk for the exposure route of dust drift seems possible. For hand spread granules, no risk from dust drift is expected. However, no new information was available for the evaluation of confirmatory data which can be used for further evaluation.

Overall conclusion:
As an overall conclusion, a risk to bees following dust drift from treated cereal seeds cannot be fully excluded, both for Imidacloprid seed treated wheat and barley.
The risk to bees following dust drift from treated sugar beet seeds is considered acceptable. No further data were available for granules (product Merit turf) and no data for the abrasiveness of the granules provided, thus the assessment could not be finalized for machine assisted spreading, whereas the risk of dust drift is considered low for hand spread granules.

B.9.4.7 The acute and long term risk to colony survival and development and the risk to bee brood for honey bees from ingestion of contaminated nectar and pollen

The first Tier ETR values for acute adult oral exposure in the lowest application rate of winter cereals and larval exposure in both winter cereals and potato are below the relevant trigger, indicating an acceptable risk. For all remaining scenarios a potential risk was identified. No new or appropriate information on imidacloprid residues in winter cereal or potato pollen are available. Therefore no second Tier risk assessment was performed. Furthermore no higher tier studies are available. However, as winter cereals are generally considered to be of low attractiveness for pollen the risk can be considered acceptable.
It has been a point of discussion if the potato flower is actually attractive to bees; only very few data indicate potato flowers could also be attractive to honey bees. However, the residues detected in pollen collected by bumble bees of potatoes seed treated with imidacloprid were low and clearly below residue levels detectable in pollen of other seed treated flowering crops (please refer to table 9.5.2-39 and table 9.5.2-43). The residues in combination with the fact that exposure seems to take place only in very rare circumstances leads to the conclusion of low risk to honey bees.
In the conclusion of EFSA (2013), it was concluded for the product “Merit turf” that due to the presence of flowering weeds cannot be excluded in turf, home garden lawns or public grass vegetation, a potential risk to bees foraging on flowering weeds cannot be excluded in all circumstances. For evaluation of confirmatory data presented here no further data on potential residues in nectar and pollen were provided by the applicant. The RMS agrees with the evaluation of EFSA (2013) that in highly managed amenity turf, such as golf greens and professional sports grounds, flowering weeds are unlikely to occur and hence a low risk to

pollinators could be concluded in these situations. However, as the presence of flowering weeds cannot be excluded in turf, home garden lawns or public grass vegetation, a potential risk to bees foraging on flowering weeds cannot be excluded for all of the intended uses of the product.

Overall conclusion:
As no exposure is expected to nectar and pollen from sugar beet, potatoes and winter cereals as treated crops the risk can be considered acceptable.

B.9.5 Extended study summaries

B.9.5.1 Toxicity

Honey bees
The oral and contact toxicity of imidacloprid to adult honey bees were assessed in three laboratory tests.

Report:	Sekine, T. 2014
Title:	Effects of imidacloprid FS 350A G (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory
Report No.:	89281035
Document No.:	M-500305-01-1
Guideline(s):	OECD 213 and 214 (1998)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective
The objectives of this study were to determine possible effects of Imidacloprid FS 350 (350 g a.s./L) on the honey bee (*Apis mellifera* L.), from contact and oral exposure.

Material and Methods:

Imidacloprid FS 350 G: Batch-ID: EDFL020681, Material No.: 04817397; density: 1.169 g/mL (20 °C).
Under laboratory conditions *Apis mellifera* 30 worker bees per treatment level were exposed for 96 hours to doses of 500.0, 250.0, 125.0, 62.5, 31.3, 15.6 and 7.8 ng a.s./bee by topical application (contact dose response test) and 30 worker bees per treatment level were exposed also for 96 hours to doses of 91.7, 72.5, 37.8, 17.7, 10.0, 7.2 and 3.5 ng a.s./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.

1. Test material:

Test item:	Imidacloprid FS 350 (350 g a.s./L)
Description:	Liquid, red
Lot/Batch #:	EDFL020681

Content of a.s.:	355.2 g/L imidacloprid (analysed)

2. Vehicle and/or positive control:

Vehicle:	dimethoate were applied in 50 % w/v sucrose solution, which was used as carrier (oral test) dimethoate, dissolved in tap water with 0.5 % Adhäsit* (contact)
Positive control:	0.30, 0.15, 0.08 and 0.05 µg dimethoate per bee (oral test) 0.30, 0.20, 0.15 and 0.10 µg dimethoate per bee (contact test)

3. Test organisms:

Species:	honey bee (*A. mellifera carnica* L.)
Age:	adult female worker bees
Source:	Honey bee colonies, disease-free and queen-right, bred by IBACON
Diet/Food:	50 % w/v sucrose solution (500 g/L tap water) *ad libitum;* was given directly after treatment

4. Environmental conditions:

Temperature:	25 °C
Humidity:	38 - 70 %
Photoperiod:	constant darkness

Findings:

The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.22 and 0.23 µg a.s./bee, respectively. No mortality occurred in the contact control group (water + 0.5 % Adhäsit). There was 6.7 % mortality in the oral control group (sucrose 50 % w/v solution = 500 g sucrose/L tap water).

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

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

Table 9.5.1-1: Toxicity to Honey Bees; laboratory tests

Exposure	contact (solution in Adhäsit (0.5 %)/water)	oral (50 % w/v sugar solution)
LD_{50} ng a.s./bee	24 hours: 154.0 48 hours: 60.0 72 hours: 49.5 96 hours: 47.6	24 hours: n.d.** 48 hours: 53.7 72 hours: 29.3 96 hours: 24.4
NOED ng a.s. /bee*	24 hours: 31.0 48 hours: 16.0 72 hours: 16.0 96 hours: 16.0	24 hours: < 3.5 48 hours: 7.2 72 hours: 7.2 96 hours: 10.0

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

Conclusion:
The toxicity of Imidacloprid FS 350A G was tested in both, an acute contact and an acute oral toxicity test on honey bees. The contact LD_{50} values (96 h) of Imidacloprid FS 350A G were determined to be 47.6 ng a.s./bee. The oral LD_{50} values (96 h) were 24.4 ng a.s./bee.

RMS's comments:
The validity criteria of OECD Guideline 213 and 214 are met:

- less than 10 % mortality in the control (observed: 6.7 % mortality during the 48h test period for oral toxicity test and no mortality during the contact toxicity test)
- LD_{50} for the reference item in the range of 0.10 – 0.30 µg a.s./bee for the contact test and 0.10 – 0.35 µg a.s./bee for the oral test (observed: 0.22 µg a.s./bee for the contact test, 0.23 µg a.s./bee for the oral test)

Consequently, the study is considered acceptable and suitable for use in risk assessment.

This study again shows the high toxicity from imidacloprid and imidacloprid containing formulations to honey bees.

Report:	Schmitzer, S. 2014a
Title:	Effects of clothianidin + imidacloprid FS 275 (100+175) G (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory
Report No.:	89691035
Document No.:	M-501653-01-1
Guideline(s):	GLP compliant study based on OECD 213 and 214 (1998)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective
The objectives of this study were to determine possible effects of Clothianidin + Imidacloprid FS 275 (100+175 g a.s./L) on the honey bee (*Apis mellifera* L.), from contact and oral exposure and to determine the median lethal dose (LD_{50}).

Material and Methods:

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

1. Test material:

Test item:	Clothianidin + Imidacloprid FS 275 (100 + 175 g a.s./L)
Content of a.s.:	100.3 g/L clothianidin (analysed) 176.7 g/L imidacloprid (analysed)

2. Vehicle and/or positive control:

Vehicle:	dimethoate were applied in 50 % w/v sucrose solution, which was used as carrier (oral test) dimethoate, dissolved in tap water with 0.5 % Adhäsit* (contact)
Positive control:	0.30, 0.20, 0.15 and 0.10 µg dimethoate per bee (contact test) 0.30, 0.15, 0.08 and 0.05 µg dimethoate per bee (oral test)

3. Test organisms:

Species:	honey bee (*Apis mellifera carnica* L.)
Age:	adult female worker bees

Source:	Honey bee colonies, disease-free and queen-right, bred by IBACON
Diet/Food:	50 % w/v sucrose solution (500 g/L tap water) (provided as "household sugar") *ad libitum;* was given directly after treatment.

4. Environmental conditions:

Temperature:	25 °C
Humidity:	51 – 96 %
Photoperiod:	constant darkness

Findings
The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.28 and 0.14 µg a.s./bee, respectively.
No mortality occurred in the contact control group (water + 0.5 % Adhäsit) and the oral control group (sucrose 50 % w/v solution = 500 g sucrose/L tap water), respectively.

Contact Test:
Test item dose levels of 1.0, 0.50, 0.25, 0.13, 0.063 and 0.031 µg product/bee led to dose dependent mortality, ranging from 73.3 % to 3.3 % at test end (48 hrs following treatment).
Behavioural abnormalities (e.g. moribund or affected bees, cramps) were observed in all dose level groups during the 4-hours assessment. Behavioural abnormalities were also observed during the 24-hours assessment in the 1.0, 0.5, 0.25 and 0.13 µg product/bee treatment groups. 48 hours following the application, five bees were found to be affected in the 1.0 µg product/bee dosing group. No further behavioural abnormalities were found in the other dosing groups. All other surviving bees appeared normal.

Oral Test:
Mortality occurred in all test item treated dose levels. Actual oral doses of 0.17, 0.11, 0.053, 0.027 and 0.013 µg product/bee resulted in mortality ranging from 96.7 % to 6.7 % at the end of the test (48 hours after application).
Behavioural abnormalities (e.g. moribund bees or affected bees) were found during the 4-hours assessment in the 0.17, 0.11, 0.053 and 0.027 µg product/bee treatment groups. A few bees were behaving abnormal 24 hours following treatment in the 0.17, 0.11 and 0.053 µg product/bee dose levels and one and 6 bees were found to be affected during the 48-hours assessment in the 0.17 and 0.11 µg product/bee treatment group, respectively. No behavioural abnormalities were found in the 0.013 µg product/bee dosing group during the test.

Table 9.5.1-2: Toxicity to Honey Bees; laboratory tests

Exposure	contact (solution in Adhäsit (0.5 %)/water)	oral (sugar solution)
LD_{50} µg product/bee	24 hours: 0.39 48 hours: 0.29	24 hours: 0.062 48 hours: 0.058
NOED µg product/bee*	24 hours: 0.063 48 hours: 0.063	24 hours: 0.027 48 hours: 0.027

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

Conclusion:
The toxicity of Clothianidin + Imidacloprid FS 275 (100+175) G was tested in both, an acute contact and an acute oral toxicity test on honey bees. The oral LD_{50} 48 h value was 0.058 µg product/bee (equivalent to 0.005 µg clothianidin + 0.009 µg imidacloprid/bee). The contact LD_{50} 48 h value was 0.29 µg product/bee (equivalent to 0.026 µg clothianidin + 0.046 µg imidacloprid/bee), respectively.

RMS's comments:
The validity criteria of OECD Guideline 213 and 214 are met:

- less than 10 % mortality in the control (observed: no mortality during the contact and oral toxicity test)
- LD_{50} for the reference item in the range of 0.10 – 0.30 µg a.s./bee for the contact test and 0.10 – 0.35 µg a.s./bee for the oral test (observed: 0.28 µg a.s./bee for the contact test, 0.14 µg a.s./bee for the oral test)

Consequently, the study is considered acceptable and suitable for use in risk assessment.

This study again shows the high toxicity from imidacloprid and imidacloprid containing formulations to honey bees.

Report:	Schmitzer, S. 2014b
Title:	Effects of imidacloprid + pencycuron FS 370 (120+250) G (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory
Report No.:	89661035
Document No.:	M-503109-01-1
Guideline(s):	GLP compliant study based on OECD 213 and 214 (1998)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective
The objectives of this study were to determine possible effects of imidacloprid + pencycuron FS 370 (120+250) G on the honey bee (*Apis mellifera* L.), from contact and oral exposure and to determine the median lethal dose (LD_{50}).

Material and Methods:

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

1. Test material:

Test item:	Imidacloprid + Pencycuron FS 370 (120 + 250 g a.s./L)
Content of a.s.:	119.8 g/L imidacloprid (analysed) 252.0 g/L pencycuron (analysed)

2. Vehicle and/or positive control:

Vehicle:	dimethoate were applied in 50 % w/v sucrose solution, which was used as carrier (oral test) dimethoate, dissolved in tap water with 0.5 % Adhäsit* (contact)
Positive control:	0.30, 0.20, 0.15 and 0.10 µg dimethoate per bee (contact test) 0.30, 0.15, 0.08 and 0.05 µg dimethoate per bee (oral test)

3. Test organisms:

Species:	honey bee (*Apis mellifera carnica* L.)
Age:	adult female worker bees
Source:	Honey bee colonies, disease-free and queen-right, bred by IBACON
Diet/Food:	50 % w/v sucrose solution (500 g/L tap water) *ad libitum;* was given directly after treatment

4. Environmental conditions:

Temperature:	25 °C
Humidity:	38 – 70 %
Photoperiod:	constant darkness

Findings

The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.22 and 0.23 µg a.s./bee, respectively. No mortality occurred in the contact control group (water + 0.5 % Adhäsit). 6.7 % mortality occurred in the oral control group (50 % w/v sucrose solution = 500 g sucrose/L tap water).

Contact Test:
The contact test was prolonged for a further 48 hours up to 96 hours due to increasing mortality between 24 and 72 hours. Application of 4.0, 2.0, 1.0, 0.50, 0.25 and 0.13 µg/bee of imidacloprid + 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).
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 4.0 and 0.50 µg/bee dose groups, respectively. At the last assessment (96 hours following application) one or two bees were still affected in the 2.0, 1.0 and 0.50

µg/bee dosing groups. No behavioural impairments occurred at the 0.13 µg/bee dose group at any time.

Oral Test:
The oral test was also prolonged for a further 48 hours up to 96 hours due to increasing mortality between 24 and 72 hours. In the oral test, the maximum nominal dose level of the test item (1.0, 0.50 and 0.25 µg product/bee) could not be achieved, because the bees did not ingest the full volume of treated 50 % w/v sucrose solution even when offered over a period of 6 hours. The resulting measured oral doses of 0.75, 0.39, 0.23, 0.14 and 0.07 µg product per bee resulted in mortality ranging from 53.3 % to 16.7 % at the end of the test (i.e. 96 hours after application). Behavioural abnormalities (e.g. bees were affected, moribund or apathetic) were observed in all dose groups during the 4-hours assessment. 24 and 48 hours following treatment bees were affected or apathetic in the 0.75, 0.39 and 0.23 µg/bee dose levels. During the 72-hours assessment 5 bees were still affected in the 0.75 µg/bee treatment and during the 96 hours assessment one bee was found to be affected in the 0.75 and 0.23 µg/bee dose levels, respectively.

Table 9.5.1-3: Toxicity to Honey Bees; laboratory tests

Exposure	contact (solution in Adhäsit (0.5 %)/water)	oral (50 % w/v sucrose solution)
LD_{50} µg product/bee	24 hours: 2.50 48 hours: 0.54 72 hours: 0.42 96 hours: 0.38	24 hours: > 0.75 48 hours: > 0.75 72 hours: ~~1.04~~ > 0.75 96 hours: ~~0.96~~ 0.75
NOED µg product/bee*	96 hours: 0.25	96 hours: < 0.07
Equivalent to: LD_{50} µg a.s. imidacloprid/bee	24 hours: 0.260 48 hours: 0.056 72 hours: 0.044 96 hours: 0.040	24 hours: > 0.078 48 hours: > 0.078 72 hours: ~~0.108~~ > 0.078 96 hours: ~~0.100~~ 0.078
Equivalent to: NOED µg a.s. imidacloprid/bee*	96 hours: 0.026	96 hours: < 0.0073
* The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$).		

Conclusion:
The toxicity of imidacloprid + pencycuron FS 370 (120+250) G was tested in both, an acute contact toxicity test and an acute oral toxicity test on honey bees.
The LD_{50} (96 h) of the test item was determined to be 0.38 µg product/bee (equivalent to 0.040 µg a.s. imidacloprid/bee) in the contact toxicity test. The LD_{50} (96 h) was 0.96 µg product/bee (equivalent to 0.10 µg a.s. imidacloprid/bee) in the oral toxicity test.

RMS's comments:
The validity criteria of OECD Guideline 213 and 214 are met:

- less than 10 % mortality in the control (observed: 6.7 % mortality during the 48h test period for oral toxicity test and no mortality during the contact toxicity test)
- LD_{50} for the reference item in the range of 0.10 – 0.30 µg a.s./bee for the contact test and 0.10 – 0.35 µg a.s./bee for the oral test (observed: 0.22 µg a.s./bee for the contact test, 0.23 µg a.s./bee for the oral test)

Consequently, the study is considered acceptable and suitable for use in risk assessment.

This study again shows the high toxicity from imidacloprid and imidacloprid containing formulations to honey bees.

Bumble bees

The contact toxicity of imidacloprid to adult bumble bees was assessed in three laboratory tests. No new tests on acute oral toxicity of imidacloprid to bumble bees were submitted.

Report:	Pfeiffer, S. 2014a
Title:	Imidacloprid FS 350 (350 g/L) - Acute contact toxicity to the bumble bee, *Bombus terrestris* L. under laboratory conditions
Report No.:	S13-05153
Document No.:	M-494307-01-1
Guideline(s):	No specific guidelines are available. The test design is based on OEPP/EPPO 170 (4) (2010) and OECD Guideline 214 (1998), and on the review article of van der Steen (2001)
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective

The objectives of this study were to determine possible effects of Imidacloprid FS 350 (350 g/L) on the bumble bee, *Bombus terrestris* L., from contact exposure and to determine the median lethal dose (LD_{50}) to *Bombus terrestris*.

Material and methods

The contact toxicity of Imidacloprid FS 350 (350 g a.s./L) to the bumble bee (*Bombus terrestris* L.) was determined in a dose-response test according to OEPP/EPPO 170 (4) (2010), the OECD Guideline No. 214 (1998) and the review article of VAN DER STEEN (2001).
In the laboratory, the bumble bees were exposed to 1.23, 3.70, 11.11, 33.33 and 100 µg imidacloprid a.s./bumble bee by topical application. Mortality and sub-lethal effects were assessed 24, 48, 72 and 96 hours after treatment. The control group was exposed for the same period of time under identical exposure conditions to tap water.
Pots of 10 bumble bees were anaesthetised with carbon dioxide, individually weighed and afterwards dosed with a 2 µL droplet containing the appropriate test solution placed onto the dorsal thorax of each bumble bee.

1. Test material:

Test item:	Imidacloprid FS 350 (350 g/L)
Content of a.s.:	355.2 g/L imidacloprid (analysed)

2. Vehicle and/or positive control:

Vehicle:	Dimethoate; Test item= dissolved in tap water
Positive control:	12 µg dimethoate a.s./bumble bee

3. Test organisms:

Species:	bumble bee (*Bombus terrestris* L.)
Age:	young adult worker bumble bees,
Source:	Koppert, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands
Diet/Food:	The bumble bees were supplied *ad libitum* with 50% (w/v) aqueous sucrose solution
Replicates:	3 replicates 10 bumble bees per group

4. Environmental conditions:

Temperature:	24.2 – 25.9 °C
Humidity:	48.7 – 63.5 %
Photoperiod:	constant darkness

Findings

In the control group, treated with tap water, no mortality was observed during the 96 hour test period. In the reference item group, mortality was ≥ 50 % at the end of the test.

At the dose level corresponding to 33.33 µg imidacloprid a.s./bumble bee, the highest mortality of 53.3 % was observed after 96 hours. In the test item treatment group, a mortality of 46.7 % was observed at the highest dose level corresponding to 100 µg imidacloprid a.s./bumble bee at the final assessment after 96 hours.

Moribund, affected and apathetic bumble bees were observed at all tested dose levels during the entire test period of 96 hours.

Table 9.5.1-4: Mortality in the contact toxicity test in the control, the test item (Imidacloprid FS 350 (350 g/L)) and the reference item group (Perfekthion)

Treatment Level [µg a.s./bumble bee]	**Mortality [%]**			
	24 h	**48 h**	**72 h**	**96 h**
Control (tap water)	0.0	0.0	0.0	0.0
Test item: Imidacloprid FS 350 (350 g a.s./L)				
1.23	0.0	6.67	16.67	20.0*
3.70	10.0	13.33	20.0	33.33*
11.11	6.67	6.67	16.67	26.67*
33.33	6.67	13.33	33.33	53.33*
100	10.0	23.33	36.67	46.67*
Reference item: Perfekthion				
12	70.00	73.33	76.67	76.67

*statistically significantly different compared to the control; (Fisher´s Exact Test, Bonferroni-Holmes corrected; one-sided, $p \leq 0.05$)

Table 9.5.1-5: LD_{50} values in the bumble bee contact toxicity test with Imidacloprid FS 350 (350 g a.s./L)

Imidacloprid FS 350 (350 g/L)	Contact toxicity test [µg imidacloprid a.s./bumble bee]
LD_{50} (24 h)	> 100
LD_{50} (48 h)	> 100
LD_{50} (72 h)	> 100
LD_{50} (96 h)	85.3*
NOED (96 h)	< 1.23

*Due to a weak dose-response, no meaningful confidence limits can be derived

Conclusions

The 96 hour contact LD_{50} value for Imidacloprid FS 350 (350 g a.s./L) was determined to be 85.3 µg imidacloprid a.s./bumble bee.
The NOED (No Observed Effect Dose) was determined to be < 1.23 µg imidacloprid a.s./bumble bee.

RMS's comments:
The validity criteria are met:
- less than 10 % mortality across the controls (observed: no mortality)
- more than 50 % mortality in the reference item group at the end of the test (observed: 76.67 %)

Overall, the study is considered acceptable and suitable for use in risk assessment.

Report:	Pfeiffer, S. 2014b
Title:	Clothianidin + imidacloprid FS 275 (100+175 g/L): Acute contact toxicity to the bumble bee, *Bombus terrestris* L. under laboratory conditions, M-494283-01-1
Report No.:	S13-05151
Document No.:	M-494283-01-1
Guideline(s):	No specific guidelines are available. The test design is based on OEPP/EPPO 170 (4) (2010) and OECD Guideline 214 (1998), and on the review article of van der Steen (2001)
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective

The objectives of this study were to determine possible effects of Clothianidin + Imidacloprid FS 275 (100+175 g/L) on the bumble bee, *Bombus terrestris* L., from contact exposure and to determine the median lethal dose (LD_{50}) to *Bombus terrestris*, where possible.

Material and methods

The contact toxicity of Clothianidin + Imidacloprid FS 275 (100+175 g a.s./L) to the bumble bee (*Bombus terrestris* L.) was determined in a dose-response test according to OEPP/EPPO 170 (4) (2010), the OECD Guideline No. 214 (1998) and the review article of VAN DER STEEN (2001).
In the laboratory, the bumble bees were exposed to 1.23, 3.70, 11.11, 33.33 and 100 µg total CNI/bumble bee by topical application. Mortality and sub-lethal effects were assessed 24, 48 and 72 hours after treatment. The control group was exposed for the same period of time under identical exposure conditions to tap water.

1. Test material:

Test item:	Clothianidin + Imidacloprid FS 275 (100 + 175 g/L)
Content of as:	100.3 g a.s./L clothianidin (analysed) 176.7 g a.s./L imidacloprid (analysed)

2. Vehicle and/or positive control:

Vehicle:	Dimethoate; Test item= dissolved in tap water
Positive control:	12 µg dimethoate a.s./bee

3. Test organisms:

Species:	bumble bee (*Bombus terrestris* L.)
Age:	young adult worker bumble bees
Source:	Koppert, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands
Diet/Food:	The bumble bees were supplied ad libitum with 50% (w/v) aqueous sucrose solution
Replicates	3 replicates 10 bees/group

4. Environmental conditions:

Temperature:	24.2 to 25.9 °C
Humidity:	51.3 to 63.5 %
Photoperiod:	constant darkness

Findings

In the control group, treated with tap water, no mortality was observed during the 72 hour test period. In the reference item group, mortality was ≥ 50 % at the end of the test. Thus, the test was considered to be valid.

In the test item treatment group, a mortality of 63.33 % was observed at the highest dose level corresponding to 100 µg total CNI/bumble bee at the final assessment after 72 hours.

In the test item treatment group, moribund, affected and apathetic bumble bees were observed at all tested dose levels at the 24, 48 and 72 hour assessments.

Table 9.5.1-6: Mortality in the contact toxicity test in the control, the test item (Clothianidin + Imidacloprid FS 275 (100+175 g/L)) and the reference item group (Perfekthion)

Treatment Level [µg total CNI/bumble bee]	**Mortality [%]**		
	24 h	**48 h**	**72 h**
Control (tap water)	0.0	0.0	0.0
Test item: Clothianidin + Imidacloprid FS 275 (100+175 g a.s./L)			
1.23	3.33	3.33	3.33
3.70	3.33	3.33	6.67
11.11	10.00	26.67	30.00*
33.33	13.33	26.67	33.33*
100	46.67	56.67	63.33*
Reference item: Perfekthion			
12	70.00	73.33	76.67

*statistically significantly different compared to the control; (Fisher´s Exact Test, Bonferroni-Holmes corrected; one-sided, $p \leq 0.05$)

Table 9.5.1-7: LD_{50} values in the bumble bee contact toxicity test with Clothianidin + Imidacloprid FS 275 (100 + 175 g/L)

Clothianidin + Imidacloprid FS 275 (100 + 175 g/L)	**Contact toxicity test [µg total a.s./bumble bee]**
LD_{50} (24 h)	> 100*
LD_{50} (48 h)	79.2 (43.82 – 226.69)**
LD_{50} (72 h)	54.9 (32.52 – 125.34)**
NOED (72 h)	3.70

* not determined
**lower and upper 95% confidence limits

Conclusions

The 72 hour contact LD_{50} value for Clothianidin + Imidacloprid FS 275 (100+175 g a.s./L) was determined to be 54.9 µg total a.s./bumble bee (equivalent to 19.9 µg CTD/bee + 35.0 µg IMD/bee).
The test item dose level corresponding to 3.70 µg total a.s./bumble bee was determined to be the NOED (No Observed Effect Dose) for mortality.

RMS's:
The validity criteria are met:
- less than 10% mortality across the controls (observed: no mortality)
- more than 50 % mortality in the reference item group at the end of the test (observed: 76.67 %)
Overall, the study is considered acceptable and suitable for use in risk assessment.

Report:	Pfeiffer, S. 2014c
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-494321-01-1
Guideline(s):	No specific guidelines are available. The test design is based on OEPP/EPPO 170 (4) (2010) and OECD Guideline 214 (1998), and on the review article of van der Steen (2001)
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective

The objectives of this study were to determine possible effects of imidacloprid + pencycuron FS 370 (120+250 g a.s./L) on the bumble bee from contact exposure and to determine the median lethal dose (LD_{50}).

Material and methods

The contact toxicity of Imidacloprid + Pencycuron FS 370 (120+250 g a.s./L) to the bumble bee (*Bombus terrestris* L.) was determined in a dose-response test according to OEPP/EPPO 170 (4) (2010), the OECD Guideline No. 214 (1998) and the review article of VAN DER STEEN VAN DER STEEN (2001).
In the laboratory, the bumble bees were exposed to 1.23, 3.70, 11.11, 33.33 and 100 µg imidacloprid a.s./bumble bee by topical application. Mortality and sub-lethal effects were assessed 24, 48, 72 and 96 hours after treatment. The control group was exposed for the same period of time under identical exposure conditions to tap water.

1. Test material:

Test item:	Imidacloprid + Pencycuron FS 370 (120 + 250 g/L)
Content of a.s.:	119.8 g a.s./L imidacloprid (analysed) 252.0 g a.s./L pencycuron (analysed)

2. Vehicle and/or positive control:

Vehicle:	Dimethoate; Test item= dissolved in tap water
Positive control:	12 µg dimethoate a.s./bumble bee.

3. Test organisms:

Species:	bumble bee (*Bombus terrestris* L.)
Age:	young adult worker bumble bees
Source:	Koppert, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands
Diet/Food:	the bumble bees were supplied *ad libitum* with 50 % (w/v) aqueous sucrose solution
Replicates	3 replicates 10 bees/group

4. Environmental conditions:

Temperature:	24.2 – 25.9 °C
Humidity:	48.7 – 63.5 %
Photoperiod:	constant darkness

Findings

In the control group, treated with tap water, no mortality was observed during the 96 h test period. In the reference item group, mortality was ≥ 50 % at the end of the test. Thus, the test was considered to be valid.

In the test item treatment group, a mortality of 80.0 % was observed at the highest dose level corresponding to 100 µg imidacloprid a.s./bumble bee at the final assessment after 96 hours.

In the test item treatment group, moribund, affected and apathetic bumble bees were observed at all tested dose levels during the entire 96 hour test period.

Table 9.5.1-8: Mortality in the contact toxicity test in the control, the test item (Imidacloprid + Pencycuron FS 370 (120+250 g a.s./L)) and the reference item group (Perfekthion)

Treatment Level [µg a.s./bumble bee]	**Mortality [%]**			
	24 h	**48 h**	**72 h**	**96 h**
Control (tap water)	0.0	0.0	0.0	0.0
Test item: Imidacloprid + Pencycuron FS 370 (120+250 g/L)				
1.23	0.0	3.33	3.33	3.33
3.70	3.33	3.33	3.33	10.0
11.11	6.67	6.67	16.67	26.67*
33.33	10.0	13.33	36.67	53.33*
100	13.33	33.33	43.33	80.0*
Reference item: Perfekthion				
12	70.00	73.33	76.67	76.67

*statistically significantly different compared to the control; (Fisher´s Exact Test, Bonferroni-Holmes corrected; one-sided, $p \leq 0.05$)

Table 9.5.1-9: LD_{50} values in the bumble bee contact toxicity test with Imidacloprid + Pencycuron FS 370 (120+250 g/L)

Imidacloprid + Pencycuron FS 370	**Contact toxicity test**

(120 + 250 g/L)	**[µg a.s./bumble bee]**
LD_{50} (24 h)	> 100*
LD_{50} (48 h)	> 100*
LD_{50} (72 h)	> 100*
LD_{50} (96 h)	28.1 (19.1 – 44.9)**
NOED (96 h)	3.70

* not determined
** lower and upper 95 % confidence limits

Conclusions

The 96 hour contact LD_{50} value for Imidacloprid + Pencycuron FS 370 (120+250 g a.s./L) was determined to be 270 µg prod./bee (equivalent to 28.1 µg imidacloprid/bumble bee).
The test item dose level corresponding to 3.70 µg imidacloprid/bumble bee was determined to be the NOED (No Observed Effect Dose) for mortality.

RMS's comments:
The validity criteria are met:
- less than 10 % mortality across the controls (observed: no mortality)
- more than 50 % mortality in the reference item group at the end of the test (observed: 76.67 %)

Overall, the study is considered acceptable and suitable for use in risk assessment.

B.9.5.2 Higher tier studies

The potential guttation exposure and the acute and the long-term risk to colony survival and development, and the risk to bee brood resulting from such exposure

The potential guttation exposure and the acute as well as the long-term risk to colony survival and development from such exposure were assessed in seven field studies.

Report:	Hofmann, S.; Lueckmann, J. 2014
Title:	Field study to monitor potential effects on honey bees from exposure to guttation fluid of winter wheat (W-WHT), seed-treated either with an imidacloprid or a clothianidin combi-product
Report No.:	R09247-4
Document No.:	M-498939-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

Objective

The effects of winter wheat (W-WHT) seed treated with either imidacloprid or clothianidin were tested on the honey bee (*Apis mellifera*) under field conditions. The study was conducted at two test locations in Germany (North at Celle, Lower Saxony, and South near Renningen, Baden-Württemburg (in the following called Ihinger Hof)) from the beginning of October 2009

until the end of April 2010. Honey bee colonies were set up directly adjacent to fields which were then sown with 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 fields and the position of the study plots were selected according to the following criteria:

- the provision of appropriate conditions for the set-up of honey bee colonies close to the study field
- at least 300 m distance to permanent open water bodies (e.g. ditches, streams or ponds) for treatment fields

Three test groups were set up at each location consisting of a field sown with seed treated with imidacloprid, clothianidin or a control (no insecticide). At each of the six study fields under investigation, five honey bee colonies were placed along a line one to eight days before sowing, either directly adjacent or within a maximum distance of 0.5 m to the W-WHT crop, depending on the actual local field situation. As colonies were in-situ at the time of drilling they were also exposed to dust emitted from seed drilling equipment at the time of sowing.

The following parameters were monitored during the Field Phase:

- the occurrence of guttation fluid and/or dew on W-WHT under typical agricultural use conditions,
- the presence of honey bees sitting on the ground or on W-WHT in specifically segregated assessment zones around honey bee colonies, set up directly adjacent to W-WHT fields,
- the uptake of guttation fluid or dew by exposed honey bees,
- the occurrence of conspicuous behaviour displayed by exposed honey bees
- the possible impact of guttation fluid on the development of exposed honey bee colonies, located directly adjacent to W-WHT fields
- the overwintering success of exposed honey bee colonies
- where sufficient guttation fluid was observed in the morning, up to three samples of guttation fluid, (approximately 1 mL each) were collected from the W-WHT crop. Samples were deep frozen (-20°C) for analysis and analysed for imidacloprid and clothianidin.

A specified assessment area in front of the honey bee colonies was intensively monitored. The assessment area was divided into two in-Crop Zones (Zone 0 and Zone 1) and an off-Crop Zone (see figure 1.5.2-1). Zone 0 covered the immediate area in front of the bee hives and Zone 1 outside of this. The bee hives were placed into the off-Crop Zone, directly adjacent to the W-WHT crop. In addition, two 1 m² assessment plots were established to record the proportion of W-WHT displaying guttation and/or dew. Each hive was equipped with a dead bee trap, and honey bee mortality was assessed daily from 09 October 2009 by counting the number of bees present in the trap and also those found on the soil surface in front of each colony. Each “monitoring session” lasted for approximately 35 minutes and was defined as one complete observation cycle of the assessment area and its associated two segregated plots of 1 m², at which guttation- and honey bee assessments were conducted during the presence of guttation fluid on the W-WHT crop.

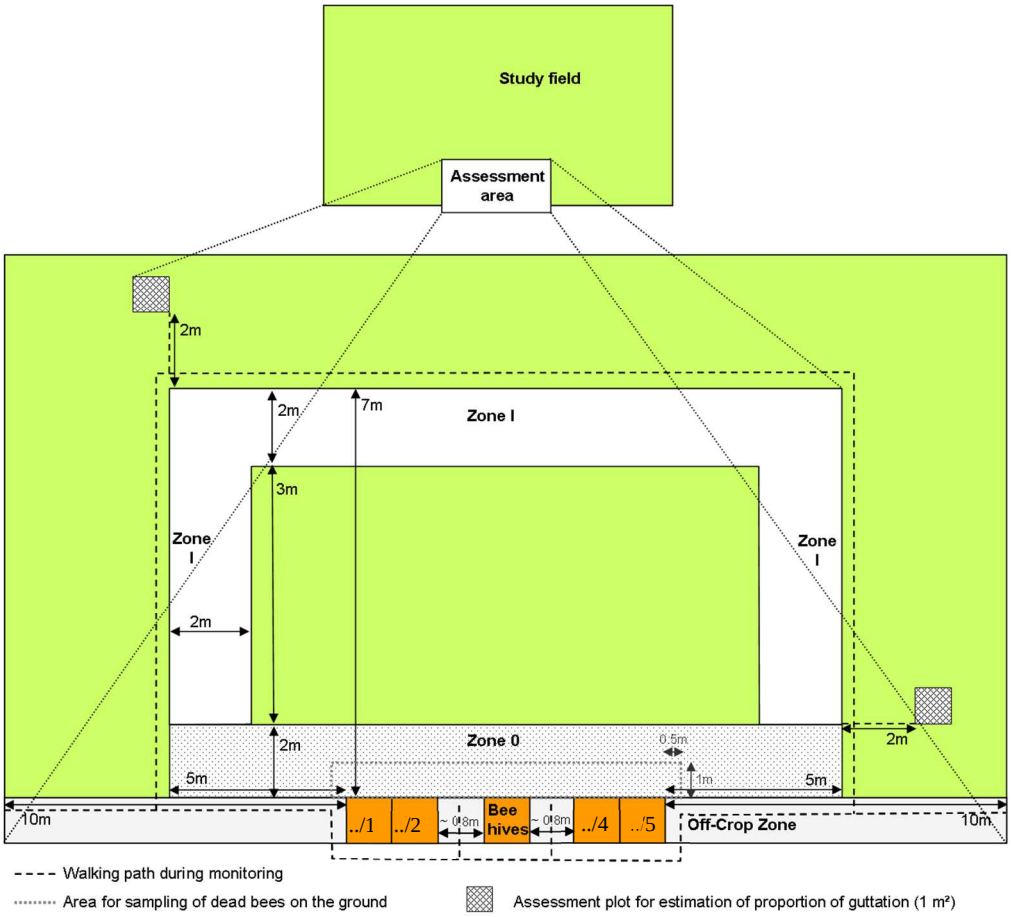


Figure 9.5.2-1: Diagram showing set up of honey bee colonies and assessment areas

Material and Methods

1. Test material:

Crop:	Winter wheat (W-WHT)
Test item:	Imidacloprid: triadimenol + imidacloprid + fuberidazol + imazalil (60 g a.s./L + 70 g a.s./L + 7.2 g a.s./L + 8 g a.s./L) Clothianidin: clothianidin + beta-cyfluthrin (375 g a.s./L + 80 g a.s./L) + EfA® (fungicide) (The seeds were seed-treated at the Seed Treatment Application Centre of Bayer CropScience AG in Monheim.)
Description:	Flowable concentrate for seed treatment
Purity:	Imidacloprid: a) triadimenol, analysed 60.95 g a.s./L (5.64 % w/w) b) imidacloprid, analysed 72.86 g a.s./L (6.74 % w/w)

c) fuberidazole, analysed 7.428 g a.s./L (0.687 % w/w)
d) imazalil, analysed 8.277 g a.s./L (0.766 % w/w)

Clothianidin:
a) clothianidin, analysed 382.9 g a.s./L (31.0 % w/w)
b) beta-cyfluthrin, analysed 82.87 g a.s./L (6.71 % w/w)

EfA®:
a) fluoxastrobin, nominally 37.5 g a.s./L
b) prothioconazole, nominally 25 g a.s./L
c) tebuconazole, nominally 3.75 g a.s./L
d) triazoxide, nominally 10.0 g a.s./L

Seeding rate: 200 kg seeds/ha
(70.00 g imidacloprid/100 kg seeds, 50.00 g clothianidin/100kg seeds)

2. Vehicle and control:

Control: EfA® (fungicide):
fluoxastrobin + prothioconazole + tebuconazole + triazoxide (37.5 g a.s./L + 25 g a.s./L + 3.75 g a.s./L + 10.0 g a.s./L)

3. Test animals:

Species: Honey bees (*Apis mellifera*)

Set up: Directly adjacent to fields, 5 honey bee colonies per field, along a line one to eight days before sowing

Source: The honey bee colonies used at the test location Ihinger Hof were provided by the State Institute of Apiculture, University of Hohenheim, August-von-Hartmann-Straße 13, 70593 Stuttgart.
The honey bee colonies used at the test location Celle were provided by the State Institute for Apiculture in Celle (LAVES), Herzogin-Eleonore-Allee 5, 29221 Celle

4. Observations:

Foraging: The number of honey bees which were foraging on guttation or dew were recorded during the assessments in the Off-Crop Zone as well as in the In-Crop Zones 0 and 1.

Behaviour: During the guttation monitoring, honey bees which foraged in the vicinity of the colonies were observed and the following observations were recorded:
uptake of guttation fluid or dew,
bees resting on W-WHT plants or on the soil surface between the W-WHT plants,
bees displaying conspicuous behaviour

Colony conditions:	Key study objectives were to evaluate and to compare the colony development and the overwintering performance of exposed honey bee colonies in three study groups (1x control, 2x treatments).
Residue analysis:	Imidacloprid and clothianidin residues in the various samples were analysed by an analytical laboratory of Bayer CropScience AG.
Study sites:	The study was conducted at two test locations in Germany: a) Northern Germany at Celle, Lower Saxony and b) Southern Germany near Renningen, Baden-Württemburg.

Results

Frequency of guttation

During the assessments in the morning, guttation fluid was observed on W-WHT at 86.4 % of all observation days in autumn 2009 and at 87.9 % of the observation days in spring 2010. No remarkable coincidence of guttation of W-WHT and bee activity in the evening in autumn 2009 and spring 2010 was observed.

Duration of guttation

Whenever guttation was observed on a respective day, it was already present in the early morning. Depending on the actual weather conditions, the time when guttation ended was variable. Under foggy or misty conditions, drizzle or slight rain, guttation lasted over longer periods as compared to dry conditions. On most observations days, guttation lasted for several hours.

Honey bee activity in the assessment area

Honey bees were observed visiting the study plots frequently. This is not unexpected as they were placed directly in front of the plots. Most of the direct honey bee observations within the assessment area were made in the in-Crop Zone 0, i.e. directly in front of the hives, followed by the Off-Crop Zone and the in-Crop Zone 1. Honey bees were observed visiting the study plots frequently. The relative proportion of honey bees observed per monitoring on plants in the respective assessment areas in both, treatments and control, was mostly higher in spring 2010 than in autumn 2009. With the exception of honey bees on soil surface: in autumn 2009 the observed relative proportion was three to four times higher in Zone 0 than in spring in the respective zone, which can be explained by the cold weather. Honey bee activity and the proportion of bees observed collecting water during the study is summarised below:

Frequency of crop guttation occurrence	86.4 % (Autumn), 87.9 % (spring)		
Crop guttation occurrence coinciding with bee activity	72.7 % (Autumn), 64.4 % (spring)		
Honey bee activity	Total no. bees observed	All areas	3276
		On soil	848 (crop) 611 (off-crop)
		On plants	1199 (crop) 618 (off-crop)
	Bees collecting water	Guttation + dew	411
		Guttation only	343
		Dew only	68
		% bees collecting guttation	10.5 % (all observations) 0.5 % (autumn) 11.9 % (spring)

Residue analysis of guttation fluid

All samples of guttation fluid collected from the treatment fields were analysed either for residues of imidacloprid or clothianidin, respectively. Selected samples of guttation fluid collected from the treatment fields were additionally analysed for their content of the clothianidin metabolites TZNG and TZMU (clothianidin treatment group) or their content of the imidacloprid metabolites imidacloprid-5-hydroxy and imidacloprid-olefin (imidacloprid treatment group).

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 range of residue levels detected is presented below:

Table 9.5.2-1: Residues of clothianidin and imidacloprid in guttation fluid

Residues in guttation (mg/L)	
Clothianidin	<LOQ – 13
TZNG	<LOQ – 0.49
TZMU	<LOQ – 0.32
Imidacloprid	<LOQ – 6.9
Imidacloprid 5-hydroxy	<LOQ – 0.61
Imidacloprid olefin	<LOQ – 0.12

Honey bee mortality

At both study sites, honey bee mortality in autumn was mostly low until a period of cold weather in October 2009. The increased mortality during this period was observed at both treated and control sites and was correlated with the weather conditions and was not influenced by the experimental setup. During springtime, the mortality found in the traps was generally low, but still variable from colony to colony and with higher mortality at the northern location compared to the southern location.

Colony development and overwintering

In Celle no monitoring was possible in autumn 2009 due to late seedling emergence.

During the autumn 2009 observation period at Ihinger Hof, 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. The winter losses were (after removal of weak colonies in the winter) 1 in 9, 2 in 10 and 1 in 7 for the clothianidin, imidacloprid and control treatments respectively. Consequently the successful overwintering rates were 89 % for clothianidin, 80 % for imidacloprid and 86 % for the control.

Table 9.5.2-2: Individual development of the study colonies in all treatment groups

Colony	**Hive development in autumn**	**Hive development in spring**
Imidacloprid treatment group		
11/1	colony was removed after last assessment (less than 5,000 bees)	-- (colony discarded in autumn)
11/2	colony was removed after last assessment (less than 5,000 bees)	-- (colony discarded in autumn)
11/3	lot of brood until late October	normal
11/4	normal	normal
11/5	normal	winter loss
17/1	normal	normal
17/2	normal	normal
17/3	normal	normal
17/4	normal	normal
17/5	normal	no brood detected during first assessment on 25 March 2010 (queen found dead in dead bee trap on 09 April 2010)
Clothianidin treatment group		
12/1	normal	normal
12/2	slight increase	normal
12/3	normal	normal
12/4	colony was removed after last assessment (less than 5,000 bees)	-- (colony discarded in autumn)
12/5	weak	winter loss
18/1	normal	normal
18/2	normal	normal
18/3	normal	normal

Colony	Hive development in autumn	Hive development in spring
18/4	normal	normal
18/5	normal	normal
Control group		
10/1	normal	normal
10/2	normal	normal
10/3	normal	normal
10/4	normal	normal
10/5	normal	winter loss
16/1	normal	normal
16/2	normal	bad overwintering, was removed after first assessment in spring
16/3	normal	normal
16/4	normal	normal
16/5	weak	winter loss

Conclusions
No treatment related differences in honey bee mortality, colony development in autumn and spring as well as in the overwintering performance (at Ihinger Hof only) was observed between the control and the treatment groups. Weak development in autumn, leading to discarding the colonies or winter losses can 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, exuded by winter wheat seedlings, seed-treated with imidacloprid or clothianidin, does not have unacceptable effects on honey bee colonies under typical commercial use conditions.

RMS's comments:
This study can be classified as generally well constructed and valid. However, due to the different methods of approach both study sites are not directly comparable. In Celle, for instances, no monitoring was possible in autumn 2009 due to late seedling emergence. Moreover, there were differences at both study sites between the frequencies of observations and the used technical tools (e.g. dead bee traps). However, these differences do not affect the reliability of the study conclusions. At both locations there was a frequent time overlap between the occurrence of guttation and bee flight activity and some honey bees were observed visiting the study plots. The honey bee colonies were in-situ at the time of drilling. It is noted there was no increased mortality. However, no detailed observations regarding potential effects of the dust emitted from the seed at the time of sowing on the colonies were conducted, thus the study is unfortunately not sufficient to be used as additional information for dust risk assessment.

No treatment-related differences in honey bee mortality, colony development in autumn and spring as well as in the overwintering performance (at Ihinger Hof only) were observed between the control and the treatment groups. Therefore, it is concluded that under the conditions of this

experiment guttation fluid, exudated by seed treated winter wheat seedlings, does not have unacceptable effects on honey bee colonies.

Report:	Hofmann, S.; Garrido, C.; Lueckmann, J.; 2012
Title:	Field study to monitor potential effects on honey bees from exposure to guttation fluid of winter barley (W-BAR), seed-treated either with an imidacloprid or a clothianidin combi-product
Report No.:	R09247-3
Document No.:	M-498922-01-1
Guideline(s):	not specified
Guideline deviation(s):	not specified
GLP/GEP:	**no**

Objective
The effects of seed treated with either imidacloprid or clothianidin were tested on the honey bee (*Apis mellifera*) under field conditions. The study was conducted at two test locations in Germany (North at Celle, Lower Saxony, and South near Renningen, Baden-Württemburg) from mid-September 2009 until mid-March 2010. Honey bee colonies were set up directly adjacent to fields which were then sown with winter barley (W-BAR) seeds, in order to investigate the potential effects from exposure to guttating W-BAR, starting from seedling emergence in autumn 2009 until beginning of winter oil-seed flowering in the respective region in spring 2010. The study fields and the position of the study plots were selected according to the following criteria:

- the provision of appropriate conditions for the set-up of honey bee colonies close to the study field
- at least 300 m distance to permanent open water bodies (e.g. ditches, streams or ponds) for treatment fields

Three test groups were set up at each location consisting of a field sown with seed treated with imidacloprid, clothianidin or a control (no insecticide). At each of the six study fields under investigation, five honey bee colonies were placed along a line one to eight days before sowing, either directly adjacent or within a maximum distance of 0.5 m to the W-BAR crop, depending on the actual local field situation. As colonies were in-situ at the time of drilling they were also exposed to dust emitted from seed drilling equipment at the time of sowing.

The following parameters were monitored during the Field Phase:

- the occurrence of guttation fluid and/or dew on W-BAR under typical agricultural use conditions,
- the presence of honey bees sitting on the ground or on W-BAR in specifically segregated assessment zones around honey bee colonies, set up directly adjacent to W-BAR fields,
- the uptake of guttation fluid or dew by exposed honey bees,
- the occurrence of conspicuous behaviour displayed by exposed honey bees
- the possible impact of guttation fluid on the development of exposed honey bee colonies, located directly adjacent to W-BAR fields
- the overwintering success of exposed honey bee colonies
- where sufficient guttation fluid was observed in the morning, up to three samples of guttation fluid, (approximately 1 mL each) were collected from the W-BAR crop.

Samples were deep frozen (-20°C) for analysis and analysed for imidacloprid and clothianidin.

A specified assessment area in front of the honey bee colonies was intensively monitored. The assessment area was divided into two in-Crop Zones (Zone 0 and Zone 1) and an off-Crop Zone. Zone 0 covered the immediate area in front of the bee hives and Zone 1outside of this. The bee hives were placed into the off-Crop Zone, directly adjacent to the W-BAR crop. In addition, two 1 m² assessment plots were established to record the proportion of W-BAR displaying guttation and/or dew. Each hive was equipped with a dead bee trap, and honey bee mortality was assessed daily from 15 September 2009 by counting the number of bees present in the trap and also those found on the soil surface in front of each colony. Each "monitoring session" lasted for approximately 35 minutes and was defined as one complete observation cycle of the assessment area and its associated two segregated plots of 1 m², at which guttation- and honey bee assessments were conducted during the presence of guttation fluid on the W-BAR crop.

Material and methods

1. Test material:

Crop:	Winter barley (W-BAR)
Test item:	Imidacloprid: triadimenol + imidacloprid + fuberidazol + imazalil (60 g a.s./L + 70 g a.s./L + 7.2 g a.s./L + 8 g a.s./L) Clothianidin: clothianidin + beta-cyfluthrin (375 g a.s./L + 80 g a.s./L) + EfA® (fungicide) (The seeds were seed-treated at the Seed Treatment Application Centre of Bayer CropScience AG in Monheim.)
Description:	Flowable concentrate for seed treatment
Purity:	Imidacloprid: a) triadimenol, analysed 60.95 g a.s./L (5.64 % w/w) b) imidacloprid, analysed 72.86 g a.s./L (6.74 % w/w) c) fuberidazole, analysed 7.428 g a.s./L (0.687 % w/w) d) imazalil, analysed 8.277 g a.s./L (0.766 % w/w) Clothianidin: a) clothianidin, analysed 382.9 g a.s./L (31.0 % w/w) b) beta-cyfluthrin, analysed 82.87 g a.s./L (6.71 % w/w) EfA®: a) fluoxastrobin, nominally 37.5 g a.s./L b) prothioconazole, nominally 25 g a.s./L c) tebuconazole, nominally 3.75 g a.s./L d) triazoxide, nominally 10.0 g a.s./L

Seeding rate:	200 kg seeds/ha (70.00 g imidacloprid/100 kg seeds, 50.00 g clothianidin/100kg seeds)
2. Vehicle and control:	
Control:	EfA® (fungicide): fluoxastrobin + prothioconazole + tebuconazole + triazoxide (37.5 g a.s./L + 25 g a.s./L + 3.75 g a.s./L + 10.0 g a.s./L)
3. Test animals:	
Species:	Honey bees (*Apis mellifera*)
Set up:	Directly adjacent to fields, 5 honey bee colonies per field, along a line one to eight days before sowing
Source:	The honey bee colonies used at the test location Ihinger Hof were provided by the State Institute of Apiculture, University of Hohenheim, August-von-Hartmann-Straße 13, 70593 Stuttgart. The honey bee colonies used at the test location Celle were provided by the State Institute for Apiculture in Celle (LAVES), Herzogin-Eleonore-Allee 5, 29221 Celle.
4. Observations:	
Foraging:	The number of honey bees which were foraging on guttation or dew were recorded during the assessments in the Off-Crop Zone as well as in the In-Crop Zones 0 and 1.
Behaviour:	During the guttation monitoring, honey bees which foraged in the vicinity of the colonies were observed and the following observations were recorded: uptake of guttation fluid or dew, bees resting on W-BAR plants or on the soil surface between the W-BAR plants, bees displaying conspicuous behaviour
Colony conditions:	Key study objectives were to evaluate and to compare the colony development and the overwintering performance of exposed honey bee colonies in three study groups (1x control, 2x treatments).
Residue analysis:	Imidacloprid and clothianidin residues in the various samples were analysed by an analytical laboratory of Bayer CropScience AG.
Study sites:	The study was conducted at two test locations in Germany: a) Northern Germany at Celle, Lower Saxony and b) Southern Germany near Renningen, Baden-Württemburg.

Results

Frequency of guttation

During the assessments in the morning, guttation fluid was observed on W-BAR at 84.2 % of all observation days in autumn 2009 and at 80.7 % of the observation days in spring 2010. No remarkable coincidence of guttation of W-BAR and bee activity in the evening in autumn 2009 and spring 2010 was observed.

Duration of guttation

Whenever guttation was observed on a respective day, it was already present in the early morning. Depending on the actual weather conditions, the time when guttation ended was variable. Under foggy or misty conditions, drizzle or slight rain, guttation lasted over longer periods as compared to dry conditions. On most observations days, guttation lasted for several hours.

Honey bee activity in the assessment area

Honey bees were observed visiting the study plots frequently. This is not unexpected as they were placed directly in front of the plots. 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. Honey bee activity and the proportion of bees observed collecting water during the study is summarized below:

Frequency of crop guttation occurrence	84.2 % (Autumn), 80.7 % (spring)		
Crop guttation occurrence coinciding with bee activity	46.6 % (Autumn), 56.3 % (spring)		
Honey bee activity	Total no. bees observed	All areas	3148
		On soil	911 (crop) 319 (off-crop)
		On plants	1386 (crop) 532 (off-crop)
	Bees collecting water	Guttation + dew	406
		Guttation only	334
		Dew only	72
		% bees collecting guttation	10.6 % (all observations) 2.6 % (autumn) 16.0 % (spring)

Residue analysis of guttation fluid

All samples of guttation fluid collected from the treatment fields were analysed either for residues of imidacloprid or clothianidin, respectively. Selected samples of guttation fluid collected from the treatment fields were additionally analysed for their content of the clothianidin metabolites TZNG and TZMU (clothianidin treatment group) or their content of the imidacloprid metabolites imidacloprid-5-hydroxy and imidacloprid-olefin (imidacloprid treatment group).

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 range of residue levels detected is presented below:

Table 9.5.2-3: Residues of clothianidin and imidacloprid in guttation fluid

Residues in guttation (mg/L)	
Clothianidin	<LOQ – 2.3
TZNG	<LOQ – 0.05
TZMU	<LOQ – 0.02
Imidacloprid	<LOQ – 15
Imidacloprid 5-hydroxy	<LOQ – 0.64
Imidacloprid olefin	<LOQ – 0.05

Honey bee mortality

During the approximately 5 week's continuous autumn exposure period, none of the treatment colonies revealed adverse effects in terms of mortality rates and/or suspicious behavioural impairments, although honey bees were frequently recorded to forage within the neonicotinoid-treated barley fields. In all treatment groups, honey bee mortality in autumn was mostly low until a period of cold weather in October. The increased mortality in all experimental groups (treatments and control) during this period was clearly correlated with the weather conditions and was not influenced by the experimental setup. During springtime, the mortality found in the traps was generally low, but still variable from colony to colony. Based on these observations, it can be concluded that guttation fluid of neonicotinoid-treated barley seedlings, although carrying an intrinsically high hazard potential, does not impair honey bee colonies, which were exposed at the field margin in direct vicinity to those fields, in an unacceptable manner.

Colony development and overwintering

The autumn- and overwintering conditions for the clothianidin treatment group were substantially less favourable as compared to the control and/or to the imidacloprid treatment group since this group includes a higher number of weak colonies at study initiation. Therefore no reliable conclusions can be drawn for this group concerning overwintering performance. However, for the other treatment groups overwintering success (total success) rate of 80 (80)% in the control group and 89 (80)% in the imidacloprid treatment group, indicating that guttating W-BAR seedlings seed treated with imidacloprid have no impact on the rate of successful overwintering of adjacently located and exposed honey bee colonies.

Table 9.5.2-4: Individual development of the study colonies in all treatment groups

Colony	Hive development in autumn	Hive development in spring
Imidacloprid treatment group		
8/1	colony was removed after last assessment (less than 5,000 bees	-- (colony discarded in autumn)
8/2	normal	normal
8/3	normal	weak development, brood activity started late, no drone brood until May
8/4	normal	normal
8/5	normal	normal
14/1	normal	strong brood activity
14/2	normal	normal
14/3	normal	winter loss
14/4	weak	normal, low colony strength until mid of May
14/5	normal	normal
Clothianidin treatment group		
9/1	weak	winter loss
9/2	slight increase, high *Varroa* load	bad overwintering, continuous decrease of colony strength up to final loss of vitality
9/3	weak	normal
9/4	very high *Varroa* load which disrupted hive vitality, colony was removed after last assessment (less than 5,000 bees)	-- (colony discarded in autumn)
9/5	normal	normal
15/1	colony was removed after last assessment (less than 5,000 bees)	-- (colony discarded in autumn)
15/2	normal	normal
15/3	normal	winter loss
15/4	normal	normal

Colony	**Hive development in autumn**	**Hive development in spring**
15/5	normal	winter loss
Control group		
9/1	weak	winter loss
9/2	slight increase, high *Varroa* load	bad overwintering, continuous decrease of colony strength up to final loss of vitality
9/3	weak	normal
9/4	very high *Varroa* load which disrupted hive vitality, colony was removed after last assessment (less than 5,000 bees)	-- (colony discarded in autumn)
9/5	normal	normal
15/1	colony was removed after last assessment (less than 5,000 bees)	-- (colony discarded in autumn)
15/2	normal	normal
15/3	normal	winter loss
15/4	normal	normal
15/5	normal	winter loss

Conclusions
No treatment related differences in honey bee mortality, colony development in autumn and spring as well as in the overwintering performance were observed between the control and the imidacloprid treatment group. Due to the substantially less favourable conditions for the clothianidin treatment group at study initiation no reliable conclusions can be drawn for this group concerning the overwintering performance.

Overall, it is concluded that guttation fluid, exuded by winter barley seedlings, seed-treated with imidacloprid, does not have unacceptable effects on honey bee colonies under typical commercial use conditions.

RMS´s comments:
This study can be classified as generally well constructed and valid. However, the study sites are not directly comparable as there were differences between the frequencies of observations and the used technical tools (e.g. dead bee traps) at both locations. Furthermore, no reliable conclusions can be drawn for the clothianidin treatment group concerning the overwintering performance as the autumn- and overwintering conditions were substantially less favourable as

compared to the control and/or to the imidacloprid treatment group. However, these differences do not affect the reliability of the study conclusions for imidacloprid.
At both locations there was a frequent time overlap between the occurrence of guttation and bee flight activity and some honey bees were observed visiting the study plots. The honey bee colonies were in-situ at the time of drilling. It is noted there was no increased mortality. However, no detailed observations regarding potential effects of the dust emitted from the seed at the time of sowing on the colonies were conducted, thus the study is unfortunately not sufficient to be used as additional information for dust risk assessment.

No treatment-related differences in honey bee mortality and colony development in autumn and spring for all test groups as well as in the overwintering performance for the control and the imidacloprid group were observed. Therefore, it is concluded that under the conditions of this experiment guttation fluid, exudated by seed treated winter barley seedlings, does not have unacceptable effects on honey bee colonies.

Report:	Hofmann, S.; Staffel, J.; Aumeier, P. 2014
Title:	Field study to monitor potential effects on honey bees from exposure to guttation fluid of winter barley (W-BAR), seed-treated with the insecticidal seed-treatment product clothianidin + imidacloprid FS 100 + 175 G in Germany in 2011/2012
Report No.:	R11130
Document No.:	M-501261-01-1
Guideline(s):	No official test guideline(s) available at present
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective
The effects of W-BAR seed treated with imidacloprid + clothianidin was tested on the honey bee (*Apis mellifera*) under field conditions. The study was conducted in ten agricultural fields located in Hesse, Germany from mid-September 2011 until early-May 2012. Five fields were sown with imidacloprid + clothianidin treated seed (treated plots) and the others received no insecticide treatment (control plots). The study fields and the position of the study plots were selected according to the following criteria:

- the provision of appropriate conditions for the set-up of honey bee colonies close to the study field
- at least 300 m distance to permanent open water bodies (e.g. ditches, streams or ponds) for treatment fields

At each of the ten study plots five honey bee colonies were set up which were then sown with winter barley (W-BAR) seeds, in order to investigate the potential effects from exposure to guttating W-BAR, starting from seedling emergence in autumn 2011 until spring 2012.

Colonies were placed either directly adjacent to the fields or approximately 4.5 m away depending on local field situation and were placed along a line six to 13 days before sowing. As colonies were in-situ at the time of drilling they were also exposed to dust emitted from seed drilling equipment at the time of sowing.

The following parameters were monitored during the Field Phase:

- the occurrence of guttation fluid and/or dew on W-BAR under typical agricultural use conditions,
- the presence of honey bees sitting on the ground or on W-BAR in specifically segregated assessment zones around honey bee colonies, set up either directly adjacent to W-BAR fields or in a distance of circa 4.5 m,
- the uptake of guttation fluid or dew by exposed honey bees,
- the occurrence of conspicuous behaviour and sign of intoxication, displayed by exposed honey bees,
- the possible impact of guttation fluid on mortality and colony development of exposed honey bee colonies, located adjacent to W-BAR fields,
- the overwintering success of exposed honey bee colonies
- where sufficient guttation fluid was observed in the morning, up to three samples of guttation fluid, each with a volume of approximately 1 mL was collected from the W-BAR crop. Samples were deep frozen ((≤18 °C) for analysis and analysed for imidacloprid and clothianidin.

A specified area (assessment area) in front of the honey bee colonies was intensively monitored. The whole assessment area was divided into two in-Crop Zones (Zone 0 and Zone 1) and an off-Crop Zone. Zone 0 (width: 5 m to each side of the hives, 2 m depth into the in-crop) covered the immediate area in front of the bee hives and Zone 1 (a 2 m broad band, shaped like an inverted ‘U’, with a vertical distance of the band to the field margin of 7 m inside the crop). The bee hives were placed into the off-Crop Zone, either directly adjacent to the W-BAR crop (Figure 1.5.2-2) or in a distance of approximately 4.5 m to the W-BAR crop (Figure 1.5.2-3). In addition, four segregated assessment plots with each 50 W-BAR plants inside in autumn 2011 respectively of one square meter in spring 2012 were established to record the proportion of W-BAR displaying guttation and/or dew.

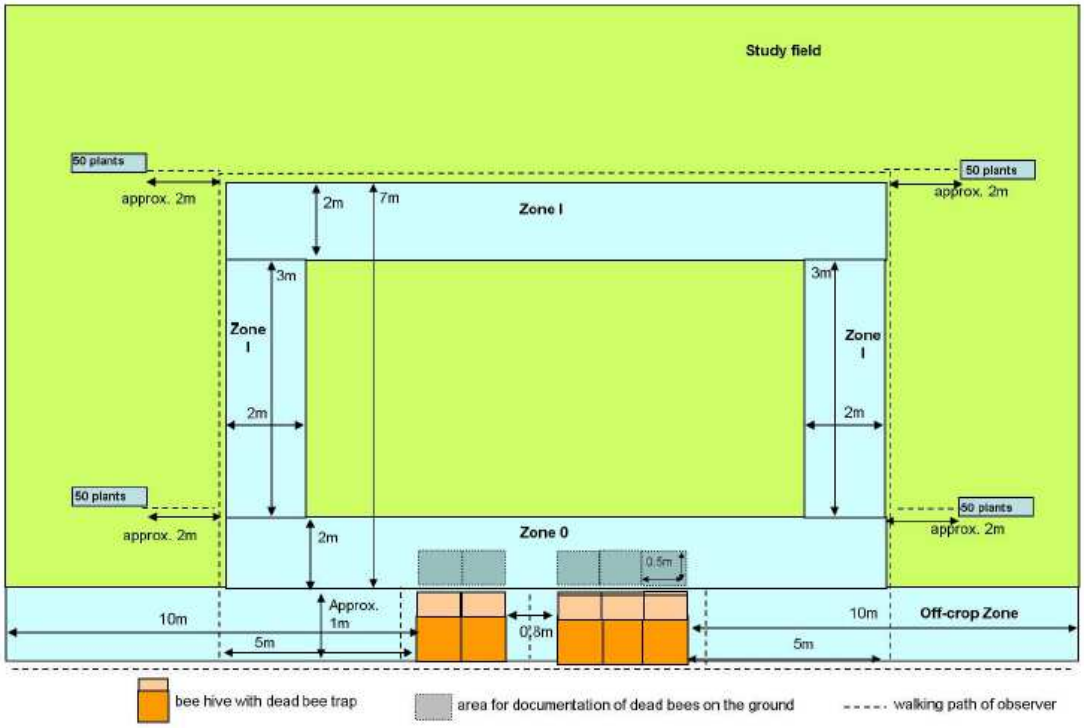


Figure 9.5.2-2: Scheme of the assessment area on a study plot with hives directly adjacent to the field border (scenario 1)

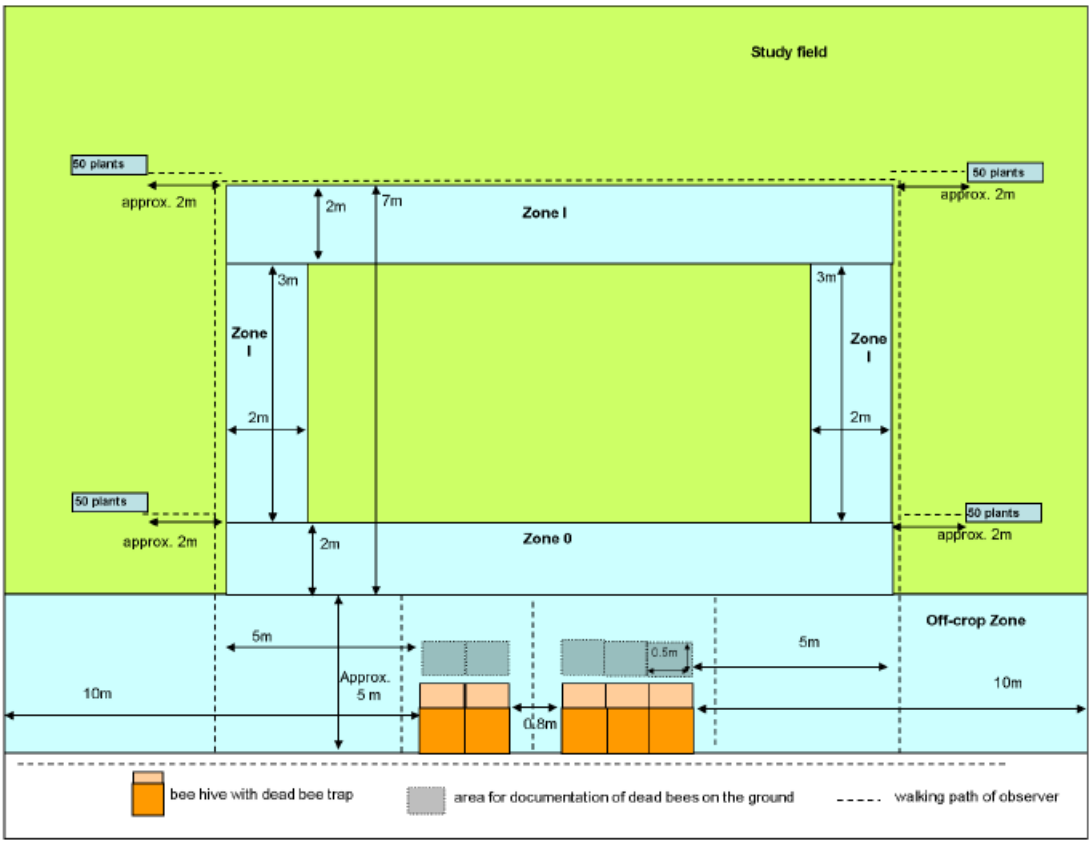


Figure 9.5.2-3: Scheme of the assessment area on a study plot with hives located at approximately 4.5 m distance from the field border within the off-crop area (scenario 2)

Material and Methods

1. Test material:

Crop:	Winter barley (W-BAR)
Test item:	Clothianidin + imidacloprid: 100 g clothianidin/L + 175 g imidacloprid/L (The seeds were seed-treated at the Seed Treatment Application Centre of Bayer CropScience AG in Monheim.)
Description:	Flowable concentrate for seed treatment
Purity:	Imidacloprid: 98.8% Clothianidin: 99.4%
Seeding rate:	183 – 207 kg seed/ha

2. Vehicle and control:

Control:	Baytan® (fungicide): fuberidazole + imazalil + triadimenol (9.0 g a.s. /L + 10.0 g a.s. /L + 75.0 g a.s. /L)

3. Test animals:

Species:	Honey bees (*Apis mellifera*)
Set up:	Honey bee colonies were set up at the study fields shortly before sowing (6 to 13 days) either directly adjacent to the crop or in a distance of approximately 4.5 m to the crop margin.
Source:	Ruhr-University Bochum, Institute of Behavioural Biology and Biology Education

4. Observations:

Foraging:	During each monitoring session, the number of honey bees foraging on guttation or dew fluid in the In-Crop Zones and in the Off-Crop Zone were recorded.
Behaviour:	Any abnormal behaviour, e.g. symptoms of intoxication like trembling, vomiting, paralysis/flight inability or aggressiveness was documented. If the number of bees with symptoms of disease or intoxication was ≥ 10 per

	observation, bee samples for potential disease analysis were taken.
Colony conditions:	Key study objectives were to evaluate and to compare the colony development and the overwintering performance of exposed honey bee colonies in two study groups (control, treatment).
Residue analysis:	Guttation fluid of W-BAR in the treatment group was collected and analysed for residues of clothianidin and imidacloprid.
Study sites:	The study was conducted in eight commercially managed agricultural fields located in the vicinity of Giessen in Hesse, Germany

Results

Frequency of guttation

During the assessments in the morning, guttation fluid was observed on W-BAR at 100 % of all observation days in autumn 2011 and at 87.6 % of the observation days in spring 2012. No remarkable coincidence of guttation of W-BAR and bee activity in the evening in autumn 2011 and there was virtually no guttation was observed in spring 2012.

Duration of guttation

Whenever guttation was observed on a respective day, it was already present in the early morning. Depending on the actual weather conditions, the time when guttation ended was variable. Under foggy or misty conditions, drizzle or slight rain, guttation lasted over longer periods as compared to dry conditions. On most observations days, guttation lasted for several hours on average up to 12 pm in both autumn and spring.

Honey bee activity in the assessment area

Honey bees were observed visiting the study plots frequently in spring, but rarely in autumn. The relative proportion of honey bees observed per monitoring on plants in the respective assessment areas in both, treatment and control, was higher in spring 2012 than in autumn 2011. Moreover, also the observed relative proportion of honey bees per monitoring taking up guttation fluid and dew in both, treatment and control, was higher in all assessment zones in spring 2012 as compared to autumn 2011, were it was a rare phenomenon. Most of the direct honey bee observations within the assessment areas were made directly in front of the hives. Accounting for all honey bees, observed during the individual assessments on the study plots throughout the entire field observation period in both, treatment and control, respectively, only a small proportion of bees was directly observed taking up guttation fluid. Honey bee activity and the proportion of bees observed collecting water during the study is summarized below:

Frequency of crop guttation occurrence	100 % (Autumn), 87.6 % (spring)		
Crop guttation occurrence coinciding with bee activity	73.1 % (Autumn), 69.7 % (spring)		
Honey bee activity	Total no. bees observed	All areas	6973
		On soil	699 (crop) 883 (off-crop)
		On plants	2160 (crop) 1717 (off-crop)
	Bees collecting water	Guttation + dew	N/A
		Guttation only	505
		Dew only	1009

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.

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 range of residue levels detected is presented below:

Table 9.5.2-5: Residues of imidacloprid and clothianidin in guttation liquid

Sample description	**Origin**	**Date of sampling**	**Plant growth period**	**Residue [mg/L]**	
				Imidacloprid	**Clothianidin**
Guttation liquid	Winter-Barley, grown from seeds dressed with Clothianidin + Imidacloprid FS 100 + 175 G	28 September to 27 October 2011	Autumn	< LOQ - 6.65	< LOQ - 8.51
		16 March to 17 April 2012	Spring	< LOD - 0.07	< LOD - 0.15

Honey bee mortality
In autumn 2011, the control and the treatment group developed in a normal and similar way, no distinct, biologically relevant differences could be detected in both, the number of adult bees and brood cells. There were no distinct, biologically relevant differences between treatment and control, irrespective whether the colonies were set-up directly adjacent to the field margins or at distance of approximately 4.5 m to the crop. This conclusion is supported by statistical analysis. In spring 2012, at the final colony assessment, there were also no distinct, biologically relevant differences in the number of adult bees and brood cells between treatment and control, irrespective whether the colonies were set-up directly adjacent to the field margins or at distance

of approximately 4.5 m to the crop, although the average number of worker bees in the treatment colonies statistically significantly exceeded the corresponding number of the control colonies.

Colony development and overwintering

Regarding honey bee mortality, brood- and colony development, colony strength and *varroa* infestation levels during autumn and spring, there were no distinct, biologically relevant differences between treatment and control, irrespective whether the colonies were set-up directly adjacent to the field margins or at distance of approximately 4.5 m to the crop. After overwintering, colony strength had decreased in both exposure groups when compared to the before-winter-evaluation, which is a typical apidological phenomenon. That equates to an average overwintering index of 57.8 ± 21.2 % in control colonies and to an average overwintering index of 67.0 ± 14.1 % in treatment colonies. There were no distinct, biologically relevant differences between treatment and control, irrespective whether the colonies were set-up directly adjacent to the field margins or at distance of approximately 4.5 m to the crop. These conclusions are supported by statistical analysis.

Conclusions

No treatment related differences in honey bee mortality, colony development in autumn and spring as well as in the overwintering performance were observed between the control and the imidacloprid + clothianidin treatment group.

Overall, it can be concluded that guttation fluid, excreted by winter barley, seed-treated with clothianidin + imidacloprid, does not have unacceptable effects on honey bee colonies under typical commercial use conditions, as there were no adverse acute, short-term or long-term effects on colony strength and -development, brood development, food storage, honey bee behaviour, queen survival, overall hive vitality, colony health, or on overwintering performance.

RMS's comments:

This study can be classified as generally well constructed and valid. There was a frequently time overlap between the occurrence of guttation and bee flight activity and some honey bees were observed visiting the study plots. The honey bee colonies were in-situ at the time of drilling. It is noted there was no increased mortality. However, no detailed observations regarding potential effects of the dust emitted from the seed at the time of sowing on the colonies were conducted, thus the study is unfortunately not sufficient to be used as additional information for dust risk assessment.

No treatment related differences in honey bee mortality and colony development in autumn and spring as well as in the overwintering performance were observed between the control and the treatment group. Therefore, it is concluded that under the conditions of this experiment guttation fluid, exudated by seed treated winter barley seedlings, does not have unacceptable effects on honey bee colonies.

Report:	Rexer, H. U.; 2014a
Title:	A long-term field study to monitor potential effects on the honey bee (*Apis mellifera* L.) from exposure to guttation fluid of sugar beets, seed-treated with the insecticides clothianidin + imidacloprid + beta-cyfluthrin in Southern Germany in 2013 and 2014
Report No.:	S13-00171
Document No.:	M-500724-01-1

Guideline(s):	OEPP/EPPO Guideline No. 170(4) (2010); SANCO/3029/99 rev. 4
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective
The objective of this study was to determine the effects of exposure of honey bees (*Apis mellifera* L.) to guttation liquid from sugar beet plants, grown under field conditions from pills treated with clothianidin, imidacloprid and beta-cyfluthrin.

The effects of honey bee exposure to guttation liquid from sugar beet plants, grown from treated sugar beet pills were examined on commercial bee colonies. The honey colonies were placed at the field sites shortly after emergence of the plants (T: BBCH 12, C: BBCH 12) and remained there for 42 days. Thereafter all honey colonies were placed at a monitoring site, without extensive agricultural crops attractive to bees (monitoring phase).
The experimental phase started with the drilling of the treated and untreated sugar beet pills in spring 2013 and ended in spring 2014 after monitoring overwintering survival, colony strength and colony development.

The influence of the test item was evaluated by comparing the results in the test item treatment to the corresponding control under consideration of the results of:

- Mean number of dead bees on the linen sheets and in the dead bee traps;
- Flight intensity in the field (mean number of forager bees/5 x 2 m²/min);
- Observation of honey bees visiting sugar beet plants displaying guttation;
- Occurrence and proportion of guttation;
- Behaviour of the bees in the crop and around the hive;
- Condition of the colonies (number of bees (colony strength), total values of the different brood stages per colony and assessment date);
- Bee health (bee disease and bee virus analysis);
- Overwintering performance

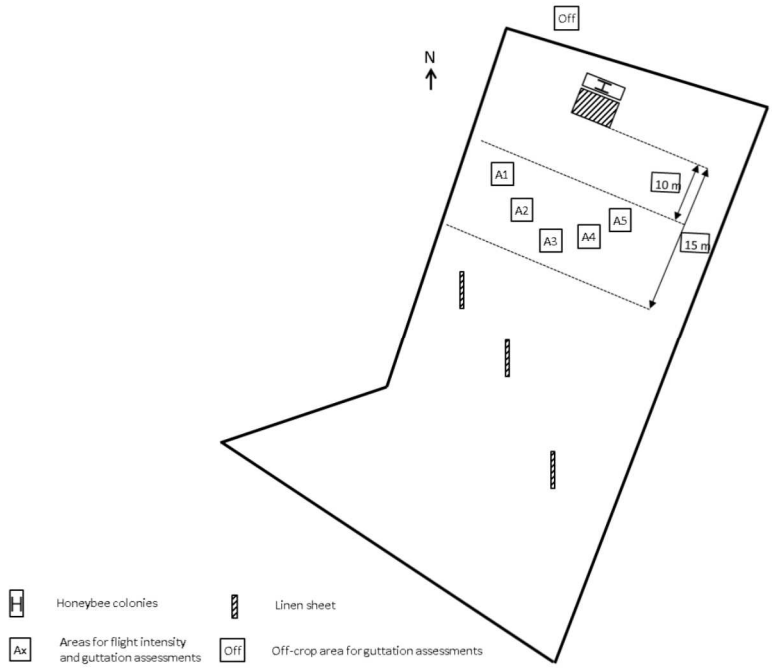


Figure 9.5.2-4: Design of the control field

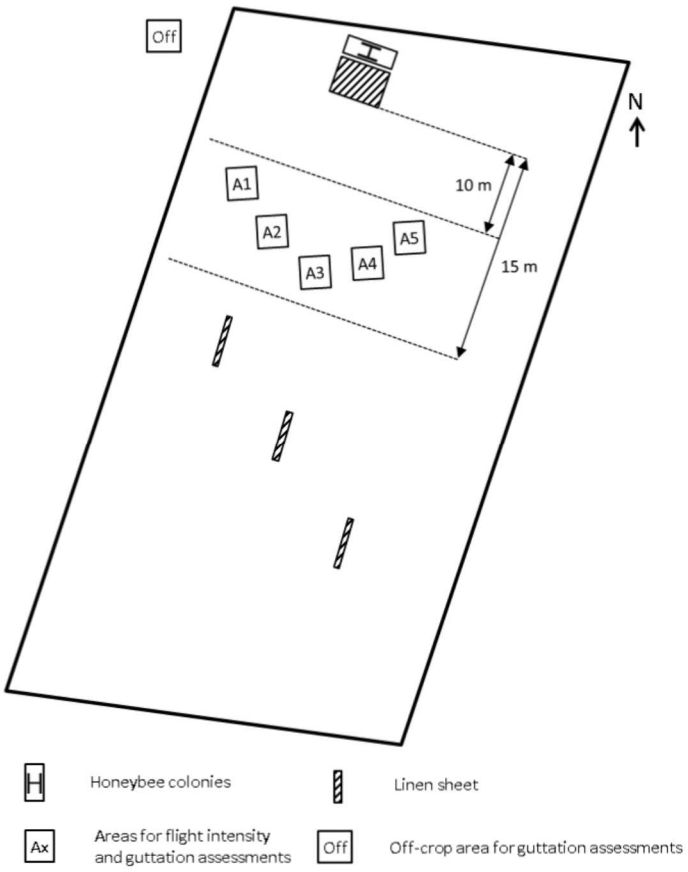


Figure 9.5.2-5: Design of the test item field

Material and Methods

1. Test material:

Crop:	Sugar beet (SB)
Test item:	Clothianidin + imidacloprid + beta-cyfluthrin + standard fungicide (Hymexazol + TMTD)
Description:	Pills/orange
Purity:	Clothianidin: 99.4% Imidacloprid: 98.8% Beta-Cyfluthrin: 98.8%
Content of a.s./pill:	Nominal Clothianidin: 0.6 mg a.s./pill Imidacloprid: 0.3 mg a.s./pill Beta-cyfluthrin: 0.08 mg a.s./pill Analysed Clothianidin: 0.6612 mg a.s./pill Imidacloprid: 0.2994 mg a.s./pill Beta-cyfluthrin: 0.0828 mg a.s./pill
Seeding rate:	130,000 pills/ha (corresponding with a target application rate of 78 g clothianidin/ha, 39 g imidacloprid/ha and 10.4 g beta-cyfluthrin/ha)

2. Vehicle and control:

Control:	Hymexazol + TMTD (fungicide)

3. Test animals:

Species:	Honey bees (*Apis mellifera* L.)
Colony size:	The 16 hives used for the purpose of this study. The colonies were prepared as homogeneous as possible and contained not less than 10000 bees per colony at the start of the test.

4. Observations:

Behaviour:	During the assessments of mortality and flight intensity , the behaviour of the honey bees in the crop and around the hive was observed with respect to the following criteria: • aggressiveness towards the observer, • aggressiveness towards other bees (filtering at the hive entrance), • intensive cleaning, • clustering of large numbers of bees at the hive entrance, • cramping,

	• locomotion problems, • trembling, • inactive, • hanging bees (holding on to plants with one or two legs)
Colony conditions:	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.
Residue analysis:	Guttation fluid of SB plants in the treatment group was collected and analysed for residues of clothianidin, imidacloprid and beta-cyfluthrin.
Study site:	The field sites were located in Neulingen-Bauschlott (C) and Pforzheim (T), both in the federal state of Baden-Württemberg, Germany. The field sites had a size of 2.47 ha (C) and 3.28 ha (T) and there were no flowering main crops within a ca. 2 km radius.

Findings

Occurrence of guttation and percentage of plants displaying guttation

In the control group, guttation of sugar beet plants in the assessment areas was observed on 1 out of 42 assessment days. In the concurrently assessed off-crop area, guttation occurred on 22 out of 42 assessment days. In the test item treatment group, guttation of sugar beet plants in the assessment areas was observed on 11 out of 42 assessment days. In the concurrently assessed off-crop area, guttation occurred on 26 out of 42 assessment days. When guttation occurred in the in-crop assessment areas in the control group, the percentage of plants exhibiting guttation per assessment area varied from 2.7 % to 5.3 %. In the test item treatment group, the percentage of plants exhibiting guttation per assessment area varied from 2.4 % to 30.0 %, when guttation was detected.

Overall, guttation occurred only infrequently in sugar beets, and the overall abundance of guttation droplets was rather low, particularly when compared to adjacent off-crop areas.

Flight intensity and observation of honey bees visiting sugar beet plants

Overall, the number of honey bees observed in the five in-crop assessment areas was on the same low level, in both, the control and the test item treatment group. There were no notable differences between the test item treatment group and the control group.

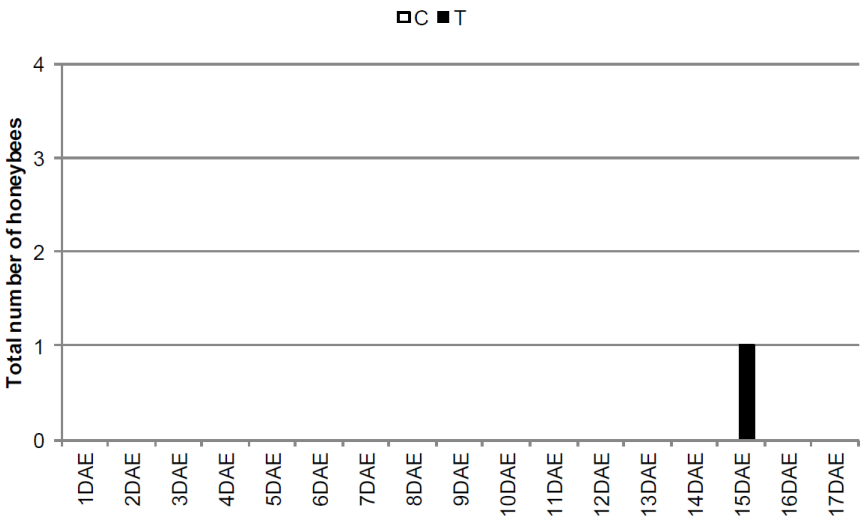


Figure 9.5.2-6: Flight Intensity: Total number of honey bees observed in the five assessment areas (total area: 10 m^2) per assessment date from 1DAE to 17DAE.
DAE: days after start of exposure

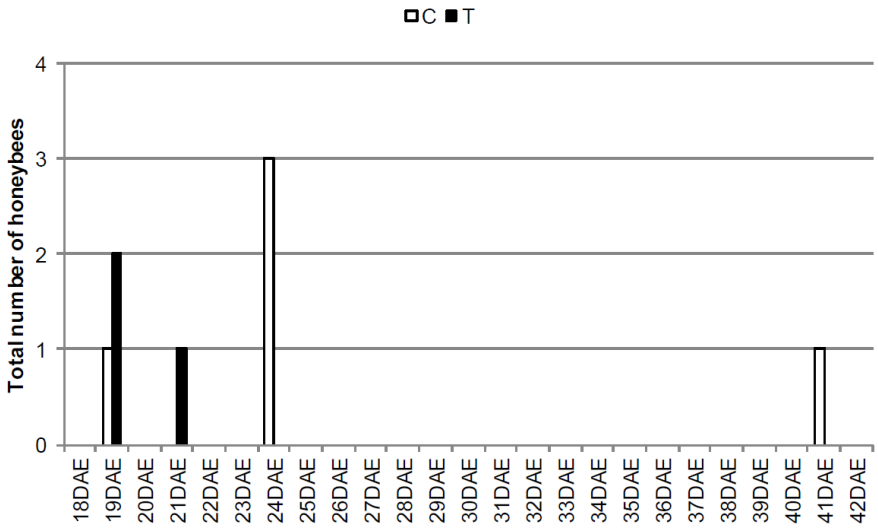


Figure 9.5.2-7: Flight Intensity: Total number of honey bees observed in the five assessment areas (total area: 10 m^2) per assessment date from 18DAE to 42DAE.
DAE: days after start of exposure

Mortality
No difference in mortality was observed between the control group and the test item treatment group during the entire exposure period.

Table 9.5.2-6: Mortality

Treatment group		Control (C)	Test item (T)
Daily mean mortality (dead bees/colony) ± STD	5DBE to 1DBE (Pre-exposure)	21.5 ± 26.2	14.8 ± 9.8
	1DAE to 42DAE (Exposure)	12.9 ± 4.7	16.6 ± 5.4

DAE: days after start of exposure; DBE: days before start of exposure; STD: standard deviation

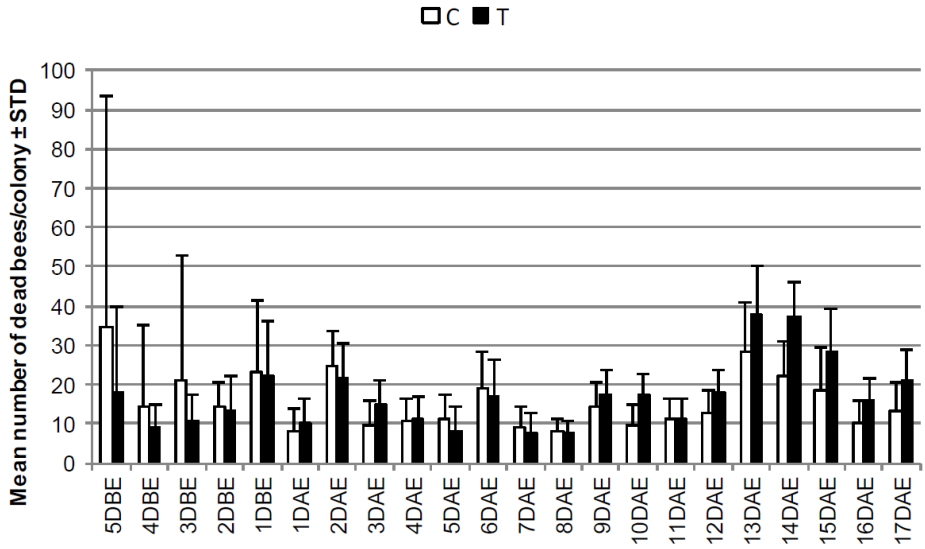


Figure 9.5.2-8: Mortality: Mean number of dead bees per colony at the monitoring site before set-up (5DBE to 1DBE) and during presence at the field sites from 1DAE to 17DAE.
DBE: days before start of exposure; DAE: days after start of exposure; STD: standard deviation

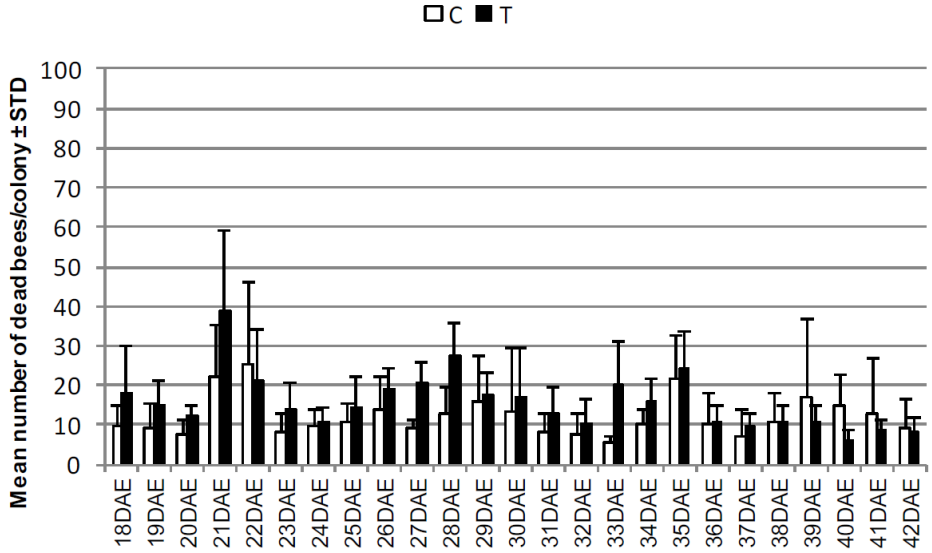


Figure 9.5.2-9: Mortality: Mean number of dead bees per colony during presence at the field sites from 18DAE to 42DAE.
DAE: days after start of exposure; STD: standard deviation

Behaviour of the bees

Overall, no notable differences in the abundance and frequency of the occurrence of abnormal behaviour were observed between the test item treatment group and the control. If abnormal behaviour was observed, it was only observed in a small number of honey bees on all assessment dates in both, in the test item treatment group and in the control group.

Condition of the colonies

Strength of the colonies

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. No test-item related adverse effects on colony strength were observed during the entire course of the study.

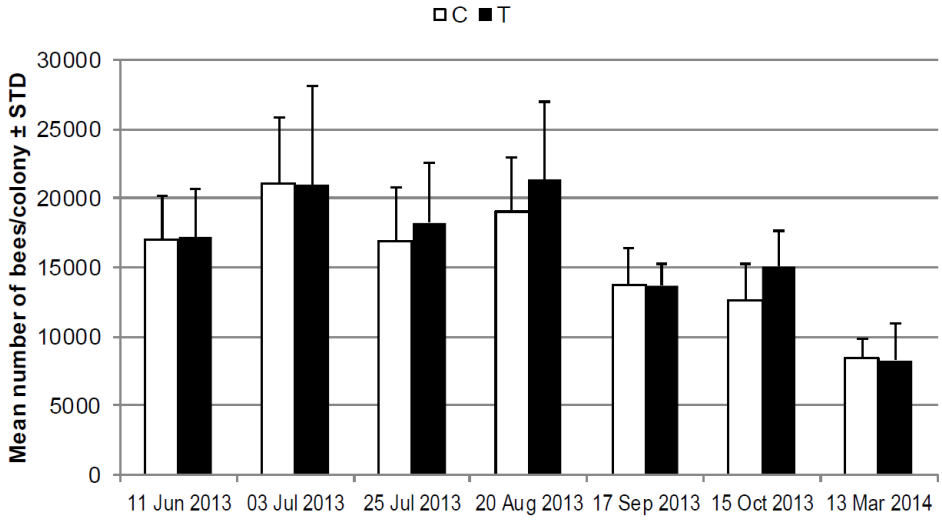


Figure 9.5.2-10: Colony strength: Mean colony strength (mean number of bees per colony) in the treatment groups C and T

Brood stages and overwintering performance

In the colonies of the control group C and the test item treatment group T, the natural and typical changes and fluctuations in the relative amount of the different pre-imaginal stages, i.e. egg stage, larval and pupal stage, occurred during the observation period. 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.

No test item-related adverse effects were observed on colony vitality and brood development, including queen survival and overwintering performance.

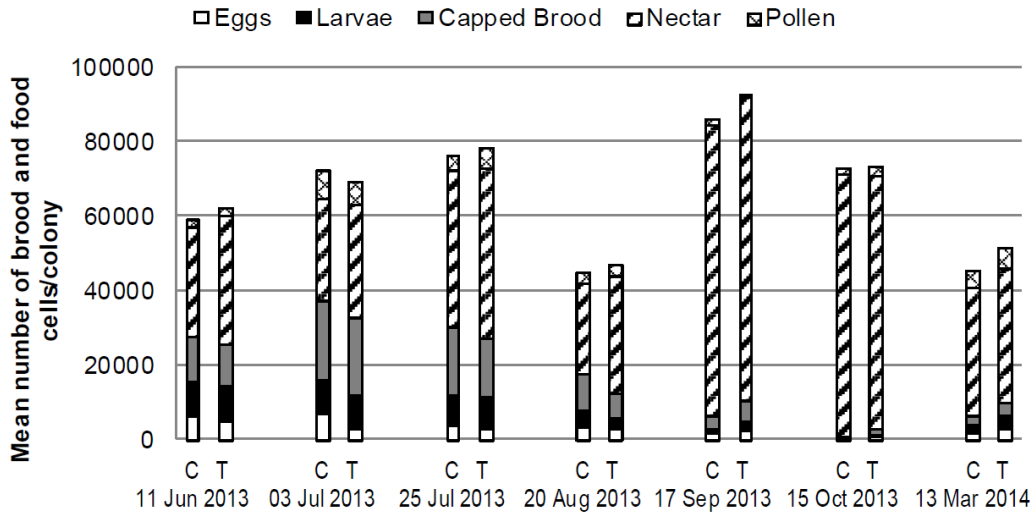


Figure 9.5.2-11: Brood stages and overwintering performance: Mean number of cells covered with brood and food in the treatment groups C and T

Food storage
In the colonies of the control group C and the test item treatment group T, respectively, the natural and typical changes and fluctuations in the relative amount of nectar and pollen storage cells occurred during the observation period. The control group C and the test item treatment group T showed approximately equal mean numbers of pollen and nectar storage cells throughout the entire observation period. No test item-related adverse effects on the food storage of the exposed colonies were observed.

Colony health
Evaluation of varroa infestation in the colonies
Varroa mite occurrence in the colonies was assessed via a '*Varroa* board' beneath the hives. The infestation level of a colony was monitored by counting dead mites on the board. 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 revealed that already the initial *Varroa* infestation level in the (future) test item treatment group (on 11 Jun 2013) was slightly to moderately higher as compared to the (future) control group before the actual set-up of the colonies on their respective exposure fields.

Bee diseases
Samples from three sampling dates in 2013 and one sampling date in 2014 were analysed for the pathogens *Nosema* sp., *Malpighamoeba mellificae*, *Varroa destructor* and *Paenibacillus larvae*. Overall, no distinct differences in the bee health status between the colonies of the control group and the test item treatment group could be observed.

Bee virus
The objective of the bee virus analysis was to determine the following bee viruses in bee samples collected at different time points of the year: DWV (deformed wing virus), SBV (sacbrood virus), ABPV (acute bee paralysis virus), CBPV (chronic bee paralysis virus), KBV (Kashmir bee virus), IAPV (Israeli acute paralysis virus), BQCV (black queen cell virus). Overall, no distinct differences in the bee health status in terms of virus infestation between the colonies of the control group and the test item treatment group could be observed.

Residue analysis

The determined clothianidin residues in guttation liquid, as analysed in the samples collected on each day where guttation droplets were actually present on the sugar beet plants in the test item treatment group T, were within the range of 153-327, 35-57 and 36-53 µg a.s./kg for parent clothianidin and its metabolites TZNG and TZMU, respectively.
The corresponding imidacloprid residues were within the range of 18-61, 6.9-16 and 1.9-4.0 µg a.s./kg for parent imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefine, respectively.
Residues of beta-cyfluthrin in all guttation liquid samples were virtually inexistent.

The Limit of Quantitation (LOQ) of clothianidin and imidacloprid in guttation fluid was 0.001 mg/L and the Limit of Detection (LOD) was 0.0003 mg/L, respectively. Due to the low compound sensitivity in the matrix guttation liquid, the LOQ for beta-cyfluthrin was set to 0.01 mg/kg. An exact and significant LOD could not be determined. Nevertheless an observation of the corresponding measurements shows no countable peaks at the expected retention time. Therefore, it was sufficiently proven that residues of beta-cyfluthrin in all guttation liquid samples were <LOQ / <LOD and as such virtually inexistent.
The range of residue levels detected is presented below:

Table 9.5.2-7: Range of residues determined in guttation liquid samples

Days after start of exposure	Residues [µg/kg]						
	CTD	TZNG	TZMU	IMD	IMD 5-hydroxy	IMD-olefine	Beta-cyfluthrin
14	222	38	36	34	13	3.7	<LOQ /<LOD
15	327	57	49	36	16	3.9	<LOQ /<LOD
22	237	37	40	39	11	2.5	<LOQ /<LOD
26	153	45	45	18	9.8	2.2	<LOQ /<LOD
27	159	39	44	26	6.9	1.9	<LOQ /<LOD
29	248	35	53	61	9.8	4.0	<LOQ /<LOD

CTD Clothianidin; IMD Imidacloprid

Conclusions

Overall, it can be concluded that guttation fluid, excreted by sugar beet plants, seed-treated with clothianidin + imidacloprid + beta-cyfluthrin, does not have unacceptable effects on honey bee colonies under typical commercial use conditions, as there were no adverse acute, short-term or long-term effects on colony strength and -development, brood development, food storage, honey bee behaviour, overall hive vitality, colony health, or on overwintering performance.

RMS's comments:

This study can be classified as generally well constructed and valid. The study is considered acceptable for use in risk assessment.

The overall occurrence of guttation droplets in the sugar beet crop was lower compared to the off-crop areas and other crops tested (winter cereals, potato).
It is noted that the duration of the observations for honey bee flight activity was very short. However, this fact is considered to be of limited consequence as these observations confirm the presence of honey bees in the field area. Thus, exposure to guttation fluid was possible. However, no treatment related differences in honey bee mortality and colony development as well as in the overwintering performance were observed between the control and the treatment group. Therefore, it is concluded that under the conditions of this experiment guttation fluid, exudated by seed treated sugar beets, does not have unacceptable effects on honey bee colonies.

Report:	Rexer, H. U.; 2014b
Title:	A long-term field study to monitor potential effects on the honey bee (*Apis mellifera* L.) from exposure to guttation fluid of sugar beets, seed-treated with the insecticides clothianidin + imidacloprid + beta-cyfluthrin in Southern Germany in 2013 and 2014
Report No.:	S13-00170
Document No.:	M-500734-01-1
Guideline(s):	OEPP/EPPO Guideline No. 170(4) (2010); SANCO/3029/99 rev. 4
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective
The objective of this study was to determine the effects of exposure of honey bees (*Apis mellifera* L.) to guttation liquid from sugar beet plants, grown from pills treated with clothianidin, imidacloprid and beta-cyfluthrin under field conditions.

The effects of honey bee exposure to guttation liquid from sugar beet plants, grown from treated sugar beet pills were examined on commercial bee colonies. Honey bees were placed at the field sites shortly after emergence of the plants (T: BBCH 12, C: BBCH 12-14). Honey bees remained at the sugar beet fields for 40 days after exposure and thereafter at a monitoring site, without extensive agricultural crops attractive to bees (monitoring phase). The experimental phase started with the drilling of the treated and untreated sugar beet pills in spring 2013 and ended in spring 2014 after monitoring overwintering survival, colony strength and colony development.

The influence of the test item was evaluated by comparing the results in the test item treatment to the corresponding control under consideration of the results of:

- Mean number of dead bees on the linen sheets and in the dead bee traps;
- Flight intensity in the field (mean number of forager bees /5 x 2 m² /min);
- Observation of honey bees visiting sugar beet plants displaying guttation;
- Occurrence and proportion of guttation;
- Behaviour of the bees in the crop and around the hive;
- Condition of the colonies (number of bees (colony strength), total values of the different brood stages per colony and assessment date);
- Bee health (bee disease and bee virus analysis);

- Overwintering performance

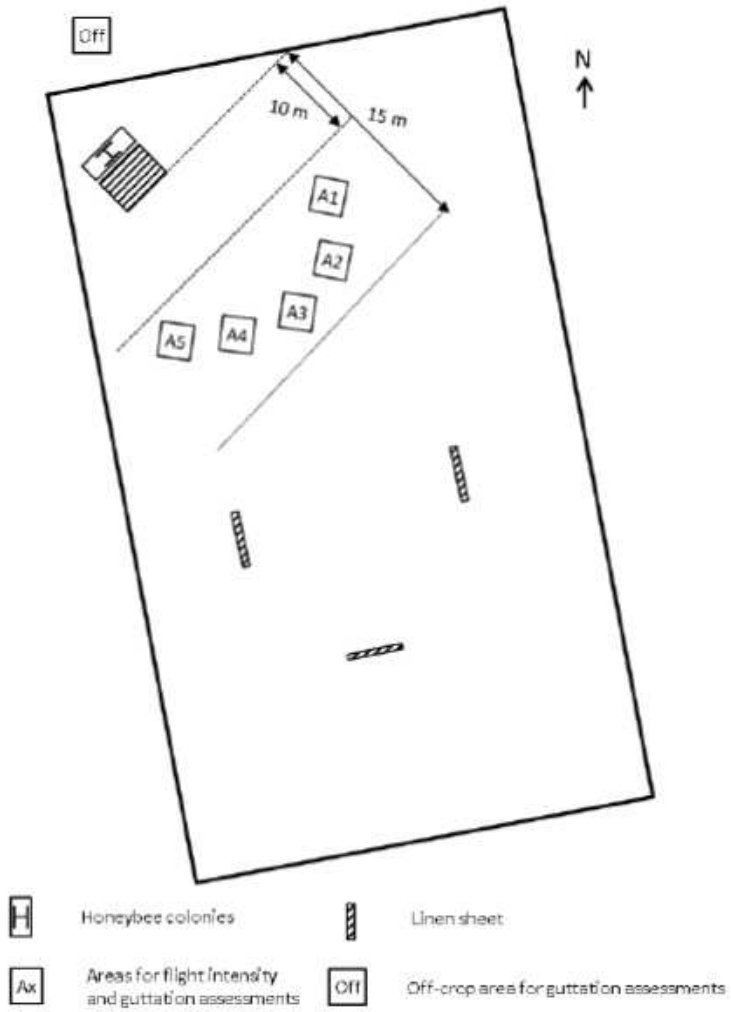


Figure 9.5.2-12: Design of the control field

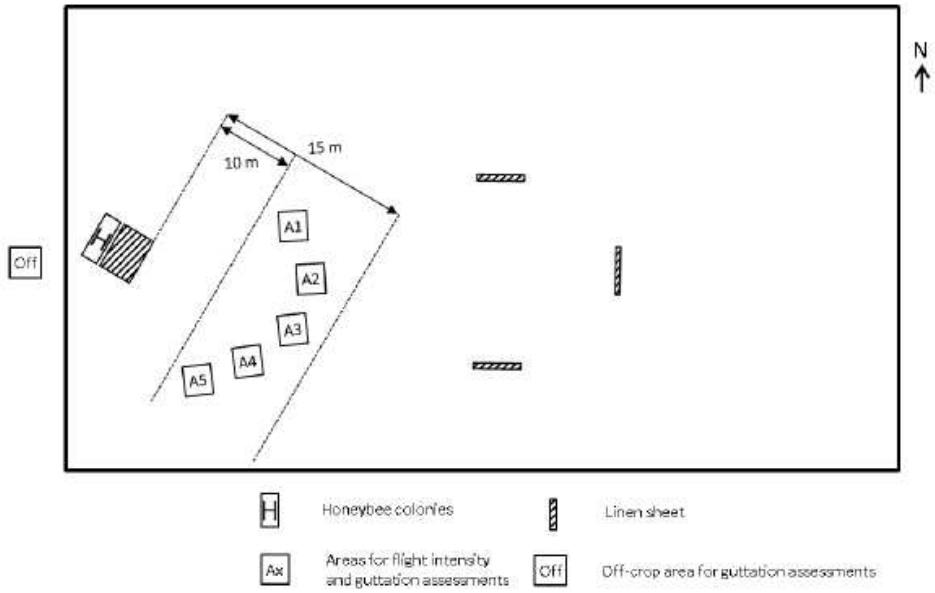


Figure 9.5.2-13: Design of the test item field

Material and Methods

1. Test material:

Crop:	Sugar beet (SB)
Test item:	Clothianidin + imidacloprid + beta-cyfluthrin + standard fungicide (Hymexazol + TMTD)
Description:	Pills /orange
Purity:	Clothianidin: 99.4% Imidacloprid: 98.8% Beta-Cyfluthrin: 98.8%
Content of a.s./pill:	Nominal Clothianidin: 0.6 mg a.s./pill Imidacloprid: 0.3 mg a.s./pill Beta-cyfluthrin: 0.08 mg a.s./pill Analysed Clothianidin: 0.6612 mg a.s./pill Imidacloprid: 0.2994 mg a.s./pill Beta-cyfluthrin: 0.0828 mg a.s./pill
Seeding rate:	130,000 pills/ha (corresponding with a target application rate of 78 g clothianidin/ha, 39 g imidacloprid/ha and 10.4 g beta-cyfluthrin/ha)

2. Vehicle and control:

Control:	Hymexazol + TMTD (fungicide)

3. Test animals:

Species:	Honey bees (*Apis mellifera*)
Colony size:	The mean number of bees per colony shortly before start of exposure was 15933 bees/colony in the control C (range: 8190 to 24635) and 15340 bees/colony in the test item treatment group T (range: 8580 to 24765).

4. Observations:

Behaviour:	During the assessments of mortality and flight intensity , the behaviour of the honey bees in the crop and around the hive was observed with respect to the following criteria: • aggressiveness towards the observer, • aggressiveness towards other bees (filtering at the hive entrance), • intensive cleaning, • clustering of large numbers of bees at the hive entrance, • cramping, • locomotion problems, • trembling, • inactive, • hanging bees (holding on to plants with one or two legs)
Colony conditions:	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.
Residues	Guttation fluid of SB plants in the treatment group was collected and analysed for residues of clothianidin, imidacloprid and beta-cyfluthrin.

Findings

Occurrence of guttation and percentage of plants displaying guttation

In the control group, guttation of sugar beet plants in the assessment areas was observed on 3 out of 40 assessment days. In the concurrently assessed off-crop area, guttation occurred on 25 out of 40 assessment days.

In the test item treatment group, guttation of sugar beet plants in the assessment areas was observed on 5 out of 40 assessment days. In the concurrently assessed off-crop area, guttation occurred on 20 out of 40 assessment days.

When guttation occurred in the in-crop assessment areas in the control group, the percentage of plants exhibiting guttation per assessment area varied from 2.9 % to 57.1 %. In the test item treatment group, the percentage of plants exhibiting guttation per assessment area varied from 3.0 % to 82.1 %, when guttation was detected.

Overall, guttation occurred only infrequently in sugar beets, and if, the overall abundance of guttation droplets was rather low, particularly when compared to adjacent off-crop areas.

Flight intensity and observation of honey bees visiting sugar beet plants

Overall, the number of honey bees observed in the five in-crop assessment areas was on the same low level, in both, the control and the test item treatment group. There were no notable differences between the test item treatment group and the control group.

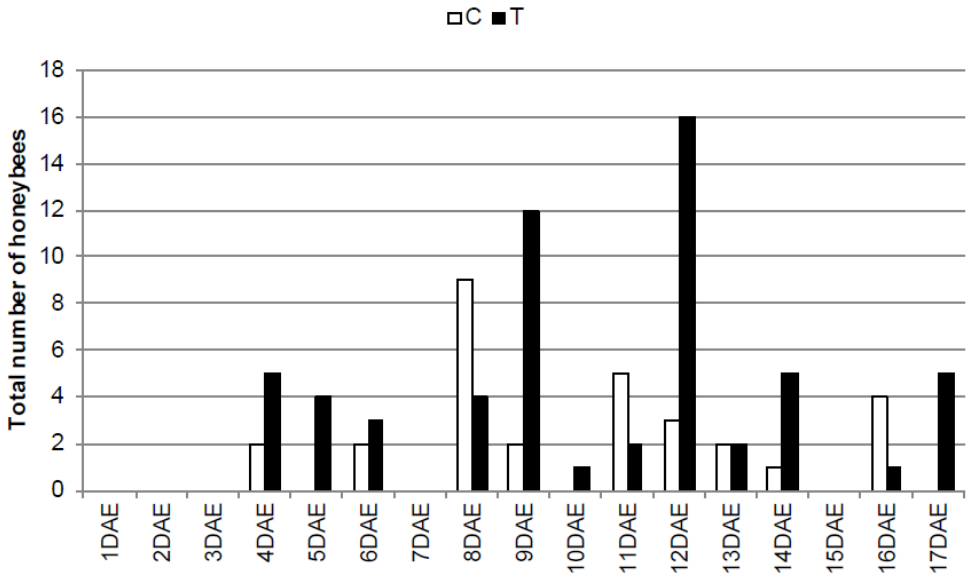


Figure 9.5.2-14: Flight Intensity: Total number of honey bees observed in the five assessment areas (total area: 10 m^2) per assessment date from 1DAE to 17DAE.
DAE: days after start of exposure

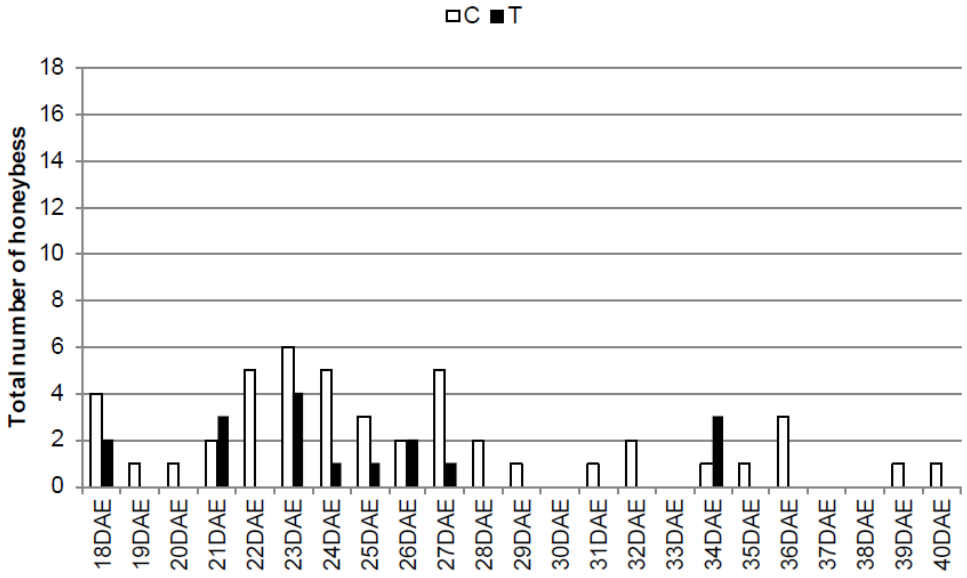


Figure 9.5.2-15: Flight Intensity: Total number of honey bees observed in the five assessment areas (total area: 10 m^2) per assessment date from 18DAE to 40DAE.
DAE: days after start of exposure

Mortality

No difference in mortality was observed between the control group and the test item treatment group during the entire exposure period.

Table 9.5.2-8: Mortality

Treatment group		Control (C)	Test item (T)
Daily mean mortality (dead bees/colony) ± STD	15DBE to 11DBE (Pre-exposure)	22.4 ± 5.7	21.5 ± 7.6
	1DAE to 40DAE (Exposure)	13.1 ± 2.9	14.1 ± 3.0

DAE: days after start of exposure; DBE: days before start of exposure; STD: standard deviation

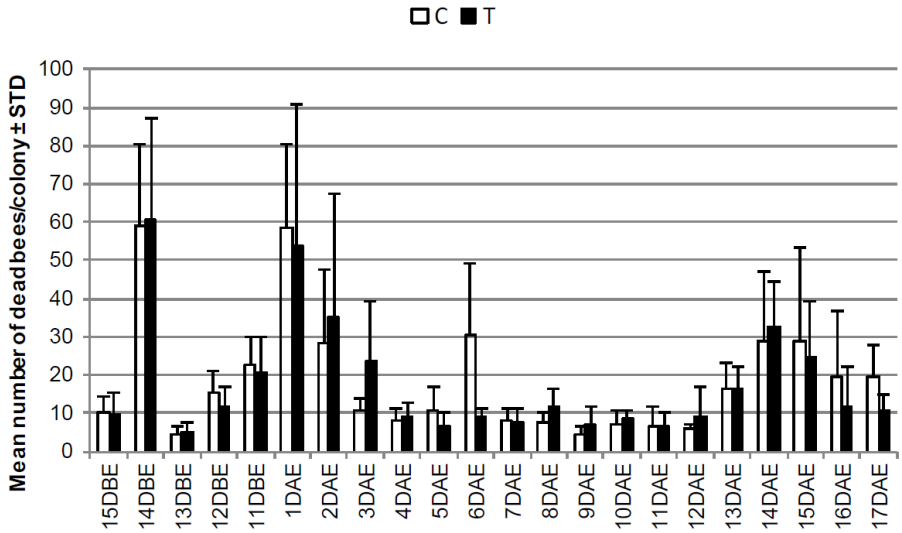


Figure 9.5.2-16: Mortality: Mean number of dead bees per colony at the monitoring site before set-up (15DBE to 11DBE) and during presence at the field sites from 1DAE to 17DAE.
DBE: days before start of exposure; DAE: days after start of exposure; STD: standard deviation

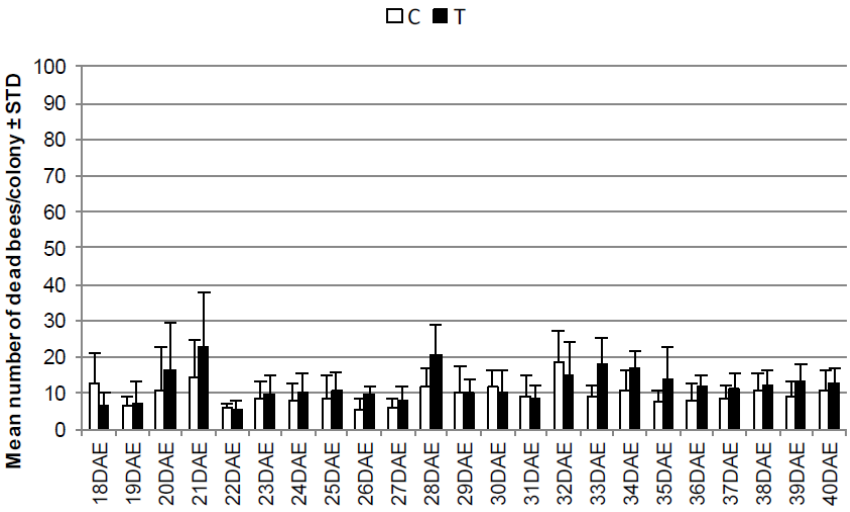


Figure 9.5.2-17: Mortality: Mean number of dead bees per colony during presence at the field sites from 18DAE to 40DAE.
DAE: days after start of exposure; STD: standard deviation

Behaviour of the bees

Overall, no notable differences in the abundance and frequency of the occurrence of abnormal behaviour were observed between the test item treatment group and the control. If abnormal behaviour was observed, it was only observed in a small number of honey bees on all assessment dates in both, in the test item treatment group and in the control group. No test-item related adverse effects on honey bee behaviour were observed.

Condition of the colonies

Strength of the colonies

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.

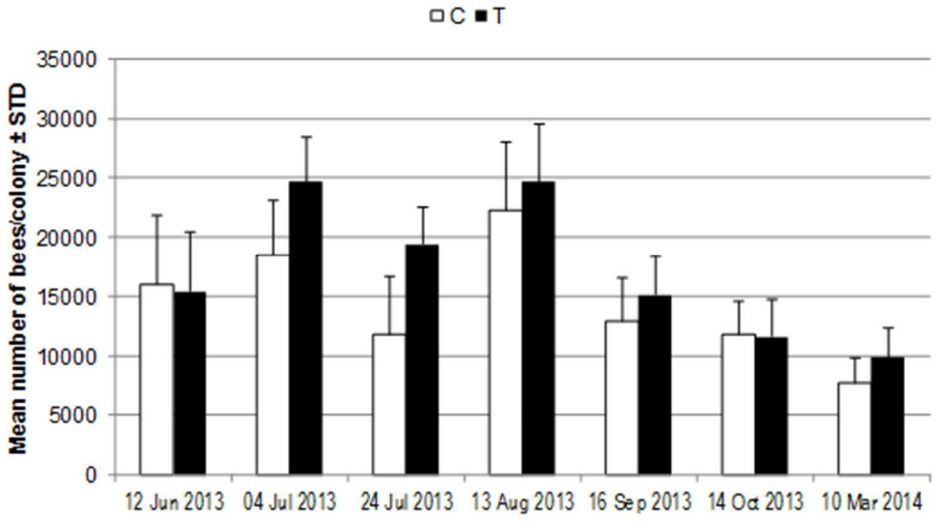


Figure 9.5.2-18: Colony strength: Mean colony strength (mean number of bees per colony) in the treatment groups C and T

Brood stages and overwintering performance
In the colonies of the control group C and the test item treatment group T the natural and typical changes and fluctuations in the relative amount of the different pre-imaginal stages, i.e. egg stage, larval and pupal stage, occurred during the observation period. The overwintering period lasted from 14 October 2013 until 10 Mar 2014. After overwintering, all colonies of the test item treatment group and the control were viable and all were found to have resumed breeding activity (except colony Cc). Thus, no test item-related adverse effects were observed on colony vitality and brood development, including queen survival and overwintering performance.

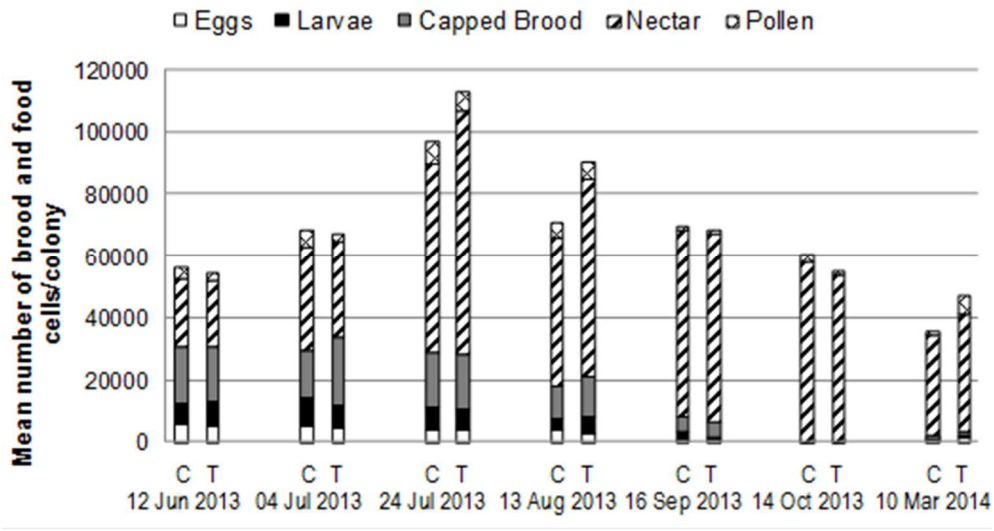


Figure 9.5.2-19: Brood Stages and Overwintering Performance: Mean number of cells covered with brood and food in the treatment groups C and T

Food storage
In the colonies of the control group C and the test item treatment group T, respectively, the natural and typical changes and fluctuations in the relative amount of nectar and pollen storage cells occurred during the observation period. The control group C and the test item treatment group T showed approximately equal mean numbers of pollen and nectar storage cells throughout the entire observation period. Thus, no test item-related adverse effects on the food storage of the exposed colonies were observed.

Colony health
Evaluation of varroa infestation in the colonies
Varroa mite occurrence in the colonies was assessed via a '*Varroa* board' beneath the hives. The infestation level of a colony was monitored by counting dead mites on the board. From the first assessment on 03 Sep 2013 (*Varroa* board was inserted on 13 Aug 2013) to 14 Oct 2013 only small numbers of mites were detected. Both the 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 honey bee season. No test item-related adverse effects were detected.

Bee diseases
Samples from three sampling dates in 2013 and one sampling date in 2014 were analysed for the pathogens *Nosema* sp., *Malpighamoeba mellificae*, *Varroa destructor* and *Paenibacillus larvae*. Overall, no distinct differences in the bee health status between the colonies of the control group and the test item treatment group could be observed.

Bee virus
The objective of the bee virus analysis was to determine the following bee viruses in bee samples collected at different time points of the year: DWV (deformed wing virus), SBV (sacbrood virus), ABPV (acute bee paralysis virus), CBPV (chronic bee paralysis virus), KBV (Kashmir bee virus), IAPV (Israeli acute paralysis virus), BQCV (black queen cell virus). Overall, no distinct differences in the bee health status in terms of virus infestation between the colonies of the control group and the test item treatment group could be observed.

Residue analysis
The determined clothianidin residues in guttation liquid, as analysed in the samples collected on each day where guttation droplets were actually present on the sugar beet plants in the test item treatment group T, were within the range of 17-64, 2.9-12 and 3.1-11 µg/kg for parent clothianidin and its metabolites TZNG and TZMU, respectively. The corresponding imidacloprid residues were within the range of 2.9-10, 1.2-4.2 and < LOQ-1.3 µg/kg for parent imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefine, respectively.
Residues of beta-cyfluthrin in all guttation liquid samples were virtually inexistent.

The Limit of Quantitation (LOQ) of clothianidin and imidacloprid in guttation fluid was 0.001 mg/L and the Limit of Detection (LOD) was 0.0003 mg/L, respectively. Due to the low compound sensitivity in the matrix guttation liquid, the LOQ for beta-cyfluthrin was set to 0.01 mg/kg. An exact and significant LOD could not be determined. Nevertheless an observation of the corresponding measurements shows no countable peaks at the expected retention time. Therefore, it was sufficiently proven that residues of beta-cyfluthrin in all guttation liquid samples were <LOQ /<LOD and as such virtually inexistent.
The range of residue levels detected is presented below:

Table 9.5.2-9: Range of residues determined in guttation liquid samples

Days after start of exposure	Residues [µg/kg]						
	CTD	**TZNG**	**TZMU**	**IMD**	**IMD 5-hydroxy**	**IMD-olefine**	**Beta-cyfluthrin**
12	17	2.9	3.1	2.9	1.2	<LOQ	<LOQ* /<LOD
16	64	12	11	9.7	4.2	1.3	<LOQ* /<LOD
17	60	7.6	7.0	10	1.9	<LOQ	<LOQ* /<LOD

CTD Clothianidin; IMD Imidacloprid

Conclusions
Overall, it can be concluded that guttation fluid, excreted by sugar beet plants, seed-treated with clothianidin + imidacloprid + beta-cyfluthrin, does not have unacceptable effects on honey bee colonies under typical commercial use conditions, as there were no adverse acute, short-term or long-term effects on colony strength and -development, brood development, food storage, honey bee behaviour, overall hive vitality, colony health, or on overwintering performance.

RMS's comments:
This study can be classified as generally well constructed and valid. The study is considered acceptable for use in risk assessment.

The overall occurrence of guttation droplets in the sugar beet crop was lower compared to the off-crop areas and other crops tested (winter cereals, potato).
It is noted that the duration of the observations for honey bee flight activity was very short. However, this fact is considered to be of limited consequence as these observations confirm the presence of honey bees in the field area. Thus, exposure to guttation fluid was possible. However, no treatment related differences in honey bee mortality and colony development as well as in the overwintering performance were observed between the control and the treatment group. Therefore, it is concluded that under the conditions of this experiment guttation fluid, exudated by seed treated sugar beets, did not have unacceptable effects on honey bee colonies.

Report:	Rexer, H. U.; 2014c
Title:	A long-term field study to monitor potential effects on the honey bee (*Apis mellifera* L.) from exposure to guttation fluid of potato plants, grown from seed tubers treated with Monceren G in southern Germany in 2014 and 2015
Report No.:	S14-01385
Document No.:	M-503349-03-1
Guideline(s):	OEPP/EPPO Guideline No. 170(4) (2010)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective
The objective of this study was to determine the effects of exposure of honey bees (*Apis mellifera* L.) to guttation liquid from potato plants, grown from seed tubers, treated with Monceren G (active ingredients: imidacloprid + pencycuron) under field conditions.

Commercial bee colonies (8 per treatment) were placed at the field sites shortly after emergence of the plants (BBCH 10). The mortality of the honey bees 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 (DAE= Days after exposure) to 58DAE. Flight intensity and behaviour as well as the number of honey bees visiting potato plants and the occurrence and proportion of guttation on potato plants was assessed daily after set-up of the bee colonies at the field sites from 0DAE to 58DAE. The condition of the colonies was assessed once before set-up of the colonies at the field sites and regularly thereafter and will be assessed until the end of overwintering. The *Varroa* infestation level was evaluated and samples of honey bees for bee disease and bee virus analysis as well as nectar for American foulbrood analysis (AFB) were collected to monitor colony health. Samples of guttation liquid from potato plants (test item treatment group T only) were collected for residue analysis. The influence of the test item was evaluated by comparing the results in the test item treatment to the corresponding control under consideration of the results of:

- Mean number of dead bees on the linen sheets and in the dead bee traps;
- Flight intensity in the field (mean number of honey bees per m2 and minute);

- Observation of honey bees visiting potato plants displaying guttation;
- Occurrence and proportion of guttation;
- Behaviour of the bees in the crop and around the hive;
- Condition of the colonies (number of bees (colony strength), total values of the different brood stages per colony and assessment date).

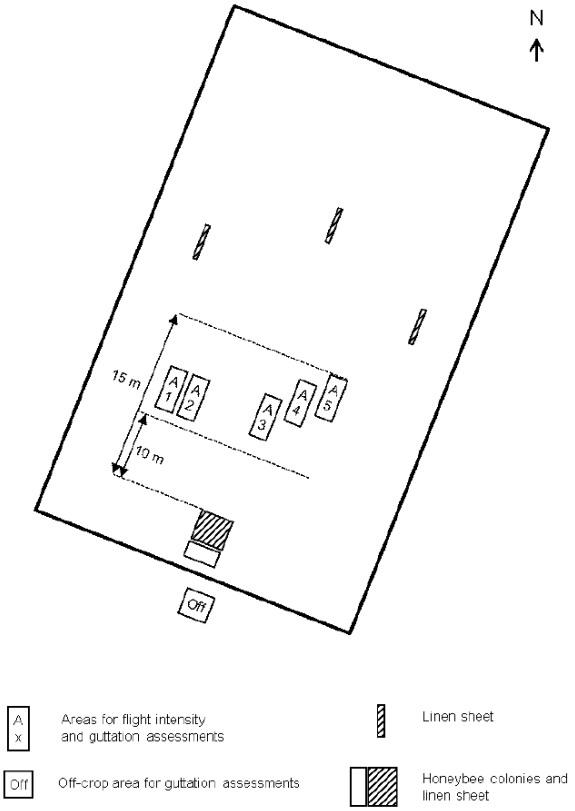


Figure 9.2.5-20: Design of the control field

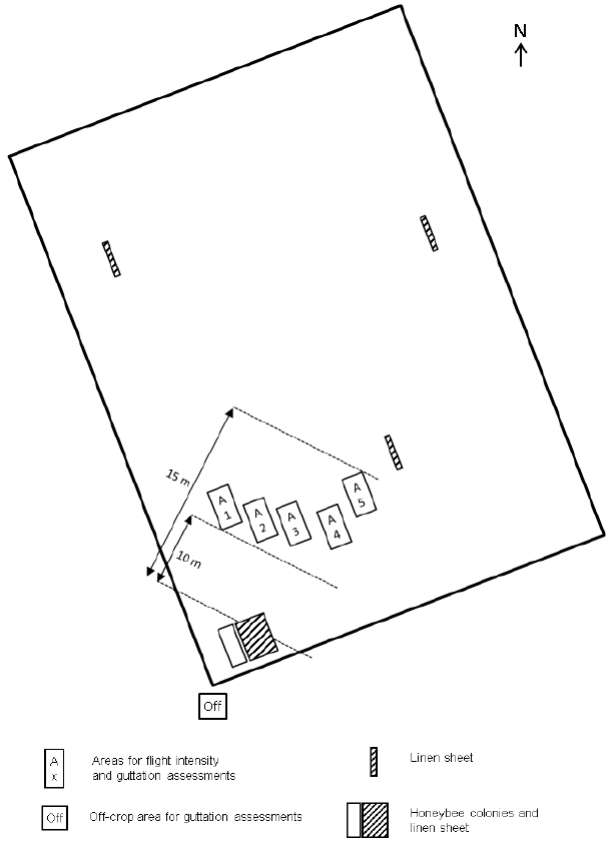


Figure 9.2.5-21: Design of the control field

Material and Methods

1. Test material:

Crop:	Potato plants (grown from seed tubers)
Test item:	Monceren G: 120 g a.s./L imidacloprid + 250 g a.s./L pencycuron (analysed: 120.5 g a.s./L imidacloprid + 251.2 g a.s./L pencycuron)
Description:	Red
Purity:	Imidacloprid: 98.8%
Application:	1.5 L product/ha (180 g imidacloprid + 375 g pencycuron)

2. Vehicle and control:

Control:	Untreated seed tubers

3. Test animals:

Species:	Honey bees (*Apis mellifera*)
Colony size:	The mean number of bees per shortly before start of exposure was 13804 bees/colony in the control C (range:

	9425 to 19305) and 13975 bees/colony in the test item treatment group T (range: 9945 to 18590)

4. Observations:

Behaviour:	During the assessments of mortality and flight intensity , the behaviour of the honey bees in the crop and around the hive was observed with respect to the following criteria: • aggressiveness towards the observer, • aggressiveness towards other bees (filtering at the hive entrance), • intensive cleaning, • clustering of large numbers of bees at the hive entrance, • cramping, • locomotion problems, • trembling, • inactive, • hanging bees (holding on to plants with one or two legs)
Colony conditions:	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.
Residues	Samples of guttation liquid from potato plants (test item treatment group T only) were collected for residue analysis

Findings

Occurrence of guttation and percentage of plants displaying guttation

In the control group, guttation of potato plants in the assessment areas was observed on 18 out of 59 assessment days. In the concurrently assessed off-crop area, guttation occurred on 29 out of 59 assessment days.

In the test item treatment group, guttation of potato plants in the assessment areas was observed on 17 out of 59 assessment days. In the concurrently assessed off-crop area, guttation occurred on 33 out of 59 assessment days.

When guttation occurred in the in-crop assessment areas, the percentage of plants exhibiting guttation per assessment area varied from 6.7 % to 100 % in the control group as well as in the test item treatment group.

Flight intensity in the field and observation of honey bees visiting potato plants

Overall, the vast majority of honey bees 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 honey bees that were only accidentally passing through the observation areas due to their close vicinity to the hives. However, virtually no honey bees were observed in direct contact with potato plants or soil in both treatment groups, with no notable differences between the test item treatment group and the control group. Moreover, uptake of guttation droplets by honey bees from potato plants (treated and untreated) did not occur during all assessments.

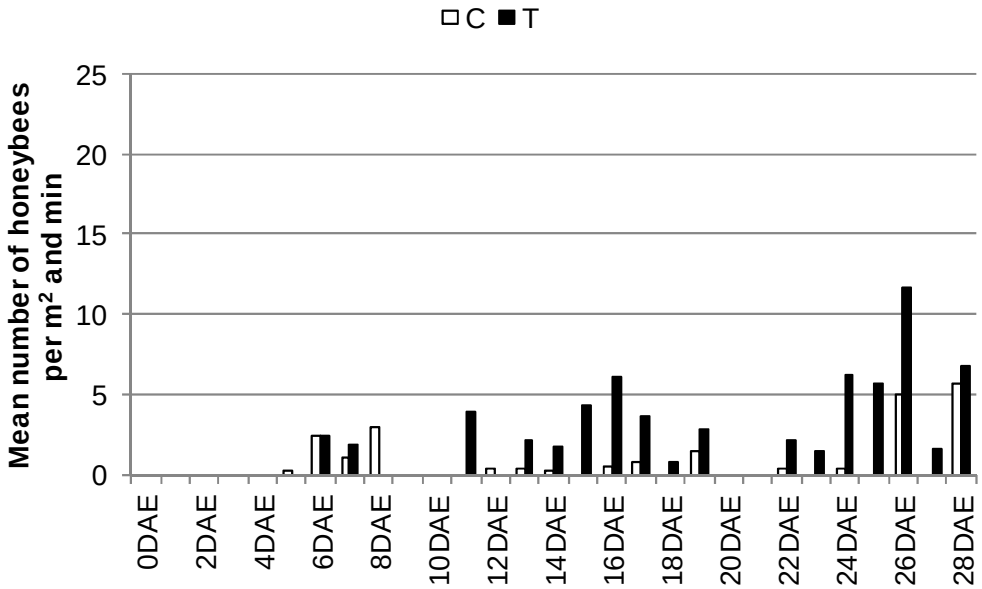


Figure 9.5.2-22: Flight Intensity: Mean number of honey bees per m² and minute observed in the field per assessment date from 0DAE to 28DAE.
DAE: days after start of exposure

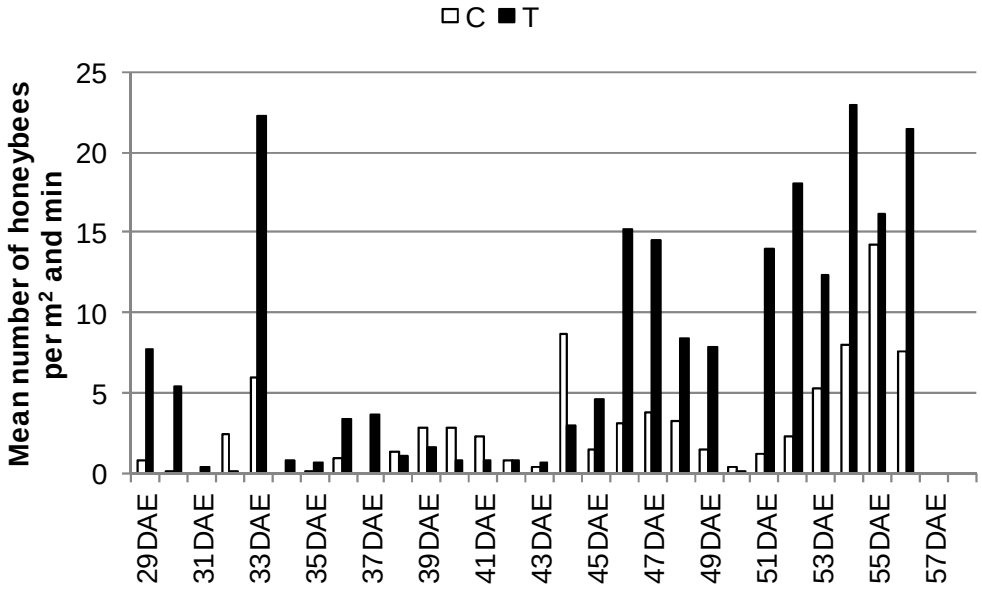


Figure 9.5.2-23: Flight Intensity: Mean number of honey bees per m² and minute observed in the field per assessment date from 29DAE to 58DAE.
DAE: days after start of exposure

Mortality

No notable difference was observed between the control and the test item treatment group concerning mortality during the exposure period.

Table 9.5.2-10: Mortality

Treatment group		Control (C)	Test item (T)
Daily mean mortality (dead bees/colony) ± STD	7DBE to 3DBE (Pre-exposure)	10.6 ± 5.4	10.5 ± 5.1
	1DAE to 58DAE (Exposure)	16.0 ± 2.8	13.8 ± 4.9

DAE: days after start of exposure; DBE: days before start of exposure; STD: standard deviation

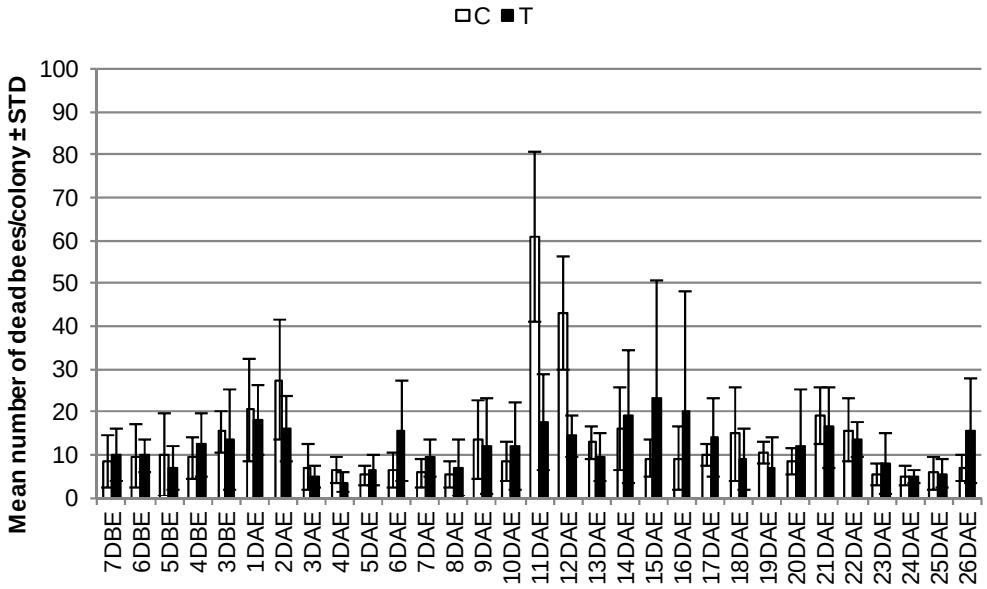


Figure 9.2.5-24: Mortality: Mean number of dead bees per colony at the monitoring site before set-up (7DBE to 3DBE) and during presence at the field sites from 1DAE to 26DAE.
DBE: days before start of exposure; DAE: days after start of exposure; STD: standard deviation

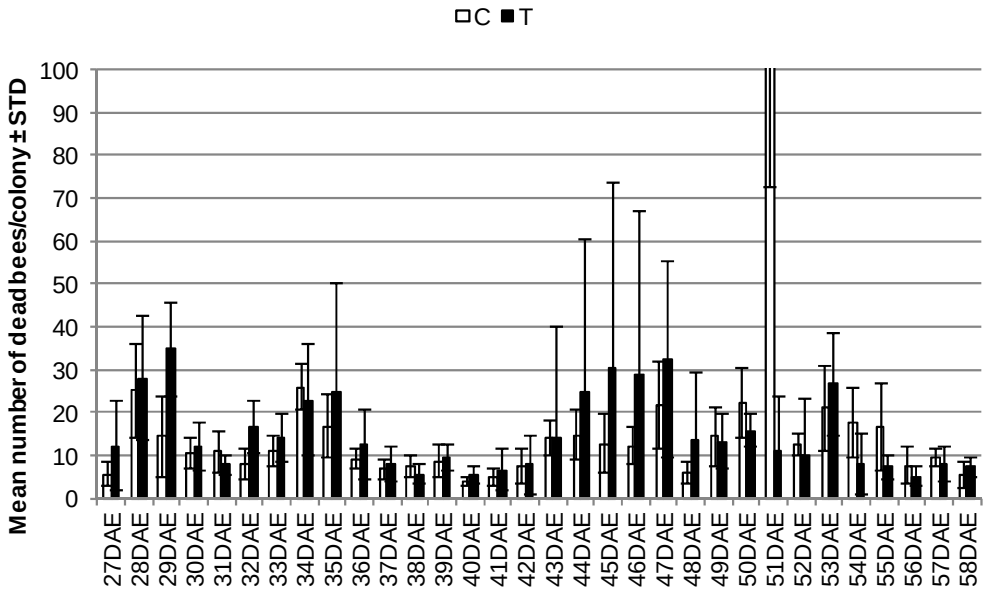


Figure 9.5.2-25: Mortality: Mean number of dead bees per colony during presence at the field sites from 27DAE to 58DAE.
DAE: days after start of exposure; STD: standard deviation

Behaviour of the bees

During the assessment period from 0DAE to 58DAE, honey bees exhibiting abnormal behaviour, 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 behaviour was recorded.

Overall, no notable differences in the abundance and frequency of the occurrence of abnormal behaviour were observed in the test item treatment group compared to the control.

Condition of the colonies

Strength of the colonies:

Throughout the entire observation period, the mean colony strength in the test item treatment group T was approximately on the same level as in the control group C without any major differences. Thus, no test-item related adverse effects on colony strength were observed during the course of the study.

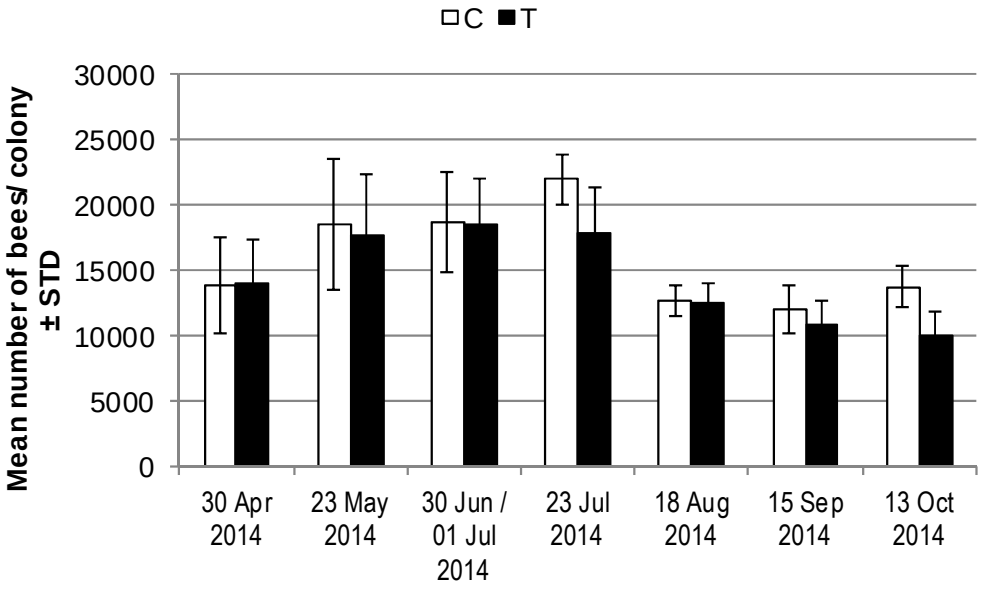


Figure 9.5.2-26: Colony strength: Mean colony strength (mean number of bees per colony) in the treatment groups C and T.
The assessment designated as 30 Jun /01 Jul 2014 was conducted on 30 Jun 2014 in the control group C and on 01 Jul 2014 in the test item treatment group T.

Brood stages:

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. On the last colony assessment before start of overwintering on 13 Oct 2014 (163DAE), the breeding activity of the colonies of the study had almost ended. Virtually no eggs and larvae, but still residual amounts of pupae were observed in the control and in the test item treatment group, respectively. No test item-related adverse effects were observed on brood development.

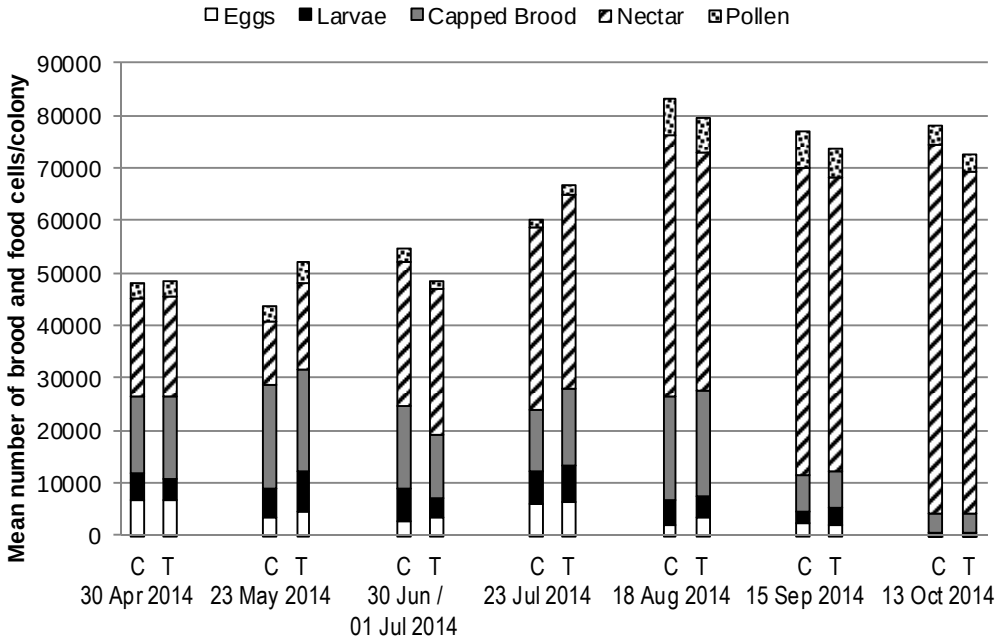


Figure 9.5.2-27: Brood Stages and Overwintering Performance: Mean number of cells covered with brood and food in the treatment groups C and T.
The assessment designated as 30 Jun /01 Jul 2014 was conducted on 30 Jun 2014 in the control group C and on 01 Jul 2014 in the test item treatment group T.

Food storage:

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 nectar and pollen storage cells occurred during the observation period. The treatment groups C and T showed approximately equal mean numbers of pollen and nectar storage cells throughout the entire observation period. Thus, no test item-related adverse effects on the food storage of the exposed colonies were observed.

Overwintering performance

Brood stages and overwintering performance

The overwintering period lasted from 13 Oct 2014 until 17 Mar 2015. After overwintering, all colonies of the test item treatment group and the control were alive and all were found to have resumed breeding activity normally (with the exception of the control colony Cc, which showed an interruption of egg-laying activity for unknown reasons).

Thus, no test item-related adverse effects were observed on colony vitality and brood development, including queen survival and overwintering performance.

Colony health:

Overall, no distinct differences in the health status between the honey bee colonies of the control group and the test item treatment group were observed either in terms of bee disease or virus.

Residue analysis

The determined imidacloprid residues in guttation liquid, as analysed in the samples collected on each day where guttation droplets were actually present on the potato plants in the test item treatment group T, are given in the Table below. The sample with high residue values at 36 DAE was contaminated with soil/dust, the results from this sample are inconsistent with the

previous and following samples and hence the soil/dust is judged to be a source of contamination.

Table 9.5.2-11: Range of residues determined in guttation liquid samples

Timing	Residues [µg/L]		
DAE: Days after start of exposure	**Imidacloprid**	**Imidacloprid-5-hydroxy**	**Imidacloprid-olefine**
7DAE	791	294	9
10DAE	522	276	6
11DAE	489	232	4
12DAE	408	202	5
13DAE	623	302	7
14DAE	488	206	5
15DAE	460	146	4
16DAE	165	70	2
17DAE	130	50	< LOQ
22DAE	88	33	< LOQ
26DAE	70	27	< LOQ
28DAE	48	22	< LOQ
31DAE	107	51	< LOQ
33DAE	106	80	2
34DAE	69	34	< LOQ
36DAE*	1958 *	583*	15*
40DAE	87	28	1
42DAE	32	13	< LOD

* The sample material was contaminated with soil/dust resulting in high value

Conclusions

Overall, it can be concluded that the exposure of honey bee colonies to guttation liquid from potato plants, grown from seed tubers, treated with Monceren G (active ingredients: imidacloprid + pencycuron) did not cause acute, short-term or long-term adverse effects on mortality, honey bee behaviour, colony strength, as well as brood and food development and overwintering performance in the exposed colonies.

RMS's comments:

This study can be classified as generally well constructed and valid. There was a frequent overlap between the occurrence of guttation and bee flight activity. Virtually no honey bees were observed in direct contact with potato plants or soil in both treatment groups. Uptake of guttation droplets by honey bees from potato plants (treated and untreated) did not occur during all assessments. However, this fact is considered to be of limited consequence as these observations confirm the presence of honey bees in the field area. Thus, exposure to guttation fluid was possible. No treatment related differences in honey bee mortality and colony development and overwintering performance were observed between the control and the treatment group. Therefore, it is concluded that under the conditions of this experiment guttation fluid, exudated by treated potato seed tubers, did not have unacceptable effects on honey bee colonies.

Report:	Rexer, H. U.; 2014d
Title:	A long-term field study to monitor potential effects on the honey bee (*Apis mellifera* L.) from exposure to guttation fluid of potato plants, grown from seed tubers treated with Monceren G in Southern Germany in 2014 and 2015
Report No.:	S14-01392
Document No.:	M-503344-03-1
Guideline(s):	OEPP/EPPO Guideline No. 170(4) (2010)
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective

The objective of this study was to determine the effects of exposure of honey bees (*Apis mellifera* L.) to guttation liquid from potato plants, grown from seed tubers, treated with Monceren G (active ingredients: imidacloprid + pencycuron) under field conditions.

The field study consisted of two treatment groups: The test item treatment group T (seed tubers treated with Monceren G) and the control group C (untreated seed tubers).
Commercial bee colonies (8 per treatment) were placed at the field sites shortly after emergence of the plants (BBCH 10). The mortality of the honey bees was assessed over a period of 5 days shortly before start of exposure and daily after set-up of the colonies at the field sites from 1DAE to 57DAE. Flight intensity and behaviour as well as the number of honey bees visiting potato plants and the occurrence and proportion of guttation on potato plants was assessed daily after set-up of the bee colonies at the field sites from 0DAE to 57DAE. The condition of the colonies was assessed once before set-up of the colonies at the field sites and regularly thereafter and will be assessed until the end of overwintering. The *Varroa* infestation level was evaluated and samples of honey bees for bee disease and bee virus analysis as well as nectar for American foulbrood (AFB) analysis were collected to monitor colony health. Samples of guttation liquid from potato plants (test item treatment group T only) were collected for residue analysis.

The influence of the test item was evaluated by comparing the results in the test item treatment to the corresponding control under consideration of the results of:

- Mean number of dead bees on the linen sheets and in the dead bee traps;
- Flight intensity in the field (mean number of honey bees per m^2 and minute);
- Observation of honey bees visiting potato plants displaying guttation;
- Occurrence and proportion of guttation;
- Behaviour of the bees in the crop and around the hive;
- Condition of the colonies (number of bees (colony strength), total values of the different brood stages per colony and assessment date).

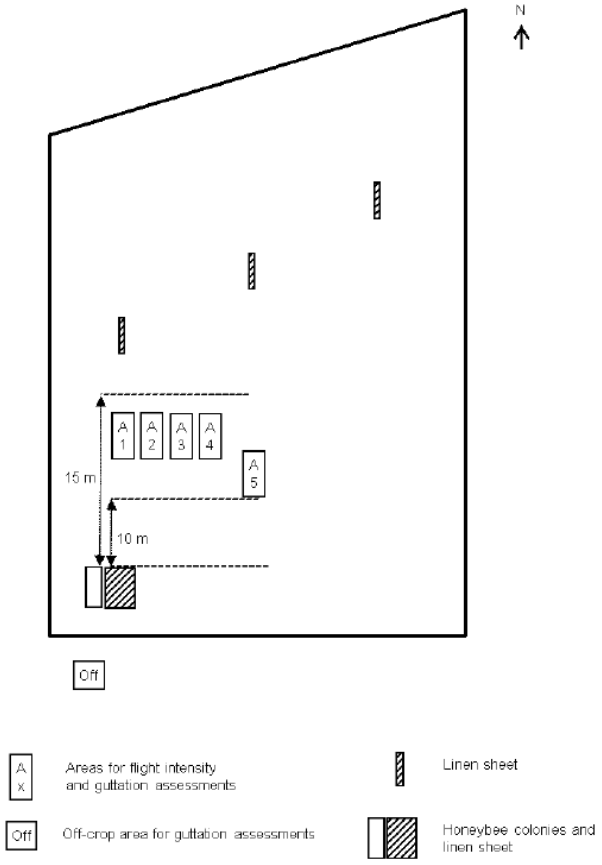


Figure 9.5.2-28: Design of the control field

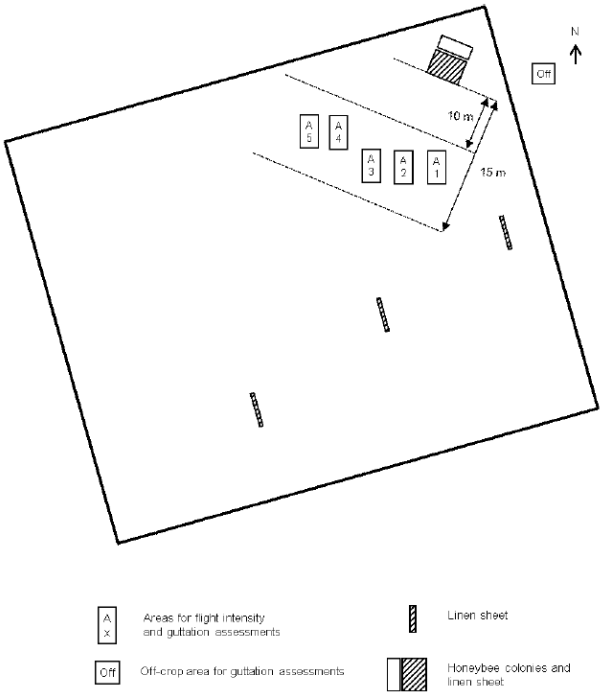


Figure 9.5.2-29: Design of the test item field

Material and methods

1. Test material:

Crop	Potato plants (grown from seed tubers)
Test item	120 g a.s./L imidacloprid + 250 g a.s./L pencycuron (analysed: 120.5 g a.s./L imidacloprid + 251.2 g a.s./L pencycuron)
Description	red
Purity	Imidacloprid: 98.8%
Application	1.5 L/ha

2. Vehicle and control:

Control	Untreated seed tubers

3. Test animals:

Species	Honey bees (*Apis mellifera*)
Colony size	The mean number of bees shortly before start of exposure was 17184 bees/colony in the control C (range: 9685 to 23140) and 17704 bees/colony in the test item treatment group T (range: 9750 to 31135).

4. Observations:

Behaviour	During the assessments of mortality and flight intensity , the behaviour of the honey bees in the crop and around the hive was observed with respect to the following criteria: • aggressiveness towards the observer, • aggressiveness towards other bees (filtering at the hive entrance), • intensive cleaning, • clustering of large numbers of bees at the hive entrance, • cramping, • locomotion problems, • trembling, • inactive, • hanging bees (holding on to plants with one or two legs)
Colony conditions	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.
Residues	Samples of guttation liquid from potato plants (test item treatment group T only) were collected for residue analysis.

Findings

Occurrence of guttation and percentage of plants displaying guttation

In the control group, guttation of potato plants in the assessment areas was observed on 33 out of 58 assessment days. In the concurrently assessed off-crop area, guttation occurred on 27 out of 58 assessment days. In the test item treatment group, guttation of potato plants in the assessment areas was observed on 37 out of 58 assessment days. In the concurrently assessed off-crop area, guttation occurred on 21 out of 58 assessment days. When guttation occurred in the in-crop assessment areas, the percentage of plants exhibiting guttation per assessment area varied from 8.3 % to 100 % in the control group as well as in the test item treatment group.

Flight intensity in the field and observation of honey bees visiting potato plants

Overall, the vast majority of honey bees 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 honey bees that were only accidentally passing through the observation areas due to their close vicinity to the hives. However, virtually no honey bees were observed in direct contact with potato plants or soil in both treatment groups, with no notable differences between the test item treatment group and the control group. Moreover, uptake of guttation droplets by honey bees from potato plants (treated and untreated) did not occur during all assessments.

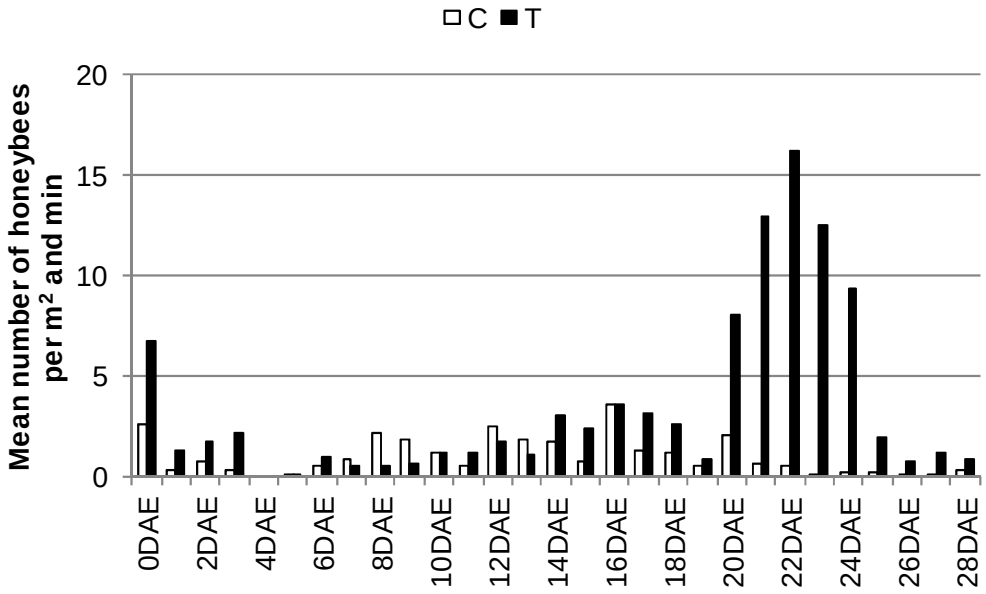


Figure 9.5.2-30: Flight Intensity: Mean number of honey bees per m^2 and minute observed in the field per assessment date from 0DAE to 28DAE.
DAE: days after start of exposure

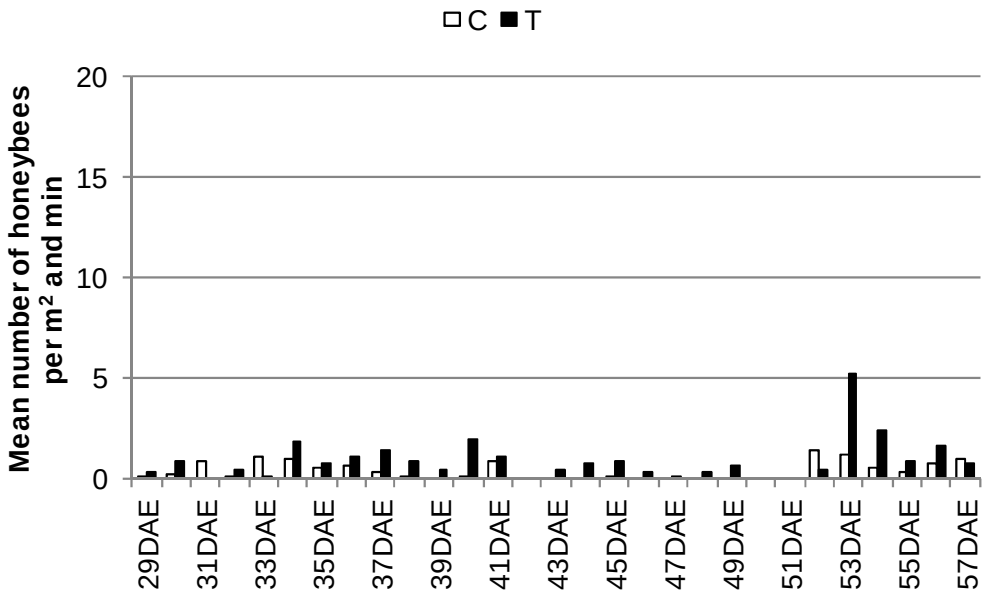


Figure 9.5.2-31: Flight Intensity: Mean number of honey bees per m^2 and minute observed in the field per assessment date from 29DAE to 58DAE.
DAE: days after start of exposure

Mortality

No notable difference in mortality was observed between the control group and the test item treatment group during the entire exposure period.

Table 9.5.2-12: Mortality

Treatment group		**Control (C)**	**Test item (T)**
Daily mean mortality (dead bees/colony) ± STD	5DBE to 1DBE (Pre-exposure)	45.9 ± 42.0	35.7 ± 20.6
	1DAE to 57DAE (Exposure)	20.7 ± 6.1	18.3 ± 3.8

DAE: days after start of exposure; DBE: days before start of exposure; STD: standard deviation

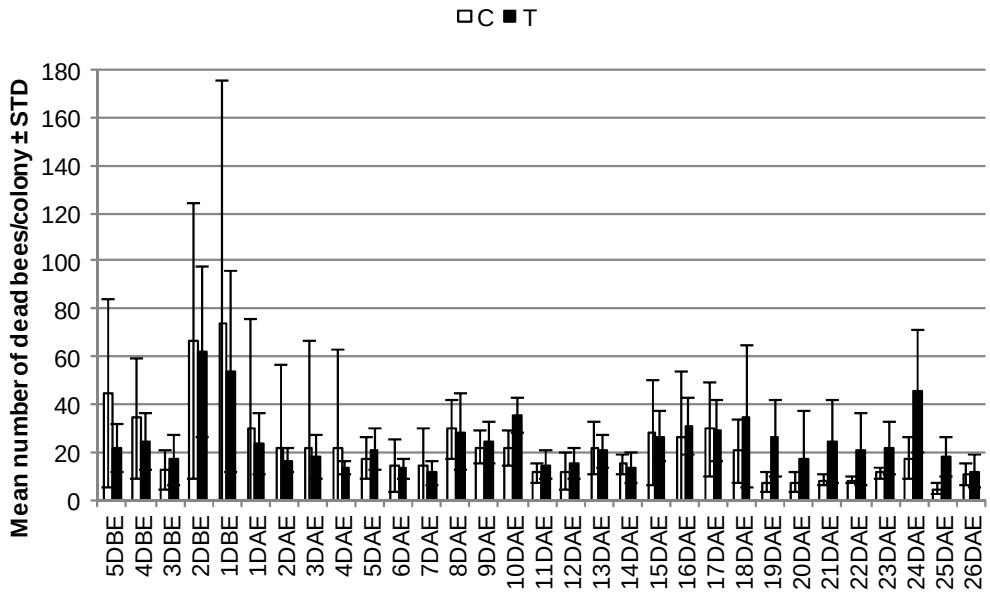


Figure 9.5.2-32: Mortality: Mean number of dead bees per colony at the monitoring site before set-up (5DBE to 1DBE) and during presence at the field sites from 1DAE to 26DAE.
DBE: days before start of exposure; DAE: days after start of exposure; STD: standard deviation

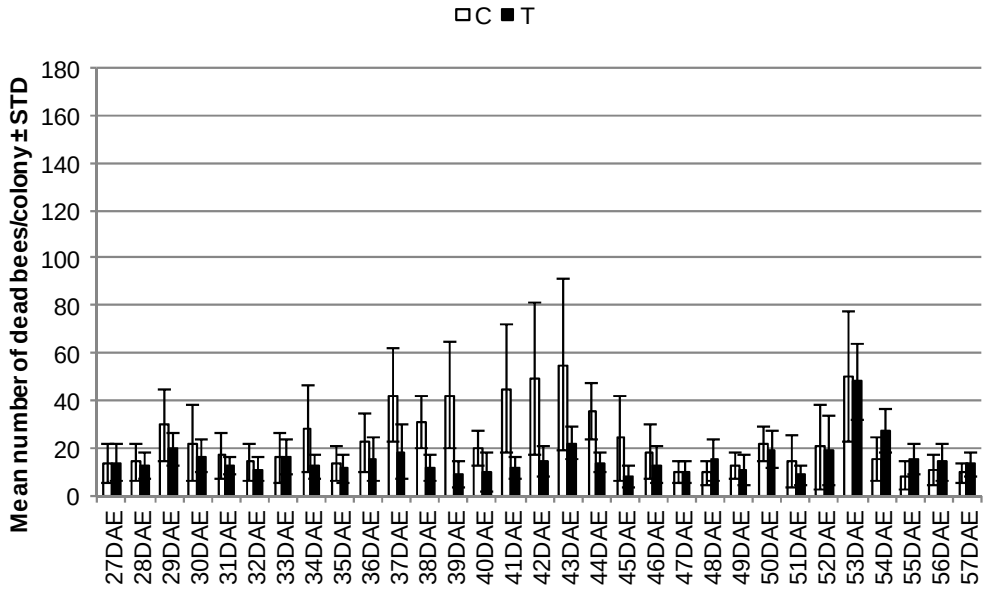


Figure 9.5.2-33: Mortality: Mean number of dead bees per colony during presence at the field sites from 27DAE to 57DAE.
DAE: days after start of exposure; STD: standard deviation

Behaviour of the bees

During the assessment period from 0DAE to 57DAE, small numbers of honey bees 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 control group. On the remaining days, only normal behaviour was recorded. Overall, no notable differences in the abundance and frequency of the occurrence of abnormal behaviour were observed in the test item treatment group compared to the control.

Condition of the colonies

Strength of the colonies
No test-item related adverse effects on colony strength were observed during the course of the study (see figure1.5.2-34).

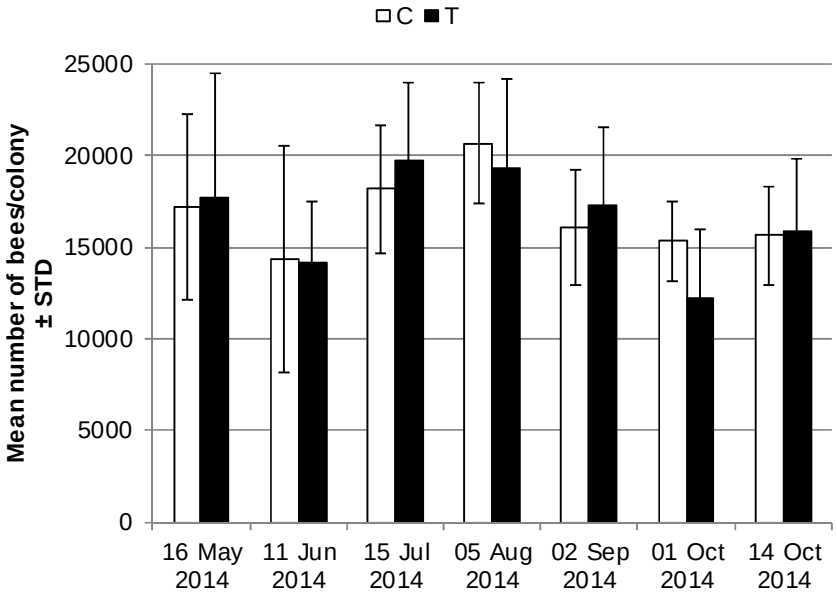


Figure 9.5.2-34: Colony strength: Mean colony strength (mean number of bees per colony) in the treatment groups C and T.
The assessment designated as 30 Jun /01 Jul 2014 was conducted on 30 Jun 2014 in the control group C and on 01 Jul 2014 in the test item treatment group T.

Brood stages
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. In early autumn, when the natural period of breeding activity of the colonies ended, the number of cells with brood had notably declined in both, the control and the test item treatment group on the day of the colony assessment on 01 Oct 2014 (135DAE). On the last colony assessment before start of overwintering, on 14 Oct 2014 (148DAE), the breeding activity of the colonies of the study had almost ended. Virtually no eggs and larvae, but still residual amounts of pupae were observed in the control and in the test item treatment group, respectively. Thus, no test item-related adverse effects were observed on brood development (see figure 1.5.2-35).

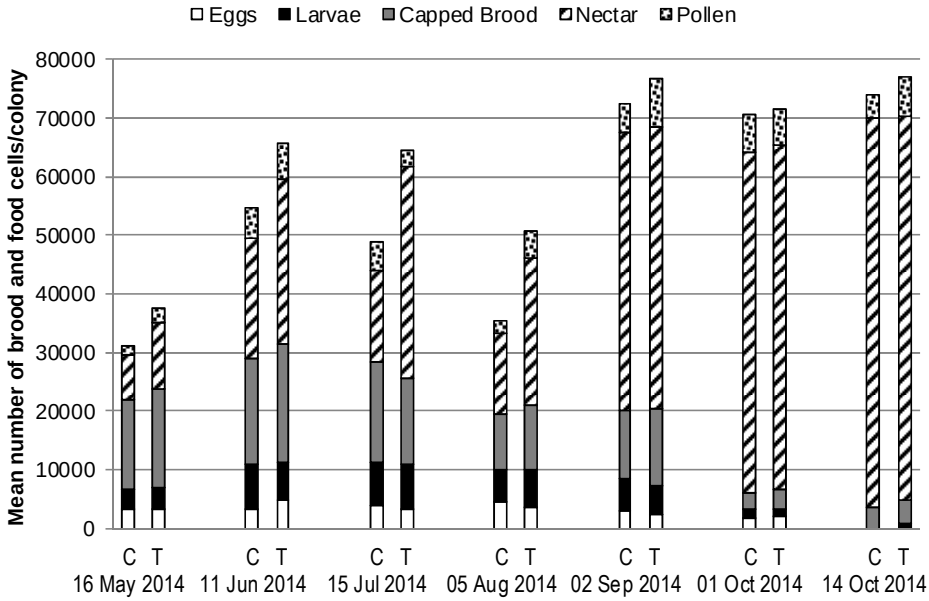


Figure 9.5.2-35: Brood Stages and Overwintering Performance: Mean number of cells covered with brood and food in the treatment groups C and T.
The assessment designated as 30 Jun /01 Jul 2014 was conducted on 30 Jun 2014 in the control group C and on 01 Jul 2014 in the test item treatment group T.

Food storage

In the colonies of the control group C and the test item treatment group T, respectively, the natural and typical changes and fluctuations in the relative amount of nectar and pollen storage cells occurred during the observation period. The treatment groups C and T showed approximately equal mean numbers of pollen and nectar storage cells throughout the entire observation period, except in the course of two assessments on 15 Jul 2014 and 05 Aug 2014, during which the mean number of nectar cells in the test item treatment colonies was remarkably higher than in the control colonies. Thus, no test item-related adverse effects on the food storage of the exposed colonies were observed.

Colony health

Evaluation of Varroa infestation in the colonies

Varroa mite occurrence in the colonies was assessed via a '*Varroa* board' beneath the hives. The infestation level of a colony was monitored by counting dead mites on the board. The *Varroa* infestation levels of the test item treatment colonies were approximately on the same level as or even lower than those of the control colonies during the course of the study and at the end of the honey bee season. No test item-related adverse effects were detected.

Overwintering performance

Brood stages and overwintering performance
After overwintering, all colonies of the test item treatment group and the control were alive. Seven out of eight colonies in the test item treatment group were found to have resumed breeding activity normally, whereas one colony (Th) did not contain any brood cells. This was most likely due to the presence of a virgin queen as a result of queen replacement by the colony

itself during overwintering, which can be considered as a naturally occurring process. In the control group, seven out of eight colonies were found to have resumed breeding activity normally, whereas one colony (Cb) did not contain any brood cells. This was due to the absence of an egg-laying queen in the colony. Consequently, no differences in terms of overwintering success and the resumption of breeding activity in early spring were observed between the test item treatment group and the control.
Thus, no test item-related adverse effects were observed on colony vitality and brood development, including queen survival and overwintering performance.

Colony health
Overall, no distinct differences in the health status between the honey bee colonies of the control group and the test item treatment group were observed either in terms of bee disease or virus.

Residue analysis

The determined imidacloprid residues in guttation liquid, as analysed in the samples collected on each day where guttation droplets were actually present on the potato plants in the test item treatment group T, are given in the Table below. In several samples contamination with soil/dust was observed, the measured residues in these samples were higher than in the samples without contamination, these samples were inconsistent with those taken before and after and hence it is likely that the soil/dust particles were the source of the high levels in the samples.

Table 9.5.2-13: Range of residues determined in guttation liquid samples

Timing	**Residues [µg/L]**		
DAE: Days after start of exposure	**Imidacloprid**	**Imidacloprid-5-hydroxy**	**Imidacloprid-olefine**
0DAE	1069	337	10
1DAE	1106	255	10
2DAE*	2411	391	14
3DAE*	4749	1042	9
4DAE	1982	313	12
5DAE	1176	189	8
6DAE	624	97	5
7DAE	324	61	3
8DAE	152	34	3
9DAE	1184	254	12
10DAE	366	94	2
11DAE	1447	319	10
12DAE	347	73	5
13DAE	367	107	4
14DAE	185	55	2
15DAE	113	28	2
16DAE	189	34	2
17DAE	105	31	< LOQ
18DAE	205	52	3
19DAE	83	24	< LOQ
20DAE	120	31	< LOQ

Timing	Residues [µg/L]		
DAE: Days after start of exposure	Imidacloprid	Imidacloprid-5-hydroxy	Imidacloprid-olefine
21DAE	208	36	1
22DAE	269	46	1
23DAE*	1157	189	6
24DAE	444	84	3
25DAE*	1950	326	11
26DAE	132	28	< LOQ
27DAE	15	5	< LOD
28DAE	70	16	< LOQ
29DAE*	2722	525	19
30DAE	14	4	< LOD
31DAE	8	3	< LOD
33DAE	15	5	< LOD
34DAE	14	6	< LOD
35DAE	9	4	< LOD
38DAE	6	3	< LOD
39DAE	5	3	< LOD
42DAE	1	1	< LOD
43DAE	2	2	< LOD
44DAE	4	2	< LOD
45DAE	5	2	< LOD
46DAE	4	2	< LOD
49DAE	2	2	< LOD
50DAE	3	2	< LOD
52DAE	4	3	< LOD
53DAE	3	2	< LOD
54DAE	4	2	< LOD
55DAE	11	5	< LOD
56DAE	10	3	< LOD
57DAE	7	3	< LOD

* The sample material was contaminated with soil/dust resulting in high value

Conclusions

Overall, it can be concluded that the exposure of honey bee colonies to guttation liquid from potato plants, grown from seed tubers, treated with Monceren G (active ingredients: imidacloprid + pencycuron) did not cause acute, short-term or long-term adverse effects on mortality, honey bee behaviour, colony strength, as well as brood and food development and overwintering performance in the exposed colonies.

RMS's comments:

This study can be classified as generally well constructed and valid.
There was a frequent time overlap between the occurrence of guttation and bee flight activity. Virtually no honey bees were observed in direct contact with potato plants or soil in both treatment groups. Uptake of guttation droplets by honey bees from potato plants (treated and untreated) did not occur during all assessments. However, this fact is considered to be of limited

consequence as these observations confirm the presence of honey bees in the field area. Thus, exposure to guttation fluid was possible. No treatment related differences in honey bee mortality and colony development and overwintering performance were observed between the control and the treatment group. Therefore, it is concluded that under the conditions of this experiment guttation fluid, exudated by treated potato seed tubers, did not have unacceptable effects on honey bee colonies.

The potential exposure to dust drift following drill and the acute and the long-term risk to colony survival and development, and the risk to bee brood resulting from such exposure

A total of three field studies which target on the determinations of imidacloprid and clothianidin residues in dust drift deposits, have been submitted. In addition, two field studies which target on the risk of residues in dust to honey bee colonies have been submitted.

Report:	Hofmann, S.; Lueckmann, J.; 2010a
Title:	Monitoring of dust drift deposits during and after sowing of winter barley (W-BAR) treated with Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 145.2 (60 + 70 + 7.2 + 8 g/L) or Clothianidin & Beta-Cyfluthrin FS 455 (375 + 80 g/L) on fields in Germany
Report No.:	R09247-1
Document No.:	M-366273-01-1
Guideline(s):	91/414/EEC of July 15, 1991, SANCO/3029/99 Rev. 4, 2000-07-11
Guideline deviation(s):	not specified
GLP/GEP:	**no**

Objective

The objective of the study was to determine the residues of imidacloprid and clothianidin in dust drift deposits during and after sowing of winter barley treated with Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 145.2 (60 + 70 + 7.2 + 8 g/L) or Clothianidin & Beta-Cyfluthrin FS 455 (375 + 80 g/L) on fields in Germany.

Material and Methods

Test item

Two different winter barley (W-BAR) varieties (i.e. Lomerit and Highlight) were purchased untreated and commercially cleaned-up from a commercial seed distributor (Gut Peterhof, D-50127 Bergheim, Germany) and were thereafter seed-treated at Bayer CropScience's Seed Treatment Application Centre in D-40789 Monheim am Rhein, Germany (non-GLP):

- Manta® Plus FS 145.2 (TOX08744-00) treated winter barley seeds, dressed with 1000mL product/100 kg seeds (= nominally 70 g imidacloprid/100 kg seeds); identification of treated seeds: TOX08780-00 (variety Lomerit); TOX08779-00 (variety Highlight)

And

- Smaragd® forte FS 455 (TOX08741-00) treated winter barley seeds, dressed with 133mL product/100 kg seeds (= nominally 50 g clothianidin/100 kg seeds);

identification of treated seeds: TOX08775-00 (variety Lomerit); TOX08774-00 (variety Highlight).

After seed-dressing, the seeds were subject to chemical analysis for the determination of the actual seed loading. Finally, the seed bags were unequivocally labelled and shipped via road transport to the respective study sites in Germany.

Study sites and sowing

The multiple site study was conducted at two different regions in Germany: one in Southern Germany in the federal state of Baden-Württemberg in Renningen, southwest of Stuttgart at the experimental station Ihinger Hof of the University Hohenheim (in the following called Ihinger Hof) and the second in Northern Germany in the federal state of Lower Saxony near Celle northeast of Hannover (in the following called Celle) with two fields per location. The sizes of the test fields sown with Manta® Plus-treated W-BAR seeds at Ihinger Hof and Celle were 4.8 ha and 8.0 ha, respectively. The fields drilled with Smaragd® forte treated W-BAR seeds at Ihinger Hof and Celle were 3.9 ha and 7.0 ha, respectively. The variety of W-BAR sown at Ihinger Hof was 'Highlight' and the variety drilled at Celle was 'Lomerit'.

A total of 200 kg seeds/ha were sown at both test locations resulting in nominal application rates of 140 g imidacloprid a.s./ha on fields drilled with Manta® Plus and 100 g clothianidin a.s./ha on fields drilled with Smaragd® forte. The seeds were drilled using two different pneumatic sowing machines.

Sampling method during sowing

Shortly before sowing the wind direction at the site was determined and ten Petri-dishes were placed in groups of two at distances of 1, 3 and 5 m from the downwind border of the field to give a total of 30 Petri-dishes per field. The actual placement of the Petri-dishes on the field edges followed the actual wind direction, in order to collect as much dust as possible. The actual situation per monitoring field, including the exact position of the sampling areas in relation to the rest of the field, the study plot dimensions (length & width of the sown area), any adaptations to the prevailing local conditions as well as the wind direction and wind speed during the sowing operation was documented in the raw data.

Each Petri-dish for sampling dust drift deposits (Ø 13.7 cm, 147.41 cm²) was filled with 70 to 80 ml of a 1:1 (v/v) glycerol/water mixture immediately before the start of the sowing. The Petri-dishes were arranged horizontally using metal racks approximately 1.5 to 2 cm above the soil or at the height of the ground vegetation surface, depending on the field boundary morphology. If necessary, the vegetation at the field border was removed to allow air to move freely across the open Petri-dishes. In order to allow any airborne dust to settle, the Petri-dishes remained open for 15 minutes following the cessation of sowing operations. The aqueous sampling medium of each Petri-dish was then individually transferred to a separate polyethylene flask. To ensure that all possible deposits of imidacloprid or respectively clothianidin from the inside of the Petri-dish were transferred to the corresponding polyethylene flask, each Petri-dish and its corresponding funnel was additionally rinsed with fresh tap water (≈ 20 mL) and the rinse was combined with the content of the respective Petri-dish within the corresponding polyethylene flask. After rinsing, each polyethylene flask was tightly closed. To avoid cross-contaminations the Petri-dishes were always approached from the downwind direction. Each polyethylene flask was labelled with the sampling date and an individual sample identification number consisting of the field number and the sampler number.

Sampling method after sowing

In order to monitor any potential dust drift during the 24h-period following sowing, a second set of ten Petri-dishes were placed in pairs at the approximate middle of each field side at a distance of 1 m to the field borders to give a total of 40 Petri-dishes per field. After 24 hours the sampling medium from each dish was individually transferred to a separate polyethylene flask following up the same workflow as described in the section above.

Residue analysis

Imidacloprid and clothianidin residues in the samples were subsequently determined by Bayer CropScience AG by High Performance Liquid Chromatography, coupled with Tandem Mass Spectrometry. Until shipment, the samples were stored at room temperature.

Results

A total number of 279 samples were collected from fields drilled with Manta® Plus or Smaragd® forte -treated seeds. One Petri-dish was inadvertently left closed. Of these 279 samples, 208 samples (74.5 %) were found to contain no quantifiable residues of imidacloprid or clothianidin, respectively (<LOQ); this included 194 samples (69.5 % of all 279 samples) with no detectable residues (<LOD). A total of 63 samples (22.6 %) were found to contain residues of imidacloprid or clothianidin above the limit of quantification (LOQ). 55 of these samples were taken at the time of sowing, the remaining 8 were taken 24h after drilling was completed. The maximum observed residue level was 0.283 g a.s./ha (see Table 9.5.2-14).

For mathematical processing, the data sets obtained with imidacloprid and clothianidin were combined and any residue value below the limit of detection (LOD = 0.004 g a.s./ha) was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification (LOQ = 0.014 g a.s./ha) was conservatively set to equal the LOQ. The calculated average residue values for samples collected during the sowing operation were 0.019 g a.s./ha for samples at a nominal distance of 1 m to the sowing border, 0.029 g a.s./ha for samples at a nominal distance from of 3 m and 0.020 g a.s./ha for samples at a nominal distance of 5 m. For the samples collected during a 24h-period after sowing, the average residue value was below the LOQ. The 90th percentile residue values during the sowing operation were 0.037 g a.s./ha, 0.031 g a.s./ha and 0.027 g a.s./ha for the nominal distance of 1 m, 3 m and 5 m, respectively. For the samples collected during a 24h-period after sowing, the 90th percentile residue value was below the LOD.

Table 9.5.2-14: Summary of residues (imidacloprid and clothianidin combined) at respective distances to the field borders

	During Sowing			24h-sampling	Total
Nominal distance (actual distance)°	**1m (1m)**	**3m (3m)**	**5m (4.5-5m)**	**1m (0.8-1m)**	
No. of samples analysed	40	40	40	159	279
No. of samples not recovered in the field	0	0	0	1	1
Residue level	**Number of samples with residue levels [n]**				
<LOQ	22	21	22	151	216
0.014-0.050 g a.s./ha	18	16	17	8	59
0.051-0.100 g a.s./ha	0	0	0	0	0
>0.100 g a.s./ha	0	3	1	0	4
Residue levels [g a.s./ha]					
Average**	0.019	0.029	0.020	<LOD	n.a.
90th percentile**	0.037	0.031	0.027	<LOD	
Maximum**	0.045	0.283	0.272	0.026	

LOD = 0.004 g a.s./ha (imidacloprid, clothianidin); LOQ = 0.014 g a.s./ha (imidacloprid, clothianidin); n.a. = not applicable

° In some cases the position of the Petri-dishes had to be adjusted from the intended distance due to the surrounding structures of the field.

** In one case due to an operator error the lid of one single Petri-dish was inadvertently not removed during the 24h-period after sowing; as such, no potentially dislodged residues could be trapped with this particular Petri-dish and consequently this sample was not considered for the mathematical processing.*

*** Calculated from the respective number of analysed samples, imidacloprid and clothianidin, combined; any residue value below the limit of detection was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification was conservatively set to equal the LOQ.*

Conclusion

The present study included 4 treatment groups, with two varieties of winter barley either treated with imidacloprid or clothianidin, sown at 4 different fields. Dust drift was monitored in Petri-dishes placed at several distances from the downwind border of the field during sowing until 15 minutes after sowing, and in Petri-dishes at 1m distance at each side of the field for 24h after sowing.

The 90th percentile calculated for the combined data set of all 4 fields was 0.037 g a.s./ha, 0.031 g a.s./ha, and 0.027 g a.s./ha for a distance of 1 m 3, and 5 m respectively. The 90th percentile for the 24 h samples was < LOD (<0.004 g a.s./ha). 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.

RMS's comments:

Currently no guideline is available. However the study-set up is considered reasonable. Overall the study is considered acceptable and suitable for use in risk assessment.

Drift to off crop areas takes place during and directly after sowing, which is confirmed by the residue analyses taken 24 hours after sowing, where rarely residues were found compared to frequent and clearly higher residues directly after sowing. This is in line with the state of knowledge, secondary drift is considered negligible compared to drift directly after sowing. The very high maximum values at 3 and 5 m should not be over-interpreted, as it is likely these are due to single emitted larger particles and result in the fact that considering mean values only no clear relation between distance of the residues in petri-dishes and the sowing area were found.

Thus the residues being on a similar level from the first meter up to 5 meters distance may be due to the nature of abradable dusts in cereals but especially to high maximum values in single petri dishes and has no impact on the acceptability of the study. As residue analyses were conducted only up to 5 m distance, it is not possible to determine at which distance no deposition of active substance takes place. It is possible but can neither be confirmed nor refused that there may be slight differences in the effects on bees from different shapes of dust particles, however this cannot be further clarified here, but may be an explanation for the observed drift patterns and the residue measurements in petri dishes.

However, no data on the seed treatment quality, the abradable dusts (Heubach g/ha) and the residue content in these dusts (Heubach g as/ha) have been provided for the treated barley seeds used in the trial. Thus it is unclear if and to which extent these data obtained in these studies are suitable to cover a 'worst case' for use in risk assessment. Data from JKI (recently submitted to EFSA as confidential data) with relatively good seed treatment quality (Heubach 0,086-0,125 g as/ha) resulted in detectable mean values for petridishes in 1-5 m distance (0,024-0,045 g Imidacloprid/ha; see Pistorius & Heimbach, 2015, Heimbach et al., 2015g), while in the study with lower Heubach-values and lower residues no effects on bees were detected (Pistorius et al, 2015g). The final risk assessment needs to keep in mind that the seed treatment quality is of major importance to reflect the potential risk in realistic conditions. The results are considered very useful supportive information, but even if no risk is clearly indicated in the submitted study, considering shortcomings of the representativeness of the seed batch used in the trials a risk cannot be excluded for the exposure route dust drift during sowing of winter wheat and barley.

Report:	Hofmann, S.; Lueckmann, J.; 2010b
Title:	Monitoring of dust drift deposits during and after sowing of winter wheat (W-WHT) treated with Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 145.2 (60 + 70 + 7.2 + 8 g/L) or Clothianidin & Beta-Cyfluthrin FS 455 (375 + 80 g/L) on fields in Germany
Report No.:	R09247-2
Document No.:	M-366277-01-1
Guideline(s):	91/414/EEC of July 15, 1991, SANCO/3029/99 Rev. 4, 2000-07-11
Guideline deviation(s):	not specified
GLP/GEP:	**no**

Objective

The objective of the study was to determine the residues of imidacloprid and clothianidin in dust drift deposits during and after sowing of winter wheat treated with Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 145.2 (60 + 70 + 7.2 + 8 g/L) or Clothianidin & Beta-Cyfluthrin FS 455 (375 + 80 g/L) on fields in Germany.

Material and Methods

Test item

Two different winter wheat (W-WHT) varieties (i.e. Hermann and Manager) were purchased untreated and commercially cleaned-up from a commercial seed distributor (Gut Peterhof, D-50127 Bergheim, Germany) and were thereafter seed-treated at Bayer CropScience's Seed Treatment Application Centre in D-40789 Monheim am Rhein, Germany (non-GLP):

- Manta® Plus FS 145.2 (TOX08744-00) treated winter wheat seeds, dressed with 1000mL product/100 kg seeds (= nominally 70 g imidacloprid/100 kg seeds); identification of treated seeds: TOX08781-00 (variety Manager); TOX08782-00 (variety Hermann)

And

- Smaragd® forte FS 455 (TOX08741-00) treated winter wheat seeds, dressed with 133mL product/100 kg seeds (= nominally 50 g clothianidin/100 kg seeds); identification of treated seeds: TOX08776-00 (variety Manager); TOX08777-00 (variety Hermann)

After seed-dressing, the seeds were subject to chemical analysis for the determination of the actual seed loading. Finally, the seed bags were unequivocally labelled and shipped via road transport to the respective study sites in Germany.

Study sites and sowing

The multiple site study was conducted at two different regions in Germany: one in Southern Germany in the federal state of Baden-Württemberg in Renningen, southwest of Stuttgart at the experimental station Ihinger Hof of the University Hohenheim (in the following called Ihinger Hof) and the second in Northern Germany in the federal state of Lower Saxony near Celle northeast of Hannover (in the following called Celle) with two fields per location. The sizes of the test fields sown with Manta® Plus-treated W-WHT seeds at Ihinger Hof and Celle were 6.0 ha and 16.21 ha, respectively. The fields drilled with Smaragd® forte treated W-WHT seeds at Ihinger Hof and Celle were 4.0 ha and 9.84 ha, respectively. The variety of W-WHT sown at both study sites was ‘Manager’. More detailed information about the study sites are given in the study report.

A total of 200 kg seeds/ha were sown at both test locations resulting in nominal application rates of 140 g imidacloprid a.s./ha on fields drilled with Manta® Plus and 100 g clothianidin a.s./ha on fields drilled with Smaragd® forte. The seeds were drilled using two different pneumatic sowing machines.

Sampling method during sowing

Shortly before sowing the wind direction at the site was determined and ten Petri-dishes were placed in groups of two at distances of 1, 3 and 5 m from the downwind border of the field to give a total of 30 Petri-dishes per field. The actual placement of the Petri-dishes on the field edges followed the actual wind direction, in order to collect as much dust as possible. The actual situation per monitoring field, including the exact position of the sampling areas in relation to the rest of the field, the study plot dimensions (length & width of the sown area), any adaptations to the prevailing local conditions as well as the wind direction and wind speed during the sowing operation was documented in the raw data.

Each Petri-dish for sampling dust drift deposits (Ø 13.7 cm, 147.41 cm²) was filled with 70 to 80 ml of a 1:1 (v/v) glycerol/water mixture immediately before the start of the sowing. The Petri-dishes were arranged horizontally using metal racks approximately 1.5 to 2 cm above the soil or at the height of the ground vegetation surface, depending on the field boundary morphology. If necessary, the vegetation at the field border was removed to allow air to move freely across the open Petri-dishes. In order to allow any airborne dust to settle, the Petri-dishes remained open for 15 minutes following the cessation of sowing operations. The aqueous sampling medium of each Petri-dish was then individually transferred to a separate polyethylene flask. To ensure that all possible deposits of imidacloprid or respectively

clothianidin from the inside of the Petri-dish were transferred to the corresponding polyethylene flask, each Petri-dish and its corresponding funnel was additionally rinsed with fresh tap water (≈ 20 mL) and the rinse was combined with the content of the respective Petri-dish within the corresponding polyethylene flask. After rinsing, each polyethylene flask was tightly closed. To avoid cross-contaminations the Petri-dishes were always approached from the downwind direction. Each polyethylene flask was labelled with the sampling date and an individual sample identification number consisting of the field number and the sampler number.

Sampling method after sowing

In order to monitor any potential dust drift during the 24h-period following sowing, a second set of ten Petri-dishes were placed in pairs at the approximate middle of each field side at a distance of 1 m to the field borders to give a total of 40 Petri-dishes per field (where necessary the distance of 1 m had to be adjusted to the field boundary morphology). After 24 hours the sampling medium from each dish was individually transferred to a separate polyethylene flask following up the same workflow as described in the section above.

Residue analysis

Imidacloprid and clothianidin residues in the samples were subsequently determined by Bayer CropScience AG by High Performance Liquid Chromatography, coupled with Tandem Mass Spectrometry. Until shipment, the samples were stored at room temperature.

Results

A total number of 280 samples were collected from fields drilled with Manta® Plus or Smaragd® forte -treated seeds. Of these 280 samples, 272 samples (97.1 %) were found to contain no quantifiable residues of imidacloprid or clothianidin, respectively (< LOQ); this included 228 samples (81.4% of all 280 samples) with no detectable residues (<LOD). A total of 8 samples (2.8 %) were found to contain residues of imidacloprid or clothianidin above the limit of quantification (LOQ). 5 of these samples were taken at the time of sowing, the remaining 3 were taken 24h after drilling was completed. The maximum observed residue level was 0.258 g a.s./ha.

For mathematical processing, the data sets obtained with imidacloprid and clothianidin were combined and any residue value below the limit of detection (LOD = 0.004 g a.s./ha) was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification (LOQ = 0.014 g a.s./ha) was conservatively set to equal the LOQ. Both, the calculated average and 90th percentile residue values for all samples collected during the sowing operation at the nominal distances of 1 m, 3 m and 5 m were below LOQ. For the samples collected during a 24h-period after sowing, the average residue value was < LOQ and the 90th percentile residue value was < LOD.

Table 9.5.2-15: Summary of residues (imidacloprid and clothianidin combined) at respective distances to the field borders

	During Sowing			24h-sampling	Total
Nominal distance (actual distance)°	**1m (1-2m)**	**3m (3-4m)**	**5m (5-6m)**	**1m (-1, 0 or 1m)**	
No. of samples analysed	40	40	40	160	280
No. of samples not recovered in the field	0	0	0	0	0
Residue level	**Number of samples with residue levels [n]**				
<LOQ	39	37	39	157	272
0.014-0.050 g a.s./ha	1	3	0	3	7
0.051-0.100 g a.s./ha	0	0	0	0	0
>0.100 g a.s./ha	0	0	1	0	1
Residue levels [g a.s./ha]					
Average**	<LOQ	<LOQ	<LOQ	<LOQ	n.a.
90th percentile**	<LOQ	<LOQ	<LOQ	<LOQ	
Maximum**	0.034	0.030	0.258	0.027	

LOD = 0.004 g a.s./ha (imidacloprid, clothianidin); LOQ = 0.014 g a.s./ha (imidacloprid, clothianidin); n.a. = not applicable

° In some cases the position of the Petri-dishes had to be adjusted from the intended distance due to the surrounding structures of the field.

*** Calculated from the respective number of analysed samples, imidacloprid and clothianidin, combined; any residue value below the limit of detection was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification was conservatively set to equal the LOQ.*

Conclusions

The present study followed the same design as study of Hofmann & Leuckmann (2010a) but winter wheat was treated and sown instead of winter barley. There were 4 treatment groups, with two varieties of winter wheat either treated with imidacloprid or clothianidin, sown at 4 different fields. Dust drift was monitored in Petri-dishes placed at several distances from the downwind border of the field during sowing until 15 minutes after sowing, and in Petri-dishes at 1m distance at each side of the field for 24h after sowing.

The 90th percentile calculated for the combined data set of all 4 fields was < LOQ (<0.014 g a.s./ha) for all 3 distances (1 m, 3 m, and 5 m). The 90th percentile for the 24 h samples was < LOD (<0.004 g a.s./ha). 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.

RMS's comments:

Currently no guideline is available, however, the study-set up is considered reasonable. Thus the study is considered acceptable and suitable for use in risk assessment.

In this study a high number of residue measurements was conducted, and demonstrates the residues off-crop may be low if good seed treatment quality is sown. Only in a very limited number of petri-dishes residues were detected, which indicates that occasionally particles are emitted, which should be considered in the evaluation, and indicates that bees may only encounter residues in a limited number of spots and do not encounter such contaminations frequently. However, a clear deficit here is that no data on the seed treatment quality, the abradable dusts and the residue content in these dusts (Heubach g as/ha) were provided for the

treated winter wheat seeds used in the trial. Thus it is unclear if and to which extent these data obtained in these studies are suitable to cover a 'worst case' for use in risk assessment.

Report:	Lueckmann, J.; 2014a
Title:	Investigation of dust drift deposits of clothianidin & imidacloprid treated winter barley seeds with pneumatic sowing machinery on fields in Germany in autumn 2011(with first and second amendment to final report)
Report No.:	R11129
Document No.:	M-502885-03-1
Guideline(s):	BBA Drift Guideline Part VII, 2-1.1
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective
This study investigates the aerial and ground dust drift deposits of clothianidin & imidacloprid after sowing of treated winter barley seeds with pneumatic sowing machinery on three study fields in Germany in autumn 2011.

Materials and method
Test item
Winter barley seeds dressed with Clothianidin + Imidacloprid FS 100 + 175 G at a nominal seed-treatment rate of 200 mL product/100 kg seeds (which corresponds to nominally 20 g clothianidin and 35 g imidacloprid/100 kg seeds).

Study sites and sowing
The study was conducted on three study fields in the district of Giessen (Hessen) 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 194.9 to 211.6 kg/ha). Each pneumatic sowing machine was filled on the farm site. Sowing of the dressed seeds was exclusively performed by typical commercial pneumatic sowing machinery, provided by the respective cooperating farmer.

Sampling method
Shortly before sowing the wind direction was determined and two different sampling devices to measure aerial and ground dust drift deposits were set up at the downwind border on each study field or its boundary (depending on the actual field boundary morphology): Petri-dishes, horizontally arranged at a height of approximately 2 cm above the soil surface (to measure ground deposition) and vertically erected gauze-netting-samplers (effective sampling area: 2 m x 3.3 m, to measure aerial deposition). 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 $\leq \pm 30°$. The border of the downwind study field side was described as "zero line".

Samples of dressed seeds were taken at the time of bagging and from the used seed bags shortly before filling of the drilling machine for Heubach analysis by the Seed Growth Center of Bayer CropScience AG (non-GLP).

Two lines of 3 x 10 Petri-dishes were set-up in pairs of two along a line of 5 m at a distance of 3 and 1 m to the zero line. The space between each row of ten Petri-dishes was approximately 9.3 m. Additionally one line of three gauze-netting-samplers were set-up in a distance of 3 m to the zero line. Sampling devices were arranged in an alternating order around the centre 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 µg, 0.1 µg, 10 µg clothianidin + imidacloprid/fortified Petri-dish sample) were established just before the start of sowing in order to investigate the stability of the samples during transport and storage.

Thirty minutes after sowing of the respective study field, the aqueous solutions of the Petri-dishes
and the gauze samples (five 50 x 50 cm squares were cut-out of each individual netting) were gathered and immediately transferred into separate polyethylene flasks.

Weather conditions during sowing and sampling
Weather was always dry during and after sowing.

For drilling at study field 1 the target wind direction was 265°. The measured mean wind direction was 280° (± 19°). The mean wind speed was 3.3 m/s (± 0.9 m/s). For study field 2 the target wind direction was 120°. The measured mean wind direction was 129° (± 33°). The mean wind speed was 2.4 m/s (± 0.9 m/s). The target wind direction for study field 3 was 140°. The measured mean wind direction was 128° (± 14°). The mean wind speed was 3.8 m/s (± 0.9 m/s).

Residue analysis
Residues of clothianidin and imidacloprid in all Petri-dishes and gauze netting samples as well as all field fortification samples, filters used in the Heubach abrasion tests obtained from the seed samples taken shortly before drilling and in soil samples were analysed by laboratory of the Analytical Test Site (BCS-D-HS-RA, Bayer CropScience AG) (Schöning R., Report # MR-12/006). Chromatography and detection by MS/MS in Heubach filters, gauze nettings and Petri-dish solutions was done according to method MR-338/00 (clothianidin) and MR-06/144 (imidacloprid). Analysis in soil samples was done according to method MR-106/02 (clothianidin) and MR-106/03 (imidacloprid).

The Limits of Quantitation (LOQ) for clothianidin and imidacloprid for the gauze samples were 0.04 g a.s./ha, respectively. The corresponding Limits of Detection (LOD) were 0.01 g a.s./ha. For the Petri-dish samples the LOQs for clothianidin and imidacloprid were 0.07 g a.s./ha, respectively, the corresponding LODs were 0.02 g a.s./ha. For the soil samples the LOQs were 5 µg a.s./kg soil for
clothianidin and imidacloprid, respectively, the corresponding LODs were 2 µg a.s./kg soil.

Results
The Heubach value determined shortly after the seed treatment process was 0.045 g/100 kg. Additional Heubach values were determined after sowing from samples taken shortly before sowing. These measurements resulted in Heubach values of 0.097 g/100 kg, 0.022 g/100 kg and

0.144 g/100 kg for study field 1, study field 2, and study field 3, respectively (Heubach dust in g). The filter from the Heubach-tests that were conducted after sowing were analysed for their content of clothianidin and imidacloprid residues. For clothianidin the mean residue content of the filters were 0.97 mg/100 kg seeds, 0.72 mg/100 kg seeds, and 0.74 mg/100 kg seeds for study field 1, study field 2, and study field 3, respectively. For imidacloprid the mean residue content of the filters were 1.05 mg/100 kg seeds, 0.80 mg/100 kg seeds, and 0.82 mg/100 kg seeds for study field 1, study field 2, and study field 3, respectively (Heubach g a.s./ha).

In 44 of the 60 Petri-dish samples from study field 1 the residue level of clothianidin was below the LOD and in 8 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 of the 60 Petri-dish samples from study field 1 the residue level of imidacloprid was below the LOD and in 8 samples below the LOQ. Eleven samples had residue values above the LOQ (range 0.08 – 2.4 g a.s./ha) In all Petri-dish samples from study field 2 and study field 3 the residue level of clothianidin and imidacloprid was below the LOD. None of the 45 gauze samples from study field 1, 2 and 3 had residue levels above the LOQ (0.04 g a.s./ha) of clothianidin or imidacloprid.

For calculations, residue values below or equal to the LOD were set conservatively to the LOD (0.02 g a.s./ha in Petri-dish samples and 0.01 g a.s./ha in gauze netting samples). Residue values below the LOQ were conservatively set to the LOQ (0.07 g a.s./ha in Petri-dish samples and 0.04 g a.s./ha in gauze netting samples). If all residue values of one sample type of one study field were <LOD or < LOQ the mean value and the 90^{th} percentile are reported as <LOD or <LOQ, respectively.

The average residue level of clothianidin found in the Petri-dishes placed at a distance of 1 m to the zero line was 0.10 g a.s./ha at study field 1 and <LOD at study field 2 and 3. At a distance of 3 m o the zero line the average residue level of clothianidin in the Petri-dishes was 0.05 g a.s./ha at study field 1 and <LOD at study field 2 and 3. For imidacloprid the average residue level in the Petri-dishes from study field 1 at 1 m distance to the zero line was 0.14 g a.s./ha and <LOD at study field 2 and 3. At a distance of 3 m to the zero line the average residue level of imidacloprid in the Petri-dishes was 0.07 g a.s./ha at study field 1 and <LOD at study field 2 and 3. The mean residue level of clothianidin and imidacloprid in the gauze netting was 0.040 g a.s./ha for all three study fields, as values >LOD and ≤LOQ were set to LOQ for calculation.

The results of the residue analysis of all samples are summarised in the table 9.5.2-15 below.

Table 9.5.2-16: Summary of clothianidin and imidacloprid residues in Petri-dishes and gauze nettings

	Residue levels of clothianidin [g a.s./ha]								
	Study field 1			Study field 2			Study field 3		
	Petri-dish		Gauze netting	Petri-dish		Gauze netting	Petri-dish		Gauze netting
	1 m	3 m		1 m	3 m		1 m	3 m	
Mean *	0.10	0.05	0.02	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
90th percentile *	0.12	0.07	0.04	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
Max *	1.66	0.50	<LOQ (0.04)	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
Min *	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
	Residue levels of imidacloprid [g a.s./ha]								
	Study field 1			**Study field 2**			**Study field 3**		
	Petri-dish		**Gauze netting**	**Petri-dish**		**Gauze netting**	**Petri-dish**		**Gauze netting**
	1 m	**3 m**		**1 m**	**3 m**		**1 m**	**3 m**	
Mean *	0.14	0.07	0.03	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
90th percentile *	0.20	0.11	0.04	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
Max *	2.41	0.75	<LOQ (0.04)	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
Min *	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ

LOD Petri-dish = 0.02 g a.s./ha; LOQ Petri-dish = 0.07 g a.s./ha;
LOD gauze netting = 0.01 g a.s./ha; LOQ gauze netting = 0.04 g a.s./ha;
** calculated from the number of analysed samples per study field with rounded values: 30 Petri-dishes per distance, 15 gauze netting samples; residue values below the LOD were conservatively set to equal the LOD, residue values above the LOD and below or equal to the LOQ were conservatively set equal to the LOQ*

Conclusion
The highest residues in Petri-dish samples were found for field one, with a 90th percentile residue level of 0.12 a.s./ha for clothianidin. In field 2 and field 3 the 90th percentile residue level in the Petri-dish samples were <LOD (<0.02 g a.s./ha). The 90th percentile residue level in gauze samples from all three fields were <LOQ (<0.04 g a.s./ha).

RMS's comments:
Currently no guideline is available, however, the study-set up is considered reasonable. Thus the study is considered acceptable and suitable for use in risk assessment.
In this study, data on the seed treatment quality, the abradable dusts and the amount of residues on Heubach filters (which is not a Heubach g as value) were provided for the treated winter barley seeds used in the trial. The study was replicated on three fields. While the study was well performed, the results also raise a number of questions and uncertainties. The LOD und LOQ

values are quite high and not sufficiently sensitive to detect residues in Petri dishes and gauze netting at rates, at which still effects on bees have to be expected.
Considering the results obtained on study field 1, a risk for bees cannot be excluded. On the other hand, the results from fields 2 and 3 indicate that no residues were detectable in off crop areas, but the LOD and LOQ values are not sufficient sensitive to make a conclusion.
In this study it is surprising that residues in petri-dishes were consistently lower than in Gauze. This is contradictory to available state of knowledge, were usually higher residues in 3-D / Gauze than in 2-D-Structures (Petri dishes). But again with lower LOD and LOQ values other results might have been achieved, because higher LOD values in Petri dishes will lead to higher residue value in the way the calculation is done. However, for interpretation of biological effects of seeds treated with both Imidacloprid and Clothianidin, the sum of the 2 actives needs to be calculated. The differences between fields again may be marginal only if sufficient sensitive residue analysis had been used. This study highlights that the issue of dust drift can only be solved in a combination of risk assessment and risk management.
However, in a final conclusion a risk for bees in practical conditions cannot be excluded.

Report:	Lueckmann, J.; Staffel, J.; 2014a
Title:	Assessment of potential impacts on honey bee colony development, their hibernation performance and concurrent monitoring of aerial dust drift during the sowing operation of imidacloprid FS 350A G - Treated winter barley with typical commercial vacuum-pneumatic sowing technology, directly adjacent to full-flowering Phacelia tanacetifolia in United Kingdom
Report No.:	R1440009
Document No.:	M-504522-02-1
Guideline(s):	none
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective

The study aims to determine residues of imidacloprid in dust drift deposits released during the pneumatic sowing operation of dressed winter barley seeds to vertically installed strips of glycerol-wetted gauze nettings. In addition, potential acute and long-term impacts of these residues on the colony development and hibernation performance of the honey bees placed at the treatment fields in comparison to those of the control fields had to be assessed.

Material and Methods

Test item
Imidacloprid FS 350A G treated winter barley seeds, Batch-ID (internal): 2014-002066 (TOX-No. 10231-00), contents nominal 70.0 g imidacloprid a.s./100 kg seeds

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 honey bees to the potential arising dust drift deposits, the winter barley sowing area was surrounded by flowering Phacelia tanacetifolia, a highly bee attractive crop. The dimension of the winter

barley-sown area inside the Phacelia tanacetifolia fields on each study field was approximately 2.0 ha (effective 1.77 to 2.11 ha). The target sowing rate was 200 kg/ha for the control and 206.4 kg/ha on the treatment fields (due to the analysed degree of insecticide loading of 96.9 %, effective 219.13 to 221.06 kg/ha) which corresponded to nominally 140 g imidacloprid/ha (effective 148.64 and 149.95 g imidacloprid/ha). In order to keep 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 honey bee hives

In total 32 honey bee colonies were monitored, eight on each study field. The honey bee colonies were placed in the assessment plots on 12 June 2014, with a distance of approximately 3 m between the edge of the winter barley sowing area and the hive entrance. When a queen died or showed significant reduced egg laying capacity, it was replaced by another sister queen. The entrance of each hive was straightened in the direction to the *Phacelia* to correspond to the apicultural practice. After the exposure period the honey bees were relocated to a monitoring site on 10 July 2014 in the region of York without intensive agricultural activities in the near vicinity.

Honey bee mortality and behaviour

The mortality of honey bees (e.g. workers, pupae, drones) was recorded at the study fields using dead bee traps. If there were ten or more dead bees in one colony after sowing, they were sampled for potential further residue analysis. Behavioural abnormalities of the honey bees at the entrance hole were recorded during the mortality assessments.

Population development and health assessment

Population strength and development (number of cells filled with eggs, larvae or capped brood) as well as food stores (i.e. pollen and nectar) were assessed every three weeks. At each assessment the percentage coverage of bees, sealed brood, open brood, eggs and food stores (pollen and nectar) on each side of each frame was recorded. This was judged by eye by an experienced assessor who carried out all of the colony assessments. The percentage coverage was given to the closest 5%. For analysis, these percentages were converted to total numbers per hive equivalents per hive. The quotient between honey bee numbers after and before hibernation was calculated as a value for the hibernation success of honey bee colonies. During the Field Phase and the Bee Health Phase, bee colonies were kept according to Good Apicultural Practice and all typical apicultural measures were respected.

Dust drift sampling

Three days before the start of the sowing activities seed samples for Heubach analysis (non-GLP) and seed loading (non-GLP) were taken from five seed bags. To measure aerial and ground dust drift deposits vertically erected gauze-netting-samplers were set up on each assessment plot at the treatment fields. The sowing was only performed when the wind speed at the beginning of the sowing was below 5 m/s. A total of eight units of gauze-netting-samplers (each with an effective sampling area of approximately 2 m x 3.3 m) were set up at a distance of approximately 3 m from the zero line. Shortly before the beginning of the sowing the gauze-netting-samplers were wetted with a 1:1 (v/v) glycerol/water mixture. Soil samples for water content and soil characterization were taken shortly before sowing. Additionally, field fortification samples (0 µg, 1 µg, 100 µg imidacloprid and clothianidin - fortified gauze sample) were established just before the start of sowing in order to investigate the stability of the samples during transport and storage. 30 minutes after the completion of sowing, the gauze samples

(five 50 cm x 50 cm squares cut out of each individual netting unit) were gathered and immediately transferred into separate polyethylene wide mouth bottles.

Residue analysis
Imidacloprid residues in the gauze samples were determined by the Analytical Test Site Bayer CropScience AG.

Results
Honey bee mortality
In the control and treatment groups, adult honey bee mortality was on the same, generally low level, mostly alternating around five dead bees per day in mean. After sowing statistically significant differences between control and treatment worker bee mortality were observed only on two single days. As the control showed also 2 times during this period an increase of the mortality and the mortality was in both groups on average on a low level (< 10 worker bees/colony) for colonies with on average approximately 11,000 to 20,000 worker bees, it can be concluded that there were no test item related effects regarding to the mortality. The mortality of the worker bee brood, i.e. pupae or larvae was also on a very low level in almost all colonies. Here on most days, in both groups a mean of ≤ one dead larva or pupa per colony was found in the dead bee traps. Therefore it can be assumed, that there was no test item related effect, also regarding to the worker bee brood mortality.

Honey bee colony development
At the pre-sowing assessment, the number of worker bees was very similar in the control and treatment group. At both groups the colony strength increased in a similar way towards the first colony assessment after sowing, which resulted in still very similar numbers of adult worker bees. Also during the following assessments in 2014 and at the assessment after hibernation in April 2015, no significant differences could be detected. Due to the good food supply at the study fields, the amount of brood increased in the period from the pre-sowing assessment towards the first assessment after sowing and remained at this level until the second assessment. From the second assessment on; the colony strength decreased as bees started preparing for hibernation. During the whole Bee Health Phase, the total amount of worker brood was approximately on the same level in both groups.
No statistically significant differences were detected between the control group and the treatment group; neither for the number of worker bees nor for the total brood amount. Also the hibernation index indicates that there is no effect of the test item, as the colonies from the test item group hibernated even slightly better than those of the control group (hibernation index of 0.516 in test item group and 0.443 in control group). Altogether, it can be concluded that the test item did not affect the honey bee colonies in any manner.
During the Field Phase and the Bee Health Phase, the queens of three colonies were replaced by another sister queen according to Good Apicultural Practice due to different reasons. As the replacements had to be done also in the control colonies, there is no hint for a test item related effect on the health of the queens.

Varroa destructor infestation
Natural daily mite fall was recorded during all colony assessments. Though it was on a generally very low level, the *Varroa* infestation was slightly higher amongst the treatment colonies, at the second assessment even statistically significant. As the values were alternating around only approximately one dead mite per day in mean, it did not influence the honey bee colonies in any manner.

Residues

No residues were found in the control gauze samples. In the field spike samples, the mean recovery at study field T1 was 102 % ± 3.2 % and at study field T2 101 % ± 2.5 %. The Limit of Quantification (LOQ) referring to the determination of imidacloprid from gauze netting samples was 1 µg imidacloprid/L gauze extract, equivalent to 0.04 g a.s./ha. The corresponding Limit of Detection (LOD) was 0.1 µg imidacloprid/L gauze extract, equivalent to 0.004 g a.s./ha.

Due to changing wind conditions and low wind speed, the association of the assessment plots at study field T1 to upwind and downwind was not as clear as on study field T2. This was demonstrated by relatively low residue levels also on the downwind assessment plots (up to 0.086 g a.s./ha). Upwind assessment plot residue levels were below the LOQ beside of the samples from assessment plot A7, were two of five samples were below the LOQ and the other three approximately on the level of the LOQ.

On study field T2, a clear wind-depending distribution of residues could be shown as the wind conditions were very stable. Downwind assessment plots residues were distinctly higher (0.18 - 0.32 g a.s./ha) compared to those determined on the upwind assessment plots, which were below the LOQ (<0.04 g a.s./ha) beside of assessment plot A3, were three of five samples were below the LOQ and the two other approximately on the level of the LOQ.

Conclusion

To assess the potential effects of Imidacloprid FS 350A G on the colony development of honey bees (*Apis mellifera* L.), Imidacloprid FS 350A G – treated winter barley seeds (nominal treatment rate 70.0 g imidacloprid/100 kg seeds) were sown during bee flight under field conditions in summer 2014. To increase the possible exposition of the bees, the winter barley was sown inside two fields of flowering *Phacelia tanacetifolia*, a highly bee attractive crop.

The dust drift measurements made during the sowing operation of imidacloprid-treated winter barley seeds on the treatment fields (nominal treatment rate 70.0 g imidacloprid/100 kg seeds) indicate that seed-treatment dust, abraded and released during the sowing operation with typical, commercial available pneumatic sowing equipment, resulted in a measurable off-field exposure, which was distinctly higher at the downwind borders of the winter barley sowing areas as compared to the corresponding upwind borders. The maximum vertical dust deposition, as measured by vertically erected gauze-netting units, directly adjacent to the winter barley sowing areas, corresponded to a maximum drift rate of 0.32 g a.s./ha. The application of Imidacloprid FS 350A G did not cause any effects on the survival of adult bees and bee pupae, foraging activity, behaviour, also not on colony development, hibernation performance and colony strength as well as on the bee brood.

Thus this study demonstrated that Imidacloprid FS 350A G – treated winter barley seeds (nominal treatment rate 70.0 g imidacloprid/100 kg seeds), sown during bee flight, did not adversely affect honey bee colonies.

RMS's comments:

Currently no guideline is available, however, the study-set up is considered reasonable. Thus the study is considered acceptable and suitable for use in risk assessment.

While in this study no petridishes were used and residues only measured in 3 D gauze samplers, it is noted that in the study of Lückmann (2014) lower residues were detected in gauze nets than in petri dishes. In the present study, residues were detected at levels which caused severe effects in JKI trials in worst-case set-up in semi-field conditions. No data on flight activity on field is available and although from climatic conditions it may be assumed bees were actively foraging

on the treated field, it remains unclear which amount of foragers were exposed during sowing. An exposure verification is only available for the day after sowing, from given meteorological conditions and observed portion nectar/pollen foragers higher flight activity on treated fields may be assumed but clearly an uncertainty needs to be concluded. On the other hand, pictures in the report seem to demonstrate that bees may have been well exposed to the dust cloud during sowing, and also no treatment related mortality, colony and brood development were observed. For field studies usually larger field sizes seem recommendable; however in this specific case of dust drift large field sizes of the adjacent flowering crop do not necessarily result in a worst case for dust scenario as larger fields dilute the potential risks. In total, this makes it difficult to conclude if the study reflects a best, realistic or worst case scenario. In addition no value for the dust quality of the seeds has been reported. In this trial no data on the dustiness of the seeds (Heubach-value) nor the content of a.s. in dusts were provided, therefore from the study no information for comparability with other studies is obtained. The lack of comparability is considered as a drawback and should in principle be reported in all future studies investigating dust deposition and potential side effects of dust drift.

Report:	Staffel, J.; Lueckmann, J.; 2014b
Title:	Final report - Assessment of potential impacts on honey bee colony development, their hibernation performance and concurrent monitoring of aerial dust drift during the sowing operation of Poncho Beta Plus - Treated sugar beet pills with typical commercial vacuum-pneumatic sowing technology, directly adjacent to full-flowering *Phacelia tanacetifolia* in Germany
Report No.:	R12261A
Document No.:	M-504065-01-1
Guideline(s):	Regulation (EC) 1107/2009 BBA Drift Guideline Part VII, 2-1.1 (1992) SANCO/825/00/rev. 8.1
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective

This study aimed to assess potential effects on honey bee colonies during and after vacuum-pneumatic sowing operation of coated sugar beet pills, sown directly adjacent to full-flowering *Phacelia tanacetifolia*. The employed sugar beet pills were commercially treated with Poncho Beta Plus (nominal rate: 0.60 mg clothianidin/pill, 0.08 mg beta-cyfluthrin/pill and 0.30 mg imidacloprid/pill). Moreover, dust drift deposits during the sowing operation of the treated sugar beet pills were concurrently monitored.

Material and Methods

Test and control item

The test item consisted of commercially prepared sugar beet pills, treated with Poncho Beta Plus, at a nominal rate of 0.60 mg clothianidin a.s./pill, 0.08 mg beta-cyfluthrin a.s./pill and 0.30 mg imidacloprid a.s./pill.

The sugar beet pills were seed-coated and bagged at KWS SAAT AG (D-37555 Einbeck, Germany) (non-GLP), by employing typical seed-treatment and bagging practises. The pills received a conventional seed treatment and were dressed in addition to Poncho Beta Plus also with the two standard fungicides Thiram 65 ZR and Hymexazol WP 70. The coated pills were

bagged into 1 Unit (=100,000 pills) cardboxes, and were labelled with a unique label and the TOX-Number.

Maize seeds, dressed with only one standard fungicidal seed-treatment (Thiram SC 700, active substance: thiram), have been used for the control group. The control fields alse served as control fields in another study ((with an active substance other than Imidacloprid, thus not reported in detail here), where maize was used as the crop of interest. Thus, in the control of the current study maize was sown. Control maize seed were dressed and bagged by the Seed Treatment Application Centre of Bayer CropScience AG in D-40789 Monheim am Rhein, Germany (non-GLP).

Study sites and sowing

The study was conducted in the vicinity of Nauen, Eastern Germany, on three study fields, two control and one treatment field. Originally, it was planned to use a second field for sowing of the test item. However, due to adverse soil conditions, the *Phacelia* plants on this study field was grown poor and patchy and did not meet the requirement of uniformly full flowering *Phacelia*, so that it could not be used.

Maize seeds were sown on the control fields and sugar beet pills were sown on the treatment field. To expose the honey bees 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 (see Figure 9.5.2-36). The dimension of the sugar beet and the control maize-drilled areas inside the *Phacelia tanacetifolia* fields on each study field were approximately 2.6 ha. The target sowing rate was 130,000 sugar beet pills and 100,000 maize seeds/ha (actual 137,708 sugar beet pills/ha and 103,189 to 101,368 maize seeds/ha). This corresponded to nominally 78.0 g clothianidin a.s./ha, 10.4 g beta-cyfluthrin a.s./ha and 39.0 g imidacloprid a.s./ha. In order to keep driving distances with filled sowing machines constant, the vacuum pneumatic sowing machines were filled on previously designated filling points at an approximate distance of 1 km from the study fields. For the sowing, a vacuum-pneumatic sowing machine (with deflector technology for the control fields and dismounted deflector technology for the treatment field, manufacturer: Amazone) were used.

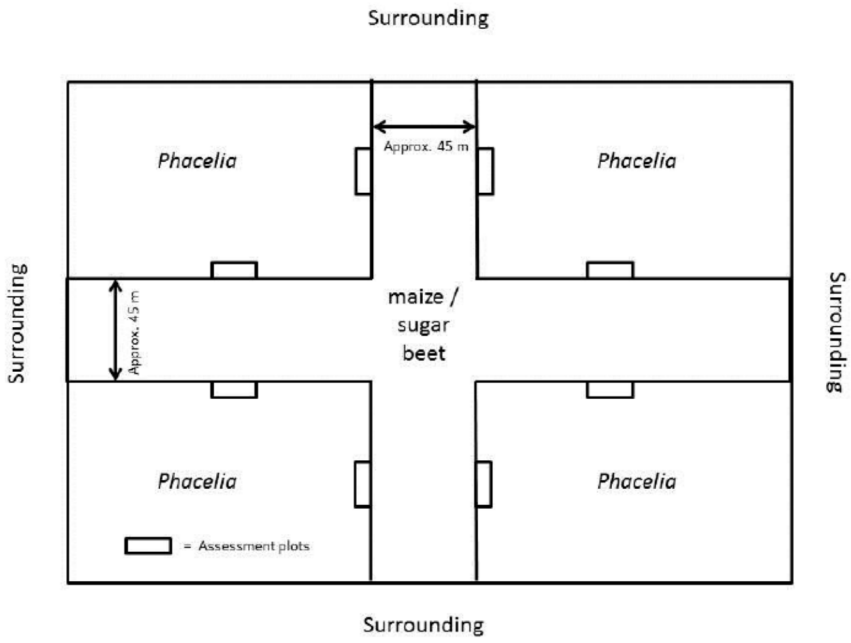


Figure 9.5.2-36: Schematic design of the study fields

After the exposure the honey bees were relocated to three monitoring sites in a region of North-Rhine-Westphalia near Gummersbach, with no intensive agricultural activities in the near vicinity. The honey bee hives were set up on these three different locations to avoid potential impacts due to a high density of honey bee hives, like a lack of food due to food concurrence or *Varroa destructor* infestation. To avoid local factors influencing the results of this study, honey bee hives from each study field were relocated randomly to the monitoring sites (one third of the hives of each study field to each monitoring site).

Set-up of honey bee hives

In total 48 honey bee colonies were monitored in the study, 16 on each study field. The honey bee colonies were placed in the assessment plots on 27.06.2013 with a distance of approximately 3 m between the edge of the maize or sugar beet sowing area and the hive entrance. When a queen died or showed significant reduced egg laying capacity, it was replaced by another sister queen. The entrance of each hive was straightened in the direction to the *Phacelia* to correspond to the apicultural practise. They were relocated to the monitoring sites in the night of 23.07.2013 to 24.07.2013 (after the end of *Phacelia flowering*).

Honey bee mortality and behaviour

The mortality of honey bees (e.g. workers, pupae, drones) was recorded using dead bee traps while the honey bees were located at the study fields. If there were ten or more dead bees in one colony after sowing, they were placed in a sample bottle and labelled unmistakably for potential further residue analysis. Since there were no sampling periods with clearly increased bee mortality no analysis of bee samples have been conducted. Behavioural abnormalities of the honey bees at the entrance hole were recorded during the mortality assessments.

Honey bee colony strength and health assessment

Population strength and development (number of cells filled with eggs, larvae or capped brood) as well as food stores (i.e. pollen and nectar) were assessed using the estimation method developed by the Bee Institute Liebefeld (Imdorf, Buehlmann et al. 1987). The precolony 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 honey bee hives.

Sampling method

To measure aerial dust drift deposits, vertically erected gauze samplers were set up on each assessment plot at the treatment field. The sowing started when the wind speed was below 5 m/s. Eight gauze samplers (each with an effective sampling area of 2 m x 3.3 m) were set up at a distance of approximately 3 m from the zero line on each assessment plot. Shortly before the beginning of the sowing the gauze samplers were wetted with a 1:1 (v/v) glycerol/water mixture. 30 minutes after the completion 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 transport and storage. Soil samples for water content analysis (non-GLP) and soil characterisation (non-GLP) were taken shortly before sowing on all study fields.

Residue analysis
Residues of clothianidin, imidacloprid and beta-cyfluthrin in gauze samples as well as all field fortification samples were analysed by Bayer CropScience AG (Schöning R. & Ballmann C., Report: MR-14/074). Chromatography and detection by MS/MS in gauze was done according to the methods 00554/M001 (clothianidin), 00537/M002 (imidacloprid) and 00922 (beta-cyfluthrin).

The Limit of Quantitation (LOQ) of the gauze samples (0.25 m^2) was 0.04 g a.s./ha for all analytes. The Limit of Detection (LOD) was 0.004 g a.s./ha for both clothianidin and imidacloprid and 0.012 g a.s./ha for beta-cyfluthrin.

Results
Honey bee mortality
In control and treatment group, worker bee mortality was on the same, generally low level, mostly around five to ten dead bees per day in mean. A statistical significant difference between control and treatment worker bee mortality could be seen on some days before the application, so that a test item related effect can be excluded. After sowing, the mean worker bee mortality in the treatment group was never significantly higher than in the control group. In contrast, on two days the worker bee mortality in the control group was significantly higher than in the treatment group. However, no test item related effect regarding to the worker bee mortality could be detected during the whole Field Phase. The mortality of the bee brood was on a very low level (mean control group: 0.52 ± 1.92; mean treatment group: 0.28 ± 0.67). On most days, no brood was found in the dead bee traps.

Honey bee colony development
Honey bee colony strength showed a similar development in the control and treatment group. It slightly increased during the first three weeks after setup of the bee colonies on the study fields. Due to the excellent food supply, the amount of brood increased in the same period. This led to a strong increase of the colony strength from the first to the second colony assessment, both in control and treatment colonies. From the second colony assessment (mid of August), the colony strength decreased towards winter and stagnated on a stable level. During winter, all colonies lost worker bees and due to the normal reduction or even stop of the breeding activity, the number of worker bees decreased towards spring. In the whole Field Phase, the mean colony strength of the control and treatment group was on the same level, no statistical significant differences were detectable.

The mean amount of honey bee brood was at the pre-colony assessment in the treatment group statistically significantly higher than in the control group. This is probably due to a slightly faster adaption of queens of the treatment group to the new colony size after assembling the colonies prior to the pre-colony assessment. This is a random factor that cannot be excluded, even if sister queens are used in this study. Also in the first colony assessment it was higher, but not statistically significant anymore. However, this indicates that the test item had no adverse effect to honey bee brood. The honey bee 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 honey bee colonies, which reduce their brood amount typically towards winter. With the beginning of the spring the honey bees 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 Müller than at the location Agger 2. After the first oxalic acid treatment, the number was also higher than at both other locations, but not statistically significantly. In contrast to this, it was statistically significantly lower after the second oxalic treatment in winter. The main reason therefore is the reduced strength of the colonies at Müller compared to the colonies at Agger 1 and Agger 2.

Residues

The results of all field spiked fortification gauze samples showed that clothianidin, imidacloprid and beta-cyfluthrin were stable during storage and transport. Residues in control samples were always below the LOD.

No residues of clothianidin, imidacloprid and beta-cyfluthrin above the LOD (0.012 g a.s./ha for beta-cyfluthrin and 0.004 g a.s./ha for clothianidin and imidacloprid) were detected in any of the gauze samples obtained from the study field during sowing of the test item.

Conclusion

To assess the potential effects of Poncho Beta Plus on the colony development of honey bees (*Apis mellifera* L.), Poncho Beta Plus-treated sugar beet pills (0.60 mg clothianidin a.s./pill, 0.08 mg beta-cyfluthrin a.s./pill and 0.30 mg imidacloprid a.s./pill) were sown (138,500 sugar beet pills/ha) during bee flight in summer 2013. To increase the possible exposition of the bees, the sugar beet was sown inside a field of flowering *Phacelia tanacetifolia*, a highly bee attractive crop.

The application of Poncho Beta Plus did not cause any effects on the survival of adult bees and bee pupae, foraging activity, behaviour, colony development and colony strength as well as on the bee brood and the hibernation success.

The dust drift measurements made during the sowing operation of Poncho Beta Plus-treated sugar beet pills on the treatment field indicate that pill-treatment dust, abraded and released during the sowing operation with non-modified (not deflected) vacuum-pneumatic sowing equipment and dismounted chassis of the discharged air system, did not result in a measurable off-field exposure as all analysed samples were below their respective LOD (0.012 g a.s./ha for beta-cyfluthrin and 0.004 g a.s./ha for clothianidin and imidacloprid).

Thus this study demonstrated that Poncho Beta Plus – treated sugar beet pills (0.60 mg clothianidin a.s./pill, 0.08 mg beta-cyfluthrin a.s./pill and 0.30 mg imidacloprid a.s./pill), sown during bee flight did not adversely affect honey bee colonies.

RMS's comments:

Currently no guideline is available, however, the study-set up is considered reasonable. Thus the study is considered acceptable and suitable for use in risk assessment.

The application rate for clothianidin in the present study was 78 g a.s./ha, slightly lower than the maximum application rate currently authorized in the EU, 90 g a.s./ha. As the potential dust emission depends on the Heubach g as/ha, the dust and the residue content of abraded dusts, but not the application rate sown in g as/ha this study can also be used to evaluate the potential risks for an application of a rate of 90 g as/ha, if the same seed treatment quality with regards

to the Heubach g as/ha is ensured. In this trial no data on the dustiness of the seeds (Heubach-value) nor the content of a.s. in dusts were provided, therefore from the study no information for comparability with other studies is obtained. The lack of comparability is considered as a drawback and should in principle be reported in all future studies investigating dust deposition and potential side effects of dust drift.
However, in this study, no residues in gauze net samples were detected above the LOD and no indication of any adverse effects in the treatment group was obtained in the bee trials. The worker mortality after sowing was low and not increased by the sowing operation. Furthermore, it is known and has been repeateadly confirmed (e.g. Draft SANCO/10553/2012) that sugar beet pills show only low abrasion values.

Overall, it is concluded that the overall the risk to bees is acceptable for the intended use of imidacloprid as a seed treatment in sugar beets.

The acute and long term risk to colony survival and development and the risk to bee brood for honey bees from ingestion of contaminated nectar and pollen

No new studies focused on imidacloprid residues in nectar and pollen of seed treated crops was submitted.

The risk to honey bees foraging nectar or pollen in succeeding crops

To determine the potential residues in succeeding crops under realistic agricultural conditions agricultural sites with a history of use of imidacloprid were selected. The residues in nectar, pollen and guttation fluid were assessed in four field studies.

Additionally, the residues in nectar, pollen and guttation fluid were assessed in two “model” studies with forced exposure with an artificially applied plateau.

Report:	Ythier, E.; 2014a
Title:	Determination of the residues of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin in bee relevant matrices collected in a succeeding crop scenario with natural aged residues of imidacloprid - Field phase conducted with *Phacelia* and maize in northern France
Report No.:	7SRFR13C1
Document No.:	M-504801-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective

The objective of the study was to determine residues of imidacloprid and its metabolites imidacloprid-5-hydroxy (hereinafter named 5-hydroxy) and imidacloprid-olefin (hereinafter called olefin) in bee relevant matrices (pollen, nectar and guttation fluid) collected from untreated flowering rotational crops cultivated as succeeding crops grown in France on fields with a history of imidacloprid use and as such with natural aged soil-residues of this active ingredient.

Material and Methods

The study was conducted on a field site near Meung-sur Loire (F-45130, France) with a known history of imidacloprid use and such with a likelihood of natural aged soil residues of this active substance. An approximately one hectare plot located within the field was marked out, and divided into two evenly sized sub-plots. One sub-plot was sown with maize (*Zea mays*) the other sub-plot was sown with *Phacelia (Phacelia tanacetifolia*).

Crops were sown according to Good Agricultural Practice (GAP). Maize and *Phacelia* without neonicotinoid seed treatment were sown in 2014, using calibrated equipment (tractor and seed drill). The target sowing rates were 10 kg seeds/ha for *Phacelia* and 100,000 kernel/ha for maize.
The plot sown with maize was later divided into three smaller sub plots, each similar in size that were large enough to have a sufficient numbers of plants available for both, sampling of guttation fluid and for maize pollen sampling.

Three bee proof tunnels (10 m long x 5 m wide x 3 m high) were placed onto the *Phacelia* plot after successful germination. A single honey bee colony was placed into each tunnel at the start of *Phacelia* flowering to collect nectar and pollen.

Soil sampling
From each of the maize sub plots and from the *Phacelia* sowing area, two different types of soil samples were collected. These samples were used for:

- Soil characterisation of the upper 10 cm soil layer.
- Determination of the residues of parent imidacloprid and its metabolites in the upper 15 cm soil layer.

Soil cores used for characterisation and residue analysis were collected from each of the three maize sub plots, during the guttation sampling phase of the trial and from inside of the *Phacelia* or mustard sowing area prior to placement of the honey bee colonies into the tunnels.

Sampling of nectar and pollen from *Phacelia* and mustard crops
Nectar and pollen sampling was conducted at three different time points during bloom of the *Phacelia* crop. Once the *Phacelia* started to bloom, honey bee colonies were placed into mesh covered tunnels erected over the crop. Honey bees were exposed to the flowering *Phacelia* or mustard under confined conditions and were exclusively used as a sampling device for both nectar and pollen.
Nectar was sampled by extracting the honey stomachs from forager bees. Therefore, the hive entrance was blocked during bee flight activity for a short period of time and the returning forager bees were collected at the hive entrance. Pollen was collected from foragers returning to the colony using a pollen trap attached to each colony. Pollen and nectar samples during bloom were analysed for residues of imidacloprid.

Sampling of guttation fluid and pollen from maize
Guttation fluid and pollen sampling was conducted in the maize crop. Samples were collected directly from the crop by hand.
Sampling of guttation fluid was carried out on a regular basis over a 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 chronology every day. Sampling took place in the same

order at each time point, starting with sub plot 1 and finishing with sub plot 3. When guttation was present it was collected from >10 plants throughout each of the sub plots. The target volume for each sample was 1 ml of guttation fluid.
Pollen sampling from three time points during bloom started when the crop started to shed pollen (BBCH scale 63) until male flowering had completed (BBCH scale 67).
At each time point ≥ 50 flowering tassels were collected from throughout each of the three sub plots and placed into paper bags. Damp tassels were air dried, in the dark at room temperature overnight.
Next day, the pollen was shaken out and cleaned with two analytical sieves (mesh size 2 mm and 1 mm), to ensure a pure pollen sample. Maize pollen in the base pan was cleaned from plant or insect debris remaining in the pollen sample by hand using forceps or a fine paint brush.

Findings/Conclusion
Residue analysis
All samples (soil samples, pollen, nectar and guttation fluid) were analysed for their content of imidacloprid and its metabolites 5-hydroxy and olefin by using high performance liquid chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection.

The limit of quantification (LOQ) and limit of detection (LOD) of imidacloprid in soil was 5 µg a.s./kg and 2 µg a.s./kg, respectively.

The LOQ levels for imidacloprid in pollen, nectar and guttation liquid were 0.6 µg a.s./kg, 0.3 µg a.s./kg and 1 µg a.s./L, respectively. The corresponding LOD were 0.2 µg a.s./kg for pollen, 0.1 µg a.s./kg for nectar and 0.3 µg a.s./L (0.0003 mg/L) for guttation liquid, respectively.

The LOQ and LOD of all metabolites were constant at 1 µg a.s./kg and 0.3 µg a.s./kg, respectively.

Maize
One set of soil samples were taken from the maize sub plots during the trial. The residue levels of imidacloprid in soils ranged from 43 µg a.s./kg to 50 µg a.s./kg dry soil. 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./L) to 5.7 µg a.s./L and are thus several orders of magnitude below neonicotinoid values measured in droplets from seed treated maize plants. The residue levels of imidacloprid in pollen, as sampled at three time points during bloom of the maize plants ranged from below the LOD (< 0.2 µg a.s./kg) to below the LOQ (< 0.6 µg a.s./kg).

Phacelia
Soil cores used for residue analysis were taken from the entire field prior to placement of the honey bee colonies into the tunnels. The residue level of imidacloprid in the *Phacelia* plot was 39 µg a.s./kg dry soil.
Residue analysis of pollen and nectar, collected at three time points during blooming of *Phacelia*, revealed generally low residue levels.
The residue levels of imidacloprid in pollen were always below the LOQ (< 0.6 µg a.s./kg).
The residue levels of imidacloprid in nectar ranged from below the LOQ (< 0.3 µg a.s./kg) to 3.5 a.s./kg. 8 out of 9 samples contained residues < 0.5 µg a.s./kg.

A summary of the analytical results as obtained by analysing samples of soil, guttation liquid, pollen and nectar samples are provided in the following tables:

Table 9.5.2-17: Residues of imidacloprid in soil

Sample material	Crop	Residue imidacloprid [µg/kg dry soil]
Soil	*Phacelia*	39
	Maize	43 - 50

LOD/LOQ in soil samples = 2 µg a.s./kg / 5 µg a.s./kg for all analytes

Table 9.5.2-18: Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in maize guttation liquid samples

Sample Material	Residue imidacloprid [µg/L]	Residue imidacloprid-5-hydroxy [µg/L]	Residue imidacloprid-olefine [µg/L]
Guttation liquid (Maize)	< LOQ - 5.7	< LOD - < LOQ	< LOQ

LOD/LOQ in Guttation fluid = 0.3 µg a.s./L / 1 µg as./L for all analytes

Table 9.5.2-19: Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in pollen from *Phacelia* and maize and nectar from *Phacelia*

Sample material	Residue imidacloprid [µg/kg]	Residue imidacloprid-5-hydroxy [µg/kg]	Residue imidacloprid-olefine [µg/kg]
Pollen (*Phacelia*)	< LOQ	< LOD - < LOQ	< LOD
Pollen (Maize)	< LOD - < LOQ	< LOD	< LOD
Nectar (*Phacelia*)*	< LOQ - 3.5	< LOD	< LOD

* 8 out of 9 samples ≤ 0.5 µg a.s./kg

LOD/ LOQ in pollen = 0.2 µg a.s./kg / 0.6 µg a.s./kg for imidacloprid, 0.3 µg a.s./kg / 1 µg a.s./kg for metabolites

LOD/LOQ in nectar = 0.1 µg a.s./kg / 0.3 µg a.s./kg for imidacloprid, 0.3 µg a.s./kg / 1 µg a.s./kg for metabolites

RMS´s comments:

The study was conducted on a field with a well-known history of regular use of imidacloprid treatment seed within the last six years (from 2008 until 2013). Therefore, the soil residues present at this site are thus considered representative for “natural” aged soil residues of imidacloprid.

Overall, the study is considered acceptable for use in risk assessment.

Report:	Ythier, E.; 2014b
Title:	Determination of the residues of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin in bee relevant matrices collected in a succeeding crop scenario with natural aged residues of imidacloprid - Field phase conducted with winter oil seed rape, *Phacelia* and maize in northern France
Report No.:	7SRFR13C2A
Document No.:	M-504806-01-1
Guideline(s):	not applicable

Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective
The objective of the study was to determine residues of imidacloprid and its metabolites imidacloprid-5-hydroxy (hereinafter named 5-hydroxy) and imidacloprid-olefin (hereinafter called olefin) in bee relevant matrices (pollen, nectar and guttation fluid) collected from flowering rotational crops cultivated as succeeding crops grown in France on fields with a history of imidacloprid use and as such with natural aged soil-residues of this active ingredient.

Material and Methods
The study was conducted on a field site near Giroux (F-36150, France) with a known history of imidacloprid use and such with a likelihood of natural aged soil residues of this active substance. On this land, non imidacloprid treated winter oil seed (*Brassica napus*) has been cultivated in 2013. During bloom in 2014, in total, three tunnels were setup for winter oil seed with one bee hive per tunnel. Samples of pollen loads (collected with pollen traps) and forager honey bees (for subsequent extraction of nectar from honey stomach) were taken. The samples were analysed for residues of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin during the analytical phase.
After sample collection and prior to sowing of non-imidacloprid treated *Phacelia* (*Phacelia tanacetifolia*) and maize (*Zea mays*) the previous crop was removed from the land.

Crops were sown according to Good Agricultural Practice (GAP). The maize and *Phacelia* plots were sown using calibrated equipment (tractor and seed drill). The target sowing rates were 10 kg seeds/ha for *Phacelia* and 100,000 kernel/ha for maize.

The sub plot sown with maize was divided into three smaller sub plots, each similar in size that were large enough to have a sufficient numbers of plants available for both guttation fluid and for maize pollen sampling.

Three bee proof tunnels (10 m long x 5 m wide x 3 m high) were placed onto the *Phacelia* plot after successful germination. A single honey bee colony was placed into each tunnel at the start of *Phacelia* flowering.

<u>Soil sampling</u>
From each of the maize sub plots and from respectively the *Phacelia* and winter oil seed rape sowing area, two different types of soil sample were collected. These samples were used for:

- Soil characterisation of the upper 10 cm soil layer.
- 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 guttation sampling phase of the trial and from inside of the *Phacelia* sowing area prior to placement of the honey bee colonies into the tunnels.

<u>Sampling of nectar and pollen from winter oilseed rape</u>

Nectar and pollen sampling was conducted at three different time points during bloom of the oilseed crop. Once the winter oilseed rape started to bloom, honey bee colonies were placed into mesh covered tunnels erected over the crop. Honey bees were exposed to the flowering winter oilseed under confined conditions and were exclusively used as a sampling device for both nectar and pollen.
Nectar was sampled by extracting the honey stomachs from forager bees. Therefore, the hive entrance was blocked during bee flight activity for a short period of time and the returning forager bees were collected at the hive entrance. Pollen was collected from foragers returning to the colony using a pollen trap attached to each colony. Pollen and nectar samples during bloom were analysed for residues of imidacloprid and metabolites.

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, honey bee colonies were placed into mesh covered tunnels erected over the crop. Honey bees were exposed to the flowering *Phacelia* under confined conditions and were exclusively used as a sampling device for both nectar and pollen.
Nectar was sampled by extracting the honey stomachs from forager bees. Therefore, the hive entrance was blocked during bee flight activity for a short period of time and the returning forager bees were collected at the hive entrance. Pollen was collected from foragers returning to the colony using a pollen trap attached to each colony. Pollen and nectar samples during bloom were analysed for residues of imidacloprid and metabolites.

Sampling of guttation fluid and pollen from maize
Guttation fluid and pollen sampling was conducted in the maize crop. Samples were collected directly from the crop by hand. Sampling of guttation fluid was carried out on a regular basis over a 40-day period. Guttation sampling started directly after emergence of the maize crop (BBCH scale 11-12) until flowering (BBCH scale 65). Guttation fluid was collected from each of the three sub-plots approximately thirty minutes after sunrise. The sampling period at each time point was approximately 30 minutes to ensure an equivalent time chronology every day. Sampling took place in the same order at each time point, starting with sub plot 1 and finishing with sub plot 3. When guttation was present it was collected from >10 plants throughout each of the sub plots. The target volume for each sample was 1 ml of guttation fluid.
Pollen sampling from three time points during bloom started when the crop started to shed pollen (BBCH scale 63) until male flowering had completed (BBCH scale 67).
At each time point ≥ 50 flowering tassels were collected from throughout each of the three sub plots and placed into paper bags. Damp tassels were air dried, in the dark at room temperature overnight.
Next day, the pollen was shaken out and cleaned with two analytical sieves (mesh size 2 mm and 1 mm), to ensure a pure pollen sample. Maize pollen in the base pan was cleaned from plant or insect debris remaining in the pollen sample by hand using forceps or a fine paint brush.
Pollen samples during bloom as well as collected guttation fluid were analysed for residues of imidacloprid and metabolites.

Findings/Conclusion

Residue analysis
All samples (soil samples, pollen, nectar and guttation fluid) were analysed for their content of imidacloprid and its metabolites 5-hydroxy and olefin by using high performance liquid chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection.

The limit of quantification (LOQ) and limit of detection (LOD) of imidacloprid in soil was 5 µg a.s./kg and 2 µg a.s./kg, respectively.

The LOQ levels for imidacloprid in pollen, nectar and guttation liquid were 0.6 µg a.s./kg, 0.3 µg a.s./kg and 1 µg a.s./L, respectively. The corresponding LOD were 0.2 µg a.s./kg for pollen, 0.1 µg a.s./kg for nectar and 0.3 µg a.s./L (0.0003 mg/L) for guttation liquid, respectively.

The LOQ and LOD of all metabolites were constant at 1 µg a.s./kg and 0.3 µg a.s./kg, respectively.

Winter oilseed rape
Soil cores used for residue analysis were taken from the entire field prior to placement of the honey bee colonies into the tunnels. The residue level of imidacloprid in the field was 43 µg a.s./kg dry soil.
Residue analysis of pollen and nectar, collected at three time points during blooming of winter oilseed rape, revealed generally low residue levels.
The residue levels of imidacloprid in pollen was always below the LOQ (< 0.6 µg a.s./kg).
The residue levels of imidacloprid in nectar ranged from below the LOQ (< 0.3 µg a.s./kg) to the LOQ (0.3 µg a.s./kg).

Maize
One set of soil samples were taken from the maize sub plots during the trial. The residue levels of imidacloprid in soils ranged from 35 µg a.s./kg to 48 µg a.s./kg dry soil during guttation.
Residues analysis of guttation fluid, collected directly after emergence until early bloom of the maize plants, revealed generally low residue levels.
The residue levels of imidacloprid in guttation fluid ranged from below the LOD (< 0.3 µg a.s.) to 1.3 µg a.s./L and are thus several orders of magnitude below values measured in droplets from seed treated maize plants.
The residue levels of imidacloprid in pollen, as sampled at three time points during bloom of the maize plants ranged from below the LOD (< 0.2 µg a.s./kg) to 2.5 µg a.s./kg). Residues in 8 of 9 samples were < LOQ

Phacelia
Soil cores used for residue analysis were taken from the entire field prior to placement of the honey bee colonies into the tunnels. The residue level of imidacloprid in the *Phacelia* plot was 46 µg a.s./kg dry soil.
Residue analysis of pollen and nectar, collected at three time points during blooming of *Phacelia*, revealed generally low residue levels.
The residue levels of imidacloprid in pollen ranged from below the LOQ (<0.6 µg a.s./kg) to 1.5 a.s./kg. Residues in 8 of 9 samples were < LOQ.
The residue levels of imidacloprid in nectar ranged from below the LOD (<0.1 µg a.s./kg) to 0.4 a.s./kg

A summary of the analytical results as obtained by analysing samples of soil, guttation liquid, pollen and nectar samples are provided in the following tables:

Table 9.5.2-20: Residues of imidacloprid in soil

Sample material	**Crop**	**Residue imidacloprid [µg/kg dry**

		soil]
Soil	Maize	35-48
Soil	*Phacelia*	46
Soil	OSR	43

LOD/LOQ in soil samples = 2 µg a.s./kg / 5 µg a.s./kg for all analytes

Table 9.5.2-21: Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in maize guttation liquid samples

Sample material	**Residue of imidacloprid [µg/L]**	**Residue of imidacloprid-5-hydroxy [µg/L]**	**Residue of imidacloprid-olefine [µg/L]**
Guttation liquid (Maize)	< LOD – 1.3	< LOD – < LOQ	< LOD – < LOQ

LOD/LOQ in Guttation fluid = 0.3 µg a.s./L / 1 µg a.s./L for all analytes

Table 9.5.2-22: Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in pollen from winter oil seed rape (OSR), *Phacelia* and maize and in nectar from winter oil seed rape (OSR) and *Phacelia*

Sample material	**Residue of imidacloprid [µg/kg]**	**Residue of imidacloprid-5-hydroxy [µg/kg]**	**Residue of imidacloprid-olefine [µg/kg]**
Pollen (OSR)	< LOQ	< LOD	< LOD
Pollen (*Phacelia*)*	< LOQ – 1.5	< LOD	< LOD
Pollen (Maize)*	< LOD – 2.5	< LOD	< LOD
Nectar (OSR)	< LOQ – 0.3	< LOD	< LOD
Nectar (*Phacelia*)	< LOD – 0.4	< LOD	< LOD

* 8 out of 9 samples < LOQ
LOD/LOQ in pollen = 0.2 µg a.s./kg / 0.6 µg a.s./kg for imidacloprid, 0.3 µg a.s./kg / 1 µg a.s./kg metabolites
LOD/LOQ in nectar = 0.1 µg a.s./kg / 0.3 µg a.s./kg for imidacloprid, 0.3 µg a.s./kg / 1 µg a.s./kg for metabolites

RMS´s comments:
The study was conducted on a field with a well-known field history for the last 7 years (from 2008 until 2014). However, in contrast to the first study (Ythier, E.; 2014a) the use of imidacloprid treatment seed was less regular in the study presented here. There was no use of imidacloprid treatment seed during spring 2014 and in the year 2011 and 2008. However, this is considered to be of limited consequence for the results as there was a regularly use of imidacloprid treatment seeds in the past three years. Therefore the soil residues present at the site are thus considered representative for “natural” aged soil residues of imidacloprid.

Overall, the study is considered acceptable for use in risk assessment.

Report: Ythier, E.; 2014c
Title: Determination of the residues of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin in bee relevant

	matrices collected in a succeeding crop scenario with natural aged residues of imidacloprid - Field phase conducted with *Phacelia* and maize in northern France
Report No.:	7SRFR13C2B
Document No.:	M-504836-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective
The objective of the study was to determine residues of imidacloprid and its metabolites imidacloprid-5-hydroxy (hereinafter named 5-hydroxy) and imidacloprid-olefin (hereinafter called olefin) in bee relevant matrices (pollen, nectar and guttation fluid) collected from flowering rotational crops cultivated as succeeding crops grown in France on fields with a history of imidacloprid use and as such with natural aged soil-residues of this active ingredient.

Material and Methods
The study was conducted on a field site near Auxy (F-45340, France) with a known history of imidacloprid use and such with a likelihood of natural aged soil residues of this active substance. An approximately one hectare plot located within the dimension of the agricultural land was marked out, and divided into two evenly sized sub-plots. One sub-plot was sown with maize (*Zea mays*) the other sub-plot was sown with *Phacelia (Phacelia tanacetifolia*).

Crops were sown according to Good Agricultural Practice (GAP). The maize and *Phacelia* plots were sown using calibrated equipment (tractor and seed drill). The target sowing rates were 10 kg seeds/ha for *Phacelia* and 100,000 kernel/ha for maize.
The sub plot sown with maize was divided into three smaller sub plots, each similar in size that were large enough to have a sufficient numbers of plants available for both guttation fluid and for maize pollen sampling.
Three bee proof tunnels (10 m long x 5 m wide x 3 m high) were placed onto the *Phacelia* plot after successful germination. A single honey bee colony was placed into each tunnel at the start of *Phacelia* flowering

<u>Soil sampling</u>
From each of the maize sub plots and from the *Phacelia* sowing area, two different types of soil sample were collected. These samples were used for:
- Soil characterisation of the upper 10 cm soil layer.
- Determination of the residues of parent imidacloprid and its metabolites in the upper 15 cm soil layer.

Soil cores used for characterisation and residue analysis were collected from each of the three segregated maize sub plots, during the guttation sampling phase of the trial and from inside of the *Phacelia* sowing area prior to placement of the honey bee colonies into the tunnels.

<u>Sampling of nectar and pollen from *Phacelia* crops</u>
Nectar and pollen sampling was conducted at three different time points during bloom of the *Phacelia* crop. Once the *Phacelia* started to bloom, honey bee colonies were placed into mesh covered tunnels erected over the crop. Honey bees were exposed to the flowering *Phacelia* under confined conditions and were exclusively used as a sampling device for both nectar and pollen.

Nectar was sampled by extracting the honey stomachs from forager bees. Therefore, the hive entrance was blocked during bee flight activity for a short period of time and the returning forager bees were collected at the hive entrance. Pollen was collected from foragers returning to the colony using a pollen trap attached to each colony. Pollen and nectar samples during bloom were analysed for residues of imidacloprid.

Sampling of guttation fluid and pollen from maize
Guttation fluid and pollen sampling was conducted in the maize crop. Samples were collected directly from the crop by hand.
Sampling of guttation fluid was carried out on a regular basis over a 42-day period. Guttation sampling started directly after emergence of the maize crop (BBCH scale 11-12) until flowering (BBCH scale 65). Guttation fluid was collected from each of the three sub-plots approximately thirty minutes after sunrise. The sampling period at each time point was approximately 30 minutes to ensure an equivalent time chronology every day. Sampling took place in the same order at each time point, starting with sub plot 1 and finishing with sub plot 3. When guttation was present it was collected from >10 plants throughout each of the sub plots. The target volume for each sample was 1 ml of guttation fluid.
Pollen sampling from three time points during bloom started when the crop started to shed pollen (BBCH scale 63) until male flowering had completed (BBCH scale 67).

At each time point ≥ 50 flowering tassels were collected from throughout each of the three sub plots and placed into paper bags. Damp tassels were air dried, in the dark at room temperature overnight.
Next day, the pollen was shaken out and cleaned with two analytical sieves (mesh size 2 mm and 1 mm), to ensure a pure pollen sample. Maize pollen in the base pan was cleaned from plant or insect debris remaining in the pollen sample by hand using forceps or a fine paint brush.
Pollen samples during bloom as well as collected guttation fluid were analysed for residues of imidacloprid.

Findings/ Conclusion
Residue analysis
All samples (soil samples, pollen, nectar and guttation fluid) were analysed for their content of imidacloprid and its metabolites 5-hydroxy and olefin by using high performance liquid chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection.

The limit of quantification (LOQ) and limit of detection (LOD) of imidacloprid in soil was 5 µg a.s./kg and 2 µg a.s./kg, respectively.

The LOQ levels for imidacloprid in pollen, nectar and guttation liquid were 0.6 µg a.s./kg, 0.3 µg a.s./kg and 1 µg a.s./L, respectively. The corresponding LOD were 0.2 µg a.s./kg for pollen, 0.1 µg a.s./kg for nectar and 0.3 µg a.s./L (0.0003 mg/L) for guttation liquid, respectively.

The LOQ and LOD of all metabolites were constant at 1 µg a.s./kg and 0.3 µg a.s./kg, respectively.

Maize
One set of soil samples were taken from the maize sub plots during the trial. The residue levels of imidacloprid in soils ranged from 41 µg a.s./kg to 59 µg a.s./kg dry soil during guttation.
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./L) to 4.1 µg a.s./L and are thus several orders of magnitude below values measured in droplets from seed treated maize plants.
The residue levels of imidacloprid in pollen, as sampled at three time points during bloom of the maize plants ranged from 0.64 µg a.s./kg to 0.91 µg a.s./kg.

Phacelia
Soil cores used for residue analysis were taken from the entire field prior to placement of the honey bee colonies into the tunnels. The residue level of imidacloprid in the *Phacelia* plot was 52 µg a.s./kg dry soil.
Residue analysis of pollen and nectar, collected at three time points during blooming of *Phacelia*, revealed generally low residue levels.
The residue levels of imidacloprid in pollen ranged from below the LOQ (<0.6 µg a.s./kg) to 1.2 µg a.s./kg. Residues in 8 out of 9 samples were < LOQ.
The residue levels of imidacloprid in nectar ranged from below the LOQ (<0.3 µg a.s./kg) to 0.4 a.s./kg.

A summary of the analytical results as obtained by analysing samples of soil, guttation liquid, pollen and nectar samples are provided in the following tables:

Table 9.5.2-23: Residues of imidacloprid in soil

Sample material	Crop	Residue imidacloprid [µg/kg dry soil]
Soil	Maize	41 - 59
Soil	*Phacelia*	52

LOD/LOQ in soil samples = 2 µg a.s./kg / 5 µg a.s./kg for all analytes

Table 9.5.2-24: Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in maize guttation liquid samples

Sample material	Residue of imidacloprid [µg/L]	Residue of imidacloprid-5-hydroxy [µg/L]	Residue of imidacloprid-olefine [µg/L]
Guttation liquid (Maize)	< LOD – 4.1	< LOD – < LOQ	< LOQ

LOD/LOQ in Guttation fluid = 0.3 µg a.s./L / 1 µg as./L for all analytes

Table 9.5.2-25: Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in pollen from *phacelia* and maize and nectar from *phacelia*

Sample material	Residue of imidacloprid [µg/kg]	Residue of imidacloprid-5-hydroxy [µg/kg]	Residue of imidacloprid-olefine [µg/kg]
Pollen (*Phacelia*)	< LOQ – 1.2	< LOD	< LOD
Pollen (Maize)	0.64 – 0.91	< LOD	< LOD
Nectar (*Phacelia*)	< LOQ – 0.4	< LOD	< LOD

LOD/LOQ in pollen = 0.2 µg a.s./kg / 0.6 µg a.s./kg for imidacloprid, 0.3 µg a.s./kg / 1 µg a.s./kg metabolites
LOD/LOQ in nectar = 0.1 µg a.s./kg / 0.3 µg a.s./kg for imidacloprid, 0.3 µg a.s./kg / 1 µg a.s./kg for metabolites

RMS´s comments:
The study was conducted on a field with a well-known field history for the last 7 years (from 2008 until 2014). In contrast to the first study (Ythier, E.; 2014a) the use of imidacloprid treatment seed was less regular in the study presented here. There was no use of imidacloprid treatment seed in the year 2010 and 2008. However, this is considered to be of limited consequence for the results as there was a regularly use of imidacloprid treatment seeds in the past four years. Therefore the soil residues present at the site are thus considered representative for "natural" aged soil residues of imidacloprid.

Overall, the study is considered acceptable for use in risk assessment.

Report:	Ythier, E.; 2014d
Title:	Determination of the residues of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin in bee relevant matrices collected in a succeeding crop scenario with natural aged residues of imidacloprid - Field phase conducted with winter oil seed rape in northern France
Report No.:	7SRFR13C2C
Document No.:	M-504810-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective

The objective of the study was to determine residues of imidacloprid and its metabolites imidacloprid-5-hydroxy (hereinafter named 5-hydroxy) and imidacloprid-olefin (hereinafter called olefin) in bee relevant matrices (pollen and nectar) collected from flowering rotational crops cultivated as succeeding crops grown in France on fields with a history of imidacloprid use and as such with natural aged soil-residues of this active ingredient.

Material and Methods
The study was conducted on a field site near Ribeaucourt (F-55290, France) with a known history of imidacloprid use and such with a likelihood of natural aged soil residues of this active substance. On this land, non imidacloprid treated winter oil seed (*Brassica napus*) has been cultivated in 2013. During bloom on 2014, in total, three tunnels were setup for winter oil seed with one bee hive per tunnel. Samples of pollen loads (collected with pollen traps) and forager honey bees (for subsequent extraction of nectar from honey stomach) were taken.

Winter oil seed rape was sown according to Good Agricultural Practice (GAP). Winter oil seed rape has been sown by the cooperating farmer. Three bee proof tunnels (10 m long x 5 m wide x 3 m high) were placed onto the winter oil seed rape plot prior to bloom. A single honey bee colony was placed into each tunnel at the start of winter oilseed rape flowering.

<u>Soil sampling</u>
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 layer.
- Determination of the residues of parent clothianidin and its metabolites in the upper 15 cm soil layer.

Soil cores used for characterisation and residue analysis were collected from inside of the winter oil seed sowing area prior to placement of the honey bee colonies into the tunnels.

Sampling of nectar and pollen from winter oilseed rape

Nectar and pollen sampling was conducted at three different time points during bloom of the oilseed crop. Once the winter oilseed rape started to bloom, honey bee colonies were placed into mesh covered tunnels erected over the crop. Honey bees were exposed to the flowering winter oilseed under confined conditions and were exclusively used as a sampling device for both nectar and pollen.
Nectar was sampled by extracting the honey stomachs from forager bees. Therefore, the hive entrance was blocked during bee flight activity for a short period of time and the returning forager bees were collected at the hive entrance. Pollen was collected from foragers returning to the colony using a pollen trap attached to each colony. Pollen and nectar samples during bloom were analysed for residues of imidacloprid.

Findings/Conclusion

Residue analysis

All samples (soil samples, pollen and nectar) were analysed for their content of imidacloprid and its metabolites 5-hydroxy and olefin by using high performance liquid chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection.

The limit of quantification (LOQ) and limit of detection (LOD) of imidacloprid in soil was 5 µg a.s./kg and 2 µg a.s./kg, respectively.

The LOQ levels for imidacloprid in pollen, nectar and guttation liquid were 0.6 µg a.s./kg, 0.3 µg a.s./kg and 1 µg a.s./L, respectively. The corresponding LOD were 0.2 µg a.s./kg for pollen, 0.1 µg a.s./kg for nectar and 0.3 µg a.s./L (0.0003 mg/L) for guttation liquid, respectively.

The LOQ and LOD of all metabolites were constant at 1 µg a.s./kg and 0.3 µg a.s./kg, respectively.

Winter oilseed rape

Soil cores used for residue analysis were taken from the entire field prior to placement of the honey bee colonies into the tunnels. The residue level of imidacloprid in the winter oilseed rape plot was 45 µg a.s./kg dry soil.
Residue analysis of pollen and nectar, collected at three time points during blooming of winter oilseed rape, revealed generally low residue levels.
The residue levels of imidacloprid in pollen ranged from below the LOQ (< 0.6 µg a.s./kg) to 1.3 µg a.s./kg.
The residue levels of imidacloprid in nectar ranged from below the LOQ (< 0.3 µg a.s./kg) to 0.7 µg a.s./kg.

A summary of the analytical results as obtained by analysing samples of soil, pollen and nectar is provided in the following tables:

Table 9.5.2-26: Residues of imidacloprid in soil samples

Sample material	Crop	Residue imidacloprid * [µg/kg dry soil]**
Soil	Winter oil seed rape	45

LOD/LOQ in soil samples = 2 µg a.s./kg / 5 µg a.s./kg for all analytes

Table 9.5.2-27: Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid olefine in oil seed rape nectar and pollen samples

Sample Material	Residue imidacloprid [µg/kg]	Residue imidacloprid-5-hydroxy [µg/kg]	Residue imidacloprid olefine [µg/kg]
Nectar (oil seed rape)	< LOD – 0.7	<LOD	<LOD
Pollen (oil seed rape)	< LOQ – 1.3	<LOD	<LOD

LOD/LOQ in pollen = 0.2 µg a.s./kg / 0.6 µg a.s./kg for imidacloprid, 0.3 µg a.s./kg / 1 µg a.s./kg for metabolites
LOD/LOQ in nectar = 0.1 µg a.s./kg / 0.3 µg a.s./kg for imidacloprid, 0.3 µg a.s./kg / 1 µg a.s./kg for metabolites

RMS´s comments:
The study was conducted on a field with a well-known field history for the last 7 years (from 2008 until 2014). In contrast to the first study (Ythier, E.; 2014a) the use of imidacloprid treatment seed was less regular in the study presented here. For instance there was no use of imidacloprid treatment seed during spring 2014 and in the year 2011, 2010, 2009 and 2008. However, this is considered to be of limited consequence for the results as there was a regularly use of imidacloprid treatment seeds in the past three years. Therefore the soil residues present at the site are thus considered representative for “natural” aged soil residues of imidacloprid. Overall, the study is considered acceptable for use in risk assessment.

Report:	Ythier, E.; 2014e
Title:	Determination of the residues of imidacloprid in bee relevant matrices collected from succeeding crops following application of imidacloprid FS 600E G via soil incorporation to plateau concentration and sowing of imidacloprid-treated winter barley seeds. Field phase conducted in southern France
Report No.:	7SRFR13C3
Document No.:	M-504842-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective

The objective of the study was to determine residues of imidacloprid and its metabolites imidacloprid-5-hydroxy (hereinafter named 5-hydroxy) and imidacloprid-olefin (hereinafter called olefin) in bee relevant matrices (pollen, nectar and guttation fluid) collected from succeeding crops following application of IMIDACLOPRID FS 600E G via soil incorporation to achieve a plateau concentration and sowing of imidacloprid-treated winter barley seeds.

Material and Methods

The study was conducted on a field site near Nîmes (F-30000, France). An approximately two hectare field located on the field site was marked out, and divided into two evenly sized plots. Three crops were cultivated on both plots of the study field: *Phacelia* (*Phacelia tanacetifolia*), mustard (*Sinapis arvensis*) and maize (*Zea mays*) (each in an area of approx. 0.2 ha).
The test item imidacloprid was applied in autumn 2013 with two different calculated plateau concentrations directly to bare soil. After incorporation of the calculated plateau concentrations, dressed winter barley seeds (again with two different seed dressing rates) were sown (see overview below):

	Application of the plateau concentration * (25.09.2013)	**Sowing of treated winter barley seeds* (10.10.2013)**
Low plateau concentration + low seed dressing rate (variant blue)	87.3 g imidacloprid/ha 0.144 L product/ha	85.8 g imidacloprid /ha 184.5 kg seeds/ha
High plateau concentration + high seed dressing rate (variant green)	154.0 g imidacloprid/ha 0.254 L product/ha	118.5 g imidacloprid/ha 189.5 kg seeds/ha

*actual concentrations

In 2014, winter barley crops were removed and untreated succeeding crops (mustard, *Phacelia* and maize) were sown on the areas with previous imidacloprid applications.
Three bee proof tunnels (10 m long x 5 m wide x 3 m high) were placed onto the *Phacelia* and the mustard plot after successful germination. A single honey bee colony was placed into each tunnel at the start of *Phacelia*, respectively mustard flowering
The sub plot sown with maize was divided into three smaller sub plots, each similar in size that were large enough to have a sufficient numbers of plants available for both guttation fluid and for maize pollen sampling.

Soil sampling
From each of the maize sub plots and from the *Phacelia* and mustard sowing areas, two different types of soil samples were collected. These samples were used for:

- Soil characterisation of the upper 10 cm soil layer.
- Determination of the residues of parent imidacloprid and its metabolites in the upper 15 cm soil layer.

Soil cores used for characterisation and residue analysis were collected from each of the three segregated maize sub plots, during the guttation sampling phase of the trial and from inside of the *Phacelia* or mustard sowing area prior to placement of the honey bee colonies into the tunnels.

Sampling of nectar and pollen from *Phacelia* and mustard crops
Nectar and pollen sampling was conducted at three different time points during bloom of the corresponding crop. Once the crop started to bloom, honey bee colonies were placed into mesh covered tunnels erected over the crop. Honey bees were exposed to the flowering *Phacelia* or mustard under confined conditions and were exclusively used as a sampling device for both nectar and pollen.
Nectar was sampled by extracting the honey stomachs from forager bees. Therefore, the hive entrance was blocked during bee flight activity for a short period of time and the returning forager bees were collected at the hive entrance. Pollen was collected from foragers returning

to the colony using a pollen trap attached to each colony. Pollen and nectar samples during bloom were analysed for residues of imidacloprid.

Sampling of guttation fluid and pollen from maize
Guttation fluid and pollen sampling was conducted in the maize crop. Samples were collected directly from the crop by hand.
Sampling of guttation fluid was carried out on a regular basis over a 37-day period. Guttation sampling started directly after emergence of the maize crop (BBCH scale 11-12) until flowering (BBCH scale 65). Guttation fluid was collected from each of the three sub-plots approximately thirty minutes after sunrise. The sampling period at each time point was approximately 30 minutes to ensure an equivalent time chronology every day. Sampling took place in the same order at each time point, starting with sub plot 1 and finishing with sub plot 3. When guttation was present it was collected from >10 plants throughout each of the sub plots. The target volume for each sample was 1 ml of guttation fluid.
Pollen sampling from three time points during bloom started when the crop started to shed pollen (BBCH scale 63) until male flowering had completed (BBCH scale 67).
At each time point ≥ 50 flowering tassels were collected from throughout each of the three sub plots and placed into paper bags. Damp tassels were air dried, in the dark at room temperature overnight.
Next day, the pollen was shaken out and cleaned with two analytical sieves (mesh size 2 mm and 1 mm), to ensure a pure pollen sample. Maize pollen in the base pan was cleaned from plant or insect debris remaining in the pollen sample by hand using forceps or a fine paint brush.
Pollen samples during bloom as well as collected guttation fluid were analysed for residues of imidacloprid.

Findings/Conclusion

The study has been performed to cover various scenarios (crop rotations) of a consecutive use of imidacloprid and to determine the potential residue level of imidacloprid and its metabolites -5-hydroxy and –olefine in bee-relevant matrices (nectar and pollen) and guttation droplets of succeeding crops. In a model approach, two levels of imidacloprid plateau concentrations were established (information about the rates to be applied were provided by the sponsor) on an agricultural site near Nîmes (F-30000, France). After incorporation of the calculated plateau concentrations in September 2013, dressed winter barley seeds (again with two different seed dressing rates) were sown.

Residue analysis
Residue analysis of imidacloprid in soil samples and samples of guttation liquid, nectar and pollen was performed by using high performance liquid chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection.

The limit of quantification (LOQ) and limit of detection (LOD) of imidacloprid in soil was 5 µg a.s./kg and 2 µg a.s./kg, respectively.

The LOQ levels for imidacloprid in pollen, nectar and guttation liquid were 0.6 µg a.s./kg, 0.3 µg a.s./kg and 1 µg a.s./L, respectively. The corresponding LOD were 0.2 µg a.s./kg for pollen, 0.1 µg a.s./kg for nectar and 0.3 µg a.s./L (0.0003 mg/L) for guttation liquid, respectively.

The LOQ and LOD of all metabolites were constant at 1 µg a.s./kg and 0.3 µg a.s./kg, respectively.

Phacelia
Residues analysis of pollen and nectar, as collected at one time during blooming of *Phacelia*, in three tunnels per test rate revealed in low residue levels. The residue levels of imidacloprid in nectar ranged from below the LOQ (< 0.3 µg a.s./kg) to 1.0 µg a.s./kg. Residue levels of imidacloprid in pollen ranged from below the LOQ (< 0.6 µg a.s./kg) to 2.0 µg a.s./kg.

Mustard
Residues analysis of pollen and nectar, as collected at three time points during blooming of mustard in three tunnels per test rate revealed in low residue levels. The residue levels of imidacloprid in nectar ranged from below the LOQ (< 0.3 µg a.s./kg) to 3.9 µg a.s./kg. Residue levels of imidacloprid in pollen ranged from 1.6 µg a.s./kg to 5.1 µg a.s./kg.

Maize
Residues analysis of guttation fluid, as collected from directly after emergence until early bloom of the Maize plants, revealed in generally low residues. The residue levels of imidacloprid in guttation fluid ranged from below the LOQ (< 1 µg a.s./L) to 88 µg a.s./L and are thus several orders of magnitude below values measured in droplets from neonicotinoid seed treated maize plants. The maximum residue level of imidacloprid in pollen, as sampled at three time points during bloom on three subplots ranged from below the LOQ (< 0.6 µg a.s./kg) to 1.2 µg a.s./kg.

Overall, transfer of imidacloprid soil residues into bee-relevant matrices and guttation droplets of succeeding crops takes place on very low levels even if calculated long-term plateau concentrations are established without ageing of residues over years. Traces of imidacloprid metabolites were only measured in single guttation or pollen samples.

A summary of the analytical results as obtained by analysing samples of soil, guttation liquid, pollen and nectar is provided in the following tables:

Table 9.5.2-28: Residues of imidacloprid in soil (blue and green plots)

Sample material	Variant	Residue imidacloprid during bloom [µg/kg dry soil]
Soil	“low” (blue plot)	34 - 82
	“high” (green plot)	25 - 93

LOD/LOQ in soil samples = 2 µg a.s./kg / 5 µg a.s./kg for all analytes

Table 9.5.2-29: Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in guttation liquid samples (blue and green plots)

Sample Material	Variant	Residue imidacloprid [µg/L]	Residue imidacloprid-5-hydroxy [µg/L]	Residue imidacloprid-olefine [µg/L]
Guttation liquid (Maize)	“low” (blue plot)	< LOQ - 88	< LOD - 9	< LOD - 2
Guttation liquid (Maize)	“high” (green plot)	< LOQ - 34	< LOD - 12	< LOQ - 2

LOD/LOQ in Guttation fluid = 0.3 µg a.s./L / 1 µg a.s./L for all analytes

Table 9.5.2-30: Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in mustard and phacelia nectar samples (blue and green plots)

Sample material	Variant	Residue imidacloprid [µg/kg]	Residue imidacloprid-5-hydroxy [µg/kg]	Residue imidacloprid-olefine [µg/kg]
Nectar (Mustard)	"low" (blue plot)	0.7 - 3.9	< LOD - < LOQ	< LOD - < LOQ
Nectar (*Phacelia*)		< LOD – < LOQ	< LOD	< LOD
Nectar (Mustard)	"high" (green plot)	< LOQ - 0.5	< LOD	< LOD
Nectar (*Phacelia*)		0.8 – 1.0	< LOD	< LOD

LOD/LOQ in pollen = 0.2 µg a.s./kg / 0.6 µg a.s./kg for imidacloprid, 0.3 µg a.s./kg / 1 µg a.s./kg metabolites
LOD/LOQ in nectar = 0.1 µg a.s./kg / 0.3 µg a.s./kg for imidacloprid, 0.3 µg a.s./kg / 1 µg a.s./kg for metabolites

Table 9.5.2-31: Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in pollen samples (blue and green plots)

Sample material	Variant	Residue imidacloprid [µg/kg]	Residue imidacloprid-5-hydroxy [µg/kg]	Residue imidacloprid-olefine [µg/kg]
Pollen (Mustard)	"low" (blue plot)	1.8 - 5.1	< LOD - <LOQ	< LOQ - 1.2
Pollen (*Phacelia*)		< LOQ - 0.6	< LOD	< LOD
Pollen (Maize)		< LOQ - 1.2	< LOD - <LOQ	< LOD
Pollen (Mustard)	"high" (green plot)	1.6 - 4.7	< LOD - < LOQ	< LOQ - 1.2
Pollen (*Phacelia*)		1.9 – 2.0	< LOD	< LOD
Pollen (Maize)		< LOQ - 0.93	< LOD	< LOD - <LOQ

LOD/LOQ in pollen = 0.2 µg a.s./kg / 0.6 µg a.s./kg for imidacloprid, 0.3 µg a.s./kg / 1 µg a.s./kg for metabolites
LOD/LOQ in nectar = 0.1 µg a.s./kg / 0.3 µg a.s./kg for imidacloprid, 0.3 µg a.s./kg / 1 µg a.s./kg for metabolites

RMS´s comments:
The study is considered acceptable for use in risk assessment.

Report:	Striffler, B.; Ballhaus, F. 2014
Title:	Residues of imidacloprid in nectar and pollen of flowering rotational crops in western Germany
Report No.:	M-504854-01-1
Document No.:	M-504854-01-1
Guideline(s):	Regulation (EC) No 1107/2009

Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective

The objective of the study was to determine residues of imidacloprid and its metabolites 5-hydroxy and olefine in nectar and pollen of flowering rotational crops (*Phacelia* and mustard) and furthermore 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.

Material and Methods

The study was conducted in the vicinity of Zuelpich, North Rhine-Westphalia in Germany. Two areas of approximately 1 ha each, were established on the study field. Three crops were cultivated on both variants of the study field: *Phacelia* (*Phacelia tanacetifolia*), mustard (*Sinapis arvensis*) (each in an area of approx. 0.2 ha) and maize (*Zea mays*) (each in an area of approx. 0.1 ha).

The test item imidacloprid was applied in two applications in autumn 2013:

	Application of the plateau concentration * (26.09.2013)	**Sowing of treated winter barley seeds* (9.10.2013)**
Low plateau concentration + low seed dressing rate (variant blue)	95.4 g imidacloprid/ha 0.157 L product/ha	63.2 g imidacloprid/ha 136 kg seeds/ha (with 46.5 g imidacloprid/dt)
High plateau concentration + high seed dressing rate (variant green)	173.4 g imidacloprid/ha 0.286 L product/ha	126.3 g imidacloprid/ha 202 kg seeds/ha (with 62.5 g imidacloprid/dt)

* Actual concentrations

In spring 2014, untreated *Phacelia*, mustard and maize were sown on the study plots which contained soil residues from the previous imidacloprid applications. During flowering, nectar and pollen of *Phacelia* and mustard were sampled by honey bees in tunnels. Maize pollen was sampled manually; the same applies to guttation droplets between maize emergence and flowering. The following ranges of imidacloprid residues were determined:

Sampling of nectar and pollen from *Phacelia* and mustard crops

Honey bee colonies were placed into mesh covered tunnels erected over *Phacelia* and mustard crops a few days prior expected bloom. Honey bees were exposed to the flowering *Phacelia* and mustard under confined conditions and were exclusively used as a sampling device for both nectar and pollen at three times (in a period of approx. 10 days) during flowering of the respective crop.

Nectar was collected by honey bulb extraction from forager bees in mustard and *Phacelia* crop. For each nectar sample about 800-1000 returning forager bees were collected with a modified vacuum sampler, deep-frozen and transported to the laboratory for nectar extraction. Targeted nectar amount per sample was ≥ 500 mg.

Pollen of *Phacelia* and mustard was collected from forager bees via pollen traps attached to the bee hive entrance. The collected pollen was stored deep-frozen until residue analysis. The target sample size per tunnel and per sampling date was approximately 1.5 g pollen with a minimum requirement of approximately 750 mg.

Sampling of guttation fluid and pollen from maize

Pollen was collected three times during flowering of maize plants (BBCH 63-65). The pollen, targeted were 1.5 g per sample, collected from at least 30 plants, was shaken out of the flowers into paper bags and cleaned by sieving (mesh size 2 mm and 1 mm).
Maize guttation fluid, target 1 ml per sample, was collected daily starting at emergence of the seedlings (BBCH 11) until early flowering (BBCH 55). The samplings started at sunrise (± 15 min) lasted for a maximum of 30 min.

Findings/Conclusion

The study has been performed to cover various scenarios (crop rotations) of a consecutive use of imidacloprid and to determine the potential residue level of imidacloprid and its metabolites -5-hydroxy and –olefine in bee-relevant matrices (nectar and pollen) and guttation droplets of succeeding crops. In a model approach, two levels of imidacloprid plateau concentrations were established (information about the rates to be applied were provided by the sponsor) on an agricultural site near Zuelpich, Germany. After incorporation of the calculated plateau concentrations in September 2013, dressed winter barley seeds (again with two different seed dressing rates) were sown (see overview below):

Phacelia
Residues analysis of pollen and nectar, as collected at three time points during blooming of *Phacelia*, in three tunnels per test rate revealed in low residue levels. The residue levels of imidacloprid in nectar ranged from below the LOD (< 0.1 µg a.s./kg) to 0.49 µg a.s./kg. Residue levels of imidacloprid in pollen ranged between from below LOD (< 0.2 µg a.s./kg) to 0.62 µg a.s./kg.

Mustard
Residues analysis of pollen and nectar, as collected at three time points during blooming of mustard in three tunnels per test rate revealed in low residue levels. The residue levels of imidacloprid in nectar ranged from below LOD (< 0.1 µg a.s./L) to 0.63 µg a.s./L. Residue levels of imidacloprid in pollen ranged between from below LOQ of < 0.6 µg a.s./kg to 1 µg a.s./kg.

Maize
Residues analysis of guttation fluid, as collected from directly after emergence until early bloom of the maize plants, revealed in generally low residues. The residue levels of imidacloprid in guttation fluid ranged from below the LOD (< 1 µg a.s./L) to 26 µg a.s./L and are thus several orders of magnitude below values measured in droplets from seed treated maize plants. Residues were primarily detected at the earliest samplings after emergence and declined over time to < LOD.

The maximum residue level of imidacloprid in pollen, as sampled at three time points during bloom on three subplots was always below the LOD (< 0.2 µg a.s./kg).

Overall, transfer of imidacloprid soil residues into bee-relevant matrices and guttation droplets of succeeding crops takes place on very low levels even if calculated long-term plateau concentrations are established without ageing of residues over years. Traces of imidacloprid metabolites were only measured in single guttation samples.

Table 9.5.2-32: Residues of imidacloprid in soil (blue and green plots)

Sample material	Residue imidacloprid * [µg/kg dry soil]	
	"low" (blue plot)	**"high" (green plot)**
Soil (2013, PEC plateau)	71	140
Soil (2014, Mustard)	12 - 18	14 - 19
Soil (2014, *Phacelia*)	9 - 13	16 - 21
Soil (2014, Maize)	9 - 13	16 - 22

* calculated to dry soil

Table 9.5.2-33: Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in guttation liquid samples (blue and green plots)

Sample Material	Variant	Residue imidacloprid [µg/L]	Residue imidacloprid-5-hydroxy [µg/L]	Residue imidacloprid-olefine [µg/L]
Guttation liquid (Maize)	"low" (blue plot)	< LOD - 13	< LOD - 2	< LOD - < LOQ
Guttation liquid (Maize)	"high" (green plot)	< LOD - 26	< LOD - 11	< LOD - 2

LOQ = Limit of Quantitation = 1 µg/L for guttation liquid samples
LOD = Limit of Detection = 0.3 µg/L for guttation liquid samples

Table 9.5.2-34: Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in mustard and phacelia nectar samples (blue and green plots)

Sample material	Variant	Residue imidacloprid [µg/kg]	Residue imidacloprid-5-hydroxy [µg/kg]	Residue imidacloprid-olefine [µg/kg]
Nectar (Mustard)	"low" (blue plot)	< LOD – 0.57	< LOD	< LOD
Nectar (Phacelia)		< LOQ – 0.43	< LOD	< LOD
Nectar (Mustard)	"high" (green plot)	< LOD – 0.63	< LOD	< LOD
Nectar (*Phacelia*)		< LOD – 0.49	< LOD	< LOD

LOQ = Limit of Quantitation = 0.3 µg/kg imidacloprid in nectar samples, 1 µg/kg for imidacloprid-5-hydroxy and imidacloprid-olefine in nectar samples

LOD = Limit of Detection = 0.1 µg/kg for imidacloprid in nectar samples, 0.3 µg/kg for imidacloprid-5-hydroxy and imidacloprid-olefine in nectar samples

Table 9.5.2-35: Residues of imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefine in pollen samples (blue and green plots)

Sample material	Variant	Residue imidacloprid [µg/kg]	Residue imidacloprid-5-hydroxy [µg/kg]	Residue imidacloprid-olefine [µg/kg]
Pollen (Mustard)	"low" (blue plot)	< LOQ - 1.0	< LOD	< LOD - < LOQ
Pollen (*Phacelia*)		< LOD - < LOQ	< LOD - < LOQ	< LOD
Pollen (Maize)		< LOD	< LOD	< LOD
Pollen (Mustard)	"high" (green plot)	< LOQ	< LOD	< LOD
Pollen (*Phacelia*)		< LOD- 0.62	< LOD - <LOQ	< LOD
Pollen (Maize)		< LOD	< LOD	< LOD

LOQ = Limit of Quantitation = 0.6 µg/kg imidacloprid in/on pollen samples, 1 µg/kg for imidacloprid-5-hydroxy and imidacloprid-olefine in/on pollen samples

LOD = Limit of Detection = 0.2 µg/kg for imidacloprid in/on pollen samples, 0.3 µg/kg for imidacloprid-5-hydroxy and imidacloprid-olefine in/on pollen samples

RMS´s comments:
The study is considered acceptable for use in risk assessment.

The risk to honey bees foraging on insect honey dew

No studies on the risk to honey bees foraging on insect honey dew were submitted. Instead, the applicant submitted a statement which informs about the possible occurrence of resistance of honeydew-producing insects against plant protection products.

Report:	Nauen, R.; 2013
Title:	Statement - Information on the occurrence or possible occurrence of the development of resistance of the plant protection product Janus Forte (for submission in Europe)
Report No.:	M-453965-01-1
Document No.:	M-453965-01-1
Guideline(s):	PP1/213(2) EU Directive 91/414 EEC According to OECD format guidance for industry data submissions on plant protection products and their active substances
Guideline deviation(s):	not specified
GLP/GEP:	**no**

Resistance in arthropod pest species comprises a change in the genetic composition of a population in response to selection by pesticides such that control in the field may be impaired repeatedly at recommended application rates. The report includes resistance management information regarding key invertebrate pests targeted in sugar beet in countries such as Belgium, Czech Republic, France, Germany, Poland, Romania, Slovakia and Serbia by seed treatments with Janus Forte® (FS 280) containing the insecticidal ingredients clothianidin, imidacloprid and beta-cyfluthrin.

Janus Forte® is a mixture of three chemically different insecticides complementing each other in numerous properties and belonging to two distinct mode of action classes, i.e. acting on different molecular target-sites not yet shown to be involved in any cross-resistance issues globally.

Beta-cyfluthrin belongs to the chemical class of synthetic pyrethroids and is a well-known contact insecticide particularly for the control of coleopteran pests, e.g. *Agriotes* ssp. other elaterid soil pests. Pyrethroid insecticides such as beta-cyfluthrin are classified by IRAC (Insecticide Resistance Action Committee) in mode of action class 3A, sodium channel modulators.

Resistance to pyrethroid insecticides has been described for different crop pests and the major mechanisms of resistance were identified as either metabolic (esterases and monooxygenases) or knock-down-resistance (kdr) due to a mutation in the IIS6 domain of the voltage-gated sodium channel. All of the pest insects intended to be targeted by Beta-cyfluthrin in Janus Forte® as a seed treatment are not listed as high risk pests within EPPO´s Std. PP1/213 on resistance risk analysis and haven´t been included for a detailed survey, primarily due to a lack of any resistance issues in the past.

Clothianidin and Imidacloprid are members of the neonicotinoid class of insecticides and well established tools for the control of sucking, chewing and soil pests in seed treatment applications due to their systemic properties. They specifically control a number of coleopteran pests in sugar beet such as elaterid larvae (*Agriotes ssp*., wireworms), weevils (*Bothynoderes*), flea beetles (Chaetocnema ssp.) and *Atomaria linearis*. Other important pests targeted in sugar beet include aphid pests such as *Aphis fabae* and *Myzus persicae*, thrips (*Thrips tabaci*), dipterans (*Pegomyia*), millipedes (e.g. *Blaniulus guttulatus*) and myriapodes (e.g. *Scutigerella immaculata*). Neonicotinoid insecticides such as clothianidin and imidacloprid are classified by IRAC in mode of action class 4A, nicotinic acetylcholine receptor (nAChR) agonists.

However, very recently *M. persicae* was shown to have locally developed resistance to neonicotinoid insecticide sprays in peaches in southern France, northern Spain and northern Italy, based on a target site mutation in the nicotinic acetylcholine receptor ß-subunit. No reports are known from any secondary host species yet, including sugar beet and vegetables.

In sugar beet no resistance to clothianidin, imidacloprid and beta-cyfluthrin seed treatments is yet described for any of the pests or pest groups mentioned above, including aphid species such as *Aphis fabae* and *Myzus persicae* (particularly targeted by systemically acting clothianidin and imidacloprid). General resistance management guidelines for neonicotinoid and pyrethroid insecticides as published by IRAC are usually followed with products such as Janus Forte® and regionally adapted as necessary.

RMS´s comments:
Resistance is usually discussed in the efficacy area under IIIA1 6.2.8 but this statement could provide useful information in support of the risk assessment.

Imidacloprid as a member of the neonicotinoid class of insecticides is classified by IRAC in mode of action class 4A, nicotinic acetylcholine receptor (nAChR) agonists. Imidacloprid has a very high efficacy on aphids and therefore no aphid population build up and relevant honeydew production has to be expected. No resistance of aphids to neonicotinoids is known yet. However, recently *Myzus persicae* was shown to have developed resistance to neonicotinoid insecticide sprays in peaches in southern Europe, based on a target-site mutation in the nicotinic acetylcholine receptor ß-subunit. No neonicotinoid resistance was detected from *M. persicae* on any secondary host species yet, including sugar beet and potatoes. However, besides aphids white fly species as pest of several vegetables can build up high populations with some honeydew production especially in the greenhouse but in summer also in the field. Neonicotinoid resistance to whitefly species is common especially in European greenhouse production systems and white flies originating from greenhouses settle on field vegetables in warm summer conditions. But farmers will use other control options to avoid damage for these high valuable crops thus the build-up of high population densities and relevant honeydew production is very unlikely.

The potential uptake via roots to flowering weeds

No studies on the potential uptake via roots to flowering weeds were submitted. Instead, the applicant submitted a statement in which the occurrence of flowering weeds in agricultural crops was evaluated.

Report:	Garside, C. M.; Miles, M.; Kriszan, M. 2014
Title:	Statement - Evaluation of the occurrence of flowering weeds in agricultural crops: Cereals, sugar beet and potatoes
Report No.:	M-505126-01-1
Document No.:	M-505126-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

Objective

In this statement, the occurrence of flowering weeds in cereals, sugar beet and potatoes has been investigated based on data from (herbicide) efficacy trials, to be able to assess the potential relevance of flowering weeds as a source of exposure for honey bees.

Material and Methods

The occurrence of weeds in insecticide efficacy trials is not recorded as a standard requirement; however the applicant also performs extensive efficacy trials on herbicidal active ingredients. In these trials the occurrence of weeds, both on control plots and in the treated plots is recorded. Parameters including the identity of the weed, the growth stage and the coverage of the test-plot are recorded.

To analyse the presence of weeds in agricultural crops the available data was extracted from the database for the crops cereals, sugar beet, and potatoes. As a conservative assessment only

the data in the control plots (i.e. no herbicide treatment) was considered to provide a worst-case situation.

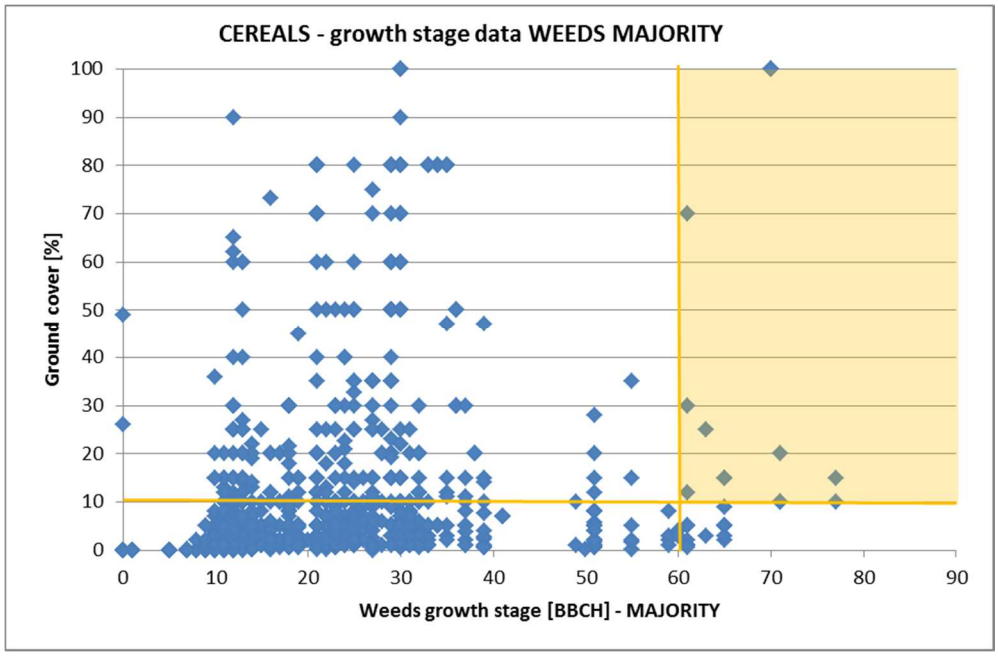
All data originate from worldwide herbicide efficacy trials testing for herbicides, in cereals (Atlantis® and Herold®), in sugar beet data (Betanal MAXXPro®), and in potatoes (Metribuzin) conducted between 2004 and 2014 has been compiled. The majority of the studies were carried out in Europe; however for completeness of the datasets trials performed outside Europe were also included. Information on weed species, weed growth stages (BBCH), weed diameter (cm), weed ground cover (%), and weed plants/m^2 were recorded. Each weed species per trial was recorded separately, thus there are several data set entries per trial. All data are mean values out of 2 to 4 plot replicates.

Since not all information was consistently provided in all trials, data was sorted to consider only cases including at least information on growth stage and ground cover. The weed growth stage classification "Majority", which represents the growth stage of the majority of the weed species on the plot, was taken into account. The cereals data were combined to make a single dataset.

Results

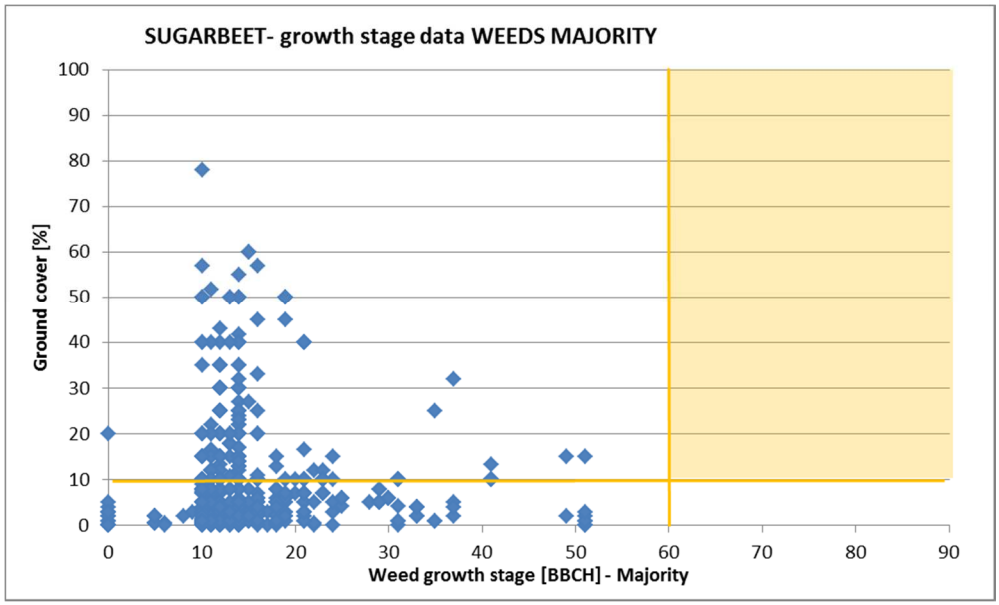
To show how often and to which extent flowering weeds cover the plots the dataset was edited for graphical representation. Hence the weed growth stage data was plotted against the corresponding ground cover data.

Data points in the yellow labelled box at top right indicate weeds at BBCH stage ≥ 60 (flowering) and ≥ 10 % ground cover (the EFSA guidance states that if <10% of the area of use is flowering weeds then the exposure route is not relevant in the 90th %ile case, and thus does not need to be considered). This combination of weed growth stage and coverage was considered to be the minimum requirement to identify situation which have the potential to be attractive to foraging bees.

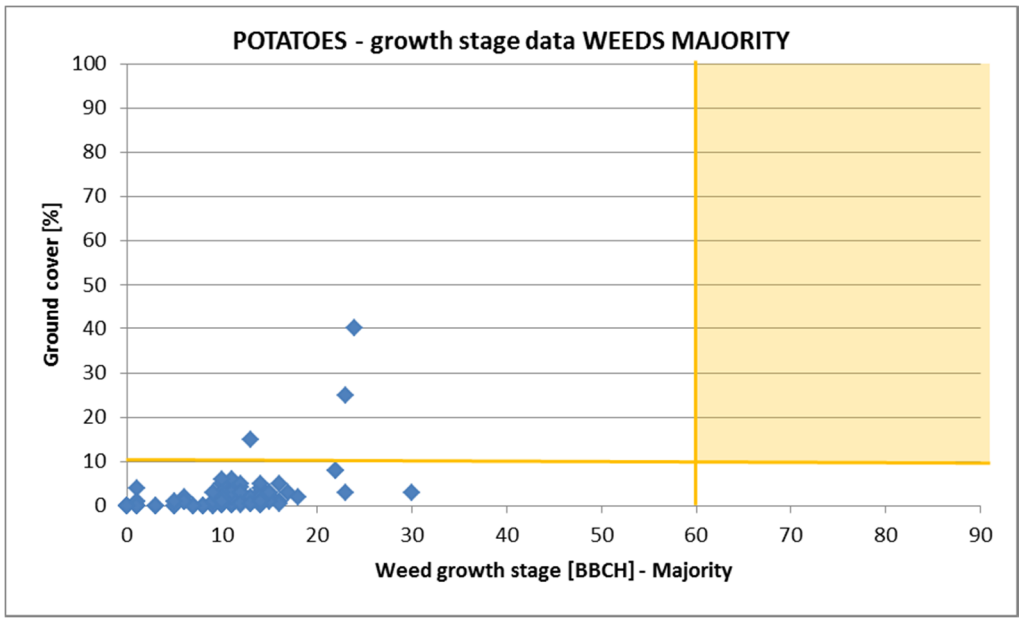


Flowering weeds exceeding 10% ground cover were only observed in 14 incidents out of 2327 observations (i.e. 0.6 %). In the majority of these cases (13 out of 14) the weeds present were small species that did not rely on bee pollination for reproduction or produce sufficient quantities of pollen and nectar to be considered a food source. Only one case was possibly

relevant but only under certain circumstances and represented only 0.04 % of all cases observed. Consequently, exposure via flowering weeds is confirmed not to be a relevant route of exposure for honey bees or non-*Apis* bees in cereal crops.



In the trials with sugar beet there were no flowering weeds present on the control plots, where no herbicide was used, confirming that this is not a relevant route of exposure for honey bees or non-*Apis* bees in these crops.



In the trials with potatoes there were no flowering weeds present on the control plots, where no herbicide was used, confirming that this is not a relevant route of exposure for honey bees or non-*Apis* bees in these crops.

Conclusions

The analysis performed here indicates that even on experimental plots not treated with herbicide (considered to be a worst case situation), cereal, sugar beet and potato fields do not provide sufficient floral food resources for bees. In sugar beet and potato flowering weeds greater than 10 % ground cover were not observed at all and only observed in 0.6 % of the trials in cereals.

The possible reason for the difference between cereals and sugar beet and potato scenarios is most likely due to the cultivation and seed bed preparation techniques required for each crop. Cereals can be grown on a wide variety of soils and do not require extensive cultivation to establish a suitable seed bed. In contrast sugar beet and potato crops have more specific requirements in terms of soil and seed bed preparation. For sugar (and other) beets deep ploughing is necessary prior to sowing to create the right growing conditions. For potatoes good ground preparation (harrowing, ploughing and rolling) is always needed and the ground can be ploughed up to three times to create the correct growing conditions. These cultivation practices reduce the presence of flowering weeds in sugar beet and potato crops.

It is concluded that exposure to flowering weeds present in cereal, sugar beet and potato crops is not a relevant route of exposure for honey bees or non-*Apis* bees.

Note: At the PPR Meeting 145 certain questions could not be answered regarding the database of the statement on the evaluation of the occurrence of flowering weeds in agricultural crops (Garside et al., M-505126-01-1). Therefore, the RMS for clothianidin and the RMS for imidacloprid kindly asks the applicant to provide a response related to the issues listed below.

1. The number of plots taken into account for the analysis and graphical representation of the data
2. Number of observations and observation timing (crop BBCH stage)
3. The graphical representation of the results

Report:	Exeler, N. 2016
Title:	Statement - Clothianidin / Imidacloprid confirmatory data: Bayer CropScience response to questions following Pesticides Peer Review Meeting 145 – Flowering weeds
Report No.:	M-505126-01-1
Document No.:	M-557823-01-1

1. The number of plots taken into account for the analysis and graphical representation of the data

Of available trials only those which recorded the BBCH stage of the weed, as well as the percentage of cover of the weeds, have been included in the analysis. This resulted in the following number of trials being included in the analysis:

Table 9.5.2-35b: Number of trials being included in the analysis

Crop	Number of trials	Number of weeds
Cereals	344	2327
Sugar beet	45	972
Potatoes	44	236

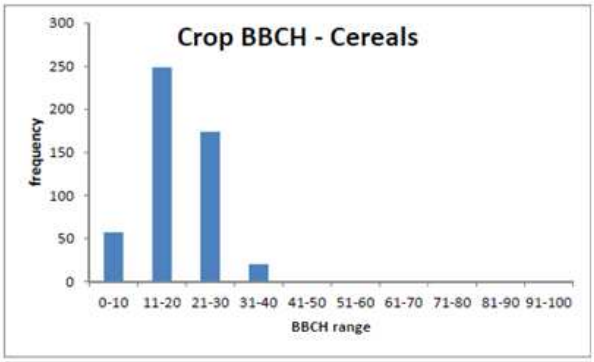
Table 9.5.2-35c: Number of trials in EU and non-EU countries

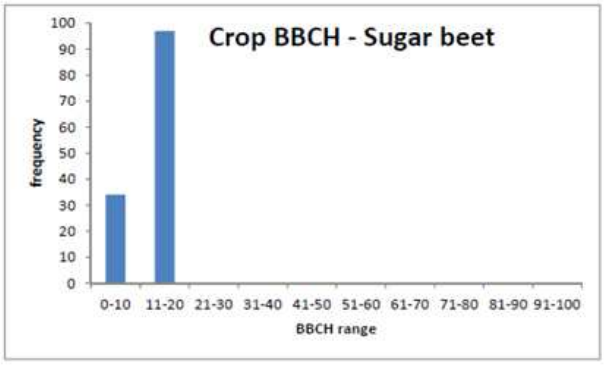
	Cereals	Sugar beet	Potatoes	Total
Austria	8	4	0	12
Belgium	17	1	0	18

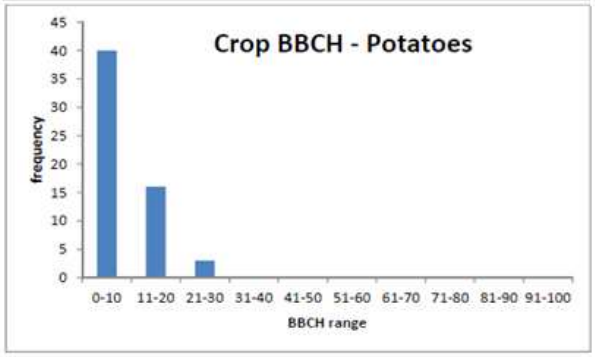
Bulgaria	2	0	0	2
Czech Republic	4	1	2	7
France	25	7	3	35
Germany	216	11	17	244
Greece	9	0	3	12
Italy	11	1	0	12
Lithuania	0	3	0	3
Poland	31	8	4	43
Slovakia	1	0	0	1
Spain	3	1	0	4
Sweden	1	0	2	3
Switzerland	1	0	1	2
Ukraine	0	1	0	1
United Kingdom	14	7	7	28
Brazil	0	0	1	1
Canada	1	0	4	5

2. Number of observations and observation timing (crop BBCH stage)

The number of assessments per trial in cereals was between 1 and 4. In sugar beet 1 to 5 assessments per trial were conducted and in potatoes 1 to 4 assessments were performed per trial.







3. The graphical representation of the results

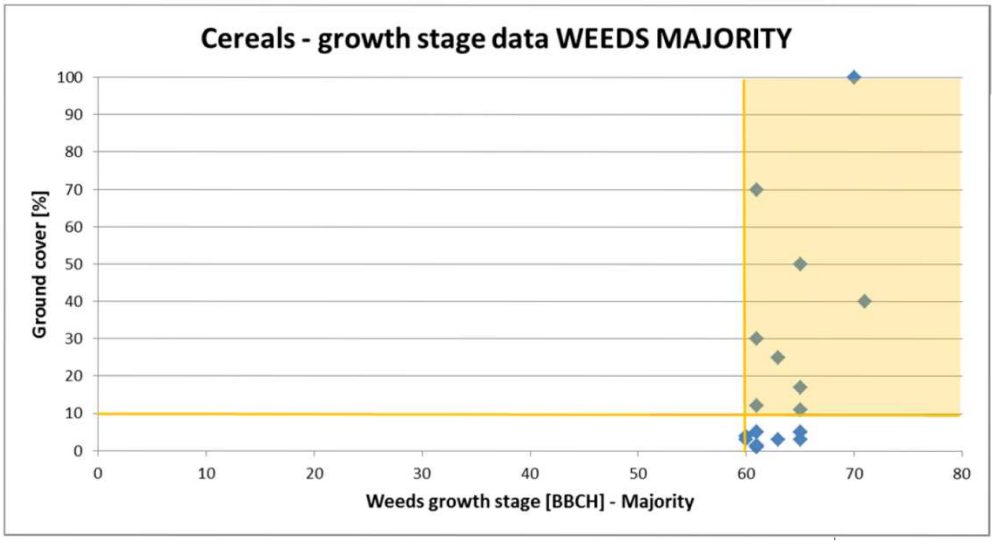
First question RMS: Based on the information in the study report, it is not clear whether the data points in the graphs represent the total ground cover (%) for one individual weed species or the average ground cover for all weed species present at one trials site.

Response applicant: Each trial has 1-4 replicate plots, the points in the graph represent the average ground cover (%) of a weed species that was recorded at one assessment.

Secound question RMS: To compare the data with the 10% trigger from the EFSA Guidance Document for bees (not a relevant route of exposure if < 10% of the area of use is covered in

attractive weeds), the total ground cover of all flowering weeds (all species) present at each field site should be known instead of the ground cover for each individual weed species. Could you therefore please calculate the total ground cover of all flowering weeds (at a certain BBCH stage) present at each field site, and provide a graphical representation of these data (similar to the graphs already included in the report).

Response applicant: Only in the trials conducted for cereals, flowering weeds = BBCH > 60 were present and thus attractive for bees. For these trials (n=23) the total ground cover of all weed species (BBCH >60) recorded in one trial was calculated and shown in the following figure. This alternative analysis resulted in only 9 trials, out of the total of 344 having > 10% coverage of flowering weeds (i.e. <3%), the nature of the flowering weeds present was discussed in the original report. These trials are not shown on the following figure, however it should be considered that 321 trials would be outside of the yellow box.



RMS´s comments:

Usually these data will be discussed in the efficacy area under B.3.1 or B.3.2 but in this case it could provide useful information in support of the risk assessment.

The conclusions are based on a large quantity of data and principle, the methodology is valid. It was noted that most of the studies were carried out in Germany and some of the data were collected outside of Europe. However, since the experiments were conducted in order to investigate effectiveness, no data were collected right before harvest (last scoring: cereals BBCH 40, sugar beet BBCH 20, potatoes BBCH 30). Hence, there is no information on what happened to flowering weeds during the period after the last sampling and before harvest. For the methodologically correct determination of the probability and abundance of flowering weed, a monitoring is necessary.

The risk to pollinators other than honey bees

Two new higher tier studies with bumble bees were submitted. These studies examined the effects of potential exposure of bumble bees to residues of imidacloprid following the use of the active substance as an in-furrow application on potatoes.

Report:	Klein, O.; 2014a
Title:	Final report - A field study to evaluate effects of Monceren G on the bumble bee (*Bombus terrestris* L; Hymenoptera, Apidae) in potato in southern Germany in 2014

Report No.:	S14-03553
Document No.:	M-503597-01-1
Guideline(s):	No specific guidelines are available. The test design is based on: SETAC/ESCORT recommendations (BARRETT et al. 1994) OEPP/EPPO Guideline No. 170 (4), 2010
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective

The objective of this study was to determine the effects of exposure of bumble bees (*Bombus terrestris* L.) to Monceren G (active ingredients: imidacloprid + pencycuron) under field conditions on potato in Germany 2014.

Potato plants (*Solanum tuberosum* L.), grown from seed tubers, treated with Monceren G (active ingredients: imidacloprid + pencycuron) at a rate corresponding to nominally 1.5 L product/ha (equivalent to 180 g imidacloprid/ha and 375 g pencycuron/ha), were planted on a field plot near Stutensee-Blankenloch, in the region Baden-Württemberg, Germany, in spring 2014. This treated field plot was matched with a similar-sized control field plot near Stutensee-Spöck, in the region Baden-Württemberg, Germany. Untreated seed tubers were planted on the control field. The sizes of the field plots were 1.84 ha for the control field and 1.85 ha for the test item treated field. Planting of the potato seed tubers was conducted on 4 Apr 2014 at both fields. The field plots were separated by approximately 3.6 km in order to exclude that bees from one treatment group visit the field of the control group and vice versa.

Bumble bee colonies (6 per treatment) reared for commercial purposes, with modifications by the supplier to account for the needs of this study (without cotton cover and specific number of workers), were placed at the field sites (C and T) as soon as the potato plants started flowering (BBCH 62) and were exposed to the flowering potato crop until end of flowering (BBCH 69). The mortality, flight activity within the crop, flight activity at the entrances of the hives, sugar consumption of the bumble bees and the weight of the hives were assessed regularly after set up of the colonies at the field sites during the exposure phase. During the monitoring phase (end of potato flowering until peak development of the colonies), bumble bee mortality was determined and production of young queens, drones and workers was assessed. The conditions of the bumble bee colonies were evaluated by an initial brood assessment before set up of the colonies, determination of the sugar consumption and assessment of the colony weight during the exposure phase and at the end of the monitoring phase by a final brood assessment.

Potato pollen samples collected by forager bumble bees (taken from four separate bumble bee colonies for residue sampling) were taken 3 times (5DAE, 12DAE, 15DAE) after start of flowering for subsequent residue- and palynological analysis.

The influence of the test item was evaluated by comparing the results in the test item treatment to the corresponding control.

Material and methods

1. Test material:

Crop	Potato plants (grown from seed tubers)

Test item	Monceren G (active ingredient: 120 g/L imidacloprid + 250 g/L pencycuron)
Description	FS, liquid, red
Purity	Imidacloprid nominal: 120.0 g a.s./L analysed: 120.5 g a.s./L Pencycuron nominal: 250.0 g a.s./L analysed: 251.2 g a.s./L
Application	Applied as an in-furrow application at planting at a rate corresponding to nominally 1.5 L product/ha
2. Vehicle and control:	
Control	Untreated potato seed-tubers
3. Test animals:	
Species	Bumble bee (*Bombus terrestris* L.)
Colony size	The bumble bee colonies (12 colonies, 6 per field site) that were used for biological assessments contained in average 100.5 alive workers. The four colonies per field site that were used for residue sampling contained at least 100 workers each.
Source	Name of supplier: Sven Behr (Pollination Management) Moorweg 18 21261 Welle, Germany Origin: Koppert B.V. Postbus 155 2650 AD Berkel en Rodenrijs The Netherlands
4. Observations:	
Foraging	During the exposure period, the foraging activity in the crop of bumble bees were assessed. At each assessment date, the number of bumble bees that was both foraging on flowers and flying over the crop in the three observation areas was counted for 10 minutes per marked square (4 m^2).
Behaviour	During the exposure period, the flight activity at the entrance of the colonies and behaviour of bumble bees was assessed. Assessments started at 0 DAE and were continued at 1, 2, 5, 8, 11 and 14 DAE until the end of potato flowering.

Colony conditions	Condition of the colony, weight of the hives and sugar solution consumption Condition of the colony after end of monitoring phase (number of eggs, larvae, pupae, queens, males, filled/empty nectar and pollen cells)

Results

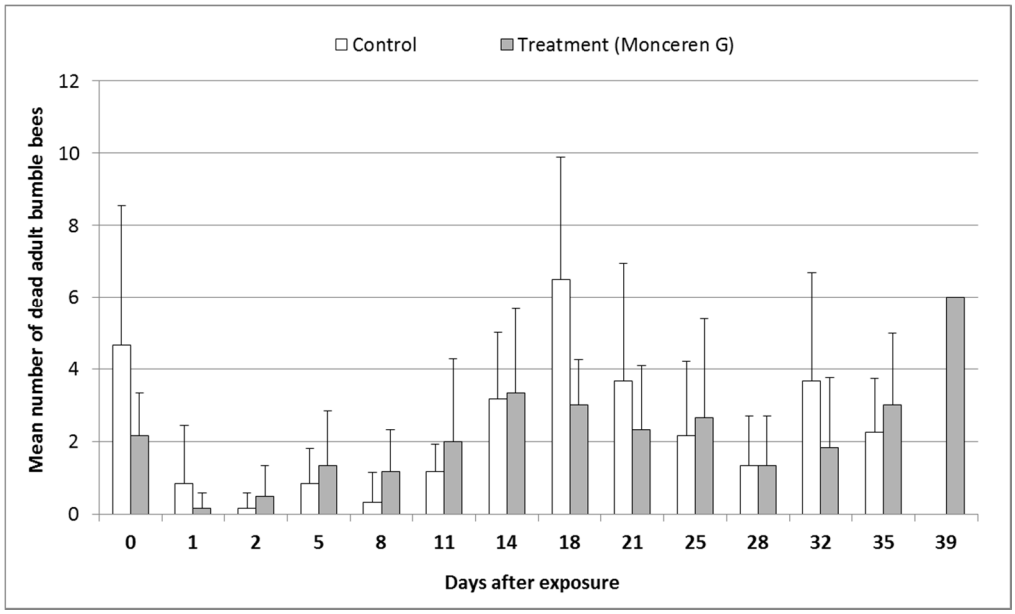
Mortality

Mortality of Adult Bumble Bees

Generally, mortality values were low in both treatment groups. At the beginning of the exposure phase, the mortality of adult bees was higher probably due to the stress caused by transport and initial brood assessment. The mortality values did not indicate any statistically significant differences between the control and the test item treatment. Thus, no statistically significant treatment related adverse effects on bumble bee mortality were observed (see table and figure below)**.**

Table 9.5.2-36: Mean number of dead adult bumble bees

Treatment group	Control (C)	Test item (T)
Mean exposure phase	1.6	1.5
Total sum of means exposure phase	11.2	10.7
Mean post-exposure phase	3.3	2.9
Total sum of means post-exposure phase	19.6	20.2
Total mean over all phases	2.4	2.2
Total sum of means over all phases	30.8	30.8



* =statistically significant difference to control (t-test ($p \leq 0.05$))

Figure 9.5.2-37: Mortality of adult bumble bees: Mean numbers of dead bumble bees within the hives

Mortality of Larvae

At the beginning of the exposure phase, the mortality was rather low for the first seven assessment dates. At the end of the exposure phase, the mortality increased slightly. This increase of dead larvae might be caused by the decreased food availability at the end of potato flowering. Higher larval mortality was observed at the monitoring site in both treatment groups. The larval mortality values did not show any statistically significant differences between the control and the test item treatment. Thus, no treatment related adverse effects on mortality of larvae were observed (see table and figure below)**.**

Table 9.5.2-37: Mean number of dead bumble bee larvae

Treatment group	Control (C)	Test item (T)
Mean exposure phase	0.5	0.9
Total sum of means exposure phase	3.3	6.5
Mean post-exposure phase	14.4	12.9
Total sum of means post-exposure phase	86.6	90.0
Total mean over all phases	6.9	6.9
Total sum of means over all phases	89.9	96.5

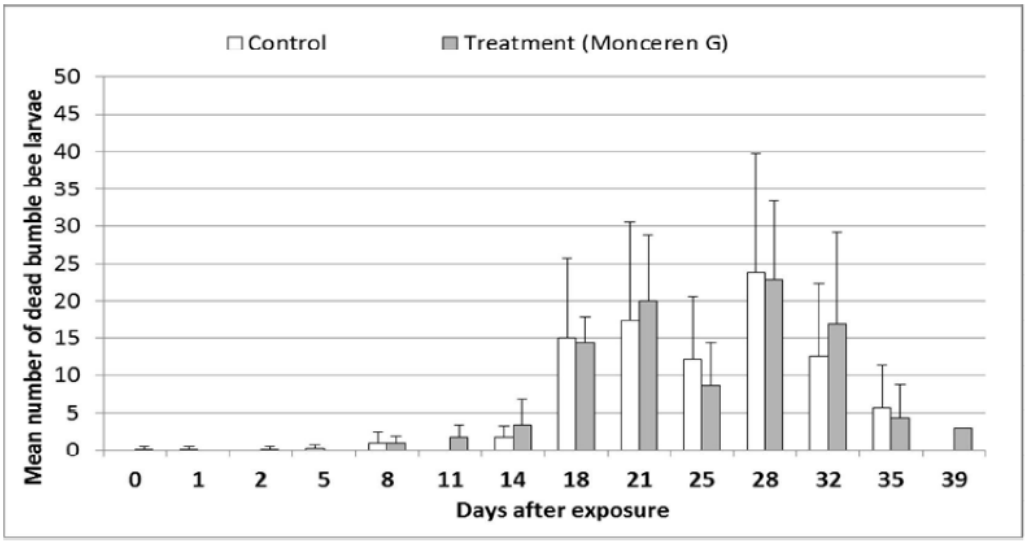
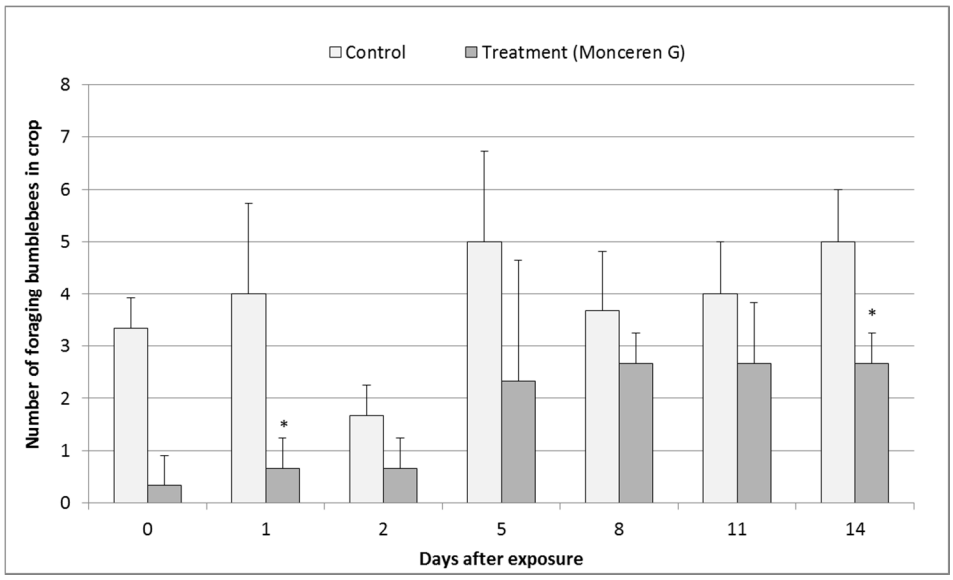


Figure 9.5.2-38: Mortality of larvae: Mean numbers of dead bumble bee larvae within the hives

Flight activity in the crop

The mean number of the flight activity in the crop for was 3.8 bumble bees/4 m^2/10 minutes and 1.7 bumble bees/4 m^2/10 minutes for the control site and the treated field respectively. The overall flight activity showed statistically significant lower flight activity for the test item treatment. Two statistically significant differences were observed at single assessment dates (1 DAE and at 14 DAE) where the foraging activity was statistically significant higher at the control field (see figure below)**.**

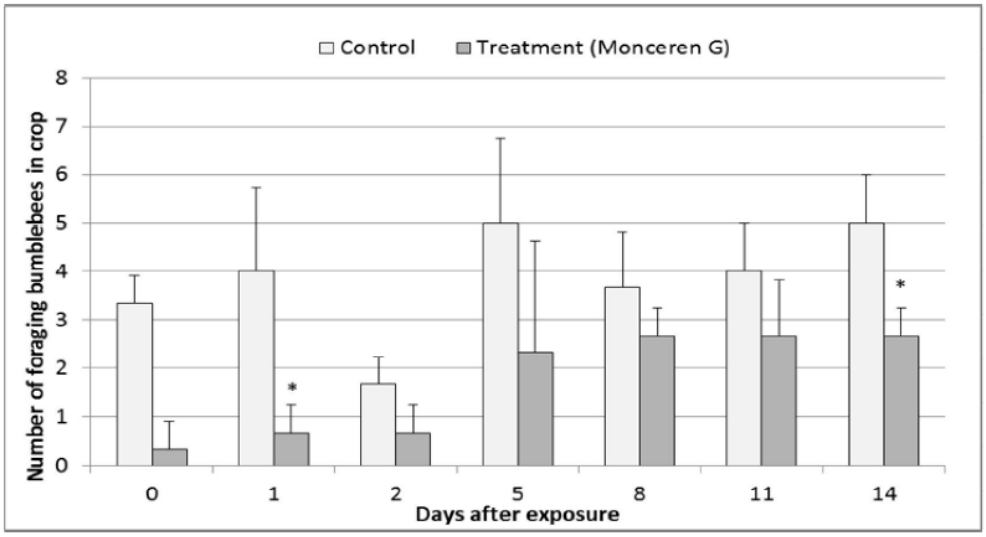


* = statistically significant difference to control (t-test ($p \leq 0.05$))

Figure 9.5.2-39: Flight activity in the crop: Mean flight activity in the crop per 4 m^2/10 min during the exposure phase

Foraging activity at the entrances of the hives

The mean number of bumble bees entering the hives was 10.5 bumble bees/hive for the control field and 7.4 bumble bees/hive for the treated field per 15 minutes. Statistically significant differences were observed at single assessment dates (2 DAE and 14 DAE) where the number of entering bees was higher at the control field site. For the other assessment days no significant differences were observed. The overall mean flight activity was slightly lower for the test item but no statistically significant difference was found (see figure below).

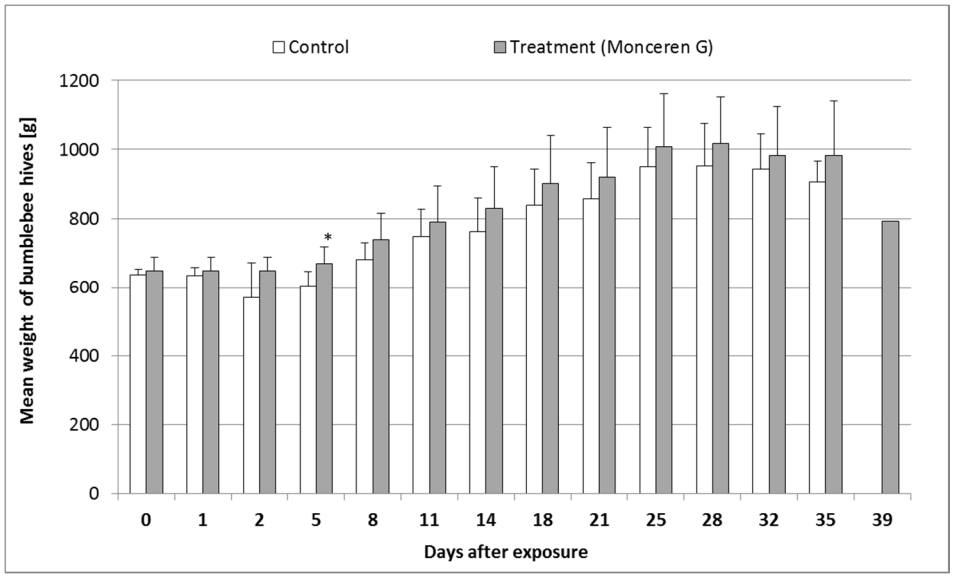


* = statistically significant difference to control (t-test ($p \leq 0.05$))

Figure 9.5.2-40: Flight activity at the entrances of the hives: Mean numbers of bumble bees entering the colonies

Assessment of the hive weight

The mean weight of the hives in the control field was 661.9 g and 710.3 g for the hives in the treated field site during the whole exposure phase (0-14 DAE). The weight increase during the exposure phase was 123.5 g and 181.8 g for the control and test item treatment, respectively. During the post exposure phase the mean weight of the hives in the control field was 907.6 g and 943.0 g for the hives in the treated field site. The weight increase during the post-exposure phase was 93.0 g and 61.7 g for the control and test item treatment, respectively. Total mean weight increases (means calculated from weight increases of single hives) were 294.3 g for the control field site and 314.8 g for the test item treatment. The weight development of the hives showed no statistically significant treatment related adverse effects. 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. It can be concluded that as 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 rather than worker brood.



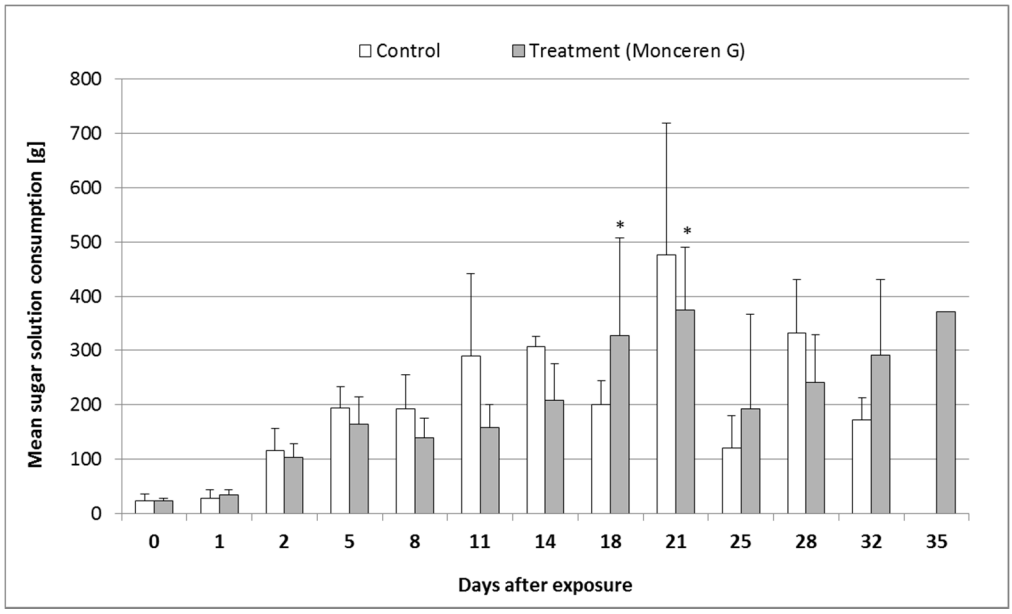
* = statistically significant difference to control (t-test ($p \leq 0.05$))

Figure 9.5.2-41: Assessment of hive weight: Mean weights of the hives of the control and the test item treatment

Assessment of sugar consumption

The mean sugar solution consumption was 840.0 g in the control field and 621.7 g for the hives in the treated field site during the exposure phase (until 14 DAE) and 1607.5 g in control and 1951.0 g for the hives in the test item treated during the post-exposure phase (18 to 39 DAE). The total mean sugar solution consumption until the end of the monitoring phase was 2447.5 g and 2572.7 g for the colonies of the control and the test item treatment, respectively.

At two assessment dates statistically significant differences in sugar solution consumption were observed (18 DAE and 21 DAE). As a significant decrease was followed by a significant increase in sugar solution consumption in the test item treatment, and due to honey bees observed in the bumble bee colonies consuming sugar solution, it is concluded that, no treatment related adverse effects on sugar solution consumption were observed.



* = statistically significant difference to control (14 DAE t-test ($p \leq 0.05$), 21 DAE Mann Whitney Exact ($p \leq 0.05$))

Figure 9.5.2-42: Assessment of sugar consumption: Mean consumption of sugar solution

Condition of the colonies

The results of the final brood evaluation did not show any statistically significant differences between the control and the test item treatment. A slight but not significant trend was observed for the number of bumble bee individuals, which was slightly higher in test item colony group. Also with regard to the queen production, the number of produced young queens (larvae, pupae and adults) was slightly higher in the test item treatment. No statistically significant treatment related adverse effects on the numbers of young queens, workers, males, eggs, larvae (queen and worker) and pupae (queen and worker) were observed. No statistically significant treatment related adverse effects on the number of filled nectar and pollen cells, total number of live brood, live adults, the total queen reproduction (larvae, pupae and adults) and the total number of living individuals were observed.

Pollen source analysis

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. 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 had taken place in the treated field site. The differences between the control and treated sites in terms of in-crop activity and foraging activity measured at hive entrance could relate to the collection of potato pollen observed at the treated site.

Table 9.5.2-38: Results of the forager bumble bee pollen analysis

% of potato pollen in pollen samples of forager bumble bees		
Sampling date	**C**	**T**
5 DAE	0.0	24.8
12 DAE	0.0	56.3
15 DAE	0.0	54.8

Residue analysis

Residue analysis was carried out on pollen samples collected from forager bumble bees at 5, 12 and 15 days after exposure (DAE). No residues of imidacloprid and its metabolites (imidacloprid-5-hydroxy and imidacloprid olefine) were detected in pollen from the control field (<LOD (< 0.2/0.3 µg/kg)). Residue levels in samples from the treated field at the sampling dates 5 DAE and 12 DAE were below the limit of quantification (LOQ=0.6 µg/kg) for the parent imidacloprid and below the limit of detection (LOD=0.3 µg/kg) for the analysed metabolites. The maximum residue level of imidacloprid of 1.4 µg/kg was found at the sampling date 15 DAE. At that sampling date, the residue level of imidacloprid-5-hydroxy and imidacloprid olefine was below LOQ and below LOD, respectively.

Table 9.5.2-39: Residues of imidacloprid and its metabolites in potato pollen

Treatment group	Sampling date	Residues [µg/kg]		
		Imidacloprid	Imidacloprid-5-hydroxy	Imidacloprid olefine
C	5 DAE	< LOD	< LOD	< LOD
	12 DAE	< LOD	< LOD	< LOD
	15 DAE	< LOD	< LOD	< LOD
T	5 DAE	< LOQ	< LOD	< LOD
	12 DAE	< LOQ	< LOD	< LOD
	15 DAE	1.4	< LOQ	< LOD

DAE = days after exposure
LOQ = limit of quantification = 0.6 µg/kg for imidacloprid, 1.0 µg/kg for imidacloprid metabolites
LOD = limit of detection = 0.2 µg/kg for imidacloprid, 0.3 µg/kg for imidacloprid metabolites

Conclusions

It can be concluded that the use of Monceren G (applied at rates of 180 g imidacloprid/ha and 375 g pencycuron/ha) at potato planting has no adverse effects on the behaviour and development of bumble bee colonies exposed during bloom.

RMS´s comments:

While there are currently no official guidelines are available for higher tier tests with bumble bees, the study is considered well performed and suitable for risk assessment. In the study with in-furrow treatment of potatoes at a rate of 170 g imidacloprid/ha and 355 g pencycuron/ha, no clear test item related effects were observed. However, in the study, higher numbers of bumble bees foraging on the potato crop were observed in the control compared to the treatment, but in stored and analysed pollen stores no potato pollen was found in the controls, while in the treatment group up to 56,3% of pollen was found. The reason for this is unknown, but is unlikely to have an adverse effect on the study or the interpretation itself, apart from the fact this study cannot distinguish between effects of potato pollen and imidacloprid. The imidacloprid residue levels in pollen measured in the treatment field were low. For each variant, the control field and the test field only one sample per sampling day and only three sampling days were analysed. Further clarification on the origin of the analysed pollen is sought from the applicant. As the palynological determination in the hive demonstrated also other pollen sources, clarification is needed if palynological determination of the pollen collected from homing foragers was conducted. Both during the exposure phase as well at study termination in the final brood assessment no treatment related differences were observed for mortality of adults and larvae and for the total amount of brood stages and total queen and drone production at study termination.

Since there were no adverse effects observed in the treated colonies as compared to colonies not feeding on potato, it can be concluded that exposure to Monceren-G-treated potato pollen did not result in adverse effects on bumble bee colonies under the conditions of this study.

Report:	Klein, O.; 2014b
Title:	A field study to evaluate effects of Monceren G on the bumble bee (*Bombus terrestris* L; Hymenoptera, Apidae) in potato in southern Germany in 2014
Report No.:	S14-03554
Document No.:	M-504174-01-1
Guideline(s):	No specific guidelines are available. The test design is based on: SETAC/ESCORT recommendations (BARRETT et al. 1994) OEPP/EPPO Guideline No. 170 (4), 2010
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:
The objective of this study was to determine the effects of exposure of bumble bees (*Bombus terrestris* L.) to Monceren G (active ingredients: imidacloprid + pencycuron) under field conditions on potato in Germany 2014.

Potato plants (*Solanum tuberosum* L.), grown from seed tubers, treated with Monceren G (active ingredients: imidacloprid + pencycuron) at a rate corresponding to nominally 1.5 L product/ha (equivalent to 180 g imidacloprid/ha and 375 g pencycuron/ha), were planted on a field plot near Neckarwestheim, in the region Baden-Württemberg, Germany, in spring 2014. This treated field plot was matched with a similar-sized control field plot near Brackenheim, in the region Baden-Württemberg, Germany. Untreated seed tubers were planted on the control field. The sizes of the field plots were 1.6 ha for the control field and 1.6 ha for the test item treated field. Planting of the potato seed tubers was conducted on 16 Apr 2014 at both fields. The field plots were separated by approximately 8.5 km in order to exclude that bees from one treatment group visit the field of the control group and vice versa.

Bumble bee colonies (6 per treatment) reared for commercial purposes, with modifications by the supplier to account for the needs of this study (without cotton cover and specific number of workers), were placed at the field sites (C and T) as soon as the potato plants started flowering (BBCH 62) and were exposed to the flowering potato crop until end of flowering (BBCH 69). The mortality, flight activity within the crop, flight activity at the entrances of the hives, sugar consumption of the bumble bees and the weight of the hives were assessed regularly after set up of the colonies at the field sites during the exposure phase. During the monitoring phase (end of potato flowering until peak development of the colonies), bumble bee mortality was determined and production of young queens, drones and workers was assessed. The conditions of the bumble bee colonies were evaluated by an initial brood assessment before set up of the colonies, determination of the sugar consumption and assessment of the colony weight during the exposure phase and at the end of the monitoring phase by a final brood assessment.

Potato pollen samples collected by forager bumble bees (taken from four separate bumble bee colonies for residue sampling) were taken 3 times (5DAE, 12DAE, 16DAE) after start of flowering for subsequent residue- and palynological analysis.

The influence of the test item was evaluated by comparing the results in the test item treatment to the corresponding control.

Material and methods

1. Test material:

Crop	Potato plants (grown from seed tubers)
Test item	Monceren G (active ingredient: 120 g/L imidacloprid + 250 g/L pencycuron)
Description	FS, liquid, red
Purity	Imidacloprid nominal: 120.0 g a.s./L analysed: 120.5 g a.s./L Pencycuron nominal: 250.0 g a.s./L analysed: 251.2 g a.s./L
Application	The application was done at a separate study S14-01392. The insecticide Monceren G was applied as in-furrow application at planting at a rate corresponding to nominally 1.5 L product/ha (equivalent to 180 g imidacloprid/ha and 375 g pencycuron/ha) under field conditions on potato (*Solanum tuberosum* L.).

2. Vehicle and control:

Control	Untreated potato seed-tubers

3. Test animals:

Species	Bumble bee (*Bombus terrestris* L.)
Colony size	Biological assessments contained in average 41.5 alive workers. The four colonies per field site that were used for residue sampling contained at least 100 workers each.
Source	Name of supplier: Sven Behr (Pollination Management) Moorweg 18 21261 Welle, Germany Origin: Koppert B.V. Postbus 155 2650 AD Berkel en Rodenrijs The Netherlands

4. Observations:

Foraging	At each assessment date, the number of bumble bees that were both foraging on flowers and flying over the crop in

	the three observation areas was counted for 10 minutes per marked square (4 m2). Assessments started at 0 DAE and were continued at 1, 2, 5, 8, 11 and 14 DAE until the end of potato flowering.
Behaviour	At each assessment date, the number of bumble bees that were entering the colony entrance was counted on two occasions. The observation time was fifteen minutes per occasion and per colony.
Colony conditions	Condition of the colony, weight of the hives and sugar solution consumption Condition of the colony after end of monitoring phase (number of eggs, larvae, pupae, queens, males, filled/empty nectar and pollen cells)

Findings

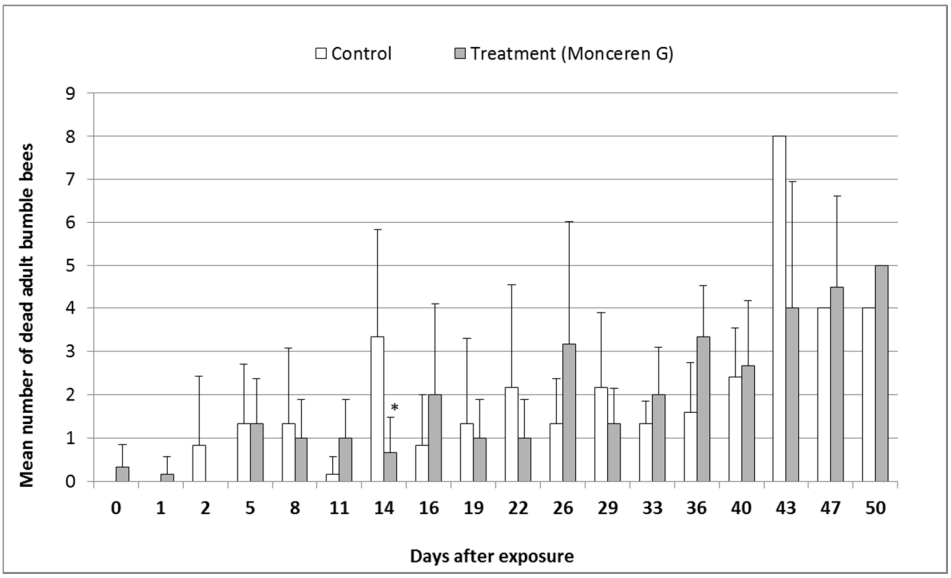
Mortality

Mortality of Adult Bumble Bees

Generally, mortality values during the exposure were low in both treatment groups. Mortality increased during the monitoring phase. This mortality is considered to be caused by the age of the bumble bee hives and their advanced development including the reproduction phase. Thus, no statistically significant treatment related adverse effects on adult bumble bee mortality were observed. For the last assessment date during the exposure phase (14 DAE), a statistically significant lower mortality of adult bumble bees was observed in the test item treatment. If the total mortality (adults and larvae) is considered, no significant difference was observed (see table and figure below).

Table 9.5.2-40: Mean number of dead adult bumble bees

Treatment group	Control (C)	Test item (T)
Mean exposure phase	1.0	0.6
Total sum of means exposure phase	7.0	4.5
Mean post-exposure phase	2.6	2.7
Total sum of means post-exposure phase	28.8	30.0
Total mean over all phases	2.0	1.9
Total sum of means over all phases	35.8	34.5



* =statistically significant difference to control (t-test ($p \leq 0.05$))

Figure 9.5.2-43: Mortality of adult bumble bees: Mean numbers of dead bumble bees within the hives

Mortality of Larvae:
At the beginning of the exposure phase and for the first three assessment dates during the monitoring phase the mortality was generally low. Higher larval mortality was observed at the monitoring site in both treatment groups. The mortality values per assessment date did not show any statistically significant differences between the control and the test item treatment, thus no statistically significant treatment related adverse effects on mortality of larvae were observed (see table and figure below). 29 Days after exposure an unusual high mortality was observed in all colonies of the control, however the reason remains unclear and this phenomenon was only observed on this specific assessment data.

Table 9.5.2-41: Mean number of dead bumble bee larvae

Treatment group	Control (C)	Test item (T)
Mean exposure phase	0.7	0.8
Total sum of means exposure phase	4.8	5.7
Mean post-exposure phase	9.5	5.6
Total sum of means post-exposure phase	104.5	61.6
Total mean over all phases	6.1	3.7
Total sum of means over all phases	109.3	67.3

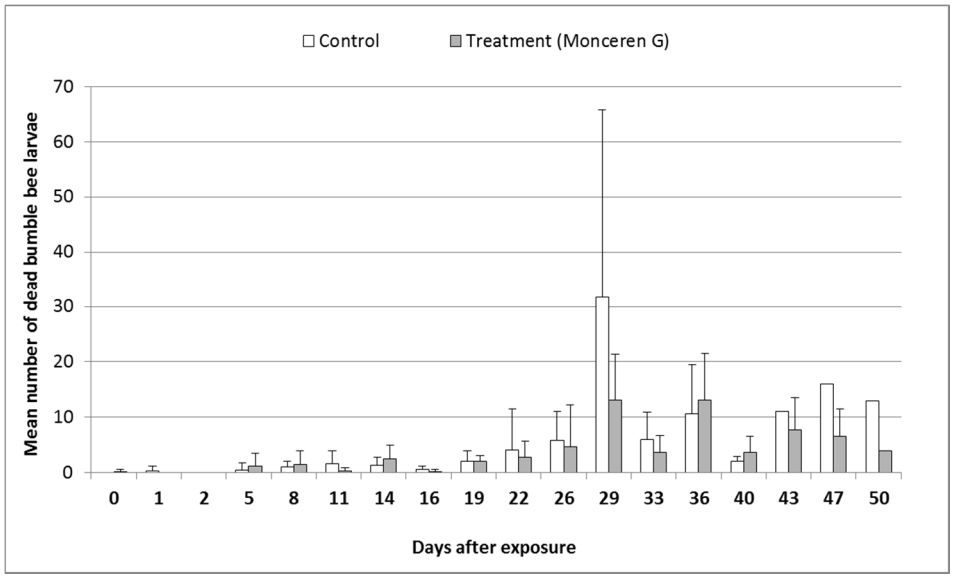
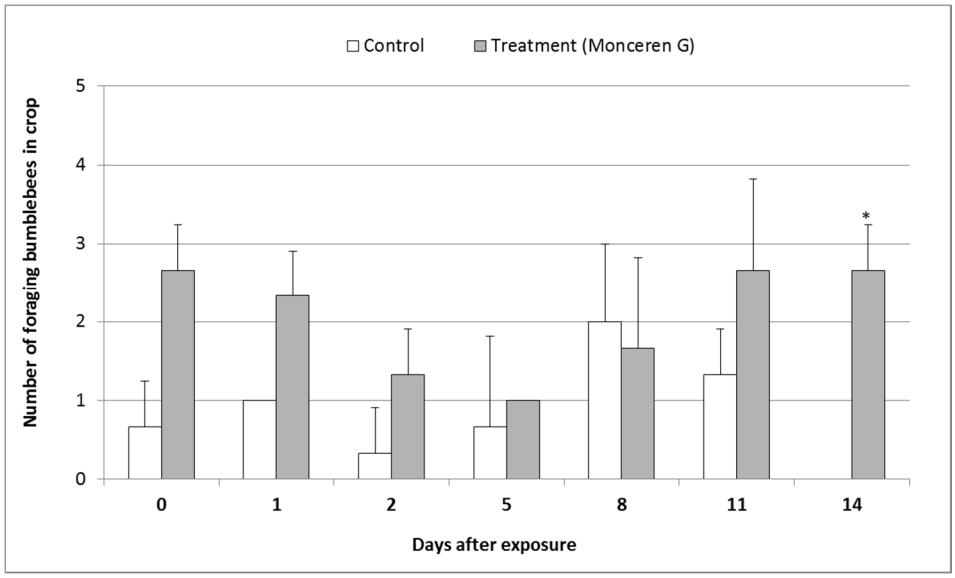


Figure 9.5.2-44: Mortality of larvae: Mean numbers of dead bumble bee larvae within the hives

Flight activity in the crop

The mean number of the flight activity in the crop for the control field was 0.9 bumble bees/4 m^2/10 minutes and 2.0 bumble bees/4 m^2/10 minutes for the treated field. One statistically significant difference was detected at 14 DAE where the flight activity at the treated field site was statistically significant higher compared to the control field site. Generally, the flight activity at the treated field site was higher.



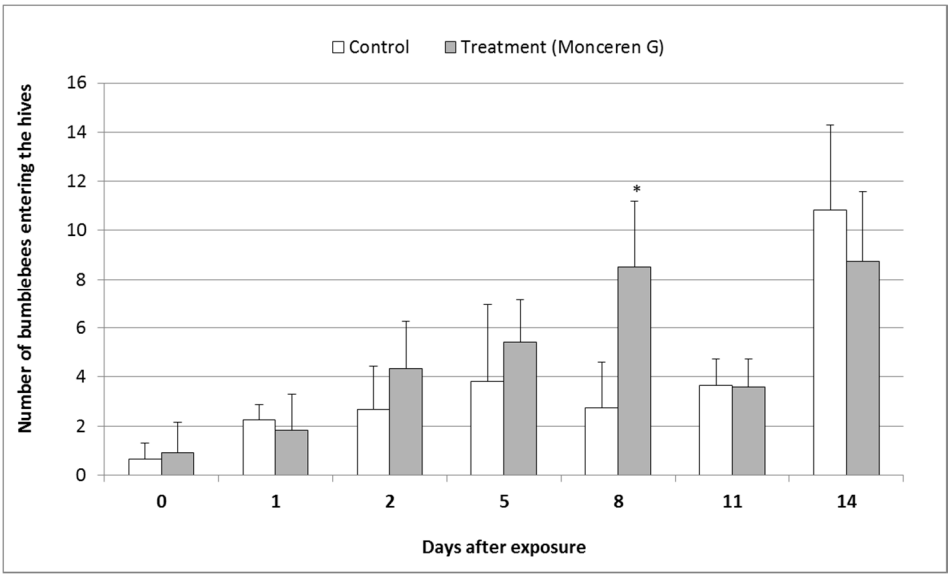
* = statistically significant difference to control (t-test ($p \leq 0.05$))

Figure 9.5.2-45: Flight activity in the crop: Mean flight activity in the crop per 4 m^2/10 min during the exposure phase

Flight activity at the entrances of the hives

The mean number of bumble bees entering the hives was 3.8 bumble bees/hive for the control field and 4.8 bumble bees/hive for the treated field. One statistically significant difference was

detected at 8 DAE where the flight activity at the treated field site was statistically significant higher compared to the control field site.



* = statistically significant difference to control (t-test ($p \leq 0.05$))

Figure 9.5.2-46: Flight activity at the entrances of the hives: Mean numbers of bumble bees entering the colonies

Assessment of the hive weight

The mean weight of the hives in the control field was 592.7 g and 585.1 g for the hives in the treated field site during the whole exposure phase (0-14 DAE). The weight increase during the exposure phase was 96.0 g and 83.3 g for the control and test item treatment, respectively. During the post exposure phase the mean weight of the hives in the control field was 993.8 g and 855.1 g for the hives in the treated field site. The weight increase during the post-exposure phase was 435.3 g and 455.6 g for the control and test item treatment, respectively. Total mean weight increases (means calculated from weight increases of single hives) were 567.8 g for the control and 567.8 g for the test item treatment. No statistically significant treatment related adverse effects on the weight of the colonies were observed. The weight of the hives increased during the exposure phase, the bumble bee colonies developed well and reached the "switchpoint", when colonies start the reproduction of young queens and drones rather than worker brood.

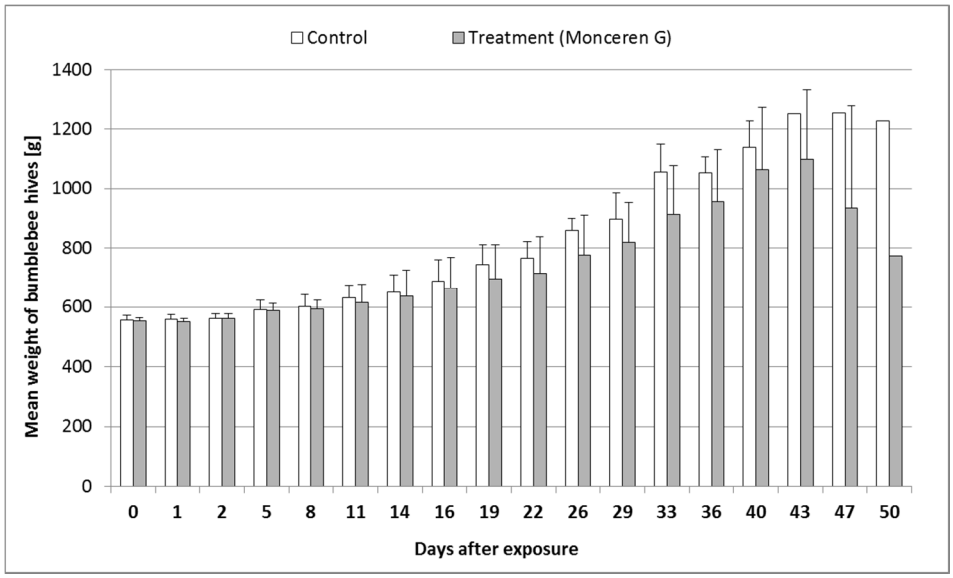


Figure 9.5.2-47: Assessment of hive weight: Mean weights of the hives of the control and the test item treatment

Assessment of sugar consumption

The mean sugar solution consumption was 545.0 g in the control field and 493.3 g for the hives in the treated field site during the exposure phase (until 14 DAE) and 3876.4 g for the control and 3384.8 g for the test item treatment for the post-exposure phase. The total mean sugar solution consumption until the end of the monitoring phase was 4421.4 g and 3878.2 g for the colonies of the control and the test item treatment, respectively. No statistically significant treatment related adverse effects on weight of the sugar consumption were observed.

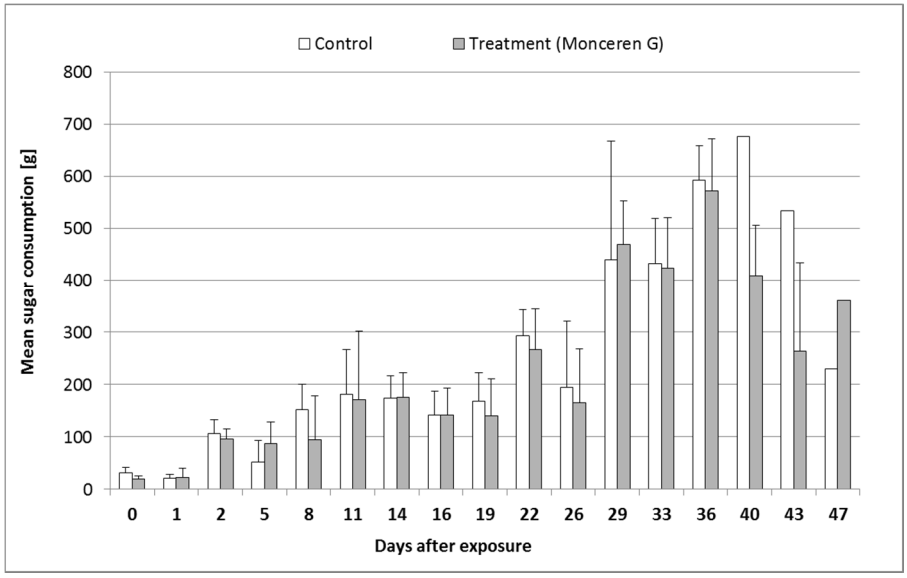


Figure 9.5.2-48: Assessment of sugar consumption: Mean consumption of sugar solution

Condition of the colonies

The results of the final brood evaluation showed a statistically significant difference in one out of all parameters assessed, a lower number of live young queen larvae. However, the number of live young queens and live 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 the other parameters, no statistically significant treatment related adverse effects on number of live young queens, workers, males, eggs, larvae (queen and worker) and pupae (queen and worker) were observed. No statistically significant treatment related adverse effects on the number of filled nectar and pollen cells, total number of live brood, live adults, the total queen reproduction (larvae, pupae and adults) and the total number of alive individuals were observed.

Pollen source analysis

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. It is assumed that the exposure to potato pollen was given in the treated field site.

Table 9.5.2-42: Results of the forager bumble bee pollen analysis

% of potato pollen in pollen samples of forager bumble bees		
Sampling date	**C**	**T**
5 DAE	47.4	1.6
12 DAE	2.2	23.5
16 DAE	0.0	29.2

Residue analysis

Residue analysis was carried out on pollen samples collected from forager bumble bees at 5, 12 and 16 days after exposure (DAE). No residues of imidacloprid and its metabolites (imidacloprid-5-hydroxy and imidacloprid olefine) were detected in pollen from the control field. Residue levels of imidacloprid in samples from the treated field were below the limit of quantification at sampling date 5 DAE and below the limit of detection at 16 DAE. The maximum residue level of 0.71 µg/kg was found at the sampling date 12 DAE. At all sampling dates, the residue levels of imidacloprid-5-hydroxy and imidacloprid olefin were below LOD.

Table 9.5.2-43: Residues of imidacloprid and its metabolites in potato pollen

Treatment group	**Sampling date**	**Residues [µg/kg]**		
		Imidacloprid	**Imidacloprid-5-hydroxy**	**Imidacloprid olefine**
C	5 DAE	< LOD	< LOD	< LOD
	12 DAE	< LOD	< LOD	< LOD
	16 DAE	< LOQ	< LOD	< LOD
T	5 DAE	< LOQ	< LOD	< LOD
	12 DAE	0.71	< LOD	< LOD
	16 DAE	< LOD	< LOD	< LOD

DAE = days after exposure

LOQ = limit of quantification = 0.6 µg/kg for imidacloprid, 1.0 µg/kg for imidacloprid metabolites

LOD = limit of detection = 0.2 µg/kg for imidacloprid, 0.3 µg/kg for imidacloprid metabolites

Conclusions

It can be concluded that the use of Monceren G (applied at rates of 180 g imidacloprid/ha and 375 g pencycuron/ha) during potato planting has no adverse effects on the behaviour and development of bumble bee colonies exposed during bloom.

RMS´s comments for bumble bees (commercially used):
The imidacloprid residue levels in pollen from the treated field were very low, only in one out of 3 sampling dates the parent compound was detected. However, only one control field and one test field were used, with only one sample per sampling day.
Due to the lack of replicates there is uncertainty as to whether this study represents a best-case, realistic or worst-case situation for residues; on the other hand the results in the study of Klein, 2014a are similar to the results in Klein, 2014b.
~~Furthermore, as in the study of Klein 2014a it is unclear if the pollen samples used for residue analysis consisted exclusively of potato pollen. The applicant may be able to provide more information on this.~~
Both during the exposure phase as well at study termination in the final brood assessment no treatment related differences were observed for mortality of adults and larvae and for the total amount of brood stages and total queen and drone production at study termination.
It can be concluded that under the conditions of this study, after in-furrow treatment at planting of potatoes with Monceren-G at actual doses of 192 g imidacloprid/ha and 400 g pencycuron/ha, no test item related adverse effects on bumble bee flight activity on mortality and colony development including production of queen larvae were seen.

Conclusion of the RMS with regard to the risk for wild bumble bees:

1. Comparability of results from the two submitted bumble field studies
Two field studies were submitted assessing the effects of potential residues in pollen in Monceren G treated potato fields to artificial *Bombus terrestris* colonies.
Both studies were carried out in southern Germany.

	S14-03553	S14-03554
Study location	Near Karlsruhe	Near Heilbronn (
Flowering period	11-27.06. 2014 (15 days)	01.-18.07. 2014 (17 days)
Monitoring time	24 days	34 days
Application rate	1.42 L/ha	1.61 L/ha

Moreover, both studies differed in their number of individuals per hive at study start. In study S14-03553, the 12 colonies used for the biological assessment (6 per field site) contained in average 100.5 alive workers, whereas colonies in study S14-03554 contained in average 41.5 alive workers only. Thus, the population was significantly different at start.
Thus, results of both studies are not directly comparable.

2. General evaluation of the study design /short comings:
a) Studies were conducted with *B. terrestris*. However, its representativeness for other bumble bee species has to be questioned.
   i. Foraging distance and foraging behaviour – *B. terrestris* is known to have a wide foraging home range compared to other bumble bee species like *Bombus pascuorum*, Scopoli, *Bombus sylvarum* L. or *Bombus muscorum* L [Walther-

Hellwig, K. & Frankl, R. (2000)[6], Darvill, B., Knight, M.E. & Goulson, D. (2004)[7], Knight, M.E. et al, 2005[8]]. Thus, *B. terrestris* may gather food from greater distances beyond treated areas while the home range of other species is restricted to smaller areas. Therefore, these species might be more exposed to residues of flowering treated plants than *B. terrestris*.

ii. Toxicological sensitivity – it is not clear whether other bumble bee species will be more susceptible to the pesticide

b) Post exposure period at uncontaminated sites
Subsequent to the exposure period at potato field sites, colonies were further observed at special monitoring sites providing sufficient food sources (e.g. wild flowers) without intensive agriculture. Natural bumble bee hives are located at one site during the total season. Thus, wild bumble bee colonies in the agricultural landscape may be subject to food shortage as well as multiple pesticides, which may hinder their recovery from an initial stress.

c) Provision of sugar solution as additional food
Bumble bees were additionally fed with sugar solution. Although this approach was reasoned with the lacking or reduced nectar production in potato flowers, it is not appropriate when assessing effects to wild bumble bees. Provision of additional nutritional value may have decreased the foraging effort of *B. terrestris* in the treated crop and consequently the exposure compared to *B. terrestris* and other bumble bee species under realistic conditions

d) As both studies were carried out with only one control and one treatment plot, it is not possible to distinct between effects caused by environmental site conditions and effects attributed to the exposure to pollen from imidacloprid treated potato plants.

3. Summary of effects significantly deviating from control and several shortcomings in the experimental design when determining theses parameters

a) Flight activity in crop and at the entrance of the hives

In study S14-03553, the flight activity in the treatment plot was lower than in the control plot. Deviations were statistically significant at two specific days as well as in regard to the arithmetic mean over all days.
Foraging activity at the entrance of the hives in the treatment plot was generally lower than in the control plot. Significant differences were found on two days.

This effect was not seen in study S14-03554. However, the measured flight and foraging activity in this study was generally lower than in study S14-03553. This might be partly due to a lower statistical population of observed individuals (please refer to 1.). However, the weather conditions seemed to be different between study S14-03553 and study S14-03554 with 11 days of rain in S14-03554 but only 5 days of rain in study S14-03553. Since precipitation influences the flight and foraging activity of bumble bees, the generally reduced activity of bumble bees in study S14-03554 might be also attributed to the different weather conditions.

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[6] Walther-Hellwig, K. & Frankl, R. (2000) Foraging habitats and foragingdistances of bumble bees, *Bombus* spp. (Hym., apidae), in an agricultural landscape. Journal of Applied Entomology, 124, 299–306.

[7] Darvill, B., Knight, M.E. & Goulson, D. (2004) Use of genetic markers to quantify bumble bee foraging range and nest density. Oikos, 107, 471–478.

[8] Knight, M.E., Bishop, S.E., Martin, A.P., Osborne, J.L., Hale, R.J., Sanderson, R.A. & Goulson, D. (2005) An interspecific comparison of foraging rangeand nest density of four bumble bee (Bombus) species. Molecular Ecology,14, 1811–1820.

Short-comings in terms of:

1. Flight activity was measured on three observation areas of 4 m² for 10 min per day only. The area of observation is considered to be too small and the observation time is regarded too short to produce reliable data on the flight activity of mobile species like bumble bees.
2. The Foraging activity at the entrance of the hives has been observed for only 15 min/day. This observation time is regarded too short as the activity of bumble bees is strongly influenced by external parameters as rainfall.
3. Detailed information on precipitation at the actual plots was not recorded in both studies. Thus, important data influencing these parameters are lacking.

b) Final brood assessment

In study S14-03554, the number of brood cells with larvae (queens) was significantly reduced in the treatment colonies. This effect was not observed in study S14-03553.

Short-comings:
Standard deviations are high for each measured parameter. This might be attributed to the high influence of external parameters (weather, food resources within the total foraging range) that are not sufficiently described.

4. Further peculiarities in the study results and study design

a) Pollen source identification and residues

Pollen from three control hives and three treatment hives at three sample days (4, 12 and 15 DAE) was analysed.
No control sample in study S14-03553 contained pollen from potatoes (*S. tuberosum*. The pollen samples consisted of pollen from butterfly bush (*Buddleja*), *Rosa* sp., St. John' wort (*Hypericum*), cornflowers (*Centaurea cyanus*), asparagus and lime (*Tilia*).
In the treatment groups, the portion of potato pollen was variable 24.8 % (5 DAE), 56.3 % (12 DAE) and 54.8 % (15 DAE). Comparatively high portions of pollen from other sources as chestnut trees (29.4 %) and lime (26.0 %) were found at 5 DAE. Further sources were St. John's wort (*Hypericum*), roses (*Rosa* sp.), cornflowers and plantains (*Plantago*).
However, the total lack of potato pollen in the control hive samples is striking and is contradictory to the significantly higher flight activity in the control plot compared to the treatment plot.

In the study, it was concluded that "the exposure to potato pollen was given in the treated field side". The lack of exposure to potato pollen in the control field side was not discussed.
Furthermore, exposure of the treatment group to potato pollen does not necessarily mean exposure to pollen from treated plants. The field sides are small (1.84 ha and 1.85 ha). The foraging home range of the bumble bee species *Bombus terrestris* may be much higher spanning several km. (Goulson & Stout, 2001[9]). Thus, it is possible that potato pollen is also gathered from other untreated potato plants from greater distances.

The portion of potato pollen in the control group of study S14-03554 were 47.4 %, 2.2 % and 0.0 % at 5 DAE, 12DAE and 16 DAE, respectively.

[9] Goulson, D., and J. C. Stout. 2001. Homing ability of the bumble bee Bombus terrestris (Hymenoptera: Apidae). Apidologie 32: 105–111.

Pollen from the treatment group comprised 1.6 %, 23.5 % and 29.2 % potato pollen at 5 DAE, 12DAE and 16 DAE, respectively. The strong variability in these results is also probably due to the small fields sides compared to the foraging home ranges of *Bombus terrestris*.

In both studies, the abundance of flowering wild and agricultural plants was only described for the direct not further defined surrounding of the study plots. Information on flowering plants in greater distances covering the potential foraging home ranges of bumble bees is not given.
Thus, it is not possible to conclude that the exposure with treated pollen was sufficient in terms of realistic worst case field situations.

b) Determination of residues in pollen
Study S14-03553:
The imidacloprid concentration in the three control samples (5, 12 and 15 days) was measured to be below the limit of detection (0.2 µg/kg). Imidacloprid concentrations in two samples from the treatment hives (5 DAE and 12 DAE) were detected above the LOD, but below the limit of quantification (<0.6 µg/kg), whereas the concentration on day 15 after exposure was 1.4 µg/kg. As the portion of potato pollen at 12 DAE sample was approx. as high as at 15 DAE, the analytical results from samples of 12 DAE and 15 DAE are contradictory and suggest that potato pollen found in the treatment hives are not only form treatment crops, but also from other untreated field sides from greater distances.
Study S14-03554:
The imidacloprid concentration in two of the three control samples (5 and 12 DAE) was measured to be below the limit of detection (0.2 µg/kg). However, measurements of the 16 DAE control sample revealed an imidacloprid concentration below the limit of quantification (0.6 µg/kg), but above the limit of detection (0.2 µg/L). Hence, this control sample was slightly contaminated with imidacloprid. In the pollen source identification sample, potato pollen was not determined in the 16 DAE control sample. The source of imidacloprid contamination in the control sample was not clarified.
The residue results from the treatment hives are not coherent with the outcome of the pollen source identification. On day 5 after exposure 1.6 % potato pollen were found, but the measured concentration of imidacloprid was above the limit of detection (< LOQ).
A concentration of 0.71 µg/kg imidacloprid was found in the 12 DAE sample. The imidacloprid concentration on day 16 after exposure was below the LOD. Since the portion of potato pollen in the treatment sample on day 16 after exposure was even higher than on day 12 after exposure, these residue data are contradictory.

Moreover, no information on imidacloprid residues in pollen from the study sides is given. Therefore, it is not possible to predict possible residues in pollen samples from the bumble bee hives and compare with the measured values in gathered pollen.
Furthermore, the exposure of bumble bees to imidacloprid via potato pollen in the treatment plots cannot be verified as well as the exposure cannot be excluded in the control plots due to the lacking residue data from field sides.

c) Surrounding of the treatment sides compared with the control sides
Treatment and control field sides and their surrounding were recorded by aerial photography.
In both studies, it is striking that the treatment plot borders on greater wood sides, whereas the control plots are surrounded by fields.
Wood sides may provide good food resources for bumble bees. Data on pollen source identification partly reflect this assumption. In study S14-03553 (conducted in June during the blooming period of several trees), pollen from the treatment plots on day 5 after exposure

comprised 29.4 % pollen of chestnut trees and 26.0 % pollen of lime trees, whereas pollen of these species was not found in the control sample (5DAE).
Treatment plots and control plots should basically be comparable. If treatment plots border on wood sides providing several attractive food resources and control plots do not, comparability is not given.

B.9.6 References relied on

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP/GEP status, published or not	Data protect. claimed	Owner
1.2 /01	Pfeiffer, S.	2014	Clothianidin + imidacloprid FS 275 (100+175 g/L): Acute contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions Eurofins Agroscience Services, EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S13-05151, Edition Number: M-494283-01-1 Date: 2014-05-05 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
1.2 /02	Schmitzer, S.	2014	Effects of clothianidin + imidacloprid FS 275 (100+175) G (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 89691035, Edition Number: M-501653-01-1 Date: 2014-11-10 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
1.2 /03	Pfeiffer, S.	2014	Imidacloprid FS 350 (350 g/L) - Acute contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions Eurofins Agroscience Services, EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S13-05153, Edition Number: M-494307-01-1 Date: 2014-05-05 GLP/GEP: yes, unpublished	Yes	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP/GEP status, published or not	Data protect. claimed	Owner
1.2 /04	Sekine, T.	2014	Effects of imidacloprid FS 350A G (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 89281035, Edition Number: M-500305-01-1 Date: 2014-10-27 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
1.2 /05	Pfeiffer, S.	2014	Imidacloprid + pencycuron FS 370 (120+250 g/L) - Acute contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions Eurofins Agroscience Services, EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S13-05154, Edition Number: M-494321-01-1 Date: 2014-05-09 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
1.2 /06	Schmitzer, S.	2014	Effects of imidacloprid + pencycuron FS 370 (120+250) G (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 89661035, Edition Number: M-503109-01-1 Date: 2014-11-10 GLP/GEP: yes, unpublished	Yes	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP/GEP status, published or not	Data protect. claimed	Owner
1.2 /07	Klein, O.	2014	Final report - 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 Eurofins-GAB GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S14-03553, Report includes Trial Nos.: S14-03553-01 S14-03553-L1 S14-03553-L2 Edition Number: M-503597-01-1 Date: 2014-11-28 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
1.2 /08	Klein, O.	2014	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 eurofins-GAB GmbH, Niefern-Oeschelbronn, Germany Report No.: S14-03554, Report includes Trial Nos.: S14-03554-01 S14-03554-L1 S14-03554-L2 Edition Number: M-504174-01-1 Date: 2014-11-28 GLP/GEP: yes, unpublished	Yes	

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1.3 /01	Ythier, E.	2014	Determination of the residues of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin in bee relevant matrices collected in a succeeding crop scenario with natural aged residues of imidacloprid - Field phase conducted with Phacelia and maize in northern France SynTech Research France SAS, Nimes, France Bayer CropScience, Report No.: 7SRFR13C1, Report includes Trial Nos.: P-672134728 SRFR13-001-7IC1 SRFR13-002-7IC1 Edition Number: M-504801-01-1 Date: 2014-12-09 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
1.3 /02	Ythier, E.	2014	Determination of the residues of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin in bee relevant matrices collected in a succeeding crop scenario with natural aged residues of imidacloprid - Field phase conducted with winter oil seed rape, Phacelia and maize in northern France SynTech Research France SAS, La Chapelle de Guinchay, France Bayer CropScience, Report No.: 7SRFR13C2A, Report includes Trial Nos.: P-672144710 SRFR13-001-7IC2A SRFR13-002-7IC2A SRFR13-003-7IC2A Edition Number: M-504806-01-1 Date: 2014-12-09 GLP/GEP: yes, unpublished	Yes	Bayer CropScience

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1.3 /03	Ythier, E.	2014	Determination of the residues of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin in bee relevant matrices collected in a succeeding crop scenario with natural aged residues of imidacloprid - Field phase conducted with Phacelia and maize in northern France SynTech Research France SAS, Nimes, France Bayer CropScience, Report No.: 7SRFR13C2B, Report includes Trial Nos.: P672144711 SRFR13-001-7IC2B SRFR13-002-7IC2B Edition Number: M-504836-01-1 Date: 2014-10-09 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
1.3 /04	Ythier, E.	2014	Determination of the residues of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefin in bee relevant matrices collected in a succeeding crop scenario with natural aged residues of imidacloprid - Field phase conducted with winter oil seed rape in northern France SynTech Research France SAS, La Chapelle de Guinchay, France Bayer CropScience, Report No.: 7SRFR13C2C, Report includes Trial Nos.: P 672144712 Edition Number: M-504810-01-1 Date: 2014-12-09 GLP/GEP: yes, unpublished	Yes	Bayer CropScience

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1.3 /05	Hammel, K.; Vrbka, L.	2014	Calculation of plateau concentrations in soil for imidacloprid and clothianidin Bayer CropScience, Report No.: EnSa-14-1318, Edition Number: M-503458-01-1 Date: 2014-11-28 GLP/GEP: n.a., unpublished	Yes	Bayer CropScience
1.3 /06	Ythier, E.	2014	Determination of the residues of imidacloprid in bee relevant matrices collected from succeeding crops following application of imidacloprid FS 600E G via soil incorporation to plateau concentration and sowing of imidacloprid-treated winter barley seeds. Field phase conducted in southern France SynTech Research France SAS, Nimes, France Bayer CropScience, Report No.: 7SRFR13C3, Report includes Trial Nos.: P 672134724 SRFR13-001-7IC3 SRFR13-002-7IC3 SRFR13-003-7IC3 Edition Number: M-504842-01-1 Date: 2014-12-09 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
1.3 /07	Striffler, B.; Ballhaus	2014	Residues of imidacloprid in nectar and pollen of flowering rotational crops in western Germany tier3 solutions GmbH, Leverkusen, Germany Bayer CropScience, Report No.: P13068-1, Edition Number M-504854-01-1 Date: 2014-12-10 GLP/GEP: yes, unpublished	Yes	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP/GEP status, published or not	Data protect. claimed	Owner
1.4 /01	Garside, C. M.; Miles, M.; Kriszan, M.	2014	Statement - Evaluation of the occurrence of flowering weeds in agricultural crops: Cereals, sugar beet and potatoes Bayer CropScience, Report No.: M-505126-01-1, Edition Number: M-505126-01-1 Date: 2014-12-10 GLP/GEP: n.a., unpublished	Yes	Bayer CropScience
1.5 /01	Nauen, R.	2013	Statement - Information on the occurrence or possible occurrence of the development of resistance of the plant protection product Janus Forte (for submission in Europe) Bayer CropScience Bayer CropScience, Report No.: M-453965-01-1, Edition Number: M-453965-01-1 Date: 2013-05-20 GLP/GEP: n.a., unpublished	Yes	Bayer CropScience
1.6 /01	Hofmann, S.; Lueckmann, J.	2014	Field study to monitor potential effects on honey bees from exposure to guttation fluid of winter wheat (W-WHT), seed-treated either with an imidacloprid or a clothianidin combi-product RifCon GmbH, Heidelberg, Germany Bayer CropScience, Report No.: R09247-4, Edition Number: M-498939-01-1 Date: 2014-07-14 GLP/GEP: no, unpublished	Yes	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP/GEP status, published or not	Data protect. claimed	Owner
1.6 /02	Hofmann, S.; Garrido, C.; Lueckmann, J.	2012	Field study to monitor potential effects on honey bees from exposure to guttation fluid of winter barley (W-BAR), seed-treated either with an imidacloprid or a clothianidin combi-product RifCon GmbH, Heidelberg, Germany Bayer CropScience, Report No.: R09247-3, Edition Number: M-498922-01-1 Date: 2012-10-17 GLP/GEP: no, unpublished	Yes	Bayer CropScience
1.6 /03	Hofmann, S.; Staffel, J.; Aumeier, P.	2014	Field study to monitor potential effects on honey bees from exposure to guttation fluid of winter barley (W-BAR), seed-treated with the insecticidal seed-treatment product clothianidin + imidacloprid FS 100 + 175 G in Germany in 2011/2012 RIFCON GmbH, Hirschberg, Germany Bayer CropScience, Report No.: R11130, Edition Number: M-501261-01-1 Date: 2014-11-04 GLP/GEP: yes, unpublished	Yes	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP/GEP status, published or not	Data protect. claimed	Owner
1.6 /04	Rexer, H. U.	2014	A long-term field study to monitor potential effects on the honey bee (Apis mellifera L.) from exposure to guttation fluid of sugar beets, seed-treated with the insecticides clothianidin + imidacloprid + beta-cyfluthrin in Southern Germany in 2013 and 2014 Eurofins Agrosciences Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S13-00171, Report includes Trial Nos.: S13-00171-00171-L3 S13-00171-01 S13-00171-L1 S13-00171-L2 Edition Number: M-500724-01-1 Date: 2014-09-30 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
1.6 /05	Rexer, H. U.	2014	A long-term field study to monitor potential effects on the honey bee (Apis mellifera L.) from exposure to guttation fluid of sugar beets, seed-treated with the insecticides clothianidin + imidacloprid + beta-cyfluthrin in Southern Germany in 2013 and 2014 Eurofins Agrosciences Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S13-00170, Report includes Trial Nos.: S13-00170-00170-L3 S13-00170-01 S13-00170-L1 S13-00170-L2 Edition Number: M-500734-01-1 Date: 2014-09-30 GLP/GEP: yes, unpublished	Yes	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP/GEP status, published or not	Data protect. claimed	Owner
1.6 /06	Rexer, H. U.	2014	A long-term field study to monitor potential effects on the honey bee (Apis mellifera L.) from exposure to guttation fluid of potato plants, grown from seed tubers treated with Monceren G in southern Germany in 2014 and 2015 eurofins-GAB GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S14-01385, Report includes Trial Nos.: S14-01385-01 S14-01385-L1 S14-01385-L2 S14-01385-L3 Edition Number: M-503349-01-1 Date: 2014-11-26 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
1.6 /07	Rexer, H. U.	2014	A long-term field study to monitor potential effects on the honey bee (Apis mellifera L.) from exposure to guttation fluid of potato plants, grown from seed tubers treated with Monceren G in Southern Germany in 2014 and 2015 eurofins-GAB GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S14-01392, Report includes Trial Nos.: S14-01392-01 S14-01392-L1 S14-01392-L2 S14-01392-L3 Edition Number: M-503344-01-1 Date: 2014-11-26 GLP/GEP: yes, unpublished	Yes	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP/GEP status, published or not	Data protect. claimed	Owner
1.7 /01	Hofmann, S.; Lueckmann, J.	2010	Monitoring of dust drift deposits during and after sowing of winter barley (W-BAR) treated with Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 145.2 (60 + 70 + 7.2 + 8 g/L) or Clothianidin & Beta-Cyfluthrin FS 455 (375 + 80 g/L) on fields in Germany RifCon GmbH, Heidelberg, Germany Bayer CropScience, Report No.: R09247-1, Edition Number: M-366273-01-1 Date: 2010-03-09 GLP/GEP: no, unpublished	Yes	Bayer CropScience
1.7 /02	Hofmann, S.; Lueckmann, J.	2010	Monitoring of dust drift deposits during and after sowing of winter wheat (W-WHT) treated with Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 145.2 (60 + 70 + 7.2 + 8 g/L) or Clothianidin & Beta-Cyfluthrin FS 455 (375 + 80 g/L) on fields in Germany RifCon GmbH, Heidelberg, Germany Bayer CropScience, Report No.: R09247-2, Edition Number: M-366277-01-1 Date: 2010-03-09 GLP/GEP: no, unpublished	Yes	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP/GEP status, published or not	Data protect. claimed	Owner
1.7 /03	Lueckmann, J.	2014	Second amendment to final report - Investigation of dust drift deposits of clothianidin & imidacloprid treated winter barley seeds with pneumatic sowing machinery on fields in Germany in autumn 2011 RifCon GmbH, Heidelberg, Germany Bayer CropScience, Report No.: R11129, Edition Number: M-502885-03-1 Date: 2014-11-20 **...Amended: 2014-12-05** GLP/GEP: yes, unpublished	Yes	Bayer CropScience
1.7 /04	Lueckmann, J.; Staffel, J.	2014	Interim report - Assessment of potential impacts on honey bee colony development, their hibernation performance and concurrent monitoring of aerial dust drift during the sowing operation of imidacloprid FS 350A G - Treated winter barley with typical commercial vacuum-pneumatic sowing technology, directly adjacent to full-flowering Phacelia tanacetifolia in United Kingdom RIFCon GmbH, Hirschberg, Germany Bayer CropScience, Report No.: GLP200, Edition Number: M-504522-01-1 Date: 2014-12-04 GLP/GEP: yes, unpublished	Yes	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP/GEP status, published or not	Data protect. claimed	Owner
1.7 /05	Staffel, J.; Lueckmann, J.	2014	Final report - Assessment of potential impacts on honey bee colony development, their hibernation performance and concurrent monitoring of aerial dust drift during the sowing operation of Poncho Beta Plus - Treated sugar beet pills with typical commercial vacuum-pneumatic sowing technology, directly adjacent to full-flowering Phacelia tanacetifolia in Germany RIFCon GmbH, Hirschberg, Germany Bayer CropScience, Report No.: 195, Edition Number: M-504065-01-1 Date: 2014-11-28 GLP/GEP: yes, unpublished	Yes	Bayer CropScience