European Commission

Addendum to Draft Assessment Report
prepared in the context of the assessment of the
Confirmatory Information requested by Reg. (EU) No 485/2013,
in view of maintenance of approval of clothianidin according to Regulation (EC) N° 1107/2009

Clothianidin

Volume 3

B.9 (Ecotoxicology)

Data submitter/owner: Sumitomo Chemical Agro Europe S.A.S.

Rapporteur Member State: Belgium

Version History

When	What
31 August 2015	Addendum to the initial DAR (May 2003, corr. April 2005) in the context of the assessment of the Confirmatory Information requested by Reg. (EU) No 485/2013, in view of maintenance of approval of Clothianidin according to Reg. (EC) No 1107/2009.
24 May 2016	Addendum revised in light of the comments from Member States, the applicant and EFSA as collated in the reporting table, which was submitted to EFSA on 25 November 2015. *Note: Changes in the original text are highlighted by means of yellow shading.*
06 July 2016	Addendum revised considering the outcome of the experts' consultation during Pesticides Peer Review Meeting 145, 7-9 June 2016 *Note: Changes compared to the previous version are highlighted by means of green shading.*

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A. INTRODUCTION

The active substance clothianidin was included in Annex I to Directive 91/414/EEC on 1 August 2006 by Commission Directive 2006/41/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 amended by Commission Directive 2010/21/EU, to permit use as a seed treatment only where 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 where adequate drilling equipment is used to ensure a high degree of incorporation in soil, minimisation of spillage and minimisation of dust emission.

In spring 2012, new scientific information on the sub-lethal effects of neonicotinoids on bees was published. The Commission, in accordance with Article 21(2) of regulation (EC) No 1107/2009, asked the European Food Safety Authority (EFSA) for scientific and technical assistance to assess this new information and to review the risk assessment of clothianidin (and the other neonicotinoid active substances imidacloprid and thiametoxam) as regards their impact on bees. EFSA presented its conclusions on the risk assessment on 16 January 2013. High acute risks for bees from plant protection products containing clothianidin were identified for bees from exposure via dust as regards several crops, from consumption of residues in contaminated pollen and nectar as regards some crops and from exposure via guttation fluid as regards maize. In addition, unacceptable risks due to acute or chronic effects on colony survival and development could not be excluded for several crops. Furthermore the EFSA identified a number of data gaps for each of the evaluated crops. In particular as regards long term risk to honeybees from dust exposure, from residues in pollen and nectar and from exposure from guttation fluid.

In the light of the new scientific and technical knowledge, the Commission considered that there are indications that the approved uses of clothianidin, thiamethoxam and imidacloprid no longer satisfy the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009 with respect to their impact on bees and that the high risk for bees could not be excluded except by imposing further restrictions. Commission Implementing Regulation (EU) No 485/2013 amended the conditions of inculsion of the active substances clothianidin, thiamethoxam and imidacloprid, by limiting the use of plant protection products containing those active substances to professional uses. Further, uses as seed treatment and soil treatment of plant protection products containing clothianidin, thiametoxam or imidacloprid were prohibited for crops attractive to bees and for cereals, excepts for uses in greenhouses and for winter cereals. Foliar treatments with plant protection products containing clothianidin, thiametoxam or imidacloprid were prohibited for crops attractive to bees and for cereals with the exception of uses in greenhouses and uses after flowering. Crops which are harvested before flowering are not considered attractive to bees.

Concerning applications of clothianidin, thiametoxam and imidacloprid which remained authorized under Regulation (EC) 1107/2009, confirmatory information was requested by Regulation (EU) No 485/2013:

The notifier shall submit confirmatory information as regards:

- *(a) the risk to pollinators other than honeybees;*
- *(b) the risk to honeybees foraging in nectar or pollen in succeeding crops;*
- *(c) the potential uptake via roots to flowering weeds;*
- *(d) the risk to honeybees 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 honeybees from ingestion of contaminated nectar and pollen.

The notifier shall submit that information to the Commission, the Member States and the Authority by 31 December 2014.'

On 7 January 2015, Sumitomo Chemical Agro Europe S.A.S. (who was the sole data submitter supporting Annex I inclusion of clothianidin), provided the RMS with a dossier containing study reports in view of addressing the above-mentioned confirmatory data requirements, for the clothianidin uses supported by Sumitomo Chemical Agro Europe S.A.S. as well as the clothianidin uses supported by Bayer CropScience. Additional data and updated study reports were submitted by Sumitomo Chemical Agro Europe S.A.S. on 19 March and 1 June 2015 and by Bayer Cropscience on 17 March 2015.

On request of both notifiers, and to guarantee data protection and intellectual property, the data submitted by Sumitomo Chemical Agro Europe S.A.S. and Bayer CropScience were evaluated separately in 2 different Addenda to the original DAR. This Addendum presents the evaluation performed by the RMS Belgium of the confirmatory data that were submitted by the notifier Sumitomo Chemical Agro Europe S.A.S. The assessment mainly concerns the section Ecotoxicology.

This assessment has been performed in line with the EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* sp. and solitary bees), published on 4 July 2013[1]. Throughout this assessment, this document will be referred to as 'The EFSA Guidance Document on bees'. It should be noted that this Guidance Document has not been been noted by the Standing Committee of Plants, Animals, Feed and Food and that it thus is not legally adopted for use in risk assessment. However, it was the choice of RMS Belgium to base the current assessment on this Guidance Document for the following reasons:

- The assessment deals with the confirmatory information as requested in Implementing Regulation (EU) No 485/2013; as explained in its preamble, this Regulation has been adopted following the publication of the EFSA Conclusions on the risk assessments for clothianidin, thiamethoxam and imidacloprid; for these assessments, EFSA has been requested by the European Commission to make use of the Scientific Opinion on the science behind the development of a risk assessment scheme for bees (Commission's mandate letter of 25 April 2012); the request for confirmatory information is to a large extent the result of the use of the Scientific Opinion;
- The Scientific Opinion on the science behind the development of a risk assessment scheme for bees has led to the publication, on 4 July 2013, of the EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* sp. and solitary bees); the Guidance Document is building further on the principles developed in the Scientific Opinion;
- In its mandate to EFSA, dated 20/06/13, the Commission requested EFSA again to use the Scientific Opinion on the science behind the development of a risk assessment scheme for bees, for the assessment of the uses other than those considered in the first set of conclusions (i.e. other than seed treatment and granular uses); for this assessment, EFSA made use of the Guidance Document instead of the Scientific Opinion, as the Guidance Document was published shortly after the receipt of the mandate;
- The Guidance Document, whilst implementing the principles as laid out in the Scientific Opinion, offers a more developed and readily usable tool for performing the risk assessment; it was therefore judged justified, and considering the whole context also logical, to use the Guidance Document for the benefit of the present assessment.

[1] European Food Safety Authority (2013). Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bompus* spp. and solitary bees). EFSA Journal 2013; 11(7):3295. doi:10.2903/j.efsa.2013.3295

- Further, the Guidance Document is the only guidance currently available that includes a risk assessment scheme for non-*Apis* bees and for the different routes of exposure for which confirmatory data was requested. Using this Guidance Document, a clear and consistent tiered risk assessment could be performed.

The uses that are supported by the Confirmatory Data of Sumitomo Chemical Agro Europe S.A.S. are the currently registered uses as granular treatment in potato, maize, sorghum and tree nursery. A list of these uses is presented here below for the sake of reference.

CLOTHIANIDIN – LIST OF USES SUPPORTED BY AVAILABLE DATA

The confirmatory data is required to address the existing, currently permitted, registrations in the EU. The applicant has registrations for clothianidin (in a number of different products) as a granular treatment at sowing in potato, maize, sorghum and forestry nursery. Table A-1 shows the GAP of the currently registered uses in the EU. These use rates will be considered in the risk assessment performed in the context of the confirmatory information.

During Peer Review of the original version of the present Addendum, there was some confusion regarding the uses to which the confirmatory data apply. During Pesticides Peer Review Meeting 145, it was however clarified that the uses of clothianidin currently authorized in Member States should be considered within the present assessment, as it is stated in Regulation (EU) 485/2013, §12 that "*Concerning applications of clothianidin, thiamethoxam or imidacloprid which may be authorised under the present Regulation, it is appropriate to require further confirmatory information."*

At Pesticides Peer Review Meeting 145, it was noted that for the indoor uses in maize and sweet maize, there is no information in the GAP table (Table A-1) on the kind of structure in which these crops are grown (e.g. permanent structures, open protected structures, …). For clarification, more details on the type(s) of protected structures in which clothianidin is applied were requested from France and the applicant. According to the information available in France, permanent structures are used for indoor cultivation of maize and sweet maize. According to the applicant, maize and sweet maize are cultivated indoor in glasshouses closed during the whole growing cycle.

At Pesticides Peer Review Meeting 129 (March 2015), exposure to bees from 'protected uses' was discussed. It was acknowledged that exposure to bees cannot be fully excluded for permanent greenhouses (i.e. bees entering the glasshouse through open vents) especially in areas with extensive glasshouse production. However, overall it was agreed that the exposure will be low, and that therefore a risk assessment for bees is not necessary for uses restricted to permanent greenhouses. This conclusion was also confirmed Pesticides Peer Review Meeting 133 (September 2015). The risk assessment for the use in Maize and Sweet Maize included in the original version of this Addendum assumed that the exposure to bees for the indoor use in these crops was similar to the exposure for outdoor use of clothianidin. However, based on the additional information received, this risk assessment is updated taking into account the differences in exposure between outdoor and indoor use.

During Peer Review, it was noted that the risk assessment in the original version of this Addendum did not specifically address the use in forestry nursery. This was due to the fact that no data or Tier I assessment was provided in the dossier submitted by the applicant. Based on the information available in the GAP table (Table A-1), it is not possible to perform a risk assessment for this use (only the dose in g a.s./plant is available, no information on the plant density and dose in g a.s./ha). Therefore, at Pesticides Peer Review Meeting 145, EFSA considered it necessary to set a data gap for this use in the absence of any information.

Table A-1: Detail of national GAPs per crop for which the applicant has a registration with the formulation clothianidin 0.7G

Crop and/ or situation (a)	Country	Product name	F G or I (b)	Pests or group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	Method kind (f-h)	Growth stage & season (j)	Number min max (k)	Interval between applications (min)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Potato	BU	Santana 0.7G	F	*Wireworms*	GR	0,7%	soil application	00	1	n.a.	n.a.	n.a.	0.07	n.a.	Minor use registration
Maize	FR	Cheyenne or Santana	G	*wireworms*	GR	0,7%	soil application	00	1	n.a.	n.a.	n.a.	0.05	n.a.	
Sweet maize				*wireworms*	GR	0,7%	soil application	00	1	n.a.	n.a.	n.a.	0.05	n.a.	
Sorghum			F	*wireworms*	GR	0,7%	soil application	00	1	n.a.	n.a.	n.a.	0.05	n.a.	Sowing forbidden between the 1st of January and the 30th of June
Forestry nursery	HU	Santana 1G Or Cheyenne 1G	F	Soil pests	GR	1%	soil application	00	1	n.a.	n.a.	n.a.	1-2 g / plant (planting hole) 4 g / m	n.a.	

Remarks:

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (e.g. fumigation of a structure)

(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)

(c) e.g. biting and suckling insects, soil born insects, foliar fungi, weeds

(d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)

(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989

(f) All abbreviations used must be explained

(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench

(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated

(i) g/kg or g/l

(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application

(k) The minimum and maximum number of application possible under practical conditions of use must be provided

(l) PHI - minimum pre-harvest interval

(m) Remarks may include: Extent of use/economic importance/restrictions

B. EVALUATION AND RISK ASSESSMENT

B.9. ECOTOXICOLOGY

B.9.1. THE RISK TO POLLINATORS OTHER THAN HONEYBEES

B.9.1.1. Laboratory toxicity studies

A laboratory study on acute oral and contact toxicity of the active substance clothianidin to bumblebees was performed, and is summarized below.

In the absence of validated test guidelines and since method development is still ongoing in this area, no studies with other (solitary) bee species were submitted.

Report:	**IIIA 10.4a/01; Harkin, S.; 2014**
Title:	**Clothianidin: Acute contact an oral toxicity to bumblebee (*Bombus terrestris*)**
Report No.:	B2AK1000
Document No.:	THW-0376
Guideline(s):	Principles of Van der Steen (2013) Draft OECD Guidelines 2013.
Guideline deviation(s):	not applicable
GLP/GEP:	yes

Objective

The objectives of this study were to determine possible effects of Clothianidin on the bumblebee, *Bombus terrestris* L., from contact exposure and oral exposure.

Material and Methods

There are currently no agreed guidelines for testing the toxicity of pesticides to bumblebees. However, the study takes into account the recommendations of the draft guidelines under development by the International Commission for Plant-Pollinator Relationships 'Bee Protection Group' (ICPPR) and the principles of Van der Steen (2013) Draft OECD Guidelines 2013.

Test item:	Clothianidin batch no.: EDFL018305 purity: 99.2% w/w a.s.
Toxic reference item:	Dimethoate
Test species:	Bumblebee *(Bombus terrestris terrestris)*
Stage:	Adult stage
Source:	Bees were obtained from commercial suppliers Biobest, sourced through Agralan Ltd. (for both the contact tests and oral test 1) and Syngenta Bioline (for oral test 2).
Replicates	3 replicate unit of 10 bumblebees/treatment level
Contact	
Treatment	300, 150, 75, 37.5 and 18.8 ng clothianidin/bumblebee in acetone
Toxic reference	10.0, 5.0, 2.5 and 1.25 µg a.s./bumblebee
Controls	I Acetone II Wetting agent control; Triton x 100 µg/L
Oral	
Treatment	30, 15, 7.5, 3.75 and 1.9 ng a.s./bee (actual mean uptake per treatment group of 19.6, 8.9, 4.0, 3.6 and 1.7 ng a.s./bumblebee or 87.97, 39.57, 17.90, 16.37, and 7.53 ng a.s./g of bumblebee

	respectively)
Toxic reference	10, 5, 2.5, 1.25, and 0.65 µg a.s./bee (actual mean uptake values per treatment group of 8.7, 4.5, 1.9, 1.1 and 0.5 µg a.s./bumblebee or 40.27, 20.86, 8.83, 4.73 and 2.52 µg a.s./g of bumblebee respectively).
Controls	I. Undosed control; 50% w/v sucrose solution II. Solvent control; 0% w/v sucrose solution with 1% acetone
Test conditions:	
Temperature:	25 ± 2°C
Relative humidity:	Contact test: 60 ± 10% Oral test:: 65 – 76%
Photoperiod:	The test units were held in darkness (except during assessments)
Test Duration:	96 hours
Toxicity endpoints:	Mortality rate after 24, 48, 72 and 96 hours
Dates of work:	30 April 2014 – 21 June 2014

Test system: Adult worker bees of similar size were collected from colonies by anaesthetisation using nitrogen. They were placed in the appropriate test units with access to an ad libitum supply of 50% w/v aqueous sucrose solution and left over night for an acclimatisation period.
Contact dosing: Pots of 10 bumblebees were anaesthetised with carbon dioxide, individually weighed and afterwards dosed with a 1 µL droplet containing the appropriate test solution placed onto the dorsal thorax of each bumblebee.
Oral dosing: On day -1 the bees were anaesthetised using nitrogen gas, collected from the nest boxes and individually weighed. Each bee was placed into a Nicot queen rearing cage, with a 1 ml syringe inserted at the end to act as a feeder. After the acclimatisation period the bees were starved for 4-5 hours then provided with pre-weighed feeders filled with 40 µL of the appropriate test solution. The cages were placed back into the incubator for a feeding period of 2 – 2 hrs 40 minutes. Three pre-weighed feeders were filled with test solution and placed into the incubator with the bees to find ou how much feed was lost due to evaporation in order to correct the feed uptake calculations. After the feeding period, the bumblebees were removed from the Nicot cages and placed into 3 pots of 10 bees for each treatment. The feeders were then re-weighed to allow the uptake of the test solution to be calculated.
For both tests the bumblebees were observed after 4 hours and then every 24 hours (±1 hour) after dosing up to 96 hours to record mortality and behavioural abnormalities.

Findings
Contact: Test: Run 1 of the contact test failed to meet the control validity criterion. The test was repeated successfully the data obtained from Run 2 are reported here.

Table B.9.1.1-1: Percent cumulative mortality of bumblebees in the Control and Test Item treated groups over 96 hours - Contact Test

Treatment/Dose	**Time (hours)**					
(ng a.s./bumblebee)	**0 (set-up)**	**4**	**24**	**48**	**72**	**96**
Acetone control	0	0	0	0	0	0
Wetting agent control	0	0	0	0	0	0
18.8	0	0	0	3	3	3
37.5	0	0	0	7	7	7
75	0	0	13	23	27	27
150	0	0	27	47	47	47
300	0	0	60	80	80	80

Table B.9.1.1-2: Percent cumulative mortality of bumblebees in the Toxic Reference treated groups over 96 hours - Contact Test

Treatment/Dose (µg a.s./bumblebee)	Time (hours)					
	0 (set-up)	4	24	48	72	96
1.25	0	0	0	10	13	13
2.5	0	0	13	30	33	37
5.0	0	0	57	63	63	63
10.0	0	0	97	97	97	100

Oral Test: Run 1 of the oral test failed to meet the control validity criterion. The test was repeated successfully the data obtained from Run 2 are reported here.

Table B.9.1.1-3: Percent cumulative mortality of bumblebees in the Control and Test Item treated groups over 96 hours - Oral Test

Treatment/Dose (ng a.s./bumblebee)	Time (hours)					
	0 (set-up)	4	24	48	72	96
Acetone control	0	0	0	0	3	3
Wetting agent control	0	0	0	7	7	7
1.7	0	10	31	31	34	34
3.8	0	63	90	90	90	90
4.0	0	77	83	93	100	100
8.9	0	80	83	97	100	100
19.6	0	87	100	100	100	100

Table B.9.1.1-4: Percent cumulative mortality of bumblebees in the Toxic Reference treated groups over 96 hours - Oral Test

Treatment/Dose (µg a.s./bumblebee)	Time (hours)					
	0 hrs (set-up)	4	24	48	72	96
0.5	0	23	33	37	40	40
1.1	0	83	87	93	93	93
1.9	0	87	87	87	87	87
4.5	0	97	100	100	100	100
8.7	0	73	97	97	97	97

Conclusions

Table B.9.1.1-5: LD_{50} values in the bumblebee contact and oral toxicity test with Clothianidin

Timepoint	Contact toxicity (ng a.s./bumblebee)	Oral toxicity (ng a.s./bumblebee)
LD_{50} (24 h)	240.1 (193.6 - 326.9)*	1.841 (0.7227 - 2.689)*
LD_{50} (48 h)	148.3 (109.5 - 221.3)*	1.911 (1.237 - 2.396)*
LD_{50} (72 h)	145.1 (106 - 220.1)*	1.943 (1.595 - 2.242)*
LD_{50} (96 h)	145.1 (106 - 220.1)*	1.943 (1.595 - 2.242)*

*lower and upper 95% confidence limits

RMS Comments

For the second run of both the oral and contact toxicity test for which the results are presented above, the validity criteria are met:

Oral test: - less than 10% mortality across the controls
- a 24h LD_{50} for the toxic reference item between 2 and 10 µg a.s./bee

Contact test: - less than 10% mortality across the controls
- a 48h LD_{50} for the toxic reference item between 0.2 and 2.5 µg a.s./bee

Although the bumblebees were weighed and actual mean food uptake was not only determined in ng a.s./bumblebee but also in ng a.s./g of bumblebee, the LD_{50} expressed as ng a.s/g of bumblebee was

not calculated in the study report. However, in the protocol discussed in the EFSA Techical report on the bee study protocols submitted by Bayer CropScience AG (EFSA Supporting publication 2014:EN-599), the applicant stated that the LD_{50} would be expressed both in terms of ng a.s./bee and ng a.s./g of bee to attempt to express the toxicity in relative terms (due to the variation in bumblebee size).

Overall, the study is considered acceptable and suitable for use in risk assessment. To be consistent with the endpoints used for honeybees, the toxicity endpoints after 48h will be used in the risk assessment:

- Contact toxicity: **$LD_{50,contact}$ = 148.3 ng a.s./bumblebee**
- Oral toxicity: **$LD_{50,oral}$ = 1.911 ng a.s./bumblebee**

B.9.1.2. Semi-field and field studies

No semi-field or field studies have been conducted to assess the effect of the use of clothianidin as seed treatment in cereals and sugar beet on non-*Apis* bees (bumblebees and solitary bees).

B.9.1.3. Summary of the available toxicity data

B.9.1.3.1. Toxicity of the active substance

The available toxicity endpoints for honeybees and bumblebees are summarized in Table B.9.1.3-1 and B.9.1.3-2, respectively. These endpoints were derived from the studies described in the DAR for Clothianidin (2003), the EFSA Conclusion on the risk assessment for bees for clothianidin for seed treatment and granule products (2013)[2], the EFSA Conclusion on the risk assessment for bees for clothianidin considering all uses other than seed treatments and granules (2015)[3] and in section B.9.1.1 of the present addendum to the DAR.

For honeybees, the active substance endpoints for both acute oral and contact toxicity will be used in the risk assessment. The available chronic oral toxicity data on adults and larvae were re-evaluated by EFSA in 2015[3]. However, the endpoints were not expressed in terms of µg a.s./bee per day (i.e. 10-day LD_{50}) or as µg a.s./larvae per developmental period. These two studies were further considered at the Pesticides Peer Review Experts' Meeting 129. Regarding the chronic oral toxicity study, it was agreed to reanalyse the raw data and recalculate the endpoint in terms of 10-day LDD_{50} (µg a.s./bee per day). This reanalysis was performed by EFSA (for details, reference is made to the study evaluation note 01_THW-0174) and the recalculated 10-day LDD_{50} was 0.00138 µg a.s./bee per day.

During Peer Review, the applicant made reference to an amendment to the study report of the chronic toxicity study by Kling (2005) (Report No. M-255911-03-01), in which an LDD_{50} of 0.00183 µg a.s./bee/day was calculated, based on the raw data available in the original study report. The applicant argued that this value should be used instead of the value of 0.00138 µg a.s./bee/day as calculated by EFSA. RMS evaluated both the reanalysis performed by EFSA and by the study authors. In both cases, the performed calculations are scientifically sound and acceptable. In the amendment to the study report, the accumulated intake values (µg a.s./bee) are based on the nominal clothianidin concentrations in the sucrose feeding solution. However, as the clothianidin concentration was measured daily in each treatment group, the intake was recalculated by EFSA using actual concentrations. The fact that the accumulated intake values based on measured concentration are slightly lower than those based on nominal concentrations resulted in a slightly lower LDD_{50} as calculated by EFSA. During Pesticides Peer Review Meeting 145, it was noted that the calculation

[2] European Food Safety Authority (2013). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin. EFSA Journal 2013;11(1):3066. doi:10.2903/j.efsa.2013.3066.
[3] European Food Safety Authority (2015). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin considering all uses other than seed treatments and granules. EFSA Journal 2015; 13(8):4210. doi:10.2903/j.efsa.2015.4210

method used by EFSA was already agreed at Pesticides Peer Review Meeting 129. As it is considered more correct to base the endpoint on the measured concentrations of clothianidin in the sucrose feeding solution, the experts at Pesticides Peer Review Meeting 145 confirmed the conclusion of the earlier meeting, and agreed that the LDD_{50} of 0.00138 µg a.s./bee/day should be used in the risk assessment.

Regarding the study on honeybee larvae (12_THW-0272), it was agreed to derive from this study a 7-day NOED of 40 µg a.s./kg diet, which, expressed in terms of µg a.s./larvae, corresponds to a NOEL of 0.00528 µg a.s./larvae (nominal dose). It is acknowledged that the 7-day NOED was selected by the experts instead of the 22-day NOED of 10 µg a.s./kg diet (i.e. NOEL of 0.00132 µg a.s./larvae, nominal dose), to be in line with the endpoint used for risk assessment according to the EFSA Guidance Document on bees. It was agreed that this endpoint should be used only as provisional endpoint for risk assessment because the study is not fully in line with the proposed protocol in the EFSA Guidance Document (i.e. exposure duration in the study was over 3 days rather than 5 days as recommended by EFSA). In addition, the actual food consumption of larvae was not reported. Therefore it was only possible to express the endpoint in terms of nominal dose.

As there is no agreed testing strategy or validated test guideline for the assessment of sublethal effects, no sublethal endpoints are available for clothianidin, including data on HPG. However, several sublethal effects were reported in the systematic literature search report, including behaviour, locomotion, navigation or orientation (Fryday et al., 2015)[4]. For example, Fischer et al. (2014)[5] reported that clothianidin at 2.5 ng a.s./bee resulted in a significant difference in the flight direction compared to the control group ($p < 0.05$) and significantly longer flight path length and duration compared to the controls ($p < 0.05$). Di Prisco et al. (2013)[6] demonstrated that clothianidin at sublethal dose (i.e. ≤ 21 ng a.s./bee topical exposure and 0.1-10 ppb oral exposure) reduces immune defences and promotes the replication of deformed wing virus. This honeybee immune-suppression is similarly induced by imidacloprid.

A comprehensive review of sublethal effects of pesticides was reported in the EFSA PPR Panel, 2012[7]. However, it has to be noted that in the EFSA Guidance Document on bees, issues were identified that should be resolved before sublethal effects other than HPG for honeybees can be fully integrated in a risk assessment scheme, such as definition of the protection goal, interpretation of the sublethal effects in terms of impact on the colony. The EFSA Guidance Document provided a proposal for a sublethal risk assessment scheme. However, for the purposes of this evaluation it was considered premature to apply such proposal.

[4] Fryday S, Tiede K and Stein J (2015). Scientific services to support EFSA systematic reviews: Lot 5 Systematic literature review on the neonicotinoids (namely active substances clothianidin, thiamethoxam and imidacloprid) and the risks to bees. EFSA supporting publication 2015:EN-756, 656 pp.

[5] Fischer J, Mueller T, Spatz A-K, Greggers U, Gruenewald B and Menzel R (2014). Neonicotinoids Interfere with Specific Components of Navigation in Honeybees. Plos One, 9(3): e91364.

[6] Di Prisco G, Cavaliere V, Annoscia D, Varricchio P, Caprio E, Nazzi F, Gargiulo G and Pennacchio F (2013). Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honeybees. Proceedings of the National Academy of Sciences of the United States of America, 110(46): 18466-18471.

[7] European Food Safety authority (2012). Panel on Plant Protection Products and their residues (PPR): Scientific opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus spp.* and solitary bees). EFSA Journal 2012;10(5):2668. doi:10.2903/j.efsa.2012.2668.

Table B. 9.1.3-1: Summary of the available toxicity endpoints for clothianidin for honeybees (*Apis mellifera*)

	Test substance	Toxicity endpoint	Reference
Acute oral toxicity 48h-LD_{50}	Clothianidin (technical active substance)	**0.00379 µg CTD/bee**	EFSA, 2013[2] and 2015[3]
Acute contact toxicity 48h-LD_{50}	Clothianidin (technical active substance)	**0.0275** – 0.0443 **µg CTD/bee**	EFSA 2013[2] and 2015[3]
Chronic toxicity 10-day NOEC LDD_{50}	Clothianidin (technical active substance)	10 µg CTD/L **0.00138 µg CTD/bee/d**	Kling A., 2005, (re-evaluation by EFSA, 2015[3])
Honeybee larvae 7-day NOED 22-day NOED	Clothianidin (technical active substance)	40 µg CTD/kg diet = **0.00528 µg CTD/larvae** 10 µg CTD/kg diet = 0.00132 µg CTD/larvae	Maus Ch., 2009 (re-evaluation by EFSA, 2015[3])

*Notes: CTD = clothianidin; IMD = imidacloprid; values in **bold** will be used in the risk assessment*

Table B.9.1.3-2: Summary of the available toxicity endpoints for clothianidin for bumblebees (*Bombus terrestris*)

	Test substance	Toxicity endpoint	Reference
Acute oral toxicity 48h-LD_{50}	Clothianidin (technical active substance)	**0.001911 µg CTD/bumblebee**	1.2/1 Harkin S., 2014
Acute contact toxicity 48h-LD_{50}	Clothianidin (technical active substance)	**0.1483µg CTD/bumblebee**	1.2/1 Harkin S., 2014

*Note: CTD = clothianidin; IMD = imidacloprid; N.A. = not available; values in **bold** will be used in the risk assessment*

For bumblebees, the active substance endpoints on both the acute and contact toxicity will be used in the risk assessment. Further, as there are no validated test methods available, there is no data on the chronic toxicity of clothianidin to adult bumblebees or bumblebee larvae. Similarly, there are no validated laboratory test methods available for solitary bees. Consequently, no toxicity studies were submitted as part of the confirmatory data.

According to the EFSA Guidance Document on bees, for performing a screening risk assessment, it can be assumed that the toxicity endpoints for bumblebees and solitary bees are ten times lower than that for honeybees. It should be noted that it is currently unclear how far an extrapolation from honeybee endpoints as surrogates is reliable and applicable. It is well possible that the proposed factor of ten is too conservative. This is supported by acute oral and contact toxicity data for honeybees and bumblebees, for which tests resulted in comparable endpoints for both species (oral LD_{50} for bumblebees was a factor 2 lower compared to honeybees, while the contact LD_{50} was a factor 5 higher). Based on these data, RMS suggested that a factor 1 could be used to determine a surrogate chronic endpoint for bumblebees. However, during Peer Review, some Member States did not agree with this approach (see comment 5(1), 5(5) and 5(6) in the Reporting Table). It is considered that the available data are too limited to scientifically justify this extrapolation, as data on more than one species would be needed to waive a safety factor. Further, it was argued that the chronic toxicity, with continuously feeding exposure regime, takes into account the toxicokinetics of the active substance which may lead to a chronic toxicity that might not be anticipated by the single exposure regime in acute tests. Therefore, the risk assessment for bumblebees was updated using the chronic endpoint for honeybees divided by 10 as a surrogate chronic endpoint for bumblebees. As for solitary bees no data is available, the EFSA Guidance Document is followed as a conservative approach to determine acute and chronic endpoints. Once more information on the toxicity for solitary bees becomes available, these endpoints might be adapted. For the larval toxicity for both bumblebees and solitary bees, the approach from the EFSA Guidance Document was not considered appropriate by the experts in

Pesticides Peer Review Experts' Meeting 129 for the risk assessment to solitary bee larvae, because only a provisional honeybee larvae endpoint was available.

The applicant provided an argumentation to demonstrate that the risk to non-*Apis* bees is covered by the risk assessment for honeybees, based on a similar oral and contact toxicity of clothianidin to bumblebees compared to honeybees. For contact exposure, bumblebees are approximately 5.4 times less sensitive than honeybees to technical clothianidin. For oral exposure, toxicity is indeed similar: oral LD_{50} for bumblebees of 0.001911 µg a.s./bumblebee, which is only slightly lower than the LD_{50} for honeybees of 0.00379 µg a.s./bee). However, due to differences in the trigger values used in the first tier risk assessment for bumblebees and honeybees according to the EFSA Guidance Document on bees, it is difficult to conclude that the risk to bumblebees is covered by the risk assessment for honeybees based on the endpoints alone. Further, there could be differences in exposure in the field, due to biological differences between honeybees and other bee pollinators. As there is no data available on the toxicity of clothianidin to solitary bees, no conclusions can be drawn regarding the sensitivity of solitary bees to clothianidin compared to honeybees. In general, RMS is of the opinion that there is no sufficient evidence to demonstrate that the risk to pollinators other than bees is covered by the risk assessment for honeybees. Therefore, a risk assessment for bumblebees and solitary bees will also be performed for the relevant routes of exposure, taking into account the toxicity endpoints as discussed above. Table B.9.1.3-3 provides an overview of all toxicity endpoints that will be used in the risk assessments throughout this Addendum.

Table B.9.1.3-3: Toxicity endpoints for clothianidin selected for tier 1 risk assessments

Risk assessment type	Endpoint	Honeybees	Bumblebees	Solitary bees
Acute oral	48-hour LD_{50} µg a.s./bee (technical a.s.)	0.00379	0.001911	0.000379*
Acute contact	48-hour LD_{50} µg a.s./bee (technical a.s.)	0.0275	0.1483	0.00275*
Chronic	10-day LDD_{50} µg a.s./bee per day (technical a.s.)	0.00138	0.000138*	0.000138*
Larvae	7-day NOEL mortality µg a.s./larva per development period (technical a.s.)	0.00528 (provisional endpoint)	No endpoint available	No endpoint available
Development of hypopharyngeal glands	NOEL (µg a.s./bee/day)	No endpoint available	Not relevant	Not relevant

*Notes: * Surrogate endpoint by using the honeybee toxicity endpoint divided by a factor of 10*

B.9.1.3.2. Toxicity of metabolites

Table B.9.1.3.2-1 shows the toxicity of the metabolites TMG, TZMU, MNG and TZNG of clothianidin, based on laboratory studies evaluated in the original DAR (2003). Only TZNG has a measurable oral bee toxicity, although its LD_{50} (3.9 µg a.s/bee) is 1000 times higher than the LD_{50} of clothianidin (0.00379 µg a.s./bee). For the other metabolites where acute oral toxicity studies have been performed, there is no measurable toxicity (LD_{50} > 113 µg a.s./L for TZMU and higher for the other metabolites). As the metabolites are of lower toxicity than the parent clothianidin, the risk from the metabolites is considered to be covered in the risk assessment and field studies performed with clothianidin. A specific risk assessment for metabolites is thus not considered necessary.

Table B.9.1.3.2-1: Toxicity of metabolites of clothianidin to honeybees (*Apis mellifera*)

	Test substance	Toxicity endpoint	Species	Reference
Acute oral toxicity 48h-LD_{50}	Metabolite TMG	>151 µg TMG/bee	*Apis mellifera*	Wilkins P., 2000a (as reported in the DAR, 2003)
Acute oral toxicity 48h-LD_{50}	Metabolite TZMU	>113 µg TZMU/bee	*Apis mellifera*	Wilkins P., 2000c (as reported in the DAR, 2003)
Acute oral toxicity 48h-LD_{50}	Metabolite MNG	>153 µg MNG/bee	*Apis mellifera*	Wilkins P., 2000b (as reported in the DAR, 2003)
Acute oral toxicity 48h-LD_{50}	Metabolite TZNG	3.9 µg TZNG/bee	*Apis mellifera*	Wilkins P., 2000d (as reported in the DAR, 2003)

In the studies described in the different sections below (B.9.2 to B.9.7), residues of TZMU and TZNG (together with the active substance clothianidin) were measured in soil and bee-relevant matrices. The selection of these metabolites was based on the occurrence of metabolites in plant metabolism studies as well as the measured toxicity to bees. In the plant metabolism studies, metabolites of clothianidin were found only in very low percentages (for details on these studies, reference is made to the original DAR of Clothianidin). Hence, it was considered reasonable to select only representative metabolites for the monitoring of residues in bee-relevant matrices. TZNG has been selected due to the measurable acute oral toxicity. As representative for the metabolites with low (non-measurable) toxicity, that might occur in bee-relevant matrices, TMZU was selected.

B.9.1.4. Relevant routes of exposure for honeybees and non-*Apis* bees

According to the EFSA Guidance Document on bees, the risk assessment for products applied as granule should consider both exposure via contact and oral exposure via contaminated food items. These exposure routes are essentially the same for both honeybees and non-*Apis* bees.

Exposure via contact occurs from dust particles emitted application of granules, when bees are foraging plants in the field margin and the adjacent crop. According to the EFSA Guidance Document, contact exposure can also occur when bees are foraging the treated crop and weeds in the field. These exposure routes are however not relevant for the currently registered uses of clothianidin as granule treatment, as the granules are applied at sowing. At that moment, no crop plants or weeds will be present on the field due to seed bed preparation.

Oral exposure will occur through the consumption of contaminated pollen or nectar from either the treated crop, weeds in the field, plants in the field margin, the adjacent crop or the succeeding crop/permanent crop the following year. Of the crops in which clothianidin is currently registered, maize is considered to be attractive to honeybees for consumption of pollen, but not for nectar (see Appendix D of the EFSA Guidance Document on bees). Sorghum and potato are generally considered low attractive to honeybees for pollen and nectar collection. However, pollen collection cannot be excluded at all due to controversial information found in literature. For potato, for example, data were provided by Denmark during Pesticides Peer Review Experts' Meeting 129 indicating that honeybees collect pollen from potatoes. Further, potato is considered attractive for bumblebees by the EFSA Guidance Document on bees. As attractiveness of sorghum and potato for solitary bees cannot be excluded, the risk from consumption of nectar and pollen from the treated crop will be assessed. Plants in the field margin and adjacent crops could be contaminated through dust drift, which could result in residues of clothianidin in their pollen and nectar. Therefore, this oral exposure route will be considered in the present assessment, as will the other sources of contaminated pollen and nectar.

Other potential routes for oral exposure are through the consumption of honey dew present in the treated crops and through the consumption of guttation water. Both routes are potentially relevant for both honeybees and non-*Apis* bees and will be assessed as well.

B.9.1.5. Risk assessment

A risk assessment for honeybees following the use of clothianidin as granule in different crops was performed by EFSA, and is reported in the EFSA Conclusion published in 2013[8]. This risk assessment was inclomplete (due to a number of data gaps), and was based on the EFSA Opinion on the science behind a risk assessment for bees (2012)[9]. Since then, the EFSA Guidance Document on the risk assessment for bees (2013)[10] was published and the confirmatory information evaluated in the present addendum was submitted. Therefore, the risk assessment for honeybees is updated following the EFSA Guidance Document and taking into account the newly available data.

For bumblebees and solitary bees, a detailed risk assessment was not yet performed due to the lack of appropriate toxicity and exposure data. A risk assessment following the EFSA Guidance Document on bees for these pollinators is performed in this addendum as well.

The risk assessment scheme for honeybees, bumblebees and solitary bees presented in the EFSA Guidance Document on bees starts with a screening step which, if failed, is followed by a first tier assessment. As clothianidin is a toxic substance for bees, the screening step is often skipped, in which case the assessment started at the first tier. If the risk is not acceptable at first tier, the risk assessment is refined using data from higher tier studies such as field studies, if available.

The results of the assessment for both honeybees and non-*Apis bees* for the different exposure routes are reported in the following sections throughout this Addendum:

- Exposure via contact through dust drift: Section B.9.6
- Oral exposure via consumption of pollen or nectar from:
    - The treated crop: Section B.9.7
    - Weeds in the field: Section B.9.3
    - Plants in the field margin: Section B.9.6
    - Adjacent crops: Section B.9.6
    - Succeeding crops: Section B.9.2
- Oral exposure via consumption of guttation water: Section B.9.5
- Oral exposure via consumption of honey dew in the treated field: Section B.9.4

Reference is made to the relevant sections for details on the assessment and its conclusion.

[8] European Food Safety Authority (2013). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin. EFSA Journal 2013;11(1):3066. doi:10.2903/j.efsa.2013.3066

[9] European Food Safety authority (2012). Panel on Plant Protection Products and their residues (PPR): Scientific opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera, Bombus spp.* and solitary bees). EFSA Journal 2012;10(5):2668. doi:10.2903/j.efsa.2012.2668.

[10] European Food Safety Authority (2013). Guidance on the risk assessment of plant protection products on bees (*Apis mellifera, Bompus* spp. and solitary bees). EFSA Journal 2013; 11(7):3295. doi:10.2903/j.efsa.2013.3295

B.9.2. THE RISK TO HONEYBEES FORAGING IN NECTAR OR POLLEN IN SUCCEEDING CROPS

B.9.2.1. Studies

The potential exposure of bees to residues of clothianidin in succeeding, bee attractive crops could be investigated based on two different approaches. First, studies can be performed under "forced" conditions, where clothianidin was specifically applied to the soil surface to create an artificial plateau concentration followed by the sowing of an untreated crop. This situation is, however, not completely representative of the exposure situation under field conditions, where any accumulated residues arise from the multi-year use of clothianidin and therefore residues will have been exposed to natural ageing processes in the soil. Therefore, a second approach can be used, where the untreated succeeding crops are sown in soil with a history of several years use of clothianidin, and thus exposed to "natural" resudues in the soil.

The applicant submitted one study that used the "forced" approach (Lebrun, 2015), and one study that followed the "natural residues" approach (Harrington, 2013).

"Natural residues" studies

Report:	**IIIA 10.4b/01; Harrington, P; 2013**
Title:	**Santana (a.s. clothianidin 1%): Exposure of honeybee colonies to clothianidin in pollen and nectar from sunflowers grown as a follow-on crop.**
Report No.:	V7YD1000
Document No.:	THW-0338
Guidelines:	Not applicable.
GLP	Yes (certified laboratory)

Objective

This aim of this study was to determine the extent of any exposure of honeybee colonies to clothianidin in nectar and pollen from sunflowers grown on fields treated over three years up to 2010 with Santana (1% w/w a.s. 110 g clothianidin/ha).

Materials and Methods

Test item:	Santana (CAGR8) 1% w/w clothianidin granules batch no. and purity: 2008: 08GR027 EQ1, 0.9597 ± 0.0494% w/w (analysed) 2009 and 2010: GR014, 1.053 ± 0.002% w/w (analysed)
Dates of work:	Experimental start: 4 August 2011 Experimental finish: 2 December 2011 (completion of analytical phase

Trial set up and test site history:

The study was conducted in southern France at the treated site (FR03) used in the 3-year maize study (see IIIA 10.4g/01). The field had been treated with the test item Santana (1% w/w granules; 110 g a.s./ha) by simulating application of granules in-furrow during the mechanical sowing of maize seeds in 2008, 2009 and 2010. The plots on the treated field were drilled with sunflowers at a commercial density sufficient to provide suitable forage for honeybees and three polytunnels (each 200 m^2) were erected on the planted area. Sowing of the sunflowers took place on 21st April 2011. One honeybee colony was placed in each of the three tunnels at the start of flowering and remained in place for the duration of the flowering period. Each colony comprised approximately 6000 adult bees, at least one frame of brood (eggs, larvae and sealed brood) and at least one frame of nectar and pollen.

Sampling of soil, pollen and nectar:

Six soil samples were taken from each tunnel at the start of the exposure phase. For each sample an area of soil approximately 5 x 5 cm and 10 cm deep was samples in six different areas of the tunnels

and these were combined to provide a single sample. Other samples collected from within the tunnel were:

- Pollen and wax as separate samples from the combs inside the colony (at full flowering and at the end of flowering);
- Pollen collected by placing a pollen trap onto each colony for a period long enough to collect sufficient pollen (once during the exposure phase);
- Nectar from the combs inside the colony (at full flowering and at the end of flowering);
- Wax from the combs inside the colony (at full flowering and at the end of flowering).

A single combined pollen sample was collected from the flowering crop within the tunnels (collected as a combined sample from all three tunnels. It was intended to collect a single combined nectar sample from the flowering crop within the tunnels. This could however not be collected, but this was considered to have no significant impact on the study as freshly collected nectar was sampled from the colonies confined within the tunnels.

Samples were transferred to a freezer facility in a portable freezer prior to transfer to the analytical laboratory.

Analytical work:
Sample extraction and determination of residues was performed according to an analytical procedure based on the multi-residue QuEChERS method with LC-MS/MS, modified for the matrices nectar, pollen and soil. The analytical method had been previously successfully validated – in accordance with SANCO/825/00 rev.7 – for the determination of clothianidin, TZNG and TZMU in soil, (maize) pollen and nectar in studies BAY-0803V[11] and SUM-1015V[12].

The analytical method for residues of clothianidin, TZNG and TZMU in wax was based on an internal laboratory method (M01-033[13], Eurofins), modified for beeswax as matrix and had also been successfully validated in study BAY-0803V. The method consisted of dissolving the wax sample in cyclohexane under gentle warming and addition of ethyl acetate followed by overnight frozen storage. After centrifugation, the supernatant was isolated, evaporated to dryness with reconstitution of the residue in methanol/0.05% acetic acid (1/1, v/v) and finally, separation and quantification via LC-MS/MS.

The validated LOQ for each of the analytes according to these analytical procedures was 1.0 µg/kg, and the LOD was determined to be at 0.3 µg/kg.

Findings
The combined soil sample from the three tunnels contained residues of clothianidin at 10 µg/kg. Levels of the two metabolites TZNG and TZMU in the soil sample were below the limit of quantitation and detection, respectively.

The residues of clothianidin, TZNG and TZMU detected in the different nectar and pollen samples are shown in Table B.9.2.1-1.

[11] Lindner, M. (2008). Method development and validation for the determination of clothianidin and its metabolites TZNG and TZMU in crop, soil and honeybee products. Study plan no BAY-0803V [study sponsor: Bayer CropScience AG]; Report no S08-02714

[12] Lindner, M. (2010). Method development and validation for the determination of clothianidin and its metabolites TZNG and TZMU in nectar. Study plan SUM-1015V [study sponsor: Sumitomo Chemical Agro Europe S.A.S.]; Report no S10-01756; Company code THA-0059

[13] Eurofins Dr. Specht GLP GmbH (2009). Extraction and cleanup of wax. Standard Operating Procedure M01-033_01 (adopted from Eurofins Dr. Specht Laboratorien SOP referenced as L-15.023.01).

Table B.9.2.1-1: Residues of clothianidin, TYZNG and TZMU in the different nectar and pollen samples.

Sample	Sample point	Date	Tunnel	Measured residue (µg/kg)		
				Clothianidin	TZNG	TZMU
Pollen Trap	1st visit	05/08/2011	1	1	< LOD	< LOD
Pollen Trap	1st visit	05/08/2011	2	< LOQ	< LOD	< LOD
Pollen Trap	1st visit	05/08/2011	3	< LOQ	< LOD	< LOD
Pollen Hive	1st visit	05/08/2011	1	< LOQ	< LOD	< LOD
Pollen Hive	1st visit	05/08/2011	2	< LOQ	< LOD	< LOD
Pollen Hive	1st visit	05/08/2011	3	< LOQ	< LOD	< LOD
Wax Hive	1st visit	05/08/2011	1	< LOD	< LOD	< LOD
Wax Hive	1st visit	05/08/2011	2	< LOD	< LOD	< LOD
Wax Hive	1st visit	05/08/2011	3	< LOD	< LOD	< LOD
Nectar Hive	1st visit	05/08/2011	1	< LOD	< LOD	< LOD
Nectar Hive	1st visit	05/08/2011	2	< LOD	< LOD	< LOQ
Nectar Hive	1st visit	05/08/2011	3	< LOD	< LOD	< LOD
Pollen Flower	1st visit	05/08/2011	All	< LOQ	< LOD	< LOD
Pollen Hive	2nd visit	18/08/2011	1	< LOQ	< LOD	< LOD
Pollen Hive	2nd visit	18/08/2011	2	1	< LOD	< LOD
Pollen Hive	2nd visit	18/08/2011	3	1	< LOD	< LOD
Wax Hive	2nd visit	18/08/2011	1	< LOD	< LOD	< LOD
Wax Hive	2nd visit	18/08/2011	2	< LOD	< LOD	< LOD
Wax Hive	2nd visit	18/08/2011	3	< LOD	< LOD	< LOD
Nectar Hive	2nd visit	18/08/2011	1	< LOD	< LOD	< LOD
Nectar Hive	2nd visit	18/08/2011	2	< LOD	< LOD	< LOD
Nectar Hive	2nd visit	18/08/2011	3	< LOD	< LOD	< LOD

LOD = 0.3 µg/kg; LOQ = 1.0 µg/kg

Conclusion

Analysis of the samples collected showed that residues of clothianidin and its metabolites TZNG and TZMU in pollen sampled from plants and pollen, nectar and wax sampled from inside the hives were at or below the limit of quantitation, 1 µg/kg, in all samples.

RMS Comments

The test field was only treated for three consecutive years with clothianidin at a rate of 110 g a.s./ha prior to the sowing of sunflower. This application history resulted in a measured concentration of clothianidin in soil at the moment of sunflower sowing of 10 µg/kg. In theoretical (model calculated) $PEC_{soil,plateau}$ concentrations, the maximum concentration in soil is usually obtained after 3 or 4 years of application (depending on the model used). Therefore, in the original version this Addendum, the test field used in the present study and the measured concentration of 10 µg/kg were considered to be representative for a 'natural' soil concentration of clothianidin (at least for soil type and climate at the test site).

At Pesticides Peer Review Meeting 145, it was argued that there are evidences suggesting that a 3 years period of clothianidin use is not enough to reach a top soil residue comparable to the expected soil $PEC_{plateau}$:

- only 10 µg/kg of clothianidin was measured, which is less than the estimated value (This expected accumulation was calculated by EFSA using the current approach for PECsoil accumulation (ESCAPE model, based on the available DegT50 in the field))
- taking into account the field dissipation data currently available in the dossier and agreed at the EU level, the experts suggest that the $PEC_{plateau}$ will only be reached after 10-15 years.

Moreover, it was noted that only the top 10 cm of the soil were sampled for residues measurement, which cannot be considered representative of the root zone uptake (an acceptable depth would be not less than 20 cm).

It was noted that it might be more appropriate not to assess the absolute worst case as it would not be realistic (e.g., due to risk management implications it would be unlikely that clothianidin would be used for a period of 15 years). However it was argued that this would not be a GAP procedure (the residue measured in the root zone should be representative of the uses in GAP regardless of the number of years of product use). Overall, it was agreed that this study conducted as *"natural"* exposure design, is not suitable for use in the risk assessment.

"Forced exposure" studies

Report:	**IIIA 10.4b/02; Lebrun, F.; 2015**
Title:	**Magnitude of the residue of clothianidin and its metabolites in pollen and nectar in succeeding crop. Northern and Southern Europe - 2014**
Report No.:	14SG019
Document No:	THW-0386
Guidelines:	Commission Regulation (EU) No. 283/2013
GLP	Yes (certified laboratory)

Objective

This study, located in Northern and Southern Europe (Northern France, United Kingdom, Germany, Spain, Italy) was conducted to generate specimens of pollen and nectar of several crops following one application of clothianidin on bare soil to quantify residues of clothianidin and its metabolites TZNG and TZMU in pollen and nectar under good agricultural practices in several succeeding crops (maize, mustard, oilseed rape, zucchini, sunflower, field beans).

In the analytical phase, samples of pollen and nectar were analysed for residues of clothianidin and its metabolites TZNG and TZMU and samples of soil were analysed for residues of clothianidin, TZNG, TZMU, thiamethoxam and imidacloprid.

Materials and Methods

Test item:	Clothianidin 50 WG batch no.: 2690651003 purity: 500 g/kg (nominal), 492.2 g/kg (analysed)
Dates of work:	Field phase: 16 April 2014 – 27 October 2014 Analytical phase: 9 October 2014 – 13 December 2014

Trials set up:

Five field trials (Table B.9.2.1-2) were performed in several succeeding crops grown in a way typical of the producing region in the test countries: maize, mustard, oilseed rape, zucchini, sunflower, field beans (all flowering crops) in Northern Europe (Northern France, Germany and United Kingdom) and Southern Europe (Spain and Italy).

Table B.9.2.1-2: Identification of the field trials

Trial number	Study type	Zone	Country (region)	Trial city	Zip code	Field Contractor
14SGS019 FR01	Pollen/nectar trial	Northern Europe	France (Pays de La Loire)	Gennes	49350	Testapi
14SGS019 GE02	Pollen/nectar trial	Northern Europe	Germany (North Rhine-Westphalia)	Goch-Nierswalde	47574	Biochem Agrar
14SGS019 SP03	Pollen/nectar trial	Southern Europe	Spain (Alicante)	Biar	03410	Trialcamp
14SGS019 IT04	Pollen/nectar trial	Southern Europe	Italy (Sicily)	Augusta	96011	Agrigeos
14SGS019 UK05	Pollen/nectar trial	Northern Europe	United Kingdom (Essex)	Manningtree	CO112NF	AgroChemex

The trial sites were typical for the intended use of clothianidin. Only sites that had not been treated in 2012, 2013 and 2014 with any product containing clothianidin and thiamethoxam were used as test sites for the field phase of this study (except trial SP03 – see further below). The sites were representative of Maize or Potato crops and their following potential flowering succeeding crops:

- In Northern Europe: maize, sunflower, spring OSR/mustard, field beans;
- In Southern Europe: maize, sunflower, spring OSR/mustard, zucchini

Each trial consisted of one untreated plot U and one treated plot T, well separated by a minimum of 100 m distance. Each untreated and treated plot of each trial was divided into four subplots for each of the four succeeding crops used in each trial (untreated subplots Ua, Ub, Uc, Ud and treated subplots Ta, Tb, Tc, Td). Each subplot consisted of a minimum of 500 m². The size of each subplot was adapted to the pollen/nectar production of each succeeding crop and could allow the use of a bee tunnel for the pollen/nectar sampling.

Application of the test item on the treated plot:
The soil surface was well prepared as a "seed bed" and application of the test item occurred on the bare soil. A "long-term plateau concentration of the test item" was applied 0 to 6 days before sowing/planting of the succeeding crops, using boom sprayer and following good agricultural practices. Immediately after application, the applied test item was incorporated into the uppermost (approx.) 20 cm of the soil.

The long-term plateau concentration target dose rate of the test item Clothianidin 50WG for the study was 0.2422 kg/ha of formulated product (FP) per application, equivalent to 121.1 g a.s./ha of clothianidin. In the 5 trials performed, the amount of formulated product ranged from 0.231 to 0.253 kg/ha and the amount of active substance applied actually ranged from 115.5 to 126.5 g a.s./ha of clothianidin. The deviations calculated on the amount of formulated product per hectare were all between +/-5% (actually ranging from -4.6 to +4.5%). The spray water volume applied ranged from 220.6 to 418.5 L/ha.

The plateau concentration of clothianidin in soil was estimated based on pre-emergence application of the Santana 0.7G formulation at a rate of 1 x 0.080 kg a.s./ha to soil for growing maize crops. The Santana 0.7G maize use was selected as a worst-case use for soil exposure and accumulation since it involves direct application to the soil without crop interception and annual applications due to the monoculture of maize crops. Bi-phasic (double first-order in parallel, DFOP) modelling parameters (consistent with available field dissipation studies) were used to determine the plateau concentration in soil following continuous, annual applications. Simulations were performed using the EFSA PEARL (version 1.1.1) model. The resultant worst-case plateau PEC_{SOIL} over a twenty year period was for

Central Europe and was 0.0404 mg/kg. This was equivalent to an application rate of 121.1 g clothianidin/ha, based on a soil density of 1.5 g/cm^3 and uniform mixing in the top 20 cm of soil.

Sampling of soil, pollen and nectar:
In all trials:

- One soil core sampling before and after application (depth 0-20 cm / 20 acetate tubes per plot) were performed.
- One soil core sampling at flowering (depth 0-20 cm) and for each of the four crops and in each untreated and treated subplots (20 acetate tubes per plot) were performed.

Sampling of pollen and nectar was performed either by hand or by using bees or bumblebees. When using bees or bumblebees, mesh-covered tunnels were set up over the crop. Specimens were sampled in triplicate (with the exception of the retain specimens).

In Northern Europe Trials (United Kingdom, Germany, France) sampling took place during main flowering periods:

- In maize one sampling of pollen occurred.
- In oilseed rape and mustard one sampling of pollen and nectar occurred.
- In sunflowers two samplings of pollen and nectar were performed with a target interval of 7±1 days between sampling.

In Southern Europe Trials (Italy and Spain) sampling took place during main flowering periods:

- In maize one sampling of pollen occurred.
- In sunflowers one sampling of pollen and nectar occurred.
- In oilseed rape and mustard one sampling of pollen and nectar occurred.
- In zucchini four samplings of pollen and nectar occurred with a target interval of 7±1 days between sampling.

All specimens were deep frozen after collection and stored at a target temperature below -18°C (during storage at the test sites, during shipment to the analytical laboratory Eurofins AgroScience Services (EAS Chem) in Germany and during storage at the analytical laboratory). Maximum storage period from sampling until extraction was 217 days.

Analytical work:
Sample extraction and determination of residues was performed according to an analytical procedure based on the multi-residue QuEChERS method with LC-MS/MS. Matrix-matched standards were used for quantification, as matrix effects were found to be significant (>20% response suppression or enhancement) for most matrix/analyte combinations.

The analytical method had been previously successfully validated – in accordance with SANCO/825/00 rev.7 – for the determination of clothianidin, TZNG and TZMU in soil, (maize) pollen and nectar in studies BAY-0803V[14] and SUM-1015V[15], with a validated LOQ of 1 µg/kg for each of the analytes. The analytical method for the determination of clothianidin, TZNG, TZMU, thiamethoxam and imidacloprid in soil – also based on QuEChERS methodology – was successfully validated in study THA-0099 (Report No. SGS-1435V) with a LOQ of 1 µg/kg for each of the analytes. Reduced validation sets for pollen (maize, sunflower, mustard, field beans and zucchini), nectar (sunflower, oilseed rape, mustard, field beans and zucchini) and soil were generated within the analytical phase of the present study, by fortification of control (untreated) test portions of the

[14] Lindner, M. (2008). Method development and validation for the determination of clothianidin and its metabolites TZNG and TZMU in crop, soil and honeybee products. Study plan no BAY-0803V [study sponsor: Bayer CropScience AG]; Report no S08-02714

[15] Lindner, M. (2010). Method development and validation for the determination of clothianidin and its metabolites TZNG and TZMU in nectar. Study plan SUM-1015V [study sponsor: Sumitomo Chemical Agro Europe S.A.S.]; Report no S10-01756; Company code THA-0059

respective matrix and subsequent determination of the procedural recoveries (n=3 per fortification level; at LOQ 1 µg/kg and at 10xLOQ). Procedural recoveries were mostly within acceptable limits (mean recoveries in range 71-125%; RSD ≤ 35%) and adequately supported the applicability of the method.

In the analytical phase, samples of pollen and nectar were analysed for residues of clothianidin and its metabolites TZNG and TZMU and samples of soil were analysed for residues of clothianidin, TZNG, TZMU, thiamethoxam and imidacloprid.

Findings

The residues detected in the treated and untreated samples of soil are shown in Table B.9.2.1-3.

Table B.9.2.1-3: Residues detected in treated and untreated samples of soil

Sampling Occasion	Study Plan Sample Code 14SGS019	Specimen Type	Plot	Clothianidin Found (rounded) [µg/kg]	TZNG Found (rounded) [µg/kg]	TZMU Found (rounded) [µg/kg]	Thiamethoxam Found (rounded) [µg/kg]	Imidacloprid Found (rounded) [µg/kg]
TRIAL 14SGS019FR01								
S1: Before application	FR01 1	Soil	U	n.d.	n.d.	n.d.	n.d.	< 1
S2: Just after application	FR01 3	Soil	U	n.d.	n.d.	n.d.	n.d.	< 1
S7: Maize plot at flowering	FR01 23	Soil	U	n.d.	n.d.	n.d.	n.d.	< 1
S8: Sunflower plot at flowering	FR01 25	Soil	U	n.d.	n.d.	n.d.	n.d.	< 1
S9: Mustard plot at flowering	FR01 27	Soil	U	n.d.	n.d.	n.d.	n.d.	< 1
S10 Field beans plot at flowering	FR01 29	Soil	U	n.d.	n.d.	n.d.	n.d.	< 1
S1: Before application	**FR01 2**	**Soil**	**T**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
S2: Just after application	**FR01 4**	**Soil**	**T**	**34**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
S7: Maize plot at flowering	**FR01 24**	**Soil**	**T**	**19**	**1**	**n.d.**	**n.d.**	**n.d.**
S8: Sunflower plot at flowering	**FR01 26**	**Soil**	**T**	**20**	**1**	**n.d.**	**n.d.**	**< 1**
S9: Mustard plot at flowering	**FR01 28**	**Soil**	**T**	**41**	**1**	**n.d.**	**n.d.**	**n.d.**
S10 Field beans plot at flowering	**FR01 30**	**Soil**	**T**	**30**	**< 1**	**n.d.**	**n.d.**	**n.d.**
TRIAL 14SGS019FGE02								
S1: Before application	GE02 1	Soil	U	n.d.	n.d.	n.d.	1	n.d.
S2: Just after application	GE02 3	Soil	U	n.d.	n.d.	n.d.	1	n.d.
S7: Maize plot at flowering	GE02 23	Soil	U	n.d.	n.d.	n.d.	< 1	n.d.
S8: Sunflower plot at flowering	GE02 25	Soil	U	n.d.	n.d.	n.d.	< 1	n.d.
S9: Mustard plot at flowering	GE02 27	Soil	U	n.d.	n.d.	n.d.	1	n.d.
S10 Field beans plot at flowering	GE02 29	Soil	U	n.d.	n.d.	n.d.	< 1	n.d.
S1: Before application	**GE02 2**	**Soil**	**T**	**2**	**n.d.**	**n.d.**	**< 1**	**6**
S2: Just after application	**GE02 4**	**Soil**	**T**	**46**	**n.d.**	**n.d.**	**< 1**	**7**
S7: Maize plot at flowering	**GE02 24**	**Soil**	**T**	**44**	**< 1**	**n.d.**	**< 1**	**9**
S8: Sunflower plot at flowering	**GE02 26**	**Soil**	**T**	**39**	**< 1**	**n.d.**	**< 1**	**9**
S9: Mustard plot at flowering	**GE02 28**	**Soil**	**T**	**30**	**< 1**	**n.d.**	**< 1**	**8**
S10 Field beans plot at flowering	**GE02 30**	**Soil**	**T**	**24**	**n.d.**	**n.d.**	**< 1**	**4**
TRIAL 14SGS019FSP03								
S1: Before application	SP03 1	Soil	U	< 1	n.d.	n.d.	4	8
S2: Just after application	SP03 3	Soil	U	< 1	n.d.	n.d.	5	7
S7: Maize plot at flowering	SP03 31	Soil	U	n.d.	n.d.	n.d.	< 1	3
S8: Sunflower plot at flowering	SP03 33	Soil	U	n.d.	n.d.	n.d.	< 1	2
S9: Mustard plot at flowering	SP03 35	Soil	U	< 1	n.d.	n.d.	1	4

Sampling Occasion	Study Plan Sample Code 14SGS019	Specimen Type	Plot	Clothianidin Found (rounded) [µg/kg]	TZNG Found (rounded) [µg/kg]	TZMU Found (rounded) [µg/kg]	Thiamethoxam Found (rounded) [µg/kg]	Imidacloprid Found (rounded) [µg/kg]
S11: Zucchini plot at flowering	SP03 37	Soil	U	< 1	n.d.	n.d.	2	4
S1: Before application	**SP03 2**	**Soil**	**T**	**< 1**	**n.d.**	**n.d.**	**3**	**3**
S2: Just after application	**SP03 4**	**Soil**	**T**	**17**	**n.d.**	**n.d.**	**3**	**4**
S7: Maize plot at flowering	**SP03 32**	**Soil**	**T**	**28**	**< 1**	**< 1**	**3**	**7**
S8: Sunflower plot at flowering	**SP03 34**	**Soil**	**T**	**7**	**n.d.**	**n.d.**	**< 1**	**2**
S9: Mustard plot at flowering	**SP03 36**	**Soil**	**T**	**16**	**n.d.**	**n.d.**	**2**	**8**
S11: Zucchini plot at flowering	**SP03 38**	**Soil**	**T**	**27**	**n.d.**	**< 1**	**2**	**4**
TRIAL 14SGS019IT04								
S1: Before application	IT04 1	Soil	U	< 1	n.d.	n.d.	n.d.	< 1
S2: Just after application	IT04 3	Soil	U	1	n.d.	n.d.	n.d.	< 1
S7: Maize plot at flowering	IT04 31	Soil	U	1	n.d.	n.d.	n.d.	< 1
S8: Sunflower plot at flowering	IT04 33	Soil	U	< 1	n.d.	n.d.	n.d.	< 1
S9: Oilseed rape plot at flowering	IT04 35	Soil	U	< 1	n.d.	n.d.	n.d.	< 1
S11: Zucchini plot at flowering	IT04 37	Soil	U	< 1	n.d.	n.d.	n.d.	< 1
S1: Before application	**IT04 2**	**Soil**	**T**	**< 1**	**n.d.**	**n.d.**	**n.d.**	**< 1**
S2: Just after application	**IT04 4**	**Soil**	**T**	**104**	**n.d.**	**n.d.**	**n.d.**	**< 1**
S7: Maize plot at flowering	**IT04 32**	**Soil**	**T**	**43**	**< 1**	**< 1**	**n.d.**	**< 1**
S8: Sunflower plot at flowering	**IT04 34**	**Soil**	**T**	**25**	**< 1**	**n.d.**	**n.d.**	**< 1**
S9: Oilseed rape plot at flowering	**IT04 36**	**Soil**	**T**	**30**	**< 1**	**n.d.**	**n.d.**	**1**
S11: Zucchini plot at flowering	**IT04 38**	**Soil**	**T**	**75**	**n.d.**	**n.d.**	**n.d.**	**1**
TRIAL 14SGS019FUK05								
S1: Before application	UK05 1	Soil	U	n.d.	n.d.	n.d.	n.d.	< 1
S2: Just after application	UK05 3	Soil	U	n.d.	n.d.	n.d.	n.d.	< 1
S7: Maize plot at flowering	UK05 23	Soil	U	n.d.	n.d.	n.d.	n.d.	< 1
S8: Sunflower plot at flowering	UK05 25	Soil	U	n.d.	n.d.	n.d.	n.d.	< 1
S9: Oilseed rape plot at flowering	UK05 27	Soil	U	n.d.	n.d.	n.d.	n.d.	n.d.
S10 Field beans plot at flowering	UK05 29	Soil	U	n.d.	n.d.	n.d.	n.d.	n.d.
S1: Before application	**UK05 2**	**Soil**	**T**	**2**	**n.d.**	**n.d.**	**n.d.**	**< 1**
S2: Just after application	**UK05 4**	**Soil**	**T**	**30**	**n.d.**	**n.d.**	**n.d.**	**< 1**
S7: Maize plot at flowering	**UK05 24**	**Soil**	**T**	**35**	**2**	**< 1**	**n.d.**	**2**
S8: Sunflower plot at flowering	**UK05 26**	**Soil**	**T**	**23**	**1**	**n.d.**	**n.d.**	**< 1**
S9: Oilseed rape plot at flowering	**UK05 28**	**Soil**	**T**	**33**	**1**	**< 1**	**n.d.**	**n.d.**
S10 Field beans plot at flowering	**UK05 30**	**Soil**	**T**	**24**	**1**	**n.d.**	**n.d.**	**< 1**

S1 = any time before application, S2 = Just after application, S7 = Maize plot at flowering, S8 = Sunflower plot at flowering, S9 = Oilseed rape/Mustard plot at flowering, S10 = Field beans plot at flowering, S11 = Zucchini plot at flowering
U = untreated, T = treated, n.d. not detected (below LOD, < 0.3 µg/kg)
Residues are given as "dry matter", i. e. corrected for their moisture content

A deviation was reported in the Spanish trial SP03 as the farmer informed the trialist, after the end of the field phase, that thiamethoxam based product was applied in the field trial site in 2012. This explains the residues of thiamethoxam found in the soil before application (*vide supra* – Table B.9.2.1-3**)**. This may have had an impact on the study results (as thiamethoxam is degraded into clothianidin). Although clothianidin levels in soil (before and after application) were not really higher compared to the other trials, slightly higher levels were measured in pollen from several treated crops sampled in

this trial (compared to the other trials) and also the 'contamination' observed in the mustard control sample from this trial may be due to the previous use of thiamethoxam on the field.

The residues detected in the treated and untreated samples of pollen are shown in Table B.9.2.1-4.

Table B.9.2.1-4: Residues detected in treated and untreated samples of pollen

Sampling Occasion	Lab. Internal Sample No.	Study Plan Sample Code 14SGS019	Specimen Type	Plot	Clothianidin Found (rounded) [µg/kg]	TZNG Found (rounded) [µg/kg]	TZMU Found (rounded) [µg/kg]
FR01							
S3	103	FR01 5A	Maize (Pollen)	U	4 (*)	n.d.	n.d.
S3	103-W	FR01 5A	Maize (Pollen)	U	8 (*)	n.d.	n.d.
S3	104	FR01 5B	Maize (Pollen)	U	< 1	n.d.	n.d.
S3	105	FR01 5C	Maize (Pollen)	U	3 (*)	n.d.	n.d.
S3	105-W	FR01 5C	Maize (Pollen)	U	3 (*)	n.d.	n.d.
S3	167	FR01 5R	Maize (Pollen)	U	5 (*)	n.d.	n.d.
S3	136	FR01 6A	Maize (Pollen)	T	< 1	n.d.	n.d.
S3	137	FR01 6B	Maize (Pollen)	T	2	n.d.	n.d.
S3	138	FR01 6C	Maize (Pollen)	T	8	n.d.	n.d.
S3	109	FR01 9A	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S3	110	FR01 9B	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S3	111	FR01 9C	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S3	142	FR01 10A	Sunflower (Pollen)	T	3	n.d.	n.d.
S3	143	FR01 10B	Sunflower (Pollen)	T	< 1	n.d.	n.d.
S3	144	FR01 10C	Sunflower (Pollen)	T	< 1	n.d.	n.d.
S4	115	FR01 13A	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S4	116	FR01 13B	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S4	117	FR01 13C	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S4	148	FR01 14A	Sunflower (Pollen)	T	3	n.d.	n.d.
S4	149	FR01 14B	Sunflower (Pollen)	T	2	n.d.	n.d.
S4	150	FR01 14C	Sunflower (Pollen)	T	3	n.d.	n.d.
S3	121	FR01 17A	Mustard (Pollen)	U	n.d.	n.d.	n.d.
S3	122	FR01 17B	Mustard (Pollen)	U	n.d.	n.d.	n.d.
S3	123	FR01 17C	Mustard (Pollen)	U	n.d.	n.d.	n.d.
S3	154	FR01 18A	Mustard (Pollen)	T	8	1	n.d.
S3	155	FR01 18B	Mustard (Pollen)	T	8	1	n.d.
S3	156	FR01 18C	Mustard (Pollen)	T	9	1	n.d.
S3	127	FR01 21A	Field Beans (Pollen)	U	n.d.	n.d.	n.d.
S3	128	FR01 21B	Field Beans (Pollen)	U	n.d.	n.d.	n.d.
S3	129	FR01 21C	Field Beans (Pollen)	U	n.d.	n.d.	n.d.
S3	160	FR01 22A	Field Beans (Pollen)	T	< 1	n.d.	n.d.
S3	161	FR01 22B	Field Beans (Pollen)	T	2	n.d.	n.d.
S3	162	FR01 22C	Field Beans (Pollen)	T	4	n.d.	n.d.
GE02							
S3	203	GE02 5A	Maize (Pollen)	U	n.d.	n.d.	n.d.
S3	204	GE02 5B	Maize (Pollen)	U	n.d.	n.d.	n.d.
S3	205	GE02 5C	Maize (Pollen)	U	n.d.	n.d.	n.d.

Sampling Occasion	Lab. Internal Sample No.	Study Plan Sample Code 14SGS019	Specimen Type	Plot	Clothianidin Found (rounded) [µg/kg]	TZNG Found (rounded) [µg/kg]	TZMU Found (rounded) [µg/kg]
S3	236	GE02 6A	Maize (Pollen)	T	< 1	n.d.	n.d.
S3	237	GE02 6B	Maize (Pollen)	T	< 1	n.d.	n.d.
S3	238	GE02 6C	Maize (Pollen)	T	< 1	n.d.	n.d.
S3	209	GE02 9A	Sunflower (Pollen)	U	< 1	n.d.	n.d.
S3	210	GE02 9B	Sunflower (Pollen)	U	< 1	n.d.	n.d.
S3	211	GE02 9C	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S3	242	GE02 10A	Sunflower (Pollen)	T	2	n.d.	n.d.
S3	243	GE02 10B	Sunflower (Pollen)	T	8	n.d.	n.d.
S3	244	GE02 10C	Sunflower (Pollen)	T	3	n.d.	n.d.
S4	215	GE02 13A	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S4	216	GE02 13B	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S4	217	GE02 13C	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S4	248	GE02 14A	Sunflower (Pollen)	T	< 1	n.d.	n.d.
S4	249	GE02 14B	Sunflower (Pollen)	T	< 1	n.d.	n.d.
S4	250	GE02 14C	Sunflower (Pollen)	T	3	n.d.	n.d.
S3	221	GE02 17A	Mustard (Pollen)	U	n.d.	n.d.	n.d.
S3	222	GE02 17B	Mustard (Pollen)	U	n.d.	n.d.	n.d.
S3	223	GE02 17C	Mustard (Pollen)	U	n.d.	n.d.	n.d.
S3	254	GE02 18A	Mustard (Pollen)	T	3	< 1	n.d.
S3	255	GE02 18B	Mustard (Pollen)	T	3	< 1	n.d.
S3	256	GE02 18C	Mustard (Pollen)	T	3	< 1	n.d.
S3	227	GE02 21A	Field Beans (Pollen)	U	n.d.	n.d.	n.d.
S3	228	GE02 21B	Field Beans (Pollen)	U	n.d.	n.d.	n.d.
S3	229	GE02 21C	Field Beans (Pollen)	U	n.d.	n.d.	n.d.
S3	260	GE02 22A	Field Beans (Pollen)	T	< 1	n.d.	n.d.
S3	261	GE02 22B	Field Beans (Pollen)	T	n.d.	n.d.	n.d.
S3	262	GE02 22C	Field Beans (Pollen)	T	n.d.	n.d.	n.d.
SP03							
S3	303	SP03 5A	Maize (Pollen)	U	< 1	n.d.	n.d.
S3	304	SP03 5B	Maize (Pollen)	U	< 1	n.d.	n.d.
S3	305	SP03 5C	Maize (Pollen)	U	< 1	n.d.	n.d.
S3	348	SP03 6A	Maize (Pollen)	T	5	< 1	1
S3	349	SP03 6B	Maize (Pollen)	T	5	< 1	1
S3	350	SP03 6C	Maize (Pollen)	T	6	< 1	1
S3	309	SP03 9A	Sunflower (Pollen)	U	< 1	n.d.	n.d.
S3	310	SP03 9B	Sunflower (Pollen)	U	< 1	n.d.	n.d.
S3	311	SP03 9C	Sunflower (Pollen)	U	< 1	n.d.	n.d.
S3	354	SP03 10A	Sunflower (Pollen)	T	5	n.d.	< 1
S3	355	SP03 10B	Sunflower (Pollen)	T	5	n.d.	< 1
S3	356	SP03 10C	Sunflower (Pollen)	T	5	n.d.	< 1
S3	315	SP03 13A	Mustard (Pollen)	U	2 (*)	< 1	n.d.
S3	315-W	SP03 13A	Mustard (Pollen)	U	2 (*)	n.d.	n.d.
S3	316	SP03 13B	Mustard (Pollen)	U	2 (*)	< 1	n.d.
S3	316-W	SP03 13B	Mustard (Pollen)	U	2 (*)	n.d.	n.d.

Sampling Occasion	Lab. Internal Sample No.	Study Plan Sample Code 14SGS019	Specimen Type	Plot	Clothianidin Found (rounded) [µg/kg]	TZNG Found (rounded) [µg/kg]	TZMU Found (rounded) [µg/kg]
S3	317	SP03 13C	Mustard (Pollen)	U	2 (*)	n.d.	n.d.
S3	317-W	SP03 13C	Mustard (Pollen)	U	2 (*)	< 1	n.d.
S3	391	SP03 13R	Mustard (Pollen)	U	2 (*)	n.d.	n.d.
S3	360	SP03 14A	Mustard (Pollen)	T	10	1	< 1
S3	361	SP03 14B	Mustard (Pollen)	T	9	< 1	< 1
S3	362	SP03 14C	Mustard (Pollen)	T	11	1	n.d.
S3	321	SP03 17A	Zucchini (Pollen)	U	< 1	< 1	n.d.
S3	322	SP03 17B	Zucchini (Pollen)	U	< 1	n.d.	n.d.
S3	323	SP03 17C	Zucchini (Pollen)	U	< 1	n.d.	< 1
S3	366	SP03 18A	Zucchini (Pollen)	T	6	2	< 1
S3	367	SP03 18B	Zucchini (Pollen)	T	4	2	< 1
S3	368	SP03 18C	Zucchini (Pollen)	T	8	2	n.d.
S4	327	SP03 21A	Zucchini (Pollen)	U	< 1	n.d.	n.d.
S4	328	SP03 21B	Zucchini (Pollen)	U	< 1	n.d.	n.d.
S4	329	SP03 21C	Zucchini (Pollen)	U	< 1	n.d.	< 1
S4	372	SP03 22A	Zucchini (Pollen)	T	2	< 1	n.d.
S4	373	SP03 22B	Zucchini (Pollen)	T	2	< 1	n.d.
S4	374	SP03 22C	Zucchini (Pollen)	T	2	< 1	n.d.
S5	333	SP03 25A	Zucchini (Pollen)	U	n.d.	n.d.	n.d.
S5	334	SP03 25B	Zucchini (Pollen)	U	n.d.	n.d.	n.d.
S5	335	SP03 25C	Zucchini (Pollen)	U	< 1	n.d.	n.d.
S5	378	SP03 26A	Zucchini (Pollen)	T	2	< 1	n.d.
S5	379	SP03 26B	Zucchini (Pollen)	T	2	< 1	n.d.
S5	380	SP03 26C	Zucchini (Pollen)	T	2	< 1	n.d.
S6	339	SP03 29A	Zucchini (Pollen)	U	n.d.	n.d.	n.d.
S6	340	SP03 29B	Zucchini (Pollen)	U	n.d.	n.d.	n.d.
S6	341	SP03 29C	Zucchini (Pollen)	U	n.d.	n.d.	n.d.
S6	384	SP03 30A	Zucchini (Pollen)	T	2	1	< 1
S6	385	SP03 30B	Zucchini (Pollen)	T	2	1	< 1
S6	386	SP03 30C	Zucchini (Pollen)	T	2	< 1	< 1
IT04							
S3	403	IT04 5A	Maize (Pollen)	U	n.d.	n.d.	n.d.
S3	404	IT04 5B	Maize (Pollen)	U	n.d.	n.d.	n.d.
S3	405	IT04 5C	Maize (Pollen)	U	n.d.	n.d.	n.d.
S3	448	IT04 6A	Maize (Pollen)	T	< 1	n.d.	n.d.
S3	449	IT04 6B	Maize (Pollen)	T	< 1	n.d.	n.d.
S3	450	IT04 6C	Maize (Pollen)	T	< 1	n.d.	n.d.
S3	409	IT04 9A	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S3	410	IT04 9B	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S3	411	IT04 9C	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S3	454	IT04 10A	Sunflower (Pollen)	T	< 1	n.d.	n.d.
S3	455	IT04 10B	Sunflower (Pollen)	T	< 1	n.d.	n.d.
S3	456	IT04 10C	Sunflower (Pollen)	T	< 1	n.d.	n.d.
S3	415 [a, b]	IT04 13A	Oilseed Rape (Pollen)	U	-	n.d.	n.d.

Sampling Occasion	Lab. Internal Sample No.	Study Plan Sample Code 14SGS019	Specimen Type	Plot	Clothianidin Found (rounded) [µg/kg]	TZNG Found (rounded) [µg/kg]	TZMU Found (rounded) [µg/kg]
S3	416 [a, b]	IT04 13B	Oilseed Rape (Pollen)	U	-	n.d.	n.d.
S3	417 [a, b]	IT04 13C	Oilseed Rape (Pollen)	U	-	n.d.	n.d.
S3	491	IT04 13R	Oilseed Rape (Pollen)	U	n.d.	n.d.	n.d.
S3	460 [a]	IT04 14A	Oilseed Rape (Pollen)	T	-	< 1	< 1
S3	460-W	IT04 14A	Oilseed Rape (Pollen)	T	5	< 1	n.d.
S3	461 [a]	IT04 14B	Oilseed Rape (Pollen)	T	-	< 1	< 1
S3	461-W	IT04 14B	Oilseed Rape (Pollen)	T	3	n.d.	n.d.
S3	462 [a]	IT04 14C	Oilseed Rape (Pollen)	T	-	< 1	< 1
S3	462-W	IT04 14C	Oilseed Rape (Pollen)	T	4	< 1	n.d.
S3	492	IT04 14R	Oilseed Rape (Pollen)	T	5	< 1	n.d.
S3	421	IT04 17A	Zucchini (Pollen)	U	n.d.	n.d.	n.d.
S3	422	IT04 17B	Zucchini (Pollen)	U	n.d.	n.d.	n.d.
S3	423	IT04 17C	Zucchini (Pollen)	U	n.d.	n.d.	n.d.
S3	466	IT04 18A	Zucchini (Pollen)	T	2	1	n.d.
S3	467	IT04 18B	Zucchini (Pollen)	T	1	< 1	n.d.
S3	468	IT04 18C	Zucchini (Pollen)	T	1	1	n.d.
S4	427	IT04 21A	Zucchini (Pollen)	U	n.d.	n.d.	n.d.
S4	428	IT04 21B	Zucchini (Pollen)	U	n.d.	n.d.	n.d.
S4	429	IT04 21C	Zucchini (Pollen)	U	n.d.	n.d.	n.d.
S4	472	IT04 22A	Zucchini (Pollen)	T	< 1	< 1	n.d.
S4	473	IT04 22B	Zucchini (Pollen)	T	< 1	n.d.	n.d.
S4	474	IT04 22C	Zucchini (Pollen)	T	< 1	n.d.	n.d.
S5	433	IT04 25A	Zucchini (Pollen)	U	n.d.	n.d.	n.d.
S5	434	IT04 25B	Zucchini (Pollen)	U	n.d.	n.d.	n.d.
S5	435	IT04 25C	Zucchini (Pollen)	U	n.d.	n.d.	n.d.
S5	478	IT04 26A	Zucchini (Pollen)	T	n.d.	n.d.	n.d.
S5	479	IT04 26B	Zucchini (Pollen)	T	n.d.	n.d.	n.d.
S5	480	IT04 26C	Zucchini (Pollen)	T	n.d.	n.d.	n.d.
S6	439	IT04 29A	Zucchini (Pollen)	U	n.d.	n.d.	n.d.
S6	440	IT04 29B	Zucchini (Pollen)	U	n.d.	n.d.	n.d.
S6	441	IT04 29C	Zucchini (Pollen)	U	n.d.	n.d.	n.d.
S6	484	IT04 30A	Zucchini (Pollen)	T	< 1	n.d.	n.d.
S6	485	IT04 30B	Zucchini (Pollen)	T	n.d.	n.d.	n.d.
S6	486	IT04 30C	Zucchini (Pollen)	T	< 1	n.d.	n.d.
UK05							
S3	503	UK05 5A	Maize (Pollen)	U	< 1	n.d.	n.d.
S3	503-W	UK05 5A	Maize (Pollen)	U	< 1	n.d.	n.d.
S3	504	UK05 5B	Maize (Pollen)	U	1 (*)	n.d.	n.d.
S3	504-W	UK05 5B	Maize (Pollen)	U	1 (*)	n.d.	n.d.
S3	505	UK05 5C	Maize (Pollen)	U	1 (*)	n.d.	n.d.
S3	505-W	UK05 5C	Maize (Pollen)	U	< 1	n.d.	n.d.
S3	567	UK05 5R	Maize (Pollen)	U	< 1	n.d.	n.d.
S3	536	UK05 6A	Maize (Pollen)	T	3	n.d.	n.d.
S3	536-W	UK05 6A	Maize (Pollen)	T	2	n.d.	n.d.

Sampling Occasion	Lab. Internal Sample No.	Study Plan Sample Code 14SGS019	Specimen Type	Plot	Clothianidin Found (rounded) [µg/kg]	TZNG Found (rounded) [µg/kg]	TZMU Found (rounded) [µg/kg]
S3	537	UK05 6B	Maize (Pollen)	T	3	n.d.	n.d.
S3	537-W	UK05 6B	Maize (Pollen)	T	2	n.d.	n.d.
S3	538	UK05 6C	Maize (Pollen)	T	2	n.d.	n.d.
S3	538-W	UK05 6C	Maize (Pollen)	T	2	n.d.	n.d.
S3	572	UK05 6R	Maize (Pollen)	T	2	n.d.	n.d.
S3	509	UK05 9A	Sunflower (Pollen)	U	2 (*)	n.d.	n.d.
S3	509-W	UK05 9A	Sunflower (Pollen)	U	2 (*)	n.d.	n.d.
S3	510	UK05 9B	Sunflower (Pollen)	U	1 (*)	n.d.	n.d.
S3	510-W	UK05 9B	Sunflower (Pollen)	U	1 (*)	n.d.	n.d.
S3	511-W	UK05 9C	Sunflower (Pollen)	U	1 (*)	n.d.	n.d.
S3	511	UK05 9C	Sunflower (Pollen)	U	1 (*)	n.d.	n.d.
S3	568	UK05 9R	Sunflower (Pollen)	U	1 (*)	n.d.	n.d.
S3	542	UK05 10A	Sunflower (Pollen)	T	< 1	n.d.	n.d.
S3	542-W	UK05 10A	Sunflower (Pollen)	T	1	n.d.	n.d.
S3	543	UK05 10B	Sunflower (Pollen)	T	1	n.d.	n.d.
S3	543-W	UK05 10B	Sunflower (Pollen)	T	1	n.d.	n.d.
S3	544	UK05 10C	Sunflower (Pollen)	T	1	n.d.	n.d.
S3	544-W	UK05 10C	Sunflower (Pollen)	T	1	n.d.	n.d.
S3	573	UK05 10R	Sunflower (Pollen)	T	< 1	n.d.	n.d.
S4	515	UK05 13A	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S4	515-W	UK05 13A	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S4	516	UK05 13B	Sunflower (Pollen)	U	< 1	n.d.	n.d.
S4	516-W	UK05 13B	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S4	517	UK05 13C	Sunflower (Pollen)	U	< 1	n.d.	n.d.
S4	517-W	UK05 13C	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S4	569	UK05 13R	Sunflower (Pollen)	U	n.d.	n.d.	n.d.
S4	548	UK05 14A	Sunflower (Pollen)	T	1	n.d.	n.d.
S4	548-W	UK05 14A	Sunflower (Pollen)	T	1	n.d.	n.d.
S4	549	UK05 14B	Sunflower (Pollen)	T	1	n.d.	n.d.
S4	549-W	UK05 14B	Sunflower (Pollen)	T	< 1	n.d.	n.d.
S4	550	UK05 14C	Sunflower (Pollen)	T	2	n.d.	n.d.
S4	550-W	UK05 14C	Sunflower (Pollen)	T	1	n.d.	n.d.
S4	574	UK05 14R	Sunflower (Pollen)	T	1	n.d.	n.d.
S3	521 [a]	UK05 17A	Oilseed Rape (Pollen)	U	-	n.d.	n.d.
S3	521-W	UK05 17A	Oilseed Rape (Pollen)	U	23 (**)	n.d.	n.d.
S3	522 [a]	UK05 17B	Oilseed Rape (Pollen)	U	-	n.d.	n.d.
S3	522-W	UK05 17B	Oilseed Rape (Pollen)	U	22 (**)	n.d.	n.d.
S3	523 [a]	UK05 17C	Oilseed Rape (Pollen)	U	-	n.d.	n.d.
S3	523-W	UK05 17C	Oilseed Rape (Pollen)	U	33 (**)	n.d.	n.d.
S3	570	UK05 17R	Oilseed Rape (Pollen)	U	27 (**)	n.d.	n.d.
S3	554 [a]	UK05 18A	Oilseed Rape (Pollen)	T	-	2	2
S3	554-W	UK05 18A	Oilseed Rape (Pollen)	T	28 (**)	1	2
S3	555 [a]	UK05 18B	Oilseed Rape (Pollen)	T	-	2	2

Sampling Occasion	Lab. Internal Sample No.	Study Plan Sample Code 14SGS019	Specimen Type	Plot	Clothianidin Found (rounded) [µg/kg]	TZNG Found (rounded) [µg/kg]	TZMU Found (rounded) [µg/kg]
S3	555-W	UK05 18B	Oilseed Rape (Pollen)	T	27 (**)	2	2
S3	556 [a]	UK05 18C	Oilseed Rape (Pollen)	T	-	6	4
S3	556-W	UK05 18C	Oilseed Rape (Pollen)	T	80 (**)	4	6
S3	527	UK05 21A	Field Beans (Pollen)	U	11 (**)	n.d.	n.d.
S3	527-W	UK05 21A	Field Beans (Pollen)	U	2 (**)	n.d.	n.d.
S3	528	UK05 21B	Field Beans (Pollen)	U	n.d.	n.d.	n.d.
S3	528-W	UK05 21B	Field Beans (Pollen)	U	n.d.	n.d.	n.d.
S3	529	UK05 21C	Field Beans (Pollen)	U	< 1	n.d.	n.d.
S3	529-W	UK05 21C	Field Beans (Pollen)	U	n.d.	n.d.	n.d.
S3	571	UK05 21R	Field Beans (Pollen)	U	n.d.	n.d.	n.d.
S3	560	UK05 22A	Field Beans (Pollen)	T	2	n.d.	n.d.
S3	560-W	UK05 22A	Field Beans (Pollen)	T	2	n.d.	n.d.
S3	561	UK05 22B	Field Beans (Pollen)	T	2	n.d.	n.d.
S3	561-W	UK05 22B	Field Beans (Pollen)	T	2	n.d.	n.d.

S3 = At main flowering, S4 = 7 ± 1 days after first pollen sampling, S5 = 7 ± 1 days after 2nd pollen sampling, S6 = 7 ± 1 days after 3rd pollen sampling
U = untreated, T = treated, W = repeated sample preparation, n.d. not detected (below LOD, < 0.3 µg/kg)
Residues are not corrected for procedural recoveries
[a] Clothianidin: contaminated controls (UK05) used for mixed control sample (at about 24 µg clothianidin/kg) for fortification experiments and matrix-matched standards; analytical set not valid;
[b] insufficient amount for re-analysis

(*): Contaminated samples: according to the analytical results obtained, slight cross-contaminations were highlighted in some untreated samples of trials FR01, SP03, UK05. Cross-contaminations could have occurred during sampling in the field or during analytical process (samples preparation) in the laboratory. These results are still reported but should not be taken into account for the field crop trial concerned.

(**): Contaminated samples and outlier results: obvious cross-contaminations were highlighted by the analytical results obtained for Field Beans and the OSR in trial UK05. Furthermore, these values (for treated and also untreated plots) are out of the range of residue values obtained in the other trials and for the same crops. Therefore, these values can be considered outliers and should not be taken into account for the field crop trial concerned.

The residues detected in the treated and untreated samples of nectar are shown in Table B.9.2.1-5.

Table B.9.2.1-5: Residues detected in treated and untreated samples of nectar

Sampling Occasion	Laboratory Sample No.	Study Plan Sample Code 14SGS019	Specimen Type	Plot	Clothianidin Found (rounded) [µg/kg]	TZNG Found (rounded) [µg/kg]	TZMU Found (rounded) [µg/kg]
FR01							
S3	106	FR01 7A	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S3	107	FR01 7B	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S3	108	FR01 7C	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S3	139	FR01 8A	Sunflower (Nectar)	T	n.d.	n.d.	n.d.
S3	140	FR01 8B	Sunflower (Nectar)	T	n.d.	n.d.	n.d.
S3	141	FR01 8C	Sunflower (Nectar)	T	n.d.	n.d.	n.d.
S4	112	FR01 11A	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S4	113	FR01 11B	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S4	114	FR01 11C	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S4	145	FR01 12A	Sunflower (Nectar)	T	n.d.	n.d.	n.d.
S4	146	FR01 12B	Sunflower (Nectar)	T	n.d.	n.d.	n.d.
S4	147	FR01 12C	Sunflower (Nectar)	T	n.d.	n.d.	n.d.
S3	118	FR01 15A	Mustard (Nectar)	U	n.d.	n.d.	n.d.
S3	119	FR01 15B	Mustard (Nectar)	U	n.d.	n.d.	n.d.
S3	120	FR01 15C	Mustard (Nectar)	U	n.d.	n.d.	n.d.
S3	151	FR01 16A	Mustard (Nectar)	T	n.d.	n.d.	n.d.
S3	152	FR01 16B	Mustard (Nectar)	T	n.d.	n.d.	n.d.
S3	153	FR01 16C	Mustard (Nectar)	T	n.d.	n.d.	n.d.
S3	124	FR01 19A	Field Beans (Nectar)	U	n.d.	n.d.	n.d.
S3	125	FR01 19B	Field Beans (Nectar)	U	n.d.	n.d.	n.d.
S3	126	FR01 19C	Field Beans (Nectar)	U	n.d.	n.d.	n.d.
S3	157	FR01 20A	Field Beans (Nectar)	T	n.d.	n.d.	n.d.
S3	158	FR01 20B	Field Beans (Nectar)	T	n.d.	n.d.	n.d.
S3	159	FR01 20C	Field Beans (Nectar)	T	n.d.	n.d.	n.d.
GE02							
S3	206 [a]	GE02 7A	Sunflower (Nectar)	U	-	-	-
S3	207	GE02 7B	Sunflower (Nectar)	U	< 1	n.d.	n.d.
S3	208	GE02 7C	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S3	239	GE02 8A	Sunflower (Nectar)	T	< 1	n.d.	n.d.
S3	240	GE02 8B	Sunflower (Nectar)	T	< 1	n.d.	n.d.
S3	241	GE02 8C	Sunflower (Nectar)	T	n.d.	n.d.	n.d.
S4	212	GE02 11A	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S4	213	GE02 11B	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S4	214	GE02 11C	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S4	245	GE02 12A	Sunflower (Nectar)	T	n.d.	n.d.	n.d.
S4	246	GE02 12B	Sunflower (Nectar)	T	n.d.	n.d.	n.d.
S4	247	GE02 12C	Sunflower (Nectar)	T	< 1	n.d.	n.d.
S3	218	GE02 15A	Mustard (Nectar)	U	n.d.	n.d.	n.d.
S3	219	GE02 15B	Mustard (Nectar)	U	n.d.	n.d.	n.d.
S3	220	GE02 15C	Mustard (Nectar)	U	n.d.	n.d.	n.d.
S3	251	GE02 16A	Mustard (Nectar)	T	< 1	n.d.	n.d.
S3	252	GE02 16B	Mustard (Nectar)	T	n.d.	n.d.	n.d.

Sampling Occasion	Laboratory Sample No.	Study Plan Sample Code 14SGS019	Specimen Type	Plot	Clothianidin Found (rounded) [µg/kg]	TZNG Found (rounded) [µg/kg]	TZMU Found (rounded) [µg/kg]
S3	253	GE02 16C	Mustard (Nectar)	T	< 1	n.d.	n.d.
S3	224	GE02 19A	Field Beans (Nectar)	U	n.d.	n.d.	n.d.
S3	225 [a]	GE02 19B	Field Beans (Nectar)	U	-	-	-
S3	226	GE02 19C	Field Beans (Nectar)	U	n.d.	n.d.	n.d.
S3	257	GE02 20A	Field Beans (Nectar)	T	< 1	n.d.	n.d.
S3	258	GE02 20B	Field Beans (Nectar)	T	< 1	n.d.	n.d.
S3	259	GE02 20C	Field Beans (Nectar)	T	< 1	n.d.	n.d.
			SP03				
S3	306	SP03 7A	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S3	307	SP03 7B	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S3	308	SP03 7C	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S3	351	SP03 8A	Sunflower (Nectar)	T	< 1	n.d.	n.d.
S3	352	SP03 8B	Sunflower (Nectar)	T	< 1	n.d.	n.d.
S3	353	SP03 8C	Sunflower (Nectar)	T	< 1	n.d.	n.d.
S3	312	SP03 11A	Mustard (Nectar)	U	n.d.	n.d.	n.d.
S3	313	SP03 11B	Mustard (Nectar)	U	n.d.	n.d.	n.d.
S3	314	SP03 11C	Mustard (Nectar)	U	n.d.	n.d.	n.d.
S3	357	SP03 12A	Mustard (Nectar)	T	n.d.	n.d.	n.d.
S3	358	SP03 12B	Mustard (Nectar)	T	n.d.	n.d.	n.d.
S3	359	SP03 12C	Mustard (Nectar)	T	n.d.	n.d.	n.d.
S3	318	SP03 15A	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S3	319	SP03 15B	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S3	320	SP03 15C	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S3	363	SP03 16A	Zucchini (Nectar)	T	1	< 1	< 1
S3	364	SP03 16B	Zucchini (Nectar)	T	1	< 1	< 1
S3	365	SP03 16C	Zucchini (Nectar)	T	1	< 1	< 1
S4	324	SP03 19A	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S4	325	SP03 19B	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S4	326	SP03 19C	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S4	369	SP03 20A	Zucchini (Nectar)	T	1	n.d.	< 1
S4	370	SP03 20B	Zucchini (Nectar)	T	1	n.d.	< 1
S4	371	SP03 20C	Zucchini (Nectar)	T	1	n.d.	< 1
S5	330	SP03 23A	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S5	331	SP03 23B	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S5	332	SP03 23C	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S5	375	SP03 24A	Zucchini (Nectar)	T	< 1	n.d.	n.d.
S5	376	SP03 24B	Zucchini (Nectar)	T	< 1	n.d.	n.d.
S5	377	SP03 24C	Zucchini (Nectar)	T	1	n.d.	n.d.
S6	336	SP03 27A	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S6	337	SP03 27B	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S6	338	SP03 27C	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S6	381	SP03 28A	Zucchini (Nectar)	T	2	< 1	< 1
S6	382	SP03 28B	Zucchini (Nectar)	T	1	< 1	< 1
S6	383	SP03 28C	Zucchini (Nectar)	T	2	1	< 1

Sampling Occasion	Laboratory Sample No.	Study Plan Sample Code 14SGS019	Specimen Type	Plot	Clothianidin Found (rounded) [µg/kg]	TZNG Found (rounded) [µg/kg]	TZMU Found (rounded) [µg/kg]
IT04							
S3	406	IT04 7A	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S3	407	IT04 7B	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S3	408	IT04 7C	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S3	451	IT04 8A	Sunflower (Nectar)	T	n.d.	n.d.	n.d.
S3	452	IT04 8B	Sunflower (Nectar)	T	n.d.	n.d.	n.d.
S3	453	IT04 8C	Sunflower (Nectar)	T	n.d.	n.d.	n.d.
S3	412	IT04 11A	OSR (Nectar)	U	n.d.	n.d.	n.d.
S3	413	IT04 11B	OSR (Nectar)	U	n.d.	n.d.	n.d.
S3	414	IT04 11C	OSR (Nectar)	U	n.d.	n.d.	n.d.
S3	457	IT04 12A	OSR (Nectar)	T	2	n.d.	< 1
S3	458	IT04 12B	OSR (Nectar)	T	3	n.d.	< 1
S3	459	IT04 12C	OSR (Nectar)	T	3	n.d.	< 1
S3	418	IT04 15A	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S3	419	IT04 15B	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S3	420	IT04 15C	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S3	463	IT04 16A	Zucchini (Nectar)	T	< 1	< 1	n.d.
S3	464	IT04 16B	Zucchini (Nectar)	T	< 1	< 1	n.d.
S3	465	IT04 16C	Zucchini (Nectar)	T	< 1	< 1	n.d.
S4	424	IT04 19A	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S4	425	IT04 19B	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S4	426	IT04 19C	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S4	469	IT04 20A	Zucchini (Nectar)	T	< 1	n.d.	n.d.
S4	470	IT04 20B	Zucchini (Nectar)	T	n.d.	n.d.	n.d.
S4	471	IT04 20C	Zucchini (Nectar)	T	n.d.	n.d.	n.d.
S5	430	IT04 23A	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S5	431	IT04 23B	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S5	432	IT04 23C	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S5	475	IT04 24A	Zucchini (Nectar)	T	n.d.	n.d.	n.d.
S5	476	IT04 24B	Zucchini (Nectar)	T	n.d.	n.d.	n.d.
S5	477	IT04 24C	Zucchini (Nectar)	T	n.d.	n.d.	n.d.
S6	436	IT04 27A	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S6	437	IT04 27B	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S6	438	IT04 27C	Zucchini (Nectar)	U	n.d.	n.d.	n.d.
S6	481	IT04 28A	Zucchini (Nectar)	T	n.d.	n.d.	n.d.
S6	482	IT04 28B	Zucchini (Nectar)	T	n.d.	n.d.	n.d.
S6	483	IT04 28C	Zucchini (Nectar)	T	n.d.	n.d.	n.d.
UK05							
S3	506	UK05 7A	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S3	507	UK05 7B	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S3	508 [b]	UK05 7C	Sunflower (Nectar)	U	< 1	< 1	< 1
S3	539	UK05 8A	Sunflower (Nectar)	T	n.d.	n.d.	n.d.
S3	540	UK05 8B	Sunflower (Nectar)	T	n.d.	n.d.	n.d.
S3	541	UK05 8C	Sunflower (Nectar)	T	n.d.	n.d.	n.d.

Sampling Occasion	Laboratory Sample No.	Study Plan Sample Code 14SGS019	Specimen Type	Plot	Clothianidin Found (rounded) [µg/kg]	TZNG Found (rounded) [µg/kg]	TZMU Found (rounded) [µg/kg]
S4	512	UK05 11A	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S4	513	UK05 11B	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S4	514	UK05 11C	Sunflower (Nectar)	U	n.d.	n.d.	n.d.
S4	545	UK05 12A	Sunflower (Nectar)	T	< 1	n.d.	n.d.
S4	546	UK05 12B	Sunflower (Nectar)	T	< 1	n.d.	n.d.
S4	547	UK05 12C	Sunflower (Nectar)	T	< 1	n.d.	n.d.
S3	518	UK05 15A	OSR (Nectar)	U	n.d.	n.d.	n.d.
S3	519	UK05 15B	OSR (Nectar)	U	n.d.	n.d.	n.d.
S3	520	UK05 15C	OSR (Nectar)	U	n.d.	n.d.	n.d.
S3	551	UK05 16A	OSR (Nectar)	T	9 (**)	< 1	1
S3	552	UK05 16B	OSR (Nectar)	T	16 (**)	< 1	2
S3	553	UK05 16C	OSR (Nectar)	T	13 (**)	n.d.	2
S3	524	UK05 19A	Field Beans (Nectar)	U	2 (*)	n.d.	n.d.
S3	524-W	UK05 19A	Field Beans (Nectar)	U	2 (*)	n.d.	n.d.
S3	525	UK05 19B	Field Beans (Nectar)	U	< 1	n.d.	n.d.
S3	525-W	UK05 19B	Field Beans (Nectar)	U	< 1	n.d.	n.d.
S3	526	UK05 19C	Field Beans (Nectar)	U	n.d.	n.d.	n.d.
S3	526-W	UK05 19C	Field Beans (Nectar)	U	n.d.	n.d.	n.d.
S3	575	UK05 19R	Field Beans (Nectar)	U	5 (*)	n.d.	n.d.
S3	557	UK05 20A	Field Beans (Nectar)	T	< 1	n.d.	n.d.
S3	558	UK05 20B	Field Beans (Nectar)	T	< 1	n.d.	n.d.
S3	559	UK05 20C	Field Beans (Nectar)	T	< 1	n.d.	n.d.

S3 = At main flowering, S4 = 7 ± 1 days after first nectar sampling, S5 = 7 ± 1 days after 2nd nectar sampling, S6 = 7 ± 1 days after 3rd nectar sampling
U = untreated, T = treated, W = repeated sample preparation, n.d. not detected (below LOD, < 0.3 µg/kg)
[a] sample weight was too low (39 mg), results will not be reported
[b] sample weight was too low (27 mg), results will not be reported
[c] sample weight was less than 75 mg but higher than 30 mg, results will be reported as "< 1 µg/kg (LOQ)"

(*): Contaminated samples: according to the analytical results obtained, slight cross-contaminations were highlighted in some untreated samples of trial UK05. Cross-contaminations could have occurred during sampling in the field or during analytical process (samples preparation) in the laboratory.
These results are still reported but should not be taken into account for the field crop trial concerned.

(**): Outlier results: high values were found for the residues in nectar of OSR in trial UK05, which are out of the range of residue values in nectar obtained in the other trials for the same crop. Further, residues in nectar of OSR from trial UK05 are extremely high compared to the trials for other crops (especially compared to other cruciferes such as mustard), for which residues in nectar are all below the LOQ. Consequently, these results can be considered outliers. These results are still reported but should not be taken into account for the field crop trial concerned.

Conclusion
In the study carried out in Northern and Southern Europe (Northern France, United Kingdom, Germany, Spain, Italy) samples of pollen and nectar were collected from several different crops (maize, mustard, oilseed rape, zucchini, sunflower, field beans) sown following one application of clothianidin on bare soil (at a rate designed to give a theoretical long-term plateau concentration, i.e.

121 g a.s./ha) and analysed for residues of clothianidin and its metabolites TZNG and TZMU. Samples of soil were analysed for residues of clothianidin, TZNG, TZMU, thiamethoxam and imidacloprid.
Table B.9.2.1-6 below summarizes the range of residue levels of clothianidin obtained for the pollen and nectar specimens from each crop in the treated plots, after application of the plateau dose rate, excluding the outlier values identified on the basis of presumed cross-contamination (*vide supra*). Residue levels of TZNG and TZMU in nectar and pollen were below the LOD in most cases.

Table B.9.2.1-6: Range of residue levels of clothianidin obtained for pollen and nectar specimens from several crops after application of clothianidin on bare soil (at a theoretical long-term plateau concentration of 121 g a.s./ha)

In µg/kg	Maize	Sunflower	Mustard	Field Beans	OSR (*)	Zucchini
Pollen	<LOD to 8	<LOQ to 8	3 to 11	<LOD to 4	3 to 5	<LOD to 8
Nectar	-	<LOD to <LOQ	<LOD to <LOQ	<LOD to <LOQ	2 to 3	<LOD to 2

LOQ=1 µg/kg; LOD=0.3 µg/kg; (*) Relatively high levels in nectar and pollen in UK05 trial were excluded, as they are considered outliers.

RMS Comments

The theoretical long-term plateau concentration in soil was calculated based on an annual pre-emergence application to soil for growing maize crops at a rate of 1 x 0.080 kg a.s./ha. This application rate covers the currently registered application rates in maize, sorghum and potato (application rates ranging from 0.050 to 0.070 kg a.s./ha). The calculations to determine the plateau concentration were performed using the SOIL_PEARL model in accordance with Tier 2A of the relevant EFSA opinion[16]. As mentioned on the PEARL and SOIL_PEARL website[17], it should be noted that the SOIL_PEARL version of PEARL is a beta release which is not intended for regulatory submissions. However, as it is already used by EFSA, its use can be accepted, but only if the PEC values calculated by this model are more critical than the PECs obtained with other models currently in use for active substance evaluation at European level (such as ESCAPE Version 2).

Further, it is noted that the calculation of the concentration of clothianidin to be applied on the field was performed by implementating bi-phasic (DFOP) parameters in PEARL version 1.1.1. RMS considers that the choice of a bi-phasic decline could overestimate the degradation of the active substance during the fast degradation phase.

Nevertheless, the measured clothianidin residues in soil in the "natural exposure" study by Harrington (2013), summarized above does not exceed 10 µg/kg soil after 3 years of consecutive application of clothianidin at a rate of 110 g a.s./ha, with no indication of accumulation of 3 years. The $PEC_{soil,plateau}$ calculated in the present study is 40.4 µg/kg soil, and exceeds this measured value. Therefore, and despite the limitations for the $PEC_{soil,plateau}$ concentrations described above, RMS considers the calculated value of 40.4µg/kg soil to be an acceptable worst case value for the evaluation of the magnitude of residues of clothianidin and its metabolites in pollen and nectar in succeeding crops under "forced" exposure.

According to the applicant, the trial was repeated in oilseed rape in 2015 on four sites (FR, SP, GE, IT), to confirm that the level of residues in oilseed rape nectar are low (and that the high residue levels measured in trial UK05 in oilseed rape are indeed outliers). Results from this additional study are expected to be available beginning of 2016. For the time being, RMS agrees that outliers identified on the basis of presumed cross-contamination are excluded when determining the range of residue levels in nectar and pollen.

[16] EFSA Panel on Plant Protection Products and their Residues (2012). Scientific Opinion on the science behind the guidance for scenario selection and scenario parameterization for predicting environmental concentrations in soil. EFSA Journal 2012; 10(2):2562.
[17] http://www.pesticidemodels.eu

After peer review of the study protocol (see EFSA Technical Report, 2014)[18], it was recommended by the experts to take triplicate samples at 3 sampling dates (start of the flowering, middle and end of the flowering). While samples were taken in triplicate, it is noted that samples were only taken at one sampling date (two for oilseed rape).

Residues were determined on 5 sites in Northern and Southern Europe, as suggested in the EFSA Guidance Document on the risk assessment for bees. Consequently, the results can be considered sufficiently reliable for use in risk assessment.

During Peer Review, it was argued that it might not be appropriate to consider the high residue levels in pollen and nectar measured in trial UK05 in oilseed rape as outliers, without further justification (see comment 5(10), 5(13) and 5(14) in the Reporting Table). Further, as the dataset for oilseed rape is only limited to two sites, making determination of whether the data from 1 site can be considered outliers is problematic. The applicant provided the following explanation of how cross contamination may have occurred for the pollen samles of trial UK05 in oilseed rape (*text in italic*):

Untreated and treated soil specimens sampled during the flowering period of each crops did not show any evidence of external contamination during the duration of the trial (eg: reported pesticides applied on / in the neighbourhood were in line with restrictions of the study plan). The sampling method used by the UK team was more sensitive to cross-contamination, as the samples were collected with micro-pipettes. It means that flowers need to be first collected in the field, then brought back to the facilities (using dedicated containers) where the extraction of pollen and nectar is then performed using several people and dedicated area of work for Untreated and Treated workers. Because it is a long sampling process, the risk of contamination exist and although no mistake was recorded, it was concluded that cross-contamination had occurred either in the laboratory or during the sampling+preparation of the samples in the field <u>only</u>.

The following justification of why the high residue values for the UK05 trial in oilseed rape could be considered outliers was provided by the applicant (*text in italic*):

High values were found for the residues in nectar of OSR in trial UK05, which are out of the range of residue values in nectar obtained in the other trials for the same crop. Further, residues in nectar of OSR from trial UK05 are extremely high compared to the trials for other crops (especially compared to other cruciferes such as mustard), for which residues in nectar are all below the LOQ. In the UK trial untreated samples provide residue values sometimes higher than in treated samples, They cannot be explained by an exchange between Untreated and Treated samples before analysis since residues are found in all samples at different levels. Cross contamination has occured but we were not able to identify at which stage. Consequently, although reported, these results can be considered as outliers.
Lastly, a new study which is in the reporting phase consistently shows the same low levels of clothianidin residues in OSR nectar and pollen in France, Spain, Germany and Italy.

At Pesticides Peer Review Meeting 145, the exclusion of the pollen and nectar residues considered as "outliers" or the result of contamination was discussed. It was argued that the dataset is not sufficient to verify that the values can be classified as outliers according to the Dixon Q-test. Moreover it was noted that quantifiable levels of metabolites were measured in the samples classified as outliers (e.g., UK oilseed rape pollen samples). It is therefore unlikely that the measured residues in those samples are only due to cross-contamination (as suggested by the applicant). It was also considered that the measured "outliers" values are not outside the range of the dataset provided in the appendix F of the EFSA Guidance Document for bees (2013).

EFSA considered that the study should not be rejected because it is quite well designed and, in general, well conducted. Additionally the results (including the values quoted as outliers) are in line

[18] European Food Safety Authority (2014). Outcome of the peer review of bee study protocols submitted by Sumitomo to assess the effects of clothianidin on bees. EFSA Supporting Publication 2014:EN-598.

with the existing dataset. The residue values considered as outliers should be included in the exposure characterisation. However, some Member States were reluctant to consider the study suitable for the exposure characterisation due to fundamental issues (cross-contamination and low sampling number) which may indicate that the study is not reliable.

Overall the experts agreed that the absolute highest values for pollen and nectar (80 and 16 µg a.s./kg respectively) by considering all the trials from this study should be considered as the more suitable values. The values to be used for the risk assessment for the risk from exposure to residues in succeeding crops are further discussed in Section B.9.2.2.

B.9.2.2. Exposure

Exposure from contaminated nectar and pollen from succeeding crops is considered a relevant route of exposure for honeybees, bumblebees and solitary bees. The applicant submitted a number of studies in which the concentration of clothianidin in nectar and pollen of bee attractive crops (maize, sunflower, mustard, field beans, zucchini or oilseed rape) were measured under conditions of 'natural' soil residues (succeeding crops grown on soils with a history of clothianidin use) or 'forced' soil residues (succeeding crops grown on soils treated with clothianidin to obtain a theoretical plateau concentration of clothianidin in the soil). The results from these studies show that there are low but measurable residues of clothianidin in pollen and nectar of succeeding crops, and hence exposure to bees is possible.

One 'natural exposure' study was performed, of which the results are summarized in Table B.9.2.2-1. This study was performed at one field site in southern France, where maize that had been treated with clothianidin containing granules was grown for three consecutive years prior to the trial (the field site monitored in study IIIA 10.4g/01, Thompson 2011). As only one field site was tested instead of five as suggested by the EFSA Guidance Document on bees, this study alone cannot be used to determine a representative 90th percentile residue value for use in the risk assessment. However, in the initial version of this Addendum, the results were considered useful to indicate that only low levels of clothianidin are found in pollen and nectar of sunflower as succeeding crop. The mean, median and 90th percentile values for this study were calculated, and are reported in Table B.9.2.2-3.

At Pesticides Peer Review Meeting 145, it was however agreed that this study by Harrington (2014) is not suitable for use in the risk assessment, based on evidence suggesting that a 3 years period of clothianidin use is not enough to reach a top soil concentration comparable to the expected soil $PEC_{plateau}$ (only 10 µg/kg of clothianidin was measured, which is less than the estimated value; based on the field dissipation data currently available in the dossier and agreed at EU level, the $PEC_{plateau}$ will only be reached after 10-15 years). The results from this study are only shown here for information.

Table B.9.2.2-1: Range of residues in soil, pollen and nectar measured in 'natural exposure' study in the succeeding crop sunflower

Reference	Succeeding crop	Residue in soil at flowering (µg/kg dry soil)	Residue in pollen (µg/kg)	Residue in nectar (µg/kg)
10.4b/0.1 Harrington, P. 2014	Sunflower	10	<LOQ – 1	<LOD

LOD = 0.3 µg/kg and LOQ = 1.0 µg/kg for both nectar and pollen

The results from the 'forced exposure' study are summarized in Table B.9.2.2-2 below. This study reports measured residues in nectar and pollen from different succeeding crops at 5 field sites spread over Europe (France, Germany, Spain, Italy and the UK). Each field site was divided into four subplots on which one succeeding crop was sown. That way, the six crops tested were cultivated on two to five different sites. On each test site, the soil was treated with clothianidin at a rate corresponding to a theoretical long-term plateau concentration (i.e. 121 g a.s./ha), resulting from years of consecutive use of clothianidin at a rate of 80 g a.s./kg. As the test was conducted at 5 field sites, as

recommended by the EFSA Guidance Document on bees, the results were considered sufficiently reliable to derive a 90th percentile residue value for use in the risk assessment in the initial version of this Addendum. The mean, median and 90th percentile values for each succeeding crop were calculated, and are reported in Table B.9.2.2-3.

At Pesticides Peer Review Meeting 145, it was however considered not appropriate to use 90th percentile residue values from this study in the risk assessment, as for some crops results from only 2 field site were available. Further, there were some uncertainties regarding the study results due to supposed cross-contamination. Some measured residue values in pollen and nectar were identified as outliers in the study report. The experts at the Meeting however did not agree with the study authors that these values could be considered as outliers (for details on the rationale, please refer to the study summary in Seciton B.9.2.1). Overall, it was agreed that the study should not be rejected because it is quite well designed and, in general, well conducted. It was agreed that the absolute highest values for pollen and nectar (80 µg/kg and 16 µg/kg, respectively, both for oilseed rape) should be considered as the more suitable values for use in risk assessment (instead of 90th percentile values).

Table B.9.2.2-2: Range of residues in soil, pollen and nectar measured in 'forced exposure' studies in the succeeding crops maize, Sunflower, Mustard, Field beans, Zuchini and Oilseed rape.

Reference	Succeeding crop	Residue in soil at flowering (µg/kg dry soil)	Residue in pollen (µg/kg)	Residue in nectar (µg/kg)
10.4b/02 Lebrun, F. 2015	Maize	19 – 44	<LOQ – 8	-
	Sunflower	7 – 39	<LOQ – 8	<LOD - <LOQ
	Mustard	16 – 41	3 – 11	<LOD - <LOQ
	Field beans	24 – 30	<LOD – 4	<LOD - <LOQ
	Zucchini	30 – 33	<LOD – 8	<LOD – 2
	Oilseed rape	27 – 75	3 – 80	2 – 16

LOD = 0.3 µg/kg for both nectar and pollen; LOQ = 1.0 µg/kg forboth nectar and pollen

In the 3-year maize study (IIIA 10.4g/01, Thompson 2011), soil samples were taken in 2010 before the maize crop was sown and the clothianidin applied for the third year. These indicated very little carry over of residues in the soil from the previous two seasons, with levels of 20 µg/kg or less. In the 'natural exposure' succeeding crop study performed the following year on the same field site, measured clothianidin residues in soil of 10 µg/kg further indicate that there is little carry over of residues in the soil from the previous years. In the 'forced exposure' study, clothianidin was applied at a rate designed to give a theoretical long-term plateau concentration i.e. 121 g a.s./ha. Over a depth of 10 cm (the same sampling depth as used in the 3-year maize study 10.4g/01) this is equivalent to a theoretical concentration of 81 µg a.s./kg (consistent with the analysed soil residues, where the average concentrations obtained in all trials was 29.6 µg/kg, over a depth of 20 cm). This is clearly worst-case in relation to the carry-over experienced under field conditions.

Table B.9.2.2-3: Mean, median and 90th percentile concentration of clothianidin (µg a.s./kg), measured in nectar and pollen in the succeeding crops maize, sunflower, mustard, field beans, zucchini and oilseed rape in the 'natural exposure' and 'forced exposure' studies.

'Natural exposure' studies								
Crop	**Residues in pollen (µg/kg)**				**Residues in Nectar (µg/kg)**			
	No. of value >LOQ /Total	**Mean**	**Median**	**90th percentile**	**No. of value >LOQ /Total**	**Mean**	**Median**	**90th percentile**
Sunflower [1]	3/10	1.0	1.0	1.0	0	<LOD	<LOD	<LOD
'Forced exposure' studies								
Crop	**Residues in pollen (µg/kg)**				**Residues in Nectar (µg/kg)**			
	No. of value >LOQ /Total	**Mean**	**Median**	**90th percentile**	**No. of value >LOQ /Total**	**Mean**	**Median**	**90th percentile**
Maize	12/19	2.6	2	5.2	-	-	-	-
Sunflower	12/32	2.0	1.0	4.8	0/24	<LOQ	<LOD	<LOQ
Mustard	9/9	7.1	8.0	10.2*	0/9	<LOQ	<LOD	<LOQ
Field beans	6/10	1.7	2.0	2.2	0/9	<LOQ	<LOQ	<LOQ
Zucchini	13/24	1.9	2.0	3.4	2/24	<LOQ	<LOQ	<LOQ
Oilseed rape	4/4	4.3	4.5	5.0	3/3	2.7	3.0	3.0*

*Note: [1]The results from this study were considered not suitable for use in the risk assessment at Pesticides Peer Review Meeting 145, and are only shown here for information; for the calculation of the mean, median and 90th percentile values, concentrations reported as <LOD were assigned the value of the LOD (0.3 µg/kg for both nectar and pollen) as a conservative approach. Values reported as <LOQ were assigned the value of the LOQ (1.0 µg/kg for both nectar and pollen); *values used in the risk assessment*

For sunflower, the soil residues measured in the 'natural exposure' study are comparable to those measured in the sunflower plots in the 'forced exposure' study (at least for the lower measured soil residues). However, the residues in pollen and nectar of sunflower are much higher in the 'forced exposure' study compared the 'natural exposure' study. This could be explained by the fact that in the latter studies, the clothianidin residues in soil had already undergone ageing processes, making them less available for plant uptake as compared to the 'forced exposure' studies. This further indicates that the 'natural exposure' studies are a more realistic representation of exposure under field condition than 'forced exposure' studies.

As 'natural exposure' studies are more realistic, it seems appropriate to use the measured residues in pollen and nectar from these studies in the risk assessment for bees, instead of the more worst case values from the 'forced exposure' studies. However, as the available 'natural exposure' study by Harrington (2013) was not considered suitable for use in the risk assessment, results from the 'forced exposure' study would have to be used instead.

At Pesticides Peer Review Meeting 145, the experts considered it scientifically justitfied to consider all available studies, from both the use of clothianidin as seed treatment (Bayer Crop Science dossier) and as granular application (Sumitomo dossier) together in the exposure assessment. For both uses, the accumulation in soil is expected to be similar (application to bare soil, with no interception in both cases), which results in the same $PEC_{plateau}$. Based on the complete dataset, it was agreed that the "natural exposure" studies could be considered more realistic (more representative of the accumulation over years, as natural ageing processes are taken into account, which may lead to a lower bioavailability of clothianidin). Therefore, they should be considered more suitable for the exposure assessment rather than the 'forced exposure' studies. As the geographical spread of the available 'natural exposure' studies was limited, with only studies performed in Germany and the UK available, it was agreed that the 90th percentile residue value from these studies cannot be used, in line with the EFSA Guidance Document for bees. Overall, the majority of the experts agreed that the highest residue level in pollen and nectar from the "natural exposure" studies could be used in the risk

assessment to address the succeeding crop scenarios for all uses under evaluation, except for the use in forestry nursery. As for forestry nursery the soil cultivation will be different (i.e. no ploughing and mixig of the residues in soil), the results from the available studies cannot be extrapolated to this use. The residue values to be used in the risk assessment are 1.5 µg a.s./kg for pollen (measured in maize pollen) and 0.6 µg a.s./kg for nectar (measured in *Phacelia* nectar). For detailed assessment of the studies from which these values were derived, reference is made to Section B.9.2.2 of the Addendum for the Bayer Crop Science data.

It was highlighted at the Meeting that this approach may not fully address the attractiveness of the crop as foreseen in the EFSA Guidance Document for bees as well as the different potential uptake from succeeding crops other than those investigated. However, even if the uncertainty with respect to the recommendation of the EFSA Guidance Document cannot be addressed with the available data, the experts agreed that this was the best way to make use of the available data.

B.9.2.3. Risk assessment

B.9.2.3.1. Risk assessment for honeybees

The risk assessment was performed following the risk assessment scheme for honeybees as proposed in the EFSA Guidance Document on bees. Due to the potential risk to honeybees from the consumption of pollen and nectar from succeeding crops, the screening step was not performed, and the risk assessment started at the first tier. As based on the information available in the GAP table (Table A-1) it is not possible to perform a risk assessment for the use in forestry nursery (only the dose in g a.s./plant is available, no information on the plant density and dose in g a.s./ha), a risk assessment for this use is not included.

It is noted that for the indoor use of clothianidin in maize and sweet maize (which is restricted to permanent greenhouses), exposure to bees from nectar and pollen in succeeding crops can be considered low. Therefore, in line with the decision of Pesticides Peer Review Meeting 129, no risk assessment needs to be performed for this indoor use.

First tier risk assessment

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 products applied as granules at sowing (incorporated into the soil). The relevant shortcut values (and the methodology used to determine these 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.

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 and/or mg/seed
SV = 0.70 (shortcut value for acute exposure to forager honeybees, taken from Table J6 in Appendix J of the Guidance Document)
E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for the succeeding crop scenario)
$LD_{50,oral}$ is expressed as µg a.s./bee

If this ETR > 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 *ETR for the chronic adult oral exposure* is calculated by the following equation:

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

Where: AR = application rate in kg a.s./ha and/or mg/seed
SV = 0.54 (shortcut value for chronic exposure to forager honeybees, taken from Table J6 in Appendix J of the Guidance Document)
E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for the succeeding crop scenario)
twa = 1
LDD_{50} is expressed as µg a.s./bee per day

If this ETR > 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.

The *ETR for larvae* is calculated by the following equation:

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

Where: AR = application rate in kg a.s./ha and/or mg/seed
SV = 0.40 (shortcut value for honeybee larvae, taken from Table J6 in Appendix J of the Guidance Document)
E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for the succeeding crop scenario)
twa = 1
NOED is expressed as µg a.s./larva/development period

If this ETR > 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.

According to the EFSA Guidance Document, an ETR for effects on the development of the hypopharyngeal glands (HPG) should also be calculated. As there is currently no validated methodology for the assessment of sublethal effects, no endpoint for the effects on the hypopharyngeal glands of honeybees is available for clothianidin. Therefore, the first tier risk assessment for honeybees based on HPG was not performed.

The first tier risk assessment has been performed using the authorized 'maximum application rate' for potato, maize and sorghum (see Table B.9.2.3.1-1). The relevant toxicity endpoints are taken from Table B.9.1.3.1-3. The calculated Tier 1 ETR values are shown in Table B.9.2.3.1-2.

Table B.9.2.3.1-1: currently authorized 'maximum application rate' of clothianidin containing formulations for use as a granule treatment at sowing in potato, maize and sorghum.

Crop	authorized 'maximum application rate'
Potato	70 g a.s./ha
Maize/ Sweet maize/ Sorghum	50 g a.s./ha

Table B.9.2.3.1-2: Tier 1 ETR calculations for acute adult oral, chronic adult oral and larval exposure for the highest authorized 'maximum application rate' of clothianidin in potato, maize and sorghum.

Acute adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Potato	0.070	1	0.70	-	0.00379	12.93	0.2
Maize/ Sweet maize/ Sorghum	0.050	1	0.70	-	0.00379	9.23	0.2
Chronic adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	LDD_{50} (µg a.s./bee/day)	ETR	Trigger
Potato	0.070	1	0.54	1	0.00138	27.39	0.03
Maize/ Sweet maize/ Sorghum	0.050	1	0.54	1	0.00138	19.57	0.03
Larval exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	NOED (µg a.s./larva /development period)	ETR	Trigger
Potato	0.070	1	0.40	1	0.00528	5.30	0.2
Maize/ Sweet maize/ Sorghum	0.050	1	0.40	1	0.00528	3.79	0.2

As all ETR values exceed the relevant trigger values, a potential risk is identified for all honeybee 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, 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 in which the clothianidin residues in nectar and pollen in several succeeding crops were measured. In the original version of this Addendum, the highest 90th percentile residue values from the submitted 'forced exposure' studies were used to refine the risk assessment. As discussed under Section B.9.2.2, the complete data set, with all available studies from both the use of clothianidin as seed treatment (Bayer Crop Science dossier) and as granular application (Sumitomo dossier), was considered at Pesticides Peer Review Meeting 145. Based on this dataset, it was considered more appropriate to use the highest available residue values from the 'natural exposure' studies in the tier 2 risk assessment. The residue values to be used in the risk assessment are 1.5 µg a.s./kg for pollen (measured in maize pollen) and 0.6 µg a.s./kg for nectar (measured in *Phacelia* nectar). As these values were obtained by exposing a number of succeeding crops to a soil concentration exceeding the theoretical soil plateau concentration of clothianidin resulting from an annual use according to GAP, the selected residue values cover the succeeding crop scenarios for all registered uses of clothianidin as granule (except the use in forestry nursery).

In table J1 of appendix J of the EFSA Guidance Document on bees, data on the consumption of nectar and pollen by forager and nurse honeybees and honeybee larvae are reported. These values are shown in Table B.9.2.3.1-3. Since the energy demand of the 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, some data from the literature is available. There is however little known about the distribution and frequency of the sugar content carried by bees. Awaiting further research in this field, it was considered that the worst case values (i.e. nectar with the lowest sugar content from the ranges which may be foraged by

bees), namely 15% for honeybees, 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 B.9.2.3.1-3.

Table B.9.2.3.1-3: Pollen, sugar and nectar consumption of honeybees

Honeybee stage	Pollen consumption (mg/bee/day or mg/larva)	Sugar consumption (mg/bee/day or mg/larva)	Nectar consumption[1] (mg/bee/day or mg/larva)
Forager bee	0	32 – 128	213 – 853
Nurse bee	6.5 – 12	34 – 50	227 – 333
Larva	1.5 – 2	59.4	396

[1]*Nectar consumption was calculated based on a worst case sugar concentration of 15% in nectar*

According to Appendix N of the EFSA Guidance Document for bees, the daily residu uptake for adult bees and the total residue uptake for larvae can be calculated based on the nectar and pollen consumption, 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)
R_n is the residue level in nectar (in mg/kg)
R_p is the residue level in pollen (in mg/kg)
C_n is the consumption of nectar in mg (mg/bee/day or mg/larva)
C_p is the consumption of pollen in mg (mg/bee/day or mg/larva)

In the initial version of this Addendum, the worst case values for pollen consumption from Table B.9.2.3.1-3 were used for the calculation of the residue intake (RI). For nectar consumption, the worst case values were used for the acute exposure for adult honeybees, while the mean from the minimum and maximum value was used for the chronic adult exposure. At Pesticides Peer Review Meeting 145, it was noted that this approach is acceptable, but represents a worst case. A tool for calculating refined shortcut values based on compound or crop specific input parameters (SHVAL Tool, see Appendix Z of the EFSA Guidance Document on bees and EFSA supporting publication 2014:EN-623[19]) has been developed by EFSA. The SHVAL tool, which is an application developed in R, allows for inputting raw data as well as reference values (central tendency measurements / ranges). It first fits theoretical distributions to the data, where possible, and then it runs a Monte Carlo simulation mimicking an hypothetical field study on 1000 fields with 1000 hives in each field and 1000 bees in each hive. The SHVAL tool returns the probabilistic distributions fitted to the data and the empirical density distribution of the Shortcut Value's 90th percentile over the 1000 iterations (fields). This way, this tool allows for the estimation of the Shortcut Value's 90th percentile and its 95% confidence interval. The refined Shortcut Values obtained by using the SHVAL tool are considered more representative than a calculation only based on maximum or mean value for pollen and nectar consumption. The experts agreed that this SHVAL tool should be used to update the Tier 2 risk assessment based on the agreed residue values for pollen and nectar in succeeding crops. The calculation of refined shortcut values was therefore updated using the EFSA Shortcut Values calculation model (EFSA SHVAL model), version 1.0. This application interface can be made available upon request to amu@efsa.europa.eu.

As discussed above, clothianidin residues of 1.5 µg/kg in pollen and 0.6 µg/kg in nectar were used, as agreed at Pesticides Peer Review Meeting 145. Regarding these residues values, it should be noted that these are single, maximum values without distribution. Further, these values are not RUD values as they originate from 'natural exposure' studies, where field sites with a history of clothianidin use over several years were used. The application rates of the treated crops in the year prior to the residue trials

[19] European Food Safety Authority (2014). A small application developed in R for the estimation of the residue intake rate for certain bee species under given conditions: the SHVAL tool. EFSA supporting publication 2014:EN-623. 15 pp.

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 calculations without any modification.

For the calculations made with the SHVAL tool, two 'test' calculations were made in a first step 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 as those that had been used for the calculation of the tier 1 Shortcut Values for nurse honeybees, honeybee larva and forager honeybees chronic for the seed dressing use and the granular use (before emergence). The other calculations were made for clothianidin for the different bees and risk categories with the chemical specific residue values. Nurse bees were considered since the agreed residue level was higher for pollen than for nectar. 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 shown in Table B.9.2.3.1-4. Table B.9.2.3.1-5 shows a summary of all the input parameters inserted in the SHVAL tool for the different bee categories. The values for pollen and nectar consumption were derived from Table B.9.2.3.1-3.

Table B.9.2.3.1-4: Residue levels used as input for the calculation of the refined Shortcut Values using the EFSA SHVAL tool.

Relevance	Residue level in mg/kg	Ln
Test	1	0
Clothianidin pollen	0.0015	-6.50229
Clothianidin nectar	0.0006	-7.41858

Table B.9.2.3.1-5: Input parameters used for the calculations with the SHVAL tool for the different honeybee categories.

No.	bee type & category	Pollen consumption (mg/bee/day or mg/larvae)	Sugar consumption (mg/bee/day or mg/larvae)	Sugar content of nectar (mg/mg)	chemical conc. in pollen[1]	chemical conc. in nectar[1]	Relevance
1	HB nurse	12	34-50	0.15	0	0	Test
2	HB larva	2	59.4	0.15	0	0	Test
3	HB forager acute	0	80-128	0.15	-6.50229	-7.41858	Clothianidin
4	HB forager chronic	0	32-128	0.15	-6.50229	-7.41858	Clothianidin
5	HB nurse	12	34-50	0.15	-6.50229	-7.41858	Clothianidin
6	HB larva	2	59.4	0.15	-6.50229	-7.41858	Clothianidin
7	HB forager chronic	0	32-128	0.15	0	0	Test

[1]*See Table B.9.2.3.1-4; HB: honeybee*

The resulting refined Shortcut Values (SV) are shown in Table B.9.2.3.1-6. These Tier 2 SVs are about three orders of magnitude lower than the Tier 1 SVs.

Table B.9.2.3.1-6: Calculated Tier 2 Shortcut Values (SV) for the different scenarios and honeybee stages

No.	Relevance	bee type & category	Tier 2 SV (µg/bee or µg/bee/day or µg/larva)	Comment
1	test	HB nurse	0.293	Expected value was 0.29
2	test	HB larva	0.398	Expected value was 0.4
3	Clothianidin	HB forager acute	0.00042	
4	Clothianidin	HB forager chronic	0.00032	
5	Clothianidin	HB nurse	0.00019	As forager's intake is higher, this value is not needed for the RA
6	Clothianidin	HB larva	0.00024	Value was confirmed by 'hand' calculation (as no variability in input parameters)
7	test	HB forager chronic	0.540	Expected value was 0.54

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 exposure factor (E_f) and the twa values are supposed to be 1 in the risk assessment for the succeeding crop scenario. Therefore, the formula to calculate the ETR values in this case can be simplified as:

$$ETR = \frac{SV}{LD_{50\ oral}\ /\ LDD_{50}\ /\ NOED}$$

The calculated ETR values are shown in Table B.9.2.3.1-7. Taking into account the rerpresentative measured residue values, the ETR values for acute risk to adult honeybees and chronic risk to honeybee larvae are below the relevant trigger, indicating an acceptable risk. However, the ETR for chronic risk to adult honeybees still exceed the trigger. Further consideration is this necessary.

Table B.9.2.3.1-7: Tier 2 ETR calculations for acute adult oral, chronic adult oral and larval exposure from nectar and pollen in succeeding crops following application of clothianidin in potato and maize.

Scenario	Honeybee stage	Tier 2 SV (µg/bee or µg/bee/day or µg/larva)	Toxicity endpoint (µg/bee or µg/larva)	ETR	Trigger
Acute adult oral	Forager	0.00042	0.00379	0.1108	0.2
Chronic adult oral	Forager	0.00032	0.00138	**0.2319**	0.03
Larvae	Larva	0.00024	0.00528	0.0454	0.2

Higher tier risk assessment

Further refinements to the risk assessment could be based on field effect studies. However, no higher tier effect studies specifically assessing the risk to honeybees from the consumption of nectar and pollen in succeeding crops are available. However, field effect studies with treated crops could be used as a surrogate for succeeding crop studies, provided that it is demonstrated that exposure from the treated crop was higher compared to what is expected from succeeding crops.

For maize, a field study on the effects of clothianidin residues in pollen on honeybee colonies is available (10.4g/01, Thompson 2011b). The measured residues in the maize pollen in this study were higher than those measured in the succeeding crops studies (up to 15 µg/kg in pollen sampled in the field, and up to 19 µg/kg in pollen sampled in tunnels). The results from the study by Thompson (2011b) indicated that there were no detectable effects of exposure to clothianidin residues in maize pollen on the colony development in the 3 sites over the 3 years. Based on these results, it was concluded in the original version of this Addendum that the risk from exposure to clothianidin residues

in pollen in succeeding crops (in which residues are lower compared to a treated crop), is likely to be low as well.

However, during Peer Review of the original version of this Addendum, several concerns were raised regarding the field study on maize by Thompson (2011b) and the study re-analysis by Lewis (2014). These issues were discussed at Pesticides Peer Review Meeting 145. The statistical power was discussed in relation to the high inter-colony variability observed. It was argued that the study has a low statistical power (assuming that the observed variability is a suitable estimation of the real natural variability). It was noted that most of the variability (c. 90%) was due to the inter-colony factor rather than inter-site and temporal factors. This may mean that the number of hives per site is more relevant in terms of statistical power than the number of sites. However, it was argued that the analysis was performed on a limited numbers of hives and sites and that therefore the variability partitioning observed in this study may not represent the real natural variability. Further, it was noted that the RMS pointed out the relevance of the biological interpretation of field trials.

It was concluded that, generally, when the results are highly variable (which is the case for the study by Thompson, 2011b) it is difficult to draw any conclusion on a cause – effect relationship (i.e. treatment or non-treatment related effects). Overall, it was agreed that the re-analysis provided for the study is not sufficient to address the concerns already identified in the conclusion of EFSA 2013 (i.e., the Thompson study cannot be considered sufficient to draw a firm conclusion on the cause-effect relationship).

Generally, it was acknowledged that the availability of several pieces of evidence (e.g. several comparable field studies) can be useful to make a trend analysis to be used as a weight of evidence for the risk assessment. However, apart from the study by Thompson (2011b) no other field studies are available. Consequently, no acceptable risk to honeybees following exposure to contaminated nectar and pollen in succeeding crops could be demonstrated.

In the original verison of the addendum, the results from the study by Thompson (2011b) in maize were also extrapolated to other succeeding crops. As maize does not produce nectar, it is difficult to extrapolate the results from this study to nectar producing succeeding crops that are attractive to bees. However, the succeeding crop residue data shows that, except for oilseed rape, measured residues in nectar of succeeding crops are below the LOQ. As the residue levels in pollen found in the three year maize study largely exceed the 90th percentile residue values in nectar in most succeeding crops, it was considered justified to extrapolate the results from the maize study to succeeding crops that also produce nectar.

During Peer Review, it was however argued that differences in pollen and nectar consumption should be taken into account when considering the extrapolation from the maize field trial to nectar producing succeeding crops, as nectar consumption is likely to exceed the pollen consumption (see comment 5(17) in the Reporting Table). This issue was also discusses at Pesticides Peer Review Meeting 145. It was agreed that a study in maize may be considerd of weak representativeness for succeeding crops that produce nectar. Consequently, even if the study by Thompson (2011b) would have been sufficient to conclude on an acceptable risk to honeybees from maize as a succeeding crop, an extrapolation of this conclusion to other, nectar producing succeeding crops would not be acceptable.

Conclusions

The risk to honeybees from consumption of contaminated pollen and nectar in succeeding crops was not acceptable at tier 1. Refinement of the assessment based on measured clothianidin residues in a number of succeeding crops did not result in an acceptable risk. The available higher tier field effect studies were not considered sufficient to demonstrate an acceptable risk.

B.9.2.3.2. Risk assessment for bumblebees

The risk assessment was performed following the risk assessment scheme for bumblebees as proposed in the EFSA Guidance Document on bees. Due to the potential risk to bumblebees from the consumption of pollen and nectar from succeeding crops, the screening step was not performed, and the risk assessment started at the first tier. As based on the information available in the GAP table (Table A-1) it is not possible to perform a risk assessment for the use in forestry nursery (only the dose in g a.s./plant is available, no information on the plant density and dose in g a.s./ha), a risk assessment for this use is not included.

It is noted that for the indoor use of clothianidin in maize and sweet maize (which is restricted to permanent greenhouses), exposure to bees from nectar and pollen in succeeding crops can be considered low. Therefore, in line with the decision of Pesticides Peer Review Meeting 129, no risk assessment needs to be performed for this indoor use.

First tier risk assessment

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 products applied as granules at sowing (incorporated into the soil). The relevant shortcut values (and the methodology used to determine these 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.

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 and/or mg/seed
SV = 0.90 (shortcut value for acute exposure to adult bumblebees, taken from Table J6 in Appendix J of the Guidance Document)
E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for the succeeding crop scenario)
$LD_{50,oral}$ is expressed as µg a.s./bee

If this ETR > 0.036, 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 *ETR for the chronic adult oral exposure* is calculated by the following equation:

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

Where: AR = application rate in kg a.s./ha and/or mg/seed
SV = 0.78 (shortcut value for chronic exposure to adult bumblebees, taken from Table J6 in Appendix J of the Guidance Document)
E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for the succeeding crop scenario)
twa = 1
LDD_{50} is expressed as µg a.s./bee per day

If this ETR > 0.0048, 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 *ETR for larvae* is calculated by the following equation:

$$ETR_{larvae} = \frac{AR * E_f * SV * 10 * twa}{NOED}$$

Where: AR = application rate in kg a.s./ha and/or mg/seed
SV = 0.20 (shortcut value for bumblebee larvae, taken from Table J6 in Appendix J of the Guidance Document). Factor 10 is to consider the food consumption of larvae over a 10-day developmental period
E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for the succeeding crop scenario)
twa = 1
NOED is expressed as µg a.s./larva/development period

If this ETR > 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 has been performed using the authorized 'maximum application rate' for potato, maize and sorghum (see Table B.9.2.3.2-1). The relevant toxicity endpoints are taken from Table B.9.1.3.1-3. As discussed in that section, there is no larval toxicity endpoint available for bumblebees, and it is also not possible to determine a surrogate endpoint based on that larval toxicity endpoint for honeybees. As a result, the risk assessment for bumblebee larvae could not be performed. The Tier 1 ETR values calculated for adult bumblebees are shown in Table B.9.2.3.2-2.

Table B.9.2.3.2-1: currently authorized 'maximum application rate' of clothianidin containing formulations for use as a granule treatment at sowing in potato, maize and sorghum.

Crop	**authorized 'maximum application rate'**
Potato	70 g a.s./ha
Maize/ Sweet maize/ Sorghum	50 g a.s./ha

Table B.9.2.3.2-2: Tier 1 ETR calculations for acute adult oral and chronic adult oral exposure for the highest authorized 'maximum application rate' of clothianidin in potatoe, maize and sorghum.

Acute adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Potato	0.070	1	0.90	-	0.00191	32.98	0.036
Maize/ Sweet maize/ Sorghum	0.050	1	0.90	-	0.00191	23.56	0.036
Chronic adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	LDD_{50} (µg a.s./bee/day)	ETR	Trigger
Potato	0.070	1	0.78	1	0.000138	395.7	0.0048
Maize/ Sweet maize/ Sorghum	0.050	1	0.78	1	0.000138	282.6	0.0048

As all ETR values exceed the relevant trigger values, a potential risk is identified for adult bumblebees 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, 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 in which the clothianidin residues in nectar and pollen in several succeeding crops were measured. In the original version of this Addendum, the highest 90th percentile residue values from the submitted 'forced exposure' studies were used to refine the risk assessment. As discussed under Section B.9.2.2, the complete data set, with all available studies from both the use of clothianidin as seed treatment (Bayer Crop Science dossier) and as granular application (Sumitomo dossier), was considered at Pesticides Peer Review Meeting 145. Based on this dataset, it was considered more appropriate to use the highest available residue values from the 'natural exposure' studies in the tier 2 risk assessment. The residue values to be used in the risk assessment are 1.5 µg a.s./kg for pollen (measured in maize pollen) and 0.6 µg a.s./kg for nectar (measured in *Phacelia* nectar). As these values were obtained by exposing a number of succeeding crops to a soil concentration exceeding the theoretical soil plateau concentration of clothianidin resulting from an annual use according to GAP, the selected residue values cover the succeeding crop scenarios for all registered uses of clothianidin as granule (except the use in forestry nursery).

In table J1 of appendix J of the EFSA Guidance Document on bees, data on the consumption of nectar and pollen by bumblebee adults and larvae are reported. These values are shown in Table B.9.2.3.2-3. Since the energy demand of the bumblebees 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, some data from the literature is available. There is however little known about the distribution and frequency of the sugar content carried by bees. Awaiting further research in this field, it was considered that the worst case values (i.e. nectar with the lowest sugar content from the ranges which may be foraged by bees), namely 15% for bumblebees, 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 B.9.2.3.2-3.

Table B.9.2.3.2-3: Pollen, sugar and nectar consumption of bumblebees

Bumblebee stage	Pollen consumption (mg/bee/day or mg/larva)	Sugar consumption (mg/bee/day or mg/larva)	Nectar consumption[1] (mg/bee/day or mg/larva)
Adult bees	26.6 – 30.3	73 – 149	487 - 993
Larva	10.3 – 39.5	23.8	159

[1]*Nectar consumption was calculated based on a worst case sugar concentration of 15% in nectar*

According to Appendix N of the EFSA Guidance Document for bees, the daily residu uptake for adult bees and the total residue uptake for larvae can be calculated based on the nectar and pollen consumption, 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)
R_n is the residue level in nectar (in mg/kg)
R_p is the residue level in pollen (in mg/kg)
C_n is the consumption of nectar in mg (mg/bee/day or mg/larva)
C_p is the consumption of pollen in mg (mg/bee/day or mg/larva)

In the initial version of this Addendum, the worst case values for pollen consumption from Table B.9.2.3.2-3 were used for the calculation of the residue intake (RI). For nectar consumption, the worst case values were used for the acute exposure for adult honeybees, while the mean from the minimum and maximum value was used for the chronic adult exposure. At Pesticides Peer Review Meeting 145, it was noted that this approach is acceptable, but represents a worst case. A tool for calculating refined shortcut values based on compound or crop specific input parameters (SHVAL Tool, see Appendix Z

of the EFSA Guidance Document on bees and EFSA supporting publication 2014:EN-623[20]) has been developed by EFSA. The SHVAL tool, which is an application developed in R, allows for inputting raw data as well as reference values (central tendency measurements / ranges). It first fits theoretical distributions to the data, where possible, and then it runs a Monte Carlo simulation mimicking an hypothetical field study on 1000 fields with 1000 hives in each field and 1000 bees in each hive. The SHVAL tool returns the probabilistic distributions fitted to the data and the empirical density distribution of the Shortcut Value's 90th percentile over the 1000 iterations (fields). This way, this tool allows for the estimation of the Shortcut Value's 90th percentile and its 95% confidence interval. The refined Shortcut Values obtained by using the SHVAL tool are considered more representative than a calculation only based on maximum or mean value for pollen and nectar consumption. The experts agreed that this SHVAL tool should be used to update the Tier 2 risk assessment based on the agreed residue values for pollen and nectar in succeeding crops. The calculation of refined shortcut values was therefore updated using the EFSA Shortcut Values calculation model (EFSA SHVAL model), version 1.0. This application interface can be made available upon request to amu@efsa.europa.eu.

As discussed above, clothianidin residues of 1.5 µg/kg in pollen and 0.6 µg/kg in nectar were used, as agreed in Pesticides Peer Review Meeting 145. Regarding these residues values, it should be noted that these are single, maximum values without distribution. Further, these values are not RUD values as they originate from 'natural exposure' studies, where field sites with a history of clothianidin use over several years were used. The application rates of the treated crops in the year prior to the residue trials 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 calculations without any modification.

For the calculations made with the SHVAL tool, two 'test' calculations were made in a first step 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 as those that had been used for the calculation of the tier 1 Shortcut Values for nurse honeybees, honeybee larva and forager honeybees chronic for the seed dressing use and the granular use (before emergence). The other calculations were made for clothianidin 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 shown in Table B.9.2.3.2-4. Table B.9.2.3.2-5 shows a summary of all the input parameters inserted in the SHVAL tool for the different bee categories. The values for pollen and nectar consumption were derived from Table B.9.2.3.2-3.

Table B.9.2.3.2-4: Residue levels used as input for the calculation of the refined Shortcut Values usning the EFSA SHVAL tool.

Relevance	Residue level in mg/kg	Ln
Test	1	0
Clothianidin pollen	0.0015	-6.50229
Clothianidin nectar	0.0006	-7.41858

[20] European Food Safety Authority (2014). A small application developed in R for the estimation of the residue intake rate for certain bee species under given conditions: the SHVAL tool. EFSA supporting publication 2014:EN-623. 15 pp.

Table B.9.2.3.2-5: Input parameters used for the calculations with the SHVAL tool for the different bee categories.

No.	bee type & category	Pollen consumption (mg/bee/day or mg/larvae)	Sugar consumption (mg/bee/day or mg/larvae)	Sugar content of nectar (mg/mg)	chemical conc. in pollen[1]	chemical conc. in nectar[1]	Relevance
1	HB nurse	12	34-50	0.15	0	0	Test
2	HB larva	2	59.4	0.15	0	0	Test
3	BB acute	30.3	111-149	0.15	-6.50229	-7.41858	Clothianidin
4	BB chronic	30.3	73-149	0.15	-6.50229	-7.41858	Clothianidin
5	HB forager chronic	0	32-128	0.15	0	0	Test

[1]*See Table B.9.2.3.2-4; HB: honeybee; BB: bumblebee*

The resulting refined Shortcut Values (SV) are shown in Table B.9.2.3.2-6. These Tier 2 SVs are about three orders of magnitude lower than the Tier 1 SVs.

Table B.9.2.3.2-6: Calculated Tier 2 Shortcut Values (SV) for the different scenarios and bee stages

No.	Relevance	bee type & category	Tier 2 SV (µg/bee or µg/bee/day or µg/larva)	Comment
1	test	HB nurse	0.293	Expected value was 0.29
2	test	HB larva	0.398	Expected value was 0.4
3	Clothianidin	BB acute	0.00057	
4	Clothianidin	BB chronic	0.00049	
5	test	HB forager chronic	0.540	Expected value was 0.54

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 exposure factor (E_f) and the twa values are supposed to be 1 in the risk assessment for the succeeding crop scenario. Therefore, the formula to calculate the ETR values in this case can be simplified as:

$$ETR = \frac{SV}{LD_{50\ oral} \,/\, LDD_{50} \,/\, NOED}$$

The calculated ETR values are shown in Table B.9.2.3.2-7. Taking into account the rerpresentative measured residue values, the ETR values for acute and chronic risk to adult bumblebees still exceed the relevant trigger. Further consideration is this necessary.

Table B.9.2.3.2-7: Tier 2 ETR calculations for acute adult oral and chronic adult oral exposure from nectar and pollen in succeeding crops following application of clothianidin in potato and maize.

Scenario	Tier 2 SV (µg/bee or µg/bee/day)	Toxicity endpoint (µg/bee or µg/larva)	ETR	Trigger
Acute adult oral	0.00057	0.001911	**0.298**	0.036
Chronic adult oral	0.00049	0.000138	**3.551**	0.0048

Higher tier risk assessment
Further refinements to the risk assessment could be based on field effect studies. However, no higher tier effect studies are available to assess the risk to bumblebees from the consumption of nectar and pollen in succeeding crops. Consequently, the risk assessment could not be finalized.

The applicant provided the following comment on the performed risk assessment (*text in italic*):
There are no agreed methods for conducting higher tier (field) studies with bumblebees. This means that the sequential testing pathway is incomplete, which is essential for any active substance such as clothianidin that do not pass the initial tiers, and in such circumstances it means that currently it will always be not possible to finalize the risk assessment. It is therefore considered premature to be carrying out risk assessments for bumblebees.

Conclusions
The risk to bumblebees from consumption of contaminated pollen and nectar in succeeding crops was not acceptable at tier 1. Refinement of the assessment based on measured clothianidin residues in a number of succeeding crops did not result in an acceptable risk. As there are no higher tier effect studies available, the risk assessment could not be finalized.

The risk assessment for effects on bumblebee larvae could not be finalized due to lack of a suitable toxicity endpoint for bumblebee larvae for clothianidin.

B.9.2.3.3. Risk assessment for solitary bees

The risk assessment was performed following the risk assessment scheme for solitary bees as proposed in the EFSA Guidance Document on bees. Due to the potential risk to solitary bees from the consumption of pollen and nectar from succeeding crops, the screening step was not performed, and the risk assessment started at the first tier. As based on the information available in the GAP table (Table A-1) it is not possible to perform a risk assessment for the use in forestry nursery (only the dose in g a.s./plant is available, no information on the plant density and dose in g a.s./ha), a risk assessment for this use is not included.

It is noted that for the indoor use of clothianidin in maize and sweet maize (which is restricted to permanent greenhouses), exposure to bees from nectar and pollen in succeeding crops can be considered low. Therefore, in line with the decision of Pesticides Peer Review Meeting 129, no risk assessment needs to be performed for this indoor use.

First tier risk assessment
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 products applied as granules at sowing (incorporated into the soil). The relevant shortcut values (and the methodology used to determine these 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.

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 and/or mg/seed
SV = 0.49 (shortcut value for exposure to adult solitary bees, taken from Table J6 in Appendix J of the Guidance Document)

E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for the succeeding crop scenario)
$LD_{50,oral}$ is expressed as µg a.s./bee

If this ETR > 0.04, 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 *ETR for the chronic adult oral exposure* is calculated by the following equation:

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

Where: AR = application rate in kg a.s./ha and/or mg/seed
SV = 0.49 (shortcut value for exposure to adult solitary bees, taken from Table J6 in Appendix J of the Guidance Document)
E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for the succeeding crop scenario)
twa = 1
LDD_{50} is expressed as µg a.s./bee per day

If this ETR > 0.0054, 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 *ETR for larvae* is calculated by the following equation:

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

Where: AR = application rate in kg a.s./ha and/or mg/seed
SV = 0.93 (shortcut value for solitary bee larvae, taken from Table J6 in Appendix J of the Guidance Document).
E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for the succeeding crop scenario)
twa = 1
NOED is expressed as µg a.s./larva/development period

If this ETR > 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 has been performed using the authorized 'maximum application rate' for potato, maize and sorghum (see Table B.9.2.3.3-1). The relevant toxicity endpoints are taken from Table B.9.1.3.1-3. As discussed in that section, there is no larval toxicity endpoint available for solitary bees, and it is also not possible to determine a surrogate endpoint based on that larval toxicity endpoint for honeybees. As a result, the risk assessment for solitary bee larvae could not be performed. The Tier 1 ETR values calculated for adult solitary bees are shown in Table B.9.2.3.3-2.

Table B.9.2.3.3-1: currently authorized 'maximum application rate' of clothianidin containing formulations for use as a granule treatment at sowing in potato, maize and sorghum.

Crop	authorized 'maximum application rate'
Potato	70 g a.s./ha
Maize/ Sweet maize/ Sorghum	50 g a.s./ha

Table B.9.2.3.3-2: Tier 1 ETR calculations for acute adult oral and chronic adult oral exposure for the highest authorized 'maximum application rate' of clothianidin in potato, maize and sorghum.

Acute adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Potato	0.070	1	0.49	-	0.000379	90.5	0.04
Maize/ Sweet maize/ Sorghum	0.050	1	0.49	-	0.000379	64.6	0.04
Chronic adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	LDD_{50} (µg a.s./bee)	ETR	Trigger
Potato	0.070	1	0.49	1	0.000138	248.6	0.0054
Maize/ Sweet maize/ Sorghum	0.050	1	0.49	1	0.000138	177.5	0.0054

As all ETR values exceed the relevant trigger values, a potential risk is identified for adult solitary 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. 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.

The applicant submitted a number of studies in which the clothianidin residues in nectar and pollen in several succeeding crops were measured. In the original version of this Addendum, the highest 90th percentile residue values from the submitted 'forced exposure' studies were used to refine the risk assessment. As discussed under Section B.9.2.2, the complete data set, with all available studies from both the use of clothianidin as seed treatment (Bayer Crop Science dossier) and as granular application (Sumitomo dossier), was considered at Pesticides Peer Review Meeting 145. Based on this dataset, it was considered more appropriate to use the highest available residue values from the 'natural exposure' studies in the tier 2 risk assessment. The residue values to be used in the risk assessment are 1.5 µg a.s./kg for pollen (measured in maize pollen) and 0.6 µg a.s./kg for nectar (measured in *Phacelia* nectar). As these values were obtained by exposing a number of succeeding crops to a soil concentration exceeding the theoretical soil plateau concentration of clothianidin resulting from an annual use according to GAP, the selected residue values cover the succeeding crop scenarios for all registered uses of clothianidin as granule (except the use in forestry nursery).

In table J1 of appendix J of the EFSA Guidance Document on bees, data on the consumption of nectar and pollen by solitary bee adults and larvae are reported. These values are shown in Table B.9.2.3.3-3. Since the energy demand of the solitary 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, some data from the literature is available. There is however little known about the distribution and frequency of the sugar content carried by bees. Awaiting further research in this field, it was considered that the worst case values (i.e. nectar with the lowest sugar content from the ranges which may be foraged by bees), namely 10% for 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 B.9.2.3.3-3.

Table B.9.2.3.3-3: Pollen, sugar and nectar consumption of solitary bees

Solitary bee stage	Pollen consumption (mg/bee/day or mg/larva)	Sugar consumption (mg/bee/day or mg/larva)	Nectar consumption[1] (mg/bee/day or mg/larva)
Adult bees	10.2	18 – 77	180 - 770
Larva	387	54	540

[1]*Nectar consumption was calculated based on a worst case sugar concentration of 10% in nectar*

According to Appendix N of the EFSA Guidance Document for bees, the daily residu uptake for adult bees and the total residue uptake for larvae can be calculated based on the nectar and pollen consumption, 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)
R_n is the residue level in nectar (in mg/kg)
R_p is the residue level in pollen (in mg/kg)
C_n is the consumption of nectar in mg (mg/bee/day or mg/larva)
C_p is the consumption of pollen in mg (mg/bee/day or mg/larva)

In the initial version of this Addendum, the worst case values for pollen consumption from Table B.9.2.3.3-3 were used for the calculation of the residue intake (RI). For nectar consumption, the worst case values were used for the acute exposure for adult honeybees, while the mean from the minimum and maximum value was used for the chronic adult exposure. At Pesticides Peer Review Meeting 145, it was noted that this approach is acceptable, but represents a worst case. A tool for calculating refined shortcut values based on compound or crop specific input parameters (SHVAL Tool, see Appendix Z of the EFSA Guidance Document on bees and EFSA supporting publication 2014:EN-623[21]) has been developed by EFSA. The SHVAL tool, which is an application developed in R, allows for inputting raw data as well as reference values (central tendency measurements / ranges). It first fits theoretical distributions to the data, where possible, and then it runs a Monte Carlo simulation mimicking an hypothetical field study on 1000 fields with 1000 hives in each field and 1000 bees in each hive. The SHVAL tool returns the probabilistic distributions fitted to the data and the empirical density distribution of the Shortcut Value's 90th percentile over the 1000 iterations (fields). This way, this tool allows for the estimation of the Shortcut Value's 90th percentile and its 95% confidence interval. The refined Shortcut Values obtained by using the SHVAL tool are considered more representative than a calculation only based on maximum or mean value for pollen and nectar consumption. The experts agreed that this SHVAL tool should be used to update the Tier 2 risk assessment based on the agreed residue values for pollen and nectar in succeeding crops. The calculation of refined shortcut values was therefore updated using the EFSA Shortcut Values calculation model (EFSA SHVAL model), version 1.0. This application interface can be made available upon request to amu@efsa.europa.eu.

As discussed above, clothianidin residues of 1.5 µg/kg in pollen and 0.6 µg/kg in nectar were used, as agreed in Pesticides Peer Review Meeting 145. Regarding these residues values, it should be noted that these are single, maximum values without distribution. Further, these values are not RUD values as they originate from 'natural exposure' studies, where field sites with a history of clothianidin use over several years were used. The application rates of the treated crops in the year prior to the residue trials 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 calculations without any modification.

[21] European Food Safety Authority (2014). A small application developed in R for the estimation of the residue intake rate for certain bee species under given conditions: the SHVAL tool. EFSA supporting publication 2014:EN-623. 15 pp.

For the calculations made with the SHVAL tool, two 'test' calculations were made in a first step 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 as those that had been used for the calculation of the tier 1 Shortcut Values for nurse honeybees, honeybee larva and forager honeybees chronic for the seed dressing use and the granular use (before emergence). The other calculations were made for clothianidin 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 shown in Table B.9.2.3.3-4. Table B.9.2.3.3-5 shows a summary of all the input parameters inserted in the SHVAL tool for the different bee categories. The values for pollen and nectar consumption were derived from Table B.9.2.3.3-3.

Table B.9.2.3.3-4: Residue levels used as input for the calculation of the refined Shortcut Values usning the EFSA SHVAL tool.

Relevance	Residue level in mg/kg	Ln
Test	1	0
Clothianidin pollen	0.0015	-6.50229
Clothianidin nectar	0.0006	-7.41858

Table B.9.2.3.3-5: Input parameters used for the calculations with the SHVAL tool for the different bee categories.

No.	bee type & category	Pollen consumption (mg/bee/day or mg/larvae)	Sugar consumption (mg/bee/day or mg/larvae)	Sugar content of nectar (mg/mg)	chemical conc. in pollen[1]	chemical conc. in nectar[1]	Relevance
1	HB nurse	12	34-50	0.15	0	0	Test
2	HB larva	2	59.4	0.15	0	0	Test
3	SB adult	10.2	18-77	0.10	-6.50229	-7.41858	Clothianidin
4	HB forager chronic	0	32-128	0.15	0	0	Test

[1]*See Table B.9.2.3.3-4; HB: honeybee; SB: solitary bee*

The resulting refined Shortcut Values (SV) are shown in Table B.9.2.3.3-6. These Tier 2 SVs are about three orders of magnitude lower than the Tier 1 SVs.

Table B.9.2.3.3-6: Calculated Tier 2 Shortcut Values (SV) for the different scenarios and bee stages

No.	Relevance	bee type & category	Tier 2 SV (µg/bee or µg/bee/day or µg/larva)	Comment
1	test	HB nurse	0.293	Expected value was 0.29
2	test	HB larva	0.398	Expected value was 0.4
3	Clothianidin	SB adult	0.00030	
4	test	HB forager chronic	0.540	Expected value was 0.54

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 exposure factor (E_f) and the twa values are supposed to be 1 in the risk assessment for the succeeding crop scenario. Therefore, the formula to calculate the ETR values in this case can be simplified as:

$$ETR = \frac{SV}{LD_{50\ oral}\ /\ LDD_{50}\ /\ NOED}$$

The calculated ETR values are shown in Table B.9.2.3.3-7. Taking into account the rerpresentative measured residue values, the ETR values for acute and chronic risk to adult solitary bees still exceed the relevant trigger. Further consideration is this necessary.

Table B.9.2.3.3-7: Tier 2 ETR calculations for acute adult oral and chronic adult oral exposure from nectar and pollen in succeeding crops following application of clothianidin in potato and maize.

Scenario	Tier 2 SV (µg/bee or µg/bee/day)	Toxicity endpoint (µg/bee or µg/larva)	ETR	Trigger
Acute adult oral	0.00030	0.000379	**0.792**	0.04
Chronic adult oral	0.00030	0.000138	**2.174**	0.0054

Higher tier risk assessment

Further refinements to the risk assessment could be based on field effect studies. However, no higher tier effect studies are available to assess the risk to solitary bees from the consumption of nectar and pollen in succeeding crops. Consequently, the risk assessment could not be finalized.

The applicant provided the following comment on the performed risk assessment (*text in italic*):
There are no agreed methods for conducting higher tier (field) studies with solitary bees. This means that the sequential testing pathway is incomplete, which is essential for any active substance such as clothianidin that do not pass the initial tiers, and in such circumstances it means that currently it will always be not possible to finalize the risk assessment. It is therefore considered premature to be carrying out risk assessments for solitary bees.

Conclusions

The risk to solitary bees from consumption of contaminated pollen and nectar in succeeding crops was not acceptable at tier 1. Refinement of the assessment based on measured clothianidin residues in a number of succeeding crops did not result in an acceptable risk. As there are no higher tier effect studies available, the risk assessment could not be finalized.

The risk assessment for effects on solitary bee larvae could not be finalized due to lack of a suitable toxicity endpoint for solitary bee larvae for clothianidin.

B.9.3. THE POTENTIAL UPTAKE VIA ROOTS TO FLOWERING WEEDS

B.9.3.1. Studies

The applicant submitted a large scale monitoring study to determine the presence of weeds and honey dew in potato and maize during the growing season.

Report:	**IIIA 10.4c/01, Negrini, P.; 2014**
Title:	**Identification of weeds population and honey dew presence in maize and potato fields during the growing season**
Report No.:	SCAE-2014-01
Document No:	THW-0383
Guidelines:	Not applicable (EU monitoring)
GLP	Not applicable

Objective

A study was conducted to assess the weeds population present in maize and potato fields, both characterized by relevant farmer maintenance with chemical programs. In addition, in each specific location, the presence of honey dew was also recorded. This was to allow the estimation the potential uptake via roots to flowering weeds and the risk to honeybees foraging on insect honey dew.

Materials and Methods

The study was performed in 2014 in a number of European countries. Fifty-three locations were involved in the monitoring on maize split between France, Italy and Hungary. These three countries were selected because they are representative of the main maize growing areas. The monitoring on potato focused on fifty-five locations split between France, Italy, Spain, Germany, United-Kingdom, Hungary and Poland which cover the main potato growing areas. The division of the locations between the different countries for the two crops is shown in Table B.9.3.1-1. The sites where the assessments were done mainly in commercial fields, but some were carried out as part of efficacy trials or registration studies.

Table B.9.3.1-1: Distribution of the monitored locations

Crop	Country	Number of sites
Maize	France	14
	Italy	26
	Hungary	13
	Total 1:	53
Potato	France	16
	Italy	11
	Spain	1
	Germany	11
	United-Kingdom	5
	Hungary	1
	Poland	10
	Total 2:	55

Protocol:

A common protocol was established for both maize and potato fields to assess the weed populations and the honey dew presence.

In each field, 8 observation plots were selected for monitoring. In the case of commercial fields, there were 4 plots of 6m x 10m (60m²) located at a distance of 20 m from the border and 4 plots of 6m x 10m (60m²) located in the middle of the field with a distance of 20m from each other. In the case of efficacy studies or registration trials, each untreated plot in the trial area was observed (4 plots in total.

Further, 4 plots of 6m x 10m (60m²) located at a distance of 20m from the trial area in each direction were observed.

For each observation plot at the field site, the number of weeds/plot was assessed by counting the weeds present over the whole plot. Each weed present was identified, so that the number of each species was recorded was well as its development stage (using the BBCH scale). In addition, the % surface covered by honey dew on the maize or potato crop was assessed (on 5 consecutive plants x 4 rows on each plot).

The observations were carried out on three occasions: one month after sowing of the crop; at crop flowering; about mid-September.

Findings

The observation plots were located in different places inside the field (edge, middle, inside the trial/study area, …). The results presented below focus on each site as a whole (i.e. the data integrates and combines all different assessments generated from each specific location inside the field). For each monitored crop (maize and potato), the results are presented by the timing of assessments with a list the flowering weeds (weeds present at BBCH60 or more) identified in all specific locations; their occurrence (% sites where the weed is present) and their average density (average number per m² when present). In addition, the presence or absence of honey dew is noted.

Maize

Three weed species were identified at the flowering stage one month after sowing (Table B.9.3.1-2), with a very low occurrence (2 to 4%) and in the case of *Chenopodium sp.* it was only found at one site. In addition, honey dew was never reported in any of the fifty-three locations monitored at this observation time.

Table B.9.3.1-2: Results on maize (one month after sowing)

Bayer code	Scientific name	No. of sites present	% sites where the weed is present	When present, average density (nb/m²)
CHEAL	*Chenopodium album*	1	2	2,52
CHEHY	*Chenopodium hybridum*	1	2	4,88
POAAN	*Poa annua*	2	4	0,02

Fourteen weed species were identified at the flowering stage at the maize flowering period (see Table B.9.3.1-3). Overall, the occurrence of the weeds was low (2 to 11% of locations). In addition, taking the species individually, the average density was also relatively low, although in a few cases (e.g. *Chenopodium album*, *Convolvulus arvensis*, *Polygonum persicaria* and *Setaria glauca*) the number is was greater than one plant per m². In addition, honey dew was never reported in any of the fifty-three locations monitored at this observation time.

Table B.9.3.1-3: Results on maize (at crop flowering)

Bayer code	Scientific name	No. of sites present	% sites where the weed is present	When present, average density (nb/m²)
ABUTH	*Abutilon theophrasti*	3	6	0,07
AGRRE	*Elytrigia repens*	1	2	0,92
AMARE	*Amaranthus retroflexus*	1	2	0,05
CHEAL	*Chenopodium album*	6	11	2,69
CIRAR	*Cirsium arvense*	1	2	0,92
CONAR	*Convolvulus arvensis*	5	9	1,38
LOLPE	*Lolium perenne*	1	2	0,34
POLPE	*Polygonum persicaria*	1	2	9,06
PHTAM	*Phytolacca americana*	1	2	0,002
RUMSS	*Rumex sp.*	1	2	0,01
SETPU	*Setaria glauca*	3	6	3,29
SETVI	*Setaria viridis*	2	4	0,07
SOLNI	*Solanum nigrum*	1	2	0,09
SORHA	*Sorghum halepense*	2	4	0,15

Twenty-seven weed species were identified at the flowering stage around mid-September (Table B.9.3.1-4). Several species were present at a frequency level between 15-30% of locations but their average density was relatively low or very low, except for *Chenopodium album* (3.68 plants/m²) and *Convolvulus arvensis* (1.69 plants/m²). In addition, honey dew was reported in only two sites out of the fifty-three locations monitored at this observation time.

Table B.9.3.1-4: Results on maize (mid-September)

Bayer code	Scientific name	No. of sites present	% sites where the weed is present	When present, average density (nb/m²)
ABUTH	*Abutilon theophrasti*	12	23	0,21
AGRRE	*Elytrigia repens*	1	2	0,92
AMARE	*Amaranthus retroflexus*	8	15	0,13
AMBAR	*Ambrosia artemisiifoila*	1	2	0,03
CHEAL	*Chenopodium album*	13	25	3,68
CHEHY	*Chenopodium hybridum*	1	2	0,02
CIRAR	*Cirsium arvense*	3	6	0,07
CONAR	*Convolvulus arvensis*	11	21	1,69
CYNDA	*Cynodon dactylon*	1	2	0,21
DATST	*Datura stramonium*	1	2	0,03
DIGSA	*Digitaria sanguinalis*	3	6	1,00
ECHCG	*Echinochloa crus-galli*	10	19	0,15
LOLPE	*Lolium perenne*	1	2	0,27
MERAN	*Mercurialis annua*	3	6	2,50
MERPE	*Mercurialis perennis*	1	2	0,11
PERMA	*Persicaria maculosa*	2	4	0,05
PHTAM	*Phytolacca americana*	1	2	0,002
POLAV	*Polygonum aviculare*	1	2	0,09
POLLA	*Polygonum lapathifolium*	1	2	0,09
POLPE	*Polygonum persicaria*	5	9	5,48
PTLRE	*Potentilla reptans*	2	4	0,11
RUBSS	*Rubus sp.*	1	2	4,47
RUMSS	*Rumex sp.*	1	2	0,002
SETPU	*Setaria glauca*	9	17	0,65
SETVI	*Setaria viridis*	3	6	0,11
SOLNI	*Solanum nigrum*	7	13	0,24
SORHA	*Sorghum halepense*	8	15	0,05

Potato

Three weed species were identified at the flowering stage one month after sowing (Table B.9.3.1-5), with a very low occurrence. In the case of *Diplotaxis erucoides*, which occurred at a relatively high density this was found at only one site. In addition, honey dew was never reported in any of the fifty-five locations monitored at this observation time.

Table B.9.3.1-5: Results on potato (one month after sowing)

Bayer code	Scientific name	No. of sites present	% sites where the weed is present	When present, average density (nb/m²)
CAGSE	Calystegia sepium	1	2	0,12
DIPER	Diplotaxis erucoides	1	2	4,33
FUMOF	Fumaria officinalis	1	2	0,80

Twenty-five weed species were identified at the flowering stage at the potato flowering period (Table B.9.3.1-6). However, the occurrence of the weeds was very low (2 to 7% of locations) as well as their average density (96% of the weeds had an average density equal to or less than 0,5 plant per m²). Only *Datura stramonium* and *Galium aparine* had a density of greater than one plant per m². In addition, honey dew was reported in only two sites out of the fifty-five locations monitored at this observation time.

Table B.9.3.1-6: Results on potato (at crop flowering)

Bayer code	Scientific name	No. of sites present	% sites where the weed is present	When present, average density (nb/m²)
AMARE	*Amaranthus retroflexus*	1	2	0,25
CAGSE	*Calystegia sepium*	1	2	0,21
CHEAL	*Chenopodium album*	3	5	0,09
CIRAR	*Cirsium arvense*	3	5	0,04
CIRVU	*Cirsium vulgare*	1	2	0,01
CONAR	*Convolvulus arvensis*	1	2	0,01
DATST	*Datura stramonium*	1	2	1,35
ECHCG	*Echinochloa crus-galli*	1	2	0,51
FUMOF	*Fumaria officinalis*	1	2	0,004
GALAP	*Galium aparine*	2	4	0,57
LAPCO	*Lapsana communis*	1	2	0,03
MATCH	*Matricaria chamomilla*	3	5	0,03
MERAN	*Mercurialis annua*	1	2	0,11
POAAN	*Poa annua*	2	4	0,01
POLAV	*Polygonum aviculare*	2	4	0,009
POLCO	*Polygonum convolvulus*	2	4	0,09
SINAR	*Sinapis arvensis*	1	2	0,006
SONAR	*Sonchus arvensis*	1	2	0,17
SONOL	*Sonchus oleraceus*	4	7	0,02
SORHA	*Sorghum halepense*	1	2	0,37
TAROF	*Taraxacum officinale*	1	2	0,006
TRZAX	*Triticum aestivum*	1	2	0,006
VERPE	*Veronica persica*	2	4	0,16
VICCR	*Vicia cracca*	1	2	0,008
VIOAR	*Viola arvensis*	1	2	0,002

Twenty-nine weed species were identified at the flowering stage around mid-September (Table B.9.3.1-7). A few species were present at a relatively high number of sites (11-16%) but their average density was relatively low (0.67 to 0.80 plants per m²). In addition, honey dew was reported in only two sites out of the fifty-five locations monitored at this observation time.

Table B.9.3.1-7: Results on potato (mid-September)

Bayer code	Scientific name	Present in … sites (number)	% sites where the weed is present	When present, average density (nb/m²)
AGRRE	*Elytrigia repens*	2	4	0,05
AMARE	*Amaranthus retroflexus*	4	7	0,17
AVEST	*Avena sterilis*	1	2	0,10
CAGSE	*Calystegia sepium*	1	2	0,16
CAPBP	*Capsella bursa-pastoris*	1	2	0,002
CHEAL	*Chenopodium album*	8	15	0,68
CIRAR	*Cirsium arvense*	2	4	1,45
CONAR	*Convolvulus arvensis*	6	11	0,67
DAUCA	*Daucus carota*	1	2	0,03
ECHCG	*Echinochloa crus-galli*	9	16	0,80
EQUAR	*Equisetum arvense*	1	2	2,50
FUMOF	*Fumaria officinalis*	1	2	0,10
GALAP	*Galium aparine*	3	5	0,78
GASCI	*Galinsoga quadriradiata*	1	2	0,01
GASPA	*Galinsoga parviflora*	1	2	4,26
GERPU	*Geranium pusillum*	2	4	1,30
MATCH	*Matricaria chamomilla*	1	2	0,008
MATIN	*Matricaria inodora*	1	2	1,30
POLAV	*Polygonum aviculare*	1	2	0,004
POLCO	*Polygonum convolvulus*	1	2	0,004
POLLA	*Polygonum lapathifolium*	1	2	0,002
RASRU	*Rapistrum rugosum*	1	2	0,03
SOLNI	*Solanum nigrum*	2	4	0,54
SONAR	*Sonchus arvensis*	1	2	0,05
SORHA	*Sorghum halepense*	1	2	0,05
TRZAW	*Triticum aestivum*	2	4	0,005
VERPE	*Veronica persica*	2	4	0,15
VICCR	*Vicia cracca*	1	2	0,008
VIOAR	*Viola arvensis*	3	5	1,80

Conclusion

This large scale monitoring study in maize (53 sites) and potato (55 sites) shows that the incidence of flowering weeds in these crops, under relevant maintenance with chemical herbicides, is low. For most weed species, the number of plants per m² is below 0.5 plants per m². Only for a small number of species, more than 1 plant of the same species per m² was present.

Honey dew was rarely found in maize: one month after sowing and at crop flowering, no honey dew was detected at any of the fields. Mid-september, honey dew was reported at two sites out of 53. Similarly, no honey dew was found on potato plants at any site after sowing. At crop flowering and mid-september, honey dew was reported at two sites out of fify-five.

RMS Comments

Maize and potato are the two major crops in which clothianidin containing products are authorized for use as a granule treatment. The field sites where monitoring took place for potato are well spread over

Central and Southern Europe. The field sites for maize monitoring are rather concentrated in southern Europe. However, the area where clothianidin is currently authorized for granule applications is covered by the monitoring sites. Therefore, the results of this study are considered representative for field uses of clothianidin as granule. Consequently, the study is acceptable for use in risk assessment.

At Pesticides Peer Review Meeting 145, this study by Negrini (2014) was further discussed. It was noted that in this study the presence of weeds at different crop growth stages was investigated, which was considered essential for a study to be useful to assess the relevance of the weed scenario for clothianidin.

It was noted that the information available in the study report was limited to the number weed species present at each site, the number of plants from each species present and the total area of the plots. In the study report, the percentage of sites with flowering weeds and the average weed density (number per m^2) is reported and discussed. No information on the weed ground cover was available (i.e. no data available regarding the area occupied by each species). Therefore, it is difficult to compare the results from this study to the 10% trigger for weed ground cover from the EFSA Guidance Document for bees. However, it was considered that a rough estimation of the weed ground cover could be made by using the weed density and plot area reported in the study.

It was also noted that the results were presented and discussed for each weed species separately. However, at most field sites, more than 1 weed species was present. As the 10% trigger from the EFSA Guidance Document refers to the total ground cover for weeds (including all species present), it was considered more appropriate to use the total weed density (for all weed species present) in the assessment for the relevance of the weed scenario for clothianidin (at least in a first step of the assessment, when it is considered that all flowering weeds are attractive to bees).

Considering the above, RMS was requested to re-evaluate the raw data and to provide a rough estimation of the total weed ground cover at the field sites monitored in this study. The re-evaluation is summarized below.

<u>Summary of additional evaluation of the data</u>
Based on the raw data available in the study report, the total number of weeds (for all species) counted at each observation time (1 month after sowing, at crop flowering, mid-september) was determined for each field site. A distinction was made between weeds that wer not flowering (BBCH < 60) and weeds at the flowering stage (BBCH ≥ 60). For the further evaluation, only flowering weeds were considered as they are the only ones attractive to bees.

For both maize and potato, the number of sites with flowering weeds was determined for each observation time (see Table B.9.3.1-8). The data shows that for both crops weeds are present at approximately half of the tested field sites one month after sowing. However, flowering weeds were only found on a limited number of occasions at that time: at only 7.5% of the sites for maize and at 3.6% of the sites for potato. During the course of the growing season, weeds in general become more abundant, with weeds recorded at 92.2 and 84.0 % of the sites for maize and potato, respectively, in September. The number of sites where flowering weeds were found also increased throughout the growing season, to 84.3% of the sites for maize and 60.0% of the sites for potato.

Table B.9.3.1-8: Number of sites for which data is available, number of sites where weeds were recorded and number of sites where flowering weeds were recorded, for each observation time in maize and potato.

Crop	Timing	Total number of sites	sites with weeds		sites with flowering weeds	
			number	%	number	%
Maize	1 month after sowing	53	28	52.8	4	7.5
	at crop flowering	53	37	69.8	14	26.4
	mid-September	51	47	92.2	43	84.3
Potato	1 month after sowing	55	34	61.8	2	3.6
	at crop flowering	55	41	74.5	17	30.9
	mid-September	50	42	84.0	30	60.0

For each site where flowering weeds were present, the total flowering weed density (weeds/m²) was calculated, based on the total number of flowering weeds recorded (for all weed species) and the total area of the field site. As mentioned in the study summary above, several observation areas (or plots) were assessed per field site, which were located in different places inside the field (e.g. edge, middle, inside the trial area, etc.). The results presented below focus on each site as a whole, i.e. the data integrates and combines all the different assessments generated from each specific location inside the field. Table B.9.3.1-9 shows, for those field sites where flowering weeds were present, the minimum and maximum weed density recorded at each observation time. These results show that not only the number of fields where flowering weeds were found increased throughout the growing season, but also the maximum recorded weed density increased from 1 month after sowing to mid-September. Further, the maximum density of flowering weeds in potato is consistently lower compared to the maximum density in maize.

Table B.9.3.1-9: Minimum and maximum density of flowering weeds, for those field sites where flowering weeds were present.

Crop	Timing	Minimum density (weeds/m²)	Maximum density (weeds/m²)
Maize	1 month after sowing	0.00625	14.79167
	at crop flowering	0.00762	14.09574
	mid-September	0.02286	30.05319
Potato	1 month after sowing	0.11458	4.404762
	at crop flowering	0.00208	3.088889
	mid-September	0.00417	15.81875

A direct estimation of the percentage of the ground surface covered by flowering weeds at the tested field sites is not possible, as no information is available in the study report on the weed ground cover. Therefore, it was calculated for each field site how much soil surface an individual weed plant would need to cover in order to obtain a total weed cover of 10% (the trigger mentioned by the EFSA Guidance Document for bees). This was done by calculating the area of 10% of the field site monitored (total area of the site, divided by 10), and dividing this value by the total number of flowering weeds recorded at that site. If the resulting value is irrealistically high for 1 weed plant (e.g. 1 m²), it can be concluded that for that site the ground cover by the flowering weeds will be well below 10% of the soil surface. In contrast, a very small value (e.g. a few cm²) would indicate that the ground cover by the flowering weeds is likely to exceed 10% of the soil surface. Table B.9.3.1-10 and B.9.3.1-11 show the calculated values for maize and potato, respectively.

Table B.9.3.1-10: Calculation of the soil surface an individual weed plant would need to cover to obtain a total weed cover of 10% of each respective field site for maize.

Trial ID	Field surface		1 month after sowing		at flowering		September	
	total (m^2)	10% of total (m^2)	Number of weeds BBCH ≥ 60	Ground cover per plant if 10% trigger is reached (m^2)	Number of weeds BBCH ≥ 60	Ground cover per plant if 10% trigger is reached (m^2)	Number of weeds BBCH ≥ 60	Ground cover per plant if 10% trigger is reached (m^2)
Location 01	240	24	0	-	0	-	13	1.8462
Location 02	240	24	3550	0.0068	0	-	43	0.5581
Location 03	240	24	0	-	1500	0.016	4825	0.0050
Location 04	240	24	0	-	0	-	26	0.9230
Location 05	120	12	0	-	1080	0.011	46	0.2609
Location 06	360	36	0	-	0	-	0	-
Location 07	360	36	0	-	0	-	0	-
Location 08	360	36	0	-	0	-	0	-
Location 09	360	36	0	-	0	-	0	-
Location 10	240	24	0	-	0	-	198	0.1212
Location 11	240	24	0	-	0	-	15	1.60
Location 12	240	24	0	-	0	-	37	0.6486
Location 13	240	24	0	-	440	0.0545	520	0.0462
14 GRM I PO AN 500	376	37.6	0	-	5300	0.0071	11300	0.0033
14 GRM I PO AN 600	376	37.6	0	-	8	4.70	18	2.0889
14 GRM I PO AN 700	376	37.6	0	-	17	2.2118	20	1.88
14PHI6025CN604	360	36	0	-	0	-	57	0.6316
14SGS021 FR02	540	54	0	-	0	-	-	-
14SGS021 IT04	360	36	0	-	258	0.1395	-	-
A14-081-36HX	480	48	0	-	0	-	815	0.0589
A14-082-36HX	480	48	0	-	0	-	0	-
A14-083-36HX	480	48	0	-	0	-	82	0.5854
A14-084-36HX	480	48	0	-	0	-	146	0.3288
A14-169-36HX	480	48	0	-	0	-	381	0.1260
A14-267-36HX	480	48	0	-	0	-	1299	0.0370
A14-268-36HX	480	48	0	-	0	-	96	0.50
A14-306-36HX	480	48	0	-	0	-	0	-
A14-307-36HX	480	48	0	-	0	-	40	1.20
A14-308-36HX	480	48	0	-	0	-	32	1.50
A14-309-36HX	480	48	0	-	0	-	204	0.2353
A14-310-36HX	480	48	0	-	0	-	322	0.1491
A14-311-36HX	480	48	0	-	0	-	217	0.2212
A14-312-36HX	480	48	0	-	45	1.0667	208	0.2308
A14-313-36HX	480	48	0	-	51	0.9412	210	0.2286
A14-314-36HX	480	48	0	-	0	-	58	0.8276
A14-315-36HX	480	48	0	-	0	-	0	-
A14-316-36HX	480	48	0	-	0	-	145	0.3310
A14-317-36HX	480	48	16	3.00	0	-	78	0.6154
A14-318-36HX	480	48	3	16.00	43	1.1163	88	0.5455
A14-319-36HX	480	48	0	-	0	-	113	0.4248
A14-320-36HX	480	48	0	-	0	-	239	0.2008
A14-321-36HX	480	48	0	-	0	-	78	0.6154
A14-322-36HX	480	48	0	-	0	-	0	-
A14-323-36HX	480	48	0	-	0	-	0	-
CTE-14-17694-FR01	393.6	39.36	0	-	4	9.84	9	4.3733
CTE-14-17698-FR01	393.6	39.36	0	-	304	0.1295	68	0.5788
CTE-14-17698-FR02	393.6	39.36	0	-	3	13.12	170	0.2315

Trial ID	Field surface		1 month after sowing		at flowering		September	
	total (m^2)	10% of total (m^2)	Number of weeds BBCH ≥ 60	Ground cover per plant if 10% trigger is reached (m^2)	Number of weeds BBCH ≥ 60	Ground cover per plant if 10% trigger is reached (m^2)	Number of weeds BBCH ≥ 60	Ground cover per plant if 10% trigger is reached (m^2)
CTE-14-17700-FR01	393.6	39.36	0	-	260	0.1514	170	0.2315
ST14GC05-I14GC02RJK01	393.6	39.36	0	-	0	-	1007	0.0391
ST14GC05-I14GC03RJK01	393.6	39.36	0	-	0	-	3279	0.0120
ST14GC05-I14GC04RJK01	393.6	39.36	33	1.1927	0	-	377	0.1044
P1405AR01-01	360	36	0	-	0	-	0	-
S14-03918-01	384	38.4	0	-	0	-	462	0.0831

Table B.9.3.1-11: Calculation of the soil surface an individual weed plant would need to cover to obtain a total weed cover of 10% of each respective field site for potato.

Trial ID	Field size		1 month after sowing		at flowering		September	
	total (m^2)	10% of total (m^2)	Number of weeds BBCH ≥ 60	Ground cover per plant if 10% trigger is reached (m^2)	Number of weeds BBCH ≥ 60	Ground cover per plant if 10% trigger is reached (m^2)	Number of weeds BBCH ≥ 60	Ground cover per plant if 10% trigger is reached (m^2)
A14-324-36HX	475.2	47.52	0	-	0	-	227	0.2093
A14-325-36HX	432	43.2	0	-	0	-	22	1.9636
A14-326-36HX	480	48	0	-	0	-	277	0.1733
A14-327-36HX	480	48	0	-	0	-	191	0.2513
A14-328-36HX	160	16	0	-	0	-	23	0.6957
A14-329-36HX	480	48	0	-	0	-	0	-
A14-330-36HX	480	48	0	-	0	-	0	-
A14-331-36HX	480	48	0	-	0	-	0	-
A14-332-36HX	240	24	0	-	306	0,0784	451	0.0532
A14-333-36HX	240	24	0	-	0	-	356	0.0674
BDR-14-19633-FR01	480	48	0	-	0	-	4	12.0
BDR-14-19633-FR02	240	24	0	-	0	-	0	-
BDR-14-19633-FR03	480	48	0	-	0	-	0	-
BDR-14-19633-FR04	480	48	0	-	25	1.92	0	-
BDR-14-19633-FR05	480	48	0	-	0	-	0	-
BDR-14-19633-FR06	480	48	0	-	0	-	0	-
BDR-14-19633-FR07	432	43.2	0	-	0	-	0	-
BDR-14-19633-FR08	432	43.2	0	-	0	-	0	-
BDR-14-19633-FR09	480	48	0	-	0	-	0	-
BDR-14-19633-FR10	480	48	0	-	2	24	0	-
BDR-14-19633-FR11	480	48	55	0.8727	222	0.2162	152	0.3158
BDR-14-19633-FR12	480	48	0	-	0	-	0	-
BDR-14-19633-FR13	504	50.4	0	-	0	-	0	-
BDR-14-19633-FR14	504	50.4	0	-	0	-	0	-
BDR-14-19633-FR15	504	50.4	0	-	0	-	0	-
BDR-14-19634-DE01	480	48	0	-	0	-	0	-
BDR-14-19634-DE02	480	48	0	-	2	24.0	0	-

Trial ID	Field size		1 month after sowing		at flowering		September	
	total (m^2)	10% of total (m^2)	Number of weeds BBCH ≥ 60	Ground cover per plant if 10% trigger is reached (m^2)	Number of weeds BBCH ≥ 60	Ground cover per plant if 10% trigger is reached (m^2)	Number of weeds BBCH ≥ 60	Ground cover per plant if 10% trigger is reached (m^2)
BDR-14-19634-DE03	480	48	0	-	1	48.0	6	8.0
BDR-14-19634-DE04	480	48	0	-	0	-	55	0.8727
BDR-14-19634-DE05	480	48	0	-	0	-	16	3.0
BDR-14-19634-DE06	480	48	0	-	5	9.60	8	6.0
BDR-14-19634-DE07	480	48	0	-	0	-	2	24.0
BDR-14-19634-DE08	480	48	0	-	4	12.0	4	12.0
BDR-14-19634-DE09	240	24	0	-	0	-	12	2.0
BDR-14-19634-DE10	480	48	0	-	76	0.6316	13	3.6923
BDR-14-19635-UK01	480	48	0	-	23	2.0870	0	-
BDR-14-19635-UK02	480	48	0	-	13	3.6923	0	-
BDR-14-19635-UK03	480	48	0	-	17	2.8235	0	-
BDR-14-19635-UK04	480	48	0	-	60	0.80	0	-
BDR-14-19635-UK05	480	48	0	-	0	-	0	-
BDR-14-19636-PL01	480	48	0	-	0	-	9	5.3333
BDR-14-19636-PL02	480	48	0	-	0	-	955	0.0503
BDR-14-19636-PL03	480	48	0	-	0	-	0	-
BDR-14-19636-PL04	480	48	0	-	0	-	460	0.1043
BDR-14-19636-PL05	480	48	0	-	0	-	0	-
BDR-14-19636-PL06	480	48	0	-	0	-	4267	0.0112
BDR-14-19636-PL07	480	48	0	-	0	-	2413	0.0199
BDR-14-19636-PL08	480	48	0	-	90	0.5333	155	0.3097
BDR-14-19636-PL09	480	48	0	-	0	-	2026	0.0237
BDR-14-19636-PL10	480	48	0	-	0	-	7593	0.0063
14SGS021 FR02	360	36	0	-	0	-	-	-
14SGS021 IT04	360	36	0	-	151	0.2384	-	-
14SGS021 GE01	540	54	0	-	33	1.6364	-	-
14SGS021 HU05	900	90	0	-	2780	0.0324	-	-
14SGS021 SP03	420	42	1850	0.0227	0	-	-	-

In the majority of cases, the soil surface each flowering weed plant should cover to reach a total weed ground cover of 10% of the total surface area is irrealistically high (e.g. 0.25 m^2 or more). However, to actually be able to estimate the number of sites where the weed ground cover will exceed 10% of the total surface area, an estimate of the average ground cover for one weed plant should be available. Making such an estimate is very difficult as it is very much dependent on the properties of each weed species (e.g. perennials vs. annual plants, monocotyledons vs. dicotyledons). To aid the interpretation of the results, Table B.9.3.1-12 shows the number of field sites where more than 10% of the soil surface would be covered with flowering weeds for different assumptions regarding the average ground cover of one flowering weed plant. It should be noted that these assumptions are not based on experimental data.

Table B.9.3.1-12: Number and percentage of the tested field sites where the total flowering weed ground cover would exceed 10% of the total field site surface area, if an average ground cover for one weed plant of 0.0225 m², 0.04 m² or 0.09 m² is assumed.

Crop	Timing	Assumed average area covered by one flowering weed plant					
		0.0225m² (15x15cm)		0.04 m² (20x20cm)		0.09 m² (30x30cm)	
		number	%	number	%	number	%
Maize	1 month after sowing	1	1.89	3	5.66	3	5.66
	at crop flowering	1	1.89	3	5.66	5	9.43
	mid-September	1	1.96	4	7.84	8	15.69
Potato	1 month after sowing	0	0	0	0	3	5.45
	at crop flowering	1	1.82	1	1.82	4	7.27
	mid-September	1	2.0	2	4.0	7	14.0

According to the data shown in Table B.9.3.1-12, the total flowering weed ground cover only exceeds 10% of the total surface area in a low number of cases. Even with a relatively worst-case assumption that a flowering weed plant on average covers an area of 30x30 cm, the 10% trigger is exceeded in less than 10% of the field sites for both potato and maize 1 month after sowing and at crop flowering. Under the same assumption, the 10% trigger is exceeded in about 15% of the field sites for both maize and potato monitored mid-September. These results will further be discussed under Section B.9.3.2.

B.9.3.2. Exposure

Theoretically, residues in weeds in the treated field could be a route of exposure to honeybees and non-*Apis* bees. As currently registered granule products containing clothianidin are applied at sowing, no weeds will be present on the field (due to seed bed preparation) at the time of application. Therefore, contact exposure from dust deposits on bee attractive weeds on the field is not considered a relevant route of exposure.

For uses of clothianidin as seed treatment, the EFSA Conclusion on the risk assessment for bees for clothianidin (2013)[22] concluded that root uptake of soil residues of clothianidin and translocation of these residues to nectar and pollen of flowering weeds can be considered negligible, as the substance in concentrated around the treated seed and therefore considerable uptake via roots of weeds is unlikely. This is however not the case for uses as granules, and therefore exposure through nectar and pollen of flowering weeds in the field is a potentially relevant route of exposure for these uses.

At Pesticides Peer Review Meeting 145, the relevance of the weeds in the treated field scenario was discussed. It was noted that the EFSA Guidance Document for bees states that the weeds in the treated field are unlikely to be an issue in view of the application via the seed treatment, in that no weeds will be present in the field when the crop is sown. Also, uptake via the roots of weeds is likely to be negligibly small in the application year because the stubstance is concentrated around the treated seed. However, given the high soil persistence of neonicotinoids such as clothianidin, in combination with the high toxicity and systemicity, the majority of the experts agreed that the weed scenario should be considered relevant for the application of clothianidin as seed treatment. The same conclusion was drawn for the granular use of clothianidin.

The applicant did not investigate the potential uptake of clothianidin via roots of flowering weeds, but submitted a monitoring study that investigated the occurrence of flowering weeds in potato and maize (Negrini, 2014; see section B.9.3.1). This monitoring study indicates that the occurrence of flowering weeds in potato and maize is low, reflecting the agronomic practices (weed control) associated with

[22] European Food Safety Authority (2013). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin. EFSA Journal 2013;11(1):3066. doi:10.2903/j.efsa.2013.3066

these crops. In the case of maize, fifty-three locations were monitored in France, Italy and Hungary and one month after sowing only three flowering species were found to be present. Only two of these (*Chenopodium* spp.) occurred at densities > 2 per m^2 and this was for one site for each of two species. At crop flowering, which is potentially the most susceptible timing for the presence of bees in the field, there were 14 flowering weed species present but only in 2 to 11% of sites and generally the density was low. In a few cases (*Chenopodium album*, *Convolvulus arvensis*, *Polygonum persicaria* and *Setaria glauca*) the density observed was greater than one plant per m². Twenty-seven weed species were identified at the flowering stage later in the season (mid-September) and while several species were present at a frequency level between 15-30% of locations their average density was relatively low or very low, except for *Chenopodium album* (3.68 plants/m²) and *Convolvulus arvensis* (1.69 plants/m²).

In the case of potatoes, fifty-five locations were monitored in France, Italy, Spain, Germany, United-Kingdom, Hungary and Poland and again one month after sowing only three flowering weed species were found, at one site each (and only in the case of one, *Diplotaxis erucoides*, was the density greater than 1 per m^2). At crop flowering, there were 25 flowering weed species present but the occurrence of the weeds was very low (2 to 7% of sites) as well as their average density (96% of the weeds had an average density equal to or less than 0,5 plant per m² and only *Datura stramonium* and *Galium aparine* had a density of greater than one plant per m²). Twenty-nine weed species were identified at the flowering stage later in the season (mid-September). A few species were present at a relatively high number of sites (11-16%) but their average density was low (0.67 to 0.80 plants per m²).

At Pesticides Peer Review Meeting 145, the study by Negrini (2014) was discussed. It was agreed that the study is useful to address the relevance of the weeds scenario for this specific case. The EFSA Guidance Document on bees (2013) states that if less than 10% of the area of use of a substance is covered by weeds at the application time, no weeds will occur in the 90th percentile case and thus their exposure can be ignored (see Appendix N of the EFSA Guidance Document). However, it was noted that in the study report the percentage of sites with flowering weeds and the average weed density (number per m²) was reported and discussed, but that no information on the weed ground cover was available. Therefore, it is difficult to compare the results from this study to the 10% trigger for weed ground cover from the EFSA Guidance Document for bees. However, it was considered that a rough estimation of the weed ground cover could be made by using the weed density and plot area reported in the study.

It was also noted that the results were presented and discussed for each weed species separately. However, at most field sites, more than 1 weed species was present. As the 10% trigger from the EFSA Guidance Document refers to the total ground cover for weeds (including all species present), it was considered more appropriate to use the total weed density (for all weed species present) in the assessment for the relevance of the weed scenario for clothianidin (at least in a first step of the assessment, when it is considered that all flowering weeds are attractive to bees).

Considering the above, RMS performed an additional evaluation of the data from the study by Negrini (2014) to provide a rough estimation of the total weed ground cover at the field sites monitored in this study. For details on this additional evaluation, please refer to the study summary in Section B.9.3.1.

A direct estimation of the percentage of the ground surface covered by flowering weeds at the tested field sites was not possible, as no information is available in the study report on the weed ground cover. Therefore, it was calculated for each field site how much soil surface an individual weed plant would need to cover in order to obtain a total weed cover of 10% (the trigger mentioned by the EFSA Guidance Document for bees). This was done by calculating the area of 10% of the field site monitored (total area of the site, divided by 10), and dividing this value by the total number of flowering weeds recorded at that site. If the resulting value is irrealistically high for 1 weed plant (e.g. 1 m²), it can be concluded that for that site the ground cover by the flowering weeds will be well below 10% of the soil surface. In contrast, a very small value (e.g. a few cm²) would indicate that the ground cover by the flowering weeds is likely to exceed 10% of the soil surface.

In the majority of cases, the soil surface each flowering weed plant should cover to reach a total weed ground cover of 10% of the total surface area was irrealistically high (e.g. 0.25 m² or more). However, to actually be able to estimate the number of sites where the weed ground cover will exceed 10% of the total surface area, an estimate of the average ground cover for one weed plant should be available. Making such an estimate is very difficult as it is very much dependent on the properties of each weed species (e.g. perennials vs. annual plants, monocotyledons vs. dicotyledons). To aid the interpretation of the results, Table B.9.3.2-1 shows the number of field sites where more than 10% of the soil surface would be covered with flowering weeds for different assumptions regarding the average ground cover of one flowering weed plant. It should be noted that these assumptions are not based on experimental data.

Table B.9.3.2-1: Number and percentage of the tested field sites where the total flowering weed ground cover would exceed 10% of the total field site surface area, if an average ground cover for one weed plant of 0.0225 m², 0.04 m² or 0.09 m² is assumed.

Crop	Timing	Assumed average area covered by one flowering weed plant					
		0.0225m² (15x15cm)		0.04 m² (20x20cm)		0.09 m² (30x30cm)	
		number	%	number	%	number	%
Maize	1 month after sowing	1	1.89	3	5.66	3	5.66
	at crop flowering	1	1.89	3	5.66	5	9.43
	mid-September	1	1.96	4	7.84	8	15.69
Potato	1 month after sowing	0	0	0	0	3	5.45
	at crop flowering	1	1.82	1	1.82	4	7.27
	mid-September	1	2.0	2	4.0	7	14.0

According to the data shown in Table B.9.3.2-1, the total flowering weed ground cover only exceeds 10% of the total surface area in a low number of cases. Based on the assumption that a flowering weed plant on average covers an area of 30x30 cm, the 10% trigger is exceeded in less than 10% of the field sites for both potato and maize 1 month after sowing and at crop flowering. Under the same assumption, the 10% trigger is exceeded in about 15% of the field sites for both maize and potato monitored mid-September. It should however be noted that an average ground cover of 30x30 cm for an individual flowering weed plant is a worst-case assumption, which is likely to be an overestimation. For example monocotyledonous weeds are likely to cover only a smaller area.

The analytical results from the guttation studies presented in Section B.9.5.1 indicate that residues of clothianidin would potentially be at their highest early in the season. At that moment, however, the incidence of flowering weeds will be very low, resulting in a negligible exposure to clothianidin residues through weeds. At the time of crop flowering, when the crop could potentially be attractive to honeybees or other bee pollinators, the number of flowering weeds has increased, resulting in an increased exposure potential compared to earlier in the season. However, as shown in Table B.9.3.2-1, even under worst case assumptions of ground cover (average ground cover of 30x30 cm for an individual weed), less than 10% of the tested field sites will have a flowering weed coverage of 10% of the soil surface at that time. As a result, exposure of clothianidin residues through flowering weeds remains low, based on the 10% trigger suggested by the EFSA Guidance Document. At the end of the season (mid-September), the occurrence of flowering weeds has further increased. Depending on the assumptions made regarding the ground cover of an individual weed plant, the 10% trigger of the EFSA Guidance Document for bees is breached in 7.84 to 15.69% of the sites for maize and 4 to 14% of the sites for potato. At that time, residue levels of clothianidin in soil will however have declined and bee activity will be declining as the end of the season approaches. Thus, while more flowering weeds were found mid-September, this will probably not represent a significant route of exposure especially as the crop is not flowering and so will not offer any attraction to bees.

The presence of flowering weeds by itself does not necessarily indicate a potential risk to bees i.e. in terms of exposure as a pollen/nectar source. Relatively little information is however available with regards to the attraction of different weed species to bees (in terms of nectar and/or pollen sources). Some of the species found in the study by Negrini (2014) have been recognized as a possible source of nectar and/or pollen e.g. *Convolvulus* spp., *Polygonum* spp. and *Setaria* spp. (Hawes, 1979)[23]. However, some of the other, more abundant species have not (e.g. *Chenopodium* spp. and *Galium* spp.). In addition, a species like *Datura stramonium* (Jimson weed), that was also found by Negrini (2014), is known to be toxic with hallucinogenic properties.

Overall, exposure of honeybees and non-*Apis* bees to clothianidin through nectar and pollen of flowering weeds in the treated fields can be considered negligibly low. It has to be noted that this conclusion is based on a monitoring study in fields where weed control following standard agricultural practices (use of herbicides) is applied. Sufficient weed control is thus necessary for the exposure to be negligible.

At Pesticides Peer Review Meeting 145, it was noted that no data is available on the occurrence of flowering weeds in fields cultivated with sweet maize and sorghum, or for the use of clothianidin in forestry nursery. However, the experts agreed that for sweet maize and sorghum the data on maize can be used to conclude on a negligible exposure. For the use in forestry nursery, this is however not the case, and it was considered necessary to set a data gap.

B.9.3.3. Risk assessment

As the exposure of honeybees and non-*Apis* bees through nectar and pollen of flowering weeds is considered negligible, a risk assessment for this route of exposure is not considered necessary. It has to be noted that this conclusion is based on a monitoring study in fields where weed control following standard agricultural practices (use of herbicides) is applied. Sufficient weed control is thus necessary for the risk to be acceptable.

[23] Hawes, FN (1979). Plants and beekeeping. Faber & Faber, UK.

B.9.4. THE RISK TO HONEYBEES FORAGING ON INSECT HONEY DEW

B.9.4.1. Studies

The applicant submitted a large scale monitoring study to determine the presence of weeds and honey dew in potato and maize during the growing season (IIIA 10.4c/01; Negrini, 2014). A summary of this study is provided in section B.9.3.1.

B.9.4.2. Exposure

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

The EFSA Conclusion on the risk assessment for bees for clothianidin (2013)[24] states that honeybees could potentially forage on insect honey dew present in the treated crops, making this a theoretically possible exposure route. As clothianidin is an insecticide, and the purpose of a granule treatment with this substance is to prevent crop pests, including aphids, it can be expected that the presence of honey dew will be very limited in clothianidin treated crops. However, as no information was available to demonstrate that the clothianidin granule treatment will prevent the formation of insect honey dew, a data gap was concluded. It is however noted that in the EFSA Guidance Document on bees, the honey dew exposure scenario was not included in the risk assessment scheme.

The applicant did not provide any data regarding the presence of honey dew specifically in clothianidin treated crops, but submitted a large scale monitoring study in potatoes and maize fields characterized by relevant farmer maintenance with chemical programs in general. This monitoring study (Negrini, 2014; See Section B.9.3.1) indicates that the occurrence of honey dew in potato and maize is very low. In the case of maize, fifty-three locations were monitored in France, Italy and Hungary. One month after sowing as well as at flowering of the crop no honey dew was seen. Only two sites out of the fifty-three monitored were observed to have honey dew present at the end of season (mid-September). In the case of potatoes, fifty-five locations were monitored in France, Italy, Spain, Germany, United-Kingdom, Hungary and Poland. As for maize, no honey dew was observed in the crop one month after sowing. Two sites out of the fifty-five locations monitored were observed to have honey dew present at flowering and at the end of season (mid-September). It is noted that no data is available on the occurrence of honey dew in fields cultivated with sorghum. However, it is considered that this will be comparable to the results for maize and potatoes, and that thus honey dew will be present in only a very limited number of sorghum fields.

The analytical results from the guttation studies presented in Section B.9.5 indicate that residues of clothianidin would potentially be at their highest early in the season. At that time, however, honey dew will be absent, resulting in no exposure through honey dew for bees. This situation also applies at flowering for maize. For potatoes, honey dew was reported only at a verly limited number of sites at flowering, indicating that exposure will also be low. At the end of the season (mid-September), honey dew was also recorded at a limited number of sites for both potato and maize. As residue levels of clothianidin will decline throughout the season, and bee activity will decline as the end of the season approaches (from mid-September on), exposure of bees from clothianidin residues in honey dew can be considered negligible.

[24] European Food Safety Authority (2013). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin. EFSA Journal 2013;11(1):3066. doi:10.2903/j.efsa.2013.3066

Overall, exposure of honeybees and non-*Apis* bees to clothianidin through honey dew present in the treated field can be considered negligibly low for the currently registered uses of clothianidin as granule treatment in crops such as maize and potatoes.

During Peer Review it was argued that in the field monitoring study by Negrini (2014), probably the investigation of the occurrence of honey dew should have been conducted in a late growth stage where the presence of aphids (and honey dew) is more likely (see comment 5(20) in the Reporting Table). It is noted that the residue level of clothianidin in honeydew might be lower, but it should be characterized. The response of the applicant to this remark was the following (*text in italic*):

The observations on aphid honeydew were carried out on three occasions: one month after sowing of the crop; at crop flowering; about mid-September. This will have covered a representative range of crop growth stages and in all cases the presence of honeydew was extremely limited. It is also noted that not only will clothianidin residues decline during the season, but at the end of the season bee activity will also decline. The conclusion of negligible exposure is therefore valid.

The study Negrini (2014) was discussed at Pesticides Peer Review Meeting 145. It was noted that this study investigated the occurrence of honeydew in the potato and maize at different crop growth stages. The conclusion of the study authors and RMS was that, considering the overall limited occurrence of honeydew in potato and maize, it may be considered as a not relevant route of exposure for treated crops. The experts agreed with this conclusion for all the granular uses of clothianidin under evaluation, including sweet corn/sorghum and forestry nursery.

B.9.4.3. Risk assessment

Based on the data from the monitoring study submitted by the applicant (Negrini, 2014), the exposure of honeybees and non-*Apis* bees to clothianidin through honey dew present in the treated field can be considered negligibly low. Therefore, a risk assessment for this route of exposure is not considered necessary.

B.9.5. THE POTENTIAL GUTTATION EXPOSURE AND THE ACUTE AND LONG-TERM RISK TO COLONY SURVIVAL AND DEVELOPMENT, AND THE RISK TO BEE BROOD FROM SUCH EXPOSURE

B.9.5.1. Studies

The applicant submitted one study on the acute and long-term risk to colony survival due to exposure to guttation in maize, and two studies on the acute and long-term risk to colony survival due to exposure to guttation in potatoes. In potatoes, clothianidin was applied either as spray application or as in-furrow granule application at sowing. Further, a study measuring the residue on potato leaves after spray application of a clothianidin containing formulation was submitted.

Report:	**IIIA 10.4e/01, Thompson, H; 2011a**
Title:	**Santana: Evaluation of potential effects to honeybee colonies of guttation in corn grown following in-furrow application of Santana (a.s. clothianidin, 1% w/w)**
Report No.:	V7GP1000
Document No:	THW-0269
Guidelines:	OEPP/EPPO 170; CEB 230 Deviations: not applicable
GLP	Yes (certified laboratory)
Previous evaluation:	This study has previously been submitted to EFSA (2012) and was included in their Peer Review Report on clothianidin (2012)[25]. It has not been evaluated by the RMS (Belgium).

Objective

The study was designed to determine the effects of Santana (a soil incorporated granular formulation containing the neonicotinoid compound clothianidin 1% w/w) applied in-furrow at sowing of maize seeds during the first few weeks post-emergence, on honeybee (*Apis mellifera*) colonies in th field.

Potential exposure may arise via guttation fluid if used as a source of water by the bees. Guttation is a natural phenomenon in which water from xylem fluid is exuded through pores on the leaf during periods when root pressure is high and transpiration is low, e.g. occurring overnight and early hours of the morning. There is the potential for the fluid exuded to contain molecules such as systemic pesticides. During the exposure phase, the colonies were monitored at the maize fields with assessment for immediate post-exposure effects e.g. mortality, behaviour of the bees in the field (both at the hive and on the crop) and colony development. Furthermore, the residue levels of clothianidin to which honeybees were exposed during the exposure phase was determined

Material and methods

The study was conducted as field monitoring (open field) under local conditions (e.g. beekeeping, agronomic and climate) in a representative corn-growing area in south-west France. This study was conducted in accordance with the guideline of the European and Mediterranean Plant Protection Organization No. 170 (3) (OEPP/EPPO, 2001) but adapted for use with systemic compounds expressed in guttation water exuded from leaves. In addition, sublethal and behavioural effects on the bees were recorded using criteria based on the behavioural categories given in CEB 230 (2007).

Test and control item

Test item: the soil incorporated granular formulation Santana (CAGR8; 1% w/w clothianidin granules (GR); purity*:* 09GR014 - 1.053 ± 0.002% w/w (analysed)), applied in-furrow during mechanical sowing of maize seeds at 110 g a.s./ha (nominal).

[25] European Food Safety Authority (2012). Peer Review Report on clothianidin. (key background document to the EFSA Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin (2013), containing the study evaluation notes, the report of the scientific consultation with Member State experts and the comments received on the draft EFSA conclusion)

At the control field, untreated maize seeds were sown. Further, the control field had not been treated with neonicotinoid insecticides (including seed treatments) in the preceding two cropping seasons.

Study sites

The study was conducted in southern France (the Landes department) on two fields; one treated and one control. These fields were also used for the 3-year maize study (IIIA 10.4g/01, see Section B.9.7.1) and were subject to staggered sowing in 2 plots on each field with drilling approximately 2 weeks apart. The first sown plot was used for this study (the effects of drilling on the second plot were limited to the latter stages of the study due to the time from drilling to emergence). However, as the second plot on each field was sown while the colonies were present on the fields, the guttated fluid present on the second plot was also available for the bees during this latter part of the study.

The two different fields of 1.2 ha (treated) and 2.3 ha (control) for each of the first sown plots and control (untreated) were separated by at least 4 km to avoid bees foraging on the other field. The test fields were surrounded by woodland and maize field (pre-flowering but at a more advanced growth stage that did not guttate).

The only sources of nectar and pollen from the control field were identified as a small number of flowering weeds in the field margins (less than 0.5%). The test item was applied by simulating application of the granules in-furrow during the mechanical sowing of maize seeds at a rate of 110 g a.s./ha.

Honeybee hives

Six small, queen-right bee colonies were used per treatment and control site, i.e. a total of 12 colonies. The colonies were typical of those used in each region (sourced from a local beekeeper) and were as similar to one another as possible, containing approximately 8,000 to 14,000 bees and appropriate amounts of brood and food stores.

The colonies were placed within the crop on each site, 5 days prior to emergence, so that to access water they had to fly over the crop. At the start of the study the plants in the control and treated fields were 75% BBCH 11 and by day 30 the plants in the control field were 90% BBCH 32 (stem elongation) with 10% BBCH 18 and the plants in the treated field were all at BBCH 32.

Monitoring activities

During the exposure phase, the colonies were monitored at the maize fields with assessment for immediate post-exposure effects e.g. mortality, behaviour of the bees in the field (both at the hive and on the crop) and colony development. Furthermore, the residue levels of clothianidin to which honeybees were exposed during the exposure phase was determined.

During the first few days after plant emergence, initial assessments were made of the time of the start of guttation and the start of activity of the bees at the hives during the morning. These data were used to identify the most appropriate time for assessments of bee activity. Subsequent assessments were aimed at collecting guttation fluid and bee behaviour observations on alternating days with the exact timing determined both by the presence of guttation fluid and suitable weather (e.g. assessments were not made during periods of rainfall).

Plants

Every day of the exposure phase the growth stage of plants (BBCH) was recorded together with an assessment of guttation (presence/absence on plants) and the time that guttation fluid dried as well as the presence of any dew on the plants (small droplets over the surface of the leaf). On the days the guttation samples were collected (see below), records were made of the presence of species of other plants (weeds) guttating at the field edges and other sources of water, e.g. puddles. As during the latter stages the second plot to be drilled emerged, the growth stage and the presence of guttation fluid on this plot was also recorded.

Honeybee mortality
During the exposure period, dead bee traps were fitted to each hive and water-permeable sheets (1.5 m x 3 m) were spread out in front of the hives. On each day of the exposure period the number of dead bees at each hive was recorded and the dead bees collected (the bees in the dead bee trap and on the sheets in front of each hive were combined) and stored at -15 to-25ºC.

Mortality was also recorded daily at four places within the crop by spreading water permeable sheets (ca. 15 m²) between the rows of the crop on which any dead bees were counted and collected (dead bees combined from all sheets) and stored at -15 to-25ºC. These sheets were placed at approximately 20 m and 40 m of the front of the hives and 10-20 m either side of this as this is the area was where most water collection by the bees was expected to occur.

Water collection and behaviour of bees in the field
Bee activity was recorded at the hive every 2-3 days (alternating with the guttation fluid collection). Bee activity was assessed by counting the number of any bees leaving the hive for 30 seconds each every 30 minutes until activity started (an average of at least 1 bee/30 seconds for the 6 hives) and then once per hour until guttation ceased.

On the same occasions, observations of the water collection in the field took place (on days when guttation occurred) by walking along the rows for a total of 30 minutes per plot (2 people for 15 minutes) each hour that guttation fluid was present and the bees were active. Assessments took into account that the rows immediately in front of the hive were likely to be the most attractive to water-collecting bees so that 15 minutes was used to assess the rows immediately adjacent to the hives (an area approximately 20 m into the crop and 50 m wide immediately in front of the hives) and the remaining 15 minutes was used to assess the remainder of the crop (including the first 20 m from the hives into the 2nd sowing plot when this has emerged).

At each assessment time the number of bees on maize plants (on leaf, at leaf axil or within flag leaf) and on the soil was assessed, noting location of the plant in field (approximate distance from hive (within 10m) and behaviour e.g. drinking, resting. In addition on each occasion sublethal and behavioural effects on the bees were recorded using criteria based on the behavioural categories given in CEB 230.

Conditions of the colonies, development of the bee brood
The condition of all colonies was recorded and the development of the bee brood was checked on the day before expected emergence of the maize and every 7±1 days until the end of the exposure period. The following parameters were assessed: weight of each colony; strength of the colony (number of combs covered with bees); presence of a healthy queen (presence of eggs, presence of queen cells); visual assessment of the pollen storage area and area with nectar (in %); visual assessment of the area containing eggs, larvae and capped cells (in %). Visual inspections of the colonies were undertaken for signs of bee disease and assessments of Varroa numbers were also made.

<u>Exposure assessment</u>
Every 2-3 days (alternating with the bee behaviour assessments) duplicate samples of guttated fluid were collected from plants by collecting droplets using a disposable Pasteur pipette, and then stored at -15 to -25ºC. Guttation droplets were distinguished from dew by the fact that droplets gathered on the edges/ tips of the leaves or in the leaf axil. The guttation droplets were collected from the edges/tips of leaves if possible and the primary source was recorded as this may have influenced the residues present. Pooled samples from plants in each area were collected to provide duplicate samples from 4 different areas of the treated field, and one sample (in duplicate) for the control field (4 treated and 1 control samples on 15 sampling days).

Samples were taken from all colonies for residue analysis during the exposure phase at the same time as the colony assessments. Duplicate samples were collected from each hive, and stored at –15 to -

25ºC after transfer from the field. Samples collected from each hive (in duplicate) were taken 1x before set-up of the hives and 4x during exposure phase: pollen from the combs; wax from combs inside hives; nectar (uncapped cells); unsealed brood from comb. In addition to the dead bees that were collected, samples of foragers were taken on the days of guttation fluid collection. Duplicate samples of at least 10 bees were taken of bees returning to the hives (one sample from all six hives combined on 8 occasions during the exposure phase at the time of guttation).

Results

Mortality

The levels of worker bee mortality in the dead bee traps and on the sheets immediately in the front of the hives for the treated and control sites remained low throughout the exposure period with a maximum mean mortality peak of only 55 bees per treated and control fields. Within this overall pattern of low mortality, there was a slightly higher level of mortality in the treated than the control plots. In addition, a number of peaks in mortality occurred and these were not always associated with the colony assessment, which could have been the cause of the increase.

However, peaks occurred on both the treated and control fields and on several occasions they occurred at the same time, particularly with the two largest peaks, indicating that a common environmental factor affecting both fields was responsible i.e. they were not treatment-related. The numbers of dead bees found on the sheets within the crop and on the soil surface were very low throughout the exposure period and again showed no treatment-related effects.

Colony assessment

There were no apparent differences between the treated and control colonies in colony development during the study (levels of brood, pollen and nectar and numbers of bees). The queen in one treated colony stopped laying towards the end of the study although the queen was present and seen after the last colony assessment. Varroa numbers based on mite fall data were low during the course of the study.

Behaviour

The numbers of bees leaving the hive were counted until the guttation fluid had dried. The timing of the observations compared with the mean numbers of bees leaving the hives shows that most observations of guttation fluid occurrence were early in the morning whereas the activity of the bees was low between 0600 and 0800 and then rose to a peak from 0930 onwards. Guttation fluid had dried by 1200 on most days (hence the lower numbers of observations after this time) and when guttation fluid was present after 1200 the same weather conditions that prevented evaporation of guttation fluid probably also resulted in fewer bees foraging. Thus although bees were active when the guttation fluid was present the degree of overlap was low.

The activity of bees on plants and on the soil was recorded. A summary of the number of observations of bees on plants and soil in both fields shows that for a total of nearly 20 hrs of observations in the control field and 24 hrs of observations in the treated field, at times when bees were active and guttation fluid was present, a total of 2 bees were observed drinking guttation fluid in each field. In the control field both were identified within 10 m of the hives and in the treated field one was within 5 m of the hives and the other was at least 20 m from the hives.

Residue data

The residue data show high initial levels in the guttation fluid with a mean of 9.1 mg/kg clothianidin on the day after emergence (9 days after sowing) decreasing to 0.53 mg/kg 29 days after emergence (38 days after sowing). The metabolites TZNG and TZMU were also present but at significantly lower levels. An apparent dip in residue levels (during the overall decline) around days 14-16 after emergence appears to have been related to an episode of prolonged heavy rainfall, which may have diluted the residues either in the plant or on the surface of the leaves. No quantifiable residues of clothianidin, TZNG or TZMU were detected in samples from the control field (LOQ=1 µg/ kg).

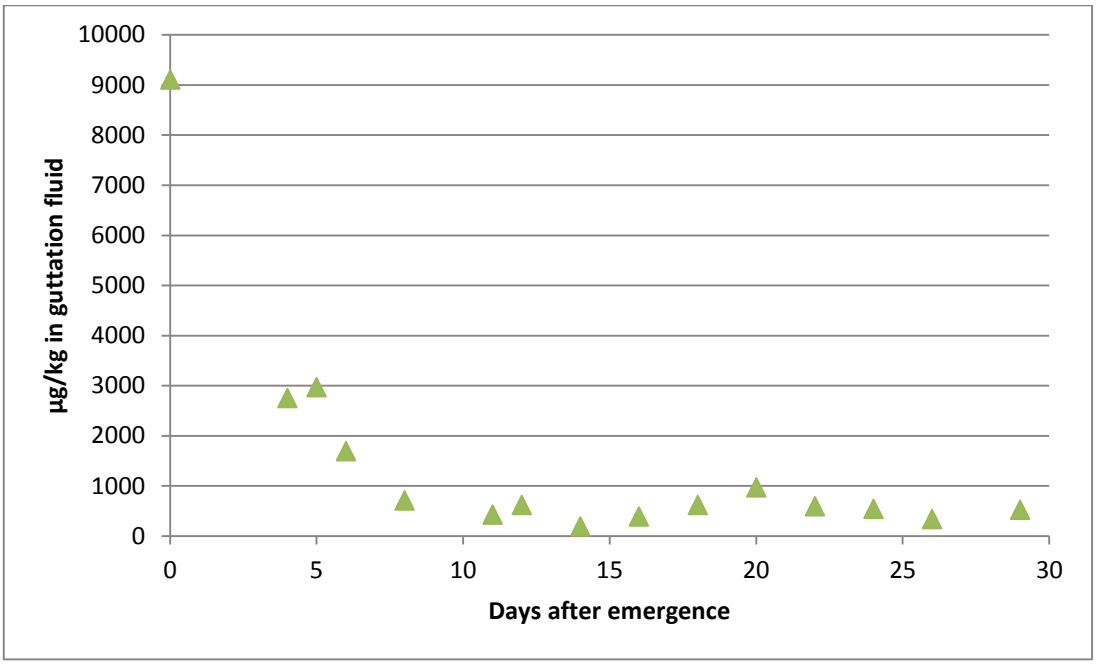


Figure B.9.5.1-1: Mean residues of clothianidin in guttation fluid from treated plants

Similarly, no residues of clothianidin, TZNG or TZMU were detected in samples of foragers from the control field. The data from bees caught returning to the hives on the treated field show only one sample (18 days after emergence) had levels of clothianidin above the limit of quantitation. This sample contained 6 µg/kg bee and comprised about 10 bees (1 g sample, assuming 0.1 g/bee). Therefore the 10 bees in the sample contained 0.006 µg whereas the guttation sample taken from the plants on day 18 after emergence contained a mean of 620 µg/kg or approximately 0.62 µg/ml. The residues found in the bees therefore corresponds to about 10 µl guttation fluid and such a low level suggests incidental (contact) exposure rather than the amount that may be carried during water collection.

No residues of clothianidin, TZNG or TZMU were detected in dead bees from the control field. Samples of dead bees from the treated field were analysed separately for each hive where there were peaks in mortality observed (days 1, 11, 20, 25 and 26 after emergence (06/06/2010)) while samples from other days were bulked samples from all hives. Over the course of the exposure period, mean residues of 6.0 µg/kg and 90th percentile residues of 12.2 µg/kg were found in the dead bees. Figure B.9.5.1-1 shows a comparison of the residue levels found with the numbers of dead bees observed on each day. There is little indication of any correlation between the numbers of dead bees collected and the residue levels found, either across the whole exposure period or in terms of individual peaks. Thus, the variable residue levels were fairly uniformly distributed across the exposure period and the peaks in residue levels tended not to coincide with the mortality peaks. In addition, the data also show residues were not higher at the start of the exposure period then decreasing over time, as might be expected if the bees were drinking guttation fluid during the study. This indicates that any direct lethal effects on the bees were marginal and that the residues found were probably largely as a result of incidental contact on the maize plants.

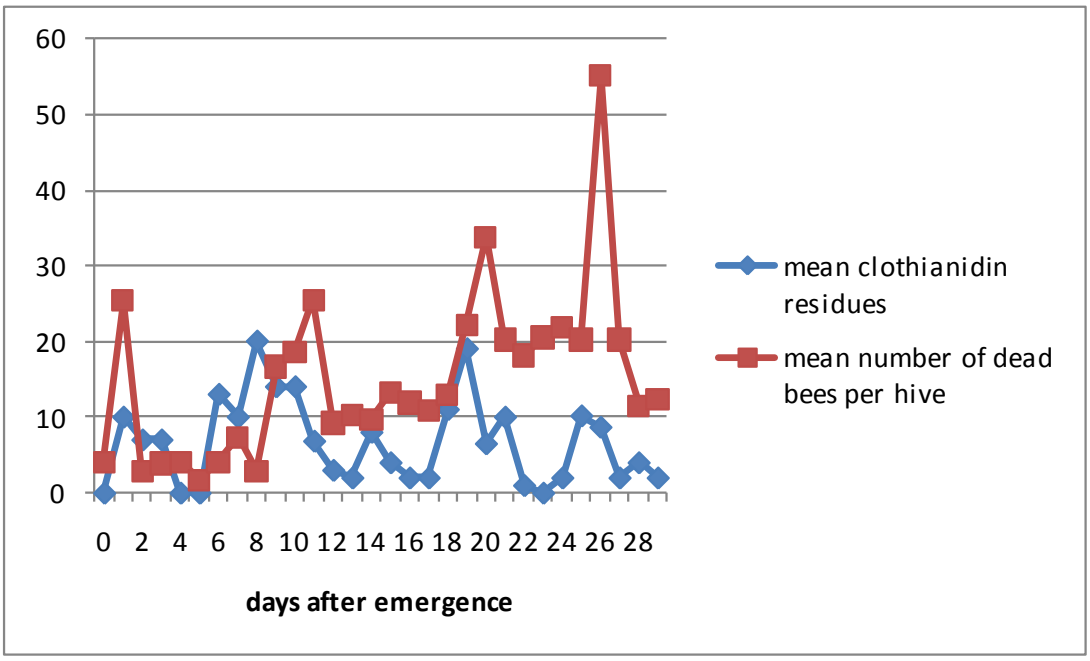


Figure B.9.5.1-2: Mean residues of clothianidin (µg/kg) in dead bees from the treated field compared with the mean number of dead bees per hive on each day

No residues of clothianidin, TZNG or TZMU were detected in any of the brood samples from the control field. The results from the analysis of the brood samples from hives in the treated field showed very low levels of residues were present with samples from only 2 of the 6 treated hives with residues at or below the limit of quantification. Similarly, no residues of clothianidin, TZNG or TZMU were detected in samples of nectar and pollen taken from hives in the control field. In the nectar and pollen samples taken from hives in the treated field no detectable residues were identified in most samples with 3 samples of nectar at or below the limit of quantitation by day 26 and only one sample of pollen showing detectable but low residues by day 26 (2 µg/kg). Levels in wax from hives on the treated field were also low (all below limit of quantitation except one hive at 2 µg/kg) with again no residues being found in any of the control samples. These results suggest there is no significant accumulation of residues of clothianidin or bee relevant metabolites within the colonies on the treated field during the 4 weeks of exposure.

Conclusions

Honeybee colonies experienced worst-case exposure to young maize plants for 30 consecutive days with guttation on 28 days as well as additional exposure from a 2nd sowing (after it had emerged) together with any dust at the actual time of the sowing. There were no other significant sources of water available in and around the test fields (except for puddles after rain). High levels of clothianidin were present in guttation fluid during the study with levels decreasing from 9.1 mg/kg to 0.53 mg/kg. However, there was only slight overlap between peak times for bee activity and the time when guttation fluid droplets were present on the crop and only 2 bees were observed drinking in each field (treated and control) during the course of the study (from a total of 20 to 24 hours of observations).

Low levels of mortality (maximum peak of 55 bees/day, mean of 6 hives) were observed at the hives on the treated field during the study and although these levels were slightly higher than for the control hives there were no effects on colony development. Analysis of the dead bees showed mean residues of 6.0 µg/kg and 90th percentile residues of 12.2 µg/kg. However, there was no correlation between the residue level and the number of dead bees in each sample, indicating that the residues found were largely as a result of incidental contact exposure. Analysis of brood, pollen, nectar and wax from the hives showed only very low levels of residues present (1-2 µg/kg) with the majority of samples below the limit of detection.

Although high residues of clothianidin were present in guttation fluid it is clear that it is not a significant source of water for honeybee colonies, even in the absence of other significant water sources. Consequently, exposure at the colony level is negligible and there are no adverse effects on the condition of the test colonies.

RMS Comments

This study has previously been evaluated by EFSA (see Study Evaluation Notes supporting the EFSA Conclusion on the risk assessment for bees for clothianidin, 2013). EFSA commented on this study that it was not excluded that the residues detected in dead bees are not linked to the guttation exposure, because the authors cannot think of any other way exposure may have occurred. Therefore this study was not considered useful to address the risk from guttation. The applicant did no submit any additional information since the previous evaluation, only the following argumentation (text in italic):

In the study by Thompson (2011a), there was no correlation between the residue level and the number of dead bees in each sample, which together with the lack of observations of bees drinking guttation fluid clearly indicates that the mortality was not linked to guttation exposure. The report does state that the residues found in the bees probably resulted from incidental exposure. This is not surprising as the colonies were placed within the crop so bees may have settled on foliage around the hives and this is demonstrated by the behavioural observations (20 bees were seen resting on plants over the study period with a further 39 seen resting/walking on the soil surface). There was clearly a very limited but finite potential for this incidental exposure.

RMS agrees that there is no correlation between the residue level and the number of dead bees in each sample and that there is a potential for incidental exposure of bees to clothianidin residues in the crop. While due to the nature of guttation, and as a result the set-up of guttation studies, it is not possible to exclude that the residues detected in dead bees are not linked to guttation exposure, RMS is of the opinion that there are sufficient indications from the study that exposure to guttation did not lead to an increased bee mortality. Overall, RMS considers this study suitable for risk assessment purposes.

The measured residue values in guttation fluid are considered reliable and can be used in the risk assessment.

During Peer Review, it was argued that the analysis of the bee mortality data focuses on the potential for correlation with active substance residues in guttation fluid. Further consideration of the treatment mortality relative to the control and whether the clothianidin residues in dead bees were consistent with exposure to this active as being the cause of dead was considered useful (see comment 5(24) and 5(25) in the Reporting Table) . The applicant provided the following response to these comments (*text in italic*):

The studies presented for the assessment of the risk to honey bees from guttation fluid in maize and potatoes Thompson (2011a), Thompson (2013a) and Ansaloni 2015 are consistent in showing that this is not a significant source of drinking water and so exposure is negligible. Also, as is noted, the guttation fluid is not the only source of exposure as this may have resulted from incidental contact e.g. bees settling on the treated crop.
For Thompson (2011a), mortality on the control and treated plots was relatively low throughout the exposure period and although there was a slightly higher level in the treated plots the actual numbers are low with a maximum mean mortality peak of only 55 bees. A mortality inferior to 100 bees is commonly accepted as non significant. In addition, any peaks in mortality occurred at both sites on the same days suggesting it was not treatment related. The maximum residue level found in foragers was 6 µg/kg, equivalent to about 0.0006 µg/bee i.e. less than the oral LD_{50}, indicating it may not have been the cause of death.

At Pesticides Peer Review Meeting 145, it was considered that as no new data were submitted triggering the re-assessment of this study, the previous conclusion regarding this study from EFSA (2013) is still valid.

Report:	**IIIA 10.4e/02; Thompson, HM; 2013a**
Title:	**Dantop 50WG: Effects of a spray application of clothianidin in potatoes on honeybees.**
Report No.:	V7XW1004
Document No:	THW-0337
Guidelines:	OEPP/EPPO 170(3)
GLP	Yes

Objective
This study was designed to determine the effects of clothianidin as the formulation Dantop 50 WG applied to control aphid populations in potatoes and potentially exuded in guttation fluid, on honeybee (*Apis mellifera* L.) colonies in the field.

Guttation is a natural phenomenon in which water from xylem fluid is exuded through pores on the leaf during periods when root pressure is high and transpiration is low, e.g. overnight and in the early hours of the morning. There is the potential for the fluid exuded to contain molecules such as systemic pesticides. During the exposure phase, the honeybee colonies were monitored at the potato fields with assessment for immediate post-exposure effects (mortality and behaviour) and continued monitoring until the start of the over-wintering period (108 days after application). In addition, residues were analysed in guttation fluid collected from the crop for 2 weeks after the application.

Material and methods
The study was conducted as field monitoring (open field) under local practical conditions in a representative potato-growing area in the UK. This study was conducted in accordance with the guideline of the European and Mediterranean Plant Protection Organization No. 170 (3) (OEPP/EPPO, 2001) but adapted for use with systemic compounds expressed in exuded water from leaves.

Test and control item
Test item: Dantop 50WG (batch no. P05-8E901; purity: 50.00% w/w a.s. (clothianidin)).

The test item application was made in the morning with calibrated equipment and the bee colonies were left open during the application. The treatment as 150 g product/ha (75 g a.s./ha) with 400 L/ha water (test item on treated field) was applied by tractor mounted spray application between 0830 and 0930 on 26 June 2012. The application was made with calibrated equipment and the applied rate was measured by determining the volume of the test item before and after application with an application tolerance of ± 10%.

A the control field, potato plants were untreated. Further, the control field had not been treated with neonicotinoid insecticides (including seed treatments) in the preceding two cropping seasons.

Study sites
The study was conducted in North Yorkshire, UK. There were two test plots of approximately 1ha. The control and treated sites were separated by at least 2 km to avoid bees foraging on the other field. Due to the nature of guttation it was not possible to locate the study fields away from other crops (although flowering crops were avoided) and weeds which may guttate under the same conditions as the test field. The control field had not been treated with neonicotinoid insecticides (including seed treatments) in the preceding two cropping seasons. The pesticide use history of all fields used in this study was documented for at least the two previous cropping seasons before the start of the study.

Agronomic records for the plots and their environment (including the presence of any weeds around the test plots and location of other crops) are available and meteorological data for the experimental period were recorded.

Honeybee hives

Six normally developed queen-right bee colonies sourced from the National Bee Unit, Fera, were used per treatment and control site, i.e. a total of 12 colonies. The colonies were as similar to one another as possible. The hives contained 10 frames including at least 2 of stores and at least 10,000 bees had an average of 3.7 and 3.8 frames of brood, for the control and treatment colonies, respectively.

The colonies were moved to the test sites 7 days before spray application. A Tinytag datalogger was placed on each site to record air temperature and humidity during the course of the exposure phase. The hives were placed on the plot such that water-collecting bees would need to fly over the plot to any other source of guttation, e.g. weeds in margins or neighbouring crops.

Monitoring activities

During the exposure phase, the honeybee colonies were monitored at the potato fields with assessment for immediate post-exposure effects (mortality and behaviour) and continued monitoring until the start of the over-wintering period (108 days after application). In addition, residues were analysed in guttation fluid collected from the crop for 2 weeks after the application.

During the first two days after placing the colonies on the plot, before spray application, the activity of the bees at the hive was assessed from 0715 to 1600hrs. This was assessed by counting the number of bees leaving the hive for 30 seconds each every 30 minutes until activity had started (at least 1 bee/30 secs/ 2 hives) and then once per hour. These data were used to identify the most appropriate times for subsequent assessments of bee activity. Subsequent assessments were aimed at collecting bee behaviour observations on alternating days but the exact timing was determined by suitable weather (e.g. assessments were not made during periods of rainfall).

Plants

On every assessment day the growth stage of the potato plants (BBCH) was recorded together with an assessment of guttation (presence/absence in plants) and the time that guttation fluid dried. The presence of dew on the plants (small droplets over the surface of the leaf) was also recorded, if visible. When possible, guttation fluid droplets were collected for analysis. On the days the guttation samples were collected records were made of the presence of species of other plants (weeds) guttating at the test field edge as well as other sources of water e.g. puddles.

Honeybee mortality

On the afternoon after the application dead bee traps were fitted to each hive and water-permeable sheets of 1.5 m width and about 3 m length were spread out in front of the hives. Each day of the exposure period the number of dead bees was recorded and the dead bees collected (the bees in the dead bee trap and on the sheets in front of the hives were combined) and stored at -15 to -25ºC for subsequent analysis if required.

Mortality was also recorded daily at six places within the crop by spreading water permeable sheets (area: approximately 15 m²) between the rows of the crop. Any dead bees found were counted and collected (and stored at -15 to-25ºC until analysis if required). The location of the sheets within the field was biased towards the crop within 40 m of the front of the hives and 10-20 m either side of this as this was the area where most water collection was expected to occur.

Behaviour of bees in the field

Bee activity and behaviour were recorded at the hive and in the field every 2-3 days. Activity at the hive was assessed by counting the number of bees leaving the hive for 30 seconds three times per day (timing based on the assessments on the day before spray application).

The observations in the field were undertaken by walking along the rows for a total of 30 minutes per plot three times per day (timing based on the pre-spray activity assessment at the hive but ensuring that two were during the period that guttation was likely to occur based on observations of the plants pre-spray). Assessments took into account that the rows immediately in front of the hive were likely to be the most attractive to any water-collecting bees so that 15 minutes was used to assess the rows immediately adjacent to the hives (an area approximately 20m into the crop and 50m wide immediately in front of the hives) and the remaining 15 minutes was used to assess the rest of the crop.

At each assessment time the number of bees on plants (on leaves or at the leaf axil) and on the soil and behaviour (drinking/resting) were assessed. In addition on each occasion, any sublethal and behavioural effects on the bees were recorded using criteria based on the behavioural categories given in CEB 230.

Conditions of the colonies, development of the bee brood
The condition of all colonies was recorded and the development of the bee brood checked 2±2 days before spray application, every 7±1 days until the end of the 14-day exposure period and then monthly until October. The following parameters were assessed: weight of each colony; strength of the colony (number of combs covered with bees); presence of a healthy queen (presence of eggs, presence of queen cells); visual assessment of the pollen storage area and area with nectar (in %); visual assessment of the area containing eggs, larvae and capped cells (in %). Visual inspections of the colonies were undertaken for signs of bee disease and assessments of Varroa numbers were also made.

Exposure assessment
On every assessment day the, an assessment of guttation (presence/absence in plants) was recorded, together with the potato plants growth stage (see above). When possible, guttation fluid droplets were collected for analysis using a disposable Pasteur pipette on the same day as the bee behaviour assessments. These were stored at -15 to-25ºC at for subsequent analysis. On the days the guttation samples were collected records were made of the presence of species of other plants (weeds) guttating at the test field edge as well as other sources of water e.g. puddles.

Results
Foraging activity
The timing of foraging activity on each field prior to treatment shows that activity of the bees started around 7:30 and ceased around 14:00 and therefore assessments were concentrated between these times. Assessment of activity at the hives on the two sites from the day after application to 13 days after treatment with three observations each day showed no consistent difference in activity at the hives in the two fields.

Only low numbers of bees were observed on the potato plants (9 at the treated site and 5 on the control site over the 14-day exposure period); the majority appeared to be resting on the leaves and none were observed drinking. Bees were observed drinking from a puddle near an irrigation outlet on the treated field on one day (day 6) post-application.

Mortality
The mortality observed at each site is shown in Figure B.9.5.1-3. Only low levels of mortality were observed in the colonies in the control site although there was an increase after the colony assessment on day 7 (mean 79 bees). Mortality in colonies in the treated field was slightly higher in several colonies (D2, D3, D4 and D6) on day 1 (mean 28 bees) and increased on day 7 (mean 36 bees), again after a colony assessment on day 6.

Figure A Mortality at each of the colonies on the control field

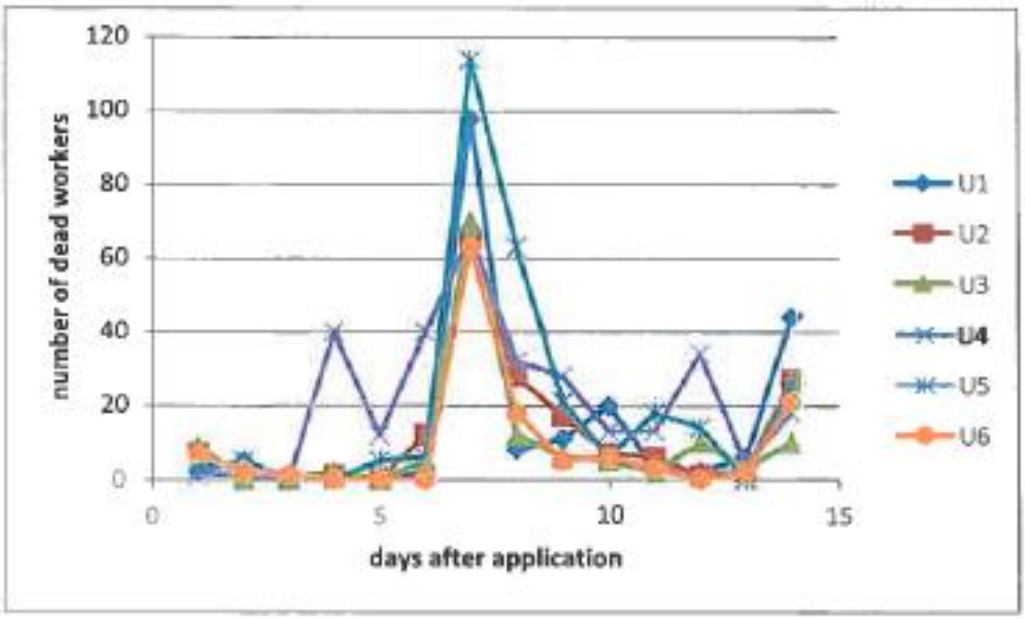


Figure B Mortality at each of the colonies on the treated field

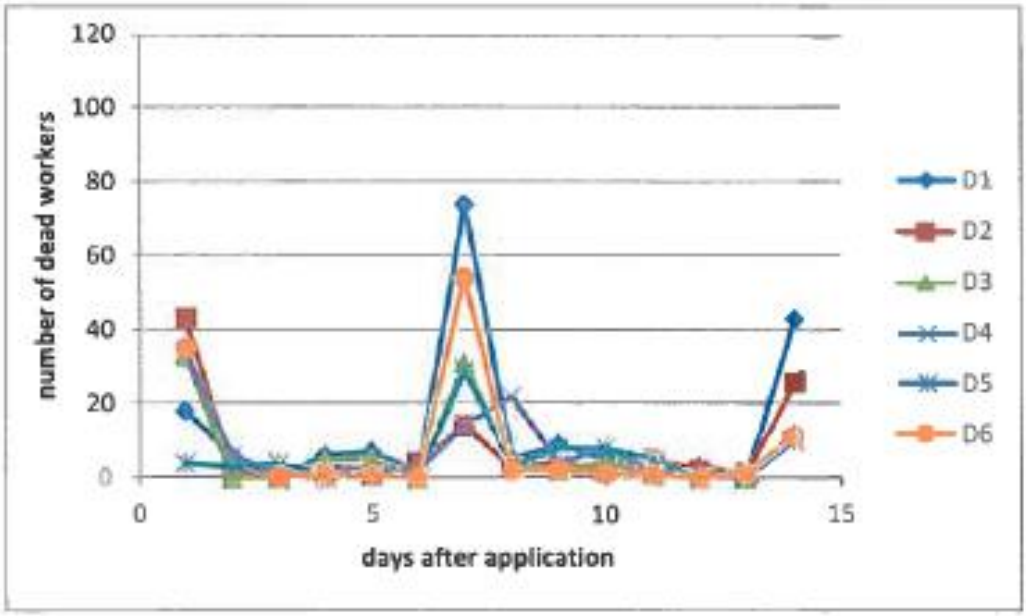


Figure B.9.5.1-3: Mortality at each of the colonies on the control (U) and treated (T) fields

Colony assessment

Colonies were assessed pre- and post- application and there were no adverse effects of treatment on the numbers of bees or levels of brood, pollen and nectar in the colonies on the treated site compared to the control site. One control colony (U4) was re-queened during the study but this was not considered to have had a significant impact. One control colony (U6) died after the end of the exposure period with dead sealed brood present in the frames with no obvious cause. The Varroa mite drop results show low numbers of mites were present at the start of the study. All surviving colonies were treated with Apivar (2 strips) on 13/09/2012.

Observations of the plants

During the exposure period there were guttation droplets observed on the leaves of the potato plants on the treated site on all of the 7 observation days spread over the 2 week post-application period. On the control plot there was no guttation observed on two days but one of these was due to heavy rain.

Residue data

The residues in the guttation fluid collected from potato leaves in the morning is shown in Figure B.9.5.1-4. This shows that the residues declined rapidly from 1317 µg clothianidin/kg on day 1 to 36 µg clothianidin/kg by day 10. Only low levels of the metabolites TZNG (maximum 53 µg/kg on day 2) and TZMU (maximum 32 µg/kg on day 1) were detected. Due to the application pattern of the test material (direct over spray on plants), the residues in the guttation fluid collected soon after application may include residues from the surface of the leaves.

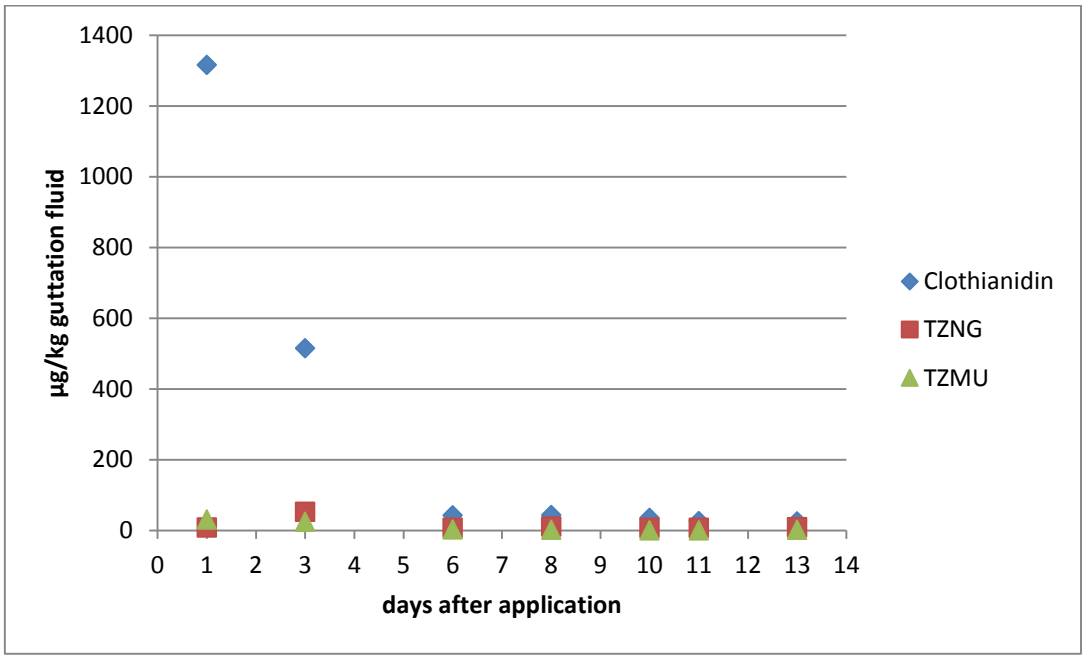


Figure B.9.5.1-4: Residues of clothianidin and its metabolites in guttation fluid from treated plants

Conclusion

During the exposure period there were guttation droplets observed on the leaves of the potato plants on the treated site on all of the 7 observation days spread over the 2-week post-application period. On the control plot there was no guttation observed on 2 of the 7 observation days but one of these was due to heavy rain. Analysis of the residues in the guttation fluid collected from potato leaves on the treated plot showed that the residues declined rapidly from a peak of 1317 µg clothianidin/kg on day 1 to 36 µg clothianidin/kg by day 10 and only low levels of the metabolites TZNG (maximum 53 µg/kg on day 2) and TZMU (maximum 32 µg/kg on day 1) were detected.

Only low levels of mortality were observed in the colonies on the control site although there was an increase after the colony assessment on day 6 (mean 79 bees). Mortality in colonies on the treated field was slightly higher in several colonies on day 1 (mean 28 bees) and increased on day 7 (mean 36 bees), again after a colony assessment on day 6.

Only 9 bees were observed on the potato plants on the treated site and 5 on the control site over the 14-day exposure period; the majority appeared to be resting on the leaves and none were observed drinking. Bees were observed drinking from a puddle near an irrigation outlet on the treated field on one day (day 6) post-application.

There were no adverse effects of treatment on the numbers of bees or levels of brood, pollen and nectar in the colonies on the treated site compared to the control site. One control colony (U4) was re-queened during the study but this was not considered to have had a significant impact. One control colony (U6) died after the end of the exposure period with dead sealed brood present in the frames with no obvious cause.

RMS Comments

In general, the study followed the recommendations from the EFSA Guidance Document on the risk assessment for bees (Appendix O and U) e.g. use of colonies with a good health status, of uniform size and similar genetic origin. However, in contrast to the recommendations of the EFSA Guidance Document, the field sites were approximately 1 ha in size instead of 2 ha. However, this is considered to be a minor deviation and does not influence the validity of the study (a 2 ha field is the size considered for flowering crops to provide sufficient forage and to isolate from other flowering areas. For guttation studies even smaller plot sizes would be appropriate and valid as bees fly only short distances to collect water as due to the high energetic cost of flying, bees will collect water from their immediate vicinity (Joachimsmeier *et al.*[26], 2012)) . Further, only 6 pairs of colonies were set-up, which might potentially be too low to achieve sufficient statistical power.

In this study, clothianidin was applied as spray application. As a consequence, the residues in the guttation fluid collected directly after application may include spray residues from the surface of the leaves. It can therefore be questioned if this study is representative for residues in guttation fluid after application of clothianidin as a granule treatment. This will be further considered in section B.9.5.2 and B.9.5.3 below.

Despite the deviations discussed above, the study is considered acceptable for use in risk assessment.

At Pesticides Peer Review Meeting 145, it was noted that this study was also considered by EFSA in 2015 for the evaluation of the risk to bees for foliar spray uses of clothianidin (see EFSA, 2015[27]). Following that evaluation, this study was not considered suitable for risk assessment by EFSA, as it does not comply with the recommendations of the EFSA Guidance Document for bees.

[26] Joachimsmeier, I.; Pistorius, J.; Heimbach, U.; Schenke, D.; P.; Kirchner, W. (2012). Water collection by honey-bees – How far will foragers fly to use water resources like guttation drops? A first distance trial using cereals and oil seed rape. Hazards of pesticides to bees: 11th International Symposium of the ICP-BR Bee Protection Group; Wageningen, (The Netherlands), November 2 - 4, 2011.

[27] European Food Safety Authority (2015). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin considering all uses other than seed treatments and granules. EFSA Journal 2015; 13(8):4210. doi:10.2903/j.efsa.2015.4210

Report:	**IIIA 10.4e/03; Thompson, HM; 2013b**
Title:	**Dantop 50WG: Residues on the surface of leaves after a spray application in potatoes.**
Report No.:	V7XW1005
Document No:	THR-0775
Guidelines:	Not applicable
GLP	Yes

Objective
This study was designed to determine the residues of clothianidin on the surface of leaves (from an initial foliar application and potentially exuded in guttation fluid) following a spray application of the formulation Dantop 50 WG to control aphid populations in potatoes.

Guttation is a natural phenomenon in which water from xylem fluid is exuded through pores on the leaf during periods when root pressure is high and transpiration is low, e.g. overnight and in the early hours of the morning. There is the potential for the fluid exuded to contain molecules such as systemic pesticides.

Material and methods
Test item application
Test item: Dantop 50WG (batch no. P05-8E901; purity: 50.00% w/w a.s. (clothianidin))

The application was made in the morning at 150 g Dantop 50% WG/ha (75 g a.s./ha) using tractor mounted spray equipment. The application was made with calibrated equipment and the applied rate was measured by determining the volume of the test item before and after application with an application tolerance of ± 10%.

Study site
The study was conducted as field monitoring (open field) under local practical conditions in a representative potato-growing area in the UK. The study was conducted in North Yorkshire, UK. There was a single test plot, of approximately 1ha, comprising clothianidin treated potato plants. Agronomic records for the plots and their environment (including the presence of any weeds around the test plots and location of other crops) are available and meteorological data for the experimental period were recorded.

Following the application, when sprayed residues had dried, 4 areas of the plot each comprising a suitable number of plants (e.g. 10) were covered with thin cotton material to provide leaves that could be sampled from plants protected from direct rainfall. That way, residues could be determined over time in the presence and absence of rainfall/irrigation.

Sampling
Individual leaves were sampled to assess the residues on the surface and were free of excess surface moisture when collected. Leaves were selected from the area of the plants where the leaves were mature. Young leaves that would not have been sprayed or where growth had diluted the residues, were avoided. On sampling, the end of the stem on each leaf was sealed with petroleum jelly to prevent leaching of the xylem fluid into the washing solution. Five replicate leaf samples were taken from each of the uncovered and covered areas of the plot. Each replicate comprised 1 leaf from a compound leaf from each of 10 plants at every time point. The leaf samples were returned to the Fera laboratory at sub-ambient temperature and processed within 12 hours.

The first sample was collected after the spray application had dried (5hrs after application had finished) – this represented the Day 0 sample. Further sampels were taken at weekly intereval until 8 weeks after the spray application.

Sample processing

0.01% (v/v) dioctylsulfsuccinate in water was added to each leaf sample and placed on an orbital shaker to thoroughly wash the leaves but not disrupt the structure of the leaf, for a period of 20 minutes. The solution was transferred into a glass bottle and this extraction process was repeated twice more, pouring the supernatant into the bottle so as to generate a total leaf wash sample for each replicate leaf sample. The 10 leaves in each replicate sample were then placed on a sheet of paper, together with a standard sized grid, and scanned to allow assessment of the total leaf area for each replicate. Leaves were discarded at the end of the process.

Results and conclusion

During the exposure period there were guttation droplets observed on the leaves of the potato plants on the treated site.

Immediately after application of 150 g Dantop 50WG/ha to a crop of potato plants, residues on the surface of unprotected leaves were on average 205 ± 12 ng clothianidin/cm^2, 0.8 ± 0.1 ng TZNG /cm^2 and 2.8 ± 0.4 ng TZMU /cm^2. Residues on the surface of protected leaves were on average 217 ± 41 ng clothianidin /cm^2, 0.9 ± 0.2 ng TZNG /cm^2 and 2.5 ± 0.6 ng TZMU /cm^2.

The residues declined rapidly on both protected and unprotected plants (see Table B.9.5.1-1) with a DT_{50} on protected plants of 1.2 days and on unprotected plants of 1.5 days. The DT_{90} on protected plants was 3.9 days and on unprotected plants was 5.1 days.

A single combined soil sample taken from the treated field 1 month after application contained 25 µg clothianidin/kg dry weight, 0.19 µg TZNG/kg dry weight and 0.29 µg TZMU/kg dry weight.

Table B.9.5.1-1: Mean (± SE) clothianidin residues on the surface of potato leaves after application of 150 g Dantop 50% WG/ha

Week	Clothianidin (ng/cm^2) on unprotected potato leaves	Clothianidin (ng/cm^2) on protected potato leaves
0	205 ± 12	217 ± 41
1	6.5 ± 6.2	2.8 ± 0.9
2	0.094 ± 0.025	0.52 ± 0.18
3	0.162 ± 0.044	0.38 ± 0.07
4	0.058 ± 0.033	0.40 ± 0.13
6	0.016 ± 0.006	0.02 ± 0.02
8	0.046 ± 0.004	0.02 ± 0.02

RMS Comments

The first sampling occurred 5h after the application had finished. While the application occurred in the morning and thus could have coincided with the occurrence of guttation, measured residues will reflect the amount of spray residues rather than the clothianidin residues present in guttation fluid. It could be argued that guttation droplets present at the time of application would be contaminated by the sprayed product, and thus the measured residues reflect the concentration in guttation fluid. However, this argument is not valid for granule applications of clothianidin.

Further, clothianidin residues were measured from the total leaf surface of the sampled potato leaves, and expressed as ng/cm². For the risk assessment as proposed by the EFSA Guidance Document on bees, residue values expressed as mg/kg are needed. As there is no information on how the measured values could be transformed to mg/kg, they cannot be used in the risk assessment.

While the study report mentions that guttation droplets were observed on the leaves of the potato plants on the treated site, it is not clearly stated whether the leaves on which residues were measured actually guttated or not. Therefore, the measured residues could potentially underestimate the concentration in guttation fluid.

In conclusion, RMS is of the opinion that the results from the present study are not representative for clothianidin residues in guttation fluid from potato plants, especially not for potato plants treated with clothianidin as granule at sowing. Consequently, these results will not be considered in the risk assessment.

Report:	**IIIA 10.4e/04; Ansaloni, T; 2015**
Title:	**Effects of Clothianidin 0.7 GR in guttation water on bees (*Apis mellifera* L.) colony under field conditions.**
Report No.:	TRC14-038BA
Document No.:	THW-0398
Guidelines:	OEPP/EPPO 170(3)
GLP	Yes (certified laboratory)

Objective
A study was conducted in order to evaluate the effects on honeybee (*Apis melifera* L.) colonies of Clothianidin 0.7 GR (a soil incorporated granular formulation containing clothianidin 0.7% w/w) applied in-furrows at sowing of potato seeds as a result of exposure to the guttation fluid used as a source of water.

The study was located in Biar (control) and Villena (treatment), Valencia (Spain) an area of intensive vegetable-cultivation of East Spain. The interaction between flight and foraging activity of the bees and the presence of guttation on potato plants, the mortality of the bees, and the condition of the colonies (adult worker population, brood population, presence of healthy queen and areas with pollen and nectar storages) were studied.

Material and methods

Test and control item

Test item: Clothianidin 0.7 GR (batch no. 050313/02; purity: 0.6796% w/w (analysed)).

In the treatment field, one application at a nominal rate of 80 g a.s./ha was performed at the time of potato sowing by means of an in-furrow granular insecticide application with a calibrated potato planting machine, simulating normal good agricultural practices (actual applied rate, 83.94 g a.s./ha, based on nominal content).

At the control field, the potato plants were untreated.

Study sites

The study was conducted in two potato fields (one for the treatment with the test product and one for the untreated control) of approximately 1 ha of surface area and approximately 12 km away from each other. The selected fields were located in Biar (control) and Villena (treatment), Valencia (Spain), in a representative potato-growing area of Spain, and were similar in environmental conditions. Potato plants on the experimental fields started to flower on the 30th of May, 18 days after the beginning of the exposure period (≈ 75% of plants emergence) and 17 days before the end of the exposure period.

Closest crops to the treatment field were a small vineyard, cereals that were spray irrigated and some vegetables (i.e. carrot) that were not in bloom. No bees were observed drinking on the irrigation water of the spray irrigated cereals. Guttation of crops or weeds adjacent to the treated field experimental field was not observed. Closest crops to the control field were potatoes which were not treated at sowing with any plant protection product, cereals, olive trees and an abandoned almond orchard with dead trees. Potatoes adjacent to the experimental field were at a later BBCH than the experimental potatoes and flowering when the exposure period of the bees started; nevertheless, no bees were observed on potato flowers during the duration of the exposure period. Guttation was observed on

potato plants adjacent to the control experimental field but no bees were observed drinking from these guttation fluids.

The fields had no adjacent standing water sources (i.e. ponds, streams, ditches etc.). Temporary water sources such as puddles were detected occasionally, and bees were observed drinking from these sources.

Honeybee hives

Twelve healthy, well-fed, queen-right bee colonies provided by a professional beekeeper were used for the study, six for the control and six for the treatment. Colonies were homogeneous in size, physiological status and treatments against *Varroa destructor*. The colonies were prepared with at least 5 brood combs and a number of worker bees between approximately 11000 and 18000.

Bees were foraging on wild food sources until the colonies were moved to the trial site. The hives were placed in the middle of the respective field 30 days after the application of the test product and approximately three days before 75% emergence of the crop. The colonies were exposed in the fields for 38 days, after which period the hives were transferred to a holding apiary for the remaining of the study free to forage in the field.
When needed, the colonies used were provided with supplementary diets.

Assessments

Initial assessments on start of guttation and start of hive activity in the morning were carried out for two days after the placements of the hives in the experimental fields. Bees' activity was monitored by counting the number of bees leaving the hive for 30 seconds every 30 minutes until activity started (at least 1 bee / 30 secs / 2 hives) and then once every hour until guttation ceased. These preliminary assessments were performed to establish the time of highest exposure of the bees to guttation of potato plants (coincidence of guttation with a relatively high flight activity). Based on the results of these preliminary assessments, flight activity at the hives entrance and within the fields was monitored three times between approximately 08:30 and 11:30 on a daily basis. Additional assessments of these parameters were performed at midday and late afternoon every three days.

Fligh activity at hive entrance

Flight activity at hives entrance was assessed by counting the number of bees entering (with and without pollen) and exiting the hive for thirty seconds at each assessment time. Behaviour of the bees at hives entrance was also monitored.

Flight activity within the field and guttation

Flight within the fields was assessed by means of an active transects method, i.e. walking at normal pace across the rows of each field while counting the bees flying over the crop. Fifteen minutes of each flight assessment were spent to monitor the rows immediately adjacent to the hives, within an area comprised between approximately 20 m into the crop and 50 m wide around the hives. The rest of the field was assessed for approximately 30 minutes.

Guttation activity was monitored using the same method and at the same time of within field flight activity.

Mortality of honeybees

Each hive was equipped with a dead bee trap at the entrance to count the number of dead bees expelled from the colonies. To collect dead individuals within the fields, thirteen linen sheets (1.5 x 10 m; area covered 195 m^2 approx.) were placed in each field: one in front of the hives and twelve scattered within the experimental field. Assessments of mortality within the colony (number of individuals in dead bee traps) and within each field (dead individuals on linen sheets) were carried out daily for the duration of the test.

Condition of the colonies
Condition of the colonies (adult worker population, brood population, presence of healthy queen and areas with pollen and nectar storages) was assessed before their placement in the fields and 4 times on a weekly basis between day +10 and day +32 of exposure in the fields. Four additional assessments in the holding apiary were carried out every three weeks until September 2014 and one final assessment was performed in March 2015 after overwintering of the colonies.

Residue sampling
Samples of dead bees (both in the dead bee traps and on the linen sheets) for residue analysis were collected on each assessment day throughout the exposure period.

Samples of guttation fluids were collected on each assessment day when the phenomenon occurred.
Samples of pollen, nectar, wax and unsealed brood were collected from each hive on the days of the first and the last colonies assessment within the experimental fields. Samples were collected both from old frames and new empty frames placed in the hives just before the beginning of the exposure period.
Soil samples were collected just before the application and after the 38-day exposure period.

Residue analysis
The method for sample extraction and determination of clothianidin and its metabolites TZNG and TZMU in guttation fluid, bees, pollen, nectar and brood was based on the multi-residue method QuEChERS. Wax was analysed using an internal laboratory method. For both methods quantification was performed by use of LC-MS/MS detection.

The limit of quantification (LOQ) of both analytical methods was 1 µg/kg for each analyte and each matrix with a limit of detection (LOD) set at 0.3 µg/kg (30 % of the LOQ).

Results
Guttation and flight activity
From the preliminary assessments at the hive entrances it was established that flight activity of the bees started relatively early in the morning, it reached a peak at around 11:30 and started to decrease again at around 12:30. From this preliminary assessment it could be appreciated already that overall activity (especially of bees entering the hives with and without pollen) was higher in the treatment field than in the control field. In general, flight activity at the hives entrance was higher in the treatment fields than that observed in the control fields throughout the exposure period in the experimental field. While activity in the treatment hives was more or less continuous and following a constant pattern throughout the exposure period, activity of the control hives was discontinuous and relatively low until the evaluation at 21 days after the beginning of the exposure period (≈ 75% of crop emergence). Starting on day 22, probably as a consequence of the rainfall occurred in the previous days, and up to the end of exposure period, activity at the entrance of the control hives started to increase and to follow a more regular pattern.

Guttation occurred on 25 days of the exposure period in the control field and on 28 days of the exposure period in the treatment field. When present, maximum level (% of plants with guttation) and intensity (number and dimensions of the droplets in the plants) of guttation were observed early in the morning and gradually decreased in time, with the total disappearance of the phenomenon at approximately midday. No guttation was observed in the afternoon.

Coincidence of guttation with flight observed during the assessments in the area close to the hives occurred in 45 occasions in the control field and in 52 occasions in the treatment field. Coincidence of guttation with flight observed during the assessments in the rest of the fields occurred in 36 occasions in the control field and in 58 occasions in the treatment field.

Overall, 31 and 11 bees were observed resting on the plants adjacent to the hives and 4 and 0 were observed drinking on guttation produced by these plants in the control and the treatment field,

respectively. In the portion of the fields farther from the hives, 85 and 11 bees were observed resting on the plants and 12 and 0 bees were observed drinking on guttation water in the control and the treatment field, respectively. Of the individuals counted in the control field, on a single assessment (second assessment in the morning of the 28th of May 2014, between 9:40 and 10:20) 39 were observed resting on the plants and 10 were observed drinking from guttation water.

Mortality

Mortality of worker bees, drones and immature in the dead bee traps was always relatively low for all the studied colonies and not related to the exposure to guttation in the treatment field. The exception to this was for assessment performed on the days after colony evaluations and one episode of one treatment colony with 75 dead adults (14 June 2014). However, this occurred towards the end of the exposure period and was not reflected by the five other colonies on the treatment field, where mortality ranged from 1 to 9 bees (see Figure B.9.5.1-5 below). Dead bees were recovered on the linen sheets only occasionally and always in relatively low numbers.

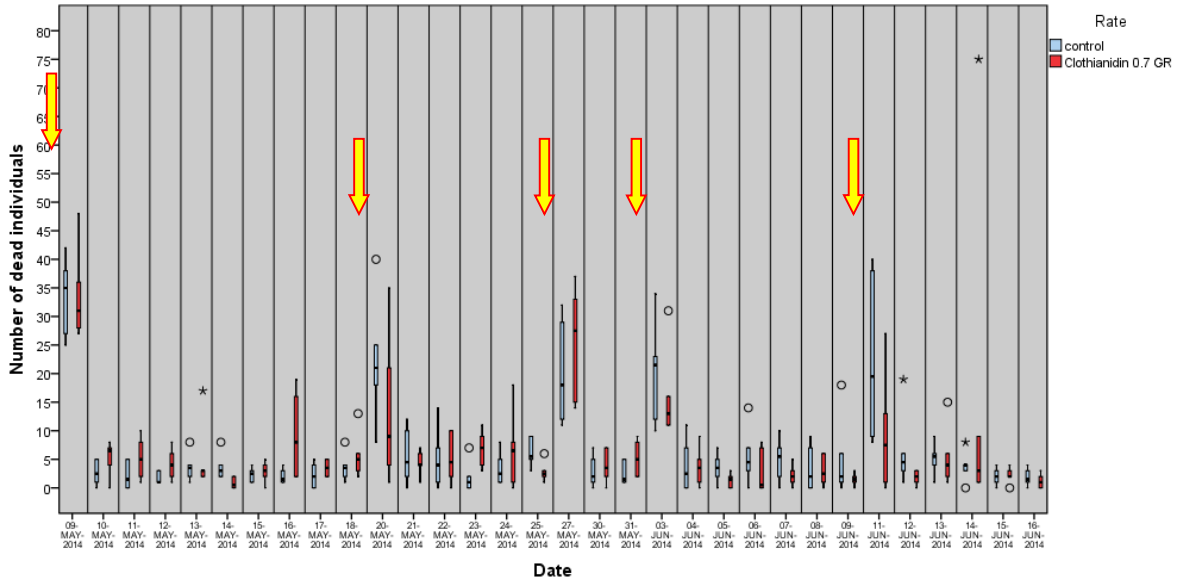


Figure B.9.5.1-5: Mortality of workers in the dead bee traps. ⇨ = Colony evaluation

Condition of the colonies

On average, after a first reduction of nectar cells with respect to the initial colony evaluation during the first two weeks of exposure in the experimental fields, nectar stores started to increase in similar trends in the control and the treated colonies. The mean number of nectar cells was more or less stable at the colony evaluations performed in the holding apiary. No significant differences in variation of nectar cells were observed between the treatment and the control hives at any colony assessment.

Reduction of pollen stores during the first two weeks of exposure in the experimental fields was on average higher in the control hives with respect to the treatment hives. Due to rainfall between the end of May and the beginning of June 2014, the mean number of pollen cells increased between the second and the third colony evaluation in both fields, but while they reached levels that were higher than the initial number in the treatment colonies, they remained at levels well below the initial numbers in the control colonies. Pollen stores decreased abruptly again between the third and the fourth colony evaluation within the experimental fields in both groups of colonies and, after a slight recovery at the first off field evaluation in the holding apiary, it progressively decreased in the following evaluations.

The different trends in brood population (mean number of brood cells) observed in the control and the treatment hives were most probably related to the different trends in pollen stores observed for the two

groups. Brood population remained approximately constant in the treatment hives during the first three weeks of exposure in the field, while it decreased in the control hives. An increase in cells with brood, probably related to the increase in pollen due to rain in the previous evaluation, was observed in the last in-field colony assessments, the variation being significantly higher in the treatment colonies than in the control colonies. Brood population decreased again abruptly starting with the first off-field evaluations in the holding apiary, and it remained relatively low up to the last evaluation performed in September 2014.

Evolution of worker populations followed similar trends in the control colonies and the colonies exposed to the test product, with no significant differences in variation with respect to the mean initial populations at any colony assessment.

The last colony assessment performed after overwintering showed similar trends in brood population, food stores and workers population in the control and the treatment colonies, with no significant differences between the two groups for any of the assessed parameters. At this assessment time, while worker populations was on average similar to that observed on the previous assessment performed in September 2014, brood populations and pollen stores had increased considerably with respect to the previous pre-overwintering assessment. Nectar stores showed a similar decrease in the control and the treatment colonies with respect to the previous evaluation. This decrease was probably due to consumption of the nectar accumulated by the bees' populations waking up after overwintering.

Residue samples

In samples of wax and nectar no residues above the limit of detection (0.3 µg/kg) were detected in any treated or untreated sample. In samples of pollen and brood no residue above the limit of detection (0.3 µg/kg) were detected in any untreated sample and no residue above the limit of quantification (1 µg/kg) were detected in any treated sample.

The highest residues of clothianidin and its metabolites TZNG and TZMU which were detected in untreated samples of guttation fluid were 22 µg/kg, 4 µg/kg and 2 µg/kg respectively. The highest residues of clothianidin and its metabolites TZNG and TZMU which were detected in treated samples of guttation fluid were 2045 µg/kg, 429 µg/kg and 182 µg/kg respectively.

At the beginning of the exposure period there was a clear reduction in residues of clothianidin and its metabolites TZNG and TZMU in guttation fluids between day 34 and day 38 after the application (from an average of 1003.25 µg clothianidin/kg at 34 DAA to an average of 191 µg clothianidin/kg at 38 DAA). Levels in guttation fluids subsequently fluctuated from sample day to sample day with no appreciable pattern up to 54 days after the application. At this assessment time, a further reduction in clothianidin, TZNG and TZMU concentrations in guttation could be seen (to an average of 15.5 µg clothianidin/kg). Subsequently levels of in guttation fluids again fluctuated from sample day to sample day with no appreciable pattern up to the last sample day 69 days after the application.

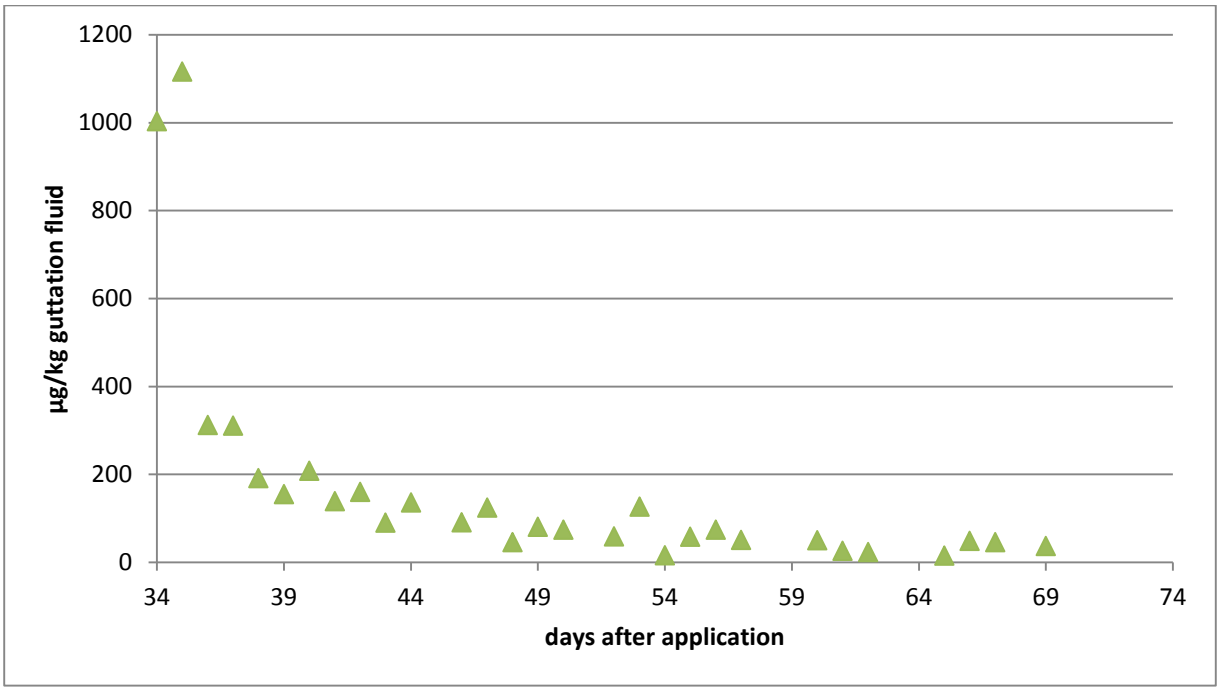


Figure B.9.5.1-6: Mean residues of clothianidin in guttation fluid from treated plants

No direct correlation between bees' mortality and residues detected in the dead bees could be established. The occurrence and concentrations of residues of clothianidin and its metabolites TZNG and TZMU in samples of dead bees collected within the bee traps of the treated field seemed to be sporadic and irregular, and samples of dead bees on the linen sheets that provided sufficient material for analysis always gave residues < 1 µg/kg (LOQ).

The highest residues of clothianidin and its metabolites TZNG and TZMU which were detected in untreated samples of bees were 17 µg/kg, 2 µg/kg and < 1 µg/kg respectively. The highest residues of clothianidin and its metabolites TZNG and TZMU which were detected in treated samples of bees were 69 µg/kg, 8 µg/kg and 35 µg/kg respectively.

Conclusion

Under the conditions of the present study, no correlation between the exposure to guttation fluids of the treated potato plants and mortality within colonies (dead workers, drones or immature within the dead bee traps) and in the fields (dead foragers on the linen sheets) was observed. Evolution of the condition of the colonies was marked by the environment and the meteorological conditions of the experimental setup. No differences in the evolution of worker populations and of nectar stores were observed between the control and the treatment colonies both during the exposure period in the experimental fields and in the holding apiary. A direct correlation between the number of cells with brood and pollen reservoirs was observed: when colonies were capable of finding and accumulating these food sources offspring population increased proportionally; on the other hand, brood production dropped consistently when accumulation of pollen in the hives was reduced. No direct correlation between bees' mortality and residues detected in the dead bees could be established. The occurrence and concentrations of residues of clothianidin and its metabolites TZNG and TZMU in samples of dead bees collected within the bee traps of the treated field seemed to be sporadic and irregular, and samples of dead bees on the linen sheets that allowed analysis always gave residues < 1 µg/kg (LOQ).

RMS comments

The study followed the recommendations of the EFSA Technical report on the bee study protocols submitted by Sumitomo (EFSA Supporting publication 2014:EN-598) and of the EFSA Guidance Document on the risk assessment for bees (Appendix O and U); e.g. use of colonies with a good health status, of uniform size and similar genetic origin. However, in contrast to the recommendations of the EFSA Guidance Document, the field sites were approximately 1 ha in size instead of 2 ha. This is

considered to be a minor deviation and does not influence the validity of the study (a 2 ha field is the size considered for flowering crops to provide sufficient forage and to isolate from other flowering areas. For guttation studies even smaller plot sizes would be appropriate and valid as bees fly only short distances to collect water as due to the high energetic cost of flying, bees will collect water from their immediate vicinity (Joachimsmeier *et al.*[28], 2012)). Further, only 6 pairs of colonies were set-up, which might potentially be too low to achieve sufficient statistical power.

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

B.9.5.2. Exposure

Guttation is a natural phenomenon in which water from xylem fluid is exuded through pores on the leaf during periods when root pressure is high and transpiration is low, e.g. overnight and in the early hours of the morning. There is the potential for the fluid exuded to contain molecules such as systemic pesticides. Exposure from contaminated guttation water is therefore considered a potentially relevant route of exposure for honeybees, bumblebees and solitary bees.

The applicant submitted studies performed in maize and potatoes on the effects on colony survival due to exposure to guttation water. In these studies, the guttation frequency of the crop, the honeybee activity in the guttating crop and the residues present in guttation fluid were assessed. Maize and potatoes are sown in spring, and hence bees could bee exposed to guttation fluid in spring and early summer. At all test locations and for each crop guttation was observed. Table B.9.5.2-1 shows a summary of the frequency to which guttation was observed, the extent of bee exposure and the levels of residues encountered in guttation fluid.

Table B.9.5.2-1: Crop guttation frequency, exposure of honeybees to guttation and measured residues in guttation fluid for the available studies.

Crop	Crop Guttation frequency	Guttation coincides with bee flight	%Bees collecting guttation fluid in crop	Residues in guttation fluid (treated crop) (mg/kg)	Reference
Maize	Control: 93% Treatment: 93%	Yes	Control: 4.7% Treatment: 2.7%	CTD: 0.19 – 9.1 TZNG: 0.0028 – 0.114 TZMU: 0.0043 – 0.181	IIIA 10.4e/01 Thompson, 2011a
Potato*	Control: 86% Treatment:100%	Yes	Control: 0% Treatment: 0%	CTD: 0.026 – 1.317 TZNG: 0.008 – 0.053 TZMU: <LOQ – 0.032	IIIA 10.4e/02 Thompson, 2013a
Potato	Control: 65.8% Treatment: 73.7	Yes	Control: 13.8 % Treatment: 0%	CTD: 0.015 – 1.117 TZNG: 0.002 – 0.198 TZMU: <LOQ – 0.090	IIIA 10.4e/04 Ansaloni. 2015

*Notes: CTD = Clothianidin, TZNG and TZMU are metabolites of clothianidin; *clothianidin was applied through spray application instead of granular application; LOD = 0.3 µg/kg; LOQ = 1 µg/kg*

In maize, guttation was a fairly common event, which was observed in both treated and untreated crops on 28 of the 30 observation days. Most observations of guttation fluid occurrence were early in the morning, and guttation fluid had dried by 12:00 on most days. The activity of the bees was low between 06:00 and 08:00 and then rose to a peak from 09:30 onwards. While the overlap was limited, bees were thus observed to be active when guttation fluid was present. The number of bees observed to collect guttation fluid was low: 2 out of 43 bees (4.7%) in the control field and 2 out of 75 (2.7%) in the treated field.

[28] Joachimsmeier, I.; Pistorius, J.; Heimbach, U.; Schenke, D.; P.; Kirchner, W. (2012). Water collection by honey-bees – How far will foragers fly to use water resources like guttation drops? A first distance trial using cereals and oil seed rape. Hazards of pesticides to bees: 11th International Symposium of the ICP-BR Bee Protection Group; Wageningen, (The Netherlands), November 2 - 4, 2011.

Residue levels of clothianidin in guttation fluid produced by maize showed high initial levels with a mean of 9.1 mg/kg on the day of emergence. Afterwards, the clothianidin residue decreases to 0.53 mg/kg 29 days after emergence, with a minimum measured value of 0.19 mg/kg measured in the period in between. The metabolites TZNG and TZMU were also present but at significantly lower levels. These findings are in line with a number of other guttation studies in maize, in which a similar rapid decrease of clothianidin residues in guttation fluid was found (for details, see the EFSA Conclusion on the risk assessment for bees for clothianidin, 2013[29]).

Two studies on guttation are available for potatoes: one in which clothianidin was applied through spray application (Thompson, 2013a) and one in which clothianidin was applied as granule in furrow at sowing (Ansaloni, 2015). As in maize, guttation was a common event, which was frequently observed. In the study by Thompson (2013a), guttation was observed on all of the 7 observation days spread over the 2 week post-application period on the treated field and on 6 of the 7 observation days on the control field. In the study by Ansaloni (2015), guttation was observed on 25 days out of the 38 days exposure period in the control field and on 28 days in the treated field. In both studies, the maximum intensity of guttation was observed early in the morning and gradually decreased over time, with total disappearance of the phenomenon at approximately midday. The activity of the bees started around 7:30 and decreased around 14:00 in the study by Thompson (2013a). In the study by Ansaloni (2015), bee activity started early in the morning as well, reached a peak around 11:30 and started to decrease again around 12:30. As for maize, bees are thus active when guttation fluid in potatoes is present. Thompson (2013a) observed only low numbers of bees on the potato plants (8 at the treated site and 5 on the control site over the 14-day exposure period). The majority appeared to be resting on the leaves, and none were observed drinking. Ansaloni (2015) observed 116 bees at the control field and only 22 at the treated field, of which 16 (13.8%) and 0 (0%) were drinking guttation water, respectively.

Residue levels of clothianidin in guttation fluid produced by potatoes showed initial levels of on average 1.317 mg/kg (Thompson, 2013a) and 1.117 mg/kg (Ansaloni, 2015), which are lower compared to those measured in maize. As for maize, the clothianidin residues declined relatively rapidly with time. For example, after four days, the residues measured by Ansaloni (2015) had already decreased to 0.191 mg/kg. In the study by Thompson (2013a), clothianidin residues decreased to 0.036 mg/kg 10 days after application. The metabolites TZNG and TZMU were also detected in both studies but at significantly lower levels.

In the study by Thompson (2013a), clothianidin was applied to the potato plants as a spray application. Due to the application pattern of the test material, the residues in the guttation fluid collected directly after application may have included residues from the surface of the leaves. It can therefore be questioned if this study is representative for residues in guttation fluid after application of clothianidin as a granule treatment. However, the residues measured by Thompson (2013a) are comparable with those measured by Ansaloni (2015), who applied clothianidin as an in furrow granular application at sowing. Further, as the occurrence of guttation and the behaviour of the bees was also similar between both studies, it is considered that the results from Thompson (2013a) are also relevant for granular applications of clothianidin.

In conclusion, guttation is a fairly common phenomenon in both maize and potatoes and bees were observed to consume guttation water in both crops (although in relatively small numbers). Consequently, consumption of contaminated guttation fluid is a considered a relevant route of exposure for bees. Therefore, a risk assessment will be performed for both the use in maize and in potatoes.

It is noted that no data is available for the exposure to bees to guttation water in sorghum. As a consequence, no risk assessment for the use in sorghum could be performed.

[29] European Food Safety Authority (2013). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin. EFSA Journal 2013;11(1):3066. doi:10.2903/j.efsa.2013.3066

It is noted that for the indoor use of clothianidin in maize and sweet maize (which is restricted to permanent greenhouses), exposure to bees from guttation water from maize can be considered low. Therefore, in line with the decision of Pesticides Peer Review Meeting 129, no risk assessment needs to be performed for this indoor use.

B.9.5.3. Risk assessment

B.9.5.3.1. Risk assessment for honeybees

The risk assessment was performed following the risk assessment sequence as proposed in the EFSA Guidance Document on bees. The first tier calculations of this assessment scheme are based on several worst-case assumptions, e.g. it is assumed that guttation fluid contains the active substance at a proportion of the water solubility. Further, it is unknown to what extent honeybees collect and consume guttation water, incorporate it into brood food and feed it to larvae. Therefore, the initial tiers of the scheme are precautionary and hence are likely to result in many failures and the need for higher tier studies. As measured values of clothianidin in guttation water are available, the first tier calculations were not performed, and the assessment started with a second tier, in which the measured residue values were used.

It is noted that no data was submitted by the applicant for the use in sorghum. Consequently, no risk assessment could be performed for that use.

It is noted that for the indoor use of clothianidin in maize and sweet maize (which is restricted to permanent greenhouses), exposure to bees from guttation water from maize can be considered low. Therefore, in line with the decision of Pesticides Peer Review Meeting 129, no risk assessment needs to be performed for this indoor use.

Tier 2 risk assessment based on measured residues

The ETR values for adult and larvae consuming guttation water are calculated based on the equations listed below. According to the EFSA Guidance Document, it is considered not necessary to include contact exposure, because the calculations for oral exposure are based on worst-case assumptions and will identify highly bee-toxic substances for higher tier assessments. In higher tier studies, bees will be exposed by oral uptake and contact exposure.

The *ETR value for acute adult oral exposure* is calculated as follows:

$$ETR_{acute\ adult} = \frac{W \times PEC}{LD_{50}}$$

Where: W = the water uptake of adult bees (11.4 µL/bee per day)
PEC = concentration in the guttation water in µg/µL
LD_{50} = oral LD_{50} in µg per adult bee.

If this ETR > 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 *ETR for chronic adult exposure* is calculated by the following equation:

$$ETR_{chronic\ adult} = \frac{W \times PEC}{LDD_{50}}$$

Where: W = the water uptake of adult bees (11.4 µL/bee per day)

PEC = concentration in the guttation water in µg/µL
LDD_{50} = oral LDD_{50} in µg/bee per day based on and exposure period of 10 days.

If this ETR > 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.

The *ETR for larvae* is calculated by the following equation:

$$ETR_{chronic\ larvae} = \frac{W \times PEC}{NOED}$$

Where: W = the water uptake of larvae (111 µL for larvae, consumed over 5 days)
PEC = concentration in the guttation water in µg/µL
NOED, in µg/bee, is based on an exposure period of five days.

If this ETR > 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.

According to the EFSA Guidance Document, an ETR for effects on the development of the hypopharyngeal glands (HPG) should also be calculated. As there is currently no validated methodology for the assessment of sublethal effects, no endpoint for the effects on the hypopharyngeal glands of honeybees is available for clothianidin. Therefore, the first tier risk assessment for honeybees based on HPG was not performed.

The PEC values used in the ETR calculations are derived from field studies in maize and potatoes submitted by the applicant. It is noted that only one study is available for maize and two for potatoes, which, according to the EFSA Guidance Document, is not sufficient to obtain reliable residue values representative for the complete area of use. For maize, some data is available from studies with maize seed treated with clothianidin (see EFSA Conclusion on the risk assessment for bees for clothianidin, 2013[30]). Measured residues in these studies are generally in line with those measured in the study submitted for granular uses (Thompson, 2011a), supporting the use of measured residues from Thompson (2011a). For potatoes, no other studies are available, and the residue values from the submitted uses are used for the time being.

For the acute risk assessment, the maximum initial measured residue value in guttation water will be used as PEC. In both the studies in maize and potatoes, there was a relatively rapid decline of the clothianidin residues in guttation water with time. The EFSA Guidance Document suggests that in the case of an exponential decline, a time-weighted-average concentration can be used in the chronic risk assessment. As the NOED for adult bees and larvae is based on an exposure period of 10 and 5 days, respectively, the mean residue values measured over the first 10 and 5 days will be used as PEC in the chronic risk assessment. Table B.9.5.3.1-1 shows the different residue values used in the risk assessment for both maize and potatoes. For potatoes, the highest values from the two available studies will be used.

During Peer Review, it was argued that there was not sufficient consideration of whether exposure represents a 90th percentile situation (see comment 5(26) in the Reporting Table). During Pesticides Peer Review Meeting 145 it was agreed that the available dataset is not sufficient for selecting the 90th percentile of exposure as suggested by the EFSA Guidance Document for bees. It was however noted that for guttation it might be more relevant to have a study in worst case environmental conditions that may maximize this phenomenon. As this seems to be the case for the available studies, it was agreed that the residue values obtained from these studies can be used in the risk assessment. However,

[30] European Food Safety Authority (2013). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin. EFSA Journal 2013;11(1):3066. doi:10.2903/j.efsa.2013.3066.

maximum residue values should be used instead of 90th percentile values (at least for the acute risk assessment).

During Peer Review, it was also argued out that the use of mean residue values in the chronic adult and larval assessments is not in line with the EFSA Guidance Document. However, according to the EFSA Guidance Document, initial (maximum) PEC values should be used for chronic assessment, unless it is scientifically justified to use the TWA PEC. It was noted that a rapid decline of clothianidin residues in guttation fluid was observed in the available studies. Moreover, it was pointed out that decline of the active substance in guttation fluid is also taken into account in the Tier 1 calculations for guttation exposure proposed by the EFSA Guidance Document (i.e. Tier 1 PEC for acute risk is based on 100% of water solubility of the active substance, where for chronic risk to adult honeybees and honeybee larvae 54% and 72% of water solubility is considered to determine the PEC). Therefore, it was considered justified to use a TWA active substance concentration in guttation for the chronic assessment. Overall, the experts considered the approach followed by RMS acceptable (i.e. use of maximum residues values in the acute assessment and the TWA concentration over 5 and 10 days in the chronic assessment for larvae and adults, respectively).

Table B.9.5.3.1-1 Maximum and mean concentration of clothianidin (mg/L) measured in guttation fluid from maize and potatoes.

Crop	Residues of clothianidin (mg/L)			Reference
	Maximum	Mean over first 5 days	Mean over first 10 days	
Maize	**9.109**	**4.943**	**3.446**	IIIA 10.4e/01 Thompson, 2011a
Potatoes	**1.317**	**0.917**	**0.391**	IIIA 10.4e/02 Thompson, 2013a
Potatoes	1.117	0.587	0.389	IIIA 10.4e/04 Ansaloni. 2015

Note: To be in line with the units used in the ETR calculations, residue values were transformed from mg/kg to mg/L. As no information on the volumetric mass density of guttation fluid is available from the study reports, it is assumed that guttation fluid has te same density of water (ρ = 1000 kg/m^3); Values in bold are used in the risk assessment

The calculated ETR values for both the use in maize and potatoes are shown in Table B.9.5.3.1-2. The relevant toxicity endpoints are taken from Table B.9.1.3.1-3.

Table B.9.5.3.1-2: Tier 2 ETR calculations for acute adult oral, chronic adult oral and larval exposure through the consumption of clothianidin contaminated guttation water in maize and potatoes.

Acute adult oral exposure					
Crop	W (µL/bee/day)	PEC (µg/µL)	$LD_{50,oral}$ (µg a.s/bee)	ETR	Trigger
Maize	11.4	0.009109	0.00379	27.4	0.2
Potatoes	11.4	0.001317	0.00379	4.0	0.2
Chronic adult exposure					
Crop	W (µL/bee/day)	PEC (µg/µL)	LDD_{50} (µg a.s/bee/ day)	ETR	Trigger
Maize	11.4	0.004943	0.00138	40.8	0.03
Potatoes	11.4	0.000917	0.00138	7.6	0.03
Larval exposure					
Crop	W (µL/bee/day)	PEC (µg/µL)	NOED (µg a.s./larva /development period)	ETR	Trigger
Maize	111	0.003446	0.00528	72.4	0.2
Potatoes	111	0.000391	0.00528	8.2	0.2

For both the use in maize and potatoes, the ETR values largely exceed the relevant trigger, due to the relatively high clothianidin residues measured in the first weeks after emergence. Consequently, a potential risk is identified for all honeybee developmental stages and for all uses. Further consideration is thus necessary.

Higher tier risk assessment based on field studies

Further refinements to the risk assessment could be based on field effect studies. Three studies on the effects on colony survival due to exposure to guttation water were submitted by the applicant. These studies cover the maximum application rate for clothianidin (CTD) used as granular treatment in maize (50 g a.s./ha) and in potatoes (70 g a.s./ha). Therefore, the available studies are considered representative for the currently registered uses. Table B.9.5.3.1-3 provides an overview of the different guttation studies available.

In one study in potatoes (Thompson, 2013a), clothianidin was applied to the potato plants as a spray application. Due to the application pattern of the test material, the residues in the guttation fluid collected directly after application may have included residues from the surface of the leaves. It can therefore be questioned if this study is representative for residues in guttation fluid after application of clothianidin as a granule treatment. However, the residues measured by Thompson (2013a) are comparable with those measured by Ansaloni (2015), who applied clothianidin as an in furrow granular application at sowing. Furthermore, the occurrence of guttation and the behaviour of the bees was also similar between both studies. It is therefore considered that the results from Thompson (2013a) are also relevant for granular applications of clothianidin.

Table B.9.5.3.1-3: Overview of the available field studies that address the risk to honeybees of exposure to guttation

Crop	Test item(s)	Treatments	No. sites	Colonies/ site	Colony exposure	Guttation period	Reference
Maize	Granular treatment (in-furrow during sowing): Santana	CTD 110 g/ha Control	1 1	6 6	Guttation from BBCH 11 (30 days)	Spring/ summer 2009	IIIA 10.4e/01 Thompson, 2011a
Potatoes	Foliar application: Dantop 50 WG	CTD 75 g/ha Control	1 1	6 6	Guttation from BBCH 39 to 66 (14 days)	Spring/ summer 2012	IIIA 10.4e/02 Thompson, 2013a
Potatoes	Granular treatment (in-furrow during planting): Clothianidin 0.7 GR	CTD 80 g/ha Control	1 1	6 6	Guttation from emergence (38 days)	Spring/ summer 2014	IIIA 10.4e/04 Ansaloni. 2015

Notes: CTD = Clothianidin

The study in **maize** (Thompson, 2011a) was performed in southern France in 2009, with the formulated product Santana. One clothianidin treated and one untreated maize field were studied. At both the control and treated field, 6 colonies were exposed to guttation in maize crops. The colonies experienced exposure for 30 consecutive days with guttation for 28 days. There were no other significant sources of water available in and around the test fields, except for puddles after rain. There was an overlap between bee activity (bees leaving the hives) and the presence of guttation water on the crop, but only a small number of bees was observed on the crop with only very few actually drinking guttation water (for details: see Section B.9.5.2). Initial levels of residues in guttation fluid were high (9.1 mg/kg on the day after emergence), but declined relatively rapidly. The colony assessments indicated that there was no impact of the treatment on colony development (levels of brood, pollen and nectar and number of bees). The level of dead bees both in the dead bee traps at the hive entrance and on linen sheets in the field was low throughout the exposure period, with no significant treatment-related differences (although the treated colonies showed consistently slightly higher levels). While no residues of clothianidin were detected in dead bee samples from the control, residues with a mean value of 6.0 µg/kg and 90th percentile of 12.2 µg/kg were found in dead bees from the treated field. However, no correlation was found between the numbers of dead bees collected and the residue levels found, either across the whole exposure period or in terms of individual peaks.

The study was also evaluated by EFSA (see the EFSA Conclusion on the risk assessment for bees for clothianidin 2013[31]). EFSA did not consider this study useful to address the risk from guttation, since it was not excluded that the residues detected in dead bees were not linked to exposure via guttation. However, RMS considers that there is enough evidence from the study that exposure to guttation did not lead to an increased bee mortality, based on the fact that no correlation was found between the residue level and the number of dead bees. It is also highly probable that these residues could be the result of incidental exposure in the treated maize crop. This study is thus considered suitable for risk assessment purposes.

In the EFSA Conclusion on the risk assessment for bees for clothianidin (2013), it stated that granular formulations give the same level of residues in guttation droplets as seed treatment products, but with indications of delay, based on guttation experiments performed in Germany on maize. As a consequence, EFSA took guttation experiments with treated maize seeds into account in their

[31] European Food Safety Authority (2013). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin. EFSA Journal 2013;11(1):3066. doi:10.2903/j.efsa.2013.3066

assessment for granular applications. Based on these studies, the EFSA Conclusion (2013) states that under the experimental circumstances that the guttation studies were performed, the risk from exposure via guttation was considered low for maize. However, since guttation is a phenomenon that is dependent on crop and environmental conditions, further information is needed to extrapolate this outcome to other EU agricultural situations for the uses on maize. Compared to the evaluation by EFSA in 2013, no new studies were submitted by the applicant. Therefore, this conclusion was supported by RMS.

During Peer Review, the applicant provided the following argumentation to demonstrate that no further data is needed for maize (see comment 5(27) in the Reporting Table) (*text in italic*):

In the study provided for guttation in maize, honey bee colonies experienced worst-case exposure to young maize plants for 30 consecutive days with guttation on 28 days as well as additional exposure from a 2nd sowing. While guttation is a phenomenon that is dependent on crop and environmental conditions the actual risk to bees depends on whether they use the guttation fluid as a source of drinking water. The available study on maize clearly demonstrates that they do not to any significant extent and this is consistent with the studies on potatoes. While the source of drinking water may also depend on environmental conditions, the conditions for guttation require sufficient levels of soil moisture that indicate other sources would also be available. Thus, the available study on maize represents a realistic worst-case assessment of the risk to bees from guttation fluid and no further data is necessary.

At Pesticides Peer Review Meeting 145, it was agreed that as no new data were submitted that triggered the re-assessment of the available study in maize, the previous conclusion on the risk from guttation for maize is still valid.

The two studies on **potatoes** were performed either in the UK (Thompson, 2013a) and in Spain (Ansaloni, 2015). In these studies, six colonies were placed in each of the four fields (two treated and two untreated). Consequently, a total of 24 colonies were exposed to guttation in potatoes (12 treated and 12 untreated). While the number of sites is limited, they are well spread over Europe (north and south). Therefore, they are considered to provide a good indication of the potential influence of guttation water from treated crops on honeybee colonies.

Guttation was observed in nearly all of the 7 observation days in the study by Thompson (2013a) and on 25 to 28 of the 38 observation days in the study by Ansaloni (2015). There was an overlap between bee activity (bees leaving the hives) and the presence of guttation water on the crop, but only a small number of bees was observed on the crop with only very few actually drinking guttation water (for details: see Section B.9.5.2). When temporary puddles were present closed to the hives, bees readily foraged on these water sources. Analysis of the residues in the guttation fluid collected from potato leaves on the treated plot showed that the residues declined rapidly. For example in the study by Thompson (2013a), a peak of 1317 µg clothianidin/kg was measured on day 1, which declined to 36 µg clothianidin/kg by day 10.

In the study by Thompson (2013a), only low levels of mortality were observed in the colonies on the control site although there was an increase after the colony assessment on day 6 (mean 79 bees). Mortality in colonies on the treated field was slightly higher in several colonies on day 1 (mean 28 bees) and increased on day 7 (mean 36 bees), again after a colony assessment on day 6. There were no adverse effects of treatment on the numbers of bees or levels of brood, pollen and nectar in the colonies on the treated site compared to the control site. Similar results were found by Ansaloni (2015). The use of guttation water produced by potato plants by the bees occurred irregularly, with no real pattern in behaviour of the bees in relation to its availability. There was no correlation between the exposure to guttation fluids of the treated potato plants and mortality within colonies (dead workers, drones or immature within the dead bee traps) and in the fields (dead foragers on the linen sheets) and development of the colonies was determined by other environmental and meteorological factors.

As no data was available for **sorghum** during the assessment by EFSA in 2013, a data gap was identified for this use. However, no studies performed in sorghum were submitted by the applicant. Therefore, this data gap remains.

During Peer Review the applicant argued that it is not necessary to generate guttation data for every crop e.g. in this case with sorghum. They consider that it should be possible to extrapolate between similar crop types. In this case sorghum is a relatively unattractive crop to honey bees that only produces pollen (no nectar). Thus, they consider that the available studies with maize and potatoes, which are similar in this respect, are sufficient for the assessment of the risk from guttation fluid for this crop. RMS would like to point out that in the assessment for the risk through guttation water as proposed by the applicant upon submission, no reference was made to the use in sorghum. Further, no argumentation to demonstrate that the submitted studies also cover the use in sorghum was provided during the drafting phase of this Addendum. Whether or not the results from the available studies in maize and potato could be extrapolated to other crops such as sorghum was discussed at Pesticides Peer Review Meeting 145 (see below for the outcome of this discussion).

When following the risk assessment scheme for exposure from guttation water as suggested by the EFSA Guidance Document on bees, an unacceptable acute and chronic risk is found for both maize and potatoes, even with calculations based on measured clothianidin residues at tier two. Although the measured concentrations of clothianidin in guttation fluid are high enough to theoretically pose an unacceptable risk to bees, acute and chronic colony level effects were not observed in the available field studies. There are a few reasons that could potentially explain the lack of any observed effect. First of all, guttation water is not highly attractive to bees and has virtually no energetic value (Goatley and Lewis, 1966[32]). Second, potatoes are not attractive to bees and do not provide a food source for the colony. Consequently, bees do not visit the crops in large numbers. While maize is attractive for pollen, only low number of bees were found in the maize crop, probably as the studies were performed at the pre-flowering stage. Third, water collected for use by the colony can come from a variety of sources located close to the colony and not just from guttation fluid. Fourth, as the plant grows the frequency of guttation events declines. Similarly, insecticide concentrations in guttation fluid tend to decline during spring. As shown in the available studies, the initial high residue levels tend to rapidly decline in the first weeks after emergence. Overall, the exposure of honeybee colonies to clothianidin present in guttation fluid from maize and potatoes seems to be limited.

At **Pesticides Peer Review Meeting 145**, it was noted that the study by Thompson (2013a) in potato was also considered by EFSA in 2015 for the evaluation of the risk to bees for foliar spray uses of clothianidin (see EFSA, 2015[33]). Following that evaluation, this study was not considered suitable for risk assessment by EFSA, as it does not comply with the recommendations of the EFSA Guidance Document for bees. Besides this study, only one other study is available (Ansaloni, 2015). It was argued that one single study might be not sufficiently informative and representative of the worst-case. The geographical representativeness of the study by Ansaloni (2015) was also considered low (only one study location in Spain cannot be considered sufficiently representative for both Southern and Northern Europe). Furthermore it was noted that the environmental conditions in the study location (Spain) were not likely to represent the worst case (water saturation in soil and high humidity did not occur). However, it was noted that the study conditions might be a worst case for other water sources (the water demand for the honeybee colony is likely to be higher in Southern Europe). Further, the statistical power of the study was also questioned.

[32] Goatley JL & Lewis RW (1966) Composition of guttation fluid from rye, wheat and barley seedlings. *Plant physiology* 41:373-375. Available online at http://www.plantphysiol.org/content/41/3/373.full.pdf+html

[33] European Food Safety Authority (2015). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin considering all uses other than seed treatments and granules. EFSA Journal 2015; 13(8):4210. doi:10.2903/j.efsa.2015.4210

Regarding the extrapolation of the available data to sorghum, it was argued that ther are substantial differences in potato and maize with regard to guttation, and that the same can be expected for sorghum. Therefore, it was concluded that further data are needed to make this extrapolation.

For drawing a conclusion, the available dataset was considered as a whole. In this discussion, both the available studies for seed treatment in cereals and sugar beet (Bayer Crop Science, see Section B.9.5.1 of the Addendum for the Bayer Crop Science data) and for granular use in potato and maize (Sumitomo, see Section B.9.5.1 of this Addendum) were considered together. This is considered justified as in the EFSA Conclusion for seed treatment and granular uses of clothianidin (2013)[34], a similar conclusion regarding the risk from guttation exposure was drawn for both seed treatment and granular uses, based on the fact that in the available studies granular formulations gave the same level of residues in guttation droplets as seed treatment products (but with indications of a delay).

The experts agreed that the available data set is generally not sufficient to draw a firm conclusion on the non-relevance of guttation as route of exposure. Concerns were expressed as to whether the available data are sufficient to address the specific protection goals (SPG). Extrapolation to other crops would also need a larger dataset. In general, even if for some crops a good dataset is available further data are needed to draw a firm conclusion. Some experts noted that there is evidence that bees are not primary collecting water from guttation fluids. The most relevant guttation plant (worst case) is maize, in which the residues are high. However, generally this route of exposure should be further investigated, because the current evidences are not sufficiently informative.

Generally, the experts considered guttation as not the primary route of exposure for bees, even if cannot fully excluded (i.e. evidence from cereals and maize data). Even if acute effects could not be excluded, the long term risk is likely to be low.

As a general line of evidence the experts noted that bees using guttation are only rarely observed. This consideration is based not only on the available data in the confirmatory data package (for both imidacloprid and clothianidin), but also on other data available at the MS level for other dossiers or literature.

It was noted that the results from the studies on maize and potato are generally in line with the results of other available studies (e.g. those reported in the EFSA Conclusion from 2013): guttation occurred but no clear effect was reported in the studies. However the statistical power was not assessed.

Taking into account all the evidences discussed during the meeting, the experts identified uncertainties driven by the lack of clear pieces of evidence (i.e., the adequacy of the dataset to address the SPG, lack of evidence demonstrating the low relevance of this route of exposure across Europe). Overall the majority of the experts considered that the risk for just the uses under evaluation can be considered low on the basis of the available data. As data is available for only the use in maize and potato, this conclusion is only valid for these uses. The minority of the experts considered that more information is needed to draw a firm conclusion (i.e., on whether the power of the available effects assessment is sufficient to conclude no effect and there is uncertainty around the exposure assessment).

Conclusions:

Overall, the acute and chronic risk to honeybee colony development and survival, resulting from exposure to residues of clothianidin in guttation fluid produced by potato and maize plants treated with clothianidin granules at the currently registered maximum application rates, is considered acceptable.

[34] European Food Safety Authority (2013). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin. EFSA Journal 2013;11(1):3066. doi:10.2903/j.efsa.2013.3066.

As no data was available for sorghum during the assessment by EFSA in 2013, a data gap was identified for this use. However, no studies performed in sorghum were submitted by the applicant. Therefore, this data gap remains.

B.9.5.3.2. Risk assessment for bumblebees and solitary bees

According to the EFSA Guidance Document on the risk assessment for bees, all bees need water for their metabolism. However, at the moment, it is not possible to quantify the level of exposure to guttation water for non-*Apis* bees. Honeybees use water to cool the colony or to dilute stored honey, and are therefore characterised by a very high level of water fluxes at the colony level. Non-*Apis* bees obtain most of their water requirements from nectar, and thus need less water from other sources. As the water fluxes for honeybees are much higher compared to non-*Apis* bees, the EFSA Guidance Document considers that the risk assessment performed for honeybees should be sufficiently protective for bumblebees and solitary bees. Therefore, no specific risk assessment for the risk to bumblebees and solitary bees from exposure to guttation water is considered necessary.

B.9.6. THE POTENTIAL EXPOSURE TO DUST DRIFT FOLLOWING DRILL AND THE ACUTE AND LONG-TERM RISK TO COLONY SURVIVAL AND DEVELOPMENT, AND THE RISK TO BEE BROOD RESULTING FROM SUCH EXPOSURE

B.9.6.1. Studies

No studies on dust drift were submitted by the applicant, based on the EFSA Conclusion on the risk assessment for bees for clothianidin (2013) [35].

B.9.6.2. Risk assessment

In the EFSA Conclusion on the risk assessment for bees for clothianidin (2013), a low risk was concluded for dust expsosure for granular formulations authorized for use in maize and sorghum, assuming that there is no air-flow in the application machinery when granules are applied in the furrow. Assuming that the same application technology is used in potatoes, RMS considers that the same conclusion can be drawn for the authorized use in potatoes.

During Peer Review, some Member States did not agree with the assumption of the EFSA Conclusion (2013), and argued that the risk assessment for dust drift cannot be considered finalised (see comment 5(32) in the Reporting Table). This issue was also discussed at Pesticides Peer Review Meeting 145. It was noted that there is evidence from some Member States showing that some contaminated dust will be emitted during the application of some granular products. The dust deposition seems to be highly related to the composition of the granules, the crop and the specialised machinery. Therefore, it was suggested that until clear information is provided with regard to the transplanting/sowing machinery to be used it should not be speculated that the exposure through dust drift is not relevant for granule applications.

It was noted that this was not the conclusion drawn in EFSA (2013) where a low risk was concluded, provided that no airflow sowing machinery are used. It was noted that the occurrence of dust drift may be substance-specific and that the Heubach value alone was not necessarily sufficient to exclude the occurrence of dust drift. However, the data referred to above have not been peer-reviewed because they were not available to the meeting and were not submitted within the confirmatory dataset. However, the issue will be reflected in the EFSA conclusion.

[35] European Food Safety Authority (2013). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin. EFSA Journal 2013;11(1):3066. doi:10.2903/j.efsa.2013.3066

B.9.7. THE ACUTE AND LONG TERM RISK TO COLONY SURVIVAL AND DEVELOPMENT AND THE RISK TO BEE BROOD FOR HONEYBEES FROM INGESTION OF CONTAMINATED NECTAR AND POLLEN

B.9.7.1. Studies

The applicant submitted a three year study in maize in which both the exposure to clothianidin residues in pollen and the effect on bee colonies were investigated. As after analysis by EFSA of this study, a number of concerns were identified, an additional analysis of the data from this study was performed by the applicant. Further, an exposure study that measured clothianidin residues in potato pollen after granular treatment was submitted.

Report:	**IIIA 10.4g/01, Thompson, H. (2011b)**
Title:	**Santana: Evaluation of potential long-term effects to honeybee colonies in France of corn grown following in-furrow application of Santana (a.s. clothianidin, 1% w/w)**
Report No.:	S3UL1000
Document No.:	THW-0280
Guidelines:	OEPP/EPPO 170; CEB 230 Deviations: not applicable
GLP	Yes (certified laboratory)
NOTE	This study has previously been submitted to EFSA (2012) and was included in their Peer Review Report on clothianidin (2012)[36]. It has not been evaluated by the RMS (Belgium).

Objective

The study was designed to determine the potential long-term effects of Santana (a soil incorporated granular formulation containing the neonicotinoid compound clothianidin 1% w/w) applied in-furrow at sowing of maize seeds on honeybee (*Apis mellifera* L.) colonies in the field. Maize may be a pollen source for honeybees. The study was conducted over 3 consecutive years using the same experimental design so far as was practical with the same colonies on the same field. During the exposure phase, the colonies were monitored at the maize fields with assessment of immediate post-exposure effects (e.g. mortality, foraging activity and behaviour). In addition, during a subsequent monitoring phase the colonies were maintained at a remote site, without extensive agricultural crops attractive to bees, where attention was paid to bee health, colony condition and development, as well as colony overwintering. Furthermore, the residue levels of clothianidin to which honeybees were exposed by foraging on maize during the exposure phase and by foraging in follow-on crops (oilseed rape and sunflowers in year 3 grown in fields which had been treated in years 1 and 2) were determined.

Material and methods

The study was conducted as field monitoring (open field) under local practical conditions in representative corn-growing areas in France. Therefore the study comprises three trials which were carried out in northern, central and southern France. This study was conducted in accordance with the guideline of the European and Mediterranean Plant Protection Organization No. 170 (3) (OEPP/EPPO, 2001) but adapted for use with systemic compounds expressed in pollen and nectar. In addition honeybee behaviour observations during flowering were undertaken as outlined in CEB 230 (2007).

[36] European Food Safety Authority (2012). Peer Review Report on clothianidin. (key background document to the EFSA Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin (2013), containing the study evaluation notes, the report of the scientific consultation with Member State experts and the comments received on the draft EFSA conclusion)

Test and control item
Test item: the soil incorporated granular formulation Santana (CAGR8; 1% w/w clothianidin granules (GR); purity: 2008 – 08GR027 EQ1, 0.9597 ± 0.0494% w/w (analysed); 2009 and 2010 – 09-GR014, 1.053 ± 0.002% w/w (analysed)).

The test item was applied by simulating application of the granules in-furrow during the mechanical sowing of maize seeds using varieties typical for the trial region. The drilling and application were made with calibrated equipment at an applied rate of 110 g a.s./ha. So that an extended flowering period could be achieved across the whole field approximately half of each field was drilled on two dates approximately 2 weeks apart.

At the control field, untreated maize seeds were sown. Further, the control field had not been treated with neonicotinoid insecticides (including seed treatments) in at least the last preceding cropping season.

Study sites
The study was conducted in 3 locations in France (North – FR01; Centre – FR02; South – FR03). The two different fields (test item and control (untreated)) in each area were separated by at least 3km to avoid bees foraging on the other field. The fields were selected so as not be close to crops flowering at the same time, which would be attractive to bees. The location of the control field at the central site was moved in 2009 (and remained the same in 2010) as the neighbouring fields to the control field used in 2008 were planted with sunflowers in 2009. The fields had not been treated with neonicotinoid insecticides (including seed treatments) in at least the preceding cropping season.

Honeybee hives
Six normally developed queen-right bee colonies were used per treatment and control site (i.e. a total of 36 colonies at all three locations). The colonies were typical of those used in each region and as similar to one another as possible. The hives contained approximately 10,000 to 20,000 bees in 2008. In 2008 one colony on the treated field at the central site lost the queen during the transfer off the colony to the site and the brood was observed to decline over the exposure period. The queen was replaced at the end of the exposure period and the impact on a single colony was not considered to have had a significant impact on the study.

In 2008 the colonies were placed in a location within the field at the start of flowering and orientated towards the flowering crop. Due to the timing of the flowering in some cases the colonies were placed on the fields (defined as set-up of colonies) shortly before or at the start of flowering rather than when a significant proportion of the field was flowering as defined in the study plan, but this was not considered to have had a significant impact on the study. In 2009 and 2010 where necessary due to over-winter losses colonies were replaced or re-queened prior to or after moving to the site where they were placed in a location within the field and orientated towards the flowering crop a few days before the start of flowering.

Effect assessment
The effect assessment took place in two phases - an exposure phase during flowering of the maize with direct exposure of bees to the crop (BBCH 59-61 to BBCH 67-69) and a monitoring phase (at the end of corn flowering BBCH 67-69, through over-wintering to the start of the new bee season, i.e. March the following year). As the exposure phase was designed to encompass two flowering periods (staggered drilling of each half of the field to result in prolonged flowering) the bees were exposed to flowering over an extended period and observations and residue sampling were designed to take this into account.

The exposure phase started when colonies were moved to the test sites (at the start of flowering in 2008 and just before the start of flowering in 2009 and 2010. At or soon after the end of flowering, the colonies were moved to a different location for post-exposure monitoring and overwintering (= start of the monitoring phase). Both control and treatment colonies were taken to the same overwintering site

in each area, but orientated to minimize drifting and robbing between treatment and control colonies. The overwintering sites were selected so as to minimize exposure to pesticides. The normal beekeeping operations outside the exposure period were performed by the beekeeper as appropriate as this was considered to more closely represent local beekeeping practices.

Exposure phase

Mortality at the bee hives and in the field

During the flowering period mortality at the hives was recorded by positioning dead bee traps on the hives and water-permeable cotton sheets were placed in front of the hive. Mortality in the field was recorded at three places distributed over the flowering area of the field by removing the crop prior to the set-up of the hives and spreading water-permeable cotton sheets between rows in approx. 8m lengths (70 cm wide) on which any dead bees found were counted.

The dead bee sheets were moved from the 1st to the 2nd flowering area of each field as the second area came into flower. The observations of mortality at the hives and in the control and test item fields were carried out daily throughout the flowering period.

Flight intensity and behaviour of the bees in the field

Observations of flight intensity in the field, which started on the day the bee hives were set-up at the test fields, took place along five marked transects (30 flowering plants) regularly distributed over the test fields as well as the control field. The transects were moved from the 1st to the 2nd flowering area of each field as the second area came into flower. Three times per day, at approximately the same time based on initial observations of foraging activity at the hive, but taking account prevailing conditions, the number of bees that are either foraging on flowering maize or flying over the crop (single, total value) were counted per transect.

In addition, on each occasion sublethal and behavioural effects on the bees were recorded using criteria based on the behavioural categories given in CEB 230 (2007). Additional assessments of the number of bees returning to the hive with pollen were undertaken to provide supporting information on the activity of bees foraging for pollen.

In 2009 the method of monitoring activity in the crop was altered to increase the representativeness of the data given the low numbers of foraging bees. The number of bees flying in the crop or foraging on the flowering maize plants were monitored during a 20 minutes period (2 observers each monitoring for 10 minutes in different parts of the crop) whilst walking continuously through the crop. An assessment was also made of the number of maize plants which were in flower along the transect. As in 2008, three assessments were undertaken per day. The same assessment methods were used in 2010.

Conditions of the colonies, development of the bee brood

The condition of all colonies was recorded and the development of the bee brood was checked. In 2008 this occurred up to 4 days before they were moved to the test sites or within 6 hours of being moved onto the test sites and then every 5-7 days until the end of flowering. In 2009 and 2010 this occurred after they were moved to the test sites but before the start of flowering and then every 5-7 days until the end of flowering.

The following parameters were assessed:

- Weight of each colony
- Strength of each colony (number of combs covered with bees)
- Presence of a healthy queen (presence of eggs, presence of queen cells)
- Visual assessment of the pollen storage area and area with nectar (in %)
- Visual assessment of the area containing eggs, larvae and capped cells (in %).

Samples of at least 100 bees were taken frm all colonies up to 4 days before and 6hrs after they were moved to the test sites for assessment of *Nosema* sp., *Acarapisi woodi* and viruses. *Varroa* assessments were undertaken by placing a sticky sheet on the floor of each colony and counting the *Varroa* mite fall.

Residue sampling
Samples for residue analysis were taken from all colonies during the exposure phase at the peak of maize flowering for the two halves of each field. In 2008 the following samples were collected: pollen was collected by placing a pollen trap under each on colony for a period long enough to collect at least 200 mg pollen per colony; forager bees with pollen loads from each colony (minimum 200 bees per sample); pollen collected from flowering maize plants within the crop. In 2009 and 2010 the following samples were collected: pollen was collected from pollen traps under each on colony (at least 300 mg pollen per colony); maize pollen loads (identified by yellow colour) from forager bees from each colony (minimum 20 bees per sample); pollen collected from flowering maize plants within the crop.

Monitoring phase
Conditions of the colonies, development of the bee brood
The condition of all colonies was recorded and the development of the bee brood was checked every 3-4 weeks until the end of the bee season (early November) and was assessed again at the end of the over-wintering phase (March).

The following parameters were assessed:
- weight of each colony
- strength of the colony (number of combs covered with bees)
- presence of a healthy queen (presence of eggs, presence of queen cells)
- visual assessment of the pollen storage area and area with nectar (in %)
- visual assessment of the area containing eggs, larvae and capped cells (in %)

Visual inspections of the colonies were undertaken for signs of bee disease (*Nosem*a sp., *Acarapis woodi*, viruses, American foulbrood and European foulbrood) every 3-4 weeks until the end of the bee season (early November) and assessed at the end of the over-wintering phase (March). *Varroa* assessments were undertaken by placing a sticky sheet on the floor of each colony and counting the *Varroa* mite fall. In addition, samples of bees were taken from all colonies at the end of the bee season (approx. end October) and again at the end of the overwintering phase (March) for assessment of *Nosema* sp., *Acarapis woodi* and viruses.

Exposure assessment
Samples of bee and plant matrices for residue analysis of clothianidin and its metabolites (TZMU and TZNG) were collected under semi-field (worst-case) conditions in order to assess honeybee exposure. Honeybee colonies were confined to the flowering maize crop and samples collected for residue analysis to determine the maximum level of exposure. A single large tunnel, comprising two tunnels placed end to end (each of approx. 100 m^2 floor area) were erected on each test site before flowering and positioned so that one tunnel was in each of the two halves of each field according to sowing date. There was a single tunnel on each control site and 3 replicate tunnels on each treatment site and a honeybee colony was placed in each one. The two constituent tunnels of each large tunnel were continuous (open at their adjacent ends) so that the bees had uninterrupted access to the flowering maize in both halves of the field, thus maximising their exposure. No behavioural or mortality assessments were conducted in the tunnels. The colonies were fed sucrose throughout the flowering period of the maize, to encourage them to collect pollen from the maize crop, and as required during the post-exposure monitoring phase.

Maize
In 2008 the sampling schedule included the following matrices for each tunnel with the time points distributed evenly (approximately) over the flowering of the two halves of the crop:
- forager bees with pollen loads from each colony, 4x during exposure phase (2x during each flowering period)

- pollen and wax as a combined sample from the combs inside the colony (1x before set-up of the hives on the field sites, 4x during exposure phase (2x during each flowering period) and every 3 weeks after end of exposure phase up to end of season
- pollen collected by placing a pollen trap onto each on colony for a period long enough to collect at least 200 mg pollen per colony, 4x during exposure phase (2x during each flowering period)
- pollen collected from flowering maize plants within the crop, 1x for each half of the tunnel
- soil, 1x from 3 days up to start of flowering of test crop, single sample for each half of the tunnel taken at right angles across the planting row
- 4 plants (top 20 cm), 1 x during flowering period (two from each half of the tunnel).

In 2009 the number of samples of pollen was reduced based on experience in 2008 and the following samples were collected:

- pollen and wax as a separate samples from the combs inside the colony (1x before set-up of the hives on the field sites; 4x during exposure phase (2x during each flowering period) and every 3 weeks after end of exposure phase up to end of season)
- pollen collected by placing a pollen trap onto each on colony for a period long enough to collect at least 300 mg pollen per colony, 2x during exposure phase (1x during each flowering period)
- soil, as for 2008
- plants, as for 2008

In addition a single pollen sample was collected from flowering maize plants within the tunnels (single sample from the control plot, combined samples from all three tunnels on the treated plot), 1x for each half of the tunnel.

"Follow-on crops"

In 2010 the tunnels at the site in the South (FR03) were sown with maize as in 2008 and 2009 but a change was made to the crops grown in the tunnels on the sites in the North (FR01) and Centre (FR02) to determine the transfer of residues in soil to "follow-on" crops. The crops were used to determine whether residues of clothianidin and its metabolites TZNG and TZMU remaining in the soil from the previous cropping season (2009) could be detected in the pollen and nectar collected from the untreated crops by honeybee colonies placed within the tunnels. In the tunnels in the North (FR01) spring oilseed rape (variety: Olindigo) and in the Centre (FR02) sunflower (variety: Heliogras) were sown at normal drilling rates, without any treatment, so as to ensure flowering of the crop at approximately the same time as maize drilled on the same fields. The area to be covered by the tunnels was sown with the "follow-on" crops so as to ensure minimum cross-contamination by the treatment applied to the maize on the remainder of the field by placing a 10m unsown barrier around the tunnels. These areas were treated with herbicide or cut to ensure flowering weeds were not present at the time bees were placed in the fields. The area used by the tunnels was minimised by placing them as close together on the treated site as possible. At site FR02/T there was significant pest damage and only one tunnel could be erected over the emerging sunflowers, which were also sporadic in distribution.

Residue samples were taken from the tunnels in which the maize was sown (FR03) as in 2009. Pollen, nectar and wax samples were taken from combs inside the colonies in the tunnels at FR01 (oilseed rape) and FR02 (sunflowers) both during the exposure phase and up to the end of the season (as well as pollen from traps on each colony during the exposure phase). It was not possible to collect nectar or pollen samples from the oilseed rape crop in the control and treated tunnels in the north or from the sunflowers on the treated plot in the centre, as intended, but they were taken from the control sunflowers.

<u>Soil sampling</u>

Soil samples were taken from each tunnel in 2008-2010 and from the main field area at each site (control and treated) during the exposure phase in 2010. Six samples were taken from each half of the tunnel just prior to flowering by sampling a 5 x 5cm area to 10 cm depth from within the planted row

between the plants and the six samples combined to provide a single sample (for each half of the tunnel). In tunnels in which maize was planted (site FR03 only in 2010) the two plots were sampled separately (i.e. 6 samples per plot). In addition, 20 samples were taken from the main fields in a W pattern for each plot in the main fields using the same procedure and combined to produce a single sample for each field. In 2010 additional soil sampling for residues were undertaken from each treated field (but not from control fields) after cultivation but prior to planting at each site. Samples were taken using a soil auger to the depth of the plough layer on each field (about x cm). Twenty soil cores were taken in a W pattern across the main part of the field (avoiding the tunnel area if this had already been sown with the follow-on crop at the time of sampling) and combined to a single sample for each field.

Results

Exposure phase

Mortality in front of the bee hives and in the field

2008: Mortality was monitored in the dead bee traps and in front of the hives daily in most cases. The mortality assessments at the hive showed no adverse effects of the treatment at any of the sites. At all sites the daily mortality did not exceed a mean of 19 bees per day on any single day. There were a few breaks in the mortality data due to the timing of the dead bee collections or due to the gap in flowering between the two sowings. However, the data is presented as the daily average for the period when daily monitoring did not occur i.e. all dead bees were collected, and so this had no impact on the study.

2009: Again, mortality was monitored in the dead bee traps and in front of the hives daily. The mortality assessments at the hive showed no adverse effects of the treatment at any of the sites. At all sites the daily mortality did not exceed a mean of 26 bees per day on any single day except on isolated occasions when robbing of hives occurred following assessments or feeding. Hornets were observed taking dead and live bees from outside the colonies at sites in the south and centre but counts of dead bees from the dead bees' traps suggests that this did not have a significant impact on the numbers of dead bees observed. Individual dead bees were found on sheets within the field at one control site (central) and one treated site (southern).

2010: The mortality assessments at the hive showed no adverse effects of the treatment at any of the sites. At all sites the daily mortality did not exceed a mean of 22 bees per day. Hornets were observed taking dead and live bees from outside the colonies at sites in the south and centre but counts of dead bees from the dead bee traps suggests that this did not have a significant impact on the numbers of dead bees observed. No dead bees were found on sheets within the field.

Flight Intensity and Behaviour of the Bees in the Field

Initial data was collected in the first few days at the start of flowering to determine when the bees were foraging most actively. This was used to determine the most appropriate times for data collection on foraging activity and behaviour within the crop in each year. However, weather was also a major determinant of the foraging activity and subsequent timings were modified as appropriate, e.g. waiting until after rain showers.

2008: There were low numbers of bees foraging on both the treated and untreated crop in the northern and central sites during the flowering period. This was also true for the southern site during the first days of flowering but foraging increased during the latter stages of the flowering period. Due to the low foraging activity an additional measure of foraging was recorded. The numbers of bees returning to the hives with pollen (all types) was also recorded and this showed that the bees were returning the hive with pollen. This together with information on the estimated percentage maize composition of forager pollen loads collected confirms that the bees were active on the maize fields.

2009 and 2010: The change in method of foraging assessment in 2009 and continued for 2010 (see materials and methods) resulted in significantly more bees observed on the flowering maize (Figure B.9.7.1-1 shows the results for 2010 as a representative year). The numbers of bees returning to the hives with pollen (all types) was also recorded (results for 2010 shown in Figure B.9.7.1-2) together

with the percentage maize pollen which allowed calculation of the number returning with maize pollen over the exposure period (results for 2010 shown in Figure B.9.7.1-3).

There was considerable variation in the numbers recorded (between fields, sites and years) although in general relatively lower numbers of foragers were recorded on the control fields. However, in some cases even where foraging numbers were low, the numbers of foragers returning with pollen loads and the % maize pollen of these shows that bees were actively foraging on the trial field (given the isolation from other fields of flowering maize). In other cases, the low numbers of foragers observed is confirmed by a corresponding low number of bees returning with pollen loads and the low maize composition in them. This reflects the inherently wide variation in foraging behaviour between sites and between bees returning to the different hives within a site. However, these data together confirm that the bees were in general active on the maize fields and particularly on all the treated fields.

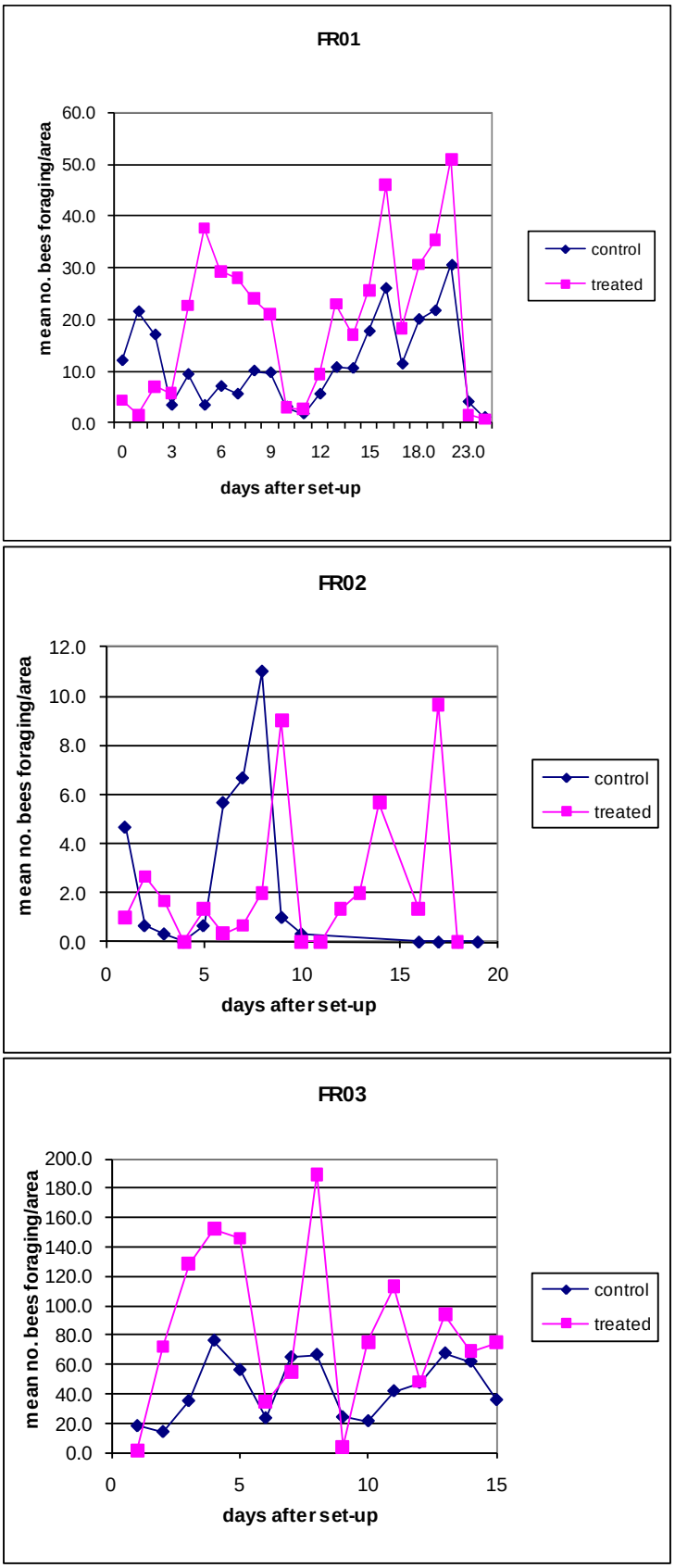


Figure B.9.7.1-1: Mean number of bees foraging on the crop during the observation period (20 minutes on each of 3 transects) in 2010

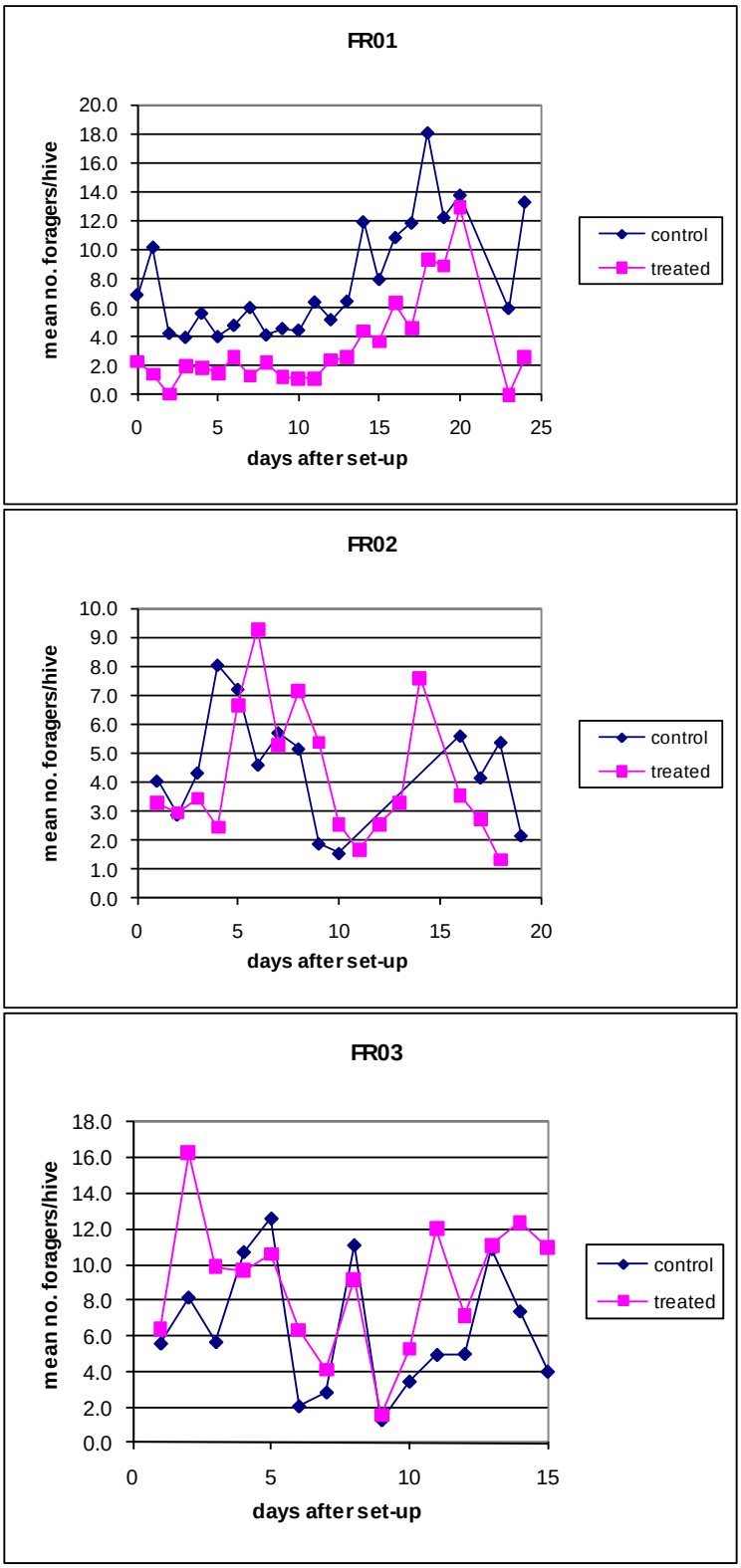
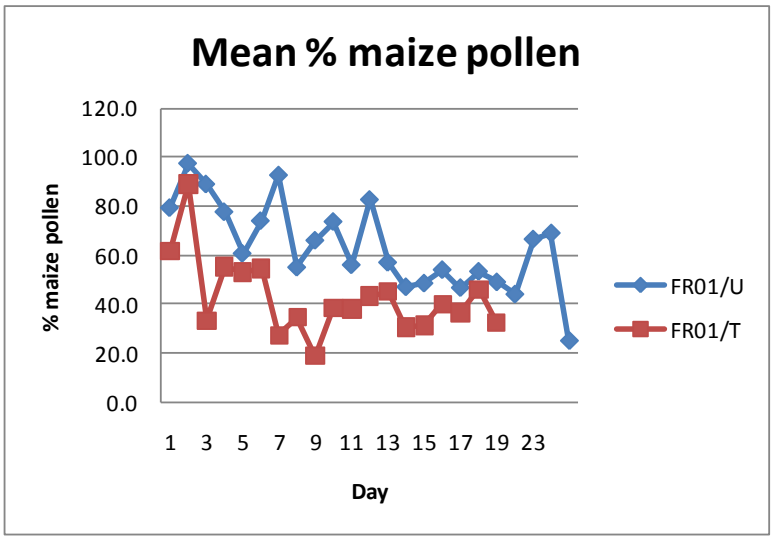
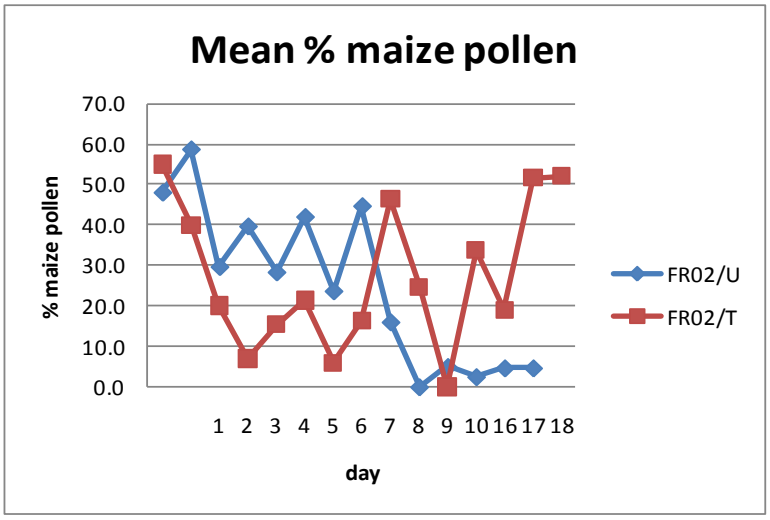


Figure B.9.7.1-2: Mean numbers returning to the hive with pollen over 30 seconds (all types) in 2010





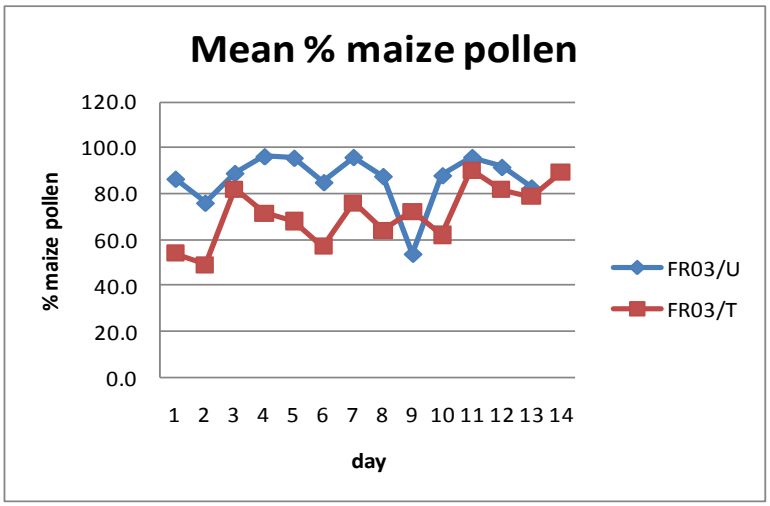


Figure B.9.7.1-3: Estimated maize composition (%) of pollen loads in 2010

Residue analysis

In 2008, due to problems in isolating pollen from the samples collected from plants (due to the presence of anthers in the samples) samples from the tunnels and main crop were combined for each time point, i.e. pollen collected from plants flowering in the 1st and 2nd sowing were kept separate. In 2009 this technical problem was overcome and data are available for both pollen from the main field plants and those from the tunnels. Collected plant samples were not analysed as the crop pollen residue data demonstrated the presence of the active ingredient and no supporting data were required.

A summary of the residue data for the three years (with the three study sites mixed), in the main field and tunnels, are shown in Table B.9.7.1-1. The data show a residue decline from maize pollen (mean 7.7 µg/kg), pollen trap in tunnel where bees are confined to maize (mean 6.5 µg/kg) to pollen trap in field (2 µg/kg). The lower figure in the field compared to in the tunnel could be explained by the relatively low attractiveness of the maize field. Residues of the metabolites TZNG and TZMU in the main field and tunnel samples were generally at or below the LOQ except in the case of the soil, reflecting the much higher parent levels although they were about an order of magnitude lower.

Analysis of pollen and wax from the hives in the tunnels showed no detectable residues of clothiandin, TZNG or TZMU in wax in all years. Residues in pollen sampled from hives declined to below detectable levels within 6-7 weeks after the start of the exposure phase in all years. Residue levels showed no evidence of accumulation over the three years of the study with mean levels in pollen (collected by the bees and brought to the hive) being consistently < 10 µg/kg or less.

Table B.9.7.1-1: Summary of measured residue data of clothianidin (µg/kg) in pollen and soil (mixed for the three sites)

Sample	Year	min	max	mean	se	median	90th percentile	n
Pollen from maize crop (main field and tunnels)	2008	4	10	7.5	1.1	8.0	10.0	6
	2009	1	12	5.4	0.9	4.5	8.9	12
	2010	5	20	11.1	1.9	10.0	17.2	8
	All years	1	20	7.7	0.9	6.5	13.5	26
Main field								
Pollen trap	2008	0.3	6	0.74	0.2	0.6	1.0	36
	2009	0.3	7	2.2	0.3	1.5	5.0	36
	2010	0.3	11	3.1	0.6	1.0	8.0	36
	All years	0.3	11	2.0	0.2	0.6	6.0	108
Pollen from foragers	2008	0.3	9	0.9	0.3	0.3	1.0	31
	2009	0.3	8	3.0	0.4	2.0	7.0	36
	2010	0.3	14	6.2	0.7	7.0	11.0	32
	All years	0.3	14	3.4	0.4	2.0	9.0	99
Soil	2010	20	318	162	48	161	288	6
Tunnels								
Pollen trap	2008	3	15	7.2	0.8	5.0	12.9	32
	2009	0.3	9	4.8	0.7	5.5	8.3	18
	2010	4	11	8.0	1.2	8.0	10.6	5
	All years	0.3	15	6.5	0.5	6.0	12.0	55
Foragers	2008	2	14	5.6	0.8	4.5	9.9	22
Soil	2008	71	373	184	22	165	279	18
	2009	3	184	35	14	6.5	141	18
	2010	6	193	47	19	16	100	10
	All years	3	373	96.0	15	71	265	46

Notes: LOD = 0.3 µg/kg, LOQ = 1 µg/kg. For calculation, values <LOD were assigned a value of 0.3 µg/kg and value <LOQ were assigned a value of 0.6 µg/kg.

Soil residue levels in 2009 showed a high level of variability, which probably reflects the high variability due to the localised granule application (in-furrow) and limitations of the sampling regime. The additional soil samples taken in 2010 before the maize crop was sown and the clothianidin applied for the third year, indicate very little carry over of residues in the soil from the previous two seasons, with levels of 20 µg/kg or less. This is relatively very low compared to the levels seen after application and unlikely to be of significance in the context of clothianidin residues expressed in plant tissues, particularly the pollen. This is reflected in the tunnels in which the follow-on crops were grown in 2010 where no residues greater than 1 µg/kg were detected in any samples of pollen or nectar returned to the hives.

Condition of the Colonies, Development of the Bee Brood

2008: The strength of the colonies during and after the exposure phase was assessed by data collected for the number of bees present in the colonies, brood strength and food stores (pollen and nectar). There were no obvious differences in the development of the colonies between the treated and control sites during the exposure phase or in the monitoring phase up to the over-wintering period. Following over-wintering (i.e. at the start of the 2009 season) a number of untreated and treated colonies died out. There was no difference in the level of colony failures between the treated and untreated groups and where this did occur it can be attributed to failing queens or related to high *Varroa* levels in all cases. For comparison the losses reported by the beekeepers in the same areas were:

Site	**Study losses**	**Beekeeper losses**
FR01 (north)	25% dead*, 6% failing queen	12% to 15% dead
FR02 (centre)	25% dead	Approx 20% dead
FR03 (south)	17% queenless	36% death colonies 6% queen less

*Identified by beekeeper as due to failing queen

2009: The strength of the colonies during and after the exposure phase was assessed as in 2008. There were no obvious differences in the development of the colonies between the treated and control sites during the exposure phase or in the monitoring phase up to the over-wintering period. High *Varroa* levels and brood damaged by *Varroa* were observed before over-wintering particularly in the southern site FR03. All colonies from the treated site had failed after the over-wintering period (i.e. by March 2010) and although only one of the control colonies had failed at this time, three of the remaining five colonies were extremely weak (<5000) bees and continued survival was marginal. The data show much higher levels of *Varroa* in 2009 throughout the exposure and monitoring phase compared to the previous year. A field test performed in 2010 was indicative of amitraz resistance in the varroa mites present which is in agreement with the high levels of varroa and brood damage observed in late 2009 as being responsible for the colony losses.

2010: The strength of the colonies during and after the exposure phase was assessed as in the previous years. There were no obvious differences in the development of the colonies between the treated and control sites during the exposure phase or in the monitoring phase up to the over-wintering period. High *varroa* levels and brood damaged by *varroa* were observed before over-wintering particularly in the central site FR02 where all but one of the treated colonies was lost and many of the control colonies were either lost or severely weakened.

Conclusions

In 2008, counts of honeybees foraging on the maize were generally low although it was higher at the southern site on both the treated and control areas, particularly during the flowering on the second sown plot. This reflects the relatively low attraction of the maize crop for bees and the large areas (>3ha) of the test sites (resulting in a low foraging density). However, exposure to the maize crop at each site was confirmed by counting the numbers of foragers returning to the hive with pollen together with information on the estimated percentage maize composition of the forager pollen loads collected. There were no apparent treatment-related effects on the honeybee colonies at the end of the first year.

In 2009 and 2010 the method for assessing foraging activity was modified and the results reflect this with significantly more bees being observed on the flowering maize with consistent foraging on both the control and treated fields. However, there was still variability in the recorded levels of foraging activity and this reflects to some extent the relatively low attraction of maize to bees and the large areas of maize on the test plots, resulting in a low density. Activity may also have been variable within each test plot reflecting the changing pattern of pollen shedding throughout the maize crop over time. Exposure to the maize crop at each site was again confirmed by counting the numbers of foragers returning to the hive with pollen together with estimated number/percentage of foragers returning with maize pollen loads. It is therefore important to consider all aspects of bee activity to assess the exposure levels.

Despite this clear pattern of exposure on the treated fields there were again no apparent treatment-related effects on the honeybee colonies at the end of the second and third years. At the beginning of the following season, after the overwintering period, some losses were seen in the colonies from the treated and control plots at all three test sites. However, in 2009 and 2010 these losses could be ascribed to failing queens or poor *Varroa* control and were similar to those experienced by the local beekeepers.

In all three years, residue analysis of samples taken from the crop, pollen loads and colonies confirmed exposure of the bees to the treatment in both the main field and tunnels. Residue levels in the pollen load and pollen trap samples from the tunnels were similar to those in the samples of pollen taken from the plants in the tunnels and main field. Samples of pollen from the main field pollen traps and pollen loads were lower than those from the tunnels or samples of pollen collected from the crop suggesting the bees were also foraging on non-maize food sources (resulting in some dilution of the residue levels). This was confirmed by visual assessment of the proportion of maize pollen being returned to the hive, particularly in the northern sites, confirming maize is not a highly attractive source of pollen for bees.

For the tunnels in which maize was grown no samples of wax from the hives in the tunnels contained residues. Pollen taken from the hives in the tunnels on untreated fields contained no residues but residues were detected after exposure of the colonies in the tunnels on treated fields at the central and southern sites in 2008 (there was very little pollen in the tunnel colonies at the northern site) and on all sites in 2009 and 2010. Residues were detected on day -1 at the southern site in 2009 but this is thought to be due to the presence of some maize flowering when the colonies were placed in the tunnels on day -1 (the tunnels may have been slightly more advanced in flowering than the main field). In all colonies from the treated field the residues in pollen declined to below levels of detection in 6-9 weeks after the start of exposure.

In summary although low level exposure within the hives could be demonstrated, samples of pollen and wax taken from the colonies showed that exposure was transient (no residues were detected in pollen 1 month after the end of the exposure period and no significant residues were detected in wax). Low levels of residues were detected in the soil in the year after application (before the next application) but these did not accumulate over the course of the 3 year study and no residues were detected in sunflower or oilseed rape follow-on crops. There were no detectable effects of exposure to clothianidin residues in maize pollen on the colony development in the 3 sites over the 3 years with the greatest impact on colony survival being *Varroa* infestations in the southern and central sites.

RMS Comments

This study has previously been evaluated by EFSA (see Study 24 in the Study Evaluation Notes supporting the EFSA Conclusion on the risk assessment for bees for clothianidin, 2013). The comments by EFSA on this study are the following:

This study (and other similar studies) shows a high level of weakness and failure which renders questionable the possibility to assess long-term effects with several overwintering phases. There is no experience with this study design. Independently to the test item (treated maize), the results indicate a

high concern with regard to the colony health and long-term survival. Care should be taken with the interpretation of these results. There is no background experience of the normal health status. Further analysis would be useful before using this study in the regulatory risk assessment.

In response to this, the applicant performed a further analysis of the available data. For details on this additional analysis, see the summary of Study 10.4g/03 (Lewis, 2014) below.

At Pesticides Peer Review Meeting 145, the study by Thompson (2011b) and the re-analysis by Lewis (2014) were discussed. Overall, it was agreed that the re-analysis provided for the study is not sufficient to address the concerns already identified in the conclusion of EFSA (2013) (i.e., the Thompson study cannot be considered sufficient to draw a firm conclusion on the cause-effect relationship). For details on this discussion, see the summary of Study 10.4g/03 (Lewis, 2014) below.

Report:	**IIIA 10.4g/03, Lewis, G. (2014)**
Title:	**Review of Long-Term (3-year) Honeybee Field Study With Santana (Clothianidin 1% w/w GR) in France**
Report No.:	THW- 0382
Document No.:	THW- 0382
Guidelines:	Not applicable
GLP	Not applicable

Objective
EFSA identified a data gap in order to be able to address the risk to honeybees for clothianidin granule treatments, including the assessment of long-term risk to colony survival and development. The EFSA clothianidin peer review considered that the long-term study in maize by Thompson (2011b) showed several deficiencies, which made questionable the possibility to assess long-term effects taking into account several overwintering periods. These deficiencies are not clearly specified rather there is a general reference made to a high concern with regard to the colony health and long-term survival. However, the EFSA review points out that these issues were independent of the test items i.e. they are concerned with the test methodology (it is stated that there is no experience with this study design).

It should be noted that, the above study was conducted in response to specific regulatory concerns from national authorities with regards to overwintering survival and possible accumulation of residues over successive years. The study was thus designed in consultation with these national authorities according to the recommendation for such non-standard higher tier studies. Indeed, the EFSA neonicotinoid review indicated that the study was well designed and reported results for several factors that may affect bee colonies. It was also noted that the studies were considered useful at Member State level to demonstrate a low acute and long-term risk to honeybees.

Overall, it was considered that on the basis of the available information, it was not possible to draw a firm conclusion. Due to the lack of background information as regards what is the normal colony survival rate under the conditions of the multi-year studies, further analysis of the available data would be needed in order to address the risk to honeybees. A data gap was therefore identified. These general issues (e.g. background disease levels, 'normal' colony survival rates and what constitutes a 'significant' effect) apply to the interpretation of all higher tier studies. In such cases, the primary comparison is normally with the controls in order to see if there are treatment effects (although additional information was provided about the level of colony losses experienced by the beekeepers involved in the studies i.e. representative for the trial areas). It is the applicant' view that this is normally considered sufficient for such studies and beyond this any data gap is a generic one i.e. it relates to the design and interpretation of higher tier studies in general. Nevertheless, the applicant will try to address this data gap in the context of this specific confirmatory data submission with further analysis of the available data.

The purpose of this study was to analyse previously collected data from a three year field study (2008-2010) Fera Study Number S3UL1000 which was run in France, at 3 sites over 3 consecutive years. The data to be analysed was restricted to the control data (6 colonies per site). The analysis aimed to determine the statistical power to detect effect on individual colony development**.**

Material and methods

The study set out to examine the power to detect changes in the mean of a number of indicators of colony status, including; colony weight, colony strength, pollen storage area and area with, the area containing eggs, larvae and capped cells. The assessment was consistent with the general approach for the assessment of statistical power described in Appendix O of the EFSA Guidance document on the risk assessment of plant protection products on bees (*Apis mellifera*, Bombus spp. and solitary bees (EFSA 2013), with the addition of a between-year effect.

Further work was also carried out in order to investigate the power of the experiment run in study S3UL1000 to detect changes in colony health. In order to do this, it was important to first define "colony health" as well as a "percentage reduction". Three levels of change were selected; 7% taken from the EFSA Guidance document (based on the Khoury model), 21% (based on an assessment of the BEEHAVE model presented at the 2014 ICPPR conference in Ghent) and finally 35% (selected as another reference level). The measures of colony health were the number of frames of bees, the number of frames of brood and the amount of nectar (used for the initial evaluation as well). These were assessed at the final observation during each season (in March, post-wintering) and, in all cases, was done on surviving colonies. It was then necessary to assess the variability of the data for those measurements. Because the objective of this work was to assess a proportional change on surviving colonies, the data were analysed after being log-transformed in order to test for an additive change.

A mixed model was used, using the treatment as a fixed effect and years and sites as random effects. The variability between colonies was used as the bottom stratum variability. Having measured these levels of variability, data were then simulated to represent a drop of 7, 21 and 35% in the response variables of interest for the treated groups, given the levels of variability (at the site, year and colony levels) observed in the collected data. A thousand such simulations were used and, with each of these three response variables, the proportion of them where the treatment effect was found to be negative (drop) and significant at the 5% significance level was the estimated power of the experiment.

An additional review on long-term colony performance was conducted using publicly available scientific papers.

Results

Number of frames of bees

When fitting the model described above to the data on number of frames of bees, the year-to-year variability (σ^2_{Year}) was estimated as 0.009, whilst the site-to-site variability (σ^2_{Site}) was estimated at 0.019 and the residual variability (σ^2_{Colony}) was estimated at 0.247. These results show that the largest variability in this set of data was found at the colony level rather than at the site or year level. The simulations were run using a year-to-year variability of 0.009, a site-to-site variability of 0.02 and a residual variability of 0.25. Based on these estimates of variability, the power of the existing experiment to detect a 7% drop in the number of frames of bees was found to be very low (20%).

Number of frames of brood

The same approach was followed for the number of frames of brood. The year-to-year variability was estimated as 0.027, whilst the site-to-site variability was estimated at 1.28×10^{-17} and the residual variability was estimated at 0.620. Again, the level where most variability was observed was between colonies. The simulations were run using a year-to-year variability of 0.03, a site-to-site variability of 1.3×10^{-17} and a residual variability of 0.62. Based on these estimates of variability, the power of the existing experiment to detect a 7% drop in the number of frames of brood was found to be lower than in the case of the number of frames of bees (15%).

Amount of nectar

When looking at the amount of nectar, the year-to-year variability was estimated as 0.039, whilst the site-to-site variability was estimated at 0.028 and the residual variability was estimated at 0.623. Again, the level where most variability was observed was between colonies. The simulations were run using a year-to-year variability of 0.04, a site-to-site variability of 0.03 and a residual variability of 0.62. Based on these estimates of variability, the power of the existing experiment to detect a 7% drop in the amount of nectar was found to be similar to that in the case of the number of frames of brood (15%).

Additional analyses

Following on from these power analyses of the design used in study S3UL1000 to detect a required 7% drop in "colony health", further work was carried out to look at the power of the design to detect larger changes i.e. what levels could have been detected with an 80% power. This was done by testing a range of changes and estimating the power as described earlier in this report. The results are presented in Table B.9.7.1-2 below.

Table B.9.7.1-2: Power of the existing design to detect a range of differences for all three "colony health" measures investigated in this report.

Difference (reduction) to be detected	Power for the "number of frames of bees"	Power for the "number of frames of brood"	Power for the "amount of nectar"
7%	20%	15%	15%
8%	22%	16%	16%
10%	27%	18%	19%
15%	44%	26%	27%
20%	63%	36%	37%
25%	80%	50%	50%
30%	91%	63%	62%
35%	97%	77%	75%
40%	99%	88%	88%

Given the existing design and the current assumptions, using the "number of frames of bees" as the indicator of "colony health" would mean that, if the experiment were required to have a power of 80% or more, the difference (reduction) that could be detected would be 25% or greater. Given that the variability observed for the "number of frames of brood" and the "amount of nectar" was even larger compared to the "number of frames of bees", in order for the experiment to have a power of 80% or more would require the reduction to be between at least 35 and 40%.

Following this analysis, a second assessment was run, to investigate the effect on the power of the experiment if only run for one year, whilst increasing the number of sites and number of colonies per site. Table B.9.7.1-3, B.9.7.1-4 and B.9.7.1-5 below present the results of the power analyses for the following scenarios: "number of frames of bees" and "number of frames of brood" for reductions of 7%, 21% and 35%, respectively for six combinations of number of sites (3, 6 and 9) and colonies per treatment at each site (10 and 15) as well as the existing design (three sites of six colonies per treatment at each site).

Table B.9.7.1-3: Power of a range of designs to detect a reduction of 7% in the "number of frames of bees" and "number of frames of brood".

Design (One year)	Power for the "number of frames of bees"	Power for the "number of frames of brood"
Current (3 sites, 6 colonies)	21%	17%
3 sites,10 colonies	21%	19%
3 sites, 15 colonies	14%	24%
6 sites, 10 colonies	24%	29%
6 sites, 15 colonies	35%	40%
9 sites, 10 colonies	33%	32%
9 sites, 15 colonies	35%	50%

Table B.9.7.1-4: Power of a range of designs to detect a reduction of 21% in the "number of frames of bees" and "number of frames of brood".

Design (One year)	Power for the "number of frames of bees"	Power for the "number of frames of brood"
Current (3 sites, 6 colonies)	44%	30%
3 sites, 10 colonies	53%	35%
3 sites, 15 colonies	73%	47%
6 sites, 10 colonies	72%	55%
6 sites, 15 colonies	87%	72%
9 sites, ten colonies	88%	66%
9 sites, 15 colonies	100.0%	94.0%

Table B.9.7.1-5: Power of a range of designs to detect a reduction of 35% in the "number of frames of bees" and "number of frames of brood".

Design (One year)	Power for the "number of frames of bees"	Power for the "number of frames of brood"
Current (3 sites, 6 colonies)	78%	50%
3 sites, 10 colonies	89%	63%
3 sites, 15 colonies	100.0%	97.0%
6 sites, 10 colonies	99%	89%
6 sites, 15 colonies	99.8%	95.4%
9 sites, 10 colonies	99.8%	93.8%
9 sites, 15 colonies	100.0%	100.0%

When running the experiment for only one year, none of the proposed six combinations of sites and colonies would be sufficient to detect a 7% reduction in numbers of frames of bees or brood if the required power were at least 80%. For the number of frames of bees, the simulations estimated a maximum power of around 30% to 35% for the three largest designs. For the number of frames of brood, the simulations estimated a maximum power of around 50% for the largest design (nine sites of 15 colonies each). When looking at a 21% reduction in the number of frames of bees, the three largest designs were estimated to be able to detect that difference with a power of at least 80%. When looking at the number of frames of brood, only the largest design (nine sites of 15 colonies each) was found to be able to detect a 21% reduction with a power greater than 80%. Lastly, when looking at a 35% reduction in the number of frames of bees, all six proposed designs were estimated to be able to detect that difference with a power greater than 80%, including five with a power greater than 90%. When looking at the number of frames of brood, five designs were estimated to be able to detect a 35% drop with 80% power (all except the smallest) and four of these had 90% power.

Long-term colony performance

It is widely recognised that the long-term performance of honeybee colonies in the EU and elsewhere is not understood very well. Thus, Chauzat et al. (2013)[37] report that the demographics of the beekeeping industry in Europe is poorly described. This is despite the fact that the health of honeybees has received considerable attention in recent years, particularly in relation to possible declines in colony numbers. There is a growing consensus that many factors contribute to the high rates of losses reported in Europe and in the United States. The degree to which particular factors contribute to loss, either on their own or in combination with other factors, is poorly understood. Accordingly the EU Reference Laboratory for bee health undertook a survey in which a questionnaire was sent to the National Reference Laboratories of the 27 European Union member states as well as contacts in Kosovo and Norway (Chauzat et al., 2013). The findings show that rates of colony mortality fell within a wide range of values (from 7.5 to 27.6%). This variation was attributed to the high heterogeneity identified (e.g. in beekeeping practice, disease prevalence etc) and to the protocols implemented to collect information, which are not standardised.

The levels of colony loss found within the 3-year maize study fall within the reported range and so are consistent with this overall pattern. Also, there were no differences between the treated and control colonies i.e. no evidence of any treatment effect thus indicating that common (regional) factors were responsible. The level of losses was also consistent with the reported contemporaneous losses from local beekeepers in the areas in which the trial was conducted. It is also consistent with fluctuating colony losses reported by Paxton (2013). Data presented for a number of countries (Denmark, Finland, Germany, Sweden and England & Wales) showed national colony loss rates of more than 5% increasing to nearly 35%. However, this was not consistent between years with levels showing considerable variation within any one country. In general, colony levels across the EU were found to be relatively stable, with losses being met by replacement. One of the main factors affecting colony numbers was attributed to sociopolitical factors i.e. the number of beekeepers present at any one time.

In their paper, Genersch et al. (2010)[38] report on a German bee monitoring project set up by nine scientific bee institutes of different Federal States in Germany following concerns about bee losses reported in the winter of 2002/2003. The four-year study was initiated in autumn 2004 and involved more than 1200 bee colonies from about 120 apiaries which were monitored for the entire study period. Bee samples were collected twice a year to analyze various pathogenic factors including the ectoparasitic mite *Varroa destructor*, fungi (*Nosema* sp., *Ascosphaera apis*), the bacterium *Paenibacillus larvae*, and several viruses. Data on environmental factors, beekeeping management practice, and pesticides were also collected. All data were statistically analyzed in respect to the overwintering mortality of the colonies. The average percentage of winter losses ranged from 3.8% (2004/05) to 15.2% in 2005/06, again consistent with the losses recorded in the 3-year maize study. However, the losses were not distributed equally among the participating beekeepers. An analysis of all data sets for the four years showed that while some beekeepers had no or only moderate colony losses during the project period but in 14.2% of the analysed cases the losses were higher than 20%. This distribution (many beekeepers with no or few colony losses and few beekeepers with high losses) were similar for all four winters. In addition to annual variations in winter losses regional variations were also observed, however, these regional differences were not consistent over the four years period. In addition, higher colony losses were not consistently related to certain beekeepers and apiaries. Several factors were significantly related to the observed winter losses of the monitored honeybee colonies: (i) high *Varroa* infestation level, (ii) infection with deformed wing virus (DWV) and acute bee paralysis virus (ABPV) in autumn, (iii) queen age, and (iv) weakness of the colonies in autumn. No effects could be observed for *Nosema* sp. or pesticides.

[37] Chauzat MP, Cauquil L, Roy L, Franco S, Hendrikx P, Ribiére-Chabert M (2013) Demographics of the European Apicultural Industry. PlosONE 8(11): e79018. doi:10.1371/journal.pone.0079018

[38] Genersch E, von der Ohe W, Kaatz H, Schroeder A, Otten C, Büchler R, Berg S, Ritter W, Mühlen W, Gisder S, Meixner M, Liebig G, Rosenkranz (2010). The German bee monitoring project: a long term study to understand periodically high winter losses of honeybee colonies. Apidologie 41 332-352.

A similar study to the one reported here with clothianidin, has been conducted with thiamethoxam (Pilling et al., 2013[39]) to investigate if exposure to residues in pollen and nectar from field treated maize and oilseed rape have a detrimental effect on colony strength and survival following repeated single treatment crop exposure each year over a four year period. Three long-term overwintering trials were established in maize in the Lorraine, Alsace and Aveyron regions of France in 2006, and two trials in oilseed rape in the Picardie and Alsace regions in 2005. At each trial site, there was one control field and one treated field of approximately 2 ha separated by approximately 2 km minimizing the movement of bees between fields. All trials used the maximum approved label rate for thiamethoxam as a seed treatment, in maize the nominal rate was 88.2 g a.s./ha and in oilseed rape the rate was 12.6 g a.s./ha. At each site, six colonies were placed adjacent to the control field and six colonies adjacent to the treated field during the entire flowering period of the crop (exposure phase). Overall, a mean loss ranging from 0 to 2.7 colonies per year across all five sites (total of 60 colonies) was reported, with similar losses observed between treated and control sites. The frequency of queen replacement and colony loss was as would be expected with this number of colonies over a four year period and considered to be in line with normal beekeeping practice. It is also consistent with the losses reported in the 3-year maize study.

Another large-scale field experiment was carried out in 2012 in southern Ontario, Canada, to determine whether exposure to clothianidin seed-treated canola (oil seed rape) had any adverse impacts on honeybees (Cutler et al., 2014)[40]. Colonies were placed in clothianidin seed-treated (1.4 L product/100 kg seed (20.4% clothianidin) at 5.6 kg seed/ha) or control canola fields during bloom, and thereafter were moved to an apiary with no surrounding crops grown from seeds treated with neonicotinoids. Colony weight gain, honey production, pest incidence, bee mortality, number of adults, and amount of sealed brood were assessed in each colony throughout summer and autumn. Samples of honey, beeswax, pollen, and nectar were regularly collected, and samples were analyzed for clothianidin residues. Several of these endpoints were also measured in spring 2013. Overall, colonies were vigorous during and after the exposure period, and we found no effects of exposure to clothianidin seed-treated canola on any endpoint measures. Bees foraged heavily on the test fields during peak bloom and residue analysis indicated that honeybees were exposed to low levels (0.5–2 ppb) of clothianidin in pollen. Low levels of clothianidin were detected in a few pollen samples collected toward the end of the bloom from control hives, illustrating the difficulty of conducting a perfectly controlled field study with free-ranging honeybees in agricultural landscapes. Overwintering success likewise did not differ significantly between treatment and control colonies. Winter colony loss rates were higher than expected, at 37% for control and 26% for treatment colonies, but overall (32%) were similar to overwintering colony loss rates reported for the winter of 2012–2013 for beekeepers in Ontario (38%) and Canada as a whole (29%). Our results suggest that exposure to canola grown from seed treated with clothianidin poses low risk to honeybees.

Conclusions

While the long-term performance of honeybee colonies in the EU and elsewhere is not understood very well, there is some long-term information available to indicate overall trends. In addition, new data is now being generated in the EU in the light of concerns about honeybee losses in recent years. Thus, the work of the European Reference Laboratory for bee health and the German bee monitoring project provide reliable indications of between year colony losses, as well as identifying possible causal factors. The level of losses found within the 3-year maize study were consistent with the reported contemporaneous losses from local beekeepers in the areas in which the trial was conducted. They also fall within the ranges reported in both of the wider ranging monitoring projects conducted in the EU, indicating that the outcome of the study was as might be expected for honeybee colonies in the

[39] Pilling E, Campbell P, Coulson M, Ruddle N, Tornier I (2013). A Four-Year Field Program Investigating Long-Term Effects of Repeated Exposure of Honeybee Colonies to Flowering Crops Treated with Thiamethoxam. PlosONE 8(10):e77193. doi:10.1371/journal.pone.0077193

[40] Cutler GC, Scott-Dupree CD, Sultan M, McFarlane AD, Brewer L (2014). A large-scale field study examining effects of exposure to clothianidin seed-treated canola on honeybee colony health, development, and overwintering success. PeerJ 2:e652 http://dx.doi.org/10.7717/peerj.652

EU in general. This is confirmed by the fact that there were no differences between the treated and control colonies i.e. no evidence of any treatment effect thus indicating that common (regional) factors were responsible.

The results of the 3-year maize study are also consistent with the other specific large scale studies looking at the effects of clothianidin and thiamethoxam treated seed and a consistent pattern emerges in all cases. Thus, while foraging bees were exposed to low levels of clothianidin residues in pollen and/or nectar and low residue levels were found in a few bee matrices but these were transient. Overall, colonies were vigorous during and after the exposure period, and no effects of exposure to the treatments were found on any endpoint measures. Overwintering success likewise did not differ significantly between treatment and control colonies in all cases.

As has been pointed out, the 3-year maize field study considered here was conducted on the basis of prevailing guidance as outlined in EPPO 170 but went well beyond what would normally be carried out i.e. assessments made at three sites in parallel over three consecutive years. The statistical analysis of the data from this study indicates that it has a relatively low power for a number of measures of colony health (number of frames of bees, the number of frames of brood and the amount of nectar). However, the power of the study to detect given levels of effects will be considerably greater than normally achieved with higher tier bee studies currently conducted. Also, the additional statistical analysis of potential study designs demonstrates that field trials by their very nature will be limited in what they can achieve from a statistical point of view. This is due to the inherent variability of real world data and the limitations of what can be practically achieved. However, while statistical analyses provide useful tools to aid in the evaluation of field trial data, their primary value is in their biological interpretation, assessing effects at the relevant organisational level (populations, colonies etc) under realistic conditions. In this sense, it can be concluded that the available 3-year maize study provided useful information to demonstrate a low acute and long-term risk to honeybees of clothianidin granules treatments in maize crop.

RMS Comments

Based on published literature data, it is sufficiently demonstrated that the overwintering losses within the three year maize study were consistent with the reported contemporaneous losses from local beekeepers in the areas in which the trial was conducted.

The additional analysis performed by the applicant on the data from the three year study in maize shows that the statistical power is relatively low for a number of measures of colony health. However, it also showed that the statistical power of field studies in general will be limited due to inherent variability of real world data. RMS agrees that the biological interpretation of field trial data is at least as important as the statistical analysis of this data. As this study was conducted on the basis of prevailing guidance (EPPO 170) and went beyond what is normally carried out, RMS considers that data from this three year study in maize provides useful information in support of the risk assessment.

During Peer Review, several concerns were raised regarding the field study on maize by Thompson (2011b) and the study re-analysis by Lewis (2014). These issues were discussed at Pesticides Peer Review Meeting 145. The statistical power was discussed in relation to the high inter-colony variability observed. It was argued that the study has a low statistical power (assuming that the observed variability is a suitable estimation of the real natural variability). It was noted that most of the variability (c. 90%) was due to the inter-colony factor rather than inter-site and temporal factors. This may mean that the number of hives per site is more relevant in terms of statistical power than the number of sites. However, it was argued that the analysis was performed on a limited numbers of hives and sites and that therefore the variability partitioning observed in this study may not represent the real natural variability (see comment 5(33) and 5(35) in the Reporting Table). Further, it was noted that the RMS pointed out the relevance of the biological interpretation of field trials (see comment 5(33) in the Reporting Table).

It was concluded that, generally, when the results are highly variable (which is the case for the study by Thompson, 2011b) it is difficult to draw any conclusion on a cause – effect relationship (i.e. treatment or non-treatment related effects). Generally, it was acknowledged that the availability of several pieces of evidence (e.g. several comparable field studies) can be useful to make a trend analysis to be used as a weight of evidence for the risk assessment.

Overall, it was agreed that the re-analysis provided for the study is not sufficient to address the concerns already identified in the conclusion of EFSA 2013 (i.e., the Thompson study cannot be considered sufficient to draw a firm conclusion on the cause-effect relationship).

Report:	**IIIA 10.4g/02, Bousquet, C. (2015)**
Title:	**Magnitude of the residue of clothianidin and its metabolites in potato pollen in Northern and Southern Europe – 2014**
Report No.:	14GS021
Document No.:	THW-0388
Guidelines:	Commission Regulation (EU) no 283/2013
GLP	Yes (certified laboratory)

Objective

The objective of the study was to generate specimens of potato pollen following one application at planting of the formulation Clothianidin 0.7 GR or two foliar applications of the formulation clothianidin 50 WG before flowering to quantify residues of clothianidin and its metabolites in potato pollen. Only the results for Clothianidin 0.7 GR are reported here (as required for confirmatory data).

Material and methods

Test items

- Clothianidin 0.7 GR; batch no. - 31LN1E; purity – 0.7% w/w (nominal), 6.852 g/kg (analysed).
- Clothianidin 50 WG; batch no. – 2690651003; purity – 500 g/kg (nominal), 492.2 g/kg (analysed).

Study sites

A study on the magnitude of the residue of clothianidin and its metabolites in potato pollen was conducted in Northern Europe and Southern Europe. The objective was to determine the residue levels of clothianidin and its metabolites TZNG and TZMU in potato pollen specimens in several trials after application of two different formulations of clothianidin:

- one application of Clothianidin 0.7 GR in furrow: 5 trials
- two foliar applications of Clothianidin 50 WG: 6 trials

Six field trials were performed on potato in Northern Europe (Northern France, Germany and Hungary) and Southern Europe (Spain and Italy). The sites were representative of potato grown in a way typical of the producing region in the test countries (see Table B.9.7.1-2). Cultivars were selected based on their flowering and pollen production power. Only sites that had not been treated in 2012, 2013 and 2014 with any product containing clothianidin and thiametoxam were used as test sites for the field phase of this study. The texture of the soil at each trial site is also indicated in the Table B.9.7.1-6 below.

Table B.9.7.1-6: Identification of the field trials

Trial number (1)	Study type	Zone	Country (region)	Trial city	Zip code	Field Contractor	Soil texture
14SGS021 GE01	Pollen trial	Northern Europe	Germany (North Rhine-Westphalia)	Weeze	47652	BioChem agrar GmbH.	Sand
14SGS021 FR02	Pollen trial	Northern Europe	Northern France (Picardie)	Lor	02190	SGS AGRI MIN	Silt loam
14SGS021 IT04	Pollen trial	Southern Europe	Italy (Veneto)	Grumolo delle Abbadesse	36040	SGS Italia	Clay loam
14SGS021 HU05	Pollen trial	Northern Europe	Hungary (Pest)	Bugyi	2347	ATC	Silt loam
14SGS021 SP06	Pollen trial	Southern Europe	Spain (Galicia)	Xunqueira de Ambia	32679	Trialcamp	Sandy clay loam
14SGS021 SP07	Pollen trial	Northern Europe	Spain (Castellón)	Viver	12460	Trialcamp	Clay

Trial 14SGS021 SP03 is not reported. It was set up but cancelled before sampling because the site was destroyed by bad weather conditions. It was replaced by trials 14SGS021 SP06 and SP07 (SP06 was for Clothianidin 50 WG only and is not reported here).

Five trials consisted of one untreated plot U and two treated plots Ta and Tb (both Clothianidin 0.7 GR and Clothianidin 50 WG). One trial (14SGS021 SP06) consisted of one untreated plot U and one treated plot Tb (Clothianidin 50 WG). Only the results for Clothianidin 0.7 GR are reported here (as required for confirmatory data).

Treated plots Ta received one application at planting of the formulation CLOTHIANIDIN 0.7 GR at the target dose rate of 80 g a.s./ha. The deviations calculated on the amount of formulated product per hectare ranged between -3.7 and +3.4 % on plot Ta. The plots consisted of minimum 1000 m², except for plot Ta in 14SGS021 HU05 which was 840 m² and plot Ta in 14SGS021 IT04 which was reduced to 750 m² for sampling because of a farmer mistake (1000 m² were treated but the farmer destroyed 250 m²). Daily weather data were collected from institutional, permanent weather recording stations situated 3.5 to 17 km from the field sites. They include historical (min. 5-year data), monthly average maximum / minimum air temperature and total rainfall.

Before the start of the trial, soil specimens were collected to be analysed for clothianidin and its metabolites, thiametoxam and imidacloprid. The results found showed no quantifiable residues of clothianidin, TZNG, TZMU, thiametoxam in the soil specimens of trials 14SGS021 GE01, FR02, HU05, SP06 and SP07. Some residues of imidacloprid were quantified in the soil specimens of trials 14SGS021 FR02 (levels around 1-2 µg/kg) and SP06 (levels around 4-32 µg/kg depending on the plot). No quantifiable residue of TZNG, TZMU and imidacloprid but some residues of clothianidin and thiametoxam were found in the soil specimens of trial 14SGS021 IT04 (levels of clothianidin between <1 / 2 µg/kg and levels of thiamethoxam between n.d. / 1 µg/kg).

In the trials, pollen and anther specimens for analyses were collected on three occasions during the flowering period: S2 - As early as possible, as soon as enough pollen was available; S3 = 7 +/-1 days after sampling S1; S4 = 14 +/-1 days after sampling S1 – if flowering was still going on. Flowers were picked in the plots avoiding the borders when it was possible. Subsequently, at the field station the pollen was extracted by rubbing the anthers with a clean tool above a petri-dish. In order to avoid losing pollen, petri-dishes were frozen with the pollen inside. Anthers were separated from the flowers by hand or with a tool, directly at the field or at the field station. Pistils were removed before sealing the anthers into the petri-dishes.

In some trials (14SGS021GE01, IT04 and HU05), it was not possible to extract pollen from flowers at some sampling dates, in part because of weather conditions not being favourable to pollen production. In this case, anthers were collected instead of pollen specimens. In order to allow comparison between the trials, anthers were also collected from other trials and sampling dates when pollen specimens could be collected to be analysed.

In addition, when pollen was available, the low availability of pollen meant that it was not possible to collect the required amounts of pollen at some sampling dates and in some trials (trials 14SGS021 GE01, IT04 and HU05). This led to some analytical difficulties. In trials 14SGS021 FR02, SP06 and SP07 the requested specimens (matrix and amount) could be collected.

All specimens were deep frozen on the day of collection and stored at a target temperature below -18°C. All specimens remained deep frozen during storage at the test sites, during shipment to the analytical laboratory Eurofins AgroScience Services (EAS Chem) in Germany and during storage at the analytical laboratory. The maximum interval from sampling to extraction was 143 days (pollen) and 168 days (anthers).

Analytical work

Residue levels of clothianidin and of its metabolites (TZNG and TZMU) were determined in the specimens of anthers and pollen, by means of the multi-residue method QuEChERS with a single extraction and single injection for determination of the three analytes and with quantification/detection by LC-MS/MS. Matrix-matched standards were used for quantification, as matrix effects were found to be significant for all analytes. The analytical method was validated for potato pollen and anthers within the analytical phase of this study, i.e. a reduced validation set for potato pollen (n=3 per fortification level) and a full validation set (n=5 per fortification level) for potato anthers was provided, demonstrating suitability of the method (mean recoveries within 70-110%; RSD<20%) with a LOQ of 1 µg/kg for each of the analytes.

In addition, for each analytical set, the method accuracy and repeatability was assessed by means of procedural recovery determinations in parallel to the analysis of residue samples, by using fortified control samples. Procedural recoveries indicated adequate performance of the method at the moment of analysis, except for clothianidin analysis in potato pollen, because the control pollen samples that were used for fortification and matrix-matched standard solutions appeared to be contaminated with significant levels of clothianidin and poor blank corrected procedural recoveries were observed. More details are reported further below under the tables with the results.

The method for soil analysis was also based on QuEChERS methodology and had been successfully validated for the determination of *i.a.* clothianidin, TZNG and TZMU in soil in study S14-05159 (SGS-1435V). In addition, procedural recoveries determined within this study (Report No.14GS021) confirmed an acceptable performance at the moment of sample analysis (mean recoveries within 70-110% and RSD<20% at LOQ 1 µg/kg and 10xLOQ; n=2 per fortification level).

Findings

Plot U – Untreated

Analyses of pollen and anther specimens sampled from plot U led to the following results summarized in Table B.9.7.1-7.

No residues of clothianidin or of its metabolites in pollen and anthers above the LOQ level were detected in the untreated specimens of trials 14SGS021 GE01 and 14SGS021 FR02. In trial IT04, no residues above the LOQ were found in the anther specimens. For the untreated pollen specimens, values are only indicative and no conclusion can be drawn from these data.

In trial HU05, very high residues (up to 741 µg/kg) were found in the untreated anther specimens, especially at the first sampling time and this was confirmed by the analysis of the spare specimens. The principal field investigator found no explanation from the field. The values are too high to reflect

just a field contamination (which could be due to a number of reasons e.g. mixing up specimens, drift, farmer application etc). Unfortunately, no residues of clothianidin or of its metabolites could be reported for the pollen specimens, because of poor recoveries obtained during the analytical set and problems in determination of the specimen weights.

In trial SP07, no residues above the LOQ were observed in the untreated anther specimens but some traces of clothianidin and TZNG above the LOQ were measured in the untreated pollen specimens. The analyses of the spare specimens did not confirm the presence of clothianidin or its metabolites. No reason could be found to explain these low residue levels found in the first analyses and the fact that no residues were found in the spare specimens indicates that the contamination occurred during processing of the specimens. Therefore, residue values found in the treated plots can be considered as valid.

Table B.9.7.1-7: Analyses of pollen and anther specimens sampled from plots U

Matrix	**14SGS021 GE01 – Plot U**									
	Residue (µg/kg) (n.d.: <0.3 µg/kg / LOQ = 1 µg/kg)									
	Sampling /DALA	Clothianidin			TZNG			TZMU		
Pollen	S2 / NAP	No pollen could be sampled at this timing								
	S3 / NAP	No pollen could be sampled at this timing								
	S4 / NAP	No pollen could be sampled at this timing								
Anthers	S2 / NAP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	S3 / NAP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	S4 / NAP	n.d.			n.d.			n.d.		

Matrix	**14SGS021 FR02 – Plot U**									
	Residue (µg/kg) (n.d.: <0.3 µg/kg / LOQ = 1 µg/kg)									
	Sampling /DALA	Clothianidin			TZNG			TZMU		
Pollen	S2 / NAP	<1 (3)	n.d. (3)	n.d. (3)	<1	n.d.	n.d.	<1	n.d.	n.d.
	S3 / NAP	n.d. (3)	n.d. (3)	/ (4)	n.d.	n.d.	/ (4)	n.d.	n.d.	/ (4)
	S4 / NAP	No pollen could be sampled at this timing								
Anthers	S2 / NAP	n.d.			n.d.			n.d.		
	S3 / NAP	n.d.			n.d.			n.d.		
	S4 / NAP	n.d.			n.d.			n.d.		

(3) Contamination of clothianidin was observed in the control samples that were used for fortification experiments and matrix-matched standard solutions above LOD (about 0.5 µg/kg, < LOQ); since no alternative potato (pollen) was available, the situation is considered to be unavoidable. Blank correction yielded reasonable procedural recoveries so that the residue values reported are considered to be accurate. Residue values are calculated by uncorrected peak areas.
(4) Despite enough pollen was collected on the field, the laboratory did no manage to collect enough material to enable an analysis.

Matrix	**14SGS021 IT04 – Plot U**			
	Residue (µg/kg) (n.d.: <0.3 µg/kg / LOQ = 1 µg/kg)			
	Sampling /DALA	Clothianidin	TZNG	TZMU
Pollen	S2 / NAP	Data not reported (5) (6)	Data not reported (5)	Data not reported (5)
	S3 / NAP	No pollen could be sampled at this timing		
	S4 / NAP	No pollen could be sampled at this timing		
Anthers	S2 / NAP	n.d.	n.d.	n.d.
	S3 / NAP	/	/	/
	S4 / NAP	/	/	/

(5) During analyses the whole pollen specimens were extracted. Sample weights could not be determined with sufficient accuracy at the analytical site. Specimen weights provided by the field investigators are not accurate enough for analytical purpose but were used for result calculations. Therefore values obtained have to be considered as indicative only. They were reported in the analytical report but not in the final report.

(6) Due to contaminated control samples (clothianidin at 2 x LOQ) used for the recoveries, the analytical set for pollen is not valid for clothianidin. Blank corrected procedural recoveries of 33% (at LOQ) and 242% (at 10% LOQ) were observed. For this reason, analytical results are not reported in the final report (but are in the analytical report).

Matrix	**14SGS021 HU05 – Plot U**												
	Residue (µg/kg) (n.d.: <0.3 µg/kg / LOQ = 1 µg/kg)												
	Sampling /DALA	Clothianidin				TZNG				TZMU			
Pollen	S2 / NAP	/				/				/			
	S3 / NAP	Data not reported (8) (9)				Data not reported (8)				Data not reported (8)			
	S4 / NAP	/				/				/			
Anthers	S2 / NAP	Implausible results				<1 (10)	n.d. (10)	n.d. (10)		n.d. (10)	n.d. (10)	n.d. (10)	
	S2 / NAP (spare)					<1 (10)				n.d. (10)			
	S3 / NAP	2	2	<1		n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	
	S3 / NAP (spare)	<1 (10)	2 (10)	/ (10)		n.d. (10)	n.d. (10)	/		n.d. (10)	n.d. (10)	/	
	S4 / NAP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

(8) During analyses the whole pollen specimens were extracted and the weights used for the calculation by the analytical laboratory are the weights measured on the field. The principal field investigator reported electrostatic problems during weighing which led to inaccurate weights. Therefore, values indicated here have to be considered as indicative only. They were reported in the analytical report but not in the final report.
(9) Due to contaminated control samples (clothianidin at 2 x LOQ) used for the recoveries, the analytical set for pollen is not valid for clothianidin. Blank corrected procedural recoveries of 33% (at LOQ) and 242% (at 10% LOQ) were observed. For this reason, analytical results are not reported in the final report (but are in the analytical report).
(10) Spare anther specimens analysed.

Matrix	**14SGS021 SP07 – Plot U**									
	Residue (µg/kg) (n.d.: <0.3 µg/kg / LOQ = 1 µg/kg)									
	Sampling /DALA	Clothianidin			TZNG			TZMU		
Pollen	S2 / NAP	3 (15)	<1 (15)	2 (15)	n.d.	n.d.	n.d.	1	n.d.	n.d.
	S2 / NAP (spare)	n.d. (16)			n.d. (16)			n.d. (16)		
	S3 / NAP	n.d. (15)	<1 (15)	n.d. (15)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	S3 / NAP (spare)	n.d. (16)			n.d. (16)			n.d. (16)		
	S4 / NAP	n.d. (15)	n.d (15)	<1 (15)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	S4 / NAP (spare)	n.d. (16)			n.d. (16)			n.d. (16)		
Anthers	S2 / NAP	n.d.			n.d.			n.d.		
	S3 / NAP	n.d.			n.d.			n.d.		
	S4 / NAP	n.d.			n.d.			n.d.		

(15) Contamination of clothianidin was observed in the control samples that were used for fortification experiments and matrix-matched standard solutions above LOD (about 0.5 µg/kg, < LOQ); since no alternative potato (pollen) was available, the situation is considered to be unavoidable. Blank correction yielded reasonable procedural recoveries so that the residue values reported are considered to be accurate. Residue values are calculated by uncorrected peak areas.
(16) Spare pollen specimens were analysed.

Plot Ta – Clothianidin 0.7 GR (80 g a.s./ha, 1 application at planting)

The results of the analyses of pollen and anther specimens sampled from plots Ta are shown in Table B.9.7.1-8.

Table B.9.7.1-8: Analyses of pollen and anther specimens sampled from plots Ta

Matrix	**14SGS021 GE01 – Plot Ta - CLOTHIANIDIN 0.7 GR – 80 g as/ha – 1 application at planting**									
	Residue (µg/kg) (n.d.: <0.3 µg/kg / LOQ = 1 µg/kg)									
	Sampling/DALA	Clothianidin			TZNG			TZMU		
Pollen (1)	S2 / 57	/			/			/		
	S3 / 63	Data not reported (2)			1 (1)	2 (1)	2 (1)	n.d. (1)	n.d. (1)	n.d. (1)
	S4 / 76	/			/			/		
Anthers	S2 / 57	<1	<1	<1	<1	<1	<1	n.d.	n.d.	n.d.
	S3 / 63	/			/			/		
	S4 / 76	n.d.			<1			n.d.		

(1) During analyses, the whole pollen specimens were extracted and the weights used for the calculation by the analytical laboratory are those measured at the field. These weights were measured under GLP with a precise balance. The principal field investigator confirmed that they were sufficiently accurate to be used for the analytical part.
(2) Due to contaminated control samples (clothianidin at 2 x LOQ) used for the fortifications, the analytical set for pollen is not valid for clothianidin since blank corrected procedural recoveries of 33% (at LOQ) and 242% (at 10% LOQ) were observed. For this reason, analytical results are not reported in the final report (but are in the analytical report).

Matrix	**14SGS021 FR02 – Plot Ta**									
	Residue (µg/kg) (n.d.: <0.3 µg/kg / LOQ = 1 µg/kg)									
	Sampling /DALA	Clothianidin			TZNG			TZMU		
Pollen	S1 / 60	10 (3)	29 (3)	19 (3)	7	19	11	<1	2	<1
	S2 / 68	13 (3)	14 (3)	5 (3)	10	11	5	<1	<1	n.d.
	S3 / 74	No pollen could be sampled at this timing								
Anthers	S1 / 60	6			8			n.d.		
	S2 / 68	4			7			n.d.		
	S3 / 74	5			7			n.d.		

(3) Contamination of clothianidin was observed in the control samples that were used for fortification experiments and matrix-matched standard solutions above LOD (about 0.5 µg/kg, < LOQ); since no alternative potato (pollen) was available, the situation is considered to be unavoidable. Blank correction yielded reasonable procedural recoveries so that the residue values reported are considered to be accurate. Residue values are calculated by uncorrected peak areas.

Matrix	**14SGS021 IT04 – Plot Ta - CLOTHIANIDIN 0.7 GR – 80 g as/ha – 1 application at planting**						
	Residue (µg/kg)						
	Sampling/DALA	Clothianidin		TZNG		TZMU	
Pollen	S2 / 87	No pollen could be sampled at this timing					
	S3 / NAV	No pollen could be sampled at this timing					
	S4 / 100	Data not reported (5) (6)		Data not reported (5)		Data not reported (5)	
Anthers	S2 / 87	5	5	7	7	<1	<1
	S3 / NAV	/		/		/	
	S4 / 100	/		/		/	

(5) During analyses the whole pollen specimens were extracted. Sample weights could not be determined with sufficient accuracy at the analytical site. Specimen weights provided by the field investigators are not accurate enough for analytical purpose but were used for result calculations. Therefore values obtained have to be considered as indicative only. They were reported in the analytical report but not in the final report.
(6) Due to contaminated control samples (clothianidin at 2 x LOQ) used for the recoveries, the analytical set for pollen is not valid for clothianidin. Blank corrected procedural recoveries of 33% (at LOQ) and 242% (at 10% LOQ) were observed. For this reason, analytical results are not reported in the final report (but are in the analytical report).

Matrix	**14SGS021 HU05 – Plot Ta**									
	Residue (µg/kg) (n.d.: <0.3 µg/kg / LOQ = 1 µg/kg)									
	Sampling /DALA	Clothianidin			TZNG			TZMU		
Pollen	S1 / 67	Data not reported (8) (9)			Data not reported (8)			Data not reported (8)		
	S2 / 71	Data not reported (8) (9)			Data not reported (8)			Data not reported (8)		
	S3 / 78	Data not reported (8) (9)			Data not reported (8)			Data not reported (8)		
Anthers	S1 / 67	1	<1	<1	2	2	2	n.d.	n.d.	n.d.
	S2 / 71	2	2	2	10	13	11	n.d.	n.d.	n.d.
	S3 / 78	<1	<1	<1	<1	<1	<1	n.d.	n.d.	n.d.

(8) During analyses the whole pollen specimens were extracted and the weights used for the calculation by the analytical laboratory are the weights measured on the field. The principal field investigator reported electrostatic problems during weighing which led to inaccurate weights. Therefore, values indicated here have to be considered as indicative only. They were reported in the analytical report but not in the final report.
(9) Due to contaminated control samples (clothianidin at 2 x LOQ) used for the recoveries, the analytical set for pollen is not valid for clothianidin. Blank corrected procedural recoveries of 33% (at LOQ) and 242% (at 10% LOQ) were observed. For this reason, analytical results are not reported in the final report (but are in the analytical report).

Matrix	**14SGS021 SP07 – Plot Ta**									
	Residue (µg/kg) (n.d.: <0.3 µg/kg / LOQ = 1 µg/kg)									
	Sampling /DALA	Clothianidin			TZNG			TZMU		
Pollen	48	15 (15)	15 (15)	29 (15)	9	15	13	2	2	3
	54	30 (15)	31 (15)	18 (15)	16	16	15	2	2	1
	61	8 (15)	5 (15)	10 (15)	11	7	8	n.d.	n.d.	<1
Anthers	48	1			3			n.d.		
	54	<1			3			n.d.		
	61	<1			2			n.d.		

(15) Contamination of clothianidin was observed in the control samples that were used for fortification experiments and matrix-matched standard solutions above LOD (about 0.5 µg/kg, < LOQ); since no alternative potato (pollen) was available, the situation is considered to be unavoidable. Blank correction yielded reasonable procedural recoveries so that the residue values reported are considered to be accurate. Residue values are calculated by uncorrected peak areas.

Conclusion:
Five trials were conducted in N-EU (3) and S-EU (2) on potatoes, with one application at planting of the formulation CLOTHIANIDIN 0.7 GR at the target dose rate of 80 g a.s./ha. Due to a number of difficulties encountered during the potato pollen sampling and during the analytical phase, reliable pollen residue data could only be obtained for 2 of the 5 trials. Maximum clothianidin levels observed in pollen were around 30 µg/kg. Maximum levels of TZNG and TZMU in pollen were 19 µg/kg and 3 µg/kg, respectively. In general, a decline was observed after 1 or 2 weeks. Due to the difficulties encountered during pollen sampling and analysis, anthers were additionally sampled and analysed. Maximum levels of clothianidin and TZNG in anthers were 6 µg/kg and 8 µg/kg, respectively (TZMU levels were < LOQ 1 µg/kg in anthers).

RMS Comments

After peer review of the study protocol (see EFSA Technical Report, 2014)41, it was recommended by the experts to take triplicate samples at 3 sampling dates (start of the flowering, middle and end of the flowering). These recommendations were followed, and samples were taken on three dates, if sufficient pollen could be sampled.

It is noted that reliable data could only be obtained from only 2 out of the 5 trials. Nevertheless, the available data from these 2 trials is considered acceptable for use in risk assessment.

B.9.7.2. Exposure

Currently, the use of clothianidin as granular treatment at sowing is registered in maize, potato and sorghum. Maize does not produce nectar, but according to the EFSA Conclusion on the risk assessment for bees for clothianidin (2013) and Appendix D of the EFSA Guidance Document on bees, maize is considered to be attractive to honeybees for the consumption of pollen. Attractiveness of maize for bumblebees and solitary bees cannot be excluded, due to the lack of sufficient data in literature. Hence, exposure is possible, and the risk from the consumption of pollen from treated maize plants will be assessed for the three groups of bee species.

The applicant submitted a three year study performed in maize on the effects on colony survival following the exposure to clothianidin contaminated pollen. In this study, the honeybee activity in the treated crop and the residues present in maize pollen were investigated. The number of honeybees visiting the maize crop was generally low, with a high variability in the recorded levels of foraging activity, which reflects to some extent the relatively low attraction of maize to bees and the large areas of maize on the test plots, resulting in a low density. However, based on the number of foragers returning to the hive with pollen together with the estimated number/percentage of foragers returning with maize pollen loads, exposure to the maize crop was confirmed at each test site and for each year of the study.

A summary of the residues present in maize pollen (sampled from the main field and tunnels) is shown in Table B.9.7.2-1 (the values presented were derived from the mixed data of the three test sites). As the study was conducted at three test sites which are geographically spread over Europe (in North, Central and Southern France), which were monitored for three consecutive years, the available data is considered to be representative for the area of use and thus to be suitable for use in the risk assessment.

Table B.9.7.2-1: Summary of measured residue data of clothianidin (µg/kg) in pollen from the maize crop (mixed for the three sites, both main field and tunnels).

Sample	Year	min	max	mean	se	median	90th percentile	n	Reference
Pollen from maize crop (main field and tunnels)	2008	4	10	7.5	1.1	8.0	10.0	6	IIIA 10.4g/01 Thompson, 2011b
	2009	1	12	5.4	0.9	4.5	8.9	12	
	2010	5	20	11.1	1.9	10.0	17.2	8	
	All years	1	20	7.7	0.9	6.5	13.5	26	

Notes: LOD = 0.3 µg/kg, LOQ = 1 µg/kg. For calculation, values <LOD were assigned a value of 0.3 µg/kg and value <LOQ were assigned a value of 0.6 µg/kg.

In the EFSA Conclusion on the risk assessment for bees for clothianidin (2013), residue data on maize pollen from two more studies with the formulated product Santana (1% w/w clothianidin granules) are referenced. The first study (Dilger, 2011a; study ID S09-00346) was performed in Italy, with a dose of 110 g clothianidin/ha. The maximum measured residue in pollen was 11 µg a.s./kg. In the second study (Dilger, 2011b; study ID 20071122/E1-FPMA) a maximum residue of 8 µg/kg was measured after applying 122.56 g a.s/ha to a study site located in Italy. The residues measured by Dilger (2011a & b) are thus comparable to those found by Thompson (2011b). Consequently, in the initial version of

[41] European Food Safety Authority (2014). Outcome of the peer review of bee study protocols submitted by Sumitomo to assess the effects of clothianidin on bees. EFSA Supporting Publication 2014:EN-598.

this Addendum, the highest available 90th percentile value from the study by Thompson (2011b) (17.2 µg/kg, from 2010) was used in the risk assessment.

At Pesticides Peer Review Meeting 145, it was discussed whether it was acceptable to use the 90th percentile residue value for clothianidin in maize pollen in the risk assessment. As data for only three maize field sites is available, which are all located in France, it was agreed that the 90th percentile residue value from these studies cannot be used, in line with the EFSA Guidance Document for bees. It was agreed that the highest value should be used instead (which is 20 µg/kg, from 2010).

It is noted that for the indoor use of clothianidin in maize and sweet maize (which is restricted to permanent greenhouses), exposure to bees from pollen from the treated crop can be considered low. Therefore, in line with the decision of Pesticides Peer Review Meeting 129, no risk assessment needs to be performed for this indoor use.

Potato plants do not produce nectar, and are not considered attractive to honeybees for the consumption of pollen by the EFSA Guidance Document on bees. However, data were provided by Denmark during Pesticides Peer Review Experts' Meeting 129 indicating that honeybees collect pollen from potatoes. Further, according to Appendix D of the EFSA Guidance Document, potato plants are considered attractive for bumblebees. Due to the lack of sufficient data in literature, attractiveness for solitary bees cannot be excluded. Consequently, the risk from the consumption of pollen from treated potato plants will be assessed.

The applicant submitted a study in which the residues in potato pollen after application of clothianidin as a granule treatment at planting, at a dose rate of 80 g a.s./ha. Five trials were conducted on study fields located in Northern and Southern Europe (Germany, Northern France, Italy, Hungary and Spain), in accordance with the recommendations of the EFSA Guidance Document on bees (which suggests that results from at least 5 field sites are necessary to determine a reliable 90th percentile residue for use in the risk assessment). However, due to practical difficulties during the course of the study, reliable results from only two locations were obtained (see Table B.9.7.2-2). The maximum measured residue level in potato was about 30 µg/kg, with an indication that residues declined to around 10 µg/kg after one week. These results are generally in line with those from the studies in maize. Therefore, despite the fact that the available data set is of limited size, the results are considered reliable and suitable for use in the risk assessment. In the original version of this Addendum, the highest available 90th percentile value (30.2 from trial SP07) was be used in the risk assessment.

At Pesticides Peer Review Meeting 145, it was discussed whether it was acceptable to use the 90th percentile residue value for clothianidin in potato pollen in the risk assessment. As reliable data for only two field sites are available, it was agreed that the 90th percentile residue value from the study by Bousquet (2014) cannot be used, in line with the EFSA Guidance Document for bees. It was agreed that the highest value should be used instead (which is 31 µg/kg, from trial SP07).

Table B.9.7.2-2: Summary of measured residues of clothianidin (µg/kg) in potato pollen, from the two trials with reliable data (FR02 and SP07).

Trial	min	max	mean	se	median	90th percentile	n	Reference
FR02	5	29	15.0	3.1	13.5	24.0	6	IIIA 10.4g/02 Bousquet, 2015
SP07	5	31	17.9	3.1	15	30.2	9	
Both trials	5	31	16.8	2.3	15	29.6	15	

Notes: LOD = 0.3 µg/kg, LOQ = 1 µg/kg. For calculation, values <LOD were assigned a value of 0.3 µg/kg and value <LOQ were assigned a value of 0.6 µg/kg.

In the EFSA Conclusion on the risk assessment for bees for clothianidin, a data gap was identified for the risk through residues in nectar and pollen for the use in sorghum, as no data was available for this crop, and it was considered to be attractive to honeybees. However, in Appendix D of the EFSA Guidance Document on bees, sorghum is identified as not attractive to honeybees for the consumption

of nectar. Further, sorghum is generally considered low attractive to honeybees for pollen, but pollen collection cannot be excluded at all due to controversial information found in literature. Due to the lack of of sufficient data in literature, attractiveness for bumblebees and solitary bees cannot be excluded. As sorghum belongs to the family of Poaceae it is related to cereals and maize, and therefore not considered to produce nectar. Consequently, the risk for consumption of pollen from treated sorghum plants will be assessed for honeybees, bumblebees and solitary bees. However, as no studies were submitted by the applicant, no data on measured residues in pollen and nectar from sorghum is available.

B.9.7.3. Risk assessment

B.9.7.3.1. Risk assessment for honeybees

The risk assessment was performed following the risk assessment scheme for honeybees as proposed in the EFSA Guidance Document on bees. Due to the potential risk to honeybees from the consumption of pollen and nectar from treated crops, the screening step was not performed, and the risk assessment started at the first tier. As there is a potential exposure to honeybees through the consumption of pollen from maize, potato and sorghum (see section B.9.7.2), the risk assessment was performed for the uses in these three crops. As based on the information available in the GAP table (Table A-1) it is not possible to perform a risk assessment for the use in forestry nursery (only the dose in g a.s./plant is available, no information on the plant density and dose in g a.s./ha), a risk assessment for this use is not included.

It is noted that for the indoor use of clothianidin in maize and sweet maize (which is restricted to permanent greenhouses), exposure to bees from nectar and pollen from the treated crop. Therefore, in line with the decision of Pesticides Peer Review Meeting 129, no risk assessment needs to be performed for this indoor use.

First tier risk assessment

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 products applied as granules at sowing (incorporated into the soil). The relevant shortcut values (and the methodology used to determine these values) are presented in Table J6 Appendix J of the EFSA Guidance Document. As maize, potato and sorghum do 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.

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 and/or mg/seed
SV = 0.012 (as forager honeybees do not consume pollen, the shortcut value for exposure to nurse honeybees is used, which is taken from Table J6 in Appendix J of the Guidance Document)
E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for granules applied at sowing)
$LD_{50,oral}$ is expressed as µg a.s./bee

If this ETR > 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 *ETR for the chronic adult oral exposure* is calculated by the following equation:

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

Where: AR = application rate in kg a.s./ha and/or mg/seed
SV = 0.012 (as forager honeybees do not consume pollen, the shortcut value for exposure to nurse honeybees is used, which is taken from Table J6 in Appendix J of the Guidance Document)
E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for granules applied at sowing)
twa = 1
LDD_{50} is expressed as µg a.s./bee per day

If this ETR > 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.

The *ETR for larvae* is calculated by the following equation:

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

Where: AR = application rate in kg a.s./ha and/or mg/seed
SV = 0.002 (shortcut value for honeybee larvae, taken from Table J6 in Appendix J of the Guidance Document)
E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for granules applied at sowing)
twa = 1
NOED is expressed as µg a.s./larva/development period

If this ETR > 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.

According to the EFSA Guidance Document, an ETR for effects on the development of the hypopharyngeal glands (HPG) should also be calculated. As there is currently no validated methodology for the assessment of sublethal effects, no endpoint for the effects on the hypopharyngeal glands of honeybees is available for clothianidin. Therefore, the first tier risk assessment for honeybees based on HPG was not performed.

The first tier risk assessment has been performed using the authorized 'maximum application rate' for potato and maize (see Table B.9.7.3.1-1). The relevant toxicity endpoints are taken from Table B.9.1.3.1-3. The calculated Tier 1 ETR values are shown in Table B.9.7.3.1-2.

Table B.9.7.3.1-1: currently authorized 'maximum application rate' of clothianidin containing formulations for use as a granule treatment at sowing in potato, maize and sorghum.

Crop	authorized 'maximum application rate'
Potato	70 g a.s./ha
Maize/ Sweet maize/ Sorghum	50 g a.s./ha

The ETR values for acute adult oral exposure in maize and sorghum, and larval exposure in potatoes, maize and sorghum are below the relevant trigger, indicating an acceptable risk. For acute adult oral exposure in potato and chronic adult oral exposure in potatoes, maize and sorghum exceed the relevant trigger values. For these scenarios, a potential risk is identified and further consideration is necessary.

Table B.9.7.3.1-2: Tier 1 ETR calculations for acute adult oral, chronic adult oral and larval exposure for the highest authorized 'maximum application rate' of clothianidin in potato and maize.

Acute adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Potato	0.070	1	0.012	-	0.00379	0.22	0.2
Maize/ Sweet maize/ Sorghum	0.050	1	0.012	-	0.00379	0.16	0.2
Chronic adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	LDD_{50} (µg a.s./bee/day)	ETR	Trigger
Potato	0.070	1	0.012	1	0.00138	0.61	0.03
Maize/ Sweet maize/ Sorghum	0.050	1	0.012	1	0.00138	0.43	0.03
Larval exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	NOED (µg a.s./larva /development period)	ETR	Trigger
Potato	0.070	1	0.002	1	0.00528	0.027	0.2
Maize/ Sweet maize/ Sorghum	0.050	1	0.002	1	0.00528	0.019	0.2

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, 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 in which the clothianidin residues in pollen from potato and maize were measured. As discussed under Section B.9.7.2, these studies are considered acceptable, and residue values suitable for use in the risk assessment were selected. As data from less than 5 study fields was available for each crop, it was agreed at Pesticides Peer Review Meeting 145 that as a conservative approach, the highest available residue values should be used (20 µg/kg for maize and 31 µg/kg for potato). As these values were obtained treating the potato or maize crop with 80 g a.s./ha and 110 g a.s./ha, the selected residue values cover the treated crop scenarios for all registered uses of clothianidin as granule. For sorghum, no studies measuring clothianidin residues in pollen are available. Consequently, it is not possible to conduct a Tier 2 assessment for this use.

In table J1 of appendix J of the EFSA Guidance Document on bees, data on the consumption of nectar and pollen by forager and nurse honeybees and honeybee larvae are reported. The values for pollen are shown in Table B.9.7.3.1-3.

Table B.9.7.3.1-3: Pollen consumption of honeybees

Honeybee stage	**Pollen consumption (mg/bee/day or mg/larva)**
Forager bee	0
Nurse bee	6.5 – 12
Larva	1.5 – 2

According to Appendix N of the EFSA Guidance Document for bees, the daily residu uptake for adult bees and the total residue uptake for larvae can be calculated based on the nectar and pollen consumption, 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)
R_n is the residue level in nectar (in mg/kg)
R_p is the residue level in pollen (in mg/kg)
C_n is the consumption of nectar in mg (mg/bee/day or mg/larva)
C_p is the consumption of pollen in mg (mg/bee/day or mg/larva)

As maize and potato do not produce nectar, this formula can be simplified to:

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

In the Initial version of this Addendum, the worst case values for pollen consumption from Table B.9.7.3.1-3 were used for the calculation of the residue intake (RI). At Pesticides Peer Review Meeting 145, it was noted that this approach is acceptable, but represents a worst case. A tool for calculating refined shortcut values based on compound or crop specific input parameters (SHVAL Tool, see Appendix Z of the EFSA Guidance Document on bees and EFSA supporting publication 2014:EN-623[42]) has been developed by EFSA. The SHVAL tool, which is an application developed in R, allows for inputting raw data as well as reference values (central tendency measurements / ranges). It first fits theoretical distributions to the data, where possible, and then it runs a Monte Carlo simulation mimicking an hypothetical field study on 1000 fields with 1000 hives in each field and 1000 bees in each hive. The SHVAL tool returns the probabilistic distributions fitted to the data and the empirical density distribution of the Shortcut Value's 90th percentile over the 1000 iterations (fields). This way, this tool allows for the estimation of the Shortcut Value's 90th percentile and its 95% confidence interval. The refined Shortcut Values obtained by using the SHVAL tool are considered more representative than a calculation only based on maximum or mean value for pollen and nectar consumption. The experts agreed that this SHVAL tool should be used to update the Tier 2 risk assessment based on the agreed residue values for pollen in maize and potatoi. The calculation of refined shortcut values was therefore updated using the EFSA Shortcut Values calculation model (EFSA SHVAL model), version 1.0. This application interface can be made available upon request to amu@efsa.europa.eu.

As discussed above, clothianidin residues of 20 µg/kg in maize pollen and 31 µg/kg in potato pollen were used, as agreed at Pesticides Peer Review Meeting 145. The application rates of the studies from which these values were obtained were 110 g a.s./ha and 80 g a.s./ha for maize and potato, respectively. Taking the application rates into account, RUD values were calculated, as shown in Table B.9.7.3.1-4.

Table B.9.7.3.1-4: Calculated RUD values for maize and potato treated with clothianidin as a granular application.

Crop	Application rate	Residue in pollen	RUD
maize	0.11 kg/ha	0.020 mg/kg	0.1818 mg/kg
potato	0.08 kg/ha	0.031 mg/kg	0.3875 mg/kg

[42] European Food Safety Authority (2014). A small application developed in R for the estimation of the residue intake rate for certain bee species under given conditions: the SHVAL tool. EFSA supporting publication 2014:EN-623. 15 pp.

For the calculations made with the SHVAL tool, some 'test' calculations were made to check whether the tool, the PC and the user perform well. In these tests the same input parameters were used as those that had been used for the calculation of the tier 1 Shortcut Values for nurse honeybees and bumblebees adult chronic for the seed dressing use and granular use (before emergence) (for the value of Ln = -20, see explanation below). The other calculations were made for clothianidin 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 shown in Table B.9.7.3.1-5. As maize and potato do not produce nectar, there are no residue values for this matrix. Since 0 mg/kg cannot be expressed in Ln form and to be able to run the model, a very low value of -20 (which is in the order of 10^{-10} mg/kg) was used for the nectar concentrations. This will have a very negligible (practically no) effect on the calculated tier 2 SVs. Table B.9.7.3.1-6 shows a summary of all input parameters inserted in the SHVAL tool for the different bee categories. The values for pollen consumption were derived from Table B.9.7.3.1-3.

Table B.9.7.3.1-5: Residue levels used as input for the calculation of the refined Shortcut Values using the EFSA SHVAL tool.

Relevance	Residue level in mg/kg	Ln
Test	1	0
Clothianidin in maize pollen	0.1818	-1.70485
Clothianidin in potato pollen	0.3875	-0.94804

Table B.9.7.3.1-6: Input parameters used for the calculations with the SHVAL tool for the different honeybee categories.

No.	bee type & category	Pollen consumption (mg/bee/day or mg/larvae)	Sugar consumption (mg/bee/day or mg/larvae)	Sugar content of nectar (mg/mg)	chemical conc. in pollen[1]	chemical conc. in nectar[1]	Relevance
1	HB nurse	12	34-50	0.15	0	0	Test
2	BB chronic	30.3	73-149	0.15	0	0	Test
3	HB nurse	12	34-50	0.15	0	-20	Test
4	BB chronic	30.3	73-149	0.15	0	-20	Test
5	HB nurse	12	34-50	0.15	-1.70485	-20	Maize
6	HB larva	2	59.4	0.15	-1.70485	-20	Maize
7	HB nurse	12	34-50	0.15	-0.94804	-20	Potato
8	HB larva	2	59.4	0.15	-0.94804	-20	Potato

[1]*See Table B.9.7.3.1-5; HB: honeybee; BB: bumblebee*

The resulting refined Shortcut Values (SV) are shown in Table B.9.7.3.1-7. The tier 2 SVs for maize and potato are considerable, but less than one order of magnitude lower than the tier 1 SVs considering the RUD values of 0.1818 mg/kg and 0.3875 mg/kg, respectively.

Table B.9.7.3.1-7: Calculated Tier 2 Shortcut Values (SV) for the different scenarios and honeybee stages

No.	Relevance	bee type & category	Tier 2 SV (µg/bee or µg/bee/day or µg/larva)	Comment
1	test	HB nurse	0.29319	Expected value was 0.29
2	test	BB chronic	0.77595	Expected value was 0.78
3	test	HB nurse	0.0120	Expected value was 0.012
4	test	BB chronic	0.0303	Expected value was 0.03
5	Maize	HB nurse	0.00218	
6	Maize	HB larva	0.00036	
10	Potato	HB nurse	0.00465	
11	Potato	HB larva	0.00078	

To calculate the Tier 2 ETR values, the same equations as for the Tier 1 risk assessment are used. As Shortcut Values (SV), the values calculated with the SHVAL tool (Table B.9.7.3.1-7) are used instead of those reported in Appendix J of the EFSA Guidance Document for bees. The same trigger values as for the Tier 1 assessment are considered. The calculated Tier 2 ETR values are shown in Table B.9.7.3.1-8.

Table B.9.7.3.1-8: Tier 2 ETR calculations for acute adult oral, chronic adult oral and larval exposure for the highest authorized 'maximum application rate' of clothianidin in potato and maize.

Acute adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Potato	0.070	1	0.00465	-	0.00379	0.086	0.2
Maize/ Sweet maize	0.050	1	0.00218	-	0.00379	0.029	0.2
Chronic adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	LDD_{50} (µg a.s./bee/day)	ETR	Trigger
Potato	0.070	1	0.00465	1	0.00138	0.327	0.03
Maize/ Sweet maize	0.050	1	0.00218	1	0.00138	0.079	0.03
Larval exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	NOED (µg a.s./larva /development period)	ETR	Trigger
Potato	0.070	1	0.00078	1	0.00528	0.010	0.2
Maize/ Sweet maize	0.050	1	0.00036	1	0.00528	0.003	0.2

The ETR values for acute adult oral exposure and for larvae are below the relevant trigger for both the use in potato and maize. However, again for both crops, the ETR for chronic adult exposure exceeds the trigger, indicating a potential risk. Further consideration is thus necessary.

Higher tier risk assessment

Further refinements to the risk assessment could be based on field effect studies. For the use in maize, a three year study on the effects on colony survival due to exposure to pollen from maize plants treated with clothianidin containing granules at sowing was submitted by the applicant. In this study, clothianidin was applied at 110 g a.s./ha, which covers the maximum application rate for clothianidin used as granular treatment in maize (50 g a.s./ha). Therefore, the available study is considered representative for the currently registered uses.

The available study in maize (Thompson, 2011b) was conducted over three consecutive years using the same experimental design so far as was practical with the same colonies on the same field. The study was performed at three test locations, situated in representative maize-growing areas in southern, central and northern France. In each area, two different fields were used: one treated with the test item and one control. At each field, six colonies were placed, resulting in a total of 36 colonies that were exposed (18 treated and 18 untreated). While the number of sites is limited (3 instead of at least 5 as recommended by the EFSA Guidance Document on bees), they are well spread over Europe (north and south) and were monitored for three consecutive years.

In all three years of the study, observations of honeybee behaviour in maize and the number of foragers returning to the hive with pollen (together with information on the composition of the forager pollen loads) showed that bees were exposed to the maize crops (see also Section B.9.7.2). Further, residue analysis of samples taken from the crop, pollen loads and colonies confirmed exposure of the bees to the treatment in both the main field and tunnels erected over the treated crop. Residue levels in the pollen load and pollen trap samples from the tunnels were similar to those in the samples of

pollen taken from the plants in the tunnels and main field. Samples of pollen from the main field pollen traps and pollen loads were lower than those from the tunnels or samples of pollen collected from the crop suggesting the bees were also foraging on non-maize food sources (resulting in some dilution of the residue levels). This was confirmed by visual assessment of the proportion of maize pollen being returned to the hive, confirming maize is not a highly attractive source of pollen for bees. A low level exposure within the hives could be demonstrated, with samples of pollen and wax taken from the colonies showing that exposure was transient (no residues were detected in pollen 1 month after the end of the exposure period and no significant residues were detected in wax).

Despite this clear pattern of exposure on the treated fields, the results for mortality show no adverse effects of the treatment at any of the sites. At all sites the daily mortality did not exceed a mean of 25 bees per day on any single day except on isolated occasions when robbing of hives occurred following assessments or feeding. The strength of the colonies during and after the exposure phase was assessed by data collected for the number of bees present in the colonies, brood strength and food stores (pollen and nectar). There were no apparent differences in the development of the colonies between the treated and control sites during the exposure phase or in the monitoring phase up to the over-wintering period. At the beginning of the following season, after the overwintering, some losses were seen in the colonies from both the treated and the control plots at all three test sites. However, these losses could be ascribed to failing queens or poor *Varroa* control and were similar to those experienced by local beekeepers. Overall, there were no detectable effects of exposure to clothianidin residues in maize pollen on honeybee mortality and colony development in the three sites over the three years.

This study was previously reviewed by EFSA (see Study 24 in the Study evaluation notes supporting the EFSA Conclusion on the risk assessment for bees for clothianidin, 2013). This review considered that the study showed several deficiencies, which made questionable the possibility to assess long-term effects taking into account several overwintering periods, and suggested that further analysis would be useful before using this study in the regulatory risk assessment. An additional statistical analysis of the data from the three year study in maize was performed by the applicant, an indicated that this study has a relatively low power for a number of measures of colony health (number of frames of bees, the number of frames of brood and the amount of nectar). However, the power of the study to detect given levels of effects will be considerably greater than normally achieved with higher tier bee studies currently conducted. Also, the additional statistical analysis of potential study designs demonstrates that field trials by their very nature will be limited in what they can achieve from a statistical point of view. This is due to the inherent variability of real world data and the limitations of what can be practically achieved. However, while statistical analysis provide useful tools to aid in the evaluation of field trial data, their primary value is in their biological interpretation, assessing effects at the relevant organisational level (populations, colonies etc) under realistic conditions. In this sense, it was concluded in the original version of this Addendum that the available 3-year maize study provided useful information to assess the impact of clothianidin granule treatments in maize on honeybee colonies. The review by EFSA also pointed out that there was a lack of background information for the normal bee and colony health status. Data available in literature was used by the applicant to demonstrate that the level of colony losses found within the three year maize study were consistent with the reported contemporaneous losses from local beekeepers in the areas in which the trial was conducted.

During Peer Review of the original version of this Addendum, several concerns were raised regarding the field study on maize by Thompson (2011b) and the study re-analysis by Lewis (2014). These issues were discussed at Pesticides Peer Review Meeting 145. The statistical power was discussed in relation to the high inter-colony variability observed. It was argued that the study has a low statistical power (assuming that the observed variability is a suitable estimation of the real natural variability). It was noted that most of the variability (c. 90%) was due to the inter-colony factor rather than inter-site and temporal factors. This may mean that the number of hives per site is more relevant in terms of statistical power than the number of sites. However, it was argued that the analysis was performed on a

limited numbers of hives and sites and that therefore the variability partitioning observed in this study may not represent the real natural variability. Further, it was noted that the RMS pointed out the relevance of the biological interpretation of field trials.

It was concluded that, generally, when the results are highly variable (which is the case for the study by Thompson, 2011b) it is difficult to draw any conclusion on a cause – effect relationship (i.e. treatment or non-treatment related effects). Overall, it was agreed that the re-analysis provided for the study is not sufficient to address the concerns already identified in the conclusion of EFSA 2013 (i.e., the Thompson study cannot be considered sufficient to draw a firm conclusion on the cause-effect relationship).

Generally, it was acknowledged that the availability of several pieces of evidence (e.g. several comparable field studies) can be useful to make a trend analysis to be used as a weight of evidence for the risk assessment. However, apart from the study by Thompson (2011b) no other field studies are available. Consequently, no acceptable chronic risk to honeybees following exposure to contaminated nectar and pollen from maize as a treated crop could be demonstrated.

For the use in potato and sorghum, no field effect studies are available. Consequently, no higher tier assessment could be performed for these uses, and no acceptable chronic risk to honeybees following exposure to contaminated nectar and pollen from potato and sorghum as a treated crop could be demonstrated.

Conclusions:
The acute risk to adult honeybees and chronic risk to honeybee larvae from consumption of contaminated pollen from treated maize and sorghum crops was acceptable at tier 1. The chronic risk to adult honeybees was however not acceptable. For potato as treated crop, the risk to honeybees was not acceptable at tier 1. Refinement of the assessment based on measured clothianidin residues in potato and maize resulted in an acceptable acute risk to adult honeybees and chronic risk to honeybee larvae for both crops. The chronic risk to adult honeybees was however still not acceptable. The available higher tier field effect studies were not considered sufficient to demonstrate an acceptable chronic risk.

B.9.7.3.2. Risk assessment for bumblebees

The risk assessment was performed following the risk assessment scheme for bumblebees as proposed in the EFSA Guidance Document on bees. Due to the potential risk to bumblebees from the consumption of pollen and nectar from succeeding crops, the screening step was not performed, and the risk assessment started at the first tier. As there is a potential exposure to bumblebees through the consumption of pollen from maize, potato and sorghum (see section B.9.7.2), the risk assessment was performed for the uses in these three crops. As based on the information available in the GAP table (Table A-1) it is not possible to perform a risk assessment for the use in forestry nursery (only the dose in g a.s./plant is available, no information on the plant density and dose in g a.s./ha), a risk assessment for this use is not included.

It is noted that for the indoor use of clothianidin in maize and sweet maize (which is restricted to permanent greenhouses), exposure to bees from nectar and pollen from the treated crop. Therefore, in line with the decision of Pesticides Peer Review Meeting 129, no risk assessment needs to be performed for this indoor use.

First tier risk assessment

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 products applied as granules at sowing (incorporated into the soil). The relevant shortcut values (and the methodology used to determine these values) are

presented in Table J6 of Appendix J of the EFSA Guidance Document. As maize, potato and sorghum do 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.

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 and/or mg/seed
SV = 0.030 (shortcut value for acute exposure to adult bumblebees, taken from Table J6 in Appendix J of the Guidance Document)
E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for the succeeding crop scenario)
$LD_{50,oral}$ is expressed as µg a.s./bee

If this ETR > 0.0036, 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 *ETR for the chronic adult oral exposure* is calculated by the following equation:

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

Where: AR = application rate in kg a.s./ha and/or mg/seed
SV = 0.030 (shortcut value for chronic exposure to adult bumblebees, taken from Table J6 in Appendix J of the Guidance Document)
E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for the succeeding crop scenario)
twa = 1
LDD_{50} is expressed as µg a.s./bee per day

If this ETR > 0.0048, 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 *ETR for larvae* is calculated by the following equation:

$$ETR_{larvae} = \frac{AR * E_f * SV * 10 * twa}{NOED}$$

Where: AR = application rate in kg a.s./ha and/or mg/seed
SV = 0.040 (shortcut value for honeybee larvae, taken from Table J6 in Appendix J of the Guidance Document). Factor 10 is to consider the food consumption of larvae over a 10-day developmental period
E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for the succeeding crop scenario)
twa = 1
NOED is expressed as µg a.s./larva/development period

If this ETR > 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 has been performed using the authorized ‘maximum application rate’ for potato, maize and sorghum (see Table B.9.7.3.2-1). The relevant toxicity endpoints are taken from

Table B.9.1.3.1-3. As discussed in that section, there is no larval toxicity endpoint available for bumblebees, and it is also not possible to determine a surrogate endpoint based on that larval toxicity endpoint for honeybees. As a result, the risk assessment for bumblebee larvae could not be performed. The Tier 1 ETR values calculated for adult bumblebees are shown in Table B.9.7.3.2-2.

Table B.9.7.3.2-1: currently authorized 'maximum application rate' of clothianidin containing formulations for use as a granule treatment at sowing in potato, maize and sorghum.

Crop	**authorized 'maximum application rate'**
Potato	70 g a.s./ha
Maize/ Sweet maize/ Sorghum	50 g a.s./ha

Table B.9.7.3.2-2: Tier 1 ETR calculations for acute adult oral and chronic adult oral exposure for the highest authorized 'maximum application rate' of clothianidin in potato, maize and sorghum.

Acute adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Potato	0.070	1	0.030	-	0.00191	1.10	0.036
Maize/ Sweet maize/ Sorghum	0.050	1	0.030	-	0.00191	0.79	0.036
Chronic adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	LDD_{50} (µg a.s./bee/day)	ETR	Trigger
Potato	0.070	1	0.030	1	0.000138	15.2	0.0048
Maize/ Sweet maize/ Sorghum	0.050	1	0.030	1	0.000138	10.9	0.0048

As all ETR values exceed the relevant trigger values, a potential risk is identified for adult bumblebees 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, 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 in which the clothianidin residues in pollen from potato and maize were measured. As discussed under Section B.9.7.2, these studies are considered acceptable, and residue values suitable for use in the risk assessment were selected. As data from less than 5 study fields was available for each crop, it was agreed at Pesticides Peer Review Meeting 145 that as a conservative approach, the highest available residue values should be used (20 µg/kg for maize and 31 µg/kg for potato). As these values were obtained treating the potato or maize crop with 80 g a.s./ha and 110 g a.s./ha, the selected residue values cover the treated crop scenarios for all registered uses of clothianidin as granule. For sorghum, no measured clothianidin residues in pollen are available. Therefore, the Tier 2 assessment could not be performed for the use in sorghum.

In table J1 of appendix J of the EFSA Guidance Document on bees, data on the consumption of nectar and pollen by bumblebee adults and larvae are reported. The values for pollen are shown in Table B.9.7.3.2-3.

Table B.9.7.3.2-3: Pollen consumption of bumblebees

Bumblebee stage	**Pollen consumption (mg/bee/day or mg/larva)**
Adult bees	26.6 – 30.3
Larva	10.3 – 39.5

According to Appendix N of the EFSA Guidance Document for bees, the daily residu uptake for adult bees and the total residue uptake for larvae can be calculated based on the nectar and pollen consumption, 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)
R_n is the residue level in nectar (in mg/kg)
R_p is the residue level in pollen (in mg/kg)
C_n is the consumption of nectar in mg (mg/bee/day or mg/larva)
C_p is the consumption of pollen in mg (mg/bee/day or mg/larva)

As maize and potato do not produce nectar, this formula can be simplified to:

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

In the Initial version of this Addendum, the worst case values for pollen consumption from Table B.9.7.3.2-3 were used for the calculation of the residue intake (RI). At Pesticides Peer Review Meeting 145, it was noted that this approach is acceptable, but represents a worst case. A tool for calculating refined shortcut values based on compound or crop specific input parameters (SHVAL Tool, see Appendix Z of the EFSA Guidance Document on bees and EFSA supporting publication 2014:EN-623[43]) has been developed by EFSA. The SHVAL tool, which is an application developed in R, allows for inputting raw data as well as reference values (central tendency measurements / ranges). It first fits theoretical distributions to the data, where possible, and then it runs a Monte Carlo simulation mimicking an hypothetical field study on 1000 fields with 1000 hives in each field and 1000 bees in each hive. The SHVAL tool returns the probabilistic distributions fitted to the data and the empirical density distribution of the Shortcut Value's 90th percentile over the 1000 iterations (fields). This way, this tool allows for the estimation of the Shortcut Value's 90th percentile and its 95% confidence interval. The refined Shortcut Values obtained by using the SHVAL tool are considered more representative than a calculation only based on maximum or mean value for pollen and nectar consumption. The experts agreed that this SHVAL tool should be used to update the Tier 2 risk assessment based on the agreed residue values for pollen in maize and potato. The calculation of refined shortcut values was therefore updated using the EFSA Shortcut Values calculation model (EFSA SHVAL model), version 1.0. This application interface can be made available upon request to amu@efsa.europa.eu.

As discussed above, clothianidin residues of 20 µg/kg in maize pollen and 31 µg/kg in potato pollen were used, as agreed at Pesticides Peer Review Meeting 145. The application rates of the studies from which these values were obtained were 110 g a.s./ha and 80 g a.s./ha for maize and potato, respectively. Taking the application rates into account, RUD values were calculated, as shown in Table B.9.7.3.2-4.

Table B.9.7.3.1-4: Calculated RUD values for maize and potato treated with clothianidin as a granular application.

Crop	Application rate	Residue in pollen	RUD
maize	0.11 kg/ha	0.020 mg/kg	0.1818 mg/kg
potato	0.08 kg/ha	0.031 mg/kg	0.3875 mg/kg

[43] European Food Safety Authority (2014). A small application developed in R for the estimation of the residue intake rate for certain bee species under given conditions: the SHVAL tool. EFSA supporting publication 2014:EN-623. 15 pp.

For the calculations made with the SHVAL tool, some 'test' calculations were made to check whether the tool, the PC and the user perform well. In these tests the same input parameters were used as those that had been used for the calculation of the tier 1 Shortcut Values for nurse honeybees and bumblebees adult chronic for the seed dressing use and granular use (before emergence) (for the value of Ln = -20, see explanation below). The other calculations were made for clothianidin 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 shown in Table B.9.7.3.2-5. As maize and potato do not produce nectar, there are no residue values for this matrix. Since 0 mg/kg cannot be expressed in Ln form and to be able to run the model, a very low value of -20 (which is in the order of 10^{-10} mg/kg) was used for the nectar concentrations. This will have a very negligible (practically no) effect on the calculated tier 2 SVs. Table B.9.7.3.2-6 shows a summary of all input parameters inserted in the SHVAL tool for the different bee categories. The values for pollen consumption were derived from Table B.9.7.3.2-3.

Table B.9.7.3.2-5: Residue levels used as input for the calculation of the refined Shortcut Values using the EFSA SHVAL tool.

Relevance	Residue level in mg/kg	Ln
Test	1	0
Clothianidin in maize pollen	0.1818	-1.70485
Clothianidin in potato pollen	0.3875	-0.94804

Table B.9.7.3.2-6: Input parameters used for the calculations with the SHVAL tool for the different bumblebee categories.

No.	bee type & category	Pollen consumption (mg/bee/day or mg/larvae)	Sugar consumption (mg/bee/day or mg/larvae)	Sugar content of nectar (mg/mg)	chemical conc. in pollen[1]	chemical conc. in nectar[1]	Relevance
1	HB nurse	12	34-50	0.15	0	0	Test
2	BB chronic	30.3	73-149	0.15	0	0	Test
3	HB nurse	12	34-50	0.15	0	-20	Test
4	BB chronic	30.3	73-149	0.15	0	-20	Test
5	BB acute	30.3	111-149	0.15	-1.70485	-20	Maize
6	BB chronic	30.3	73-149	0.15	-1.70485	-20	Maize
7	BB acute	30.3	111-149	0.15	-0.94804	-20	Potato
8	BB chronic	30.3	73-149	0.15	-0.94804	-20	Potato

[1]*See Table B.9.7.3.2-5; HB: honeybee; BB: bumblebee*

The resulting refined Shortcut Values (SV) are shown in Table B.9.7.3.2-7. The tier 2 SVs for maize and potato are considerable, but less than one order of magnitude lower than the tier 1 SVs considering the RUD values of 0.1818 mg/kg and 0.3875 mg/kg, respectively.

Table B.9.7.3.2-7: Calculated Tier 2 Shortcut Values (SV) for the different scenarios and bumblebee stages

No.	Relevance	bee type & category	Tier 2 SV (µg/bee or µg/bee/day or µg/larva)	Comment
1	test	HB nurse	0.29319	Expected value was 0.29
2	test	BB chronic	0.77595	Expected value was 0.78
3	test	HB nurse	0.0120	Expected value was 0.012
4	test	BB chronic	0.0303	Expected value was 0.03
5	Maize	BB acute	0.00551	
6	Maize	BB chronic	0.00551	
7	Potato	BB acute	0.01174	
8	Potato	BB chronic	0.01174	

To calculate the Tier 2 ETR values, the same equations as for the Tier 1 risk assessment are used. As Shortcut Values (SV), the values calculated with the SHVAL tool (Table B.9.7.3.2-7) are used instead of those reported in Appendix J of the EFSA Guidance Document for bees. The same trigger values as for the Tier 1 assessment are considered. The calculated Tier 2 ETR values are shown in Table B.9.7.3.2-8.

Table B.9.7.3.2-8: Tier 2 ETR calculations for acute adult oral and chronic adult oral exposure for the highest authorized 'maximum application rate' of clothianidin in potato and maize.

Acute adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Potato	0.070	1	0.01174	-	0.00191	0.430	0.036
Maize/ Sweet maize	0.050	1	0.00551	-	0.00191	0.144	0.036
Chronic adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	LDD_{50} (µg a.s./bee/day)	ETR	Trigger
Potato	0.070	1	0.01174	1	0.000138	5.955	0.0048
Maize/ Sweet maize	0.050	1	0.00551	1	0.000138	1.996	0.0048

The ETR values for acute and chronic adult oral exposure for both the use in maize and potato exceed the trigger, indicating a potential risk. Further consideration is thus necessary.

Higher tier risk assessment

Further refinements to the risk assessment could be based on field effect studies. However, no higher tier effect studies are available to assess the risk to bumblebees from the consumption of pollen in maize, potato and sorghum as treated crops. Consequently, the risk assessment could not be finalized.

The applicant provided the following comment on the performed risk assessment (*text in italic*):
There are no agreed methods for conducting higher tier (field) studies with bumblebees. This means that the sequential testing pathway is incomplete, which is essential for any active substance such as clothianidin that do not pass the initial tiers, and in such circumstances it means that currently it will always be not possible to finalize the risk assessment. It is therefore considered premature to be carrying out risk assessments for bumblebees.

Conclusions

The risk to bumblebees from consumption of contaminated pollen and nectar in maize, potato and sorghum as treated crops was not acceptable at tier 1. Refinement of the assessment based on measured clothianidin residues in a maize and potato pollen did not result in an acceptable risk. As there are no higher tier effect studies available, the risk assessment could not be finalized.

The risk assessment for effects on bumblebee larvae could not be finalized due to lack of a suitable toxicity endpoint for bumblebee larvae for clothianidin.

B.9.7.3.3. Risk assessment for solitary bees

The risk assessment was performed following the risk assessment scheme for solitary bees as proposed in the EFSA Guidance Document on bees. Due to the potential risk to solitary bees from the consumption of pollen and nectar from succeeding crops, the screening step was not performed, and the risk assessment started at the first tier. As there is a potential exposure to solitary bees through the consumption of pollen from maize, potato and sorghum (see section B.9.7.2), the risk assessment was performed for the uses in these three crops. As based on the information available in the GAP table

(Table A-1) it is not possible to perform a risk assessment for the use in forestry nursery (only the dose in g a.s./plant is available, no information on the plant density and dose in g a.s./ha), a risk assessment for this use is not included.

It is noted that for the indoor use of clothianidin in maize and sweet maize (which is restricted to permanent greenhouses), exposure to bees from nectar and pollen from the treated crop. Therefore, in line with the decision of Pesticides Peer Review Meeting 129, no risk assessment needs to be performed for this indoor use.

First tier risk assessment

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 products applied as granules at sowing (incorporated into the soil). The relevant shortcut values (and the methodology used to determine these values) are presented in Table J6 of Appendix J of the EFSA Guidance Document. As maize, potato and sorghum do 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.

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 and/or mg/seed
SV = 0.010 (shortcut value for exposure to adult solitary bees, taken from Table J6 in Appendix J of the Guidance Document)
E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for the succeeding crop scenario)
$LD_{50,oral}$ is expressed as µg a.s./bee

If this ETR > 0.04, 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 *ETR for the chronic adult oral exposure* is calculated by the following equation:

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

Where: AR = application rate in kg a.s./ha and/or mg/seed
SV = 0.010 (shortcut value for exposure to adult solitary bees, taken from Table J6 in Appendix J of the Guidance Document)
E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for the succeeding crop scenario)
twa = 1
LDD_{50} is expressed as µg a.s./bee per day

If this ETR > 0.0054, 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 *ETR for larvae* is calculated by the following equation:

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

Where: AR = application rate in kg a.s./ha and/or mg/seed
SV = 0.39 (shortcut value for solitary bee larvae, taken from Table J6 in Appendix J of the Guidance Document).
E_f = 1 (According to Appendix X of the Guidance Document, no exposure factor (or an E_f of 1) should be used for the succeeding crop scenario)
twa = 1
NOED is expressed as µg a.s./larva/development period

If this ETR > 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 has been performed using the authorized 'maximum application rate' for potato, maize and sorghum (see Table B.9.7.3.3-1). The relevant toxicity endpoints are taken from Table B.9.1.3.1-3. As discussed in that section, there is no larval toxicity endpoint available for solitary bees, and it is also not possible to determine a surrogate endpoint based on that larval toxicity endpoint for honeybees. As a result, the risk assessment for solitary bee larvae could not be performed. The Tier 1 ETR values calculated for adult solitary bees are shown in Table B.9.7.3.3-2.

Table B.9.7.3.3-1: currently authorized 'maximum application rate' of clothianidin containing formulations for use as a granule treatment at sowing in potato, maize and sorghum.

Crop	**authorized 'maximum application rate'**
Potato	70 g a.s./ha
Maize/ Sweet maize/ Sorghum	50 g a.s./ha

Table B.9.7.3.3-2: Tier 1 ETR calculations for acute adult oral and chronic adult oral exposure for the highest authorized 'maximum application rate' of clothianidin in potato, maize and sorghum.

Acute adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Potato	0.070	1	0.010	-	0.000379	1.85	0.04
Maize/ Sweet maize/ Sorghum	0.050	1	0.010	-	0.000379	1.32	0.04
Chronic adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	LDD_{50} (µg a.s./bee/day)	ETR	Trigger
Potato	0.070	1	0.010	1	0.000138	5.07	0.0054
Maize/ Sweet maize/ Sorghum	0.050	1	0.010	1	0.000138	3.62	0.0054

As all ETR values exceed the relevant trigger values, a potential risk is identified for adult solitary 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. 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.

The applicant submitted a number of studies in which the clothianidin residues in pollen from potato and maize were measured. As discussed under Section B.9.7.2, these studies are considered acceptable, and residue values suitable for use in the risk assessment were selected. As data from less than 5 study fields was available for each crop, it was agreed at Pesticides Peer Review Meeting 145 that as a conservative approach, the highest available residue values should be used (20 µg/kg for maize and 31 µg/kg for potato).As these values were obtained treating the potato or maize crop with 80 g a.s./ha and 110 g a.s./ha, the selected residue values cover the treated crop scenarios for all

registered uses of clothianidin as granule. For sorghum, no measured clothianidin residues in pollen are available. Therefore, the Tier 2 assessment could not be performed for the use in sorghum.

In table J1 of appendix J of the EFSA Guidance Document on bees, data on the consumption of nectar and pollen by solitary bee adults and larvae are reported. The values for pollen are shown in Table B.9.7.3.3-3.

Table B.9.7.3.3-3: Pollen consumption of solitary bees

Solitary bee stage	Pollen consumption (mg/bee/day or mg/larva)
Adult bees	10.2
Larva	387

According to Appendix N of the EFSA Guidance Document for bees, the daily residu uptake for adult bees and the total residue uptake for larvae can be calculated based on the nectar and pollen consumption, 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)
R_n is the residue level in nectar (in mg/kg)
R_p is the residue level in pollen (in mg/kg)
C_n is the consumption of nectar in mg (mg/bee/day or mg/larva)
C_p is the consumption of pollen in mg (mg/bee/day or mg/larva)

As maize and potato do not produce nectar, this formula can be simplified to:

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

In the Initial version of this Addendum, the worst case values for pollen consumption from Table B.9.7.3.1-3 were used for the calculation of the residue intake (RI). At Pesticides Peer Review Meeting 145, it was noted that this approach is acceptable, but represents a worst case. A tool for calculating refined shortcut values based on compound or crop specific input parameters (SHVAL Tool, see Appendix Z of the EFSA Guidance Document on bees and EFSA supporting publication 2014:EN-623[44]) has been developed by EFSA. The SHVAL tool, which is an application developed in R, allows for inputting raw data as well as reference values (central tendency measurements / ranges). It first fits theoretical distributions to the data, where possible, and then it runs a Monte Carlo simulation mimicking an hypothetical field study on 1000 fields with 1000 hives in each field and 1000 bees in each hive. The SHVAL tool returns the probabilistic distributions fitted to the data and the empirical density distribution of the Shortcut Value's 90th percentile over the 1000 iterations (fields). This way, this tool allows for the estimation of the Shortcut Value's 90th percentile and its 95% confidence interval. The refined Shortcut Values obtained by using the SHVAL tool are considered more representative than a calculation only based on maximum or mean value for pollen and nectar consumption. The experts agreed that this SHVAL tool should be used to update the Tier 2 risk assessment based on the agreed residue values for pollen in maize and potato. The calculation of refined shortcut values was therefore updated using the EFSA Shortcut Values calculation model (EFSA SHVAL model), version 1.0. This application interface can be made available upon request to amu@efsa.europa.eu.

[44] European Food Safety Authority (2014). A small application developed in R for the estimation of the residue intake rate for certain bee species under given conditions: the SHVAL tool. EFSA supporting publication 2014:EN-623. 15 pp.

As discussed above, clothianidin residues of 20 µg/kg in maize pollen and 31 µg/kg in potato pollen were used, as agreed at Pesticides Peer Review Meeting 145. The application rates of the studies from which these values were obtained were 110 g a.s./ha and 80 g a.s./ha for maize and potato, respectively. Taking the application rates into account, RUD values were calculated, as shown in Table B.9.7.3.3-4.

Table B.9.7.3.3-4: Calculated RUD values for maize and potato treated with clothianidin as a granular application.

Crop	Application rate	Residue in pollen	RUD
maize	0.11 kg/ha	0.020 mg/kg	0.1818 mg/kg
potato	0.08 kg/ha	0.031 mg/kg	0.3875 mg/kg

For the calculations made with the SHVAL tool, some 'test' calculations were made to check whether the tool, the PC and the user perform well. In these tests the same input parameters were used as those that had been used for the calculation of the tier 1 Shortcut Values for nurse honeybees and bumblebees adult chronic for the seed dressing use and granular use (before emergence) (for the value of Ln = -20, see explanation below). The other calculations were made for clothianidin 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 shown in Table B.9.7.3.3-5. As maize and potato do not produce nectar, there are no residue values for this matrix. Since 0 mg/kg cannot be expressed in Ln form and to be able to run the model, a very low value of -20 (which is in the order of 10^{-10} mg/kg) was used for the nectar concentrations. This will have a very negligible (practically no) effect on the calculated tier 2 SVs. Table B.9.7.3.3-6 shows a summary of all input parameters inserted in the SHVAL tool for the different bee categories. The values for pollen consumption were derived from Table B.9.7.3.3-3.

Table B.9.7.3.3-5: Residue levels used as input for the calculation of the refined Shortcut Values using the EFSA SHVAL tool.

Relevance	Residue level in mg/kg	Ln
Test	1	0
Clothianidin in maize pollen	0.1818	-1.70485
Clothianidin in potato pollen	0.3875	-0.94804

Table B.9.7.3.3-6: Input parameters used for the calculations with the SHVAL tool for the different solitary bee categories.

No.	bee type & category	Pollen consumption (mg/bee/day or mg/larvae)	Sugar consumption (mg/bee/day or mg/larvae)	Sugar content of nectar (mg/mg)	chemical conc. in pollen[1]	chemical conc. in nectar[1]	Relevance
1	HB nurse	12	34-50	0.15	0	0	Test
2	BB chronic	30.3	73-149	0.15	0	0	Test
3	HB nurse	12	34-50	0.15	0	-20	Test
4	BB chronic	30.3	73-149	0.15	0	-20	Test
5	SB adult	10.2	18-77	0.10	-1.70485	-20	Maize
6	SB adult	10.2	18-77	0.10	-0.94804	-20	Potato

[1]*See Table B.9.7.3.3-5; HB: honeybee; BB: bumblebee; SB: solitary bee*

Table B.9.7.3.1-7: Calculated Tier 2 Shortcut Values (SV) for the different scenarios and solitary bee stages

No.	Relevance	bee type & category	Tier 2 SV (µg/bee or µg/bee/day or µg/larva)	Comment
1	test	HB nurse	0.29319	Expected value was 0.29
2	test	BB chronic	0.77595	Expected value was 0.78
3	test	HB nurse	0.0120	Expected value was 0.012
4	test	BB chronic	0.0303	Expected value was 0.03
5	Maize	SB adult	0.00185	
6	Potato	SB adult	0.00395	

To calculate the Tier 2 ETR values, the same equations as for the Tier 1 risk assessment are used. As Shortcut Values (SV), the values calculated with the SHVAL tool (Table B.9.7.3.3-7) are used instead of those reported in Appendix J of the EFSA Guidance Document for bees. The same trigger values as for the Tier 1 assessment are considered. The calculated Tier 2 ETR values are shown in Table B.9.7.3.3-8.

Table B.9.7.3.3-8: Tier 2 ETR calculations for acute adult oral and chronic adult oral exposure for the highest authorized 'maximum application rate' of clothianidin in potato and maize.

Acute adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Potato	0.070	1	0.00395	-	0.000379	0.730	0.04
Maize/ Sweet maize	0.050	1	0.00185	-	0.000379	0.244	0.04
Chronic adult oral exposure							
Crop	Application rate (kg a.s./ha)	E_f	SV	twa	LDD_{50} (µg a.s./bee/day)	ETR	Trigger
Potato	0.070	1	0.00395	1	0.000138	2.004	0.0054
Maize/ Sweet maize	0.050	1	0.00185	1	0.000138	0.670	0.0054

The ETR values for acute and chronic adult oral exposure for both the use in maize and potato exceed the trigger, indicating a potential risk. Further consideration is thus necessary.

Higher tier risk assessment

Further refinements to the risk assessment could be based on field effect studies. However, no higher tier effect studies are available to assess the risk to solitarybees from the consumption of pollen in maize, potato and sorghum as treated crops. Consequently, the risk assessment could not be finalized.

The applicant provided the following comment on the performed risk assessment (*text in italic*):
There are no agreed methods for conducting higher tier (field) studies with solitary bees. This means that the sequential testing pathway is incomplete, which is essential for any active substance such as clothianidin that do not pass the initial tiers, and in such circumstances it means that currently it will always be not possible to finalize the risk assessment. It is therefore considered premature to be carrying out risk assessments for bumblebees.

Conclusions

The risk to solitary bees from consumption of contaminated pollen and nectar in maize, potato and sorghum as treated crops was not acceptable at tier 1. Refinement of the assessment based on measured clothianidin residues in a maize and potato pollen did not result in an acceptable risk. As there are no higher tier effect studies available, the risk assessment could not be finalized.

The risk assessment for effects on solitary bee larvae could not be finalized due to lack of a suitable toxicity endpoint for solitary bee larvae for clothianidin.

C. OVERALL CONCLUSIONS

Based on the information available in the GAP table (Table A-1) it is not possible to perform a risk assessment for the use in forestry nursery (only the dose in g a.s./plant is available, no information on the plant density and dose in g a.s./ha). Therefore a risk assessment for this use could not be performed, and no acceptable risk could be demonstrated.

Due to the lack of a validated methodology to test the chronic toxicity to adult bumblebees and bumblebee larvae, no such toxicity endpoints are available. For the chronic risk to adult bumblebees, the toxicity endpoint for honeybees divided by ten was used as a surrogate. For bumblebee larvae, no suitable (surrogate) toxicity endpoint is available and therefore no risk assessment could be performed.

For solitary bees, no toxicity endpoints are available, due to the lack of validated test methodology. For the acute and chronic risk to adult solitary bees, the toxicity endpoints for honeybees divided by ten were used as a surrogate. For solitary bee larvae, no suitable (surrogate) toxicity endpoint is available and therefore no risk assessment could be performed.

A potential **risk to pollinators other than honeybees** from the use of clothianidin containing products as granule through the consumption of contaminated nectar and pollen from succeeding crops was identified at tier 1 and 2. As no higher tier effect studies relevant for the currently registered uses are available, the risk assessment could not be finalized.

The risk to bumblebees and solitary bees from exposure to flowering weeds and honey dew in the treated field is considered acceptable. Further, the risk from exposure to residues of clothianidin in guttation fluid from treated winter cereals or sugar beets is covered by the risk assessment for honeybees. The risk from exposure to dust drift residues was considered acceptable in the EFSA Conclusion on the risk assessment for bees for clothianidin (2013).

A potential risk to pollinators other than honeybees from the use of clothianidin containing products as granule through the consumption of contaminated pollen from maize, potato and sorghum as treated crops was identified at tier 1 and 2. As no higher tier effect studies relevant for the currently registered uses are available, the risk assessment could not be finalized.

For the indoor use of clothianidin in maize and sweet maize (which is restricted to permanent greenhouses), exposure to bumblebees and solitary bees through nectar and pollen from the treated crop and succeeding crops can be considered low. Therefore, in line with the decision of Pesticides Peer Review Meeting 129, no risk assessment needs to be performed, and the risk for this indoor use can be considered acceptable.

The **risk to honeybees foraging in nectar or pollen in succeeding crops** was not acceptable at tier 1. At tier 2, refinements based on measured clothianidin residues in pollen and nectar in a number of succeeding crops did not result in an acceptable risk. The available higher tier field effect studies were not considered sufficient to demonstrate an acceptable risk to honeybees.

The **potential uptake via roots of flowering weeds** was not assessed. However, based on a large scale monitoring study of weeds in potato and maize fields, the exposure of honeybees and non-*Apis* bees through nectar and pollen of flowering weeds is considered negligible. As it was considered acceptable to extrapolate these results to sorghum, the same conclusion can be made for the use in sorghum. Therefore the risk for this exposure route is considered acceptable, provided that weeds are sufficiently controlled following standard agricultural practices.

The **risk to honeybees foraging on insect honey dew** is considered acceptable. Based on a monitoring study in maize and potatoes, it was demonstrated that exposure of honeybees (and no-*Apis* bees) to clothianidin through honey dew present in the treated field can be considered negligibly low,

and no risk assessment for this route of exposure was necessary. This conclusion was considered valid for all uses under evaluation (including the use in sweet corn sorghum and forestry nursery).

The **potential guttation exposure and the acute and long-term risk to colony survival and development, and the risk to bee brood from such exposure** is considered acceptable for the use in potatoes and maize. At tier one and two, a potential risk was identified. However, based on the higher tier effect studies submitted by the applicant which were performed in potatoes and maize, together with all other available studies investigating the effects from guttation exposure, the risk could be considered acceptable.

As no data was available for sorghum during the assessment by EFSA in 2013, a data gap was identified for this use. However, no studies performed in sorghum were submitted by the applicant. Therefore, this data gap remains.

The **potential exposure to dust drift following drill and the acute and long-term risk to colony survival and development, and the risk to bee brood resulting from such exposure** was assessed as low for granular formulations authorized for use in maize and sorghum in the EFSA Conclusion on the risk assessment for bees for clothianidin (2013), assuming there is no air-flow in the application machinery when granules are applied in the furrow. Assuming the same application technology is used, the same conclusion can be drawn for the use in potatoes.

The **acute and long term risk to colony survival and development and the risk to bee brood for honeybees from ingestion of contaminated nectar and pollen** was assessed for the use in maize, potato and sorghum, as these crops are potentially attractive for the consumption of pollen. At tier one, the acute risk to adult honeybees and the chronic risk to honeybee larvae was acceptable for the use in maize and sorghum. The chronic risk to adult honeybees was however not acceptable. For potato as treated crop, the risk to honeybees was not acceptable at tier 1. Refinement of the assessment based on measured clothianidin residues in potato and maize resulted in an acceptable acute risk to adult honeybees and chronic risk to honeybee larvae for both crops. The chronic risk to adult honeybees was however still not acceptable. The available higher tier field effect studies were not considered sufficient to demonstrate an acceptable chronic risk.

For the indoor use of clothianidin in maize and sweet maize (which is restricted to permanent greenhouses), exposure to honeybees through nectar and pollen from the treated crop and succeeding crops can be considered low. Therefore, in line with the decision of Pesticides Peer Review Meeting 129, no risk assessment needs to be performed, and the risk for this indoor use can be considered acceptable.

Following the risk assessment, **a number of data gaps** were identified. The following data is needed to be able to finalize the risk assessment for certain exposure routes:

Data for honeybees:

- All data necessary to demonstrate an acceptable risk to honeybees for the use of clothianidin in forestry nursery;
- Further measured residue data in nectar and pollen of oilseed rape (and other crops) as succeeding crop (to expand the available dataset and further refine the Tier 2 risk assessment for succeeding crops);
- Field effect studies that investigate the acute and long-term risk to honeybees foraging in nectar and pollen in succeeding crops;
- Exposure and effect studies that investigate the potential exposure to guttation and the acute and long-term risk to colony survival and development, and the risk to bee brood from such exposure for the registered use in sorghum (to enable performing a risk assessment for guttation in sorghum)

- Field effect studies that investigate the long-term risk to colony survival and development from ingestion of contaminated nectar and pollen from maize, potato and sorghum as treated crop

Data for pollinators other than honeybees:

- Data on the chronic toxicity of clothianidin to adult bumblebees and bumblebee larvae (to enable the execution of a chronic risk assessment for bumblebees);
- Data on the acute and chronic toxicity of clothianidin to adult solitary bees and solitary bee larvae (to enable the execution of an acute and chronic risk assessment for solitary bees);
- Higher tier effect studies to demonstrate an acceptable risk to pollinators other than honeybees from the consumption of nectar and pollen in succeeding crops;
- Higher tier effect studies to demonstrate an acceptable risk to pollinators other than honeybees from the consumption of pollen in maize, potato and sorghum as treated crops.

RMS acknowledges the fact that for pollinators other than honeybees, no validated and agreed test guidelines are currently available for the above mentioned data gaps, making it difficult to fulfil them in the near future.

D. LIST OF REFERENCES RELIED UPON

Annexpoint / reference number	**Author(s)**	**Year**	**Title** **Source *(where different from company)*** **Company, Report No., Date, GLP or GEP status *(where relevant)*, Published or not**	**Data protection claimed Y/N**	**Justification if data protection is claimed**	**Owner**
IIIA 10.4a/01	Harkin, S	2014	Clothianidin: Acute contact and oral toxicity to bumblebee (*Bombus terrestris*) The Food and Environment Research Agency, York, United Kingdom Report No.: B2AK100, Document number: THW-0376 Date: December 04, 2014 GLP, unpublished	Y	New data submitted to fulfil the data requirements according to 485/2013	Sumitomo Chemical Company
IIIA 10.4b/01	Harrington, P.	2013	Santana (a.s. clothianidin 1%): Exposure of honeybee colonies to clothianidin in pollen and nectar from sunflowers grown as a follow-on crop. Report No.: V7YD1000 Document Number: THW-0338 Date: September 09, 2013 GLP, unpublished	Y	New data submitted to fulfil the data requirements according to 485/2013	Sumitomo Chemical Company
IIIA 10.4b/02	Lebrun, F	2015	Magnitude of the residue of clothianidin and its metabolites in pollen and nectar in succeeding crop Northern and Southern Europe – 2014 Report No.: 14SG019 Document number: THW-0386 Date: February 13, 2015 GLP, unpublished	Y	New data submitted to fulfil the data requirements according to 485/2013	Sumitomo Chemical Company

Annexpoint / reference number	**Author(s)**	**Year**	**Title** **Source *(where different from company)*** **Company, Report No., Date, GLP or GEP status *(where relevant)*, Published or not**	**Data protection claimed Y/N**	**Justification if data protection is claimed**	**Owner**
IIIA 10.4c/01	Negrini, P	2014	Identification of weeds population and honeydew presence in maize and potato fields during the growing season Report No.: SCAE-2014-01 Document number: THW-0383 Date: December 19, 2014 Non- GLP, unpublished	Y	New data submitted to fulfil the data requirements according to 485/2013	Sumitomo Chemical Company
IIIA 10.4e/01	Thompson, H.	2011a	Santana: Evaluation of potential effects to honeybee colonies of guttation in corn grown following in-furrow application of Santana (a.s. clothianidin, 1% w/w) Report No.:V7GP1000 Document number: THW-0269 Date: February 03, 2011 GLP, unpublished	Y	Data previously evaluated by EFSA (2013)	Sumitomo Chemical Company
IIIA 10.4e/02	Thompson, H.	2013a	Dantop 50WG: Effects of a spray application of clothianidin in potatoes on honeybees. Report No.:V7XW1004 Document number: THW-0337 Date: June 14, 2013 GLP, unpublished	Y	New data submitted to fulfil the data requirements according to 485/2013	Sumitomo Chemical Company
IIIA 10.4e/04	Ansaloni, T	2014	Effects of CLOTHIANIDIN 0.7 GR in guttation water on bees (*Apis mellifera* L.) colony under field conditions Report No.:TRC14-038BA Document number: THW-0384 Date: December 16, 2014 GLP, unpublished	Y	New data submitted to fulfil the data requirements according to 485/2013	Sumitomo Chemical Company

Annexpoint / reference number	**Author(s)**	**Year**	**Title** **Source *(where different from company)*** **Company, Report No., Date, GLP or GEP status *(where relevant)*, Published or not**	**Data protection claimed Y/N**	**Justification if data protection is claimed**	**Owner**
IIIA 10.4g/01	Thompson, H.	2011b	Santana: Evaluation of potential long-term effects to honeybee colonies in France of corn grown following in-furrow application of Santana (a.s. clothianidin, 1% w/w) Report No.: S3UL1000 Document number: THW-0280 Date: October 26, 2011 GLP, unpublished	Y	Data previously evaluated by EFSA (2013)	Sumitomo Chemical Company
IIIA 10.4g/02	Bousquet, C	2015	Magnitude of the residue of clothianidin and its metabolites in potato pollen in Northern and Southern Europe – 2014 Report No.:14GS021 Document number: THW-0388 Date: February 13, 2015 GLP, unpublished	Y	New data submitted to fulfil the data requirements according to 485/2013	Sumitomo Chemical Company
IIIA 10.4g/03	Lewis, G	2014	Review of 2008-2010 control data from France based study (3 sites, 3 years, 6 colonies per site) to determine the statistical power to detect effect on colony development Report No. SCC/13/04 Document number: THW- 0382 Date: December 19, 2014 Non- GLP, unpublished	Y	New data submitted to fulfil the data requirements according to 485/2013	Sumitomo Chemical Company