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: Bayer CropScience (BCS)

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.*

TABLE OF CONTENT

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 Bayer CropScience. 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 Bayer CropScience are the currently registered uses as seed treatment in winter cereals and beets. 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 seed treatment in (sugar)beet and winter cereals. A summary of the range of use rates is given in table A-1 below. 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."*

Table A-1: Summary of the range of use rates of clothianidin containing formulations for use as a seed treatment in winter cereals and beet.

Crop	Use rate of CTD (range) dose/unit	Use rate of CTD (range) Dose g a.s./ha	Countries where registered
Winter cereals	27 - 50 g a.s./dt	59 - 100	BEL, CZE, GBR, HUN, IRL, ROU, SVK
Beet[#]	10 – 60 g a.s./u	10 – 108	AUT, BEL, CRO, CZE, DEU, DNK, ESP, FIN, GBR, GRC, HUN, ITA, NLD, POL, ROU, SVK

Notes: CTD = clothianidin; [#] 1 unit = 100,000 seeds

Table A-2 shows the different formulations and product names of currently registered products. Table A-3 and A-4 show the detailed national GAP for these products. The values given in Table A-3 and A-4 refer to clothianidin and not to any mixing partner.

Table A-2: Product names and formulations of currently registered products containing clothianidin

Crop	Product name	Formulation	Countries
Cereals	Deter	Clothianidin FS 250 (250 g/L)	CZE, SVK, GBR
	Argento (Redigo Deter)	Clothianidin + Prothioconazole FS 300 (250 g/L + 50 g/L)	BEL, IRL, GBR
	Yunta Quattro	Clothianidin + Imidacloprid + Prothioconazole + Tebuconazole FS 373.4 (166.7 + 166.7 + 33.3 + 6.7 g/L)	HUN, ROU
Beet	Poncho (Poncho Ungefaerbt, Poncho Bianco, Poncho FS 600 Rot)	Clothianidin FS 600 (600 g/L)	BEL, CRO, DEU, ITA, SVK, SVN, ESP
	Poncho Beta	Clothianidin + Beta-cyfluthrin FS 453.3 (400 + 53.3 g/L)	AUT, BEL, CZE, DNK, FIN, DEU, HUN, ITA, NLD, ROU, SVK, GBR
	Mundus	Clothianidin + Beta-cyfluthrin FS 380 (300 + 80 g/L)	DNK, DEU, POL
	Janus	Clothianidin + Beta-cyfluthrin FS 180 (100 + 80 g/L)	BEL, CZE, DNK, DEU, GRC, POL, SVK

Table A-3: Detail of national GAPs for clothianidin containing formulations for which the applicant still has a registration in cereals

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		
Winter Wheat, Winter Barley, Rye, Triticale, Spelt, Oats	BEL	Argento	F	EHD	FS	250	Seed treatment	00	1	na	na		0.090	na	Sowing rate: 1.8 unit seeds/ha 1 u = 100 kg Active substance dose rate: 0.050 kg /u
Winter Barley, Winter Rye, Winter Triticale	CZE	Deter	F	APHISP, PSAMAL	FS	250	Seed treatment	00	1	na	na		0.080	na	Sowing rate: 1.6 unit seeds/ha 1 u = 100 kg Active substance dose rate: 0.050 kg /u
Winter Wheat, Winter Barley	HUN	Yunta Quattro	F	AGRISP, EHD, OSCIFR	FS	166.7	Seed treatment	00	1	na	na		0.06668	na	Sowing rate: 2.0 unit seeds/ha 1 u = 100 kg Active substance dose rate: 0.03334 kg /u
Winter Wheat, Winter Barley, Winter Oat, Triticale, Rye, Durum Wheat	IRL	Redigo Deter	F	AGRISP, APHIFA, ARIOSP, DEROSP	FS	250	Seed treatment	00	1	na	na		0.100	na	Sowing rate: 2.0 unit seeds/ha 1 u = 100 kg Active substance dose rate: 0.050 kg /u
Wheat, Barley	ROU	Yunta Quattro	F	AGRISP, HYLECO, HYLESP, OSCIFR, ZABUTE	FS	166.7	Seed treatment	00	1	na	na		0.05867	na	Sowing rate: 2.2 unit seeds/ha 1 u = 100 kg Active substance dose rate: 0.02667 kg /u
Winter Wheat, Winter Barley, Winter Rye, Triticale, Durum Wheat	SVK	Deter	F	APHIFA	FS	250	Seed treatment	00	1	na	na		0.100	na	Sowing rate: 2.0 unit seeds/ha 1 u = 100 kg Active substance dose rate: 0.050 kg /u
Winter Wheat, Winter Barley, Winter Oat, Rye, Triticale, Durum Wheat	GBR	Deter	F	ACB, AGRISP, APHIFA	FS	250	Seed treatment	00	1	na	na		0.100	na	Sowing rate: 2.0 unit seeds/ha 1 u = 100 kg Active substance dose rate: 0.050 kg /u

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		
Winter Wheat, Winter Barley, Winter Oat, Rye, Triticale, Durum Wheat	GBR	Redigo Deter	F	AGRISP, APHIFA, ARIOSP, DEROSP	FS	250	Seed treatment	00	1	na	na		0.100	na	Sowing rate: 2.0 unit seeds/ha 1 u = 100 kg Active substance dose rate: 0.050 kg /u

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

Table A-4: Detail of national GAPs for clothianidin containing formulations for which the applicant still has a registration in beet

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		
Sugar beet Fodder beet	AUT	Poncho Beta	F	AGRISP ATOMLI APHIFA MYZUPE PEGOHY PHYESP	FS	400	Seed Treatment	00	1	na	na		0.060	na	Sowing rate: 1.0 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u
Beet	BEL	Poncho	F	AGRISP ATOMLI CHAESP EHD PEGOHY PEGOSP	FS	600	Seed Treatment	00	1	na	na		0.090	na	Sowing rate: 1.5 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u
Beet	BEL	Janus	F	AGRISP ATOMLI BLANSP SCUTSP	FS	100	Seed Treatment	00	1	na	na		0.015	na	Sowing rate: 1.5 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.010 kg /u
Beet	BEL	Poncho Beta	F	AGRISP ATOMLI BLANSP SCUTSP TIPUSP HALCSP	FS	400	Seed Treatment	00	1	na	na		0.090	na	Sowing rate: 1.5 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u
Sugar beet	CRO	Poncho FS 600 Rot	F	ATOMLI EHD EMB PEGOHY	FS	600	Seed Treatment	00	1	na	na		0.072	na	Sowing rate: 1.2 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u
Beet Fodder beet	CZE	Janus	F	ATOMLI	FS	100	Seed Treatment	00	1	na	na		0.011	na	Sowing rate: 1.1 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.010 kg /u
Beet Fodder beet	CZE	Poncho Beta	F	ATOMLI APHIFA CHAETI CHAECO EMB	FS	400	Seed Treatment	00	1	na	na		0.066	na	Sowing rate: 1.1 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u

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		
Sugar beet	DNK	Janus	F	ATOMLI PHDCSP	FS	100	Seed Treatment	00	1	na	na		0.010	na	Sowing rate: 1.0 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.010 kg /u
Sugar beet	DNK	Mundus	F	CHAEAR PEGOSP PHYESP	FS	300	Seed Treatment	00	1	na	na		0.030	na	Sowing rate: 1.0 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.030 kg /u
Sugar beet	DNK	Poncho Beta	F	ATOMLI AGRISP PHDCSP	FS	400	Seed Treatment	00	1	na	na		0.060	na	Sowing rate: 1.0 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u
Sugar beet	FIN	Poncho Beta	F	ATOMLI AGRISP PHDCSP	FS	400	Seed Treatment	00	1	na	na		0.060	na	Sowing rate: 1.0 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u
Fodder beet	DEU	Poncho Ungefaerbt	F	AGRISP ATOMLI APHISP PEGOHY	FS	600	Seed Treatment	00	1	na	na		0.078	na	Sowing rate: 1.3 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u
Beet	DEU	Janus	F	ATOMLI PEGOHY	FS	100	Seed Treatment	00	1	na	na		0.013	na	Sowing rate: 1.3 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.010 kg /u
Beet Fodder beet	DEU	Mundus	F	ATOMLI APHDSP PEGOHY	FS	300	Seed Treatment	00	1	na	na		0.039	na	Sowing rate: 1.3 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.030 kg /u
Beet	DEU	Poncho Beta	F	ATOMLI AGRISP APHISP BRACSP PEGOHY	FS	400	Seed Treatment	00	1	na	na		0.078	na	Sowing rate: 1.3 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u

Crop and/ or situation (a)	Country	Product name	FG 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		
Sugar beet	GRC	Janus	F	AGRISP CHAETI	FS	100	Seed Treatment	00	1	na	na		0.0154	na	Sowing rate: 1.54 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.010 kg /u
Sugar beet	HUN	Poncho Beta	F	AGRISP CLEOPL MELOME	FS	400	Seed Treatment	00	1	na	na		0.078	na	Sowing rate: 1.3 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u
Sugar beet	ITA	Poncho Bianco	F	AGRISP ATOMLI CHAETI	FS	600	Seed Treatment	00	1	na	na		0.045	na	Sowing rate: 1.5 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.030 kg /u
Sugar beet	ITA	Poncho Bianco	F	APHIFA MYZUPE	FS	600	Seed Treatment	00	1	na	na		0.090	na	Sowing rate: 1.5 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u
Sugar beet	ITA	Poncho Beta	F	AGRISP ATOMLI	FS	400	Seed Treatment	00	1	na	na		0.045	na	Sowing rate: 1.5 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.030 kg /u
Sugar beet	ITA	Poncho Beta	F	CHAETI	FS	400	Seed Treatment	00	1	na	na		0.0675	na	Sowing rate: 1.5 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.045 kg /u
Sugar beet	ITA	Poncho Beta	F	APHIFA MYZUPE PEGOHY	FS	400	Seed Treatment	00	1	na	na		0.090	na	Sowing rate: 1.5 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u

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		
Fodder beet	NLD	Poncho Beta	F	APHIFA AGRISP ATOMLI BLANGU BRACSP SCUTIM MYZUPE MYZUAS PEGOSP TIPUSP	FS	400	Seed Treatment	00	1	na	na		0.060	na	Sowing rate: 1.0 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u
Sugar beet	POL	Janus	F	APHIFA ATOMLI EMB HYLERA PHYESP	FS	100	Seed Treatment	00	1	na	na		0.010	na	Sowing rate: 1.0 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.010 kg /u
Sugar beet	POL	Mundus	F	AGRISP APHIFA ATOMLI CHAECO EMA PEGOHY	FS	300	Seed Treatment	00	1	na	na		0.036	na	Sowing rate: 1.2 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.030 kg /u
Sugar beet	ROU	Poncho Beta	F	AGRISP CHAEBR CLEOPU TANYDI	FS	400	Seed Treatment	00	1	na	na		0.084	na	Sowing rate: 1.4 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u
Beet	SVK	Poncho	F	APHISP ATOMLI LEMASP PHYESP	FS	600	Seed Treatment	00	1	na	na		0.0546	na	Sowing rate: 1.3 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.042 kg /u Once per 2 years.
Beet	SVK	Janus	F	APHISP ATOMLI PHYESP	FS	100	Seed Treatment	00	1	na	na		0.013	na	Sowing rate: 1.3 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.010 kg /u

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		
Beet Fodder beet	SVK	Poncho Beta	F	APHISP ATOMLI LEMASP PHYESP	FS	400	Seed Treatment	00	1	na	na		0.078	na	Sowing rate: 1.3 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u Once per 3 years.
Sugar beet	SVN	Poncho FS 600 Rot	F	APHISP ATOMLI EMB PEGOHY	FS	600	Seed Treatment	00	1	na	na		0.084	na	Sowing rate: 1.4 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u
Sugar beet	ESP	Poncho FS 600 Rot	F	AGRISP CHAETI EHD	FS	600	Seed Treatment	00	1	na	na		0.108	na	Sowing rate: 1.8 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u
Sugar beet Fodder beet	GBR	Poncho Beta	F	AGRISP ANURHE APHIFA ATOMLI MYZUPE PEGOHY PHYESP PSYICH	FS	400	Seed Treatment	00	1	na	na		0.078	na	Sowing rate: 1.3 unit seeds/ha 1 u = 100000 Active substance dose rate: 0.060 kg /u

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 number of laboratory studies on the toxicity of the active substance clothianidin and three clothianidin containing formulations to bumblebees were performed. In addition, the toxicity of the three clothianidin containing formulations to honeybees was also investigated, to be able to compare the toxicity to honeybees and bumblebees.

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.

Toxicity studies with bumblebees

Report:	**1.2/1; Harkin, S.; 2014**
Title:	**Clothianidin: Acute contact an oral toxicity to bumblebee (*Bombus terrestris*)**
Report No.:	B2AK1000
Document No.:	M-504345-01-1
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**

Report:	**1.2/2; Pfeiffer, S.; 2014a**
Title:	**Clothianidin + imidacloprid FS 275 (100+175 g/L): Acute contact toxicity to the bumblebee, *Bombus terrestris* L., under laboratory conditions**
Report No.:	S13-05151
Document No.:	M-494283-01-1
Guideline(s):	No specific guidelines are available. The test design is based on OEPP/EPPO 170 (4) (2010) and OECD Guideline 214 (1998), and on the review article of Van der Steen (2001)
Guideline deviation(s):	not applicable
GLP/GEP:	yes

Objective

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

Material and Methods

There are currently no agreed guidelines for testing the toxicity of pesticides to bumblebees. However, the study design is based on OEPP/EPPO 170 (4) (2010) and OECD Guideline 214 (1998), and on the review article of van der Steen (2001)[2].

Test item:	Clothianidin + Imidacloprid FS 275 (100 + 175 g/L) TOX No.: 10068-00 Specification No.: 102000025006-01
Content of a.s. (analysed)	100.3 g/L clothianidin (analysed) 176.7 g/L imidacloprid (analysed)
Toxic reference item:	Perfekthion (400 g/L dimethoate)
Test species:	Bumblebee (*Bombus terrestris* L.)
Stage:	Adult stage
Source:	Bees were collected from adequately fed, healthy, disease-free and queen-right hives, obtained from commercial supplier Koppert B.V. (The Netherlands)
Replicates	3 replicate unit of 10 bumblebees/treatment level
Treatment	1.23, 3.70, 11.11, 33.33 and 100 µg total a.s./bumblebee (the unit µg total a.s./bumblebee refers to the analysed content of total active substance, clothianidin + imidacloprid

[2] Van der Steen, J. (2001). Review of the methods to determine the hazard and toxicity of pesticides to bumblebees. *Apidologie* 32:399-406.

Toxic reference	12 µg dimethoate a.s./bumblebee
Controls	Tap water
Test conditions:	
Temperature:	24.2 – 25.9°C
Relative humidity:	51.3 – 63.5%
Photoperiod:	The test units were held in darkness (except during assessments)
Test Duration:	72 hours
Toxicity endpoints:	Mortality rate after 24, 48 and 72 hours
Dates of work:	27 November 2013 – 07 February 2014

Test system: One day prior to the test start, the bumblebees were randomly collected from the colonies, introduced in the test units and kept under test conditions at test start. The bumblebees were supplied *ad libitum* with 50% (w/v) aqueous sucrose solution. Bumblebees were weighed to ensure that overly small and overly big bumblebees were excluded from the test. The weights of the single individuals actually used for the test did not differ by more than 0.2 g.
After bumblebees had been anaesthetized with carbon dioxide for approximately 20 seconds, they were treated individually by topical application of 2 µL of the control, test and reference item solutions. After application, the bumblebees were returned to the test cages and fed with a 50% aqueous sucrose solution *ad libitum*. Mortality and sub-lethal effects were assessed 24, 48 and 72 hours after treatment.

Findings
In the control group, treated with tap water, no mortality was observed during the 72 hour test period.
In the test item treatment group, affected, apathetic or moribund bumblebees were observed at all tested dose levels at the 24, 48 and 72h assessments. A mortality of 63.33 % was observed at the highest dose level corresponding to 100 µg total a.s./bumblebee at the final assessment after 72 hours.
In the reference item group, mortality was ≥ 50 % at the end of the test.

Table B.9.1.1-6: Mortality in the contact toxicity test in the control, the test item (Clothianidin + Imidacloprid FS 275 (100+175 g/L)) and the reference item group (Perfekthion)

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

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

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

Table B.9.1.1-7: LD_{50} values in the bumblebee contact toxicity test with Clothianidin + Imidacloprid FS 275 (100 + 175 g/L)

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

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

RMS Comments
The validity criteria are met:

1. less than 10% mortality in the control (observed: no mortality during the 72h test period)
2. More than 50% mortality in the reference item group at the end of the test (observed: 76.67%)

It is not mentioned in the study report when the bumblebees used in the test were weighed. It is therefore unclear if the bees were weighed before dosing, as recommended in the EFSA Technical report on the bee study protocols submitted by Bayer CropScience AG (EFSA Supporting publication 2014:EN-599), or at the end of the test.

To attempt to express the toxicity in relative terms (and thus to take into account the variation in bumblebee size), the applicant stated in the EFSA Technical report on the bee study protocols submitted by Bayer CropScience AG (EFSA Supporting publication 2014:EN-599) that the LD_{50} would be expressed in both µg a.s./bumblebee and µg a.s./g of bumblebee. The latter was however not reported in the study report.

Overall, the study is considered acceptable and suitable for use in risk assessment. The lowest endpoints (toxicity after 72h) will be used in the risk assessment: **$LD_{50,contact}$ =** 54.9 µg total a.s./bumblebee. This corresponds to **19.9 µg clothianidin/bumblebee + 35.0 µg imidacloprid/bumblebee** (based on the analysed content of 100.3 g/L clothianidin + 176.7 g/L imidacloprid, which corresponds to 36.2% and 63.8% of total a.s., respectively).

Report:	**1.2/4; Pfeiffer, S.; 2014b**
Title:	**Clothianidin + fluopicolide + fluoxastrobin FS 510 (300+120+90 g/L) - Acute contact toxicity to the bumblebee, *Bombus terrestris* L., under laboratory conditions**
Report No.:	S13-05150
Document No.:	M-494271-01-1
Guideline(s):	No specific guidelines are available. The test design is based on OEPP/EPPO 170 (4) (2010) and OECD Guideline 214 (1998), and on the review article of van der Steen (2001)
Guideline deviation(s):	not applicable
GLP/GEP:	yes

Objective
The objectives of this study were to determine possible effects of clothianidin + Fluopicolide + Fluoxastrobin FS 510 (300+120+90 g/L) on the bumblebee, *Bombus terrestris* L., from contact exposure, and to determine the median lethal dose (LD_{50}), where possible.

Material and Methods

There are currently no agreed guidelines for testing the toxicity of pesticides to bumblebees. However, the study design is based on OEPP/EPPO 170 (4) (2010) and OECD Guideline 214 (1998), and on the review article of van der Steen (2001)[3].

Test item:	Clothianidin + Fluopicolide + Fluoxastrobin FS 510 (300+120+90 g/L) Short code: CTD+FLC+FXA FS 300+120+90 G TOX No.: 10029-00 Specification No.: 102000021198-02
Content of a.s. (analysed)	306.6 g/L clothianidin (analysed) 121.9 g/L fluopicolide (analysed) 91.06 g/L fluoxastrobin (analysed)
Toxic reference item:	Perfekthion (400 g/L dimethoate)
Test species:	Bumblebee (*Bombus terrestris* L.)
Stage:	Adult stage
Source:	Bees were collected from adequately fed, healthy, disease-free and queen-right hives, obtained from commercial supplier Koppert B.V. (The Netherlands)
Replicates	3 replicate unit of 10 bumblebees/treatment level
Treatment	0.1, 0.2, 0.4, 0.8 and 1.6 µg clothianidin a.s./bumblebee
Toxic reference	12 µg dimethoate a.s./bumblebee
Controls	Tap water
Test conditions:	
Temperature:	24.4 – 25.7°C
Relative humidity:	Oral test:: 51.3 – 61.4%
Photoperiod:	The test units were held in darkness (except during assessments)
Test Duration:	48 hours
Toxicity endpoints:	Mortality rate after 24 and 48 hours
Dates of work:	27 November 2013 – 29 November 2013

Test system: One day prior to the test start, the bumblebees were randomly collected from the colonies, introduced in the test units and kept under test conditions at test start. The bumblebees were supplied *ad libitum* with 50% (w/v) aqueous sucrose solution. Bumblebees were weighed to ensure that overly small and overly big bumblebees were excluded from the test. The weights of the single individuals actually used for the test did not differ by more than 0.2 g.

After bumblebees had been anaesthetized with carbon dioxide for approximately 13 seconds, they were treated individually by topical application of 2 µL of the control, test and reference item solutions. After application, the bumblebees were returned to the test cages and fed with a 50% aqueous sucrose solution *ad libitum*. Mortality and sub-lethal effects were assessed 24 and 48 hours after treatment.

Findings

In the control group, treated with tap water, no mortality was observed during the 48 h test period.

In the test item treatment group, an overall maximum mortality of 50.0 % was observed at the highest dose level corresponding to 1.6 µg clothianidin/bumblebee at the final assessment after 48 hours.

In the reference item group, mortality was ≥ 50 % at the end of the test.

[3] Van der Steen, J. (2001). Review of the methods to determine the hazard and toxicity of pesticides to bumblebees. *Apidologie* 32:399-406.

Table B.9.1.1-8: Mortality in the contact toxicity test in the control, the test item (Clothianidin + Fluopicolide + Fluoxastrobin FS 510 (300+120+90 g/L)) and the reference item (Perfekthion) group

Treatment Level (µg clothianidin/bumblebee)	**Time (hours)**	
	24 h	**48 h**
Control (tap water)	0.0	0.0
Test item: Clothianidin + Fluopicolide + Fluoxastrobin FS 510 (300+120+90 g/L)		
0.1	6.67	6.67
0.2	13.3	13.3
0.4	43.3	43.3*
0.8	40.0	40.0*
1.6	50.0	50.0*
Reference item: Perfekthion		
12	53.3	73.3

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

In the test item group, no remarkable sub-lethal effects were observed until the final assessment 48 hours after start of the experimental phase.

Conclusions

The 48 hour contact LD_{50} value for Clothianidin + Fluopicolide + Fluoxastrobin FS 510 (300+120+90 g/L) was determined to be 1.22 µg clothianidin/bumblebee.

The test item dose level corresponding to 0.2 µg clothianidin/bumblebee was determined to be the NOED (No Observed Effect Dose).

Table B.9.1.1-9: LD_{50} values in the bumblebee contact toxicity test with Clothianidin + Fluopicolide + Fluoxastrobin FS 510 (300+120+90 g/L)

Clothianidin + Fluopicolide + Fluoxastrobin FS 510 (300+120+90 g/L)	**Contact toxicity test (µg clothianidin/bumblebee)**
LD_{50} (24 h)	1.22 (0.76 – 3.21)*
LD_{50} (48 h)	1.22 (0.76 – 3.21)*
NOED (48 h)	0.2

** lower and upper 95% confidence limits*

RMS Comments

The validity criteria are met:

3. less than 10% mortality in the control (observed: no mortality during the 48h test period)
4. More than 50% mortality in the reference item group at the end of the test (observed: 73.3%)

It is not mentioned in the study report when the bumblebees used in the test were weighed. It is therefore unclear if the bees were weighed before dosing, as recommended in the EFSA Technical report on the bee study protocols submitted by Bayer CropScience AG (EFSA Supporting publication 2014:EN-599), or at the end of the test.

To attempt to express the toxicity in relative terms (and thus to take into account the variation in bumblebee size), the applicant stated in the EFSA Technical report on the bee study protocols submitted by Bayer CropScience AG (EFSA Supporting publication 2014:EN-599) that the LD50 would be expressed in both µg a.s./bumblebee and µg a.s./g of bumblebee. The latter was however not reported in the study report.

Overall, the study is considered acceptable and suitable for use in risk assessment. The lowest endpoints (toxicity after 48h) will be used in the risk assessment: **$LD_{50,contact}$ = 1.22 µg clothianidin/bumblebee**.

Report:	**1.2/6; Pfeiffer, S.; 2014c**
Title:	**Clothianidin + prothioconazole FS 300 (250+50 g/L) - Acute contact toxicity to the bumblebee, Bombus terrestris L. under laboratory conditions**
Report No.:	S13-05152
Document No.:	M-494300-01-1
Guideline(s):	No specific guidelines are available. The test design is based on OEPP/EPPO 170 (4) (2010) and OECD Guideline 214 (1998), and on the review article of van der Steen (2001)
Guideline deviation(s):	not applicable
GLP/GEP:	yes

Objective

The objectives of this study were to determine possible effects of Clothianidin + Prothioconazole FS 300 (250+50 g/L) on the bumblebee, *Bombus terrestris* L., from contact exposure and to determine the median lethal dose (LD_{50}) to *Bombus terrestris*, where possible.

Material and Methods

There are currently no agreed guidelines for testing the toxicity of pesticides to bumblebees. However, the study design is based on OEPP/EPPO 170 (4) (2010) and OECD Guideline 214 (1998), and on the review article of van der Steen (2001)[4].

Test item:	Clothianidin + Prothioconazole FS 300 (250 + 50 g/L) Short code: CTD+PTZ FS 250+50 G TOX No.: 10245-00 Specification No.: 102000008430
Content of a.s. (analysed)	248.2 g/L clothianidin (analysed) 50.59 g/L prothioconazole (analysed)
Toxic reference item:	Perfekthion (400 g/L dimethoate)
Test species:	Bumblebee (*Bombus terrestris* L.)
Stage:	Adult stage
Source:	Bees were collected from adequately fed, healthy, disease-free and queen-right hives, obtained from commercial supplier Koppert B.V. (The Netherlands)
Replicates	3 replicate unit of 10 bumblebees/treatment level
Treatment	0.1, 0.22, 0.48, 1.06 and 2.34 µg clothianidin a.s./bumblebee
Toxic reference	12 µg dimethoate a.s./bumblebee
Controls	Tap water
Test conditions:	
Temperature:	23.4 – 25.7°C
Relative humidity:	Oral test:: 52.9 – 62.1%
Photoperiod:	The test units were held in darkness (except during assessments)
Test Duration:	72 hours
Toxicity endpoints:	Mortality rate after 24, 48 and 72 hours
Dates of work:	17 December 2013 – 20 December 2014

Test system: One day prior to the test start, the bumblebees were randomly collected from the colonies, introduced in the test units and kept under test conditions at test start. The bumblebees were supplied *ad libitum* with 50% (w/v) aqueous sucrose solution. Bumblebees were weighed to ensure

[4] Van der Steen, J. (2001). Review of the methods to determine the hazard and toxicity of pesticides to bumblebees. *Apidologie* 32:399-406.

that overly small and overly big bumblebees were excluded from the test. The weights of the single individuals actually used for the test did not differ by more than 0.2 g.
After bumblebees had been anaesthetized with carbon dioxide for approximately 20 seconds, they were treated individually by topical application of 2 µL of the control, test and reference item solutions. After application, the bumblebees were returned to the test cages and fed with a 50% aqueous sucrose solution *ad libitum*. Mortality and sub-lethal effects were assessed 24, 48 and 72 hours after treatment.

Findings
In the control group, treated with tap water, no mortality was observed during the 72 h test period.
In the test item treatment group, a mortality of 90.0 % was observed at the highest dose level corresponding to 2.34 µg clothianidin/bumblebee at the final assessment after 72 hours. A mortality of 96.7 % was observed at the dose level corresponding to 1.06 µg clothianidin/bumblebee after 72 hours.
In the reference item group, mortality was ≥ 50 % at the end of the test.

Table B.9.1.1-10: Mortality in the contact toxicity test in the control, the test item (CTD + PTZ FS 300 (250+50 g/L) and the reference item group (Perfekthion)

Treatment Level (µg clothianidin/bumblebee)	**Time (hours)**		
	24 h	**48 h**	**72 h**
Control (tap water)	0.0	0.0	0.0
Test item: Clothianidin + Prothioconazole FS 300 (250+50 g/L)			
0.1	26.7	33.3	33.3*
0.22	26.7	46.7	46.7*
0.48	60.0	70.0	73.3*
1.06	93.3	96.7	96.7*
2.34	76.7	86.7	90.0*
Reference item: Perfekthion			
12	70.00	73.33	100

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

In the test item treatment group, affected or moribund bumblebees were observed at all tested dose levels at the 24 and 48 hours assessment. At the final assessment 72 hours after start of the experimental phase, only single affected or apathetic bumblebees were observed.

Conclusions
The 72 hour contact LD_{50} value for Clothianidin + Prothioconazole FS 300 (250+50 g/L) was determined to be 0.20 µg clothianidin/bumblebee. The NOED (No Observed Effect Dose) was determined to be < 0.1 µg clothianidin/bumblebee.

Table B.9.1.1-11: LD_{50} values in the bumblebee contact toxicity test with Clothianidin + Prothioconazole FS 300 (250+50 g/L)

Clothianidin + Prothioconazole FS 300 (250+50 g/L)	**Contact toxicity test (µg clothianidin/bumblebee)**
LD_{50} (24 h)	0.34 (0.22 to 0.50)*
LD_{50} (48 h)	0.20 (0.12 to 0.30)*
LD_{50} (72 h)	0.20 (0.12 to 0.28)*
NOED (72 h)	< 0.1

**lower and upper 95% confidence limits*

RMS Comments
The validity criteria are met:

5. less than 10% mortality in the control (observed: no mortality during the 72h test period)
6. More than 50% mortality in the reference item group at the end of the test (observed: 100%)

It is not mentioned in the study report when the bumblebees used in the test were weighed. It is therefore unclear if the bees were weighed before dosing, as recommended in the EFSA Technical report on the bee study protocols submitted by Bayer CropScience AG (EFSA Supporting publication 2014:EN-599), or at the end of the test.

To attempt to express the toxicity in relative terms (and thus to take into account the variation in bumblebee size), the applicant stated in the EFSA Technical report on the bee study protocols submitted by Bayer CropScience AG (EFSA Supporting publication 2014:EN-599) that the LD50 would be expressed in both µg a.s./bumblebee and µg a.s./g of bumblebee. The latter was however not reported in the study report.

Overall, the study is considered acceptable and suitable for use in risk assessment. The lowest endpoints (toxicity after 48h) will be used in the risk assessment: **$LD_{50,contact}$ = 0.20 µg clothianidin/bumblebee.**

Toxicity studies with honeybees

Report:	**1.2/3; Schmitzer, S.; 2014a**
Title:	**Effects of clothianidin + imidacloprid FS 275 (100+175) G (acute contact and oral) on honeybees (*Apis mellifera* L.) in the laboratory**
Report No.:	89691035
Document No.:	M-501653-01-1
Guideline(s):	GLP compliant study based on OECD 213 and 214 (1998)
Guideline deviation(s):	not specified
GLP/GEP:	yes

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

Material and Methods

Test item:	Clothianidin + Imidacloprid FS 275 (100 + 175 g/L) TOX No.: 10068-00 Specification No.: 102000025006-01
Content of a.s. (analysed)	100.3 g/L clothianidin (analysed) 176.7 g/L imidacloprid (analysed)
Toxic reference item:	Perfekthion (400 g/L dimethoate)
Test species:	Honeybee (*Apis mellifera carnica* L.)
Stage:	Adult stage (female working bees)
Source:	Honeybee colonies, disease free and queen-right, bred by IBACON
Replicates	3 replicate unit of 10 honeybees/treatment level
Contact	
Treatment	Nominal dose levels: 1.0, 0.50, 0.25, 0.13, 0.063 and 0.031 µg product/bee
Toxic reference	0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee
Controls	Tap water with 0.5% Adhäsit
Oral	
Treatment	Nominal dose levels: 0.20, 0.10, 0.05, 0.025 and 0.013 µg product/bee

	Measured dose levels: 0.17, 0.11, 0.053, 0.027 and 0.013 µg product/bee
Toxic reference	Nominal dose levels: 0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee Measured dose levels: 0.32, 0.16, 0.08 and 0.06 µg dimethoate/bee
Controls	50% w/v sucrose solution
Test conditions:	
Temperature:	25°C
Relative humidity:	51 – 96%
Photoperiod:	The test units were held in darkness (except during assessments)
Test Duration:	48 hours
Toxicity endpoints:	Mortality rate after 4, 24 and 48
Dates of work:	5 May 2014 – 8 May 2014

Test system: Adult bees were collected from the flight board or the outer honeycombs (away from the brood), without the use of smoke and without anaesthetics. Collection took place on the morning of use.
Contact dosing: The test item, reference item and control were applied as one 5 µL droplet of the solution, placed on the dorsal bee thorax using a calibrated pipette. Test item and reference item were dissolved in tap water with 0.5% Adhäsit (used to improve the spreading of the test droplet on the bee body). The control consisted only of tap water with 0.5% Adhäsit. A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item.
Oral dosing: The test item and reference item were applied in 50% w/v sucrose solution, which was used as carrier (food) in the oral test. For the control pure 50% w/v sucrose solution was offered to the bees. The treated food was offered in syringes, which were weighed before and after introduction into the cages. After a maximum of 6h, the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food. Result are given based on the measured food consumption.

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

Table B.9.1.1-12: Mortality and behavioural abnormalities of the bees in the contact toxicity test

Dosage	After 4 h		After 24h		After 48h	
	Mortality (mean%)	Behav. Abnorm. (mean %)	Mortality (mean%)	Behav. Abnorm. (mean %)	Mortality (mean%)	Behav. Abnorm. (mean %)
Water control	0.0	0.0	0.0	0.0	0.0	0.0
Test item (µg product/bee)						
1.0	20.0	80.0	63.3	36.7	73.3	16.7
0.50	6.7	93.3	53.3	36.7	60.0	0.0
0.25	6.7	93.3	60.0	40.0	70.0	0.0
0.13	16.7	53.3	26.7	26.7	26.7	0.0
0.063	0.0	3.3	6.7	0.0	6.7	0.0
0.031	0.0	3.3	3.3	0.0	3.3	0.0
Reference item (µg a.s./bee)						
0.30	0.0	53.3	50.0	0.0	50.0	0.0
0.20	0.0	6.7	33.3	0.0	46.7	3.3
0.15	0.0	0.0	10.0	0.0	20.0	3.3
0.10	0.0	0.0	0.0	0.0	3.3	0.0

Results are averages from three replicates (ten bees each) per dosage/control
Behav. abnorm. = behavioural abnormalities

Oral Test: Mortality occurred in all test item treated dose levels. Actual oral doses of 0.17, 0.11, 0.053, 0.027 and 0.013 µg product/bee resulted in mortality ranging from 96.7 % to 6.7 % at the end of the test (48 hours after application). No mortality occurred in the control group (sucrose 50 % w/v solution = 500 g sucrose/L tap water).
Behavioural abnormalities (e.g. moribund bees or affected bees) were found during the 4-hours assessment in the 0.17, 0.11, 0.053 and 0.027 µg product/bee treatment groups. A few bees were behaving abnormal 24 hours following treatment in the 0.17, 0.11 and 0.053 µg product/bee dose levels and one and 6 bees were found to be affected during the 48-hours assessment in the 0.17 and 0.11 µg product/bee treatment group, respectively. No behavioural abnormalities were found in the 0.013 µg product/bee dosing group during the test.

Table B.9.1.1-13: Mortality and behavioural abnormalities of the bees in the oral toxicity test

Dosage consumed	After 4 h		After 24h		After 48h	
	Mortality (mean%)	Behav. Abnorm. (mean %)	Mortality (mean%)	Behav. Abnorm. (mean %)	Mortality (mean%)	Behav. Abnorm. (mean %)
Water control	0.0	0.0	0.0	0.0	0.0	0.0
Test item (µg product/bee)						
0.17	80.0	20.0	96.7	3.3	96.7	3.3
0.11	20.0	80.0	73.3	16.7	73.3	20.0
0.053	0.0	40.0	40.0	3.3	46.7	0.0
0.027	0.0	3.3	10.0	0.0	10.0	0.0
0.013	0.0	0.0	3.3	0.0	6.7	0.0
Reference item (µg a.s./bee)						
0.32	0.0	73.3	100.0	0.0	100.0	0.0
0.16	3.3	0.0	56.7	43.3	66.7	33.3
0.08	0.0	0.0	6.7	0.0	6.7	0.0
0.06	0.0	0.0	3.3	0.0	3.3	0.0

Results are averages from three replicates (ten bees each) per dosage/control
Behav. abnorm. = behavioural abnormalities

Conclusions

Table B.9.1.1-14: Toxicity of Clothianidin + Imidacloprid FS 275 (100 + 175 g/L) to honeybees.

Endpoint	Contact toxicity	Oral toxicity
LD_{50} (µg product/bee)	24 hours: 0.39 48 hours: 0.29	24 hours: 0.062 48 hours: 0.058
LD_{20} (µg product/bee)	24 hours: 0.101 48 hours: 0.093	24 hours: 0.034 48 hours: 0.030
LD_{10} (µg product/bee)	24 hours: 0.050 48 hours: 0.051	24 hours: 0.025 48 hours: 0.021
NOED (µg product/bee)*	24 hours: 0.063 48 hours: 0.063	24 hours: 0.027 48 hours: 0.027

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

RMS Comments

The validity criteria of OECD Guideline 213 and 214 are met:

7. less than 10% mortality in the control (observed: no mortality during the 48h test period for both the oral and contact toxicity test)
8. LD_{50} for the reference item in the range of 0.10 – 0.30 µg a.s./bee for the contact test and 0.10 – 0.35 µg a.s./bee for the oral test (observed: 0.28 µg a.s./bee for the contact test, 0.14 µg a.s./bee for the oral test)

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

The lowest endpoint (toxicity after 48h) will be used in the risk assessment:

9. Contact toxicity: **$LD_{50,contact}$ =** 0.29 µg product/bee, which corresponds to **0.026 µg clothianidin/bee** and 0.046 µg imidacloprid/bee
10. Oral toxicity: **$LD_{50,oral}$ =** 0.058 µg product/bee, which corresponds to **0.0052 µg clothianidin/bee** and 0.0091 µg imidacloprid/bee

Report:	**1.2/5; Schmitzer, S.; 2010**
Title:	**Effects of clothianidin + fluopicolide + fluoxastrobin FS 510 (300+120+90) G (acute contact and oral) on honeybees (*Apis mellifera* L.) in the laboratory**
Report No.:	53631035
Document No.:	M-367011-01-1
Guideline(s):	OECD 213 and 214 (1998)
Guideline deviation(s):	none
GLP/GEP:	yes

Objective

The objectives of this study were to determine possible effects of CTD+FLC+FXA FS 300+120+90 G on the honeybee (*Apis mellifera* L.), from contact and oral exposure and to determine the median lethal dose (LD_{50}) where possible.

Material and Methods:

Test item: Clothianidin + Fluopicolide + Fluoxastrobin FS 510 (300+120+90 g/L)
Short code: CTD+FLC+FXA FS 300+120+90 G
TOX No.: 8454-00
Specification No.: 102000021198

Content of a.s. (analysed)	303.1 g/L clothianidin (analysed) 122.5 g/L fluopicolide (analysed) 92.78 g/L fluoxastrobin (analysed)
Toxic reference item:	Perfekthion (400 g/L dimethoate)
Test species:	Honeybee (*Apis mellifera carnica* L.)
Stage:	Adult stage (female working bees)
Source:	Honeybee colonies, disease free and queen-right, bred by IBACON
Replicates	3 replicate unit of 10 honeybees/treatment level
Contact	
Treatment	Nominal dose levels: 400, 200, 100, 50, 25 and 12.5 ng product/bee
Toxic reference	0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee
Controls	Tap water with 0.5% Adhäsit
Oral	
Treatment	Nominal dose levels: 40, 20, 10, 5.0, 2.5, and 1.3 ng product/bee (equivalent to 9.8, 4.9, 2.5, 1.2, 0.61 and 0.32 ng clothianidin/bee) Measured dose levels: 22.5, 19.8, 10.8, 5.4, 2.7 and 1.3 ng product/bee (equivalent to 5.5, 4.9, 2.6, 1.3, 0.66 and 0.32 ng clothianidin/bee)
Toxic reference	Nominal dose levels: 0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee Measured dose levels: 0.32, 0.14, 0.08 and 0.05 µg dimethoate/bee
Controls	50% w/v sucrose solution (in tap water)
Test conditions:	
Temperature:	25°C
Relative humidity:	49 – 81%
Photoperiod:	The test units were held in darkness (except during assessments)
Test Duration:	48 hours
Toxicity endpoints:	Mortality rate after 4, 24 and 48
Dates of work:	28 September 2009 – 1 October 2009

Test system: Adult bees were collected from the flight board or the outer honeycombs (away from the brood), without the use of smoke and without anaesthetics. Collection took place on the morning of use.

Contact dosing: The test item, reference item and control were applied as one 5 µL droplet of the solution, placed on the dorsal bee thorax using a calibrated pipette. Test item and reference item were dissolved in tap water with 0.5% Adhäsit (used to improve the spreading of the test droplet on the bee body). The control consisted only of tap water with 0.5% Adhäsit. A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item.

Oral dosing: Aqueous stock solutions of the test and reference item were prepared in such a way that they had the respective target concentration of the test item once they were subsequently mixed with sugar syrup at a ratio of 1 + 1. After mixing of the test solutions with ready-to-use sugar syrup (composition of the sugar component: 30% sacharose, 31% glucose, 39% fructose) the final concentration of sugar syrup in the test item solutions offered to the bees was 50%. For the controls water and sugar syrup was used at the same ratio (1 + 1). The treated food was offered in syringes, which were weighed before and after introduction into the cages. After a maximum of 6h, the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food. Result are given based on the measured food consumption.

Findings

Contact Test: Dose levels of 400, 200, 100 and 50 ng product per bee led to dose dependent mortality ranging from 96.7 to 3.3 % at the end of the test (48 hours). No mortality occurred in the 25 and 12.5 ng product per bee dose levels. There was 3.3 % mortality in the control (water + 0.5 % Adhäsit) group.

During the 4 hours assessment of the experiment behavioural abnormalities (*e.g.* movement coordination problems and/or apathy) were observed in the 400, 200 and 100 ng product per bee dose groups. After 24 hours movement coordination problems were found in the 400 and 200 ng product per bee dose levels. During the 48 hours assessment only one single bee in the 200 ng product per bee dose group showed a movement coordination problem.

Table B.9.1.1-15: Mortality and behavioural abnormalities of the bees in the contact toxicity test

Dosage	After 4 h		After 24h		After 48h	
	Mortality (mean%)	Behav. Abnorm. (mean %)	Mortality (mean%)	Behav. Abnorm. (mean %)	Mortality (mean%)	Behav. Abnorm. (mean %)
Water control	0.0	0.0	3.3	0.0	3.3	0.0
Test item (ng product/bee)						
400	10.0	73.3	93.3	3.3	96.7	0.0
200	10.0	16.7	56.7	3.3	56.7	3.3
100	0.0	3.3	13.3	0.0	13.3	0.0
50	0.0	0.0	3.3	0.0	3.3	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0
12.5	0.0	0.0	0.0	0.0	0.0	0.0
Reference item (µg a.s./bee)						
0.30	3.3	16.7	90.0	3.3	90.0	0.0
0.20	3.3	3.3	80.0	6.7	86.7	0.0
0.15	0.0	0.0	20.0	16.7	46.7	0.0
0.10	0.0	0.0	0.0	0.0	10.0	0.0

Results are averages from three replicates (ten bees each) per dosage/control
Behav. abnorm. = behavioural abnormalities

Oral Test: In the oral test, the maximum nominal dose levels of the test item (40 and 20 ng product/bee) could not be achieved, because the bees did not ingest the full volume of treated sugar solution even when offered over a period of 6 hours. Oral doses of 22.5, 19.8 and 10.8 ng product per bee resulted in mortality ranging from 100.0 % to 33.3 % at the end of the test (48 hours after application). No mortality occurred in the 5.4, 2.7 and 1.3 ng per bee dose groups and control (50% sugar solution).
During the first 4 hours, behavioural abnormalities (*e.g.* movement coordination problems and apathy) were observed in the three highest treatment groups (22.5, 19.8 and 10.8 ng product per bee). After 24 and 48 hours these behavioural impairments were not found any more in all treatment groups.

Table B.9.1.1-16: Mortality and behavioural abnormalities of the bees in the oral toxicity test

Dosage consumed	After 4 h		After 24h		After 48h	
	Mortality (mean%)	Behav. Abnorm. (mean %)	Mortality (mean%)	Behav. Abnorm. (mean %)	Mortality (mean%)	Behav. Abnorm. (mean %)
Water control	0.0	0.0	0.0	0.0	0.0	0.0
Test item (ng product/bee)						
22.5	96.7	3.3	100.0	0.0	100.0	0.0
19.8	36.7	30.0	60.0	0.0	60.0	0.0
10.8	30.0	6.7	33.3	0.0	33.3	0.0
5.4	0.0	0.0	0.0	0.0	0.0	0.0
2.7	0.0	0.0	0.0	0.0	0.0	0.0
1.3	0.0	0.0	0.0	0.0	0.0	0.0
Reference item (µg a.s./bee)						
0.32	40.0	40.0	86.7	0.0	96.7	0.0
0.14	3.3	23.3	86.7	0.0	86.7	0.0
0.08	0.0	0.0	13.3	0.0	20.0	0.0
0.05	0.0	0.0	3.3	0.0	3.3	0.0

Results are averages from three replicates (ten bees each) per dosage/control
Behav. abnorm. = behavioural abnormalities

Conclusions

Table B.9.1.1-17: Toxicity of Clothianidin + Fluopicolide + Fluoxastrobin FS 510 (300 + 120 + 90 g/L) to honeybees.

Endpoint	Contact toxicity	Oral toxicity
LD_{50} (ng product/bee)	24 hours: 188 48 hours: 183	24 hours: 13.2 48 hours: 13.2
LD_{50} (ng clothianidin/bee)	24 hours: 46.1 48 hours: 44.8	24 hours: 3.2 48 hours: 3.2

RMS Comments

The validity criteria of OECD Guideline 213 and 214 are met:

11. less than 10% mortality in the control (observed: 3.3% mortality during the 48h test period for contact toxicity test and no mortality during the oral toxicity test)
12. LD_{50} for the reference item in the range of 0.10 – 0.30 µg a.s./bee for the contact test and 0.10 – 0.35 µg a.s./bee for the oral test (observed: 0.18 µg a.s./bee for the contact test, 0.13 µg a.s./bee for the oral test)

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

The lowest endpoint (toxicity after 48h) will be used in the risk assessment:

13. Contact toxicity: **$LD_{50,contact}$ =** 183 ng product/bee, which corresponds to **44.8 ng clothianidin/bee**
14. Oral toxicity: **$LD_{50,oral}$ =** 13.2 ng product/bee, which corresponds to **3.2 ng clothianidin/bee**

Report:	**1.2/7; Schmitzer, S.; 2014b**
Title:	**Effects of clothianidin + prothioconazole FS 300 (250+50) G (acute contact and oral) on honeybees (*Apis mellifera* L.) in the laboratory**
Report No.:	89681035
Document No.:	M-501142-01-1
Guideline(s):	GLP compliant study based on OECD 213 and 214 (1998)
Guideline deviation(s):	not specified
GLP/GEP:	yes

Objective

The objectives of this study were to determine possible effects of Clothianidin + Prothioconazole FS 500 (250+50 g/L) G on the honeybee, *Apis mellifera* L., from contact and oral exposure and to determine the median lethal dose (LD_{50}) where possible.

Material and Methods

Test item:	Clothianidin + Prothioconazole FS 300 (250 + 50 g/L) Short code: CTD+PTZ FS 250+50 G TOX No.: 10245-00 Specification No.: 102000008430
Content of a.s. (analysed)	248.2 g/L clothianidin (analysed) 50.59 g/L prothioconazole (analysed)
Toxic reference item:	Perfekthion (400 g/L dimethoate)
Test species:	Honeybee (*Apis mellifera carnica* L.)
Stage:	Adult stage (female working bees)
Source:	Honeybee colonies, disease free and queen-right, bred by IBACON
Replicates	3 replicate unit of 10 honeybees/treatment level

Contact	
Treatment	Nominal dose levels:500.0, 250.0, 125.0, 62.5 and 31.3 ng product/bee (equivalent to 104.0, 52.0, 26.0, 13.0 and 6.5 ng clothianidin/bee)
Toxic reference	0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee
Controls	Tap water with 0.5% Adhäsit
Oral	
Treatment	Nominal dose levels: 100.0, 50.0, 25.0, 12.5 and 6.3 ng poduct/bee (equivalent to 20.8, 10.4, 5.2, 2.6 and 1.3 ng clothianidin/bee) Measured dose levels: 70.8, 55.6, 28.3, 13.9 and 6.8 ng product/bee (equivalent to 14.7, 11.6, 5.9, 2.9 and 1.4 ng clothianidin/bee)
Toxic reference	Nominal dose levels: 0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee Measured dose levels: 0.32, 0.16, 0.08 and 0.06 µg dimethoate/bee
Controls	50% w/v sucrose solution (500 g sucrose/L tap water)
Test conditions:	
Temperature:	25°C
Relative humidity:	51 – 96%
Photoperiod:	The test units were held in darkness (except during assessments)
Test Duration:	48 hours
Toxicity endpoints:	Mortality rate after 4, 24 and 48
Dates of work:	5 May 2014 – 8 May 2014

Test system: Adult bees were collected from the flight board or the outer honeycombs (away from the brood), without the use of smoke and without anaesthetics. Collection took place on the morning of use.

Contact dosing: The test item, reference item and control were applied as one 5 µL droplet of the solution, placed on the dorsal bee thorax using a calibrated pipette. Test item and reference item were dissolved in tap water with 0.5% Adhäsit (used to improve the spreading of the test droplet on the bee body). The control consisted only of tap water with 0.5% Adhäsit. A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item.

Oral dosing: The test item and reference item were applied in 50% w/v sucrose solution, which was used as carrier (food) in the oral test. For the control pure 50% w/v sucrose solution was offered to the bees. The treated food was offered in syringes, which were weighed before and after introduction into the cages. After a maximum of 6h, the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food. Result are given based on the measured food consumption.

Findings

Contact Test: Test item dose levels of 500.0, 250.0, 125.0, 62.5 and 31.3 ng product/bee led to dose dependent mortality, ranging from 90.0 % to 13.3 % at test end (48 hrs following treatment). No mortality occurred in the control group (water + 0.5 % Adhäsit).

Behavioural abnormalities (e.g. moribund or affected bees) were observed in all dose level groups during the 4-hours assessment. During the 24-hours assessment, in the 500.0 ng product/bee treatment group one bee was moribund. No further behavioural abnormalities were found in the other test item treatment dose groups. All other surviving bees appeared normal.

Table B.9.1.1-18: Mortality and behavioural abnormalities of the bees in the contact toxicity test

Dosage	After 4 h		After 24h		After 48h	
	Mortality (mean%)	Behav. Abnorm. (mean %)	Mortality (mean%)	Behav. Abnorm. (mean %)	Mortality (mean%)	Behav. Abnorm. (mean %)
Water control	0.0	0.0	0.0	0.0	0.0	0.0
Test item (ng product/bee)						
500.0	63.3	23.3	86.7	3.3	90.0	0.0
250.0	43.3	40.0	66.7	0.0	66.7	0.0
125.0	36.7	6.7	43.3	0.0	43.3	0.0
62.5	10.0	3.3	13.3	0.0	13.3	0.0
31.3	10.0	3.3	13.3	0.0	13.3	0.0
Reference item (µg a.s./bee)						
0.30	0.0	53.3	50.0	0.0	50.0	0.0
0.20	0.0	6.7	33.3	0.0	46.7	3.3
0.15	0.0	0.0	10.0	0.0	20.0	3.3
0.10	0.0	0.0	0.0	0.0	3.3	0.0

Results are averages from three replicates (ten bees each) per dosage/control
Behav. abnorm. = behavioural abnormalities

Oral Test: The maximum nominal dose level of the test item (100 ng product/bee) could not be achieved, because the bees did not ingest the full volume of treated sugar solution even when offered over a period of six hours. Mortality occurred in all test item treated dose levels. Actual oral doses of 70.8, 55.6, 28.3, 13.9 and 6.8 ng product/bee resulted in mortality ranging from 100.0 % to 3.3 % at the end of the test (48 hours after application). No mortality occurred in the control group (sucrose 50 % w/v solution = 500 g sucrose/L tap water).
Behavioural abnormalities (*e.g.* moribund bees or affected bees) were found during the 4-hours assessment in the 70.8, 55.6 and 28.3 ng product/bee treatment groups. 24 hours following treatment one bee was affected in the 55.6 ng/bee dose level and two and one bees were found to be affected during the 48-hours assessment in the 55.6 and 28.3 ng/bee treatment groups, respectively. No behavioural abnormalities were found in the 13.9 and 6.8 ng product/bee dosing group during the test.

Table B.9.1.1-19: Mortality and behavioural abnormalities of the bees in the oral toxicity test

Dosage consumed	After 4 h		After 24h		After 48h	
	Mortality (mean%)	Behav. Abnorm. (mean %)	Mortality (mean%)	Behav. Abnorm. (mean %)	Mortality (mean%)	Behav. Abnorm. (mean %)
Water control	0.0	0.0	0.0	0.0	0.0	0.0
Test item (ng product/bee)						
70.8	96.7	3.3	100.0	0.0	100.0	0.0
55.6	63.3	36.7	93.3	6.7	93.3	6.7
28.3	23.3	20.0	36.7	0.0	40.0	3.3
13.9	3.3	0.0	6.7	0.0	6.7	0.0
6.8	0.0	0.0	0.0	0.0	3.3	0.0
Reference item (µg a.s./bee)						
0.32	0.0	73.3	100.0	0.0	100.0	0.0
0.16	3.3	0.0	56.7	43.3	66.7	33.3
0.08	0.0	0.0	6.7	0.0	6.7	0.0
0.06	0.0	0.0	3.3	0.0	3.3	0.0

Results are averages from three replicates (ten bees each) per dosage/control
Behav. abnorm. = behavioural abnormalities

Conclusions

Table B.9.1.1-20: Toxicity to of Clothianidin + Prothioconazole FS 300 (250 + 50 g/L) to honeybees (endpoints expressed as ng product/bee)

Endpoint	Conctact toxicity	Oral toxicity
LD_{50} (ng product/bee)	24 hours: 153.3 48 hours: 148.3	24 hours: 29.9 48 hours: 27.9
LD_{20} (ng product/bee)	24 hours: 58.6 48 hours: 59.3	24 hours: 21.0 48 hours: 18.1
LD_{10} (ng product/bee)	24 hours: 35.5 48 hours: 36.7	24 hours: 17.4 48 hours: 14.5
NOED (ng product/bee)*	24 hours: 62.5 48 hours: 62.5	24 hours: 13.9 48 hours: 13.9

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

Table B.9.1.1-21: Toxicity to of Clothianidin + Prothioconazole FS 300 (250 + 50 g/L) to honeybees (endpoints expressed as ng clothianidin/bee)

Endpoint	Conctact toxicity	Oral toxicity
LD_{50} (ng clothianidin/bee)	24 hours: 31.9 48 hours: 30.8	24 hours: 6.2 48 hours: 5.8
LD_{20} (ng clothianidin/bee)	24 hours: 12.2 48 hours: 12.3	24 hours: 4.4 48 hours: 3.8
LD_{10} (ng clothianidin/bee)	24 hours: 7.4 48 hours: 7.6	24 hours: 3.6 48 hours: 3.0
NOED (ng clothianidin/bee)*	24 hours: 13.0 48 hours: 13.0	24 hours: 2.9 48 hours: 2.9

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

RMS Comments

The validity criteria of OECD Guideline 213 and 214 are met:

15. less than 10% mortality in the control (observed: no mortality during the 48h test period for both the oral and contact toxicity test)
16. LD_{50} for the reference item in the range of 0.10 – 0.30 µg a.s./bee for the contact test and 0.10 – 0.35 µg a.s./bee for the oral test (observed: 0.28 µg a.s./bee for the contact test, 0.14 µg a.s./bee for the oral test)

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

The lowest endpoint (toxicity after 48h) will be used in the risk assessment:

17. Contact toxicity: **$LD_{50,contact}$ =** 148 ng product/bee, which corresponds to **30.8 ng clothianidin/bee** and 0.046 µg imidacloprid/bee
18. Oral toxicity: **$LD_{50,oral}$ =** 27.9 ng product/bee, which corresponds to **5.8 ng clothianidin/bee** and 0.0092 µg imidacloprid/bee

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).

The report from a large scale research project, which covered about 1,800 ha of winter oil seed rape and investigated the effects and exposure of honeybees, bumblebees and solitary bees to Elado® (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed oilseed rape, was submitted by the applicant. While the use of clothianidin containing products as seed treatment in oilseed rape is currently not authorized in the EU, this research project provides useful information in support of the present risk assessment for bumblebees and solitary bees. A summary of the results for solitary bees and bumblebees is provided in Section B.9.7.1, under Study 1.8/8 (Peters, 2015) and Study 1.8/9 (Sterk & Peters, 2014).

B.9.1.3. Summary of 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.9.1.3.1-1 and B.9.1.3.1-2, 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)[5], the EFSA Conclusion on the risk assessment for bees for clothianidin considering all uses other than seed treatments and granules (2015)[6] and in section B.9.1.1 of the present addendum to the DAR.

For honeybees, both the acute contact and oral toxicity of clothianidin as active substance is comparable to the toxicity of clothianidin in a formulation. Therefore, the active substance endpoints will be used in the risk assessment. The available chronic oral toxicity data on adults and larvae were re-evaluated by EFSA in 2015[6]. 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 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

[5] 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.
[6] 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

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)[7]. For example, Fischer et al. (2014)[8] 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)[9] 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.

It should be noted that the papers referenced in the literature review by Fryday et al. (2015) were not assessed for reliability and have potential shortcomings that make it difficult to derive suitable endpoints. The applicant pointed out the following shortcomings to the studies by Fischer et al. (2015 and Di Prisco et al. (2013) (*text in italic*):

It must be noted that in Fischer et al. (2014) the test substance doses administered were excessively high and even around or above the LD_{50} and by orders of magnitude above field-realistic exposure levels. Furthermore, only individual bees have been tested so that the biological relevance of the described effects cannot be assessed for potential effects on colony level. There have been many cases in previous studies where sublethal effects observed in individual bees did not translate into adverse effects on colony level. Clothianidin has been investigated in extensive field studies, and in no case colony depopulation effects have been seen, which would have to be expected if there would be relevant effects on homing behaviour. The evidence coming from the field indicate that although Clothianidin seed treatment is used on large scale in highly bee-attractive crops (e.g. oilseed rape) there are no reports about major bee health problems of hives foraging on such crops.

The methods used in Di Prisco et al. (2013) have not been validated, so it is not clear whether the results are reproducible or might be erratic. Results of previous publications about "interactions" between neonicotinoids and pathogens in bees do not follow a similar trend that would indicate a

[7] 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.

[8] 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.

[9] 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.

causality of the interaction. In the same publication, the authors report likewise a positive correlation between Varroa infestation to DWV virus infestation;, this finding would suggest that there may be an unspecific reaction to a stressor rather than neonicotinoid-specific effect. The reported results have been generated in the laboratory and on individual bees only – there is no assessment of any potential effects on colony level, nor is it established that such effects could be reproduced under field conditions at all. It is well known that individual bees in the laboratory may react completely differently to stressors than bees in the field in the context of their colony, and that laboratory results usually cannot easily be extrapolated to the field. If the authors' hypothesis of neonicotinoids supporting DWV virus infestation was true, then a correlation should be seen in the field between colony mortality and the exposure to neonicotinoid residues, and between the prevalence of DWV (and other viruses) and the exposure to neonicotinoids. None of these correlations has, to BCS's knowledge, ever been observed in any field monitoring.

Table B. 9.1.3.1-1: 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[5] and 2015[6]
	Clothianidin + Imidacloprid FS 275 (100 + 175 g/L)	0.0144 µg total a.s./bee = 0.0052 µg CTD/bee + 0.0091 µg IMD/bee	1.2/3 Schmitzer S., 2014a
	Clothianidin + Fluopicolide+ Fluoxastrobin FS 510 (300 + 120 + 90 g/L)	0.0032 µg CTD/bee	1.2/4 Schmitzer S., 2010
	Clothianidin + Prothioconazole FS 300 (250 + 50 g/L)	0.0058 µg CTD/bee	1.2/6 Schmitzer S., 2014b
Acute contact toxicity 48h-LD_{50}	Clothianidin (technical active substance)	**0.0275** – 0.0443 **µg CTD/bee**	EFSA, 2013[5] and 2015[6]
	Clothianidin + Imidacloprid FS 275 (100 + 175 g/L)	0.072 µg total a.s./bee = 0.026 µg CTD/bee + 0.046 µg IMD/bee	1.2/3 Schmitzer S., 2014a
	Clothianidin + Fluopicolide+ Fluoxastrobin FS 510 (300 + 120 + 90 g/L)	0.0448 µg CTD/bee	1.2/4 Schmitzer S., 2010
	Clothianidin + Prothioconazole FS 300 (250 + 50 g/L)	0.0308 µg CTD/bee	1.2/6 Schmitzer S., 2014b
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[6])
Honeybee larvae 7-day NOED 22-day NOED	Clothianidin (technical active substance)	40 µg CTD/kg diet = **0.00528 µg CTD/bee** 10 µg CTD/kg diet = 0.00132 µg CTD/bee	Maus Ch., 2009 (re-evaluation by EFSA, 2015[6])

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

A comprehensive review of sublethal effects of pesticides was reported in the EFSA PPR Panel, 2012[10]. 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

[10] 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.

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.

Table B.9.1.3.1-2: 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
	Clothianidin + Imidacloprid FS 275 (100 + 175 g/L)	54.9 µg total a.s./bumblebee[1] = 19.9 µg CTD/bumblebee + 35.0 µg IMD/bumblebee	1.2/2 Pfeiffer S., 2014a
	Clothianidin + Fluopicolide+ Fluoxastrobin FS 510 (300 + 120 + 90 g/L)	1.22 µg CTD/bumblebee	1.2/4 Pfeiffer S., 2014b
	Clothianidin + Prothioconazole FS 300 (250 + 50 g/L)	0.20 µg CTD/bumblebee	1.2/6 Pfeiffer S., 2014c

*Note: CTD = clothianidin; IMD = imidacloprid; N.A. = not available; values in **bold** will be used in the risk assessment; [1] 72h-LD_{50} instead of 48h*

For bumblebees, the contact toxicity of clothianidin in a formulation is lower compared to the toxicity as active substance. Therefore, the active substance endpoints 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 lower contact toxicity of clothianidin to bumblebees compared to honeybees. It is correct that in the laboratory studies for both the active substance and the three tested formulations, bumblebees were less sensitive for contact toxicity compared to honeybees. In general, bumblebees were approximately 5.4 times less sensitive than honeybees to technical clothianidin and over 700 times less sensitive than honeybees to the formulated products. For oral toxicity, however, there is no difference in toxicity between bumblebees and honeybees. The oral LD_{50} for bumblebees is even slightly lower than the oral LD_{50} for honeybees (0.001943 µg a.s./bumblebee vs. 0.00379 µg a.s./honeybee). According to the argumentation provided by the applicant, there are differences in feeding habits between bumblebees and honeybees that make it difficult to compare oral toxicity endpoints between them. Nevertheless, a similar or even slightly higher acute oral toxicity to bumblebees cannot be ignored. Further, 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. There could also 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.9.1.3.1-3 provides an overview of all toxicity endpoints that will be used in the risk assessments throughout this Addendum.

Table B.9.1.3.1-3: 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.

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.

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)

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 seed treatment 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 during sowing of treated seeds, 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 seed treatment, as at the moment of sowing 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. For the currently registered uses for clothianidin as seed treatment in winter cereals and beets, consumption of pollen or nectar from the treated crop was not considered relevant in the original version of this Addendum, as these crops were categorized as 'non-attractive crops' to honeybees in an earlier version of Appendix D of the EFSA Guidance Document on bees. During Peer Review, it was however noted that the revised version of Appendix D states that although cereals are not attractive for nectar and are generally considered low attractive to honeybees for pollen, pollen collection from cereals cannot be excluded at all due to controversial information found in literature. At Pesticides Peer Review Meeting 145, it was therefore concluded that a risk assessment for the treated crop scenario for cereals should be included in this addendum. For beets, the revised version of Appendix D states that this crop is attractive for nectar collection and that pollen collection cannot be excluded. It was noted at Pesticides Peer Review Meeting 145 that beets are biannual crops, which only flowers in the second year. Therefore, it was concluded that the treated crop scenario is not relevant if beets are not grown for seed production. 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 seed treatment in different crops was performed by EFSA, and is reported in the EFSA Conclusion published in 2013[11]. 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)[12]. Since then, the EFSA Guidance Document on the risk assessment for bees (2013)[13] 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:

19. Exposure via contact through dust drift: Section B.9.6
20. 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
21. Oral exposure via consumption of guttation water: Section B.9.5
22. 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.

[11] 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

[12] 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.

[13] 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 two studies that used the "forced" approach (Ythier, 2014; Striffler & Ballhaus, 2014) and three studies that followed the "natural residues" approach (Jarrat 2014a, b and c). In addition to the studies performed in Europe, a study similar to the "natural residues" studies has been completed in USA (Xu & Dyer, 2014). This study was submitted as supplementary data.

"Natural residues" studies

To determine the potential residues in succeeding crops under realistic agricultural conditions agricultural sites with a history of use of clothianidin were selected. As discussed above these are considered to represent a more realistic scenario than exposure to freshly applied residues.

To perform these studies, sites in the UK with a history of the use of clothianidin were selected. Prior to use in the study the presence (and concentration) of clothianidin in the fields was measured. The sites were sown with a number of crops which have bee attractive matrices (i.e. pollen or nectar). To represent a flowering crop providing both nectar and pollen phacelia was selected, this plant has been recognised as a highly bee attractive crop which is frequently recommended for use in bee testing regimes due to its abundant flowering[14]. The second crop selected was maize, by selecting maize it was also possible to collect guttation liquid for analysis as maize has been shown to guttate frequently.

Report:	**1.3/1; Jarratt, N.; 2014a**
Title:	**Determination of clothianidin residues in bee relevant matrices, collected in a succeeding crop scenario with natural aged clothianidin residues - Field phase conducted with phacelia and maize in the UK (Goole, East Yorkshire)**
Report No.:	B2BN2000
Document No.:	M-504590-01-1
Guideline(s):	not available
Guideline deviation(s):	not available
GLP/GEP:	yes

Objective

The aim of the study was to determine residues of clothianidin and its metabolites Thiazolylnitroguanidine (TZNG) and Thiazolylmethylurea (TZMU) in bee relevant matrices (pollen, nectar and guttation fluid) collected from flowering rotational crops cultivated as succeeding crops

[14] e.g. in EFSA 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.

grown in the UK on fields with a history of clothianidin use and as such with natural aged soil-residues of this active ingredient.

Material and Methods

The study was conducted on a field site near Goole, East Yorkshire (UK) with a known history of clothianidin use (i.e. use of clothianidin and/or thiamethoxam as seed treatment in 5 crops/years within the last 10 years) and such with a likelihood of natural aged soil residues of this active substance. An approximately one hectare plot located within the dimension of the agricultural land was marked out, and divided into two evenly sized sub-plots. One sub-plot was sown with maize (*Zea mays*) the other sub-plot was sown with phacelia *(Phacelia tanacetifolia*).

Crops were sown according to Good Agricultural Practice (GAP). The maize and phacelia plots were sown on the afternoon of 21/05/2014, using calibrated equipment (tractor and seed drill). The target sowing rates were 10 kg seeds/ ha for phacelia and 23.3 kg seeds/ ha for maize. The actual sowing rates calculated based on the tractors on board drilling computer were 9.0 kg seeds/ ha for phacelia and 22.8 kg seeds/ ha for maize.

The sub-plot sown with maize was divided into three smaller sub plots, each similar in size that were large enough to have a sufficient number of plants available for both guttation fluid and for maize pollen sampling.

Three bee proof tunnels (14.6 m long x 5.5 m wide x 3 m high) were placed onto the phacelia plot after successful germination. A single honeybee colony was placed into each tunnel at the start of phacelia flowering.

Soil Sampling

From each of the maize sub plots and phacelia tunnels, two different types of soil sample were collected. These samples were used for:

23. Soil characterisation of the upper 10 cm soil layer.
24. Determination of the residues of parent clothianidin and the metabolites in the upper 15 cm soil layer.

An Edelman type combination soil auger was used to collect 12 soil cores (per sample type) at the required depth throughout the sample areas.

Soil cores used for characterisation and residue analysis were collected from each of the three segregated maize sub plots, prior to the start of the guttation sampling phase of the trial and from inside the three tunnels prior to placement of the honeybee colonies into the tunnels. Additional soil cores for residue analysis were also collected from the maize sub plots during the guttation sampling phase of the trial.

Sampling of Nectar and Pollen from Phacelia Crops

Nectar and pollen sampling was conducted at three different time points during bloom of the phacelia crop. Once the phacelia started to bloom, honeybee colonies were placed into mesh covered tunnels erected over the crop. Honeybees were exposed to the flowering phacelia under confined conditions and were exclusively used as a sampling device for both nectar and pollen.

Nectar was sampled by extracting the honey stomachs from forager bees. Therefore, the hive entrance was blocked during bee flight activity for a short period of time and the returning forager bees (without pollen loads on the corbicula) were collected at the hive entrance, using either modified vacuum cleaners or tweezers. Pollen was collected from foragers returning to the colony using a pollen trap attached to each colony. This pollen trap is a restrictive mesh through which bees must pass when entering the hive and which dislodges the corbicular pollen from the bees' hind legs. Pollen and nectar samples during bloom were analysed for residues of clothianidin.

Sampling of Guttation Fluid and Pollen from Maize

Guttation fluid and pollen sampling was conducted in the maize crop. Samples were collected directly from the crop by hand. Sampling of guttation fluid was carried out on a regular basis over a 76 day period. Guttation sampling started directly after emergence of the maize crop (BBCH scale 11 - 12) until flowering (BBCH scale 65). Guttation fluid was collected from each of the three sub-plots approximately thirty minutes after sunrise. The sampling period, except for one occasion was ≤ 30 minutes at each time point. This was to ensure an equivalent time chronology every day. Sampling took place in the same order at each time point, starting with sub plot 1 and finishing with sub plot 3. When guttation was present it was collected from > 10 plants throughout each of the sub plots. The target volume for each sample was 1 mL of guttation fluid.

Maize pollen sampling from three time points during bloom started when the crop started to shed pollen (BBCH scale 63) until male flowering had completed (BBCH scale 65). At each time point ≥ 50 flowering tassels were collected from throughout each of the three sub plots and placed into paper bags. Damp tassels were air dried, in the dark at room temperature for 24 – 48 hours. Pollen was extracted from the tassels by shaking them over a 710 µm analytical sieve and base pan. Plant or insect debris remaining in the pollen sample was removed by hand using forceps or a fine paint brush. The target sample size per sub plot, per time point was 1.5 g pollen. Pollen samples during bloom as well as collected guttation fluid were analysed for residues of clothianidin.

Sample storage and residue analysis

Samples were stored in the dark at -20°C ±10°C until processing and analysis (with the exception of guttation fluid and phacelia pollen, which were stored refrigerated instead of frozen for 1 night during shipment).

All samples (pollen, nectar and guttation fluid) were analysed for their content of clothianidin and its metabolites TZNG and TZMU via HPLC-MS/MS. Soil was analysed for clothianidin only. Processing and analysis was conducted according to the following analytical methods:

25. Soil: *Method 00540/M001 (Sommer, 2003 – BCS Report No MR-106/02)*, consisting of microwave assisted extraction with water/acetonitrile, centrifugation and quantification HPLC-MS/MS using stable isotopically labelled internal standards (SILIS).
26. Guttation fluid: an adapted version of *Method 00554/M001 (Schöning, 2001 – BCS Report No MR-338/00)*, i.e. dilution with water/acetonitrile (4/1, v/v) and SILIS, followed by quantification using reversed phase HPLC with MS/MS-detection.
27. Pollen and nectar: *Method 01433 (Schöning, R. – BCS report No MR-14/123)*, consisting of extraction with methanol/water (3/1, v/v), filtration, concentration of the extract, addition of internal standard, partitioning against dichloromethane. For i.a. pollen, further clean-up is carried out by column chromatography on silica-gel, elution with acetonitrile/water, evaporation to dryness and dilution with methanol/water. Quantification is done by reversed phase HPLC with MS/MS detection using SISIL.

These methods were validated within this study and further studies (1.3/2, 1.3/3, 1.3/6, 1.3/7 – *vide infra*) by means of fortification of control samples. Overall, the recovery results suggest that the methods were suitable for quantification of the analytes down to the LOQs mentioned below (all recoveries at all fortification levels within acceptable range 60-120%). The Limit of Quantitation (LOQ) for clothianidin was 5 µg a.s./kg in soil, 1 µg a.s./L in guttation liquid, 0.3 µg a.s./kg in nectar and 0.6 µg a.s./kg in pollen samples. The corresponding limits of detection (LOD) were 2 µg/kg in soil, 0.3 µg/L in guttation liquid, 0.1 µg/kg in nectar and 0.2 µg/kg in pollen. The limits for the metabolites are as shown in the tables below.

Findings

Soil characterisation

The soil of all subplots was characterised as silt loam, according to USDA soil classification.

Residue analysis

A summary of the analytical results as obtained by analysing samples of soil, guttation liquid, pollen and nectar is provided in the tables below. Residues are reported in terms of µg a.s./kg for pollen, nectar and soil respectively µg a.s./L for guttation fluid.

Table B.9.2.1-1: Residues of clothianidin in soil

Crop	Sample material	Residue clothianidin (µg/kg dry soil)
Phacelia	Soil	18 - 41
Maize	Soil	16 - 22

LOD/LOQ = 2 µg/kg / 5 µg/kg for clothianidin in soil

TableB.9.2.1-2: Residues of clothianidin, TZMU and TZNG in maize guttation liquid samples

Sample material	Residue of clothianidin (µg/L)	Residue of TZNG (µg/L)	Residue of TZMU (µg/L)
Guttation liquid (Maize)	< LOD – 5.6	< LOD	< LOD – < LOQ

LOD/LOQ = 0.3 µg/L / 1 µg/L for guttation liquid samples (all analytes)

Table B.9.2.1-3: Residues of clothianidin, TZMU and TZNG in pollen from *Phacelia* and maize and nectar from *Phacelia*

Sample material	Residue of clothianidin (µg/kg)	Residue of TZNG (µg/kg)	Residue of TZMU (µg/kg)
Pollen (Phacelia)	< LOQ – 0.81	< LOD	< LOD
Pollen (Maize)	< LOQ – 0.80	< LOD	< LOD
Nectar (Phacelia)	< LOD – < LOQ	< LOD	< LOD

LOD/LOQ = 0.1 µg/kg / 0.3 µg/kg for clothianidin in nectar
LOD/LOQ = 0.2 µg/kg / 0.6 µg/kg for clothianidin in pollen
LOD/LOQ = 0.3 µg/kg / 1 µg/kg for the metabolites in pollen and nectar samples.

Conclusion

The study was conducted on a field site near Goole, East Yorkshire (UK) with a known history of crops and clothianidin uses as such with natural aged soil-residues of this active substance. Therefore, this study provides realistic field data on residue levels of clothianidin within bee relevant matrices, collected from non-clothianidin treated flowering phacelia and maize plants cultivated as succeeding crops from a field with natural aged soil-residues of clothianidin.

Maize

Two sets of soil samples were taken from the maize sub plots during the trial. One was collected prior to guttation, the second during the guttation period of the maize plants. The residue levels of clothianidin in soils ranged from 16 µg a.s./kg to 21 µg a.s./kg dry soil prior to guttation and 20 µg a.s./kg to 22 µg a.s./kg dry soil during guttation.

The residue levels of clothianidin in guttation fluid ranged from below the LOD (< 0.3 µg a.s./L) to 5.6 µg a.s./L. The residue levels of clothianidin in pollen, as sampled at three time points during bloom of the maize plants ranged from below the LOQ (< 0.6 µg a.s./kg) to 0.8 µg a.s./kg.

Phacelia

Soil cores used for residue analysis were taken from inside the three tunnels prior to placement of the honeybee colonies into the tunnels. The residue levels of clothianidin in soils ranged from 18 µg a.s./kg to 41 µg a.s./kg dry soil.

The residue levels of clothianidin in pollen ranged from below the LOQ (< 0.6 µg a.s./kg) to 0.81 µg a.s./kg. The residue levels of clothianidin in nectar ranged from below the LOD (< 0.1 µg a.s./kg) to below the LOQ (< 0.3 µg a.s./kg).

RMS Comments
The study was conducted on a field with a history of use of clothianidin and/or thiamethoxam as seed treatment in 5 crops/years within the last 10 years. The soil residues present at the site are thus considered representative for 'natural' aged soil residues of this clothianidin.

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

At Pesticides Peer Review Meeting 145, it was agreed that this study was acepptable for use in the risk assessment, as the soil residue level in this study was equal or higher than the expected accumulation of use over successive years (soil $PEC_{plateau}$). Note this expected accumulation was estimated by EFSA using the current approach for PEC_{soil} accumulation (ESCAPE model, based on the available $DegT_{50}$ in the field), which resulted, in any case, lower than the value estimated by the applicant (see 1.3/5; Hammel & Vrbka, 2014). The calculation approach used by the applicant using the soil PEARL approach which is still under development is considered not appropriate in regulatory submissions.

Report:	**1.3/2; Jarratt, N.; 2014b**
Title:	**Determination of clothianidin residues in bee relevant matrices, collected in a succeeding crop scenario with natural aged clothianidin residues - Field phase conducted with phacelia and maize in the UK (Thorney, Cambridgeshire)**
Report No.:	B2BN3000
Document No.:	M-504595-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	yes

Objective
The aim of the study was to determine residues of clothianidin and its metabolites TZNG and TZMU in bee relevant matrices (pollen, nectar and guttation fluid) collected from flowering rotational crops cultivated as succeeding crops grown in the UK on fields with a history of clothianidin use and as such with natural aged soil-residues of this active ingredient.

Material and Methods
The study was conducted on a field site near Thorney, Cambridgeshire (UK) with a known history of clothianidin use (i.e. use of clothianidin as seed treatment in 6 crops/years within the last 8 years) and such with a likelihood of natural aged soil residues of this active substance. An approximately one hectare plot located within the dimension of the agricultural land was marked out, and divided into two evenly sized sub-plots. One sub-plot was sown with maize (*Zea mays*) the other sub-plot was sown with phacelia *(Phacelia tanacetifolia*).

Crops were sown according to Good Agricultural Practice (GAP). The maize and phacelia plots were sown on the afternoon of 20/05/2014, using calibrated equipment (tractor and seed drill). The target sowing rates were 10 kg seeds/ ha for phacelia and 23.3 kg seeds/ ha for maize. The actual sowing rates calculated based on the tractors on board drilling computer were 12.0 kg seeds/ ha for phacelia and 23.8 kg seeds/ ha for maize.

The sub-plot sown with maize was divided into three smaller sub plots, each similar in size that were large enough to have a sufficient number of plants available for both guttation fluid and for maize pollen sampling.

Three bee proof tunnels (14.6 m long x 5.5 m wide x 3 m high) were placed onto the phacelia plot after successful germination. A single honeybee colony was placed into each tunnel at the start of phacelia flowering.

Soil Sampling

From each of the maize sub plots and phacelia tunnels, two different types of soil sample were collected. These samples were used for:

28. Soil characterisation of the upper 10 cm soil layer.
29. Determination of the residues of parent clothianidin and the metabolites in the upper 15 cm soil layer.

An Edelman type combination soil auger was used to collect 12 soil cores (per sample type) at the required depth throughout the sample areas.

Soil cores used for characterisation and residue analysis were collected from each of the three segregated maize sub plots, prior to the start of the guttation sampling phase of the trial and from inside the three tunnels prior to placement of the honeybee colonies into the tunnels. Additional soil cores for residue analysis were also collected from the maize sub plots during the guttation sampling phase of the trial.

Sampling of Nectar and Pollen from Phacelia Crops

Nectar and pollen sampling was conducted at three different time points during bloom of the phacelia crop. Once the phacelia started to bloom, honeybee colonies were placed into mesh covered tunnels erected over the crop. Honeybees were exposed to the flowering phacelia under confined conditions and were exclusively used as a sampling device for both nectar and pollen.

Nectar was sampled by extracting the honey stomachs from forager bees. Therefore, the hive entrance was blocked during bee flight activity for a short period of time and the returning forager bees (without pollen loads on the corbicula) were collected at the hive entrance, using either modified vacuum cleaners or tweezers. Pollen was collected from foragers returning to the colony using a pollen trap attached to each colony. This pollen trap is a restrictive mesh through which bees must pass when entering the hive and which dislodges the corbicular pollen from the bees' hind legs. Pollen and nectar samples during bloom were analysed for residues of clothianidin.

Sampling of Guttation Fluid and Pollen from Maize

Guttation fluid and pollen sampling was conducted in the maize crop. Samples were collected directly from the crop by hand. Sampling of guttation fluid was carried out on a regular basis over a 70 day period. Guttation sampling started directly after emergence of the maize crop (BBCH scale 11 - 12) until flowering (BBCH scale 69). Guttation fluid was collected from each of the three sub-plots approximately thirty minutes after sunrise. The sampling period, except for one occasion was ≤ 30 minutes at each time point. This was to ensure an equivalent time chronology every day. Sampling took place in the same order at each time point, starting with sub plot 1 and finishing with sub plot 3. When guttation was present it was collected from > 10 plants throughout each of the sub plots. The target volume for each sample was 1 mL of guttation fluid.

Maize pollen sampling from three time points during bloom started when the crop started to shed pollen (BBCH scale 63) until male flowering had completed (BBCH scale 67). At each time point ≥ 50 flowering tassels were collected from throughout each of the three sub plots and placed into paper bags. Damp tassels were air dried, in the dark at room temperature for 24 – 48 hours. Pollen was extracted from the tassels by shaking them over a 710 µm analytical sieve and base pan. Plant or insect debris remaining in the pollen sample was removed by hand using forceps or a fine paint brush. The target sample size per sub plot, per time point was 1.5 g pollen. Pollen samples during bloom as well as collected guttation fluid were analysed for residues of clothianidin.

Sample storage and residue analysis

Samples were stored in the dark at -20°C ±10°C until processing and analysis (with the exception of guttation fluid and phacelia pollen, which were stored refrigerated instead of frozen for 1 night during shipment).

All samples (pollen, nectar and guttation fluid) were analysed for their content of clothianidin and its metabolites TZNG and TZMU via HPLC-MS/MS. Soil was analysed for clothianidin only. Processing and analysis was conducted according to the following analytical methods:

30. Soil: *Method 00540/M001 (Sommer, 2003 – BCS Report No MR-106/02);*
31. Guttation fluid: an adapted version of *Method 00554/M001 (Schöning, 2001 – BCS Report No MR-338/00)*;
32. Pollen and nectar: *Method 01433 (Schöning, R. – BCS report No MR-14/123);*

For a summary on description and validation of these methods: see 1.3/01 (*vide supra*).

Findings

Soil characterisation

The soil of all maize subplots was characterised as silty clay loam, according to USDA soil classification. The soil in the sub-plots used for phacelia was either silty clay or clay loam.

Residue analysis

A summary of the analytical results as obtained by analysing samples of soil, guttation liquid, pollen and nectar is provided in the following tables. Residues are reported in terms of µg a.s./kg for pollen, nectar and soil respectively µg a.s./L for guttation fluid.

Table B.9.2.1-4: Residues of clothianidin in soil

Crop	Sample material	Residue clothianidin (µg/kg dry soil)
Phacelia	Soil	64 – 78
Maize	Soil	59 – 80

LOD/LOQ = 2 µg/kg / 5 µg/kg for clothianidin in soil

Table B.9.2.1-5: Residues of clothianidin, TZMU and TZNG in maize guttation liquid samples

Sample material	Residue of Clothianidin (µg/L)	Residue of TZNG (µg/L)	Residue of TZMU (µg/L)
Guttation liquid (Maize)	< LOD – 40.3	< LOD –1.9	< LOD – 1.9

LOD/LOQ = 0.3 µg/L / 1 µg/L for guttation liquid samples (all analytes)

Table B.9.2.1-6: Residues of clothianidin, TZMU and TZNG in pollen from Phacelia and maize and nectar from Phacelia

Sample material	Residue of clothianidin (µg/kg)	Residue of TZNG (µg/kg)	Residue of TZMU (µg/kg)
Pollen (Phacelia)	< LOQ – 1.2	< LOD – < LOQ	< LOD
Pollen (Maize)	< LOQ – 1.5	< LOD	< LOD
Nectar (Phacelia)	< LOQ	< LOD	< LOD

LOD/LOQ = 0.1 µg/kg / 0.3 µg/kg for clothianidin in nectar
LOD/LOQ = 0.2 µg/kg / 0.6 µg/kg for clothianidin in pollen
LOD/LOQ = 0.3 µg/kg / 1 µg/kg for the metabolites in pollen and nectar samples.

Conclusion

The study was conducted on a field site near Thorney, Cambridgeshire (UK) with a known history of crops and clothianidin uses as such with natural aged soil-residues of this active substance. Therefore, this study provides realistic field data on residue levels of clothianidin within bee relevant matrices, collected from non-clothianidin treated flowering phacelia and maize plants cultivated as succeeding crops from a field with natural aged soil-residues of clothianidin.

Maize

Two sets of soil samples were taken from the maize sub plots during the trial. One was collected prior to guttation, the second during the guttation period of the maize plants. The residue levels of clothianidin in soils ranged from 76 µg a.s./kg to 80 µg a.s./kg dry soil prior to guttation and 59 µg a.s./kg to 64 µg a.s./kg dry soil during guttation.

The residue levels of clothianidin in guttation fluid ranged from below the LOD (< 0.3 µg a.s./L) to 40.3 µg a.s./L. The residue levels of clothianidin in pollen, as sampled at three time points during bloom of the maize plants ranged from below the LOQ (< 0.6 µg a.s./kg) to 1.5 µg a.s./kg.

Phacelia
Soil cores used for residue analysis were taken from inside the three tunnels prior to placement of the honeybee colonies into the tunnels. The residue levels of clothianidin in soils ranged from 64 µg a.s./kg to 78 µg a.s./kg dry soil.
The residue levels of clothianidin in pollen ranged from below the LOQ (< 0.6 µg a.s./kg) to 1.2 µg a.s./kg. The residue levels of clothianidin in nectar were all below the LOQ (< 0.3 µg a.s./kg).

RMS Comments
The study was conducted on a field with a history of use of clothianidin as seed treatment in 6 crops/years within the last 8 years. The soil residues present at the site are thus considered representative for 'natural' aged soil residues of this clothianidin.

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

At Pesticides Peer Review Meeting 145, it was agreed that this study was acepptable for use in the risk assessment, as the soil residue level in this study was equal or higher than the expected accumulation of use over successive years (soil $PEC_{plateau}$). Note this expected accumulation was estimated by EFSA using the current approach for PEC_{soil} accumulation (ESCAPE model, based on the available $DegT_{50}$ in the field), which resulted, in any case, lower than the value estimated by the applicant (see 1.3/5; Hammel & Vrbka, 2014). The calculation approach used by the applicant using the soil PEARL approach which is still under development is considered not appropriate in regulatory submissions.

Report:	**1.3/3; Jarratt, N.; 2014c**
Title:	**Determination of clothianidin residues in bee relevant matrices, collected in a succeeding crop scenario with natural aged clothianidin residues - Field phase conducted with phacelia and maize in the UK (Sawtry, Cambridgeshire)**
Report No.:	B2BN4000
Document No.:	M-504601-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	yes

Objective
The aim of the study was to determine residues of clothianidin and its metabolites TZNG and TZMU in bee relevant matrices (pollen, nectar and guttation fluid) collected from flowering rotational crops cultivated as succeeding crops grown in the UK on fields with a history of clothianidin use and as such with natural aged soil-residues of this active ingredient.

Material and Methods
The study was conducted on a field site near Sawtry, Cambridgeshire (UK) with a known history of clothianidin use (i.e. use of clothianidin or thiamethoxam as seed treatment in 7 crops/years within the last 8 years) and such with a likelihood of natural aged soil residues of this active substance. An approximately one hectare plot located within the dimension of the agricultural land was marked out, and divided into two evenly sized sub-plots. One sub-plot was sown with maize (*Zea mays*) the other sub-plot was sown with phacelia *(Phacelia tanacetifolia*).

Crops were sown according to Good Agricultural Practice (GAP). The maize and phacelia plots were sown on the afternoon of 06/06/2014, using calibrated equipment (tractor and seed drill). The target

sowing rates were 10 kg seeds/ ha for phacelia and 23.3 kg seeds/ ha for maize. The actual sowing rates calculated based on the tractors on board drilling computer were 11.3 kg seeds/ ha for phacelia and 25.3 kg seeds/ ha for maize.

The sub plot sown with maize was divided into three smaller sub plots, each similar in size that were large enough to have a sufficient number of plants available for both guttation fluid and for maize pollen sampling.

Three bee proof tunnels (14.6 m long x 5.5 m wide x 3 m high) were placed onto the phacelia plot after successful germination. A single honeybee colony was placed into each tunnel at the start of phacelia flowering.

Soil Sampling

From each of the maize sub plots and phacelia tunnels, two different types of soil sample were collected. These samples were used for:

33. Soil characterisation of the upper 10 cm soil layer.
34. Determination of the residues of parent clothianidin and the metabolites in the upper 15 cm soil layer.

An Edelman type combination soil auger was used to collect 12 soil cores (per sample type) at the required depth throughout the sample areas.

Soil cores used for characterisation and residue analysis were collected from each of the three segregated maize sub plots, prior to the start of the guttation sampling phase of the trial and from inside the three tunnels prior to placement of the honeybee colonies into the tunnels. Additional soil cores for residue analysis were also collected from the maize sub plots during the guttation sampling phase of the trial.

Sampling of Nectar and Pollen from Phacelia Crops

Nectar and pollen sampling was conducted at three different time points during bloom of the phacelia crop. Once the phacelia started to bloom, honeybee colonies were placed into mesh covered tunnels erected over the crop. Honeybees were exposed to the flowering phacelia under confined conditions and were exclusively used as a sampling device for both nectar and pollen.

Nectar was sampled by extracting the honey stomachs from forager bees. Therefore, the hive entrance was blocked during bee flight activity for a short period of time and the returning forager bees (without pollen loads on the corbicula) were collected at the hive entrance, using either modified vacuum cleaners or tweezers. Pollen was collected from foragers returning to the colony using a pollen trap attached to each colony. This pollen trap is a restrictive mesh through which bees must pass when entering the hive and which dislodges the corbicular pollen from the bees' hind legs. Pollen and nectar samples during bloom were analysed for residues of clothianidin.

Sampling of Guttation Fluid and Pollen from Maize

Guttation fluid and pollen sampling was conducted in the maize crop. Samples were collected directly from the crop by hand. Sampling of guttation fluid was carried out on a regular basis over a 70 day period. Guttation sampling started directly after emergence of the maize crop (BBCH scale 12) until flowering (BBCH scale 69). Guttation fluid was collected from each of the three sub-plots approximately thirty minutes after sunrise. The sampling period, except for one occasion was ≤ 30 minutes at each time point. This was to ensure an equivalent time chronology every day. Sampling took place in the same order at each time point, starting with sub plot 1 and finishing with sub plot 3. When guttation was present it was collected from > 10 plants throughout each of the sub plots. The target volume for each sample was 1 mL of guttation fluid.

Maize pollen sampling from three time points during bloom started when the crop started to shed pollen (BBCH scale 63) until male flowering had completed (BBCH scale 65). At each time point ≥ 50 flowering tassels were collected from throughout each of the three sub plots and placed into paper

bags. Pollen was extracted from the tassels by shaking them over a 710 µm analytical sieve and base pan. Plant or insect debris remaining in the pollen sample was removed by hand using forceps or a fine paint brush. The target sample size per sub plot, per time point was 1.5 g pollen. Pollen samples during bloom as well as collected guttation fluid were analysed for residues of clothianidin.

Sample storage and residue analysis
Samples were stored in the dark at -20°C ±10°C until processing and analysis (with the exception of guttation fluid and phacelia pollen, which were stored refrigerated instead of frozen for 1 night during shipment).

All samples (pollen, nectar and guttation fluid) were analysed for their content of clothianidin and its metabolites TZNG and TZMU via HPLC-MS/MS. Soil was analysed for clothianidin only. Processing and analysis was conducted according to the following analytical methods:

35. Soil: *Method 00540/M001 (Sommer, 2003 – BCS Report No MR-106/02);*
36. Guttation fluid: an adapted version of *Method 00554/M001 (Schöning, 2001 – BCS Report No MR-338/00)*;
37. Pollen and nectar: *Method 01433 (Schöning, R. – BCS report No MR-14/123);*

For a summary on description and validation of these methods: see 1.3/01 (*vide supra*).

Findings
Soil characterisation
The soil of all phacelia subplots was characterised as silty clay loam, according to USDA soil classification. The soil in the sub-plots used for maize was either silty clay (sub-plot 1) or silty clay loam (sub-plots 2 and 3).

Residue analysis
A summary of the analytical results as obtained by analysing samples of soil, guttation liquid, pollen and nectar is provided in the following tables. Residues are reported in terms of µg a.s./kg for pollen, nectar and soil respectively µg a.s./L for guttation fluid.

Table B.9.2.1-7: Residues of clothianidin in soil

Sample ID	Crop	Sample Type	Residue clothianidin (µg/kg dry soil)
Prior to Guttation – Sub plot 1	Maize	Soil	187
Prior to Guttation – Sub plot 2	Maize	Soil	118
Prior to Guttation – Sub plot 3	Maize	Soil	92
During Guttation – Sub plot 1	Maize	Soil	248
During Guttation – Sub plot 2	Maize	Soil	137
During Guttation – Sub plot 3	Maize	Soil	98
Tunnel 1	Phacelia	Soil	80
Tunnel 2	Phacelia	Soil	78
Tunnel 3	Phacelia	Soil	79

LOD/LOQ = 2 µg/kg / 5 µg/kg for clothianidin in soil

Whilst clothianidin soil residues show a low variability between the phacelia tunnel sub plots, values for the maize sub plots 1 to 3 vary quite significantly. This variability is very likely linked to varying soil properties on the study field. While the tunnel plots were located on top of a small hill and close to each other, the maize plots followed the slope of the hill, with sub-plot 3 at the top and sub-plot 1 at the bottom. In addition to topography, important soil properties also changed between the maize sub plots. Soil on maize sub-plot 1, which had the highest residues levels also had approximately 2 x more clay (44.8 %) and organic carbon content (28.3 %) than the other two plots.

Table B.9.2.1-8: Residues of clothianidin, TZMU and TZNG in maize guttation liquid samples

Sample material	Residue of clothianidin [µg/L]	Residue of TZNG [µg/L]	Residue of TZMU [µg/L]
Guttation liquid (Maize)	< LOD – 28.2	< LOD –1.8	< LOD – 1.0

LOD/LOQ = 0.3 µg/L / 1 µg/L for guttation liquid samples (all analytes)

Table B.9.2.1-9: Residues of clothianidin, TZMU and TZNG in pollen from Phacelia and maize and nectar from Phacelia

Sample material	Residue of clothianidin [µg/kg]	Residue of TZNG [µg/kg]	Residue of TZMU [µg/kg]
Pollen (Phacelia)	< LOQ – 0.84	< LOD – < LOQ	< LOD
Pollen (Maize)	< LOQ – 1.3	< LOD	< LOD
Nectar (Phacelia)	< LOD – 0.6	< LOD	< LOD

LOD/LOQ = 0.1 µg/kg / 0.3 µg/kg for clothianidin in nectar
LOD/LOQ = 0.2 µg/kg / 0.6 µg/kg for clothianidin in pollen
LOD/LOQ = 0.3 µg/kg / 1 µg/kg for the metabolites in pollen and nectar samples.

Conclusion

The study was conducted on a field site near Sawtry, Cambridgeshire (UK) with a known history of crops and clothianidin uses as such with natural aged soil-residues of this active substance. Therefore, this study provides realistic field data on residue levels of clothianidin within bee relevant matrices, collected from non-clothianidin treated flowering phacelia and maize plants cultivated as succeeding crops from a field with natural aged soil-residues of clothianidin.

Maize

The residue levels of clothianidin in guttation fluid ranged from below the LOD (< 0.3 µg a.s./L) to 28.2 µg a.s./L. The residue levels of clothianidin in pollen, as sampled at three time points during bloom of the maize plants ranged from below the LOQ (< 0.6 µg a.s./kg) to 1.3 µg a.s./kg.

Although soil residues of clothianidin varied between the three maize subplots, most likely due to a high variability of key soil properties (e.g. texture organic carbon content and clay content), there is no indication that the translocation of soil residues into guttation droplets or pollen has been influenced by these factors.

Phacelia

Soil cores used for residue analysis were taken from inside the three tunnels prior to placement of the honeybee colonies into the tunnels. The residue levels of clothianidin in soils ranged from 78 µg a.s./kg to 80 µg a.s./kg dry soil.
The residue levels of clothianidin in pollen ranged from below the LOQ (< 0.6 µg a.s./kg) to 0.84 µg a.s./kg. The residue levels of clothianidin in nectar ranged from below the LOD (< 0.1 µg a.s./kg) to 0.6 µg a.s./kg.

RMS Comments

The study was conducted on a field with a history of use of clothianidin or thiamethoxam as seed treatment in 7 crops/years within the last 8 years. The soil residues present at the site are thus considered representative for ‘natural’ aged soil residues of this clothianidin.

There was a high variation of soil residues of clothianidin between the three maize subplots, most likely due to a high variability in key soil parameters. However, RMS agrees that there is no indication that the translocation of clothianidin soil residues to pollen or guttation droplets in maize is influenced by the observed differences in soil properties.

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

At Pesticides Peer Review Meeting 145, it was agreed that this study was acepptable for use in the risk assessment, as the soil residue level in this study was equal or higher than the expected accumulation

of use over successive years (soil $PEC_{plateau}$). Note this expected accumulation was estimated by EFSA using the current approach for PEC_{soil} accumulation (ESCAPE model, based on the available $DegT_{50}$ in the field), which resulted, in any case, lower than the value estimated by the applicant (see 1.3/5; Hammel & Vrbka, 2014). The calculation approach used by the applicant using the soil PEARL approach which is still under development is considered not appropriate in regulatory submissions.

Report:	**1.3/4; Xu, T.; Dyer, Daniel; 2014**
Title:	**Clothianidin plant bioavailability and soil accumulation study - Clothianidin (TI-435)**
Report No.:	METIY004
Document No.:	M-498438-01-1
Guideline(s):	OCSPP 835.SUPP
Guideline deviation(s):	not specified
GLP/GEP:	yes

Objective
To investigate the potential accumulation of clothianidin in soil and crop matrices after multiple years of planting clothianidin treated corn and canola seeds, a plant bioavailability and soil accumulation study was conducted. The study evaluated clothianidin residues in soil, pollen and nectar from commercial agricultural fields which had historical plantings of clothianidin treated seeds.

Material and Methods
Sites, Crops, and Application Rates
Commercial agricultural fields in the corn growing regions of the United States and canola growing region of Canada were selected based primarily on crop acreage, with consideration of the distribution of the number of years of planting clothianidin treated seeds (and thiamethoxam, which degrades partly to clothianidin), and a broad geographic distribution for each crop. Fifty corn sites were identified, and represented 2 to 11 years of clothianidin use, wide geographic coverage and diverse soil types, climate, and agronomic practices. The corn seed was treated at rates of 0.25, 0.5, or 1.25 mg clothianidin/seed (PONCHO® 250, 500, or 1250, respectively), and a wide variety of treatments was represented. Each site was planted with clothianidin treated seeds in the year of sampling. Twenty sites were sampled in 2012, and 30 sites were sampled in 2013.

Fifteen canola fields were selected representing 1 to 4 years of planting clothianidin treated seeds, with 5 sites having only a single year, and one site having 4 years of treatments. Thiamethoxam treated canola seeds were planted at 7 of the sites at some time in the past few years. Sites represented a wide geographic distribution and a variety of climatic, soil, and crop rotation practices common for canola, typically, 2 to 3 year rotations with wheat. Typical treatment rates were 400 g clothianidin /100 kg seed (PROSPER®). Five sites were sampled in 2012, and 10 sites were sampled in 2013.
Statistical analysis demonstrated that the study sampled a representative range of soil and environmental factors that could influence clothianidin variability in soil.

Sampling
Replicate plots were established at each site for sampling of soil and pollen or nectar. The plots were at least 100 feet from each other and were divided into 8 sampling areas from which samples of each matrix were collected. The samples for each matrix within a replicate plot were composited into a single analytical sample (2 replicates per site, per matrix). Soil samples were collected with a 2 inch diameter auger, except for the 2012 canola samples which were collected with a 1-inch coring device (4 cores per sampling area).

Corn pollen was collected by removing tassels from the corn plant, and shaking the pollen into a paper bag (0.5 g target amount). Canola nectar was collected by cutting flowers from multiple plants, removing the flower petals and extracting the nectar with a micro-capillary tube using capillary action

(0.3-0.5 mL target amount). Due to the 2012 drought in the mid-western United States, pollen production was very poor, and only six of the twenty sites produced sufficient pollen samples, and the few available samples were of poor quality. Therefore, corn pollen results are only for the 2013 samples. Similarly, the quantity and quality of canola pollen samples was poor and therefore a decision was made to discontinue canola pollen sampling.

Analysis

Analytical methods were developed to determine residues of clothianidin and its metabolites, TZNG (N-(2-chlorothiazol-5-ylmethyl)-N'-nitroguanidine; desmethyl clothianidin) and TZMU (N-(2-chlorothiazol-5-ylmethyl)-N'-methylurea; clothianidin urea) in pollen and nectar, and to determine total and "bioavailable" concentrations of clothianidin in soil.

Table B.9.2.1-10: Limits of Detection (LOD, µg/kg) and Quantitation (LOQ, µg/kg) for clothianidin and the concerned metabolites TZNG and TZMU in the different tested matrices

Matrix	Analyte	Limit of Detection, LOD (µg/kg)	Limit of Quantification, LOQ (µg/kg)
Nectar	Clothianidin, TZNG, TZMU	0.2	1
Pollen	Clothianidin, TZNG, TZMU	0.25	1
Soil, Total	Clothianidin	1.3	5
Soil, bioavailable	Clothianidin	0.3	5

Findings

Corn Sites - Pollen

There was no indication of residues in pollen being higher from fields which had multiple years of clothianidin use. Generally, pollen residues were related to the amount of clothianidin on the treated seed from the current year – for example, three of the four highest pollen concentrations were from sites with Poncho® 1250 corn, and the fourth highest value was from a site with Poncho® 500 corn.

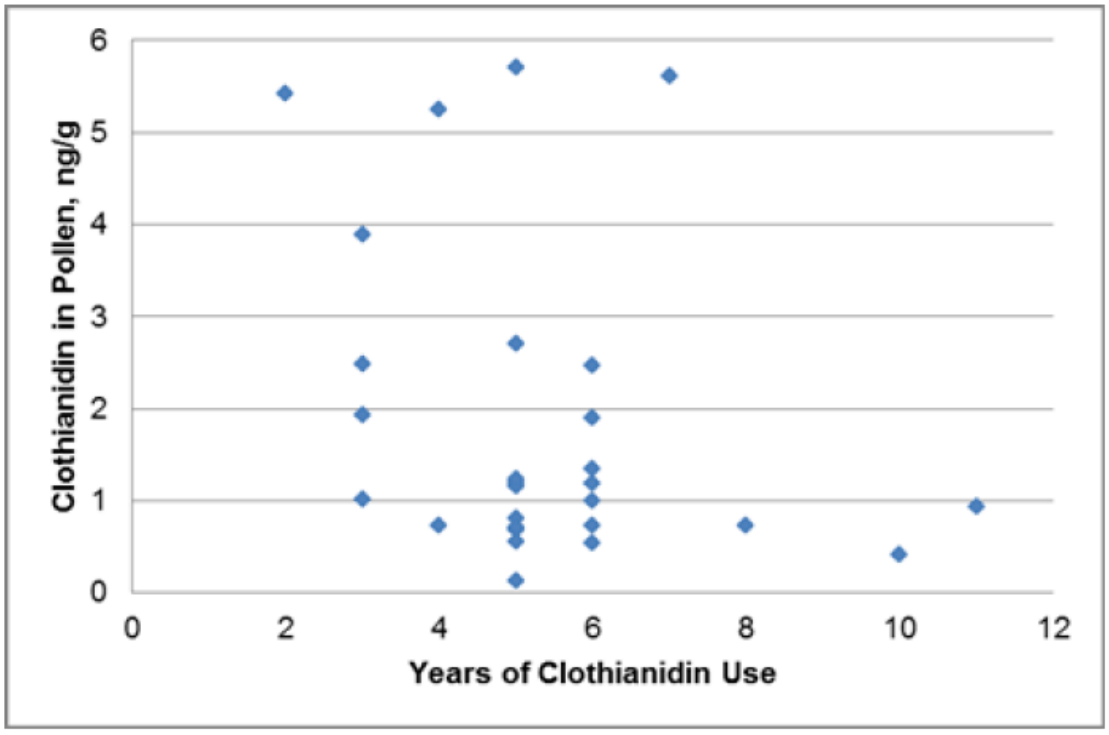

Figure B.9.2.1-1: Clothianidin in corn pollen with respect to years of use

Overall, the soil and pollen results indicate (a) there is minimal accumulation of clothianidin in soil from corn fields, (b) the majority of the clothianidin in soil is not readily bioavailable, and (c) clothianidin in pollen is not higher from fields with multiple applications.

Canola Sites - Nectar

In canola nectar, clothianidin concentrations were greater than the LOQ (>1 ng/g) in only 4 of 15 canola sites. The average concentration was 0.6 ng/g and the 90th percentile concentration was 1.7 ng/g (1/2 LOD was used as the concentration for samples <LOD). The clothianidin metabolites, TZNG and TZMU, were not detected (<0.2 ng/g) in the canola nectar samples. Clothianidin residues in canola nectar showed no correlation with the years of use of clothianidin or with clothianidin concentration in soil.

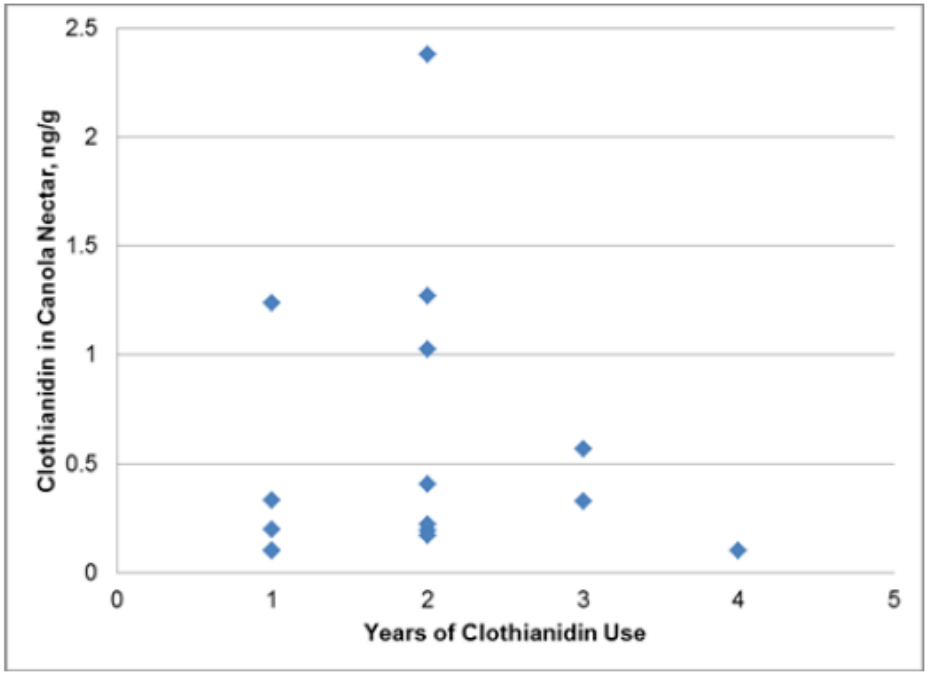

Figure 2: Clothianidin in canola nectar with respect to years of use

Conclusion

To investigate the potential accumulation of clothianidin in soil and crop matrices after multiple years of planting clothianidin treated corn and canola seeds, a plant bioavailability and soil accumulation study was conducted. The study evaluated clothianidin residues in soil, pollen and nectar from commercial agricultural fields which had historical plantings of clothianidin treated seeds. Fifty corn fields in the Midwestern United States and 15 canola fields in Western Canada were sampled in 2012 and 2013. Soil samples were taken from all sites, corn pollen was sampled in corn fields and canola nectar was sampled in canola fields. The sampled fields represented a broad geographical extent of the corn and canola growing regions, as well as the areas where clothianidin has been used. For the corn sites, the number of years of planting with clothianidin-treated corn seeds ranged between 2 and 11 years (mean: 4.7 years), with the greatest number of sites (15) having had 5 years of planting with clothianidin-treated corn seeds. Soil residues were determined using standard soil analytical methods. Clothianidin residues in soil were greater than the LOQ (5 ng/g) at 35 of the 50 corn sites, with a 90th percentile concentration of 13.5 ng/g and an average concentration of 7.0 ng/g. There was no significant accumulation of clothianidin in soil from fields with multiple years of clothianidin use. For the sites which had the longest clothianidin use histories (10 and 11 years), the clothianidin soil residues were only 16.2 ng/g and 8.9 ng/g, respectively. Even considering these were typically Poncho® 250 treatments, these low residues show there is little accumulation over these long periods of use. For sites having soil residues greater than the LOQ, a separate soil sample was extracted with water (0.01 M CaCl2) only, to represent the “bioavailable” concentration of clothianidin residues. The bioavailable concentrations were less than the LOQ (5 ng/g) in soil from all sites, with a 90th percentile concentration of 2.1 ng/g, and an average concentration of 1.0 ng/g. The average bioavailable fraction was 10% of the total soil residue. The bioavailable fraction showed no correlation with the years of clothianidin use, except for a slight decrease in the bioavailable fraction with

increasing years of clothianidin application, which is likely due to residues becoming more tightly bound to soil over time.

On canola sites, clothianidin in soil greater than LOQ (5 ng/g) were detected in 7 of 15 canola sites, and clothianidin were detected (greater than LOD (>1.3 ng/g) in the remaining 8 sites, resulting in an average concentration of 6.6 ng/g and a 90th percentile concentration of 15.8 ng/g. The bioavailable residues in soil were less than the LOQ (5 ng/g), with an average concentration of 0.7 ng/g and a 90th percentile concentration of 1.5 ng/g. The average bioavailable fraction (bioavailable residue/total residue) was 6% for the 12 samples from 8 sites where the total clothianidin concentrations were greater than LOQ.

On corn sites, there was no indication of clothianidin residues in pollen being higher from fields which had multiple years of clothianidin use or from fields with higher concentrations of clothianidin in the soil. Generally, pollen residues are related to the amount of clothianidin on the treated seed from the current year – for example three of the four highest pollen concentrations were from sites corn treated with Poncho® 1250, and the fourth highest value was from corn treated with Poncho® 500.

Overall, the soil and nectar results from canola fields indicate (a) there is minimal accumulation of clothianidin in soil, (b) the majority of clothianidin in the soil is not readily bioavailable, and (c) clothianidin concentrations in nectar are low, and not correlated to clothianidin use, or concentration in soil.

RMS comments

Even if the study was conducted in the United States and not on European soils, the range of tested fields (50 and 15 for corn and canola sites, respectively) is sufficiently large and gives a good overview of clothianidin accumulation and bioavailability in cultivated soils. The European GAPs (50-100 g a.s/ha) are equivalent to the theoretical application range tested in the present study (0,5-1,25 mg a.s/seed, typical seed density 84.000 seeds/ha, 42-105 g a.s/ha), the European GAPs are thus covered by the application rate in the study. The study is acceptable as supportive information.

In the study report, it is concluded that "results clearly show that clothianidin residues in pollen or nectar result from the clothianidin on the treated seed in the current year". This conclusion is based on the soil residue measurements that indicate that there is no trend to increasing soil concentrations with increasing time. Therefore, the applicant considers that the soil residues from previous treatments are not a significant contributor to the residues in the current crop. As seedlings from the current crop will have access to a large amount of clothianidin due to seed treatment, it is indeed reasonable to expect that the current year treatment will be the main source of clothianidin residues in aerial parts of the crop. However, from the data presented in the study, uptake from clothianidin residues from previous treatments cannot completely be excluded. Nevertheless, it is reasonable to assume that their contribution will be small compared to the current year treatment.

As the crop for which the concentration of clothianidin in nectar (canola) or pollen (maize) is measured is also treated with clothianidin, the measured residues are not representative for a "non-treated bee-attractive succeeding crop". Further, conclusions regarding the uptake of soil residues from clothianidin treatments in previous years can also not be extrapolated to non-treated succeeding crops.

"Forced exposure" studies

In a first study (Hammel & Vrbka, 2014), the theoretical plateau concentration in soil was calculated. This calculated soil plateau concentration was then applied in the two "forced exposure" studies submitted by the applicant.

The applicant provided the following argumentation for the application rates and crops considered in the calculation of the theoretical soil plateau concentration (text in italic):

Justification of soil plateau applied to the "model" crop rotational studies:

In considering the appropriate theoretical concentration of clothianidin which could occur in a succeeding crop situation the possible crops which could be treated with clothianidin and the potential rotations of these crops were elaborated. As the crop rotations may vary from country to country Bayer Crop Science (BCS) has performed a survey in a number of European countries and based on this survey the potential rotations were elaborated.

Clothianidin is used in different formulations in the same crop, frequently representing a "high" use and a "low". To take this into account two plateau concentrations were calculated, the first using the maximum rate for all relevant seed treated crops while the second accounts for a lower use rate of the seed treatment formulations.

Clothianidin is currently used as a seed treatment on winter cereals and sugar beet.

Winter cereals: Cereals may be grown as monoculture, however the use of break crops is recommended, and this break crop could be potatoes, oilseeds, sugar beet or a number of diverse crops which are not relevant for the use of neonicotinoid seed treatments. Hence the worst case is a winter cereals monoculture as this has the highest application rate.

Sugar beet: Sugar beet is most often grown with a crop rotation of 3 to 4 years, although in a few countries a two year rotation was also possible. The most common rotational crops were determine to be cereals, and possibly maize. As the maximum rate of clothianidin used as a seed treatment in sugar beet is less than that of cereals the use in this crop is also covered by the cereal monoculture described above.

Considering these common rotations a worst-case situation has been defined for the use of clothianidin seed treatment formulations:

38. *High loading: Cereal monoculture with 100g/ha/year*
39. *Low loading: 40g/ha/year (monoculture with lower annual application rates)*

Report:	**1.3/5; Hammel, K. & Vrbka, L.; 2014**
Title:	**Calculation of plateau concentrations in soil for imidacloprid and clothianidin**
Report No.:	EnSa-14-1318
Document No.:	M-503458-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	no

Introduction

Plateau concentrations in soil were calculated for the actives imidacloprid and clothianidin to assess the contribution of preceding applications of these actives to the exposure in soil. For this purpose a conservative assessment scheme was used which was recently presented by EFSA in EFSA (2010)[15] and EFSA (2012)[16] – in the following abbreviated as "EFSA approach". The plateau concentrations were used to determine the application rates of the two actives which are necessary to establish these plateau concentrations at the test sites Zülpich and Nimes.

Short Description of Approach

The EFSA approach considers three relevant geo-climatic zones for soil exposure assessment, the northern, central and southern zone. For each of these zones the environmental conditions relevant for soil exposure, i.e. PEC_{soil} of pesticides, were assessed at high spatial resolution (1 km^2 grid cell size). For a number of test compounds with different sorption and degradation behaviour, soil exposure calculations were performed with mechanistic models which provide a realistic and state-of-the-art description of solute transport in soil, such as PEARL and PELMO. Such calculations were made for every grid cell and for multiple year application (as for current PEC groundwater calculations). From the distribution of PEC_{soil} obtained from this large number of single calculations, environmental scenarios (consisting of soil and weather data) were derived which represent a defined vulnerability percentile. Thus the development of concentration plateaus is automatically included both in the derivation of the scenarios and in the results obtained with these scenarios.

As relevant for soil risk assessment, EFSA defined an overall 90th percentile soil exposure. This percentile even considers the effect of substance parameter uncertainty. Different scenarios were derived for total PEC_{soil} and liquid PEC_{soil}. In the following only total PEC_{soil} will be considered. The EFSA approach considers several tiers, of which here Tier 2a (PEC_{soil} calculation with numerical model) will be used together with a safety factor, the so-called crop extrapolation factor, accounting for the specific geographical distribution of the target crop (to which the pesticide is applied) in the zone. Here, the model SOILPEARL (http://www.pearl.pesticidemodels.eu/) was used which already contains the EFSA PEC_{soil} scenarios.

Justification of using both imidacloprid and clothianidin applied to the same plot

During review of the bee study protocols by EFSA[17], the question was raised whether an application of both imidacloprid and clothianidin to the same field would have any influence on the uptake of both substances by plants and on the measured residues in bee relevant matrices. It was decided at Pesticides Peer Review Meeting on the review of bee study protocols (April 2014) that the applicant should document (supported with data) whether the mixture of imidacloprid and clothianidin may result in a different root uptake for each individual substance.

[15] European Food Safety Authority (2010). Scientific Opinion on outline proposals for assessment of exposure of organisms to substances in soil. EFSA Journal 2010;8(1):1442.

[16] European Food Safety Authority (2012). Scientific Opinion on the science behind the guidance for scenario selection and scenario parameterisation for predicting environmental concentrations of plant protection products in soil. EFSA Journal 2012;10(2):2562.

[17] European Food Safety Authority (2014). Outcome of the peer review of bee study protocols submitted by Bayer CropScience AG to assess the effects of clothianidin on bees. EFSA supporting publication 2014:EN-599.

In the present study (and several other studies submitted), an application of both imidacloprid and clothianidin is considered, as the aim of the study is to represent a situation which could occur in the field. Bayer CropScience has parallel registrations of imidacloprid and clothianidin on cereal and sugar beet crops. As these crops can be grown in rotation, the co-occurrence of both active substances on an individual field is likely.

The limitation on the uptake of an individual active substance is not influenced by another active substance on the field but by the properties of the individual active substance. This statement is supported by the data obtained from the "natural exposure" and "forced exposure" soil residue trials. In the "natural exposure" trials (see Section B.9.2.1, studies 1.3/1 Jarrat, 2014a; 1.3/2 Jarrat, 2014b and 1.3/3 Jarrat, 2014c), the field sites were characterised by a historical use of clothianidin (and in some cases thiamethoxam), but were not treated with imidacloprid over at least the past 8 years. In the 'forced exposure' studies (see Section B.9.2.1 studies 1.3/6 Ythier, 2014 and 1.3/7 Striffler & Ballhaus, 2014), winter barley seeds dressed with both clothianidin and imidacloprid were sown before installation of the succeeding crop. Table B.9.2.1-11 shows the main results from both types of studies. The lower residues in the "natural exposure" trials indicate that the uptake of clothianidin is not reduced by the presence of imidacloprid in the "forced exposure" trials.

Table B.9.2.1-11: Mean, median and 90th percentile concentration of clothianidin (µg a.s./kg), measured in nectar and pollen in the succeeding crops maize, phacelia and mustard 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**
Maize		12/25	0.73	0.60	1.0	-	-	-	-
Phacelia		11/26	0.68	0.60	0.84	2/26	0.29	0.3	0.3
'Forced exposure' studies									
Crop	**Applied a.s.**	**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	Low dose	17/17	1.4	1.3	2.1	-	-	-	-
	High dose	18/18	3.5	3.0	5.2	-	-	-	-
Phacelia	Low dose	12/20	3.3	3.4	5.3	11/20	1.9	2.2	3.1
	High dose	12/24	5.4	4.6	9.9	9/24	3.9	6.4	5.5
Mustard	Low dose	18/18	2.6	2.0	6.4	10/18	1.0	0.3	2.7
	High dose	18/18	7.0	7.5	9.8	13/18	1.9	0.7	5.6

Note: for the calculation of the mean, median and 90th percentile values, concentrations reported as <LOD were assigned the value of the LOD (0.1 µg/kg for nectar and 0.2 µg/kg for pollen) as a conservative approach. Values reported as <LOQ were assigned the value of the LOQ (0.3 µg/kg for nectar and 0.6 µg/kg for pollen);

The conclusion that the presence of imidacloprid does not influence the uptake of clothianidin is further supported by the data from the guttation studies in winter cereals (see Section B.9.5.1). The studies 1.6/1 (Hoffmann and Leuckmann, 2014) and 1.6/2 (Hoffman, Garrido and Lueckmann, 2012) were performed with seed treated with either imidacloprid or cltohianidin, and each active substance was applied to a different field. Study 1.6/3 (Hoffmann, Straffel and Aumeier, 2014), imidacloprid and clothianidin were applied to the same seeds (via seed treatment) and field. A comparison of the data from the two sets of trials indicates that the residues of clothianidin in guttation droplets from plants treated with the combined formulation (imidacloprid + clothianidin) are in the same range as those from plants treated with the formulation containing only clothianidin (see Table B.9.2.1-12. This

further supports the conclusion that the presence of one active substance does not influence the uptake of the second active substance.

Table B.9.2.1-12: Measured residues of clothianidin in guttation droplets from winter barley plants that were seed treated either with a formulation containing only clothianidin or a formulation containing both clothianidin and imidacloprid

Treatment (rate a.s./ha)	CTD residue in guttation droplets	Reference
Clothianidin (100 g CTD/ha)	<LOQ to 13 mg CTD/L <LOQ to 2.3 mg CTD/L	1.6/01 Hoffmann & Leuckmann, 2014 1.6/02 Hoffman, Garrido & Leuckmann, 2014
Clothianidin + Imidacloprid FS 100 + 175 G (100 g CTD/ha)	<LOQ to 8.511 mg CTD/L	1.6/3 Hoffmann, Straffel & Aumeier, 2014

Substance Data

The EFSA PEC_{soil} scenarios include comprehensive environmental information with daily weather records. For this reason normalised soil degradation half-lives (DT_{50}) are to be used. Conceptually, the EFSA PEC_{soil} scenarios use the same mean substance properties as currently used in the FOCUS groundwater scenarios. This principle was applied in the calculations presented in the following. The main driver among substance properties for potential accumulation is the DT_{50} in soil. The values used are shown in the Table B.9.2.1-12. For completeness, the sorption data (Freundlich K_{om} and exponent 1/n) used are included.

Table B.9.2.1-12: Key substance properties used for the calculations

Compound	DT_{50}* [20 °C, 100% FC]	K_{om}** [mL/g]	1/n** [days]
Imidacloprid	103.4***	131.0	0.80
Clothianidin	153.1***	81.5	0.83

*Notes: *geometric mean, ** arithmetic mean, ***derived from field data*

GAPs considered

The use pattern shown in Table B.9.2.1-13 below is considered.

Table B.9.2.1-13: Use pattern considered

Compound	Application mode	Crop**	Annual application rate [g/ha]
Imidacloprid	seed treatment	winter cereals	63
Imidacloprid	seed treatment	winter cereals	126-126-180*
Clothianidin	seed treatment	winter cereals	40
Clothianidin	seed treatment	winter cereals	100

**rotation, ** chosen as surrogate crop*

The EFSA approach in Tier 2a considers a specific crop. However for the case presented here, the influence of the crop on the calculated PEC_{soil} is marginal, because there is no interception due to the application mode as seed treatment. As a common crop to which both compounds can be applied winter cereals was selected as surrogate crop for the calculations.

Simulations and Calculations

Simulations were performed for the central and southern zone for 26 years of which the last 20 years were considered for PEC_{soil}. This evaluation aimed at plateau calculations which represent the maximum concentration in soil which can occur after long-term annual use of the compound one year after the last application. As it is common agronomic practice, annual soil tillage at least down to a soil depth of 20 cm was assumed which justifies the application of a mixing depth of z_{mix} = 20 cm. The mixing depth can be supplied to SOILPEARL as an input so that PEC_{soil} are directly output for this mixing depth. The plateau concentrations were determined from the SOILPEARL daily output as

follows. The overall maximum PEC_{soil} over 20 years was identified. This value is necessarily encountered at the day of an actual application. Thus the PEC_{soil} one day before the occurrence of the PEC_{max} was defined as maximum PEC_{soil} plateau ($PEC_{soil\ plateau}$).

In a second step the application rate ($R_{soil\ plateau}$) necessary to establish this concentration at specific field sites was calculated as given below:

$$R_{soil\ plateau} = PEC_{soil\ plateau} \cdot f_c \cdot z_{mix} \cdot BD$$

including the zone specific crop extrapolation factor f_c to be applied for major crops as given in EFSA (2012)[16]. For this calculation the topsoil dry bulk density (BD) of the field sites need to defined. At the field sites standard soil properties including the content of organic matter (OM) were available but not the dry bulk density. As was also done in the development of the EFSA approach a pedotransfer function was used the estimate BD (in kg/L) from OM (as fraction) which is given below:

$$BD = 1.8\ kg/L + 1.236\ kg/L \cdot OM - 2.91\ kg/L \cdot SQRT(OM)$$

where SQRT is square root. For the test site Zülpich with an OM of 0.019 a value of BD = 1.422 kg/L was obtained and for the test site Nîmes with an OM of 0.026 a value of BD = 1.363 kg/L was obtained.

Results and conclusion

In the tables below the calculated $PEC_{soil\ plateau}$ (EFSA approach, Tier 2a) for a mixing depth of 20 cm and the resulting rates $R_{soil\ plateau}$ are shown for the two tests sites Zülpich and Nîmes.

Table B.9.2.1-14: $PEC_{soil\ plateau}$ for uses and zones considered

Compound	Annual application rate [g/ha]	Zone	$PEC_{soil\ plateau}$ [mg/kg]
Imidacloprid	63	Central	0.0274
Imidacloprid	63	Southern	0.0174
Imidacloprid	126-126-180*	Central	0.0534
Imidacloprid	126-126-180*	Southern	0.0338
Clothianidin	40	Central	0.0270
Clothianidin	40	Southern	0.0173
Clothianidin	100	Central	0.0668
Clothianidin	100	Southern	0.0426

Generally maximum $PEC_{soil\ plateau}$ were obtained for the central zone. Also the crop extrapolation factor is larger (f_c = 1.16) for the central than for the southern zone (f_c = 1.07). Thus the values for the central zone were used to calculate the application rate to be applied to the field sites ($R_{soil\ plateau}$).

Table B.9.2.1-15: $R_{soil\ plateau}$ for the two test sites Zülpich and Nîmes to establish $PEC_{soil\ plateau}$ including crop extrapolation factor

Compound	Annual application rate [g/ha]	Test site	$R_{soil\ plateau}$ [g/ha]
Imidacloprid	63	Zülpich	90.3
Imidacloprid	63	Nîmes	86.5
Imidacloprid	126-126-180*	Zülpich	176.1
Imidacloprid	126-126-180*	Nîmes	168.8
Clothianidin	40	Zülpich	89.2
Clothianidin	40	Nîmes	85.5
Clothianidin	100	Zülpich	220.5
Clothianidin	100	Nîmes	211.3

RMS comments

As mentioned on the PEARL and SOIL_PEARL website[18], 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, RMS takes note of the DT_{50} value of 153.1 days (from Hammel & Kahl, 2009) used by the applicant but considered that this DT_{50} is not acceptable for the calculation of the $PEC_{soil\ plateau}$. During the peer review of the original DAR, the acceptability of the field studies, from which data was used by Hammel & Kahl (2009) to calculate the DT_{50}, was questioned because the product was sprayed on bare soils and not applied as a seed treatment. As clothianidin is easily photolysed, the early stages of the field studies might have been influenced by photolysis on the soil surface, potentially leading to a lower DT_{50} than what could be expected from a seed treatment application.

In a reaction to this, the applicant pointed out that the field studies used to calculate the DT_{50} were indeed criticized, but their validity was not questioned. Further, the studies were performed fully in accordance with the data requirements at the time of submission and those in place today. The applicant addressed the possible influence of surface processes in accordance with the relevant kinetic guidance: the initial rapid degradation, which could be attributed to soil surface processes (including photolysis) is discounted by using bi-phasic kinetic evaluations (in this case hockey-stick evaluations) and the relevant DT_{50} is then calculated only taking into account the slow phase degradation. Hence, the applicant claims that the DT_{50} of 153.1 days is correctly used in this calculation, as it refers to degradation excluding the influence of surface processes and hence is the appropriate value. RMS agrees that it could be assumed that the soil surface processes are accounted for by the rapid phase of the hockey-stick evaluation, however, this remains a hypothesis.

The measured clothianidin residues in soil in the "natural exposure" studies summarized above do not exceed 248 µg/kg dry soil (highest residue value, measured in Study 1.3/3 Jarrat, 2014c, after multiple applications of clothianidin over a period of 10-11 years). These measured values are considered more realistic for the situation in the field than model calculations, regardless of whether a correct DT_{50} was used in the calculations or not. The calculated $PEC_{soil,plateau}$ values exceed the measured value of 248 µg/kg dry soil (at least for the "high" application scenario with annual application rate of 100 g a.s./ha). Therefore, the $PEC_{soil,plateau}$ values calculated in the present study are considered to be an acceptable worst case, and suitable as a starting point to determine the application rate of clothianidin for the "forced exposure" studies summarized below.

[18] http://www.pesticidemodels.eu

Report:	**1.3/6; Ythier, E.; 2014**
Title:	**Determination of the residues of clothianidin in bee relevant matrices collected from succeeding crops following application of clothianidin FS 600B G via soil incorporation to plateau concentration and sowing of clothianidin-treated winter barley seeds. Field phase conducted in southern France**
Report No.:	7SRFR13C4
Document No.:	M-504814-01-1
Guideline(s):	Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonization of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances (codified version). OECD Principles of Good Laboratory Practice (as revised in 1997). Series on Principles of GLP and Compliance Monitoring, No. 1. revised ENV/MC/CHEM(98)17, No. 6. Revised ENV/JM/MONO(99)22 and No.13 ENV/JM/MONO(2002)9. Regulation (EC) No.1107/2009 of the European Parliament and of the Council of 21 October 2009, concerning the placing of plant protection on the market and repealing Council Directives 79/117/EEC and 91/414/EEC
Guideline deviation(s):	not specified
GLP/GEP:	yes

Objective
The objective of the study was to determine residues of clothianidin and its metabolites TZNG and TZMU in bee relevant matrices (pollen, nectar and guttation fluid) collected from succeeding crops following application of CLOTHIANIDIN FS 600B G via soil incorporation to plateau concentration and sowing of clothianidin-treated winter barley seeds.

Material and Methods
Test item (for seed treatment and for spray application to bare soil): CLOTHIANIDIN F 600B G; TOX no.:10232-00; Batch-ID: EDFL021793; content(s) of a.i. (nominal): 600 g/L or 470 g/kg clothianidin; content(s) of a.i. (analysed): 617.4 g/L or 484 g/kg clothianidin.

The study was conducted on a field site near Nîmes (F-30000, France). An approximately two hectare field located on the field site was marked out, and divided into two evenly sized plots. Three crops were cultivated on both plots of the study field: Phacelia (*Phacelia tanacetifolia*), mustard (*Sinapis arvensis*) and maize (*Zea mays*) (each in an area of approx. 0.2 ha).

The test item clothianidin was applied in autumn 2013 with two different calculated plateau concentrations directly to bare soil. After incorporation of the calculated plateau concentrations, dressed winter barley seeds (again with two different seed dressing rates) were sown (see overview below):

	Application of the plateau concentration* (25.09.2013)	**Sowing of treated winter barley seeds* (10.10.2013)**
Low plateau concentration + low seed dressing rate (variant green)	78.4 g a.s./ha 0.127 L product/ha	38.1 g a.s./ha 189.5 kg seeds/ha (20.1 g a.s./dt seeds)
High plateau concentration + high seed dressing rate (variant blue)	212.8 g a.s./ha 0.344 L product/ha	134.7 g a.s./ha 184.5 kg seeds/ha (73 g a.s./dt seeds)

**Actual concentrations*

It is noted that the seeds were dressed, in addition to clothianidin, also with imidacloprid (IMIDACLOPRID FS 600B E) and a standard fungicide.

In 2014, winter barley crops were removed and untreated succeeding crops (mustard, phacelia and maize) were sown on the areas with previous clothianidin applications. Three bee proof tunnels (10 m long x 5 m wide x 3 m high) were placed onto the phacelia and the mustard plot after successful germination. A single honeybee colony was placed into each tunnel at the start of phacelia, respectively mustard flowering. The sub-plot sown with maize was divided into three smaller sub plots, each similar in size that were large enough to have a sufficient numbers of plants available for both guttation fluid and for maize pollen sampling.

Soil sampling

From each of the maize sub plots and from the phacelia and mustard sowing areas, two different types of soil sample were collected. These samples were used for:

40. Soil characterisation of the upper 10 cm soil layer.
41. Determination of the residues of parent clothianidin and its metabolites in the upper 15 cm soil layer.

Soil cores used for characterisation and residue analysis were collected from each of the three segregated maize sub plots, during the guttation sampling phase of the trial and from inside of the phacelia or mustard sowing area prior to placement of the honeybee colonies into the tunnels.

Sampling of Nectar and Pollen from Phacelia or Mustard crops

Nectar and pollen sampling was conducted at three different time points during bloom for mustard and one time point during bloom for phacelia of the corresponding crop. Once the crop started to bloom, honeybee colonies were placed into mesh covered tunnels erected over the crop. Honeybees were exposed to the flowering phacelia or mustard under confined conditions and were exclusively used as a sampling device for both nectar and pollen.

Nectar was sampled by extracting the honey stomachs from forager bees. Therefore, the hive entrance was blocked during bee flight activity for a short period of time and the returning forager bees were collected at the hive entrance. Pollen was collected from foragers returning to the colony using a pollen trap attached to each colony. Pollen and nectar samples during bloom were analysed for residues of clothianidin.

Sampling of Guttation Fluid and Pollen from Maize

Guttation fluid and pollen sampling was conducted in the maize crop. Samples were collected directly from the crop by hand. Sampling of guttation fluid was carried out on a regular basis over a 43-day period. Guttation sampling started directly after emergence of the maize crop (BBCH scale 11-12) until flowering (BBCH scale 65). Guttation fluid was collected from each of the three sub-plots approximately thirty minutes after sunrise. The sampling period at each time point was approximately 30 minutes to ensure an equivalent time chronology every day. Sampling took place in the same order at each time point, starting with sub plot 1 and finishing with sub plot 3. When guttation was present it was collected from >10 plants throughout each of the sub plots. The target volume for each sample was 1 ml of guttation fluid.

Pollen sampling from three time points during bloom started when the crop started to shed pollen (BBCH scale 63) until male flowering had completed (BBCH scale 67). At each time point ≥ 50 flowering tassels were collected from throughout each of the three sub plots and placed into paper bags. Damp tassels were air dried, in the dark at room temperature overnight. Next day, the pollen was shaken out and cleaned with two analytical sieves (mesh size 2 mm and 1 mm). Maize pollen in the base pan was cleaned from plant or insect debris remaining in the pollen sample by hand using forceps or a fine paint brush. Pollen samples during bloom as well as collected guttation fluid were analysed for residues of clothianidin.

Sample storage and residue analysis
Samples were stored in the dark at <-18°C until processing and analysis.
All samples (pollen, nectar and guttation fluid) were analysed for their content of clothianidin and its metabolites TZNG and TZMU via HPLC-MS/MS. Soil was analysed for clothianidin only. Processing and analysis was conducted according to the following analytical methods:

42. Soil: *Method 00540/M001 (Sommer, 2003 – BCS Report No MR-106/02);*
43. Guttation fluid: an adapted version of *Method 00554/M001 (Schöning, 2001 – BCS Report No MR-338/00)*;
44. Pollen and nectar: *Method 01433 (Schöning, R. – BCS report No MR-14/123);*

For a summary on description of these methods: see 1.3/01 (*vide supra*). The methods were successfully validated within the study (1.3/6) for the respective analytes and matrices at the appropriate LOQs, by means of performing at least 4 recovery determinations per fortification level (n=5 for soil) on spiked control samples. The recoveries at all fortification levels were well within acceptable range (60-120%) and RSD was below 20%, indicating suitability of the methods.

Findings
Soil characterisation
The soil was characterised as either silty clay loam or clay loam, according to USDA soil classification.

Residue analysis
A summary of the analytical results as obtained by analysing samples of soil, guttation liquid, pollen and nectar is provided in the following tables.

Table B.9.2.1-16: Residues of clothianidin in soil (green and blue plots)

Sample material	Variant	Residue clothianidin during bloom [µg/kg]	Moisture [%]	Residue clothianidin during bloom [µg/kg dry soil]
Soil	green plot (“low”)	19 - 59	10.2 - 18.8	21 - 71
Soil	blue plot (“high”)	45 - 78	10.7 - 17.8	51 - 90

LOD/LOQ = 2 µg/kg / 5 µg/kg for clothianidin in soil

Table B.9.2.1-17: Residues of clothianidin, TZNG and TZMU in guttation liquid samples (green and blue plots)

Sample Material	Variant	Residue clothianidin [µg/L]	Residue TZNG [µg/L]	Residue TZMU [µg/L]
Guttation liquid (Maize)	green plot (“low”)	4 - 547	< LOD - 13	< LOQ - 92
Guttation liquid (Maize)	blue plot (“high”)	< LOQ - 126	< LOD - 5	< LOD - 23

LOD/LOQ = 0.3 µg/L / 1 µg/L for guttation liquid samples (all analytes)

Table B.9.2.1-18: Residues of clothianidin, TZNG and TZMU in mustard and phacelia nectar samples (green and blue plots)

Sample material	Variant	Residue clothianidin [µg/kg]	Residue TZNG [µg/kg]	Residue TZMU [µg/kg]
Nectar (Mustard)	green plot (“low”)	< LOD - LOQ	< LOD	< LOD
Nectar (Phacelia)		0.5 - 1.0	< LOD	< LOD
Nectar (Mustard)	blue plot (“high”)	< LOD - 0.8	< LOD	< LOD - < LOQ
Nectar (Phacelia)		< LOD	< LOD	< LOD

LOD/LOQ = 0.1 µg/kg / 0.3 µg/kg for clothianidin in nectar
LOD/LOQ = 0.3 µg/kg / 1 µg/kg for the metabolites in nectar samples.

Table B.9.2.1-19: Residues of clothianidin, TZNG and TZMU in pollen samples (green and blue plots)

Sample material	Variant	Residue Clothianidin [µg/kg]	Residue TZNG [µg/kg]	Residue TZMU [µg/kg]
Pollen (Mustard)	green plot ("low")	0.72 - 2.0	< LOD	< LOD
Pollen (Phacelia)		0.9 - 1.2	< LOQ	<LOD
Pollen (Maize)*		0.65 - 1.4	< LOD - < LOQ	< LOD - < LOQ
Pollen (Mustard)	blue plot ("high")	5.3 - 10	< LOQ - 2.5	< LOQ - 1.1
Pollen (Phacelia)		2.6 - 3.0	2.2 - 2.4	< LOQ
Pollen (Maize)		2.9 - 5.7	< LOD - 1.7	< LOD - < LOQ

**One sample excluded due to contamination with green plant material (see report for details)*
LOD/LOQ = 0.2 µg/kg / 0.6 µg/kg for clothianidin in pollen
LOD/LOQ = 0.3 µg/kg / 1 µg/kg for the metabolites in pollen.

Conclusion

The study has been performed to cover various scenarios (crop rotations) of a consecutive use of clothianidin and to determine the potential residue level of clothianidin and its metabolites TZNG and TZMU in bee-relevant matrices (nectar and pollen) and guttation droplets of succeeding crops. In a model approach, two levels of clothianidin plateau concentrations were established (information about the rates to be applied were provided by the sponsor) on an agricultural site near Nîmes (F-30000, France). After incorporation of the calculated plateau concentrations in September 2013, dressed winter barley seeds (again with two different seed dressing rates) were sown.

Phacelia

Residues analysis of pollen and nectar, as collected at one time during blooming of phacelia, in three tunnels per test rate revealed in low residue levels. The residue levels of clothianidin nectar was below the LOD (< 0.1 µg a.s./kg) to 1.0 µg a.s./kg. Residue levels of clothianidin in pollen ranged from 0.9 µg a.s./kg to 3.0 µg a.s./kg.

Mustard

Residues analysis of pollen and nectar, as collected at three time points during blooming of mustard in three tunnels per test rate revealed in low residue levels. The residue levels of clothianidin in nectar ranged from below the LOQ (< 0.3 µg a.s./kg) to 3.9 µg a.s./kg. Residue levels of clothianidin in pollen ranged from 0.7 µg a.s./kg to 10 µg a.s./kg.

Maize

Residues analysis of guttation fluid, as collected from directly after emergence until early bloom of the maize plants, revealed in generally low residues. The residue levels of clothianidin in guttation fluid ranged from below the LOQ (< 1 µg a.s./L) to 547 µg a.s./L and are thus several orders of magnitude below values measured in the solution with which maize seeds were dressed (617.4 g clothianidin/L). The residue level of clothianidin in pollen, as sampled at three time points during bloom on three subplots ranged from 0.65 µg a.s./kg to 5.7 µg a.s./kg.

Overall, transfer of clothianidin soil residues into bee-relevant matrices and guttation droplets of succeeding crops takes place on low levels even if calculated long-term plateau concentrations are established without ageing of residues over years. Traces of clothianidin metabolites were only measured in single guttation or pollen samples.

RMS Comments

The treated winter barley seeds sown after incorporation of the calculated soil plateau concentrations were dressed not only with clothianidin, but also with imidacloprid and a standard fungicide. During review of the study protocol by EFSA[19] the question was raised the question was raised whether an application of both imidacloprid and clothianidin to the same field would have any influence on the

[19] European Food Safety Authority (2014). Outcome of the peer review of bee study protocols submitted by Bayer CropScience AG to assess the effects of clothianidin on bees. EFSA supporting publication 2014:EN-599.

uptake of both substances by the plants and the measured residues in bee relevant matrices It was decided at Pesticides Peer Review Meeting on the review of bee study protocols (April 2014) that the applicant should document (supported with data) whether the mixture of imidacloprid and clothianidin may result in a different root uptake for each individual substance. Data was provided and is discussed in section B.9.2.1 (1.3/5, Hammel & Vrbka 2014, *vide suppra*), and is considered acceptable to demonstrate that the limitation on the uptake of an individual active substance is not influenced by another active substance in the field.

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

Report:	**1.3/7; Striffler, B.& Ballhaus F.; 2014**
Title:	**Residues of clothianidin in nectar and pollen of flowering rotational crops in Western Germany**
Report No.:	P13068-1
Document No.:	M-504884-01-1
Guideline(s):	Regulation (EC) No 1107/2009
Guideline deviation(s):	not applicable
GLP/GEP:	yes

Objective
The objective of the study was to determine residues of clothianidin and its metabolites TZNG and TZMU in bee relevant matrices (pollen, nectar and guttation fluid) collected from succeeding crops following application of CLOTHIANIDIN FS 600B G via soil incorporation to plateau concentration and sowing of clothianidin-treated winter barley seeds.

Material and Methods
Test item (for seed treatment and for spray application to bare soil): CLOTHIANIDIN F 600B G; TOX no.:10232-00; Batch-ID: EDFL021793; content(s) of a.i. (nominal): 600 g/L or 470 g/kg clothianidin; content(s) of a.i. (analysed): 617.4 g/L or 484 g/kg clothianidin.

The study was conducted in the vicinity of Zuelpich, North Rhine-Westphalia in Western Germany. Two areas of approximately 1 ha each were established on the Study Field.
Three crops were cultivated on both variants of the Study Field: phacelia (*Phacelia tanacetifolia*), mustard (*Sinapis arvensis*) (each in an area of approx. 0.2 ha) and maize (*Zea mays*) (each in an area of approx. 0.1 ha).

The test item clothianidin was applied in autumn 2013 with two different calculated plateau concentrations directly to bare soil. After incorporation of the calculated plateau concentrations about 20 cm into the soil, dressed winter barley seeds (again with two different seed dressing rates) were sown (see overview below):

	Clothianidin Application of the Plateau Concentration* 26.09.2013	**Clothianidin Sowing of treated winter barley seeds* 09.10.2013**
Low plateau concentration + low seed dressing rate (Variant green)	88.8 g a.s./ha 0.144 L product/ha	40.4 g a.s./ha 202 kg seeds/ha (with 20 g a.s./dt)
High plateau concentration + high seed dressing rate (Variant blue)	229.6 g a.s./ha 0.372 L product/ha	99.3 g a.s./ha 136 kg seeds/ha (with 73 g a.s./dt)

**Actual concentrations*

It is noted that the seeds were dressed, in addition to clothianidin, also with imidacloprid (IMIDACLOPRID FS 600E G).

In spring 2014, the grown winter barley was desiccated (using glyphosate treatment) and incorporated into the ground. After that, untreated mustard, phacelia and maize were sown on the study plots which contained soil residues from the previous Clothianidin applications. During flowering, nectar and pollen of mustard and phacelia were sampled by honeybees in tunnels. Maize pollen was sampled manually; the same applies to guttation droplets between maize emergence and flowering.

Soil sampling and analysis

Soil samples were taken in September 2013 after spray application and incorporation. Samples were taken with a hand sampler from the upper 15 cm of at least 20 randomly selected locations and combined to one pooled sample of at least 500 g.

The additional soil loading by sowing of seed-dressed cereals has not been verified by analysis, however in Spring/Summer 2014, after drilling and emergence of the rotational crops, in total 9 soil samples were taken for residue analyses and water content determination on variant blue and 9 on variant green. From each subplot one soil sample was taken in the later sampling areas for nectar, pollen and guttation. In phacelia and mustard these sampling areas corresponded to the bee tunnels. In maize the sub-plots corresponded to the areas where guttation sampling was carried out.

From each of the maize sub plots and from the phacelia and mustard sowing areas soil sample were also collected for soil characterisation of the upper 10 cm soil layer.

Nectar and Pollen sampling from Phacelia or Mustard crops

Honeybee colonies were placed into mesh covered tunnels erected over phacelia and mustard crops a few days prior expected bloom. Honeybees were exposed to the flowering phacelia and mustard under confined conditions and were exclusively used as a sampling device for both nectar and pollen at three times (in a period of approx. 10 days) during flowering of the respective crop.

Nectar was collected by honey bulb extraction from forager bees in mustard and phacelia crop. For each nectar sample about 800-1000 returning forager bees were collected with a modified vacuum sampler, deep-frozen and transported to the laboratory for nectar extraction. Targeted nectar amount per sample was ≥ 500 mg.

Pollen of phacelia and mustard was collected from forager bees via pollen traps attached to the bee hive entrance. The collected pollen was stored deep-frozen until residue analysis. The target sample size per tunnel and per sampling date was approximately 1.5 g pollen with a minimum requirement of approximately 750 mg.

Sampling of Guttation Fluid and Pollen from Maize

Maize pollen was collected three times during flowering of maize plants (BBCH 63-65). The pollen, targeted were 1.5 g per sample, collected from at least 30 plants, was shaken out of the flowers into paper bags and cleaned by sieving (mesh size 2 mm and 1 mm).

Maize guttation fluid, target 1 ml per sample, was collected daily starting at emergence of the seedlings (BBCH 11) until early flowering (BBCH 55). The samplings started at sunrise (± 15 min) lasted for a maximum of 30 min.

Sample storage and residue analysis

Samples were stored in the dark at <-18°C until processing and analysis.

All samples (pollen, nectar and guttation fluid) were analysed for their content of clothianidin and its metabolites TZNG and TZMU via HPLC-MS/MS. Soil was analysed for clothianidin only. Processing and analysis was conducted according to the following analytical methods:

45. Soil: *Method 00540/M001 (Sommer, 2003 – BCS Report No MR-106/02);*
46. Guttation fluid: an adapted version of *Method 00554/M001 (Schöning, 2001 – BCS Report No MR-338/00)*;
47. Pollen and nectar: *Method 01433 (Schöning, R. – BCS report No MR-14/123);*

For a summary on description of these methods: see 1.3/01 (*vide supra*). The methods were successfully validated within the study (1.3/7) for the respective analytes and matrices at the

appropriate LOQs, by means of performing at least 5 recovery determinations per fortification level on spiked control samples. The recoveries at all fortification levels were well within acceptable range (60-120%) and RSD was below 20%, indicating suitability of the methods.

Findings

Residue analysis:

A summary of the analytical results as obtained by analysing samples of soil, guttation liquid, pollen and nectar is provided in the following tables.

Table B.9.2.1-20: Residues of clothianidin in soil (green and blue plots)

Sampling Date (crop/BBCH)	variant	Residue Clothianidin during bloom (calculated to dry soil*) [µg/kg]**	variant	Residue Clothianidin during bloom (calculated to dry soil) [µg/kg]
16.06.2014 (Phacelia/35)	Blue (high)	65-75	Green (low)	40-51
16.06.2014 (Mustard/53)	Blue (high)	68 - 90	Green (low)	39 - 49
06.06.2014 (Maize/13)	Blue (high)	67 - 84	Green (low)	46 - 52

LOD/LOQ = 2 µg/kg / 5 µg/kg for clothianidin in soil

Table B.9.2.1-21: Residues of clothianidin, TZNG and TZMU in guttation liquid samples (green and blue plots)

Sample Material	Variant	Residue clothianidin [µg/L]	Residue TZNG [µg/L]	Residue TZMU [µg/L]
Guttation liquid (Maize)	green plot ("low")	3 – 175	<LOD – 3	<LOD – 5
Guttation liquid (Maize)	blue plot ("high")	1 – 73	<LOD – 9	<LOD – 12

LOD/LOQ = 0.3 µg/L / 1 µg/L for guttation liquid samples (all analytes)

Table B.9.2.1-22: Residues of clothianidin, TZNG and TZMU in mustard and phacelia nectar samples (green and blue plots)

Sample material	Variant	Residue clothianidin [µg/kg]	Residue TZNG [µg/kg]	Residue TZMU [µg/kg]
Nectar (Mustard)	green plot ("low")	0.4 – 3.6	<LOD - <LOQ	<LOD
Nectar (Phacelia)		1.3 – 3.3	<LOD - <LOQ	<LOD
Nectar (Mustard)	blue plot ("high")	0.5 – 6.4	<LOD - <LOQ	<LOD
Nectar (Phacelia)		1.8 – 6.9	<LOD - <LOQ	<LOD - <LOQ

LOD/LOQ = 0.1 µg/kg / 0.3 µg/kg for clothianidin in nectar; LOD/LOQ = 0.3 µg/kg / 1 µg/kg for the metabolites in nectar samples.

Table B.9.2.1-23: Residues of clothianidin, TZNG and TZMU in pollen samples (green and blue plots)

Sample material	Variant	Residue Clothianidin [µg/kg]	Residue TZNG [µg/kg]	Residue TZMU [µg/kg]
Pollen (Mustard)	green plot ("low")	1.9 – 7.3	<LOD – 1.8	<LOD - <LOQ
Pollen (Phacelia)		2.3 – 6.1	1.1 – 5.5	<LOD - <LOQ
Pollen (Maize)*		1.2 – 2.3	<LOD - <LOQ	<LOD - <LOQ
Pollen (Mustard)	blue plot ("high")	2.3 – 11	<LOQ – 2.2	<LOD - <LOQ
Pollen (Phacelia)		1.8 – 11	1.8 - 5	<LOD - <LOQ
Pollen (Maize)		2.2 – 5.0	< LOQ	<LOD

LOD/LOQ = 0.2 µg/kg / 0.6 µg/kg for clothianidin in pollen; LOD/LOQ = 0.3 µg/kg / 1 µg/kg for the metabolites in pollen.

Conclusion

The study has been performed to simulate various scenarios (crop rotations) of a consecutive use of Clothianidin and to determine the potential residue level of Clothianidin in bee-relevant matrices (nectar and pollen) and guttation droplets of succeeding crops under unrealistic worst-case conditions. In a model approach, two levels of Clothianidin plateau concentrations were established (information about the rates to be applied were provided by the sponsor) on an agricultural site near Zuelpich, Germany. After incorporation of the calculated plateau concentrations in September 2013, dressed winter barley seeds (again with two different seed dressing rates) were sown.

Phacelia:

Clothianidin residues in pollen and nectar, as collected at three time points during blooming of phacelia, in three tunnels per test rate ranged from 1.3 µg a.s./kg to 6.9 µg a.s./kg in nectar and from 1.8 µg a.s./kg to 11 µg a.s./kg in pollen.

Mustard:

Clothianidin residues in pollen and nectar, as collected at three time points during blooming of mustard in three tunnels per test rate ranged from 0.4 µg a.s./kg to 6.4 µg a.s./kg in nectar and from 1.9 to 11 µg a.s./kg in pollen.

Maize:

Residues analysis of guttation fluid, as collected from directly after emergence until early bloom of the maize plants, revealed in generally low residues. The residue levels of clothianidin in guttation fluid ranged from 1 µg a.s./L to 175 µg a.s./L and are thus several orders of magnitude below values measured in the solution with which maize seeds were dressed (617.4 g clothianidin/L). The residue level of clothianidin in pollen, as sampled at three time points during bloom on three subplots ranged from below 1.2 µg a.s./kg to 5.0 µg a.s./kg.

Overall, transfer of clothianidin soil residues into bee-relevant matrices and guttation droplets of succeeding crops takes place on low levels even if calculated long-term plateau concentrations are established artificially without ageing of residues over years.

RMS Comments

The treated winter barley seeds sown after incorporation of the calculated soil plateau concentrations were dressed not only with clothianidin, but also with imidacloprid and a standard fungicide. During review of the study protocol by EFSA[20] the question was raised the question was raised whether an application of both imidacloprid and clothianidin to the same field would have any influence on the uptake of both substances by the plants and the measured residues in bee relevant matrices It was decided at Pesticides Peer Review Meeting on the review of bee study protocols (April 2014) that the applicant should document (supported with data) whether the mixture of imidacloprid and clothianidin may result in a different root uptake for each individual substance. Data was provided and is discussed in section B.9.2.1 (1.3/5, Hammel & Vrbka 2014, *vide suppra*), and is considered acceptable to demonstrate that the limitation on the uptake of an individual active substance is not influenced by another active substance in the field.

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

[20] European Food Safety Authority (2014). Outcome of the peer review of bee study protocols submitted by Bayer CropScience AG to assess the effects of clothianidin on bees. EFSA supporting publication 2014:EN-599.

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 (phacelia, maize or mustard) 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.

The results from the 'natural exposure' studies are summarized in Table B.9.2.2-1 below. These studies were performed at three field sites in the UK. Although a there was a high variation in measured soil residues, both between studies and within study plots, there is no clear influence of the residue of clothianidin in soil and the measured residue in nectar or pollen. The mean, median and 90th percentile values for the overall dataset from the three field sites were calculated, and are reported in Table B.9.2.2-4.

Table B.9.2.2-1: Range of residues in soil, pollen and nectar measured in 'natural exposure' studies in the succeeding crops phacelia and maize

Reference	Succeeding crop	Residue in soil (µg/kg dry soil)	Residue in pollen (µg/kg)	Residue in nectar (µg/kg)
1.3/1 Jarratt, N. 2014a	Phacelia	18 – 41	<LOQ – 0.81	<LOQ - <LOQ
	Maize	16 – 22	<LOQ – 0.80	-
1.3/2 Jarratt, N. 2014b	Phacelia	64 – 78	<LOQ – 1.2	<LOQ
	Maize	59 – 80	<LOQ – 1.5	-
1.3/3 Jarratt, N. 2014c	Phacelia	78 – 80	<LOQ – 0.84	<LOD – 0.6
	Maize	92 – 248	<LOQ – 1.3	-

LOD = 0.1 µg/kg for nectar and 0.2 µg/kg for pollen; LOQ = 0.3 µg/kg for nectar and 0.6 µg/kg for pollen

The results from the 'forced exposure' studies are summarized in Table B.9.2.2-2 below. The results from two studies are available. In each of these studies, 1 field site was divided in 2 parts. Each part was spiked with either a low or a high dose clothianidin prior to sowing of the succeeding crop. The dose applied corresponded to the theoretical plateau concentration, resulting from 20 years of consecutive use of clothianidin in either a low (40g a.s./ha/year) or high (100 g a.s./ha/year). Although studies from only two field sites instead of 5 (as recommended by the EFSA Guidance Document on bees) are available, the results provide a good indication of clothianidin residues in succeeding crops after forced exposure. The mean, median and 90th percentile values for the overall dataset from the two field sites were calculated, and are reported in Table B.9.2.2-4.

Table B.9.2.2-2: Range of residues in soil, pollen and nectar measured in 'forced exposure' studies in the succeeding crops maize, phacelia and mustard

Reference	Applied a.s.	Succeeding crop	Residue in soil (µg/kg dry soil)	Residue in pollen (µg/kg)	Residue in nectar (µg/kg)
1.3/6 Ythier, E. 2014	Low dose	Maize	21 – 71	0.65 – 1.4	-
		Phacelia		0.9 – 1.2	0.5 – 1.0
		Mustard		0.72 – 2.0	<LOD - <LOQ
	High dose	Maize	51 – 90	2.9 – 5.7	-
		Phacelia		2.6 – 3.0	<LOD
		Mustard		5.3 – 10	<LOD – 0.8
1.3/7 Striffler, B. & Ballhaus, F. (2014)	Low dose	Maize	46 – 52	1.2 – 2.3	-
		Phacelia	40 – 51	2.3 – 6.1	1.3 – 3.3
		Mustard	39 – 49	1.9 – 7.3	0.4 – 3.6
	High dose	Maize	67 – 84	2.2 – 5.0	-
		Phacelia	65 – 75	1.8 – 11	1.8 – 6.9
		Mustard	68 - 90	2.3 – 11	0.5 – 6.4

LOD = 0.1 µg/kg for nectar and 0.2 µg/kg for pollen; LOQ = 0.3 µg/kg for nectar and 0.6 µg/kg for pollen

The soil residues measured in the 'natural exposure' studies are comparable to those measured in the 'forced exposure' studies. This is especially true for the studies by Jarratt (2014b & c) compared to the high dose plots in the studies by Ythier (2014) and Striffler & Ballhaus (2014). However, the residues in pollen and nectar are much higher in the 'forced exposure' studies (by a factor of 2 to 10 fold) compared to the 'natural exposure' studies. 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. Aged residues were shown to be of lower availability to plants (see Stupp, 2001a, IIA 7.1.2/02, evaluated in the original Monograph). In laboratory studies it has been shown that the K_{OC} values of clothianidin increase with ageing and thus that bioavailability of aged soil residues decreases substantially over time. This is further supported by information from laboratory studies performed with larvae of *Poecillus cupreus* that were previously submitted and evaluated in the original DAR. In these studies, aged residues were less toxic to sensitive this sensitive soil dwelling species. At aged test concentrations of 0.074 mg a.s./kg soil, no statistically significant difference in mortality was observed (see original monograph: Maus, 2001a, IIIA 10.5.1/21), whereas in contrast 80% corrected mortality was observed in the same species in non-aged soil (see original monograph: Neumann, 2000a, IIIA 10.5.1/30). This indicates that the 'natural exposure' studies, in which soil residues have aged, are a more realistic representation of exposure under field condition than 'forced exposure' studies.

While only three succeeding crop species were investigated, they are considered to be representative for highly bee attractive flowering crops (Phacelia and mustard) on the one hand and for large grain plants potentially producing pollen (maize) on the other hand. It could be argued that data from three crop species is not sufficient, as it is difficutlt to extrapolate residue values from one crop to another due to a possible variation in clothianidin residues in pollen and nectar between different crops (due to species dependency of the systemic translocation of the a.s.). However, the results from the 'forced exposure' studies show that for Phacelia and mustard, the residues in nectar and pollen are highly comparable. Further, residues in maize pollen were lower or similar compared to those in Phacelia pollen in the 'forced exposure' and 'natural exposure' studies, respectively.

During the evaluation, the applicant submitted the results of a number of other studies that measured residues in succeeding crops, which have been previously submitted in the EU (see Table B.9.2.2-3). It has to be noted that this information was submitted without the full study reports and only a few weeks before the deadline of submission of this addendum. Therefore, these studies were not evaluated in detail. Nevertheless, the outcome of these studies is reported as supportive information. Although these previously submitted studies had a higher LOQ that the studies submitted as confirmatory data, they confirm that the exposure under field conditions with 'natural exposure' (i.e. aged residues) will be significantly lower than that measured with fresh residues.

Table B.9.2.2-3 Measured residues of clothianidin in pollen and nectar of succeeding crops from earlier submitted and evaluated studies

'Natural exposure' studies[1]						
Succeeding crop	**Country**	**Soil concentration (µg/kg dw soil)**	**Residue in pollen (µg/kg)**	**Residue in nectar (µg/kg)**	**Reference**	**Previous submission**
Oilseed rape	Germany (Hoefchen)	11.9	< 1 (max.)	< 1 (max.)	Przygoda, Schoening, Brumhard & Maus, 2007 M-291947-01-1	EFSA request for bee studies, September 2012
Oilseed rape	Germany (Laacherhof)	12.6	< 1 (max.)	< 1 (max.)	Przygoda, Schoening, Brumhard & Maus, 2007 M-291950-01-1	EFSA request for bee studies, September 2012
Maize	Germany (Laacherhof)	19.2	< 1	-	Neumann, Schoening & Brumhard, 2005 M-256474-01-1	Poncho FS600 red (Maize) European dossier OECD, BE, UK, DE, May 2006; EFSA request for bee studies, September 2012
Maize	Germany (Hoefchen)	18.0	< 1	-	Neumann, Schoening & Brumhard, 2005 M-256564-01-1	Poncho FS600 red (Maize) European dossier OECD, BE, UK, DE, May 2006; EFSA request for bee studies, September 2012
'Forced exposure' studies[2]						
Succeeding crop	**Country**	**Soil concentration (µg/kg dw soil)**	**Residue in pollen (µg/kg)**	**Residue in nectar (µg/kg)**	**Reference**	**Previous evaluation**
Oilseed rape	Germany (Hoefchen)	21.0	4.0 (max.) 3.9 (90^{th} %ile)	2.15	Neumann, Schoening & Brumhard, 2005 M-256718-01-1	EFSA request for bee studies, September 2012

Notes: [1]minimum 2 months from soil treatment to planting of succeeding crop; [2]<1month from soil treatment to planting of succeeding crop

Table B.9.2.2-4: Mean, median and 90th percentile concentration of clothianidin (µg a.s./kg), measured in nectar and pollen in the succeeding crops maize, phacelia and mustard 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**
Maize		12/25	0.73	0.60	1.0*	-	-	-	-
Phacelia		11/26	0.68	0.60	0.84	2/26	0.29	0.3	0.3*
'Forced exposure' studies									
Crop	**Applied a.s.**	**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	Low dose	17/17	1.4	1.3	2.1	-	-	-	-
	High dose	18/18	3.5	3.0	5.2	-	-	-	-
Phacelia	Low dose	12/20	3.3	3.4	5.3	11/20	1.9	2.2	3.1
	High dose	12/24	5.4	4.6	9.9	9/24	3.9	6.4	5.5
Mustard	Low dose	18/18	2.6	2.0	6.4	10/18	1.0	0.3	2.7
	High dose	18/18	7.0	7.5	9.8	13/18	1.9	0.7	5.6

*Note: for the calculation of the mean, median and 90th percentile values, concentrations reported as <LOD were assigned the value of the LOD (0.1 µg/kg for nectar and 0.2 µg/kg for pollen) as a conservative approach. Values reported as <LOQ were assigned the value of the LOQ (0.3 µg/kg for nectar and 0.6 µg/kg for pollen); *values used in the risk assessment*

From the above, it can be concluded that 'natural exposure' studies are more realistic, and that 'forced exposure studies' represent an unrealistic worst case. Consequently, it is considered justified to use the measured residues in pollen and nectar from the 'natural exposure' studies in the risk assessment for bees, instead of the more worst case values from the 'forced exposure' studies.

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). Therefore, they should be considered more suitable for the exposure assessment rather than the 'forced exposure' studies. It was discussed whether "forced" and "natural" exposure studies should be considered equally relevant as the results of the forced exposure studies could be considered worst case. Overall, the majority of the experts agreed that residues in pollen and nectar from the 'natural exposure' studies should be included in the exposure assessment. This was appropriate in this case as the soil residue levels from the 'natural' exposure studies were equal or higher than the expected accumulation of use over successive years (soil $PEC_{plateau}$). Note this expected accumulation was estimated by EFSA using the current approach for PEC_{soil} accumulation (ESCAPE model, based on the available $DegT_{50}$ in the field), which resulted, in any case, lower than the value estimated by the applicant in the dossier. The calculation approach used by the applicant using the soil PEARL approach which is still under development is considered not appropriate in regulatory submissions.

During Peer Review, it was argued that it should still be considered whether the submitted succeeding crop studies (3 studies with 'natural exposure' and 2 studies with 'forced exposure') can be considered representative for attractiveness vs. 90th percentile for establishing the spatial variation of the RUD values, and whether these studies can be considered representative for the area of use of the active substance, considering that 3 out of 5 studies were performed in the UK (see comment 5(12) in the

Reporting Table). This issue was discussed at Pesticides Peer Review Meeting 145. As only three new 'natural exposure' studies (on 3 field sites in total) were submitted instead of 5 (as suggested by the EFSA Guidance Document for bees), and because these three studies were performed in the UK, it was not considered acceptable to use 90th percentile values in the risk assessment. It was noted that additional trials carried out in Germany were available (see Table B.9.2.2-3) from a previous evaluation of clothianidin. Those data were considered realistic worst case regarding the soil concentration (the soil concentration was similar to the soil $PEC_{plateau}$ calculated by EFSA using ESCAPE). Therefore, they might be used together with the three new natural exposure studies to assess the geographical distribution of RUD values. A full assessment according to the principles of the EFSA Guidance Document of these additional studies was not available in the addendum. It was however noted that even considering the additional trials the geographical representativeness of the available data would be weak (data only from Germany and UK). Therefore, it was agreed that the 90th percentile residue values cannot be used, in line with the EFSA Guidane Document. Overall, the majority of the experts agreed that the highest residue level in pollen and nectar from the 'natural exposure' studies should be used in the 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).

During Peer Review it was argued that it has not been fully justified why the forced exposure studies on maize, *Phacelia* and mustard cover the risk to all succeeding crops (see comment 5(19) and 5(22) in the Reporting Table). It was appreciated that these crops are worst-case in terms of their attractiveness to bees but possible differences in pollen/nectar concentrations between these and other crops have not been fully considered. The concentrations of clothianidin measured in pollen and nectar are highly dependent on the crop and also on the type of soil. Based on the available results for maize, *Phacelia* and mustard, a high variance between the different crops can be assumed. The applicant provided the following response to this comment (*text in italic*):

The results do not indicate a high variability between crops, in the "natural exposure" the pollen residues in maize are 1 µg/kg and from phacelia are 0.84 µg/kg (considering the 90th percentile value), hence the results do not support the conclusion of a high variability. The results also do not indicate a dependence on the soil concentration. This is similarly shown in the "forced exposure" where the 90th percentile values for the phacelia and mustard are very similar while that for maize varies slightly from these values. Variability in the residue expected both in terms of crops and any theoretical weeds is taken into account by providing the 90th percentile residues as is standard practice for any risk assessment.

At Pesticides Peer Review Meeting 145, this issue was further discussed. It was highlighted that the agreed approach (to use the highest residue values from the available 'natural exposure' studies) may not fully address the attractiveness of the crop as foreseen in the EFSA Guidance Document 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 the 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.

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 product applied as seed treatment. 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 highest and lowest authorized 'maximum application rate' for both winter cereals and beets (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: Lowest and highest authorized 'maximum application rate' of clothianidin containing formulations for use as a seed treatment in winter cereals and beet.

Crop	Lowest 'maximum application rate'	Highest 'maximum application rate'
Winter cereals	59 g a.s./ha (27 g a.s./dt)	100 g a.s./ha (50 g a.s./dt)
Beet[#]	10 g a.s./ha (10 g a.s./u)	108 g a.s./ha (60 g a.s./u)

Notes: [#] 1 unit = 100,000 seeds

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.

Table B.9.2.3.1-2: Tier 1 ETR calculations for acute adult oral, chronic adult oral and larval exposure for the lowest and highest authorized 'maximum application rate' of clothianidin in winter cereals and sugar beet.

Acute adult oral exposure								
Crop	Application rate (kg a.s./ha)		E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Winter cereals	Lowest	0.059	1	0.70	-	0.00379	10.90	0.2
	Highest	0.100	1	0.70	-	0.00379	18.47	0.2
Sugar beet	Lowest	0.010	1	0.70	-	0.00379	1.847	0.2
	Highest	0.108	1	0.70	-	0.00379	19.94	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
Winter cereals	Lowest	0.059	1	0.54	1	0.00138	23.09	0.03
	Highest	0.100	1	0.54	1	0.00138	39.13	0.03
Sugar beet	Lowest	0.010	1	0.54	1	0.00138	3.913	0.03
	Highest	0.108	1	0.54	1	0.00138	42.26	0.03
Larval exposure								
Crop	Application rate (kg a.s./ha)		E_f	SV	twa	NOED (µg a.s./larva /development period)	ETR	Trigger
Winter cereals	Lowest	0.059	1	0.40	1	0.00528	4.47	0.2
	Highest	0.100	1	0.40	1	0.00528	7.58	0.2
Sugar beet	Lowest	0.010	1	0.40	1	0.00528	0.76	0.2
	Highest	0.108	1	0.40	1	0.00528	8.18	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 nectar and pollen in several succeeding crops were measured. In the original version of the Addendum, the highest 90th percentile residue values from the 'natural exposure' succeeding crop studies were used in 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 seed treatment.

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[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

[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.

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. 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 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.9.2.3.1-5: 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 winter cereals ans beet.

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. 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.

In the original DAR, a number of cage and tunnel studies investigating the effects of residues of clothianidin in pollen and nectar from seed treated summer rape, sunflower and maize on honeybees are available. These studies were assessed by EFSA in the EFSA Conclusion on the risk assessment for bees for clothianidin (2013). These studies were considered not suitable for the risk assessment because of a number of deficiencies (e.g. short period of exposure, lack of raw data in in the study reports, no statistical analysis performed, …). For details on the assessment by EFSA, reference is made to the EFSA Conclusion on clothianidin (2013).

Three field studies have been performed in maize, on different locations in France (Hecht-Rost S., 2009a, b & c; see study 25, 26 and 27 in the study evaluation notes from the EFSA Conclusion, 2013). In these studies, fields were sown with either maize seeds treated with clothianidin (treated sites) or untreated maize seeds (control sites). In each study, there was one treated and one control field, each with 6 bee colonies. In the EFSA Conclusion (2013) some weak points were noted, such as field size, distance between the fields, and the presence of attractive crops close to the study area. This lead to the conclusion that these studies did not represent a worst case exposure for maize as treated crop. In the original version of this Addendum, RMS considered that these studies could be useful in support of the risk assessment for succeeding crops, as the exposure was worst case compared to what is expected for the succeeding crop scenario. The measured residues of clothianidin in pollen in the studies by Hecht-Rost (2009a, b & c) were between 1 µg/kg and 5 µg/kg, depending on the study, which exceeds the value of 1 µg/kg for succeeding crops. Even though the fraction of maize pollen in the diet of bees in these studies was relatively low (between 1 and 36% of the total amount of pollen consumed), the exposure can still be considered worst case compared to the succeeding crop scenario. No treatment related effects on mortality, foraging activity, behaviour, brood development, colony strength and generally on the colony and weight development were found. As maize is not highly attractive to bees and does not produce nectar, these results can however not be extrapolated to other, more bee attractive and nectar producing crops (e.g. oilseed rape).

However, during Peer review it was argued that the studies performed in maize on different locations in France and evaluated in the EFSA Conclusion 2013 cannot be considered robust enough to draw the conclusion that there were no treatment-related effects (see comment 5(14) in the Reporting Table). At Pesticided Peer Review Meeting 145, these studies were not further discussed, as the experts agreed that the previous conclusion of EFSA (2013) is still valid for these studies. Consequently, these studies are considered not acceptable for use in the risk assessment.

Two field studies performed in Canada are available that assess the impact of clothianidin treated oilseed rape on honeybees. The first one (Scott-Dupree C.D. and Spivak M.S., 2001) was part of the original DAR. As there was no untreated control present and the treated field was treated with a mixture of three molecules, this study was not considered usefull for the risk assessment in the EFSA Conclusion on clothianidin (2013). The second study (Cutler C., 2009; see study 23 in the study evaluation notes from the EFSA Conclusion, 2013) was also not considered reliable for the risk assessment due to several deficiencies, i.e. the colony size was not reported, plot size and distance between the treated and control plots was too small, behaviour effects were not investigated, and residue was detected in some control samples.

A new field effect study which invastigated the effects of residues in nectar and pollen of clothianidin treated oilseed rape on honeybee colony development (Rolke et al. 2014; see Section B.9.7.1, Study 1.8/7) was submitted by the applicant. This field study is part of a large scale monitoring project on the effects of seed treatment of oilseed rape with clothianidin on honeybees, bumblebees and solitary bees. For this monitoring project, two study sites (treated site and control site) were selected in Northern Germany, each covering an area of about 65 km² and containing about 20 study fields sown with oilseed rape. Oilseed rape sown in the treated site were seed treated with clothianidin, while those sown in the control site were untreated. For the honeybee study, six study locations were identified at each study site within a core area (7 km diameter) where honeybee hives were set up. Of the six locations in each study site, three locations were situated at the edge of oilseed rape fields, and three location at about 400m distant from the oilseed rape fields. At each study location, 8 honeybee hives were placed, resulting in a total of 96 colonies that were exposed to nectar and pollen from oilseed rape (48 treated and 48 untreated). It could be argued that only one study is available and that the geographical spread of the study locations is limited. However, a high number of colonies was monitored, which should result in a sufficient statistical power. Overall, this honeybee field study is considered to provide a good indication of the potential influence of nectar and pollen from succeeding crops on honeybee colonies.

Residues in nectar and pollen from the treated oilseed rape fields were measured (see Persigehl, 2014; Study 1.8/6). The maximum measured concentration of clothianidin was 3.5 µg/kg in pollen and 3.6 µg/kg in nectar. For succeeding crops, realistic worst case residue values of 1.0 µg/kg in pollen and 0.3 µg/kg in nectar were identified (see section B.9.2.2). Resideus in pollen and nectar from oilseed rape as treated crop are thus clearly higher than those measured in succeeding crops. Further, oilseed rape is a highly bee attractive crop for both pollen and nectar. In the study by Rolke et al. (2014), residues of clothianidin in pollen samples collected by forager honeybees were also measured. Residues were generally low, with only one out of 48 samples with a concentration above the LOQ (1.1 µg/kg) at the first sampling, which increased to 23 out of 46 samples with measureable concentrations of up to 2.7 µg/kg at the second sampling. The percentage of oilseed rape pollen in pollen pellets collected by forager bees was very high with a mean percentage of more than 71% in all areas, and up to 91% within the hives at locations at the edge of oilseed rape fields. This indicates that honeybees were higly exposed to pollen from clothianidin treated oilseed rape fields, and that this exposure can be considered worst case compared to exposure through nectar and pollen from succeeding crops.

Rolke et al. (2014) found no treatment related effects on the development of honeybee colonies (number of adult bees and worker and drone brood), nor on the honey production, composition of the collected pollen or infestation rates with diseases and *Varroa* mites, neither during blossom in spring nor thereafter until the end of the study in autumn. The weather and the distance to the oilseed rape fields were the main influencing variables on the development of the honeybee colonies, with only marginally significant effects.

At Pesticides Peer Review Meeting 145, the large scale monitoring study in oilseed rape was discussed. It was noted that the study was performed in Germany. A similarity analysis between the study area and other oilseed rape growing areas in Europe was performed, but it seems that it does not cover the landscape composition (i.e. differences in field margin composition in oilseed rape areas other than DE may influence the proportion of pollen from different plant species entering into the hive, for example when more attractive plants are available in the filed margin). An in depth evaluation of the similarity analysis provided with the study would be appropriate to confirm this.

It was noted that the complexity of the study design and the number of analyses and observations performed and reported would require a peer review of all the original study reports. A full consideration of this study within the confirmatory data procedure was not feasible. The study will be evaluated more deeply under the review on the neonicotinoids (Ref. EFSA question number: EFSA-Q-2015-00771).

Overall, the experts considered that this study, for the time being, cannot be used to draw firm conclusions on possible extrapolation of the results to other scenarios (i.e. succeeding crops, field margin and treated crop other than oilseed rape) for honeybees.

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 for chronic adult exposure. Higher Tier field effect studies with treated primary crops (in which residues in pollen and nectar exceeded those measured in the succeeding crop studies) could potentially be used to refine the risk assessment. However, the available large scale monitoring study performed in oilseed rape requires further in depth evaluation (which will be performed within EFSA-Q-2015-00771). For the time being, this study cannot be used to extrapolate the results to other scenarios. Consequently, no acceptable risk to could be concluded.

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 the 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.

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 seed treatment. 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 a dult 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 highest and lowest authorized 'maximum application rate' for both winter cereals and beets (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: Lowest and highest authorized 'maximum application rate' of clothianidin containing formulations for use as a seed treatment in winter cereals and beet.

Crop	Lowest 'maximum application rate'	Highest 'maximum application rate'
Winter cereals	59 g a.s./ha (27 g a.s./dt)	100 g a.s./ha (50 g a.s./dt)
Beet[#]	10 g a.s./ha (10 g a.s./u)	108 g a.s./ha (60 g a.s./u)

Notes: CTD = clothianidin; [#] 1 unit = 100,000 seeds

Table B.9.2.3.2-2: Tier 1 ETR calculations for acute adult oral and chronic adult oral exposure for the lowest and highest authorized 'maximum application rate' of clothianidin in winter cereals and sugar beet.

Acute adult oral exposure								
Crop	Application rate (kg a.s./ha)		E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Winter cereals	Lowest	0.059	1	0.90	-	0.00191	27.80	0.036
	Highest	0.100	1	0.90	-	0.00191	47.12	0.036
Sugar beet	Lowest	0.010	1	0.90	-	0.00191	4.712	0.036
	Highest	0.108	1	0.90	-	0.00191	50.89	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
Winter cereals	Lowest	0.059	1	0.78	1	0.000138	333.5	0.0048
	Highest	0.100	1	0.78	1	0.000138	565.2	0.0048
Sugar beet	Lowest	0.010	1	0.78	1	0.000138	56.52	0.0048
	Highest	0.108	1	0.78	1	0.000138	610.43	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 the Addendum, the highest 90th percentile residue values from the 'natural exposure' succeeding crop studies were used in 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 seed treatment.

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[22]) 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

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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

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 winter cereals and beet.

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. No higher tier effect studies specifically assessing the risk to bumblebees from the consumption of nectar and pollen in succeeding crops are available. However, the applicant submitted a field effect study which investigated the effects of residues in nectar and pollen of clothianidin treated (seed treatment) oilseed rape on bumblebee colony development (Sterk & Peters, 2014; see section B.9.7.1, Study 1.8/9). This study could be used in support of the risk assessment for succeeding crops, and is considered below.

The field study on bumblebees (Sterk & Peters, 2014) is part of a large scale monitoring project on the effects of seed treatment of oilseed rape with clothianidin on honeybees, bumblebees and solitary bees. For this monitoring project, two study sites (treated site and control site) were selected in Northern Germany, each covering an area of about 65 km² and containing about 20 study fields sown with oilseed rape. Oilseed rape sown in the treated site were seed treated with clothianidin, while those sown in the control site were untreated. For the bumblebee study, six study locations were identified at each study site within a central area (3 km diameter) where bumblebee hives were set up. Of the six locations in each study site, three locations were situated at the edge of oilseed rape fields, and three location at about 400m distant from the oilseed rape fields. At each study location, 10 bumblebee colonies were placed, resulting in a total of 120 colonies that were exposed to nectar and pollen from oilseed rape (60 treated and 60 untreated). It could be argued that only one study is available and that the geographical spread of the study locations is limited. However, a high number of colonies was monitored, which should result in a sufficient statistical power. Overall, this bumblebee field study is

considered to provide a good indication of the potential influence of nectar and pollen from succeeding crops on bumblebee colonies.

Residues in nectar and pollen from the treated oilseed rape fields were measured (see Persigehl, 2014; Study 1.8/6). The maximum measured concentration of clothianidin was 3.5 µg/kg in pollen and 3.6 µg/kg in nectar. For succeeding crops, realistic worst case residue values of 1.0 µg/kg in pollen and 0.3 µg/kg in nectar were identified (see section B.9.2.2). Resideus in pollen and nectar from oilseed rape as treated crop are thus clearly higher than those measured in succeeding crops. Further, oilseed rape is a highly bee attractive crop for both pollen and nectar. In the study by Sterk & Peters (2014), residues of clothianidin in pollen samples from the bumblebee colonies were also measured. As bumblebees did not exclusively forage on oilseed rape (on average 43.9% of the pollen pellets consisted of oilseed rape pollen, with variation depending on the availability of alternative flowering plants), residues were lower compared those measured in pollen sampled directly from oilseed rape plants. The maximum residue value measured in pollen collected from bumblebee colonies was 1.3 µg/kg. As this value is comparable to the clothianidin residues in pollen from succeeding crops, bumblebees would have to feed exclusively on succeeding crop pollen to obtain the same level of exposure as in the study by Sterk & Peters (2014). Overall, exposure of bumblebees to residues of clothianidin in the field study by Sterk & Peters (2014) can thus be considered worst case compared to exposure through nectar and pollen from succeeding crops.

During Peer Review, it was noted that field study data from oilseed rape fields hase been extrapolated to all succeeding crops on the basis of pollen residue data. It was however argued that consideration of residues in nectar is also necessary (see comment 5(24 in the Reporting Table). In the field study by Sterk & Peters (2014), indeed only the clothianidin residues in pollen collected by bumblebees were measured. Thus, only pollen residues from this study could be directly compared to the residues measured in succeeding crop pollen. Nevertheless, in other studies that were performed in parallel to the study by Sterk & Peters (2014), which were part of the same large scale monitoring project, residues in oilseed rape nectar were measured. Bumblebees in the study by Sterk & Peters (2014) will have been exposed to similar clothianidin residues in nectar. In the paragraph above, the residues in nectar from the oilseed rape field studies were compared to residues in nectar from succeeding crops, demonstrating that the residues in the oilseed rape field study were worst case.

Sterk & Peters (2014) found no treatment related effects on the development of bumblebee hives (measured as evolution in number of workers, colony weight, brood size and number of new queens), neither during blossom in spring nor thereafter until the end of the season. The weather and the distance to the oilseed rape fields were the main influencing variables on the development of the bumblebee colonies. In the original version of this Addendum, it was considered reasonable to assume that based on these results, due to the lower exposure, no effects would be seen in studies with succeeding crops. Therefore, the acute and long-term risk to bumblebees following exposure to nectar and pollen in succeeding crops was considered acceptable.

At Pesticides Peer Review Meeting 145, the large scale monitoring study in oilseed rape was discussed. For the solitary bee, *Osmia*, the experts noted that the pollen composition indicated that oilseed rape is not a relevant source of pollen. For Bumblebees, the range of pollen composition was very high (2-100%) with an average of 50%. It was argued that in this case it could be useful to only consider the results from hives with a large proportion of oilseed rape pollen to obtain a worst-case exposure situation, but this would further reduce the power of the study. Based on the current evaluation of the data presented in the study report, extrapolation to other scenarios was considered not fully reliable because not worst-case.

It was noted that the study was performed in Germany. A similarity analysis between the study area and other oilseed rape growing areas in Europe was performed, but it seems that it does not cover the landscape composition (i.e. differences in field margin composition in oilseed rape areas other than DE may influence the proportion of pollen from different plant species entering into the hive, for example

when more attractive plants are available in the filed margin). An in depth evaluation of the similarity analysis provided with the study would be appropriate to confirm this.

It was noted that the complexity of the study design and the number of analyses and observations performed and reported would require a peer review of all the original study reports. A full consideration of this study within the confirmatory data procedure was not feasible. The study will be evaluated more deeply under the review on the neonicotinoids (Ref. EFSA question number: EFSA-Q-2015-00771).

Overall, the experts considered that this study, for the time being, cannot be used to draw firm conclusions on possible extrapolation of the results to other scenarios (i.e. succeeding crops, field margin and treated crop other than OSR) for honeybees. Further consideration for bumblebees would be needed. However, for solitary bees the experts considered that the extrapolation to other crops or scenarios could not be reliably performed because likely the conditions in the study were not worst case for these species.

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. Higher Tier field effect studies with treated primary crops (in which residues in pollen and nectar exceeded those measured in the succeeding crop studies) could potentially be used to refine the risk assessment. However, the available large scale monitoring study performed in oilseed rape requires further in depth evaluation (which will be performed within EFSA-Q-2015-00771). For the time being, this study cannot be used to extrapolate the results to other scenarios. Consequently, no acceptable risk to could be concluded.

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 the 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.

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 product applied as seed treatment. 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 highest and lowest authorized 'maximum application rate' for both winter cereals and beets (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: Lowest and highest authorized 'maximum application rate' of clothianidin containing formulations for use as a seed treatment in winter cereals and beet.

Crop	Lowest 'maximum application rate'	Highest 'maximum application rate'
Winter cereals	59 g a.s./ha (27 g a.s./dt)	100 g a.s./ha (50 g a.s./dt)
Beet[#]	10 g a.s./ha (10 g a.s./u)	108 g a.s./ha (60 g a.s./u)

Notes: CTD = clothianidin; [#] 1 unit = 100,000 seeds

Table B.9.2.3.3-2: Tier 1 ETR calculations for acute adult oral and chronic adult oral exposure for the lowest and highest authorized 'maximum application rate' of clothianidin in winter cereals and sugar beet.

Acute adult oral exposure								
Crop	Application rate (kg a.s./ha)		E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Winter cereals	Lowest	0.059	1	0.49	-	0.000379	76.28	0.04
	Highest	0.100	1	0.49	-	0.000379	129.3	0.04
Sugar beet	Lowest	0.010	1	0.49	-	0.000379	12.93	0.04
	Highest	0.108	1	0.49	-	0.000379	139.6	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
Winter cereals	Lowest	0.059	1	0.49	1	0.000138	209.5	0.0054
	Highest	0.100	1	0.49	1	0.000138	355.1	0.0054
Sugar beet	Lowest	0.010	1	0.49	1	0.000138	35.51	0.0054
	Highest	0.108	1	0.49	1	0.000138	383.5	0.0054

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 the Addendum, the highest 90th percentile residue values from the 'natural exposure' succeeding crop studies were used in 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 seed treatment.

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

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	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[23]) 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.

[23] 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. No higher tier effect studies specifically assessing the risk to solitary bees from the consumption of nectar and pollen in succeeding crops are available. However, the applicant submitted a field effect study which investigated the effects of residues in nectar and pollen of clothianidin treated (seed treatment) oilseed rape on the development and reproduction of solitary bees (see section B.9.7.1, Study 1.8/8). This study could be used in support of the risk assessment for succeeding crops, and is considered below.

The field study (Peters, 2015) was conducted with the red mason bee *Osmia bicornis*. In Appendix Q of the EFSA Guidance Document on bees, this species is proposed as test species in the risk assessment scheme for solitary bees. The study by Peters (2015) is part of a large scale monitoring project on the effects of seed treatment of oilseed rape with clothianidin on honeybees, bumblebees and solitary bees. For this monitoring project, two study sites (treated site and control site) were selected in Northern Germany, each covering an area of about 65 km² and containing about 20 study fields sown with oilseed rape. Oilseed rape sown in the treated site were seed treated with clothianidin, while those sown in the control site were untreated. For the solitary bee study, six study locations were identified at each study site where nesting shelters and solitary bee cocoons were set up. Of the six locations in each study site, three locations were situated at the edge of oilseed rape fields, and three location at about 100m distant from the oilseed rape fields. At each study location, three nesting shelters containing each two or three nesting blocks (with 200 nesting holes) were placed. This resulted in 36 nesting shelters in total (18 treated and 18 untreated). Further, 1500 cocoons of red mason bees were set up at each test location. It could be argued that only one study is available and that the geographical spread of the study locations is limited. However, a high number of nesting mason bee females was monitored, which should result in a sufficient statistical power. Overall, this solitary bee field study is considered to provide a good indication of the potential influence of nectar and pollen from succeeding crops on solitary bees.

Residues in nectar and pollen from the treated oilseed rape fields were measured (see Persigehl, 2014; Study 1.8/6). The maximum measured concentration of clothianidin was 3.5 µg/kg in pollen and 3.6 µg/kg in nectar. For succeeding crops, realistic worst case residue values of 1.0 µg/kg in pollen and 0.3 µg/kg in nectar were identified (see section B.9.2.2). Resideus in pollen and nectar from oilseed rape as treated crop are thus clearly higher than those measured in succeeding crops. Further, oilseed rape is a highly bee attractive crop for both pollen and nectar. In the study by Peters (2015), the composition of pollen samples from mason bee brood cells and clothianidin residues in similar samples were also investigated. The amount of oilseed rape pollen in the brood cells of mason bees averaged 18.0% at the control site and 10.7% at the treatment site, with a maximum of 41.2%. A higher amount of pollen was collected from other plants than oilseed rape, mainly Rosaceae. These findings are in line with Westich (2014) [24], and indicate that mason bees are food-generalists, that prefer to collect polen from the surrounding hedges and trees. As mason bees did not exclusively forage on oilseed rape, measured residues in oilseed rape were lower compared those measured in pollen sampled directly from oilseed rape plants. The maximum residue value measured in pollen from mason bee brood cells was 1.7

[24] Westrich, P (2014) Wildbienen: die anderen Bienen, Pfeil Verlag, München, 4. Auflage, 168 pp.

µg/kg. As this value is comparable to the clothianidin residues in pollen from succeeding crops, solitary bees would have to feed exclusively on succeeding crop pollen to obtain the same level of exposure as in the study by Peters (2015). Based on the pollen composition of pollen collected by mason bees, this is however not likely to occur. Overall, exposure of mason bees to residues of clothianidin in the field study by Peters (2015) can thus be considered worst case compared to exposure through nectar and pollen from succeeding crops.

The results from Peters (2015) indicate that Elado dressed oilseed rape had no impact on the development of red mason bees neither on the nest building nor on the reproduction, neither during blossom in spring nor thereafter until autumn. Also in the Study Locations which were selected at the edge of oilseed rape fields no effects of Clothianidin were measurable although mason bees at these locations were more intensively exposed to Elado dressed oilseed rape. The weather and especially the sunshine was the main influencing variable on the nest building activity and reproduction of the mason bees. In the original version of this Addendum, it was considered reasonable to assume that based on these results, due to the lower exposure, no effect would be seen in studies with succeeding crops. Therefore, the acute and long-term risk to solitary bees following exposure to nectar and pollen in succeeding crops was considered acceptable.

During Peer Review, it was argued that a field study with one species of solitary bees is not considered sufficient addressing the risk to bees taking into account the high variability between the different species of solitary bees. In addition, it should be considered that the used solitary bees (mason bees) are food-generalists. Hence, the available field study might not cover the variability between species and the realistic exposure to solitary bees (see comment 5(20) and 5(44) in the Reporting Table). In response to this comment, the applicant pointed out that the solitary bee species *Osmia bicornis* investigated in this study is the representative solitary bee species recommended by the EFSA Guidance Document on bees. Further, the applicant submitted a literature evaluation, which is summarized in Section B.9.7.1 (Exeler N., 2015; study 1.8/10). Based on this literature evaluation the applicant is of the opinion that only 2% of a regional bee species pool represents the dominant crop-visiting species. These pollinator species are generally common and polylectic, foraging on a range of different plant species. The applicant claims to be not aware of any information that refers to presence of food specialists in oilseed rape and therefore this exposure scenario appears unrealistic. As the life cycle for solitary bee species is overall comparable, the applicant considers that the field study is also representative for other species of solitary bees.

At Pesticides Peer Review Meeting 145, the large scale monitoring study in oilseed rape was discussed. For the solitary bee, *Osmia*, the experts noted that the pollen composition indicated that oilseed rape is not a relevant source of pollen. Therefore, the exposure in this study cannot be considered worst-case, and therefore extrapolation to other scenarios was considered not fully reliable.

It was noted that the study was performed in Germany. A similarity analysis between the study area and other oilseed rape growing areas in Europe was performed, but it seems that it does not cover the landscape composition (i.e. differences in field margin composition in oilseed rape areas other than DE may influence the proportion of pollen from different plant species entering into the hive, for example when more attractive plants are available in the filed margin). An in depth evaluation of the similarity analysis provided with the study would be appropriate to confirm this.

It was noted that the complexity of the study design and the number of analyses and observations performed and reported would require a peer review of all the original study reports. A full consideration of this study within the confirmatory data procedure was not feasible. The study will be evaluated more deeply under the review on the neonicotinoids (Ref. EFSA question number: EFSA-Q-2015-00771).

Overall, the experts considered that this study, for the time being, cannot be used to draw firm conclusions on possible extrapolation of the results to other scenarios (i.e. succeeding crops, field margin and treated crop other than OSR) for honeybees. Further consideration for bumblebees would

be needed. However, for solitary bees the experts considered that the extrapolation to other crops or scenarios could not be reliably performed because likely the conditions in the study were not worst case for these species.

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. Higher Tier field effect studies with treated primary crops (in which residues in pollen and nectar exceeded those measured in the succeeding crop studies) could potentially be used to refine the risk assessment. However, the available large scale monitoring study performed in oilseed rape requires further in depth evaluation (which will be performed within EFSA-Q-2015-00771). For solitary bees, extrapolation of the results from this study to other scenarios was not considered reliable because likely the conditions in the study were not worst case. Consequently, no acceptable risk to could be concluded.

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

B.9.3.1. Studies

No studies on the potential uptake via roots to flowering weeds were submitted. Instead, the applicant submitted a statement in which the occurrence of flowering weeds in agricultural crops was evaluated.

Report:	**1.4/1; Garside, C.M., Miles, M. & Kriszan, M.; 2014**
Title:	**Statement - Evaluation of the occurrence of flowering weeds in agricultural crops: Cereals, sugar beet and potatoes**
Report No.:	M-505126-01-1
Document No.:	M-505126-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	no

Objective

In this statement, the occurrence of flowering weeds in cereals, sugar beet and potatoes has been investigated based on data from (herbicide) efficacy trials, to be able to assess the potential relevance of flowering weeds as a source of exposure for honeybees.

Material and Methods

Data sources

The occurrence of weeds in insecticide efficacy trials is not recorded as a standard requirement; however Bayer CropScience also performs extensive efficacy trials on herbicidal active ingredients. In these trials the occurrence of weeds, both on control plots and in the treated plots is recorded. Parameters including the identity of the weed, the growth stage and the coverage of the test-plot are recorded.

To analyse the presence of weeds in agricultural crops the available data was extracted from the database for the crops cereals, sugar beet, and potatoes. As a conservative assessment only the data in the control plots (i.e. no herbicide treatment) was considered to provide a worst-case situation.

All data originate from worldwide herbicide efficacy trials testing for herbicides in cereals (Atlantis® and Herold®), in sugar beet data (Betanal MAXXPro®) and in potatoes (Metribuzin) conducted between 2004 and 2014. The majority of the studies were carried out in Europe; however for completeness of the datasets trials performed outside Europe were also included. Information on weed species, weed growth stages (BBCH), weed diameter (cm), weed ground cover (%), and weed plants/m^2 obtained were recorded. Each weed species per trial was recorded separately, thus there are several data set entries per trial. All data are mean values out of 2 to 4 plot replicates.

Initial data sorting

Since not all information was consistently provided in all trials, data was sorted to consider only cases including at least information on growth stage and ground cover. The weed growth stage classification "Majority", which represents the growth stage of the majority of the weed species on the plot, was taken into account. The cereals data were combined to make a single dataset.

Results

To show how often and to which extent flowering weeds cover the plots, the dataset was edited for graphical representation. Hence the weed growth stage data was plotted against the corresponding ground cover data (see Figure B.9.3.1-1, B.9.3.1-2 and B.9.3.1-3).

Data points in the yellow labelled box at top right indicate weeds at BBCH stage ≥ 60 and ≥ 10% ground cover. This combination of weed growth stage and coverage was considered to be the minimum requirement to identify situation which have the potential to be attractive to foraging bees.

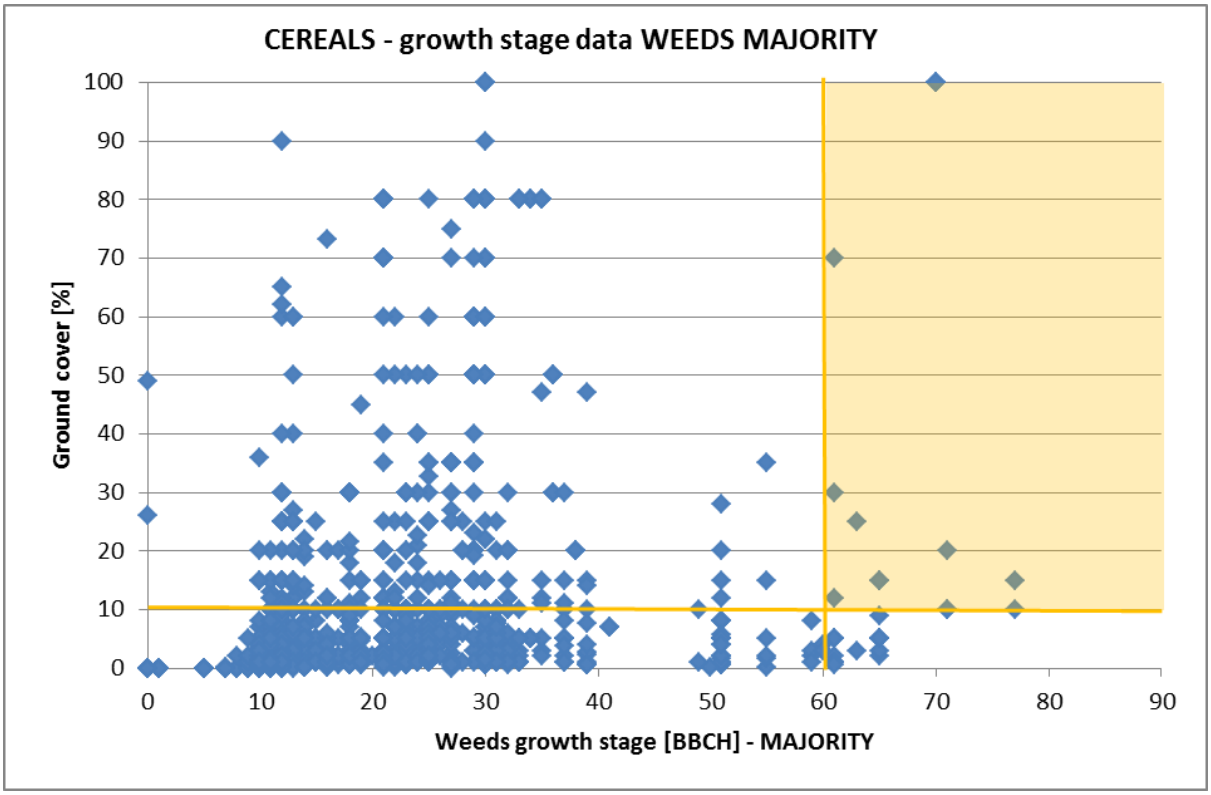


Figure B.9.3.1-1: Weed growth data against ground cover data found in cereals

Flowering weeds exceeding 10% ground cover was only observed in 14 incidents out of 2327 observations (i.e. 0.6%). In the majority of these cases (13 out of 14) the weeds present were small species that did not rely on bee pollination for reproduction or produce sufficient quantities of pollen and nectar to be considered a food source. Only one case was possibly relevant but only under certain circumstances and represented only 0.04% of all cases observed. Consequently, exposure via flowering weeds is confirmed not to be a relevant route of exposure for honeybees or non-*Apis* bees in cereal crops.

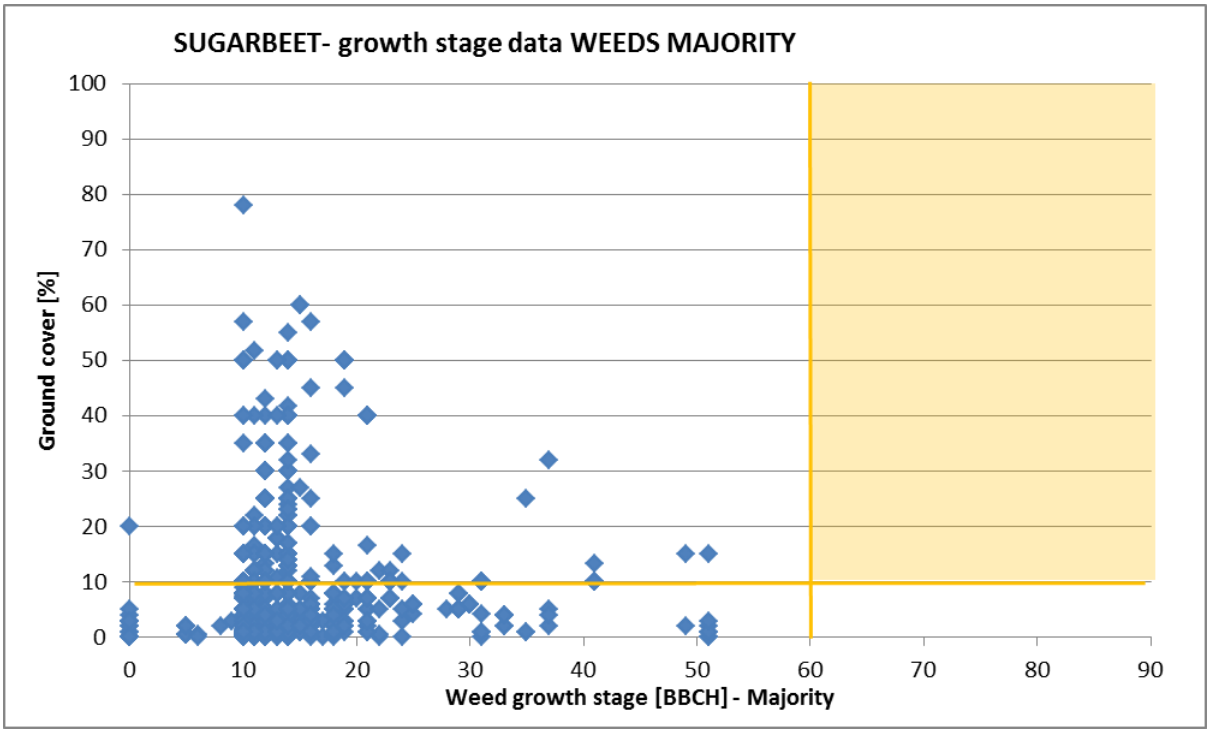


Figure B.9.3.1-2: Weed growth data against ground cover data found in sugar beets

In the trials with sugar beet there were no flowering weeds present on the control plots, where no herbicide was used, confirming that this is not a relevant route of exposure for honeybees or non-*Apis* bees in these crops.

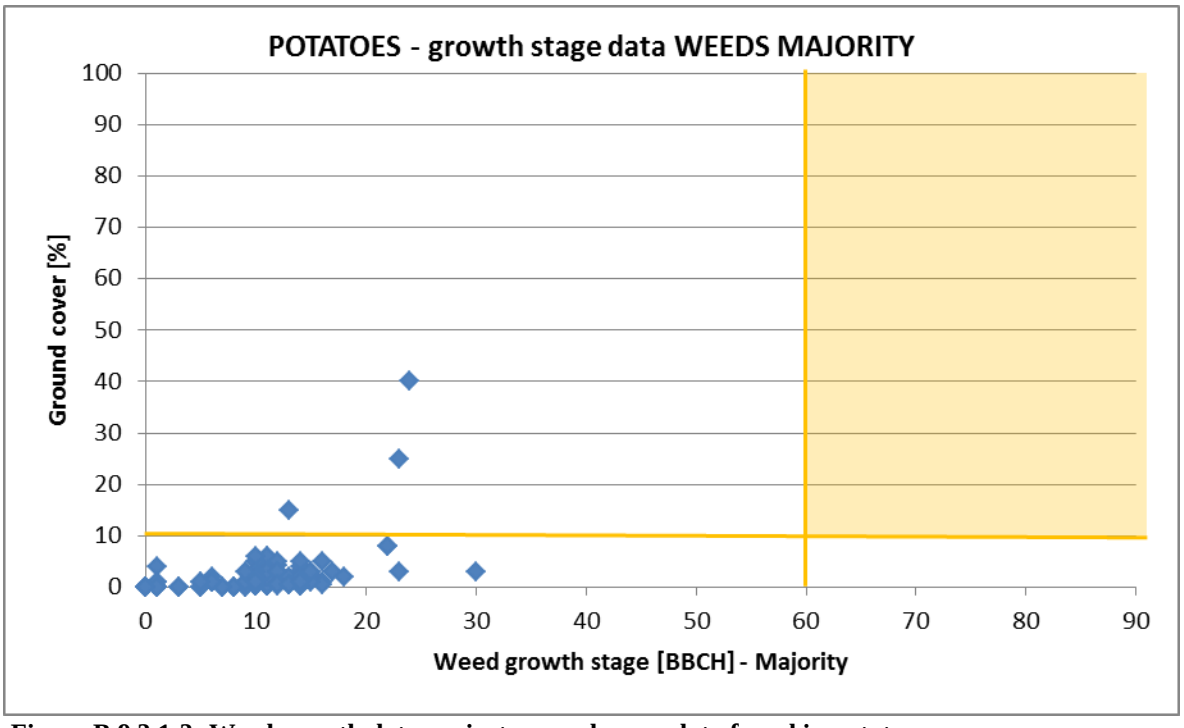


Figure B.9.3.1-3: Weed growth data against ground cover data found in potatoes

In the trials with potatoes there were no flowering weeds present on the control plots, where no herbicide was used, confirming that this is not a relevant route of exposure for honeybees or non-*Apis* bees in these crops.

Conclusions
The analysis performed here indicates that even on experimental plots not treated with herbicide (considered to be a worst case situation), cereal, sugar beet and potato fields do not provide sufficient floral food resources for bees. In sugar beet and potato flowering weeds greater than 10% ground cover were not observed and only observed 0.6% of the time in cereals.

The possible reason for the difference between cereals and sugar beet and potato scenarios is most likely due to the cultivation and seed bed preparation techniques required for each crop. Cereals can be grown on a wide variety of soils and do not require extensive cultivation to establish a suitable seed bed. In contrast sugar beet and potato crops have more specific requirements in terms of soil and seed bed preparation. For sugar (and other) beets deep ploughing is necessary prior to sowing to create the right growing conditions. For potatoes good ground preparation (harrowing, ploughing and rolling) is always needed and the ground can be ploughed up to three times to create the correct growing conditions. These cultivation practices reduce the presence of flowering weeds in sugar beet and potato crops.

It is concluded that exposure to flowering weeds present in cereal, sugar beet and potato crops is not a relevant route of exposure for honeybees or non-*Apis* bees.

RMS Comments
The present evaluation of weed growth and ground cover data is considered acceptable for use in the risk assessment.

At Pesticides Peer Review Meeting 145, this study by Garside *et al.* (2014) was further discussed. This study was considered useful to address the relevance of the weed scenario for this specific case. However, as some points were not clear from the study report, it was considered that clarification was needed with regard to:

1. The number of plots taken into account for the analysis
2. The number of observations per field trial and the timing of the observations (crop BBCH stage)
3. The graphical representation of the results: what does each data point in the graphs represent (The total ground cover (%) for one individual species or the average ground cover for all weed species present at on trial site)?

After the Meeting, the applicant was requested to provide clarification for each of these points. The response from the applicant is provided below.

<u>1. The number of plots taken into account for the analysis</u>
Of available trials only those which recorded the BBCH stage of the weed, as well as the percentage of ground cover of the weeds, have been included in the analysis. This resulted in the following number of trials being included:

- Cereals: 344 trials were evaluated; 2327 weeds were recorded
- Sugar beet: 45 trials were evaluated; 972 weeds were recorded
- Potatoes: 44 trials were evaluated; 236 weeds were recorded

Table B.9.3.1-1 summarizes the number of trials for each country, both EU and non-EU countries, that was taken into account.

Table B.9.3.1-1: Number of trials in EU and non-EU countries taken into accound in the analysis for cereals, sugar beet and potatoes.

Country	Cereals	Sugar beet	Potatoes
Austria	8	4	0
Belgium	17	1	0
Bulgaria	2	0	0
Czech Republic	4	1	2
France	25	7	3
Germany	216	11	17
Greece	9	0	3
Italy	11	1	0
Lithuania	0	3	0
Poland	31	8	4
Slovakia	1	0	0
Spain	3	1	0
Sweden	1	0	2
Switzerland	1	0	1
Ukraine	0	1	0
United Kingdom	14	7	7
Brazil	0	0	1
Canada	1	0	4

2. Number of observations and observation timing (crop BBCH stage)

The number of observations per trial in cereals was between 1 and 4. In sugar beet 1 to 5 assessments per trial were conducted and in potatoes 1 to 4 assessments were performed per trial.

The number of observations performed (frequency) at each crop BBCH stage is shown in Figure B.9.3.1-4, B.9.3.1-5 and B.9.3.1-6 for cereals, sugar beet and potatoes, respectively.

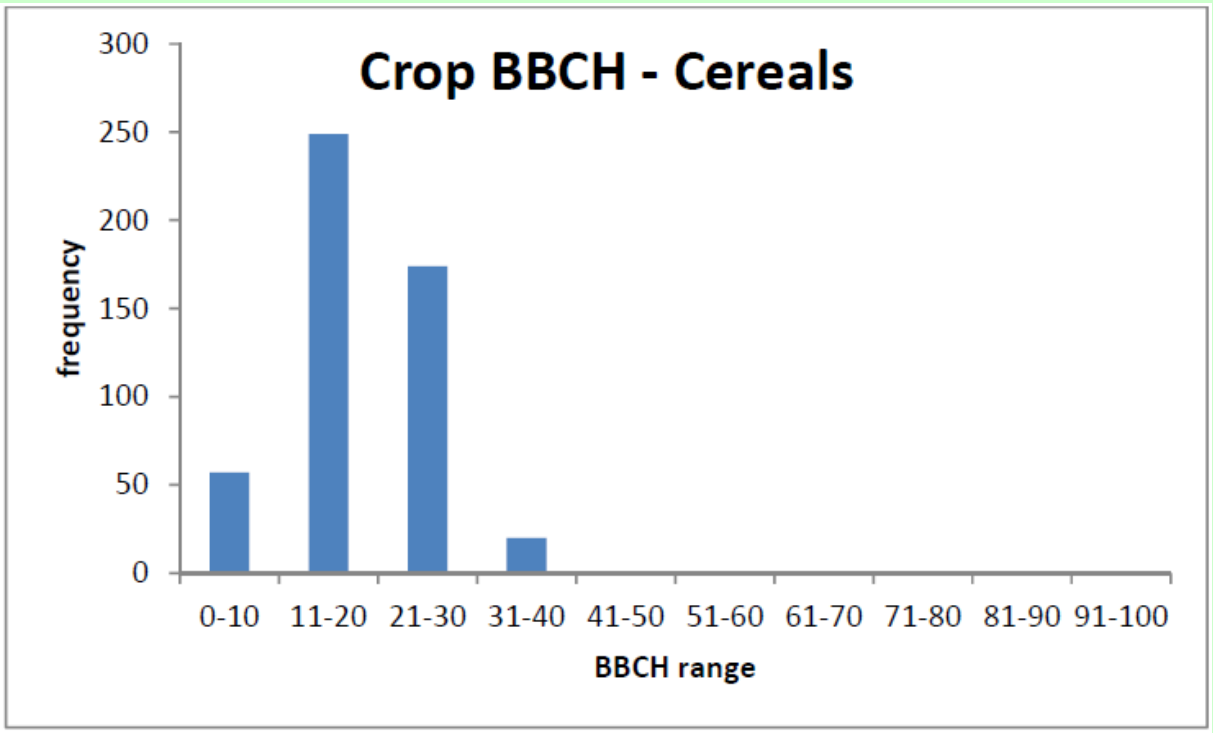


Figure B.9.3.1-4: Number of observations performed (frequency) at each crop BBCH stage monitored in the trials in cereals.

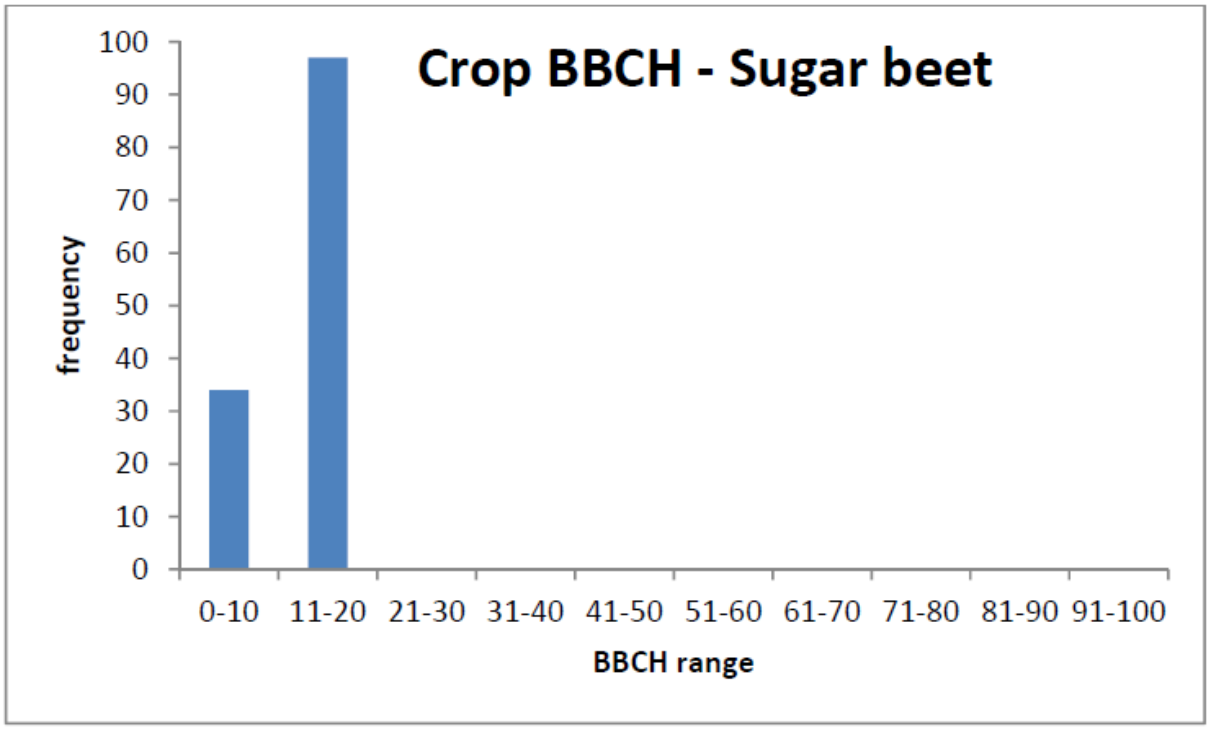


Figure B.9.3.1-5: Number of observations performed (frequency) at each crop BBCH stage monitored in the trials in sugar beet.

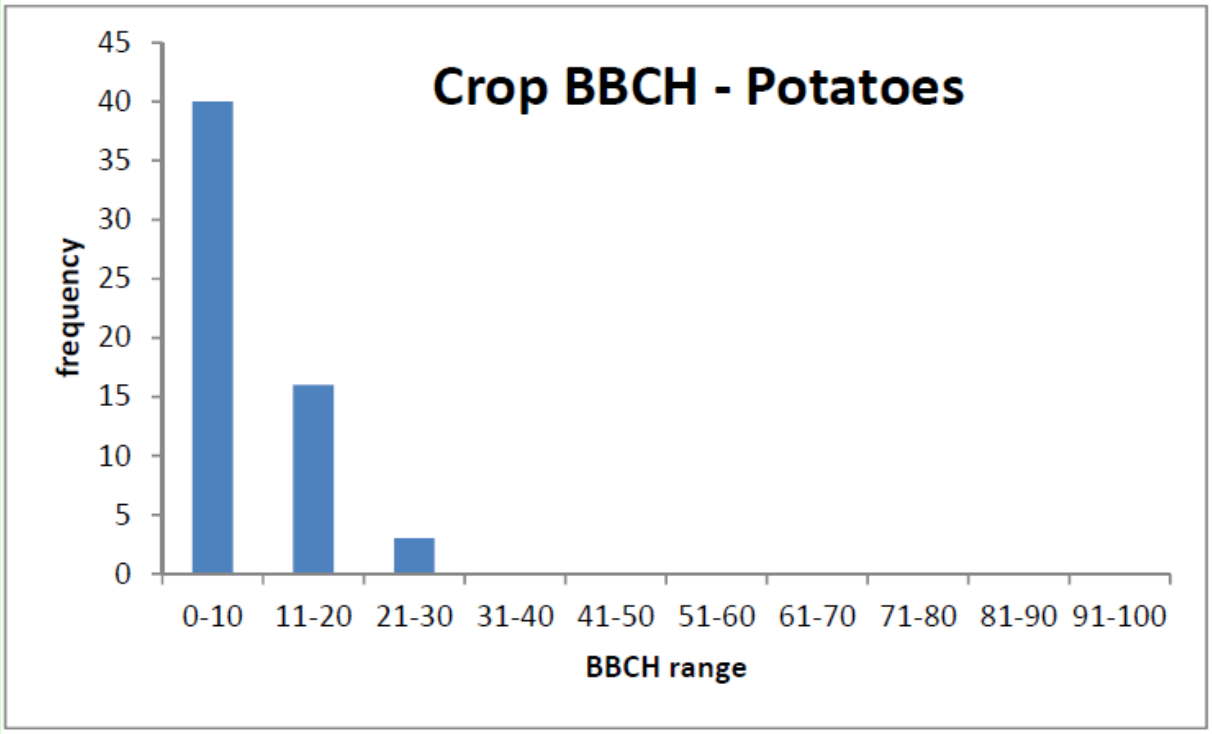


Figure B.9.3.1-6: Number of observations performed (frequency) at each crop BBCH stage monitored in the trials in potatoes.

3. Graphical representation of the results

Each trial has 1 to 4 replicate plots. The data points in Figure B.9.3.1-1 to B.9.3.1-3 represent the average ground cover (%) of one weed species that was recorded at one assessment over the different replicate plots.

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). This 10% trigger refers to the total ground cover for weeds, including all species present. As stated above, the data represented in Figure B.9.3.1-1 to B.9.3.1-3 refers to only one weed species. To be able to compare the data from this study with the 10% trigger, the total ground cover for all flowering weeds (BBCH > 60) for each trial was calculated.

Only in the trials conducted for cereals, flowering weeds (BBCH > 60) were present and thus attractive for bees. For these trials (n=23) the total ground cover of all weed species (BBCH >60) recorded in one trial was calculated and is shown in Figure B.9.3.1-7. This alternative analysis resulted in only 9 trials, out of the total of 344 having > 10% coverage of flowering weeds (which corresponds to < 3% of all trials), the nature of the flowering weeds present was discussed in the original report. The trials where only weeds at BBCH < 60 were present are not shown on the graph in Figure B.9.3.1-7, however it should be considered that these 321 trials would be outside of the yellow box.

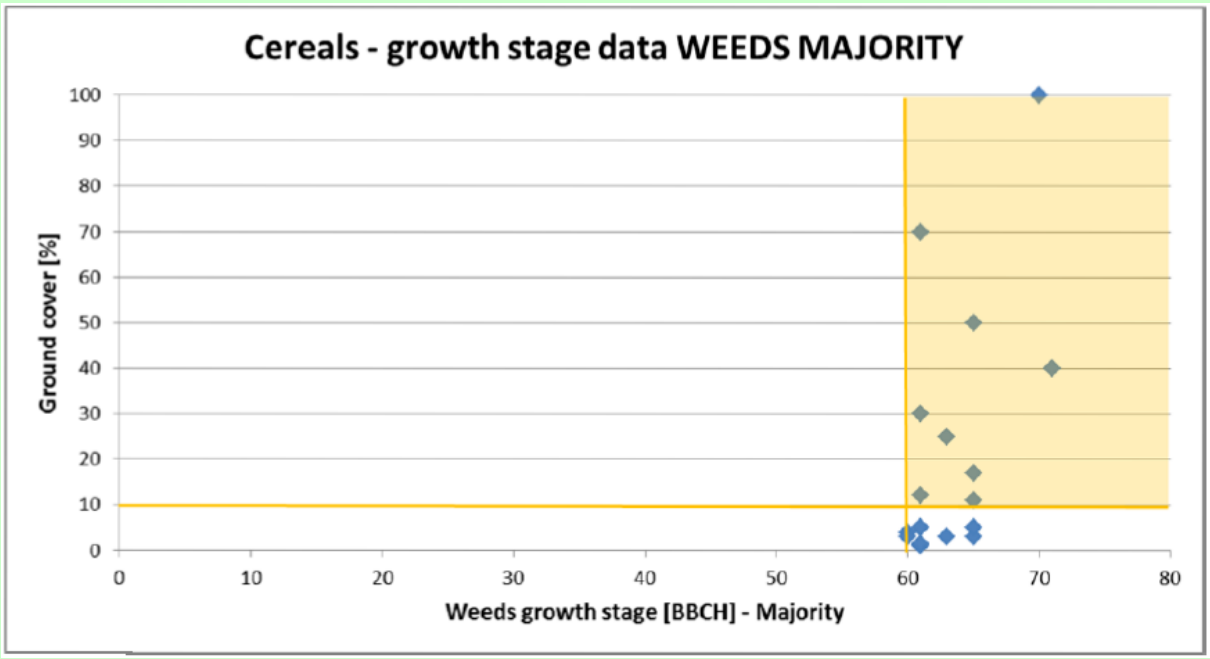


Figure B.9.3.1-7: Total ground cover of all flowering weed species present in trials with flowering weeds for cereals

The additional information provided by the applicant is further discussed in 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. For the currenltly registered uses for clothianidin as seed treatment, no weeds will be present on the field at the time of application (due to seed bed preparation). Therefore, contact exposure from dust deposits on bee atrractive weeds is not considered a relevant route of exposure. Further, the EFSA Conclusion on the risk assessment for bees for clothianidin (2013)[25] concluded that the risk through oral exposure from nectar and pollen from flowering weeds could be considered negligible for uses as seed treatment since considerable uptake via the roots in unlikely as the substance is concentrated around the treated seed. Nevertheless, a data gap was identified to further address the potential uptake of clothianidin via roots of flowering weeds.

[25] 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

During Peer Review, some Member States did not agree, at least not for the use in cereals, with the conclusion that exposure via flowering weeds is negligible, as neonicotinoids show a high potential of bioavailability (see comment 5(27) in the Reporting Table). Furthermore, solitary wild bees are highly specialised on their food plants and for this reason even small numbers of certain flowering weed species may be highly attractive for certain wild pollinators. Thus, it was argued that taking that into account, the risk for wild pollinators through consumption of nectar and pollen of flowering weeds could not be finalised. In response to this comment, the applicant submitted the following argumentation (*text in italic*):

We disagree with the comments considering that weeds occur only very rarely and that flowering weeds exceeding 10% of the groundcover were present in only 0.6% of the trials. In a publication by Maynard et al. (2015)[26], further evidence is presented regarding the low presence of weeds in the field. In a total of 1024 wheat field trials examined only 0.86% weeds were recorded at flowering growth stage. It should be noted that this figure does not yet distinguish between weeds non-attractive or attractive to bees, which can result in an even lower figure.
Based on a literature evaluation (Exeler N., 2015; study 1.8/10 summarized in Section B.9.7.1), Bayer Crop Science is of the opinion that only 2% of a regional bee species pool represents the dominant crop-visiting species. These pollinator species are generally common and polylectic, foraging on a range of different plant species and even in flowering weeds only polylectic species were recorded within the field. The life cycle for solitary bee species is overall comparable between food specialists and generalists. Consequently, conclusions drawn from studies conducted with food generalist solitary bee species can be representative for food specialists and is also more relevant to the bee species pool present in arable fields.

At Pesticides Peer Review Meeting 145, 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 applicant did not assess the potential uptake of clothianidin via roots into flowering weeds. They argue that, due to the variation in weed species (growth, habit, flowering period), the small amounts of pollen and nectar produced by many weedy species and different growing conditions and crops, this would be very difficult to measure experimentally as no standardized methods are available. Instead, a statement was provided in which the occurrence of flowering weeds in agricultural crops was assessed (Garside *et al.*, 2014).

Data extracted from efficacy trials on herbicidal active ingredients was used to evaluate the potential occurrence (and relative importance) of flowering weeds in cereals and sugar beet. Only data from the control plots was analysed as this represents a worst case scenario. The EFSA Guidance Document on bees (2013) suggests 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 thuis their exposure can be ignored (see Appendix N of the EFSA Guidance Document). Based thereon, it was investigated whether weeds at BBCH ≥ 60 (flowering stage) occurred with ≥ 10% ground cover.

The data presented in the original study report indicated that for cereals, flowering weeds exceeding 10% ground cover was only observed in 14 incidents out of 2327 observations (i.e. 0.6%). In the majority of these cases (13 out of 14) the weeds present were small species that did not rely on bee

[26] Maynard et al. (2015). Weeds in the treated field – a realistic scenario for pollinator risk assessment? Hazards of pesticides to bees - 12th International Symposium of the ICP-PR Bee Protection Group, Ghent (Belgium), September 15-17, 2014, 56 Julius-Kühn-Archiv, 450.

pollination for reproduction or produce sufficient quantities of pollen and nectar to be considered a food source. Only one case was possibly relevant but only under certain circumstances and represented only 0.04% of all cases observed. In the trials with sugar beet, there were no flowering weeds present on the control plots, where no herbicide was used.

At Pesticides Peer Review Meeting 145, the study by Garside *et al.* (2014) was discussed. It was agreed that the study was useful to address the relevance of the weeds scenario for this specific case. However, as some points were not clear from the study report, it was considered that clarification was needed. For each of these points, the applicant provided the necessary clarifications and/or performed an additional analysis of the data. Details on this additional information can be found in the study summary in Section B.9.3.1. A discussion of these points is provided below.

The number of trials included in the analysis was 344 trials for cereals and 45 trials for sugar beet, with 2327 and 972 weeds recorded in cereals and sugar beet, respectively. For both crops, the location of the different trial sites was well spread over Europe (see Table B.9.3.1-1). It has to be noted that in some cases, more than one observation was made per trial, with between 1 and 4 observations per trial in cereals and between 1 and 5 observations per trial in sugar beet. Consequently, not all weed recordings can be considered as independent measurements. Nevertheless, taking into account the number of trials and the geographical spread, it is considered that the available dataset is sufficiently representative for the area of use of clothianidin.

The analysis presented in the original study report focussed on each weed species separately. However, at most field sites, more than one weed species was present. As the 10% trigger from the EFSA Guidance Document refers to the total ground cover for weeds (considering all species present), it was considered more appropriate to base the analysis on the total weed ground cover. Therefore, an additional analysis based on the total weed ground cover at each field site was performed. In the case of cereals, the total ground cover of flowering weeds exceeded 10% of the total field surface in only 9 out of the total of 344 trials (which corresponds to < 3%). For sugar beet, no flowering weeds (BBCH > 60) were recorded in any of the trials. These results suggest that exposure to honeybees and non-*Apis* bees through nectar and pollen of flowering weeds in the treated fields will be negligibly low, even in non-herbicide treated fields.

However, according to the additional information received, the trials were all conducted relatively early in the crop growing season. For cereals, all observations were made at a crop BBCH stage below 40, with the majority of the observations made between crop BBCH 11 and 30. For sugar beet, all observations were made at a crop BBCH stage below 20. At Pesticides Peer Review Meeting 145, it was considered essential that the presence of weeds is investigated at different crop stages (also later in the season, e.g. at crop flowering) in order to fully assess the relevance of the weed scenario. As in the study by Garside *et al.* (2014) only data is available for early crop growth stages, this study is not considered sufficient to also demonstrate a negligible exposure from flowering weeds at later crop growth stages.

In the Addendum for granular uses of clothianidin in potato and maize (Sumitomo, see Section B.9.3.1 of the Addendum for the Sumitomo data), a large scale monitoring study that investigated the occurrence of flowering weeds in maize and potato fields at different crop growth stages is available (Negrini, 2014). The results from this study show that the number of flowering weeds present in the field consistently increases throughout the crop growing season, with the highest incidence mid-September. One month after sowing and at crop flowering, the 10% trigger for weed ground cover was exceeded in less than 10% of the tested fields, even under worst case assumptions. For the assessment mid-September, the 10% trigger for weed ground cover was exceeded in 7.84 to 15.69% of the maize fields and in 4.0 to 14.0% of the potato fields, depending on the assumptions made. In September the residues of clothianidin in soil will however already have declined and also bee activity will be declining as the end of the season approaches. Therefore, it was considered that while more flowering weeds were found at that time, this will probably not represent a significant route of exposure.

Assuming that the trends observed in the study by Negrini (2014) can be extrapolated to other crops such as cereals and sugar beet, it could be expected that the number of flowering weeds observed in these crops will also increase throughout the season. Consequently, the available data from Garside *et al.* (2014) are likely not to represent a worst-case situation. However, despite the increase observed, the total weed ground cover in the study by Negrini (2014) was still low at the end of the season in the majority of cases, resulting in the conclusion that exposure to bees will be negligibly low. Therefore, the same can be expected for cereals and sugar beet.

Overall, taking into account all available data, exposure of honeybees and non-*Apis* bees to clothianidin through nectar and pollen of flowering weeds in the treated field can be considered negligibly low. It has to be noted that in the study by Negrini (2014) was performed 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.

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. The risk can be considered acceptable.

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

B.9.4.1. Studies

The applicant submitted a statement in which information on the possible occurrence of the development of resistance of the plant protection product Janus Forte (containing the active substance clothianidin, imidacloprid and beta-cyfluthrin) is provided.

Report:	**1.5/1; Nauen, R.; 2013**
Title:	**Statement - Information on the occurrence or possible occurrence of the development of resistance of the plant protection product Janus Forte (for submission in Europe)**
Report No.:	M-453965-01-1
Document No.:	M-453965-01-1
Guideline(s):	PP1/213(2) EU Directive 91/414 EEC According to OECD format guidance for industry data submissions on plant protection products and their active substances
Guideline deviation(s):	not specified
GLP/GEP:	no

Objective

This statement provides information of the mode of action, known mechanisms of resistance and resistance risk of the three active substances present in the product Janus Forte (the pyrethroid beta-cyfluthrin and the neonicotinoids imidacloprid and clothianidin), used as sead treatment in sugar beet.

Summary

Resistance in arthropod pest species comprises a change in the genetic composition of a population in response to selection by pesticides such that control in the field may be impaired repeatedly at recommended application rates. The report includes resistance management information regarding key invertebrate pests targeted in sugar beet in countries such as Belgium, Czech Republic, France, Germany, Poland, Romania, Slovakia and Serbia by seed treatments with Janus Forte® (FS 280) containing the insecticidal ingredients clothianidin, imidacloprid and beta-cyfluthrin.

Janus Forte® is a mixture of three chemically different insecticides complementing each other in numerous properties and belonging to two distinct mode of action classes, i.e. acting on different molecular target-sites not yet shown to be involved in any cross-resistance issues globally.

Beta-cyfluthrin belongs to the chemical class of synthetic pyrethroids and is a well-known contact insecticide particularly for the control of coleopteran pests, e.g. *Agriotes* ssp. other elaterid soil pests. Pyrethroid insecticides such as beta-cyfluthrin are classified by IRAC (Insecticide Resistance Action Committee) in mode of action class 3A, sodium channel modulators.

Resistance to pyrethroid insecticides has been described for different crop pests and the major mechanisms of resistance were identified as either metabolic (esterases and monooxygenases) or knock-down-resistance (kdr) due to a mutation in the IIS6 domain of the voltage-gated sodium channel. All of the pest insects intended to be targeted by Beta-cyfluthrin in Janus Forte® as a seed treatment are not listed as high risk pests within EPPO´s Std. PP1/213 on resistance risk analysis and haven´t been included for a detailed survey, primarily due to a lack of any resistance issues in the past.

Clothianidin and Imidacloprid are members of the neonicotinoid class of insecticides and well established tools for the control of sucking, chewing and soil pests in seed treatment applications due to their systemic properties. They specifically control a number of coleopteran pests in sugar beet such as elaterid larvae (*Agriotes ssp.*, wireworms), weevils (*Bothynoderes*), flea beetles (*Chaetocnema* ssp.)

and *Atomaria linearis*. Other important pests targeted in sugar beet include aphid pests such as *Aphis fabae* and *Myzus persicae*, thrips (*Thrips tabaci*), dipterans (*Pegomyia*), millipedes (e.g. *Blaniulus guttulatus*) and myriapodes (e.g. *Scutigerella immaculata*). Neonicotinoid insecticides such as clothianidin and imidacloprid are classified by IRAC in mode of action class 4A, nicotinic acetylcholine receptor (nAChR) agonists.

Neonicotinoids sich as clothianidin and imidacloprid contained in Janus Forte are used on a broad range of crops but resistance reports for pests targeted in sugar beet are not known. However, very recently *M. persicae* was shown to have locally developed resistance to neonicotinoid insecticide sprays in peaches in southern France, northern Spain and northern Italy, based on a targetsite mutation in the nicotinic acetylcholine receptor ß-subunit. No reports are known from any secondary host species yet, including sugar beet and vegetables.

In sugar beet no resistance to clothianidin, imidacloprid and beta-cyfluthrin seed treatments is yet described for any of the pests or pest groups mentioned above, including aphid species such as *Aphis fabae* and *Myzus persicae* (particularly targeted by systemically acting clothianidin and imidacloprid). General resistance management guidelines for neonicotinoid and pyrethroid insecticides as published by IRAC are usually followed with products such as Janus Forte® and regionally adapted as necessary.

RMS Comments
This statement provides information on the mode of action and the occurrence and known mechanisms of resistence against the active substances beta-cyfluthrin, imidacloprid and clothianidin, present in the product Janus Forte, which is used as seed treatment in sugar beet. This statement could provide useful information in support of the risk assessment.

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) [27] 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 seed 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 seed treatment will prevent the formation of insect honey dew, a data gap was concluded.

The applicant did not provide any data regarding the presence of honey dew in crops grown from clothianidin treated seeds. Instead, a statement was submitted to demonstrate that exposure to honeydew is negligible (see text below, in italic). Further, a statement on the occurrence or possible occurrence of the development of resistance against the plant protection product Janus Forte (containing the active substances beta-cyfluthrin, imidacloprid and clothianidin) was submitted (see Section B.9.4.1).

[27] 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

Considering the use of clothianidin as a seed treatment.
A consideration of the risk to honeybees foraging on insect honey dew to cover the currently permitted registrations is presented below. A statement providing information on the occurrence or possible occurrence of the development of resistance of the Plant Protection Product Janus Forte (CYB+CTD+IMD 80+100+100 g/L) is also presented (see section B.9.4.1).

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

Need for sap feeding insect control
Deposits of honeydew on leaf surfaces can cause sooty mould growth which can be deleterious to plants in that they can indirectly damage the plant by coating the leaves to the point that it reduces or inhibits sunlight penetration affecting photosynthetic production. In addition the presence of aphids (and other sap feeding pests) can be harmful to plants as heavy infestations can weaken plants due to feeding damage. However, the most important deleterious effect of aphid infestations is the transmission of disease causing viruses on the aphid's stylets. Significant damage by virus transmission can be caused even by very light aphid infestations if virus transmission occurs. Hence aphid efficient control can be highly important to prevent the spread of many economically important virus diseases in winter cereal, beet and potato crops. Consequently it is economically important for the grower to ensure control of aphid pests on these crops, and management of aphids in these crops is recommended by official agronomical advisory organizations in several European countries.

Sap feeding insect control
Control is achieved by seed treatment by neonicotinoid insecticides and also by foliar sprays of various different effective classes of insecticides including neonicotinoids. Seed treatments offer typically around 6 to 8 weeks of protection, and thus can provide highly effective and timely control of insect pests especially during the crop establishment phase. Due to the sensitivity of aphids to neonicotinoid insecticides and other strategies employed by growers, aphid numbers are managed so as not to build up to large infestations which can provide a food source for honeybees. At later crop growth stages, the concentrations of neonicotinoid insecticides may be much lower which may not be sufficient to control aphid pests. However, at that time, the crop is usually well established and much better at resisting the effects of viruses. The aim of aphid control is then no longer to prevent virus transmission but the reduction of aphid numbers as high infestations could lead to reductions in crop yield.

In the case of autumn sown cereals following the phase of protection by CNI seed treatment the weather patterns are typically becoming more wintery and less favourable for aphids. However, during this period growers will monitor their crops for invading aphids and if thresholds are met further sprays can be made. This may involve the use of a pyrethroid insecticide in autumn. In the spring and summer the seed treatment will no longer provide protection of the cereals, however at this point the need is not for the prevention of virus transmission but to control aphids which will feed directly on the plant causing economic yield loss and, if left uncontrolled, infection by sooty mould fungus which can grow on honeydew causing a loss of photosynthetic area. Cereals are inspected according to thresholds during the spring-summer growing period and if these thresholds are met a range of pyrethroid, organophosphate (e.g. chlorpyrifos and dimethoate) and other specialist aphicide insecticides (e.g. pirmicarb and flonicamid) may be used. For examples of cereal aphid management practices and suitable product recommendations reference is made to the official websites for

France[28] and UK[29] farmers, although similar systems are in place through the EU to enable growers to efficiently manage their crops. In addition AHDB (Agriculture and Horticulture Development Board) recommend alternate mode of action sprays in the event that aphids are invading crops following the protection window of CNI seed treatment.

Sugar beet is sown in the spring and again CNI seed treatment provides essential protection from virus transmitting aphids. As for cereals, crops are monitored for aphids and if thresholds are met a range of products of different modes of action may be used (depending on country registrations) such as pyrethroid, organophosphate and pymetrozine. In addition, sprays targeted at other important pests (e.g. Mangold fly / leaf miner on sugar beet) containing thiacloprid will have a side effect against black bean aphid (Aphis fabae). In the future new modern insecticide products with novel modes of action based on lower risk chemistries such as flupyradifurone will continue to offer the grower a range of choices for aphid and vector control.

In the highly unlikely event that in the later part of the growing season for cereals and sugar beet aphids are left uncontrolled by the grower and are allowed to be present at high densities which could result in the presence of honeydew as a food source for honeybees, these conditions will not pose a risk to bees. If there are sufficient nectar sources nearby honeybees will prefer these to honeydew and indeed, as regards to honeydew collection by bees, tree dwelling homopteran species are greatly preferred over crop species (Crane & Walker[30], 1985, Sanz et al.[31], 2005). However, cases where bees will visit crops for honeydew are known and although this only forms a small part of the overall diet it could only occur during large outbreaks of aphid pests not controlled by the grower.

Consequently, due to agronomic need and support from a range of official agronomy advisory organizations, growers will protect their crops from the damaging effects of aphid infestations and keep infestations below a level at which plants will become infested so that they become attractive to bees due to excessive honeydew production.

Exposure of bees to residues of neonicotinoid in honey dew

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

Aphid resistance to Neonicotinoids

Neonicotinoid insecticides act on the insect nicotinic acetylcholine receptors (nAChR) via both contact and ingestion routes of administration the exposure route is ideal for targeted insect pest control. Imidacloprid and clothianidin are both neonicotinoid insecticides with the same mode of action (MOA) and belong to IRAC MOA class 4 A. To date, the occurrence of resistance to this class of insecticide in aphid pests is rare. Moderate imidacloprid resistance in green peach aphid Myzus persicae collected in Greek tobacco has been reported but this is possibly an adaption to nicotine-containing tobacco plants. This metabolic mechanism also confers cross-resistance to other neonicotinoids such as clothianidin and thiamethoxam.

[28]http://www.arvalis-infos.fr/pucerons-et-cicadelles-d-automne-detecter-leur-presence-pour-traiter-en-vegetation-@/view-8157-arvarticle.html

[29]http://cereals.ahdb.org.uk/media/177420/is42-controlling-aphids-and-virus-diseases-in-cereals-and-oilseed-rape.pdf

[30] Crane E., Walker P. (1985). Important honeydew sources and their honeys. Bee World Vol.66 (3) 1985 pp. 105- 112. http://www.ibra.org.uk/articles/Important-honeydew-sources-and-their-honeys

[31] Sanz ML.., Gonzalez M., de Lorenzo C, Sanz J, I. Martinez-Castro I. (2005). A contribution to the differentiation between nectar honey and honeydew honey. Food Chemistry 91 (2005) 313–317

In 2011 target-site resistance in a M. persicae clone derived from a French field population collected in peach was first described (Slater et al 2011[32]). The R81T mutation provides resistance to all neonicotinoid insecticides tested. However similar mechanisms of resistance in M. persicae populations collected in any other crop such as for example cabbage and potatoes have not been described. No reports on neonicotinoid resistance mechanisms have been described in any of the other sucking, chewing and soil pests controlled by clothianidin and imidacloprid used as seed treatment, including thrips and all major aphid species occurring in cereals (e.g Metapolophium dirhodum, Sitobion avenae and Rhopalosiphium padi) or in sugar beet such as Apis fabae. The peach-potato aphid is potentially of risk in potato cultivation but as already noted no such accounts have been documented for seed treatment uses or on potato crops for this species. In addition, anti-resistance strategies are in place which restrict the use of consecutive sprays with the same MOA and require the implementation of long-term rotation with insecticides with other MOAs. Furthermore, field performance is regularly monitored by growers and where performance is poor a repeat application with the same MOA is not permitted and an alternative class of insecticide must be used. Label instructions for anti-resistant management strategies can be crop and use specific and are hence on all product labels and adherence to them is mandatory.

The risk to honeybees foraging on insect honey dew – seed treatment uses

The risk of exposure of honeybees to neonicotinoid insecticides seed treatments via honey dew is considered to be low. The seed treatments themselves control the honey dew producing insects and hence no exposure can occur. At later stages of crop development when the levels of systemic insecticide have declined and no longer provide sap feeding insect control, these clothianidin levels will be of low risk to bees.

There is a large difference in size and body weight between aphids and honeybee foragers. Adult aphid body weights for cereal aphids and those found on beets such as Apis fabae are about 1 mg, with young aphids considerably smaller (Dixon and Kindlmann, 1994[33]). Honeybee foragers are approximately 100 – 120x larger and would be expected to be far less sensitive than aphid pests. Although oral toxicity values are not available for aphids for clothianidin, Forster et al. [34] (2008) derived a contact LD_{50} for the peach potato aphid Myzus persicae (sensitive strain US1L) in the laboratory to be 0.035 ng a.s./aphid (0.000035 µg a.s./aphid). In comparison the contact acute LD_{50} for honeybees is 0.0275 µg a.s./bee. This is a relative difference in sensitivity of approximately 800x indicating a very large margin of safety for bees if they actually do consume honeydew from aphids feeding of plants containing non-aphicidal concentrations of insecticide at the later part of the season. It is also likely that this is an underestimate of the margin of safety as aphids are probably more sensitive via the oral route of exposure. Although an oral LD_{50} for M. persicae is not available there are values for a closely related compound, imidacloprid. In this case the oral toxicity value is 166 – 375x lower than the contact toxicity (according to data from Forster et al., 2008, Elbert et al., 1991; M-110655-1-01 and Nauen, 2013; M-461449-1-01). For honeybees the differential in toxicity for imidacloprid between oral and contact routes of exposure is only 7.26x. Although this information is not for clothianidin it provides some evidence that the margin of safety of approximately 800x for clothianidin between bees and aphids is also conservative. Consequently, when levels in the plant have fallen to those which do not affect aphids they would also not be expected to impact honeybees. As there is no incidence of aphid resistance to a neonicotinoid insecticides seed treatment the risk of exposure to honeybees via honey dew produced by sap feeding insects is low. In addition, resistance management strategies are well known by growers and advisors and they are on labelled on all

[32] Slater R, Paul VL, Andrews M, Garbay M & Camblin P (2011). Identifying the presence of neonicotinoid resistant peach-potato aphid (Myzus persicae) in the peach-growing regions of Southern France and northern Spain. Pest Management Science, 68:634-638.

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

[34] Foster S.P., Cox D., Oliphant L., Mitchinson S., Denholm I. (2008). Correlated responses to neonicotinoid insecticides in clones of the peach-potato aphid, *Myzus persicae* (Hemiptera: Aphididae) Pest Manag Sci 64:1111–1114 (2008).

products. Furthermore, Bayer CropScience operates a product stewardship programme for its products.

Consequently, as sap feeding pests are controlled by neonicotinoid insecticides seed treatments, there are no current incidents of resistance to seed treatments (even after many years of use), and the implementation of anti-resistance strategies mean that the risk to bees foraging on honey dew is low.

RMS agrees in general with the statement of the applicant. For exposure of honeybees and non-*Apis* bees to contaminated honey dew to occur, aphids need to be able to feed on a treated plant without being killed by the clothianidin present in the phloem sap. Early in the season, this will only happen when aphids have developed resistance against clothianidin (meaning that the applied dose is no longer sufficient to kill the aphids). As there currently are no reports of resistance against neonicotinoid insecticides for aphids present in winter cereals or sugar beet, this situation is very unlikely to occur. The seed treatment with clothianidin will thus sufficiently control honey dew producing insects, an hence no exposure can occur.

Later in the growing season (at later stages of the crop development), concentrations of neonicotinoids in crop plants may have decreased to a level that is no longer sufficient to control aphid pests. At that moment, honey dew containing residues of clothianidin could be present in the crop, and exposure to bees is possible. The applicant argues that there are not only differences in body size between aphids and bees (honeybee foragers are approximately 100-120 times larger), but there is also a large difference in relative sensitivity to clothianidin. Consequently, as the residues in phloem sap are too low to affect aphids, they will also not be able to affect bees. However, even if these low levels of clothianidin in phloem sap and honey dew will not be acutely toxic to bees, chronic toxicity effects could potentially occur after consumption of this honey dew. As there is no data available on the actual concentration in phloem sap at that time and on the amount of honeydew consumed by bees, it is difficult to estimate the actual exposure.

However, due to the possible impacts of aphids and other sap feeding insects on crop yield, even at later stages of crop development, aphids will be chemically controlled by other insecticides if the clothianidin seed treatment turns out to be insufficient later in the season. Consequently, it is unlikely that large aphid infestations (and thus high levels of honey dew) will occur in crops grown from clothianidin treated seeds. Exposure of honeybees and non-*Apis* bees to clothianidin through honey dew present in the treated field can thus be considered negligibly low.

During Peer Review, it was noted that according to the EFSA Guidance Document for bee (EFSA, 2013) the honeydew exposure scenario was not included in the risk assessment scheme. The statement presented was considered to seem a reasonable approach to address this issue. However, it was noted that in the paper from Foster *et al*. (2008), the acute contact 72h-EC_{50} values were reported for different clones of the aphid species *Myzus persicae* in the range of 0.034-3.4 ng a.s./aphid (see comment 5(29) in the Reporting Table). By comparing these data with the acute contact toxicity of honeybees, the aphid species appears more sensitive (8-800 times). However, it was argued that by considering this high variability within different clones of the same species and by considering that such comparison would be more relevant between oral exposures, the uncertainty around this route of exposure remained unresolved. In respone to this comment, the applicant submitted the following argumentation (*text in italic*):

Aphid clones used were known highly resistant ones which were created by selecting (by topical application of CNI) from single nymph so are the worst case. Even these were concluded in the paper by Foster et al. (2008) to be controlled by CNI. The strains are artificial and under real-life circumstances in seed treatment uses in potato and cereals there is no resistance so the range of 8-800 times is not realistic to the uses. Control of aphids is only for the first 6–8 weeks after which concentrations are expected to be too low to affect aphids and certainly too low to affect bees

The statement paper by Nauen (2013) was discussed at Pesticides Peer Review Meeting 145. Generally the argumentation provided was agreed since clothianidin is intended to control sap sucking insects., and thus at least during the first weeks of crop growth the exposure of honeybees is likely to be low. It was noted that the ED_{50} in the study by Foster *et al.* (2008) was not consistent among the tested clones (varying about 2 orders of magnitude). It was agreed that neonicotinoids resistance to aphids could not be excluded (there are several reported cases of neonicotinoids resistant strains of aphids in literature, including *M. persicae*, which is an highly polyphagous species. See also Bass *et al.*, 2015[35]). Moreover it was noted that at later crop growth stages (i.e., after the 8th week) the efficacy of the aphids control will be lower, therefore a certain exposure of honeybees through honeydew might occur.

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.9.4.1 of this Addendum) and for granular use in potato and maize (Sumitomo, see Section B.9.4.1 of the Addendum for the Sumitomo data) were considered together. This is considered justified as the occurrence of honeydew is rather crop related and than related to the application method. The study by Negrini (2014) (See Section B.9.4.1 of the Addendum for the Sumitomo data) investigated the occurrence of honeydew in 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 non relevant route of exposure for treated crops. The experts agreed with this conclusion for all the granular uses of clothianidin under evaluation.

Overall, the experts agreed on the basis of the available data that honeydew can be considered as a low relevance route of exposure for the treated crop for clothianidin. This conclusion is valid for all uses under evaluation.

B.9.4.3. Risk assessment

Based on the argumentation provided by the applicant (see Section B.9.4.2), the exposure of honeybees and non-*Apis* bees to clothianidin through honey dew present in the treated field can be considered negligibly low, provided that aphids are sufficiently controlled. Therefore, a risk assessment for this route of exposure is not considered necessary.

[35] Bass, C. *et al.* (2015). The global status of insect resistance to neonicotinoid insecticides. *Pesticide biochemistry and physiology* 121:78-87.

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 five studies on the acute and long-term risk to colony survival due to exposure to guttation. The studies were performed either on winter cereals (three studies) or on sugar beet (two studies), to cover the currently permitted use of clothianidin as seed treatment in these crops.

Studies performed on winter cereals

Report:	**1.6/1; Hofmann, S. & Lueckmann, J.; 2014**
Title:	**Field study to monitor potential effects on honeybees from exposure to guttation fluid of winter wheat (W-WHT), seed-treated either with an imidacloprid or a clothianidin combi-product**
Report No.:	R09247-4
Document No.:	M-498939-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	no

Objective

Key study objectives were to evaluate and compare the colony development and the overwintering performance of honeybee colonies exposed to guttation droplets of either clothianidin or imidacloprid treated winter wheat (through seed treatment). Furthermore, the guttation behaviour of winter wheat was surveyed and it was examined whether exudation of guttation fluid of winter wheat and flight activity of honeybees occurred simultaneously. In case flight activity and guttation coincided, the bee activity in the respective study field was surveyed.

Material and Methods

Test and control item

Winter wheat (W-WHT) seeds were seed-treated at the Seed Treatment Application Centre of Bayer CropScience AG in Monheim with either:

4. Imidacloprid (Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 60 + 70 + 7.2 + 8)
5. Clothianidin (Clothianidin & Beta-Cyfluthrin FS 375 + 80).

Fungicidal seed treatments are routinely used in agriculture to prevent crop plants becoming infected with pathogenic fungi. Because the clothianidin-containing test item did not contain any fungicides, the seeds were co-treated with a standard commercial fungicidal seed treatment product (i.e. EfA, containing 37.5 g/L fluoxastrobin, 25 g/L prothioconazole, 3.75 g/L tebuconazole and 10.0 g/L triazoxide, at a nominal seed dressing rate of 200 mL/dt). In addition, seeds were additionally treated with commercial INTECO at a nominal rate of 50 mL/100 kg wheat seeds in order to minimize dust abrasion.

The control consisted of seeds that were seed-treated only with the routine fungicide EfA and INTECO, at identical rates as for the test item.

Study sites

The effects of seed treated with either imidacloprid or clothianidin were tested on the honeybee (*Apis mellifera*) under field conditions. The study was conducted at two test locations in Germany: a) Northern Germany at Celle, Lower Saxony and b) Southern Germany near Renningen, Baden-Württemburg.

The study fields and the position of the respective study plots were selected according to the following criteria:

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

Characterisation of the study location Ihinger Hof
All study fields shared similar environmental parameters (e.g. surrounding vegetation, slope, etc.). Rather fine-grained soil prevailed, i.e. clayey loam, silty clay and clays silt. The distance between individual study plots of the treatment groups was 760 m. The minimum distance between a bee hive on the study plot at the control study field to a bee hive on the study plot at a treated study field was 590 m. The area in which the study was conducted was richly structured, with small fields, woodlands bordered by shrubs and some managed grassland with fruit trees or meadows. Settled areas were found in the vicinity. Due to the proximity of all study fields, the weather conditions throughout the study period were essentially the same at each of the study fields.

In autumn 2009, the natural nectar supply of the honey bee colonies (flowering plants) was low but acceptable within a radius of 3 km around the monitoring hives in both, treatment and control. The remaining little nectar source was a small number of fields planted with mustard (*Sinapis arvensis*). These little agricultural nectar sources were supplemented by a relatively small number of flowering wild plants scattered throughout settlements, along field margins and in close proximity to some of the wooded areas.

The monitoring in spring 2010 commenced with start of the flowering of the Goat Willow *(Salix caprea)* in the region where the W-WHT fields under investigation were located. At the beginning of April, Common Osier *(Salix viminalis)* and Crack Willow *(Salix fragilis)* had started flowering. Along the field margins and on meadows, bees were foraging on a variety of flowering wild plants. These included different species of *Veronica* sp., Deadnettles *(Lamium* sp.*)* and European wood anemone *(Anemone nemorosa)* in the nearby forests. Near settlements various flowers and shrubs were also in blossom. By mid-April these were followed by various fruit trees (*Prunus* sp., *Malus* sp.). Additionally trees, shrubs and herbs like Norway Maple *(Acer platanoides)*, Common Ash *(Fraxinus excelsior)*, European Cornel *(Cornus mas)* and Blackthorn *(Pruns spinosa)*, Dandelion *(Taraxacum officinale)* and *Ranunculus* sp. were flowering along field margins and on meadows until the end of the study in April 2010, which was reached with the beginning of winter oil-seed flowering in the region.

Characterisation of the study location Celle
All study fields shared similar environmental parameters (e.g. surrounding vegetation, slope, etc.). Clayey and loamy sand prevailed as soil types. The distance between individual study plots of the treatment groups was 770 m. The minimum distance between a bee hive on the study plot at the control study field to a bee hive on the study plot at a treated study field was 7,000 m. The test location Celle was not as richly structured as Ihinger Hof. The study fields were mostly surrounded by agricultural fields, grassland or meadows as well as woodlands, bordered by shrubs. Only little settled areas were found in the vicinity. Due to the proximity of all study fields, the weather conditions throughout the study period were essentially the same at each of the study fields.

In autumn 2009 the natural nectar supply of the honey bee colonies (flowering plants) was very low within a radius of 3 km and consisted of a relatively small number of flowering plants, scattered throughout settlements, along field margins and in close proximity to some of the wooded areas and some flowering fallow land at study plot 16.

The monitoring in spring 2010 commenced with start of the flowering of the Goat Willow *(Salix caprea)* in the region where the W-WHT fields under investigation were located. At the beginning of April, also Common Osier *(Salix viminalis)* and Crack Willow *(Salix fragilis)* had started flowering. Along the field margins and on meadows, bees were foraging on some flowering plants. Near

settlements, various flowers and shrubs were also in blossom. By mid-April these were followed by various fruit trees (*Prunus* sp., *Malus* sp.). Additionally trees, shrubs and herbs like Common Ash (*Fraxinus excelsior*), European Cornel (*Cornus mas*), Blackthorn (*Prunus spinosa*), Dandelion (*Taraxacum officinale*), Deadnettles (*Lamium* sp.) and *Ranunculus* sp. were flowering along field margins and on meadows until the end of the study end of April 2010, which was reached with the beginning of winter oil-seed flowering in the region.

Sowing and set-up of honeybee hives

Honeybee colonies were set up directly adjacent to fields which were then sown with winter wheat (W-WHT) seeds, in order to investigate the potential effects from exposure to guttating W-WHT, starting from seedling emergence in autumn 2009 (October 2009) until beginning of winter oil-seed flowering in the respective region in spring 2010 (April 2010). The study fields and the position of the study plots were selected according to the following criteria:

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

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

Assessment area

A specified assessment area in front of the honeybee colonies was intensively monitored. The assessment area was divided into two in-Crop Zones (Zone 0 and Zone 1) and an off-Crop Zone (see Figure B.9.5.1-1). Zone 0 covered the immediate area in front of the bee hives and Zone 1 outside of this. The bee hives were placed into the off-Crop Zone, directly adjacent to the W-WHT crop. In addition, two 1 m² assessment plots were established to record the proportion of W-WHT displaying guttation and/or dew.

Honeybee mortality

Each hive was equipped with a dead bee trap, and honeybee mortality was assessed daily from 09 October 2009 by counting the number of bees present in the trap and also those found on the soil surface in front of each colony.

Monitoring activities

The monitoring activities started as soon as the W-WHT plants had emerged on the fields under investigation and lasted for a maximum period of four consecutive weeks until the end of October 2009. The monitoring activities in the field re-started in spring 2010 with the beginning of the inflorescence of the Goat Willow (*Salix caprea*) and lasted for a period of four consecutive weeks until beginning of the flowering of winter oil-seed in the respective region.

During the morning the respective assessment area of the study fields was systematically checked for occurrence of guttation fluid and/or dew. If guttation was still present at the start of honeybee activity, the numbers of honeybees resting or walking on the ground or on the W-WHT crop were counted and any potential uptake of guttation fluid or dew by the bees or any conspicuous bee behaviour was recorded. Field assessments were stopped after no more guttation fluid was present or after a maximum of four subsequent monitorings, whatever occurred earlier.

Beyond field assessments in the morning, the study field which was monitored in the morning was also visited in the evening. During these evening assessments, the onset of guttation and the end of bee activity was recorded.

One "monitoring session" lasted for approximately 35 minutes and was defined as one complete observation cycle of the assessment area and its associated two segregated plots of 1 m², at which guttation- and honeybee assessments were conducted during the presence of guttation fluid on the W-WHT crop.

The following parameters were monitored during the Field Phase:

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

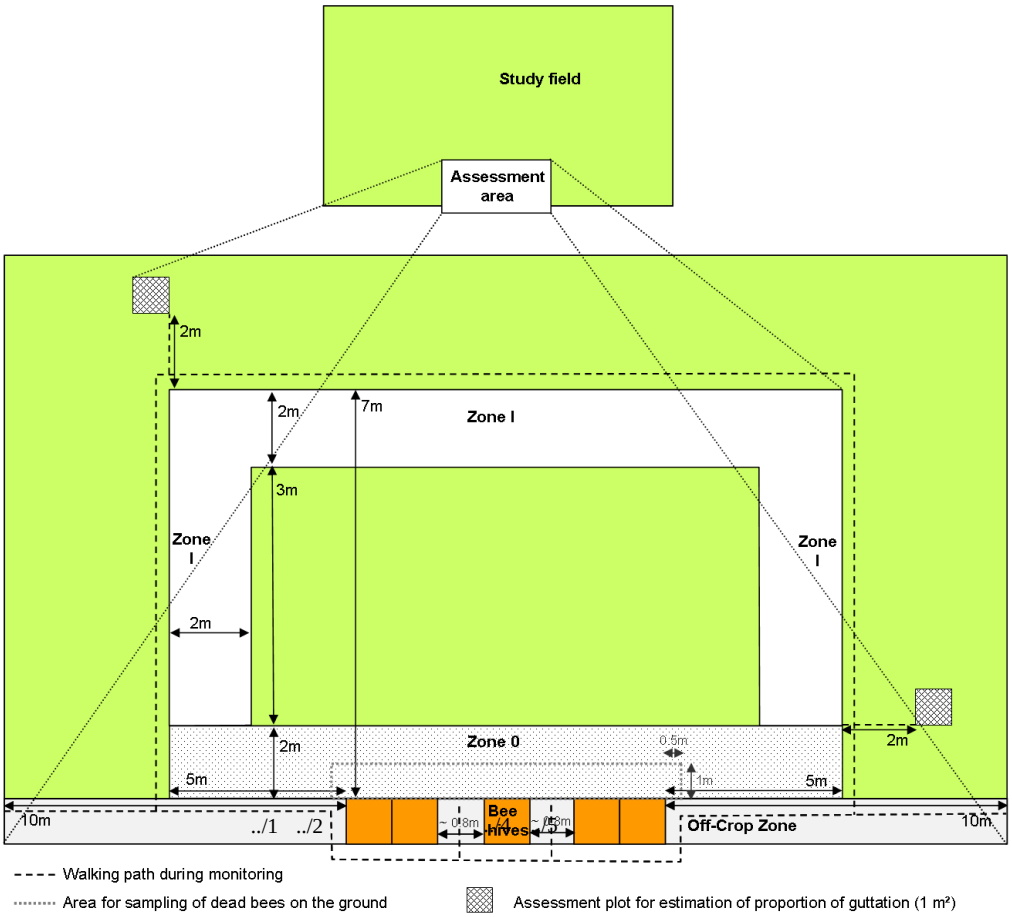


Figure B.9.5.1-1: Diagram showing set up of honeybee colonies and assessment areas

Honeybee colony strength assessment

At both test locations, the colony strength and the colony development were estimated according to the Liebefeld method. The first assessment was performed shortly before (Celle) or after (Ihinger Hof) colony set-up. Further assessments were performed every 21 days until the end of October 2009. In spring 2010 colony development was assessed in the same manner from the beginning of inflorescence of the Goat Willow (*Salix caprea*) until beginning of winter oil-seed flowering in the respective region.

Results

Frequency of guttation

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

Duration of guttation

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

Honeybee activity in the assessment area

Honeybees were observed visiting the study plots frequently. This is not unexpected as they were placed directly in front of the plots. Most of the direct honeybee observations within the assessment area were made in the in-Crop Zone 0, i.e. directly in front of the hives, followed by the Off-Crop Zone and the in-Crop Zone 1.

The relative proportion of honeybees observed per monitoring on plants in the respective assessment areas in both treatments and control, was mostly higher in spring 2010 than in autumn 2009. With the exception of honeybees on soil surface: in autumn 2009 the observed relative proportion was three to four times higher in Zone 0 than in spring in the respective zone, which can be explained by the cold weather. The observed relative proportion of honeybees per monitoring taking up guttation fluid and dew in both treatment and control, was unequivocally higher in all assessment zones in spring 2010 as compared to autumn 2009. Most of the honeybees taking up guttation fluid were observed in Zone 0, i.e. directly in front of/adjacent to the hives. Honeybee activity and the proportion of bees observed collecting water during the study is summarized in Table B.9.5.1-1 below.

Table B.9.5.1-1: Summary of observations on honeybee activity and water collection

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

Residue analysis of guttation fluid

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

The Limit of Quantitation (LOQ) of each analyte in guttation fluid was 0.01 mg/L and the Limit of Detection (LOD) of each analyte was 0.001 mg/L, respectively. The range of residue levels detected is presented in Table B.9.5.1-2 below.

Table B.9.5.1-2: Measured residues in guttation fluid

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

Honeybee mortality

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

Colony development and overwintering

During the autumn 2009 observation period, most colonies developed normally. Most colonies reduced their brood activity. However, in many colonies the number of bees was reduced, indicating that the adult bees were already winter bees which have a longer life expectancy. Three colonies had to be removed after the last assessment before overwintering, as they had less than 5,000 bees and were therefore not considered capable for overwintering.

The winter 2009/2010 was very long and cold; bees could not fly out until the beginning of April. During wintertime, four colonies died. During the spring 2010 observation period, the colony development in both treatment and control, was considered to be within the normal range in most of the exposed colonies. Two colonies had to be removed during spring, one did not recover from bad overwintering and one lost its queen. The winter losses were (after removal of weak colonies in the winter) 1 in 9, 2 in 10 and 1 in 7 colonies for the clothianidin, imidacloprid and control treatments respectively. Consequently the successful overwintering rates were 89% for clothianidin, 80% for imidacloprid and 86% for the control.

In the group at fields treated with imidacloprid, three colonies showed a good overwintering performance (overwintering index above 0.8) and four an average overwintering performance (0.5-0.8). In the following spring, most colonies in the imidacloprid treatment group developed normally and also the colonies with a bad overwintering index recovered. However, colony 17/5 lost its queen and was removed from the experiment on 09 April 2010. In the group at fields treated with clothianidin, two colonies showed a good overwintering performance (overwintering index above 0.8), five were average (0.5-0.8), while one colony overwintered only badly (less than 0.5). In the following spring, most colonies in the clothianidin treatment group developed normally and also the colonies with a bad overwintering index recovered. In the control groups, two colonies showed a good overwintering performance (overwintering index above 0.8), four an average overwintering performance (0.5-0.8) and two colonies showed a bad overwintering performance (less than 0.5). In

the following spring, most colonies in the control group developed normally. Only colony 16/2 did not recover from the bad overwintering performance of this colony.

Table B.9.5.1-3: Inidivual development and overwintering performance of study colonies in the treatment and control groups.

Treatment group	Field site	Colony	Hive development in autumn	Hive development in spring	Overwintering index
Imidacloprid treated	Ihinger hof	11/1	colony was removed after last assessment (less than 5,000 bees)	-- (colony discarded in autumn)	--
		11/2	colony was removed after last assessment (less than 5,000 bees)	-- (colony discarded in autumn)	--
		11/3	lot of brood until late October	normal	0.89
		11/4	normal	normal	0.97
		11/5	normal	Winter loss	--
	Celle	17/1	normal	normal	0.55
		17/2	normal	normal	0.57
		17/3	normal	normal	0.90
		17/4	normal	normal	0.66
		17/5	normal	No brood detected during first assessment on March 2010 (queen found dead in dead bee trap on 09 April 2010)	0.76
Clothianidin treated	Ihinger hof	12/1	normal	normal	0.53
		12/2	Slight increase	normal	0.40
		12/3	Normal	normal	0.51
		12/4	Colony was removed after last assessment (less than 5000 bees)	-- (colony discarded in autumn)	--
		12/5	weak	Winter loss	--
	Celle	18/1	normal	normal	0.67
		18/2	normal	normal	0.55
		18/3	normal	normal	0.85
		18/4	normal	normal	0.84
		18/5	normal	normal	0.75
Control	Ihinger hof	10/1	normal	normal	0.53
		10/2	normal	normal	0.82
		10/3	normal	normal	0.56
		10/4	normal	normal	0.81
		10/5	normal	Winter loss	--
	Celle	16/1	normal	normal	0.65
		16/2	normal	Bad overwintering, was removed after first assessment in spring	0.07
		16/3	normal	normal	0.70
		16/4	normal	normal	0.48
		16/5	weak	normal	--

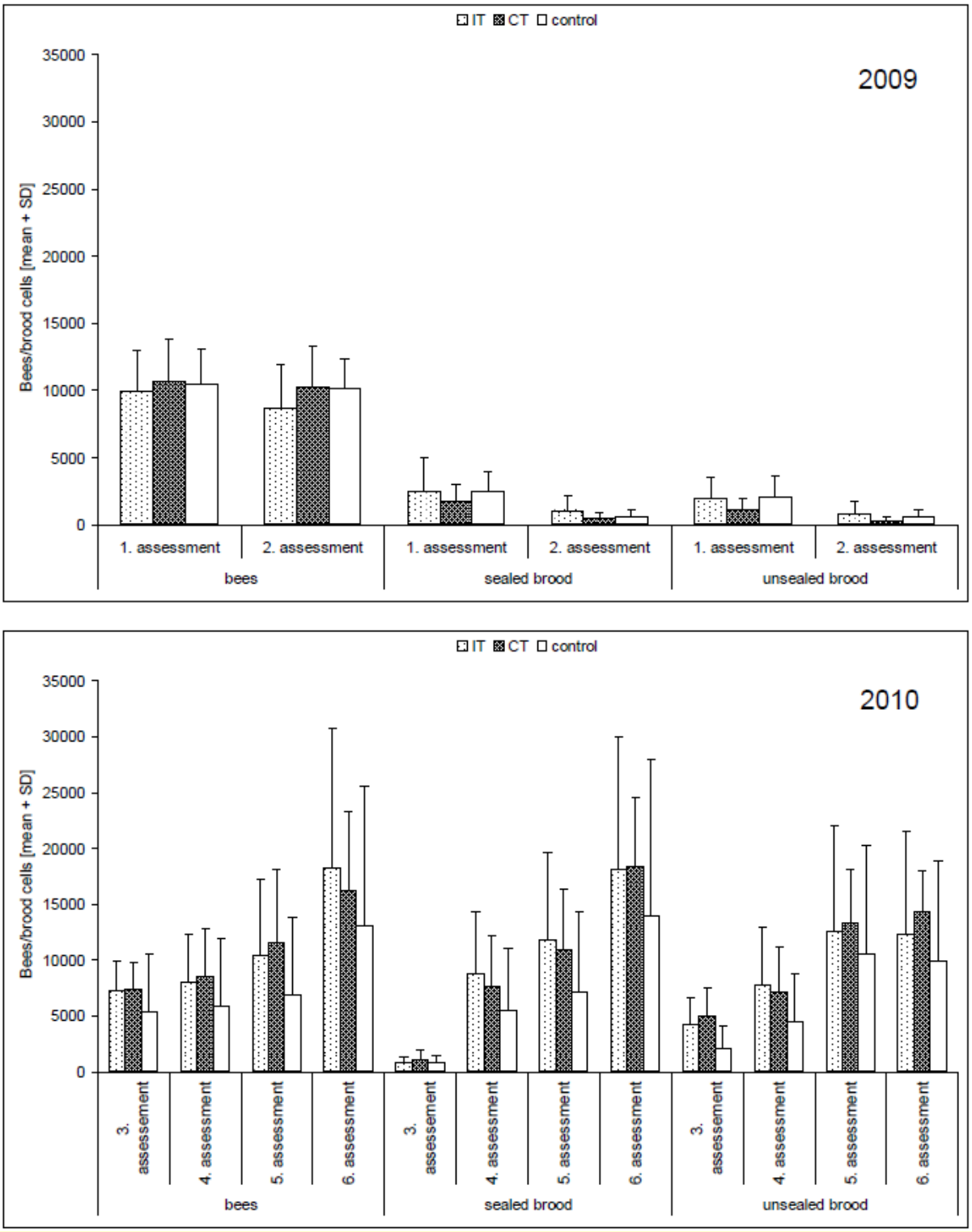


Figure B.9.5.1-2: Colony development of the study colonies in autumn 2009 and spring 2010. IT – imidacloprid-treated group; CT – clothianidin-treated group; C – control group.

Conclusions

No treatment related differences in honeybee mortality, colony development in autumn and spring as well as in the overwintering performance was observed between the control and the treatment groups (imidacloprid and clothianidin treatment group, respectively). Weak development in autumn, leading to discarding the colonies or winter losses can be explained by *varroa* loads and other diseases found in the colonies, together with the very long and cold winter 2009/10.

Overall, it is concluded that guttation fluid, exuded by winter wheat seedlings, seed-treated with nitro-substituted neonicotinoids (imidacloprid or clothianidin), does not have unacceptable effects on honeybee colonies under typical commercial use conditions.

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. field size of all study fields exceeded 2 ha, use of colonies with a good health status, of uniform size and similar genetic origin. However, in contrast to the recommendations of the EFA Guidance Document, the colonies overwintered at their respective field site instead of at the same overwintering location. Further, only 10 pairs of colonies were set-up (5 at each of the two treatment and control fields), which might potentially be too low to achieve sufficient statistical power.

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

During Peer Review, it was argued that it is not ideal that hives were overwintered on the test site, in the absence of specific information regarding the availability of nearby food sources (see comment 5(33) in the Reporting Table). Additionally, it was considered that variability in colony size and relatively high losses makes interpretation of the results from these studies problematic. Further detail regarding potential effects on colony development would aid interpretation. Additional information regarding the surrounding vegetation and on the colony assessments, present in the full study report, were therefore included in the study summary.

Report:	**1.6/2; Hofmann, S., Garrido, C. & Lueckmann, J.; 2012**
Title:	**Field study to monitor potential effects on honeybees from exposure to guttation fluid of winter barley (W-BAR), seed-treated either with an imidacloprid or a clothianidin combi-product**
Report No.:	R09247-3
Document No.:	M-498922-01-1
Guideline(s):	not specified
Guideline deviation(s):	not specified
GLP/GEP:	no

Objective
Key study objectives were to evaluate and compare the colony development and the overwintering performance of honeybee colonies exposed to guttation droplets of either clothianidin or imidacloprid treated winter barley (through seed treatment). Furthermore, the guttation behaviour of winter barley was surveyed and it was examined whether exudation of guttation fluid of winter barley and flight activity of honeybees occurred simultaneously. In case flight activity and guttation coincided, the bee activity in the respective study field was surveyed.

Material and methods
Test and control item
Winter barley (W-BAR) seeds were seed-treated at the Seed Treatment Application Centre of Bayer CropScience AG in Monheim with either:

15. Imidacloprid (Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 60 + 70 + 7.2 + 8)
16. Clothianidin (Clothianidin & Beta-Cyfluthrin FS 375 + 80).

Fungicidal seed treatments are routinely used in agriculture to prevent crop plants becoming infected with pathogenic fungi. Because the clothianidin-containing test item did not contain any fungicides, the seeds were co-treated with a standard commercial fungicidal seed treatment product (i.e. EfA, containing 37.5 g/L fluoxastrobin, 25 g/L prothioconazole, 3.75 g/L tebuconazole and 10.0 g/L triazoxide, at a nominal seed dressing rate of 200 mL/dt). In addition, seeds were additionally treated

with commercial INTECO at a nominal rate of 50 mL/100 kg wheat seeds in order to minimize dust abrasion.

The control consisted of seeds that were seed-treated only with the routine fungicide EfA and INTECO, at identical rates as for the test item

Study sites
The effects of seed treated with either imidacloprid or clothianidin were tested on the honeybee (*Apis mellifera*) under field conditions. The study was conducted at two test locations in Germany: a) Northern Germany at Celle, Lower Saxony, and b) Southern Germany near Renningen, Baden-Württemburg.

The study fields and the position of the respective study plots were selected according to the following criteria:

17. the provision of appropriate conditions for the set-up of honey bee colonies close to the study fields as well as a suitable place for the hibernation of these colonies
18. at least 300 m distance to permanent open water bodies (e.g. ditches, streams or ponds) for treatment fields

All in all, the finally selected locations for the honey bee hives were always a result of the consideration of the criteria mentioned above. To find a suitable place for the colonies to hibernate and to account for routine apicultural practice, the hives were placed next to structures which might give shelter from bad weather, i.e. next to hedges, edges of forests or slight elevations, if existing.

However, some study plots where less suitable in this aspect. At the study location Ihinger Hof, the honey bee colonies at study plot 9 were significantly more exposed to the wind than the honey bee colonies at the two other study plots (i.e. 7 and 8) due to the absence of any shelter; moreover, the hive entrance of the colonies set-up on study plot 9 were directed North (i.e. no sun), whereas the hive entrances of the colonies set-up on study plot 7 and 8 were directed to the South and East, respectively. In addition, study plot 9 suffered from a significantly higher soil dampness, which further contributed to an increased cold and damp microclimate. This situation was also reflected by the retarded emergence of the barley crop during October 2009.

Also on the study location Celle, environmental factors differed on the individual study locations. Particularly study plot 15 was affected as the honey bee colonies were placed in a slight depression. Moreover, the soil around the bee colonies was compacted, which rendered the place to be damp, which became most apparent during springtime 2010, where the area was swamped and the hives had to be supported in order to prevent the colonies from flooding. During the wintertime, also cold air could be expected to have accumulated in this depression framed by the edges of a forest.

Characterisation of the test location Ihinger Hof
All study fields shared similar environmental parameters (e.g. surrounding vegetation, slope, etc.). Silty clay or clayey silt prevailed as soil types (see Table 33). The distance between individual study plots of the treatment groups was 770 m. The minimum distance between a bee hive on the study plot at the control study field to a bee hive on the study plot at a treated study field was 900 m. The area in which the study was conducted was richly structured, with small fields, woodlands bordered by shrubs and some managed grassland with fruit trees or meadows. Settled areas were found in the vicinity. Due to the proximity of all study fields, the weather conditions throughout the study period were essentially the same at each of the study fields.

In autumn 2009, the natural nectar supply of the honey bee colonies (flowering plants) was low but acceptable within a radius of 3 km around the monitoring hives in both, treatment and control. The remaining little nectar source was a small number of fields planted with sunflower (*Helianthus annuus*) and mustard (*Sinapis arvensis*). These little agricultural nectar sources were supplemented by

a relatively small number of flowering wild plants scattered throughout settlements, along field margins and in close proximity to some of the wooded areas.

The monitoring in spring 2010 commenced with start of the flowering of the Goat Willow (*Salix caprea*) in the region where the W-BAR fields under investigation were located. At the beginning of April, Common Osier (*Salix viminalis*) and Crack Willow (*Salix fragilis*) had started flowering. Along the field margins and on meadows, bees were foraging on a variety of flowering wild plants. These included different species of *Veronica* sp., Deadnettles (*Lamium* sp.) and European wood anemone (*Anemone nemorosa*) in the nearby forests. Near settlements various flowers and shrubs were also in blossom. By mid-April these were followed by various fruit trees (*Prunus* sp., *Malus* sp.). Additionally trees, shrubs and herbs like Norway Maple (*Acer platanoides*), Common Ash (*Fraxinus excelsior*), European Cornel (*Cornus mas*) and Blackthorn (*Pruns spinosa*), Dandelion (*Taraxacum officinale*) and *Ranunculus* sp. were flowering along field margins and on meadows until the end of the study in April 2010, which was reached with the beginning of winter oil-seed flowering in the region.

Characterisation of the stud location Celle
All study fields shared similar environmental parameters (e.g. surrounding vegetation, slope, etc.). Study plot 14 was located in a more open landscape. Clayey and loamy sand prevailed as soil types. The distance between individual study plots of the treatment groups was 3,400 m. The minimum distance between a bee hive on the study plot at the control study field to a bee hive on the study plot at a treated study field was 4,500 m. The test location Celle was not as richly structured as Ihinger Hof. The study fields were mostly surrounded by agricultural fields, grassland or meadows as well as woodlands, bordered by shrubs. Settled areas were found in the vicinity. Due to the proximity of all study fields, the weather conditions throughout the study period were essentially the same at each of the study fields.

In autumn 2009 the natural nectar supply of the honey bee colonies (flowering plants) was very low within a radius of 3 km and consisted of a relatively small number of flowering plants, scattered throughout settlements, along field margins and in close proximity to some of the wooded areas and some flowering fallow land at study plots 13 and 15.

The monitoring in spring 2010 commenced with start of the flowering of the Goat Willow (*Salix caprea*) in the region where the W-BAR fields under investigation were located. At the beginning of April, also Common Osier (*Salix viminalis*) and Crack Willow (*Salix fragilis*) had started flowering. Along the field margins and on meadows, bees were foraging on some flowering plants. Near settlements, various flowers and shrubs were also in blossom. By mid-April these were followed by various fruit trees (*Prunus* sp., *Malus* sp.). Additionally trees, shrubs and herbs like Common Ash (*Fraxinus excelsior*), European Cornel (*Cornus mas*), Blackthorn (*Prunus spinosa*), Dandelion (*Taraxacum officinale*), Deadnettles (*Lamium* sp.) and *Ranunculus* sp. were flowering along field margins and on meadows until the end of the study in April 2010, which was reached with the beginning of winter oil-seed flowering in the region.

<u>Sowing and set-up of honeybee hives</u>
Honeybee colonies were set up directly adjacent to fields which were then sown with winter barley (W-BAR) seeds, in order to investigate the potential effects from exposure to guttating W-BAR, starting from seedling emergence in autumn 2009 (October 2009) until beginning of winter oil-seed flowering in the respective region in spring 2010 (April 2010). The study fields and the position of the study plots were selected according to the following criteria:

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

Three test groups were set up at each location consisting of a field sown with seed treated with imidacloprid, clothianidin or a control (no insecticide). At each of the six study fields under

investigation, five honeybee colonies were placed along a line one to eight days before sowing, either directly adjacent or within a maximum distance of 0.5 m to the W-BAR crop, depending on the actual local field situation. As colonies were in-situ at the time of drilling they were also exposed to dust emitted from seed drilling equipment at the time of sowing.

Assessment area

A specified assessment area in front of the honeybee colonies was intensively monitored. The assessment area was divided into two in-Crop Zones (Zone 0 and Zone 1) and an off-Crop Zone (see Figure B.9.5.1-2). Zone 0 covered the immediate area in front of the bee hives and Zone 1 outside of this. The bee hives were placed into the off-Crop Zone, directly adjacent to the W-BAR crop. In addition, two 1 m² assessment plots were established to record the proportion of W-BAR displaying guttation and/or dew.

Honeybee mortality

Each hive was equipped with a dead bee trap, and honeybee mortality was assessed daily from 15 September 2009 by counting the number of bees present in the trap. From 09 October 2009 also those found on the soil surface in front of each colony were recorded.

Monitoring activities

The monitoring activities started as soon as the W-BAR plants had emerged on the fields under investigation and lasted for a maximum period of four consecutive weeks until the end of October 2009. The monitoring activities in the field re-started in spring 2010 with the beginning of the inflorescence of the Goat Willow (*Salix caprea*) and lasted for a period of four consecutive weeks until beginning of the flowering of winter oil-seed in the respective region.

During the morning the respective assessment area of the study fields was systematically checked for occurrence of guttation fluid and/or dew. If guttation was still present at the start of honeybee activity, the numbers of honeybees resting or walking on the ground or on the W-BAR crop were counted and any potential uptake of guttation fluid or dew by the bees or any conspicuous bee behaviour was recorded. Field assessments were stopped after no more guttation fluid was present or after a maximum of four subsequent monitorings, whatever occurred earlier.

Beyond field assessments in the morning, the study field which was monitored in the morning was also visited in the evening. During these evening assessments, the onset of guttation and the end of bee activity was recorded.

Each “monitoring session” lasted for approximately 35 minutes and was defined as one complete observation cycle of the assessment area and its associated two segregated plots of 1 m², at which guttation- and honeybee assessments were conducted during the presence of guttation fluid on the W-BAR crop.

The following parameters were monitored during the Field Phase:

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

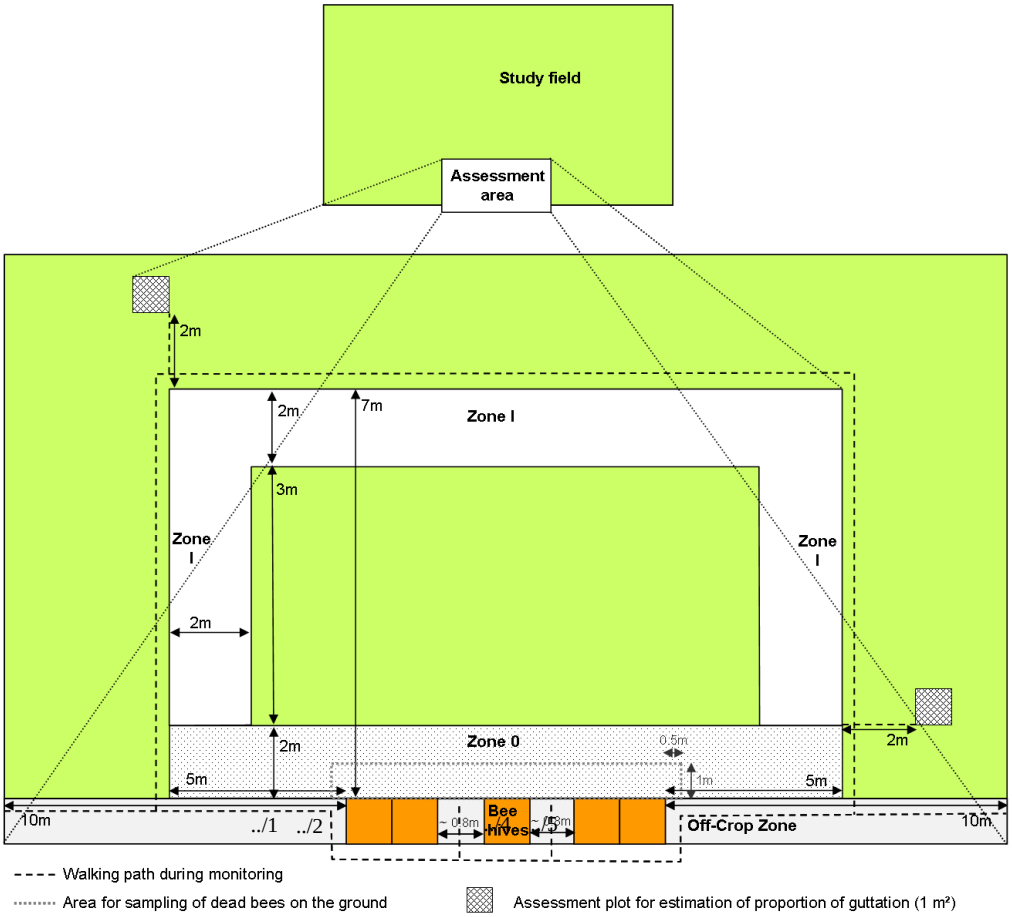


Figure B.9.5.1-3: Diagram showing set up of honeybee colonies and assessment areas

Honeybee colony strength assessment

At both test locations, the colony strength and the colony development were estimated according to the Liebefeld method. The first assessment was performed immediately after colony set-up. Further assessments were performed every 21 days until the end of October 2009. In spring 2010 colony development was assessed in the same manner from the beginning of inflorescence of the Goat Willow (*Salix caprea*) until beginning of winter oil-seed flowering in the respective region.

Results

Frequency of guttation

During the assessments in the morning, guttation fluid was observed on W-BAR at 84.2 % of all observation days in autumn 2009 and at 80.7 % of the observation days in spring 2010. No remarkable coincidence of guttation of W-BAR and bee activity in the evening in autumn 2009 was observed. A coincidence during this period of time occurred, with a few exceptions only, just on those days where guttation anyhow prevailed for the whole day due to damp or rainy weather. In spring 2010, no coincidence between presence of guttation in the evening and bee activity was observed at all.

Duration of guttation

Whenever guttation was observed on a respective day, it was already present in the early morning. Depending on the actual weather conditions, the time when guttation ended was variable. On dry, windy days, guttation stopped shortly after sunrise, whereas on cold, damp days with drizzle, it occasionally lasted until afternoon and on some occasions even until evening. On most observations days, guttation lasted for several hours.

Honeybee activity in the assessment area

Honeybees were observed visiting the study plots frequently. This is not unexpected as they were placed directly in front of the plots. Most of the direct honeybee observations within the assessment area were made in the in-Crop Zone 0, i.e. directly in front of the hives, followed by the Off-Crop Zone and the in-Crop Zone 1.

The relative proportion of honeybees observed per monitoring on plants in the respective assessment areas in both treatments and control, was mostly higher in spring 2010 than in autumn 2009. Moreover, also the observed relative proportion of honeybees per monitoring taking up guttation fluid and dew in both treatment and control, was mostly higher in all assessment zones in spring 2010 as compared to autumn 2009. Honeybee activity and the proportion of bees observed collecting water during the study is summarized in Table B.9.5.1-4 below:

Table B.9.5.1-4: Summary of observations on honeybee activity and water collection

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

Residue analysis of guttation fluid

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

The Limit of Quantitation (LOQ) of each analyte in guttation fluid was 0.01 mg/L and the Limit of Detection (LOD) of each analyte was 0.001 mg/L, respectively. The range of residue levels detected is presented in Table B.9.5.1-5 below.

Table B.9.5.1-5: Measured residues in guttation fluid

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

Honeybee mortality

During the approximately 5 week's continuous autumn exposure period, none of the treatment colonies revealed adverse effects in terms of mortality rates and/or suspicious behavioural impairments, although honeybees were frequently recorded to forage within the neonicotinoid-treated barley fields. The number of honeybees exhibiting behavioural impairments, however, did not differ

between treatment groups with 30, 48 and 13 impaired bees for the control, the imidacloprid and the clothianidin treatment, respectively. In all treatment groups, honeybee mortality in autumn was mostly low until a period of cold weather in October. The increased mortality in all experimental groups (treatments and control) during this period was clearly correlated with the weather conditions and was not influenced by the experimental setup. During springtime, the mortality found in the traps was generally low, but still variable from colony to colony.

Based on these observations, it can be concluded that guttation fluid of neonicotinoid-treated barley seedlings, although carrying an intrinsically high hazard potential, does not impair honeybee colonies, which were exposed at the field margin in direct vicinity to those fields, in an unacceptable manner.

Colony development and overwintering

During the autumn 2009 observation period, most colonies developed normally. All colonies reduced their brood activity and, consequently, also the number of bees. However, in many colonies the latter was not pronounced, indicating that the adult bees were already winter bees which have a longer life expectancy. Three colonies had to be removed after the last assessment before overwintering. Two of them had less than 5,000 bees and where therefore not considered capable for overwintering, one had a very high *Varroa* load which disrupted hive vitality.

The winter 2009/2010 was very long and cold; bees could not fly out until the beginning of April. Over wintertime, six colonies died. During the spring 2010 observation period, the colony development in both treatment and control, was considered to be within the normal range in most of the exposed colonies. One colony had to be removed during spring as it did not recover from bad overwintering. The winter losses were (after removal of weak colonies in the winter) 3 in 8, 1 in 9 and 2 in 10 colonies for the clothianidin, imidacloprid and control treatments, respectively.

In the group at fields treated with imidacloprid, one colony showed a good overwintering performance (overwintering index above 0.8), five an average overwintering performance (0.5-0.8) and two colonies showed a bad performance (less than 0.5). One colony (colony 14/3) was lost during the winter season. In the following spring, most colonies in the treatment group developed normally and also the colonies with a bad overwintering index recovered. In the group at fields treated with clothianidin, also one colony showed a good overwintering performance (overwintering index above 0.8), two were average (0.5-0.8), while two colonies overwintered only badly (less than 0.5). Three colonies (colonies 9/1, 15/3 and 15/5) were lost during the winter season. Colony 9/2 did not recover from the bad overwintering and lost vitality during the observation period in spring. The other surviving colonies from this group developed normally. In the control group, no colony showed a good overwintering performance (overwintering index above 0.8), four an average overwintering performance (0.5-0.8) and four colonies showed a bad overwintering performance (less than 0.5). Two colonies (colonies 7/4 and 13/1) were lost during the winter season. In the following spring, most colonies in the control group developed normally.

Regarding those colonies which were discontinued due to a too low colony strength after the autumn exposure period (0 colonies in control, 1 in the imidacloprid treatment group and 2 in the clothianidin treatment group), a clear correlation can be seen between colony strength in combination with available brood mass: the weaker both figures, the less the probability to reach the minimum colony strength to overwinter and/or to survive overwintering. Due to this and further experimental bias in the clothianidin treatment group no reliable conclusions can be drawn for this group concerning overwintering performance.

However, for the other treatment groups overwintering success (total success) rate of 80 (80)% in the control group and 89 (80)% in the imidacloprid treatment group, indicating that guttating W-BAR seedlings, carrying high levels of intrinsically bee-toxic neonicotinoid residues, have no impact on the rate of successful overwintering of adjacently located and exposed honeybee colonies.

Table B.9.5.1-6: Inidivual development and overwintering performance of study colonies in the treatment and control groups.

Treatment group	Field site	Colony	Hive development in autumn	Hive development in spring	Overwintering index
Imidacloprid treated	Ihinger hof	8/1	colony was removed after last assessment (less than 5,000 bees)	-- (colony discarded in autumn)	--
		8/2	normal	normal	0.60
		8/3	normal	Weak development, brood activity started late, drone brood until May	0.27
		8/4	normal	normal	0.70
		8/5	normal	normal	0.74
	Celle	14/1	normal	Strong brood activity	0.88
		14/2	normal	normal	0.55
		14/3	normal	Winter loss	--
		14/4	weak	Normal, low colony strength until mid of May	0.32
		14/5	normal	normal	0.67
Clothianidin treated	Ihinger hof	9/1	weak	Winter loss	--
		9/2	Slight increase, high *Varroa* load	Bad overwintering, continuous decrease of colony strength up to final loss of vitality	0.22
		9/3	weak	normal	0.79
		9/4	Very high *Varroa* load which disrupted hive vitality, colony was removed after last assessment (lass than 5000 bees)	-- (colony discarded in autumn)	--
		9/5	normal	normal	0.62
	Celle	15/1	Colony was removed after last assessment (less than 5000 bees)	-- (colony discarded in autumn)	--
		15/2	normal	normal	0.18
		15/3	normal	Winter loss	--
		15/4	normal	normal	0.89
		15/5	normal	Winter loss	--
Control	Ihinger hof	7/1	normal	normal	0.59
		7/2	normal	normal	0.46
		7/3	normal	normal	0.47
		7/4	Weak	Winter loss	--
		7/5	Slight increase	normal	0.54
	Celle	13/1	normal	Winter loss	--
		13/2	normal	normal	0.63
		13/3	normal	normal	0.48
		13/4	normal	normal	0.22
		13/5	normal	normal	0.54

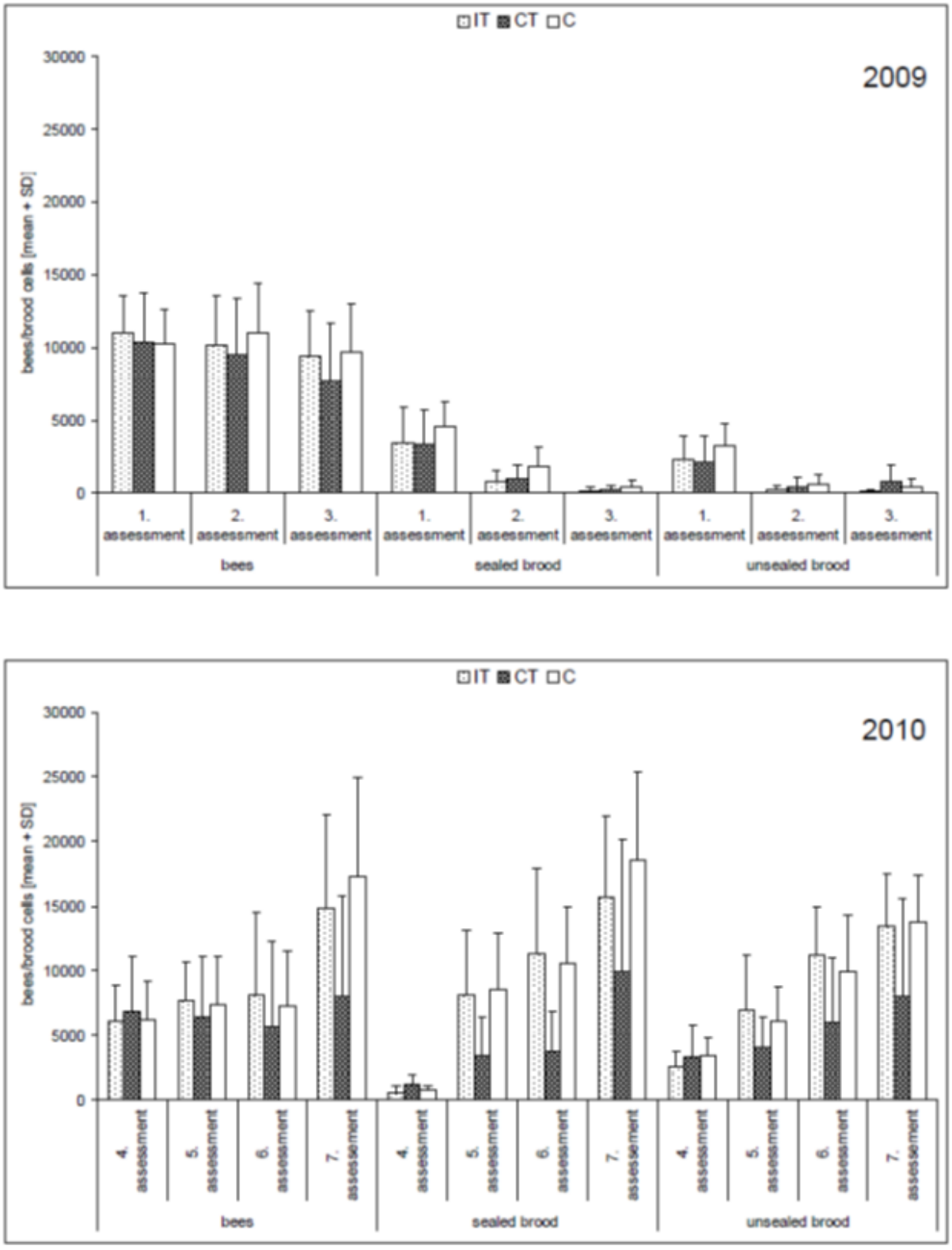


Figure B.9.5.1-4: Colony development of the study colonies in autumn 2009 and spring 2010. IT – imidacloprid-treated group; CT – clothianidin-treated group; C – control group.

Methodological deficiencies resulting in experimental biases, particularly for the clothianidin treatment group

The autumn- and overwintering conditions for the clothianidin treatment group were substantially less favourable as compared to the control and/or to the imidacloprid treatment group due to three key factors:

28. Higher number of weak colonies at study initiation:
    Colonies which have a below average colony strength in autumn will have an overall lower survival rate over winter time than stronger colonies. Considering the initial pre-exposure colony vitality of all colonies across the three treatment groups, it turned out that there was an assignment bias in the number of the weakest colonies, i.e. colonies with ≤ 8,000 bees with 2, 2 and 3 of such colonies being assigned to the control (colonies 7/2 and 7/4), the imidacloprid-treatment group (colonies 8/1 and 14/4) and the clothianidin-treatment group (colonies 9/1, 9/4 and 15/1), respectively. In the control group, one of the two weak colonies (7/4) developed badly during the course of the study and did finally not survive the winter. The second weak colony (7/2) could restore colony strength during autumn from better bee brood stores and subsequently hibernate successfully.

    In the imidacloprid-treatment group one of the two weak colonies (8/1) was removed before over-wintering as from empirical experience the number of bees was evidently too low for successful overwintering. This colony could not restore colony strength due to low bee brood stores at the time of test initiation. The second weak colony (14/4) showed a weak colony strength during autumn and overwintered badly. Although it finally overwintered successfully, the restoring of this colony during springtime would have required favourable circumstances.

    In the clothianidin-treatment group, two of the three colonies with insufficient brood for restoring colony strength (9/4 and 15/1) had to be removed before overwintering as from empirical experience the number of bees was too low for successful overwintering. The third of these colonies (9/1) developed slightly during autumn but remained too weak to finally survive the winter.

    When comparing the colony performance of all initially weak colonies, they all showed a similar pattern across experimental groups, i.e. no restore of colony strength except for control colony (7/2) due to better brood mass. Those colonies which could not restore colony strength from available brood stores experienced either early termination (at the end of the autumn exposure period) or failure during overwintering. The abandonment/loss of three colonies in the clothianidin-treatment group (i.e. colonies 9/1, 9/4 and 15/1) can be attributed to their rather low number of adult bees at the time of colony set-up in combination with below average brood stores.

29. Higher *Varroa* infestation level:
    Colonies which are infested by *Varroa* mites are heavily stressed, first, by the parasitic activity of the mites and second by the diseases vectored by the mites. It is well known that a high *Varroa* infestation rate during the autumn period significantly increases the likelihood of overwintering failure of a colony. Nonetheless, based on genetic adaptation, some colonies apparently tolerate a higher *Varroa* pressure than other colonies. Although all colonies which were employed for this study received the same anti-*Varroa* treatments (Bayvarol® before study initiation, oxalic acid (and Perizin®, additionally used in Celle) during the study), it is a matter of fact well known in apiculture that the anti-*Varroa* treatment success per individual colony is quite variable. When scrutinizing the clothianidin-treatment group with regard to *Varroa* infestation, there was one colony (9/2; study site Ihinger Hof) which showed during the pre-oxalic acid anti-*Varroa* treatment period in autumn the overall highest natural mite drop (Σ: 343 mites) and the overall highest mite drop after oxalic acid treatment (1,220 mites), which shows that this colony was heavily infested by *Varroa* between study initiation and overwintering. Also the colonies 15/3 and 15/5 (both: study site Celle) in the clothianidin-

treatment group suffered from a high *Varroa* pressure, which became apparent during the pre-anti-*Varroa* treatment period in autumn.

In the control group, only one colony (13/1; study site Celle) exhibited during both, the preoxalic acid (and Perizin®) anti-*Varroa* treatment period and the time immediately after the treatment period a mite number which was higher as compared to the colonies 15/3 and 15/5. However, the mite drop in the colony 13/1 decreased more significantly after treatment as compared to the colonies 15/3 and 15/5 (period 05 – 11 NOV versus period 29 OCT – 05 NOV) which indicated a more effective *Varroa* control as compared to the colonies 15/3 and 15/5. The poor overwintering performance of the colonies 9/2, 15/3 and 15/5 in the clothianidin treatment group, which finally resulted in winter loss, could, therefore, be attributed to the high *Varroa* infestation level of these colonies rather than an effect of an exposure to potentially acute toxic guttation fluid which, however, is not stored and should therefore, not exhibit any delayed toxicity effects.

30. Less favourable ambient conditions during hibernation:
    On top of the negatively biased colony vitality of the clothianidin treatment groups, these colonies also suffered from more unfavourable ambient conditions prevailing at the assigned study plots in comparison to the control- and the imidacloprid study sites.

    At the Ihinger Hof study site, the honeybee colonies at the clothianidin study plot were significantly more exposed to the wind due to the absence of any shelter. Moreover, the hive entrances of the colonies in the clothianidin group were directed to the North (i.e. no sun), whereas the hive entrances of the colonies set-up in the two other groups were directed to the South and East. In addition, the clothianidin study plot suffered from a significantly higher soil dampness, which further contributed to an increased cold and damp microclimate.

    Also on the study location Celle, environmental factors differed on the individual study locations. Particularly the clothianidin study plot was affected, as the honeybee colonies were placed in a slight landscape depression. The soil around the bee colonies was compacted, rendering the place to be damp, which became most apparent during springtime 2010, where the area was swamped and the hives had to be placed on elevated ground in order to prevent the colonies from flooding. During wintertime, also cold air could be expected to have accumulated in this landscape depression, framed by the edges of a forest.

    When correcting the clothianidin treatment group performance for colonies with evidently lower colony vitality at study initiation due to low colony strength, low brood stores and high *Varroa* infestation levels, the observed total performance, including overwintering performance, is not indicative for an unacceptable effect of an autumn exposure of honeybee colonies to guttating W-BAR seedlings, seed-treated with clothianidin.

The assumption of a treatment-related effect as the reason for the lower overall performance and the lower overwintering success of the clothianidin treatment group is further not supported from the following considerations:

31. Intrinsic bee toxicity and exposure levels were not different between imidacloprid and clothianidin colonies:
    The analysis of the residue situation of both neonicotinoid compounds, clothianidin and imidacloprid, in guttation fluid on both study locations did not reveal distinct differences, neither in the absolute maximum residue levels (imidacloprid: 15 mg a.s./L, clothianidin: 2.3 mg a.s/L) nor in the residue kinetics, which gives no indication that the colonies in the two nitro-substituted neonicotinoid treatment groups were exposed differently over time. Both nitro-substituted neonicotinoid compounds share an identical intrinsic honeybee toxicity (imidacloprid – lowest LD_{50} value: 3.7 ng/bee; clothianidin – lowest LD_{50} value: 2.5 ng/bee; source: Bayer CropScience).

32. Recorded symptoms during exposure to guttation exudates were comparable between imidacloprid and clothianidin colonies:
    The number of bees with behavioural abnormalities did not differ between the clothianidin (13 bees) and the imidacloprid treatment group (48 bees). There were also no distinct differences in the number of honeybees directly observed in the individual assessment areas taking up guttation fluid from seed-treated W-BAR plants, neither during the autumn period nor during springtime (control group – autumn/spring/total: 7/53/60 bees; imidacloprid treatment group – autumn/spring/total: 12/111/123 bees; clothianidin treatment group – autumn/spring/total: 5/58/63 bees).

Thus, when accounting for all of the above mentioned facts, it can be concluded that the lower performance of the clothianidin treatment group as compared to the imidacloprid treatment and control group, is in fact not treatment related, but can be attributed to a combination of adverse external factors, which affected the clothianidin group, like the allocation of a higher number of weaker colonies (colony strength and brood), higher initial *Varroa* infestation levels as well as a lower suitability of the study sites.

Conclusions
No treatment related differences in honeybee mortality, colony development in autumn and spring as well as in the overwintering performance were observed between the control and the imidacloprid treatment group. The same conclusion could be drawn for the clothianidin treatment group if appropriate corrections are made for experimental biases concerning colony vitality at study initiation.

Overall, it is concluded that guttation fluid, exudated by winter barley seedlings, seed-treated with nitro-substituted neonicotinoids (imidacloprid or clothianidin), does not have unacceptable effects on honeybee colonies under typical commercial use conditions.

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. field size of al study fields exceeded 2 ha, use of colonies with a good health status, of uniform size and similar genetic origin. However, in contrast to the recommendations of the EFA Guidance Document, the colonies overwintered at their respective field site instead of at the same overwintering location. Further, only 10 pairs of colonies were set-up (5 at each of the two treatment and control fields), which might potentially be too low to achieve sufficient statistical power.

RMS agrees with the argumentation to demonstrate that the lower performance of the clothianidin treatment group as compared to the imidacloprid treatment and control group is not treatment related, but can be attributed to a combination of adverse external factors.

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

During Peer Review, it was argued that it is not ideal that hives were overwintered on the test site, in the absence of specific information regarding the availability of nearby food sources (see comment 5(33) in the Reporting Table). Additionally, it was considered that variability in colony size and relatively high losses makes interpretation of the results from these studies problematic. Further detail regarding potential effects on colony development would aid interpretation. Additional information regarding the surrounding vegetation and on the colony assessments, present in the full study report, were therefore included in the study summary.

Report:	**1.6/3; Hofmann, S., Staffel, J. & Aumeier, P.; 2014**
Title:	**Field study to monitor potential effects on honeybees from exposure to guttation fluid of winter barley (W-BAR), seed-treated with the insecticidal seed-treatment product clothianidin + imidacloprid FS 100 + 175 G in Germany in 2011/2012**
Report No.:	R11130
Document No.:	M-501261-01-1
Guideline(s):	No official test guideline(s) available at present
Guideline deviation(s):	not specified
GLP/GEP:	yes

Objective
Key study objectives were to assess acute honeybee mortality and to evaluate and compare the long-term colony development and the overwintering performance of honeybee colonies exposed to guttation droplets of clothianidin and imidacloprid treated winter barley (through seed treatment). Furthermore, the guttation behaviour of winter barley was surveyed and it was examined whether exudation of guttation fluid of winter barley and flight activity of honeybees occurred simultaneously. In case flight activity and guttation coincided, the bee activity in the respective study field was surveyed.

Material and Methods
Test and control item
Winter barley (W-BAR) seeds were seed-treated at the Seed Treatment Application Centre of Bayer CropScience AG in Monheim with:

33. Imidacloprid + clothianidin FS 100 + 175 G

Fungicidal seed treatments are routinely used in agriculture to prevent crop plants becoming infected with pathogenic fungi. Therefore, Clothianidin + Imidacloprid FS 100 + 175 G treated winter barley seeds were additionally treated with the fungicide Baytan UFB (nominal: 9.0 g fuberidazole/L, 10.0 imazalil/L, 75.0 g triadimenol/L; dressing rate: 500 mL/dt). In addition, seeds were additionally treated with commercial INTECO at a nominal rate of 30 mL/dt in order to minimize dust abrasion.

The control consisted of seeds that were seed-treated with Baytan UFB and INTECO only, at identical rates as for the test item.

Study sites and sowing
The effects of seed treated with Imidacloprid + clothianidin FS 100 + 175 G was tested on the honeybee (*Apis mellifera*) under field conditions. The study was conducted in eight commercially managed agricultural fields located in the vicinity of Giessen in Hesse, Germany from mid-September 2011 until early-May 2012. On four study fields, five study plots were established which were assigned as Clothianidin + Imidacloprid FS 100 + 175 G treated plots. On four other study fields, five study plots were established and assigned as control plots.

The study fields and the position of the study plots were selected according to the following criteria:

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

Set-up of honeybee hives
At each of the ten study plots (i.e. five treatment and five control plots) five honeybee colonies were placed along a line shortly before sowing (6 to 13 days) the fields with winter barley (W-BAR), in order to investigate the potential effects from exposure to guttating W-BAR, starting from seedling emergence in autumn 2011 until spring 2012. Colonies were placed either directly adjacent or within a

distance of approximately 4.5m to the W-BAR crop, depending on the actual local field situation. In total, the treatment and control group comprised each 25 honeybee colonies. As colonies were in-situ at the time of drilling they were also exposed to dust emitted from seed drilling equipment at the time of sowing.

For overwintering, all colonies were set-up together at one location to prevent different local, environmental factors potentially biasing the overwintering assessment and the comparison between control and treatment performance. An old military site (well-wooded and wind protected area) near Wiehl, east of Cologne, Germany was selected as overwintering location. Due to space reasons, the bee colonies were separated on two similar locations at a distance of about 100 m, with 25 colonies each, in front of and at the rear of an old hangar.

Assessment area

A specified area (assessment area) in front of the honeybee colonies was intensively monitored. The whole assessment area was divided into two in-Crop Zones (Zone 0 and Zone 1) and an off-Crop Zone. Zone 0 (width: 5 m to each side of the hives, 2 m depth into the in-crop) covered the immediate area in front of the bee hives and Zone 1 (a 2 m broad band, shaped like an inverted 'U', with a vertical distance of the band to the field margin of 7 m inside the crop). The bee hives were placed into the off-Crop Zone, either directly adjacent to the W-BAR crop (off-Crop Zone: width: 10 m length along the field margin, 1 m depth into the off-crop) or in a distance of approximately 4.5 m to the W-BAR crop (off-Crop Zone: width: 10 m length along the field margin, 5 m depth into the off-crop), see Figure B.9.5.1-3 and B.9.5.1-4, respectively. In addition, four segregated assessment plots with each 50 W-BAR plants inside in autumn 2011 respectively of one square meter in spring 2012 were established to record the proportion of W-BAR displaying guttation and/or dew.

Honeybee mortality

Each hive was equipped with a dead bee trap. The traps were emptied daily to record the number of dead bees. Additionally, also the number of dead bees from dead bee traps located on a small plot of 0.5 x 0.5 m² in from of each dead bee trap was recorded.

Monitoring activities

The monitoring activities on the respective study plots started as soon as the W-BAR plants had emerged on the study fields and the autumn exposure period lasted up to a period of four and a half consecutive weeks until end of October 2011. The monitoring activities in the field re-started in spring 2012 with the beginning of the flowering of the Goat Willow (*Salix caprea*) and lasted for a period of five consecutive weeks until beginning of the flowering of winter oil-seed in the respective region where the study fields were located.

During the morning the respective assessment area of the study plots under investigation was systematically checked for occurrence of guttation fluid and/or dew. If guttation was still present at the start of honeybee flight activity, the numbers of honeybees resting or walking on the ground or on the W-BAR crop were counted and any potential uptake of guttation fluid or dew by the bees as well as any conspicuous bee behaviour was recorded. The monitoring sessions were stopped if no more guttation fluid was present.

Beyond field assessments in the morning, the study field which was monitored in the morning was also visited in the evening. During these evening assessments, the onset of guttation and the end of bee activity was recorded.

One "monitoring session" lasted for approximately 35 minutes and was defined as one complete observation cycle of the assessment area and its associated two segregated plots of 1 m², at which guttation- and honeybee assessments were conducted during the presence of guttation fluid on the W-BAR crop.

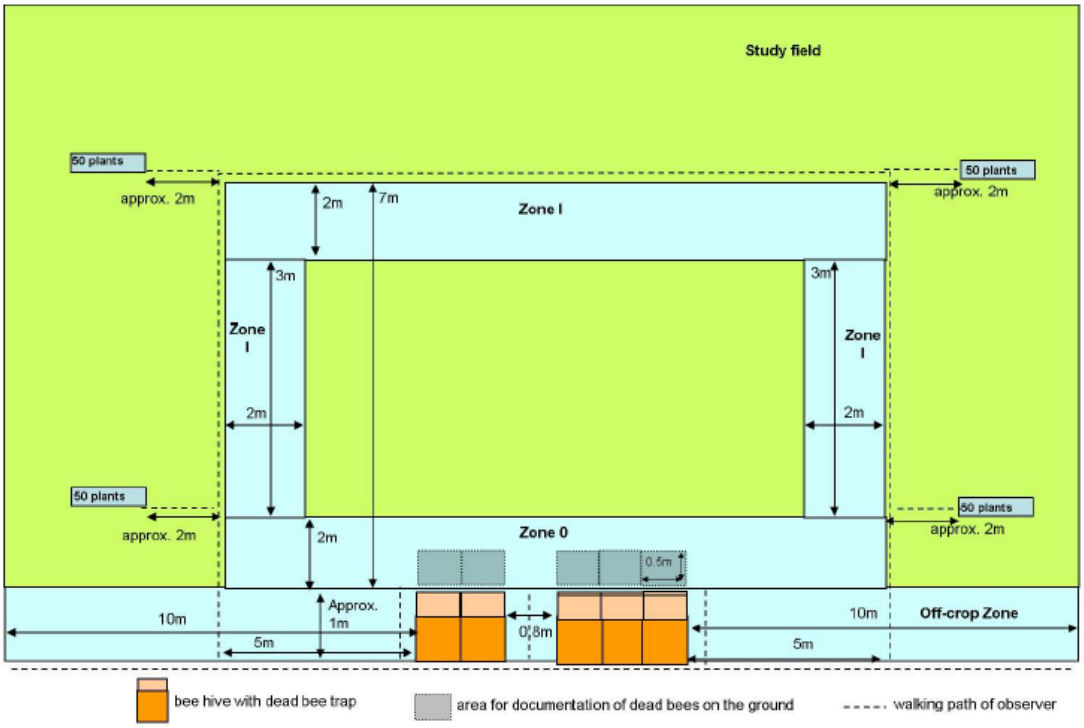


Figure B.9.5.1-5: Scheme of the assessment area on a study plot with hives directly adjacent to the field border (scenario 1)

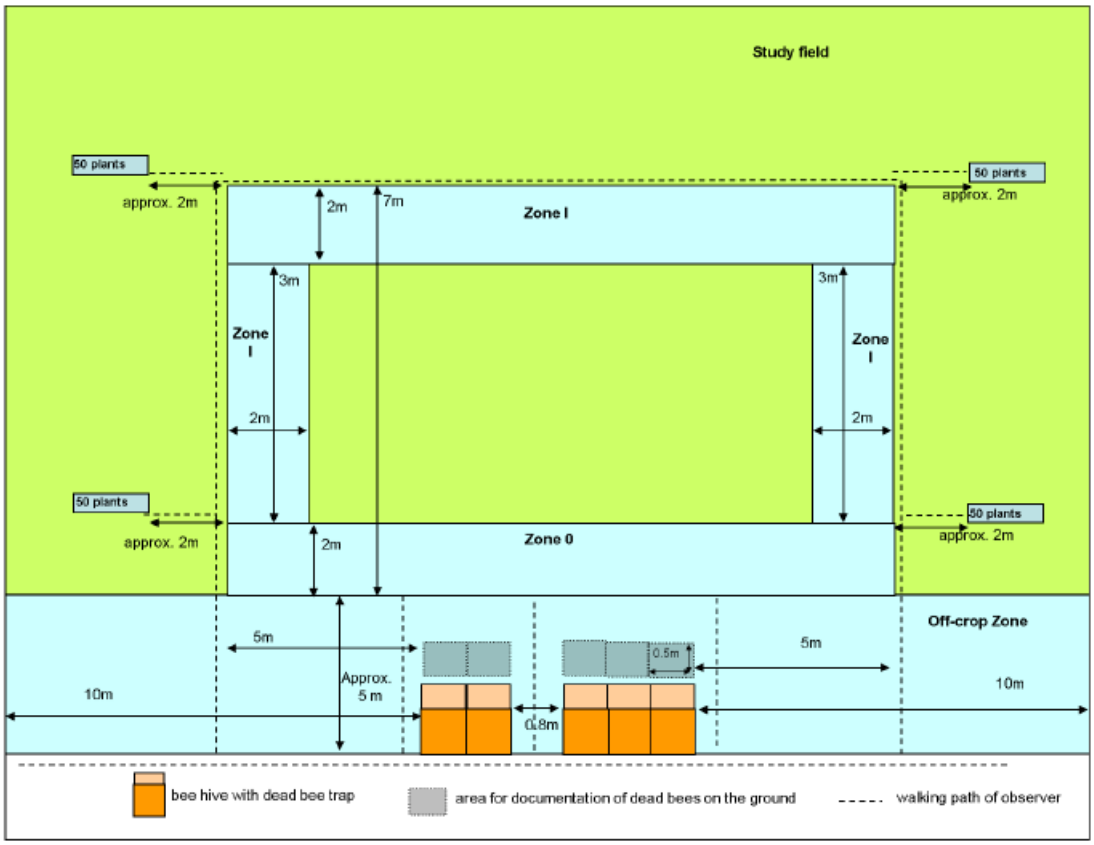


Figure B.9.5.1-6: Scheme of the assessment area on a study plot with hives located at approximately 4.5m distance from the field border within the off-crop area (scenario 2)

The following parameters were monitored during the Field Phase:

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

Honeybee colony strength assessment

The colony strength and the colony development were assessed according to the Liebefeld method. The first assessment on the study plots was performed two to three days after colony set-up. Further assessments were performed every 21 days until the end of October 2011. In spring 2012, colony development was assessed in the same manner from the beginning of flowering of the Goat Willow (*Salix caprea*) until beginning of winter oil-seed flowering in the region. From the beginning of November 2011 until the start of goat willow flowering, all colonies from treatment and control plots were overwintered on a shared overwintering location. After the last assessment on the respective study plots in spring 2012, all honeybee colonies were transferred to a monitoring site with low exposure to any pesticides and were assessed three weeks later for a final time.

Results

Frequency of guttation

Guttation was a frequent phenomenon during the Assessment Phase. During the assessments in the morning, guttation fluid was observed on W-BAR at 100 % of all observation days in autumn 2009 and at 87.6 % of the observation days in spring 2010. Guttation in the herbaceous off-crop area was observed at 66.2% in autumn 2011 and at 87.0% in spring 2012.

No remarkable coincidence of guttation of W-BAR and bee flight activity was observed in the evening. In most cases with evening guttation in autumn 2011, the guttation lasted for the whole day, due to rainy or damp weather (24.1% on W-BAR and 9.7% in off-crop zone). In spring 2012, there was virtually no guttation in the evening (4.7% on W-BAR and 4.1% in off crop zone).

Duration of guttation

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

Honeybee activity in the assessment area

Honeybees were observed visiting the study plots frequently in spring, but rarely in autumn. The relative proportion of honeybees observed per monitoring on plants in the respective assessment areas in both treatment and control, was higher in spring 2012 than in autumn 2011. Moreover, the observed relative proportion of honeybees per monitoring taking up guttation fluid and dew in both treatment and control, was higher in all assessment zones in spring 2012 as compared to autumn 2011, were it was a rare phenomenon.

Most of the direct honeybee observations within the assessment areas were made directly in front of the hives. Accounting for all honeybees, observed during the individual assessments on the study plots throughout the entire field observation period in both treatment and control, respectively, only a small proportion of bees was directly observed taking up guttation fluid. Honeybee activity and the proportion of bees observed collecting water during the study is summarized in Table B.9.5.1-7 below.

Table B.9.5.1-7: Summary of observations on honeybee activity and water collection

Frequency of crop guttation occurrence	100% (Autumn), 89.4% (spring)		
Crop guttation occurrence coinciding with bee activity	73.1% (Autumn), 69.1% (spring)		
Honeybee activity	Total no.bees observed	All areas	5325
		On soil	688 (crop) 860 (off-crop)
		On plants	1129 (crop) 1150 (off-crop)
	Bees collecting water	Guttation + dew	N/A
		Guttation only	495
		Dew only	1003
		% bees collecting guttation	9.3% (all observations) 0.6% (autumn) 14.8% (spring)

Residue analysis of guttation fluid

All samples of guttation fluid collected from the treatment fields were analysed either for residues of imidacloprid or clothianidin, respectively. The Limit of Quantitation (LOQ) of each analyte in guttation fluid was 0.01 mg/L and the Limit of Detection (LOD) of each analyte was 0.001 mg/L, respectively.

Residues of clothianidin and imidacloprid generally peaked shortly after emergence of the dressed W-BAR crop, and declined throughout the autumn observation period until end of October 2011 and were generally distinctly lower throughout the spring observation period 2012. The range of residue levels detected is presented in Table B.9.5.1-8 below.

Table B.9.5.1-8: Measured residues in guttation fluid

Sample description	**Origin**	**Date of sampling**	**Plant growth period**	**Residue (mg/L)**	
				Imidacloprid	**Clothianidin**
Guttation liquid	Winter-Barley, grown from seeds dressed with Clothianidin + Imidacloprid FS 100 + 175 G	28 September to 27 October 2011	Autumn	< LOQ - 6.645	< LOQ - 8.511
		16 March to 17 April 2012	Spring	< LOD - 0.068	< LOD - 0.150

Honeybee mortality

In autumn 2011, both in the control and treatment group, honeybee mortality was on the same, generally low level. With the beginning of October 2011, there was a slight increase in mortality in both treatment and control group, following increasing precipitation and decreasing temperatures. There was quite some variability in mortality, even among colonies at the same study plot, indicating that there are other factors than weather, location and treatment, which may influence honeybee colonies. There were no distinct, biologically relevant differences between treatment and control, irrespective whether the colonies were set-up directly adjacent to the field margins or at distance of approximately 4.5 m to the crop. This conclusion is supported by statistical analysis.

Colony development and overwintering

In both autumn 2011 and spring 2012, the control and treatment group developed in a normal and similar way. Regarding honeybee mortality, brood- and colony development, colony strength and varroa infestation levels during autumn and spring, there were no distinct, biologically relevant differences between treatment and control, irrespective whether the colonies were set-up directly adjacent to the field margins or at distance of approximately 4.5 m to the crop. The *Varroa* infestation was on a generally low level, which did not affect the colonies during this study.

After overwintering, colony strength had decreased in both exposure groups when compared to the before-winter-evaluation, which is a typical apidological phenomenon. That equates to an average overwintering index of 57.8 ± 21.2 % in control colonies and to an average overwintering index of 67.9 ± 14.1 % in treatment colonies. There were no distinct, biologically relevant differences between treatment and control, irrespective whether the colonies were set-up directly adjacent to the field margins or at distance of approximately 4.5 m to the crop. These conclusions are supported by statistical analysis. Only one colony had to be removed from the study, as it was detected to be queenless and was therefore deprieved in bees after overwintering. No colony was lost during winter time due to scarce food supply, inefficient anti-*Varroa* treatment or other factors capable of being influenced by the beekeeper.

Conclusions

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

Overall, it can be concluded that guttation fluid, excreted by winter barley, seed-treated with Clothianidin + Imidacloprid FS 100 + 175 G, does not have unacceptable effects on honeybee colonies under typical commercial use conditions, as there were no adverse acute, short-term or long-term effects on colony strength and -development, brood development, food storage, honeybee behaviour, queen survival, overall hive vitality, colony health, or on overwintering performance.

RMS Comments

The treatment in the present study consisted of barley seeds treated with both clothianidin and imidacloprid. During review of the bee study protocols by EFSA[36], the question was raised whether an application of both imidacloprid and clothianidin to the same field would have any influence on the uptake of both substances by the plants and the measured residues in bee relevant matrices. It was decided at Pesticides Peer Review Meeting on the review of bee study protocols (April 2014) that the applicant should document (supported with data) whether the mixture of imidacloprid and clothianidin may result in a different root uptake for each individual substance. Data was provided and is discussed in section B.9.2.1 (1.3/5, Hammel & Vrbka 2014), and is considered acceptable to demonstrate that the limitation on the uptake of an individual active substance is not influenced by another active substance in the field.

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, overwintering of all colonies at the same post-treatment location. However, the field size of one of the study fields (1.9 ha) was just below the recommended 2 ha. This was 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.*[37], 2012)). A total of 25 pairs of colonies were set-up (5

[36] European Food Safety Authority (2014). Outcome of the peer review of bee study protocols submitted by Bayer CropScience AG to assess the effects of clothianidin on bees. EFSA supporting publication 2014:EN-599.

[37] 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

at each of the 5 treatment and control plots), which is considered to be enough to achieve sufficient statistical power.

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

Studies performed on sugar beet

Report:	**1.6/4; Rexer, H.U.; 2014a**
Title:	**A long-term field study to monitor potential effects on the honeybee (*Apis mellifera* L.) from exposure to guttation fluid of sugar beets, seed-treated with the insecticides clothianidin + imidacloprid + beta-cyfluthrin in Southern Germany in 2013 and 2014**
Report No.:	S13-00171
Document No.:	M-500724-01-1
Guideline(s):	OEPP/EPPO Guideline No. 170(4) (2010); SANCO/3029/99 rev. 4
Guideline deviation(s):	not specified
GLP/GEP:	yes

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

Materials and methods
Test and control item

Test item: Sugar beet pills treated with clothianidin, imidacloprid and beta-cyfluthrin, and the standard fungicides Hymexazol + TMTD.
Batch-ID: ZR02931, Tox-no.: TOX10065-00.
Contents of a.s. (nominal): 0.6 mg clothianidin/pill, 0.3 mg imidacloprid/pill and 0.08 mg beta-cyfluthrin/pill
Contents of a.s. (analysed): 0.6612 mg clothianidin/pill, 0.2994 mg imidacloprid/pill and 0.0828 mg beta-cyfluthrin/pill

The target seeding rate was 130,000 pills/ha, corresponding with a target application rate of 78 g clothianidin/ha, 39 g imidacloprid/ha and 10.4 g beta-cyfluthrin/ha. The actual application rate was 94.0 g clothianidin/ha, 42.6 g imidacloprid/ha and 11.8 g beta-cyfluthrin/ha.

The control consisted of sugar beet pills that were not treated with insecticides, but only with the standard fungicides Hymexazol + TMTD.

Study and monitoring sites
The field sites were located in Neulingen-Bauschlott (C) and Pforzheim (T), both in the federal state of Baden-Württemberg, Germany. The field sites had a size of 2.47 ha (C) and 3.28 ha (T) and there were no flowering main crops within a ca. 2 km radius.

During the assessments of mortality before the start of the exposure period, the colonies were located at a monitoring site without flowering main crops attractive to honeybees in the near surroundings. The monitoring site was located at a distance of 10.7 km to the field site C and 9.4 km to the field site T.

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.

After the end of the exposure period, the colonies were relocated to a monitoring site without flowering main crops attractive to bees within a radius of 3 km. The monitoring site was located at a distance of approximately 65 km to the field site C and approximately 63 km to the field site T.

Set-up of honeybee hives

The effects of honeybee exposure to guttation liquid from sugar beet plants, grown from treated sugar beet pills were examined on commercial bee colonies. The 16 hives used for the purpose of this study (8 colonies per field, and as such 8 colonies for treatment and control, respectively), were healthy, normally developed and free of *Nosema* and *Varroa* disease symptoms and other obvious bee disease symptoms. The colonies were prepared as homogeneous as possible and contained not less than 10,000 bees per colony at the start of the test.

Honeybees were placed at the field sites shortly after emergence of the plants (T: BBCH 12, C: BBCH 12). Honeybees remained at the sugar beet fields for 42 days after exposure and thereafter at a monitoring site (see above). The experimental phase started with the drilling of the treated and untreated sugar beet pills in spring 2013 and ended in spring 2014 after monitoring overwintering survival, colony strength and colony development.

Honeybee mortality

Before set-up of the colonies at the field sites, mortality of the honeybees was recorded by counting the number of dead bees in the dead bee traps in front of the hive entrances. After set-up of the colonies at the field sites, mortality of the honeybees was recorded daily by counting the number of dead honeybees in the dead bee traps in front of the hives, and on a linen sheet that was spread out in front of the hives (size: 10 m x 1.5 m) and on three linen sheets placed in the field (size: 10 m x 0.5 m each); The numbers of dead bees on the linen sheets were equally divided and added to the number of dead bees in the dead bee traps.

Monitoring activities

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

43. Mean number of dead bees on the linen sheets and in the dead bee traps;
44. Flight intensity in the field (mean number of forager bees/ 5x2 m²/min);
45. Observation of honeybees visiting sugar beet plants displaying guttation;
46. Occurrence and proportion of guttation;
47. Behaviour of the bees in the crop and around the hive;
48. Condition of the colonies (number of bees (colony strength), total values of the different brood stages per colony and assessment date);
49. Bee health (bee disease and bee virus analysis);
50. Overwintering performance.

At least once a day during flight activity of the bees, the number of bees entering and leaving the hives by the hive entrance were observed and counted over 30 seconds to determine the flight intensity at the hive entrance. The flight intensity in the field was assessed in five marked areas per field site (size: 2 m² each). At least once a day during flight activity of the honeybees, each assessment area was observed for 1 minute. In addition, honeybees found on sugar beet plants, on the soil surface or taking up guttation liquid in the observation area were counted. During the assessments of mortality and flight intensity, the behaviour of the honeybees in the crop and around the hive was observed.

The observation of guttation in the field took place two times a day from 1DAE (days after exposure) to 21DAE and once a day from 22DAE to 42DAE, always between sunrise and noon. The occurrence of guttation was checked in five marked observation areas of 2 m² each. At each assessment, the proportion of sugar beet plants displaying guttation was assessed. If guttation droplets were present, samples of guttation liquid were collected from the sugar beet leaves. In addition, the occurrence of guttation was checked in one randomly chosen area with suitable vegetation (e.g; grass, weeds) close to the hives. For this area, the percentage of vegetation that was displaying guttation was estimated.

The condition of the honeybee colonies were assessed every three to four weeks at the monitoring site or field sites. Between colony assessments, less invasive bee keeper checks were performed every 7-19 days.

Results

Mortality

No notable difference in mortality was observed between the control group and the test item treatment group during the entire exposure period (see table and figures below).

Table B.9.5.1-9: Honeybee mortality in the control (C) and test item (T)

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

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

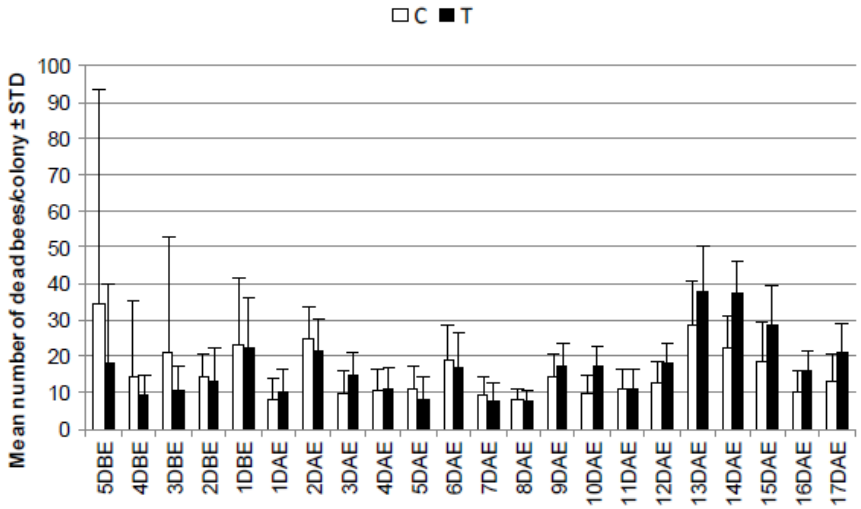


Figure B.9.5.1-7: Mortality: Mean number of dead bees per colony at the monitoring site before set-up (5DBE to 1DBE) and during presence at the field sites from 1DAE to 17DAE. DBE: days before start of exposure; DAE: days after start of exposure; STD: standard deviation.

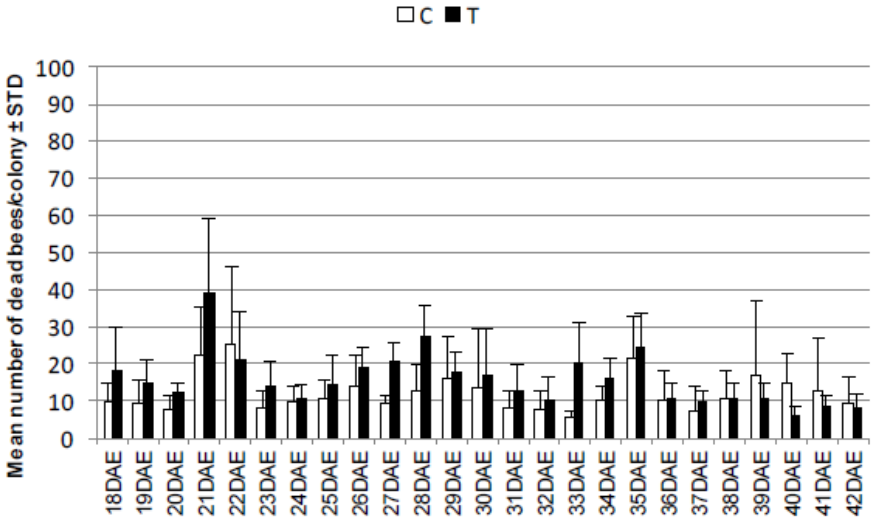


Figure B.9.5.1-8: Mortality: Mean number of dead bees per colony during presence at the field sites from 18DAE to 42DAE. DAE: days after start of exposure; STD: standard deviation

Flight intensity and observation of honeybees visiting sugar beet plants

During the entire assessment period, a total of 5 honeybees was observed in the observation areas in the control group, whereas a total of 4 honeybees was observed in the test item treatment group. In the control groups, 4 honeybees were flying over the crop and 1 honeybee was located on sugar beet plants. In the test item treatment group, 3 honeybees were flying over the crop and 1 honeybee was located on the sugar beet plants. No honeybees taking up guttation liquid were observed in both the control and test item treatment group during the entire observation period.

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

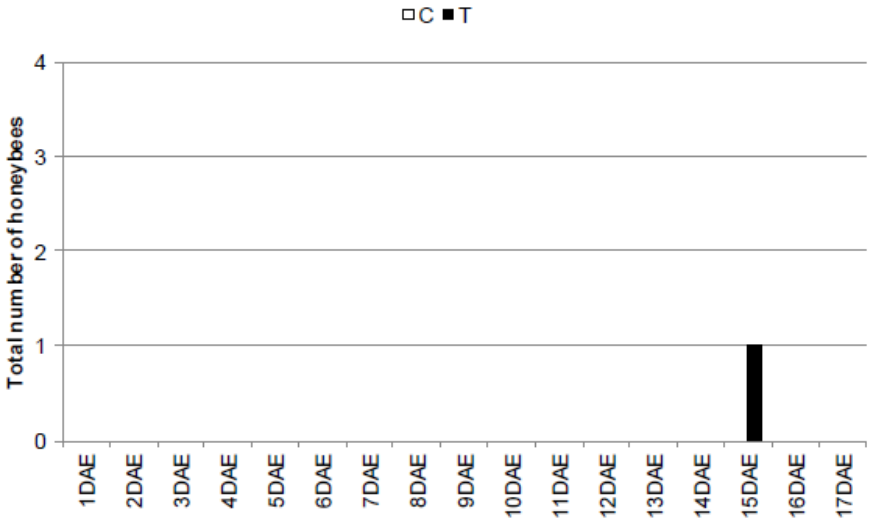


Figure B.9.5.1-9: Flight Intensity: Total number of honeybees observed in the five assessment areas (total area: 10 m^2) per assessment date from 1DAE to 17DAE. DAE: days after start of exposure.

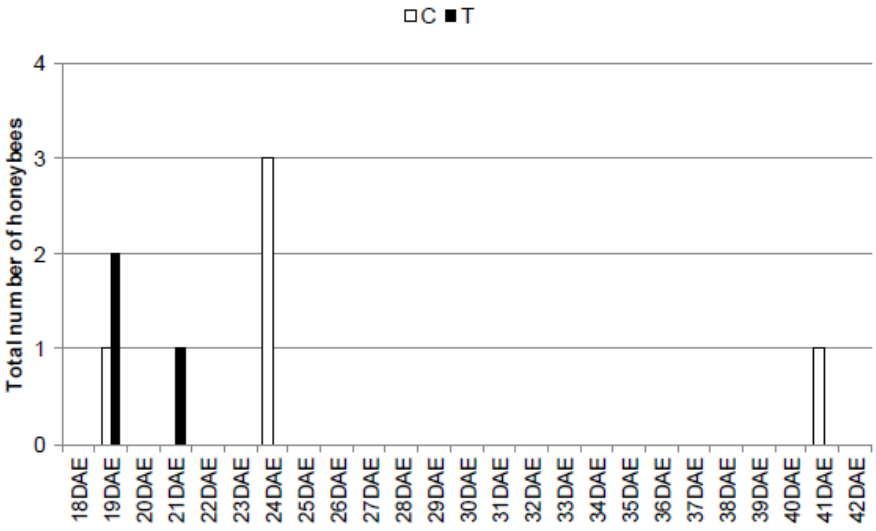


Figure B.9.5.1-10: Flight Intensity: Total number of honeybees observed in the five assessment areas (total area: 10 m^2) per assessment date from 18DAE to 42DAE. DAE: days after start of exposure.

Behaviour of the bees

During the assessment period from 1DAE to 42DAE, small numbers of honeybees exhibiting abnormal behaviour were observed on 5 out of 42 days in the test item treatment group and on 4 out of 42 days in the control group. On the remaining days, only normal behaviour was recorded in both treatment groups. Overall, no notable differences in the abundance and frequency of the occurrence of abnormal behaviour were observed between the test item treatment group and the control. If abnormal behaviour was observed, it was only observed in a small number of honeybees on all assessment dates in both in the test item treatment group and in the control group.

Occurrence of guttation and percentage of plants displaying guttation

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

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

Condition of the colonies

Strength of the colonies

Throughout the entire observation period, the mean colony strength in the test item treatment group T was on the same level as or slightly higher than in the control group C. No test-item related adverse effects on colony strength were observed during the entire course of the study (see Figure B.9.5.1-9).

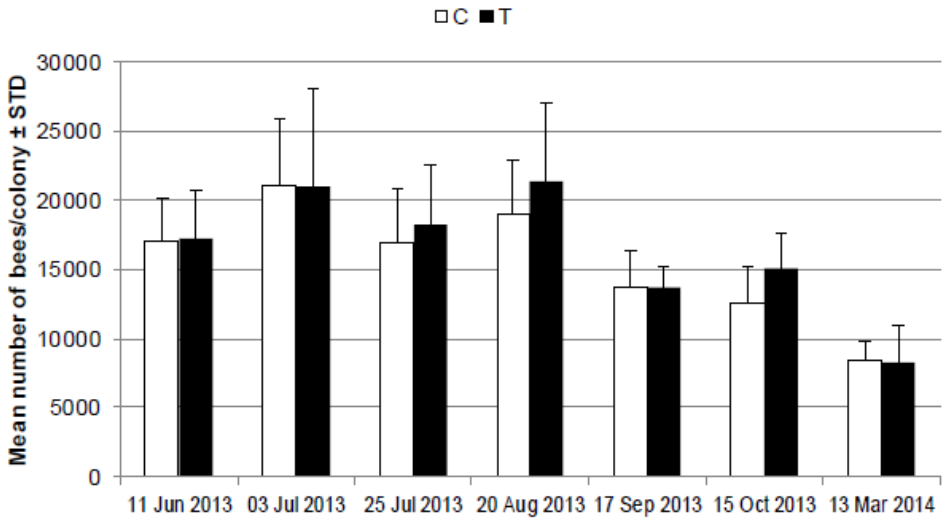


Figure B.9.5.1-11: Colony strength: Mean colony strength (mean number of bees per colony) in the treatment groups C and T

Brood stages and overwintering performance

In the colonies of the control group C and the test item treatment group T, the natural and typical changes and fluctuations in the relative amount of the different pre-imaginal stages, i.e. egg stage, larval and pupal stage, occurred during the observation period. The overwintering period lasted from 15 October 2013 until 13 Mar 2014. After overwintering, all colonies of the test item treatment group and the control were viable and all were found to have resumed breeding activity.

No test item-related adverse effects were observed on colony vitality and brood development, including queen survival and overwintering performance (see Figure B.9.5.1-10).

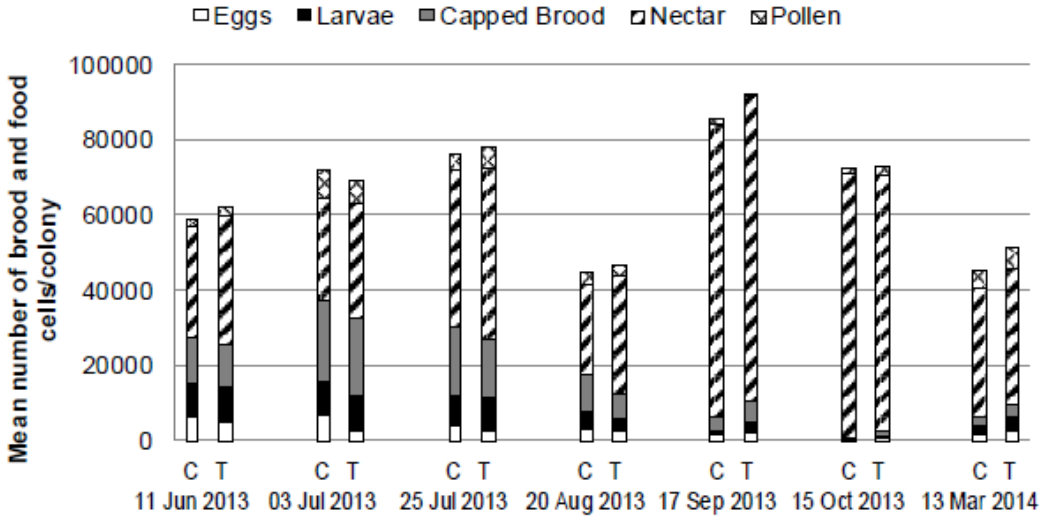


Figure B.9.5.1-12: Brood Stages and Overwintering Performance: Mean number of cells covered with brood and food in the treatment groups C and T

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

Colony health
Evaluation of Varroa infestation in the colonies
Varroa mite occurrence in the colonies was assessed via a '*Varroa* board' beneath the hives. The infestation level of a colony was monitored by counting dead mites on the board. From the first assessment on 20 Aug 2013 (*Varroa* board was inserted on 01 Aug 2013) to 15 Oct 2013, small or medium mean numbers of mites were detected. The mean *Varroa* infestation levels in the test item treatment colonies were moderately higher than in the control colonies during all assessments. However, the detailed bee disease analysis revealed that already the initial *Varroa* infestation level in the (future) test item treatment group (on 11 Jun 2013) was slightly to moderately higher as compared to the (future) control group before the actual set-up of the colonies on their respective exposure fields.

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

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

Residue analysis

The determined clothianidin residues in guttation liquid, as analysed in the samples collected on each day where guttation droplets were actually present on the sugar beet plants in the test item treatment group T, were within the range of 153-327, 35-57 and 36-53 µg/kg for parent clothianidin and its metabolites TZNG and TZMU, respectively. The corresponding imidacloprid residues were within the range of 18-61, 6.9-16 and 1.9-4.0 µg/kg for parent imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefine, respectively. Residues of beta-cyfluthrin in all guttation liquid samples were virtually inexistent (see Table B.9.5.1-10 below).

Table B.9.5.1-10: Range of residues determined in guttation liquid samples

Sample Name (Sample ID)	Days after start of exposure	Residues [µg/kg]						
		Clothianidin	TZNG	TZMU	Imidacloprid	Imidacloprid-5-hydroxy	Imidacloprid-olefine	Beta-cyfluthrin
L13-00171-T-14DAE-GL-A	14	222	38	36	34	13	3.7	<LOQ* /<LOD
L13-00171-T-15DAE-GL-A	15	327	57	49	36	16	3.9	<LOQ* /<LOD
L13-00171-T-22DAE-GL-A	22	237	37	40	39	11	2.5	<LOQ* /<LOD
L13-00171-T-26DAE-GL-A	26	153	45	45	18	9.8	2.2	<LOQ* /<LOD
L13-00171-T-27DAE-GL-A	27	159	39	44	26	6.9	1.9	<LOQ* /<LOD
L13-00171-T-29DAE-GL-A	29	248	35	53	61	9.8	4.0	<LOQ* /<LOD

LOD/LOQ = 0.3 µg/L / 1 µg/L for guttation liquid samples (clothianidin, imidacloprid and metabolites)
** = Due to the low compound sensitivity in the matrix guttation liquid, the LOQ for beta-cyfluthrin was set to 10 µg/kg. An exact and significant LOD could not be determined. Nevertheless an observation of the corresponding measurements shows no countable peaks at the expected retention time. Therefore, it was sufficiently proven that residues of beta-cyfluthrin in all guttation liquid samples were <LOQ / <LOD and as such virtually inexistent.*

Conclusions

Overall, it can be concluded that the exposure of honeybee colonies to guttation liquid from sugar beet plants, grown from pills, commercially prepared with the insecticides clothianidin, imidacloprid and beta-cyfluthrin at a rate corresponding to nominally 0.6 mg clothianidin/pill + 0.3 mg imidacloprid/pill + 0.08 mg beta-cyfluthrin/pill during the first 6 weeks after emergence, did neither cause acute, short-term nor long-term adverse effects on mortality, honeybee behaviour, colony strength, colony health and vitality, brood- and food development and overwintering performance in the exposed colonies.

RMS Comments

The treatment in the present study consisted of sugar beet seeds treated with clothianidin, imidacloprid and beta-cyfluthrin. During review of the bee study protocols by EFSA[38], the question was raised whether an application of both imidacloprid and clothianidin to the same field would have any influence on the uptake of both substances by the plants and the measured residues in bee relevant matrices. It was decided at Pesticides Peer Review Meeting on the review of bee study protocols (April 2014) that the applicant should document (supported with data) whether the mixture of imidacloprid and clothianidin may result in a different root uptake for each individual substance. Data was provided and is discussed in section B.9.2.1 (1.3/5, Hammel & Vrbka 2014), and is considered acceptable to demonstrate that the limitation on the uptake of an individual active substance is not influenced by another active substance in the field.

[38] European Food Safety Authority (2014). Outcome of the peer review of bee study protocols submitted by Bayer CropScience AG to assess the effects of clothianidin on bees. EFSA supporting publication 2014:EN-599.

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, overwintering of all colonies at the same post-treatment location, size of the study field > 2 ha. However, a total of 8 pairs of colonies were set-up (8 colonies at the control and treatment plot), which which might potentially be too low to achieve sufficient statistical power.

It is noted that the duration of the observations for honeybee flight intensity in the field and honeybees visiting sugar beet plats is very short (only 1 minutes for each of the 5 assessment areas, so 5 minutes per day in total). RMS is of the opinion that this period could be too short to obtain correct information on the frequency at which bees visit the sugar beet crop. The fact that the number of honeybees observed to visit the sugar beet plants in the second study performed in sugar beet (see 1.6/5 Rexer, 2014b) is much higher compared to the present study (despite the same short daily observation period) supports this hypothesis. However, the difference in recorded bee flight activity between the present study and study 1.6/5 is considered to be of limited consequence as overall guttation occurred only infrequently in sugar beets and the overall abundance of guttation droplets in the crop was low compared to the off-crop areas. Although more bees were observed visiting the sugar beet crop in study 1.6/5, almost no collection of guttation water was observed.

Despite the limitations discussed above, the study is considered acceptable to be used in the risk assessment.

During Peer Review it was noted that the intended application rate for clothianidin in this study was 78 g a.s./ha, which is less than the maximum value for the proposed uses (see comment 5(34) in the Reporting Table). However, although the nominal application rate was 78 g a.s./ha, the actual application rate was higher (94 g a.s./ha). The loading per pill was 0.6615 mg/pill and thus higher than the maximum registered rate (0.6 mg/seed). Due to some differences in the assumed seeding rate, the registered amount of clothianidin/ha in Spain is slightly higher than the actual application rate in this study. For all other countries, the registered rate is exceeded.

Report:	**1.6/5; Rexer, H.U.; 2014b**
Title:	**A long-term field study to monitor potential effects on the honeybee (*Apis mellifera* L.) from exposure to guttation fluid of sugar beets, seed-treated with the insecticides clothianidin + imidacloprid + beta-cyfluthrin in Southern Germany in 2013 and 2014**
Report No.:	S13-00170
Document No.:	M-500734-01-1
Guideline(s):	OEPP/EPPO Guideline No. 170(4) (2010); SANCO/3029/99 rev. 4
Guideline deviation(s):	not specified
GLP/GEP:	yes

Objective

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

Materials and methods

Test and control item

Test item: Sugar beet pills treated with clothianidin, imidacloprid and beta-cyfluthrin, and the standard fungicides Hymexazol + TMTD.
Batch-ID: ZR02931, Tox-no.: TOX10065-00.
Contents of a.s. (nominal): 0.6 mg clothianidin/pill, 0.3 mg imidacloprid/pill and 0.08 mg beta-cyfluthrin/pill

Contents of a.s. (analysed): 0.6612 mg clothianidin/pill, 0.2994 mg imidacloprid/pill and 0.0828 mg beta-cyfluthrin/pill

The target seeding rate was 130,000 pills/ha, corresponding with a target application rate of 78 g clothianidin/ha, 39 g imidacloprid/ha and 10.4 g beta-cyfluthrin/ha. The actual application rate was 79.7 g clothianidin/ha, 36.1 g imidacloprid/ha and 9.98 g beta-cyfluthrin/ha.

The control consisted of sugar beet pills that were not treated with insecticides, but only with the standard fungicides Hymexazol + TMTD.

Study and monitoring sites

The field sites were located in Gäufelden-Tailfingen (C) and Gäufelden-Öschelbronn (T), both in the federal state of Baden-Württemberg, Germany. The field sites had a size of 2.76 ha (C) and 2.37 ha (T) and there were no flowering main crops within a ca. 3 km radius.

During the assessments of mortality before the start of the exposure period, the colonies were located at a monitoring site without flowering main crops attractive to honeybees in the near surroundings. The monitoring site was located at a distance of approximately 21 km to the field site T and approximately 16 km to the field site T.

After the end of the exposure period, the colonies were relocated to an interim monitoring site without flowering main crops attractive to bees within a radius of 3 km. The interim monitoring site was located at a distance of approximately 40 km to the field sites C and T. The colonies remained at the interim monitoring site for approximately 30 hours. Thereafter, they were relocated to the final monitoring site without flowering main crops attractive to honeybees within a radius of 3 km. The final monitoring site was located at a distance of approximately 79 km to the field site T and approximately 83 km to the field site C.

Set-up of honeybee hives

The effects of honeybee exposure to guttation liquid from sugar beet plants, grown from treated sugar beet pills were examined on commercial bee colonies. The 16 hives used for the purpose of this study (8 colonies per field, and as such 8 colonies for treatment and control, respectively), were healthy, normally developed and free of *Nosema* and *Varroa* disease symptoms and other obvious bee disease symptoms. The colonies were prepared as homogeneous as possible and contained not less than 10,000 bees per colony at the start of the test.

Honeybees were placed at the field sites shortly after emergence of the plants (T: BBCH 12, C: BBCH 12-14). Honeybees remained at the sugar beet fields for 40 days after exposure and thereafter at a monitoring site (see above). The experimental phase started with the drilling of the treated and untreated sugar beet pills in spring 2013 and ended in spring 2014 after monitoring overwintering survival, colony strength and colony development.

Honeybee mortality

Before set-up of the colonies at the field sites, mortality of the honeybees was recorded by counting the number of dead bees in the dead bee traps in front of the hive entrances. After set-up of the colonies at the field sites, mortality of the honeybees was recorded daily by counting the number of dead honeybees in the dead bee traps in front of the hives, and on a linen sheet that was spread out in front of the hives (size: 8 m x 1.5 m) and on three linen sheets placed in the field (size: 10 m x 0.5 m each); The numbers of dead bees on the linen sheets were equally divided and added to the number of dead bees in the dead bee traps.

Monitoring activities

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

51. Mean number of dead bees on the linen sheets and in the dead bee traps;

52. Flight intensity in the field (mean number of forager bees/ 5x2 m²/min);
53. Observation of honeybees visiting sugar beet plants displaying guttation;
54. Occurrence and proportion of guttation;
55. Behaviour of the bees in the crop and around the hive;
56. Condition of the colonies (number of bees (colony strength), total values of the different brood stages per colony and assessment date);
57. Bee health (bee disease and bee virus analysis);
58. Overwintering performance.

At least once a day during flight activity of the bees, the number of bees entering and leaving the hives by the hive entrance were observed and counted over 30 seconds to determine the flight intensity at the hive entrance. The flight intensity in the field was assessed in five marked areas per field site (size: 2 m² each). At least once a day during flight activity of the honeybees, each assessment area was observed for 1 minute. In addition, honeybees found on sugar beet plants, on the soil surface or taking up guttation liquid in the observation area were counted. During the assessments of mortality and flight intensity, the behaviour of the honeybees in the crop and around the hive was observed.

The observation of guttation in the field took place two times a day from 1DAE (days after exposure) to 21DAE and once a day from 22DAE to 40DAE, always between sunrise and noon. The occurrence of guttation was checked in five marked observation areas of 2 m² each. At each assessment, the proportion of sugar beet plants displaying guttation was assessed. If guttation droplets were present, samples of guttation liquid were collected from the sugar beet leaves. In addition, the occurrence of guttation was checked in one randomly chosen area with suitable vegetation (e.g; grass, weeds) close to the hives. For this area, the percentage of vegetation that was displaying guttation was estimated.

The condition of the honeybee colonies were assessed every three to four weeks at the monitoring site or field sites. Between colony assessments, less invasive bee keeper checks were performed every 7-19 days.

Results

Mortality

During the pre-exposure period at the monitoring site (15DBE to 11DBE), the mean daily mortality, assessed by using dead bee traps, was on the same level in the control group and in the test item treatment group (22.4 and 21.5 dead bees/colony/day for the control group C and test item treatment group T, respectively). No notable difference in mortality was observed between the control group and the test item treatment group during the entire exposure period.

Table B.9.5.1-11: Honeybee mortality in the control (C) and test item (T)

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

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

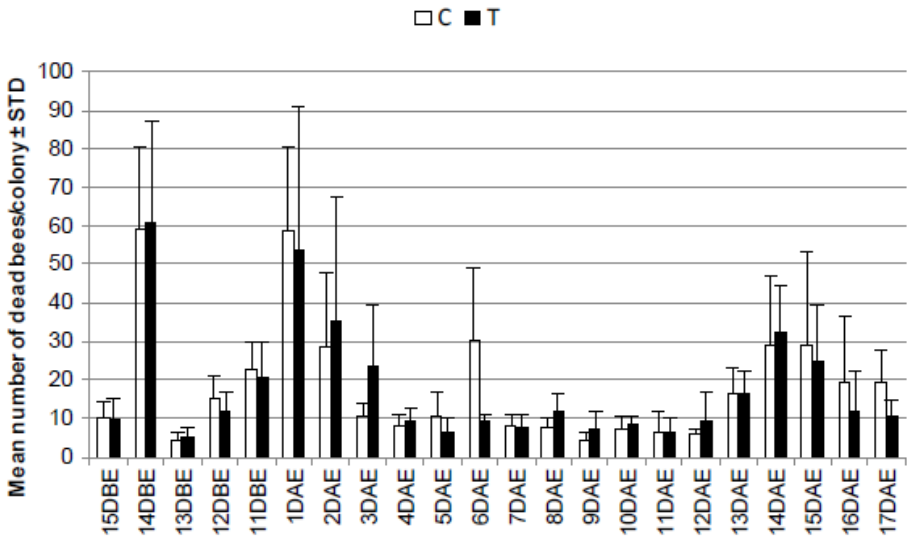
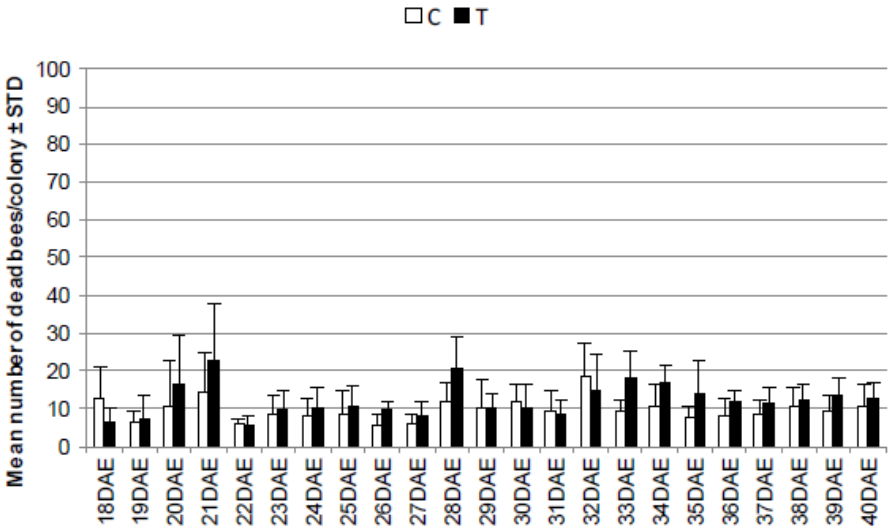


Figure B.9.5.1-13: Mortality: Mean number of dead bees per colony at the monitoring site before set-up (15DBE to 11DBE) and during presence at the field sites from 1DAE to 17DAE. DBE: days before start of exposure; DAE: days after start of exposure; STD: standard deviation



FigureB.9.5.1-14: Mortality: Mean number of dead bees per colony during presence at the field sites from 18DAE to 40DAE. DAE: days after start of exposure; STD: standard deviation

Flight intensity and observation of honeybees visiting sugar beet plants

During the entire assessment period, a total of 77 honeybees was observed in the observation areas in the control group, as well as the test item group. In the control group, 56 honeybees were flying over the crop, 14 honeybees were located on sugar beet plants and 7 honeybees were observed on the soil. In the test item treatment group, 53 honeybees were flying over the crop, 15 were located on sugar beet plants and 9 honeybees were observed on the soil. No honeybees taking up guttation liquid were observed in both the control and the test item treatment group during the entire observation period.

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

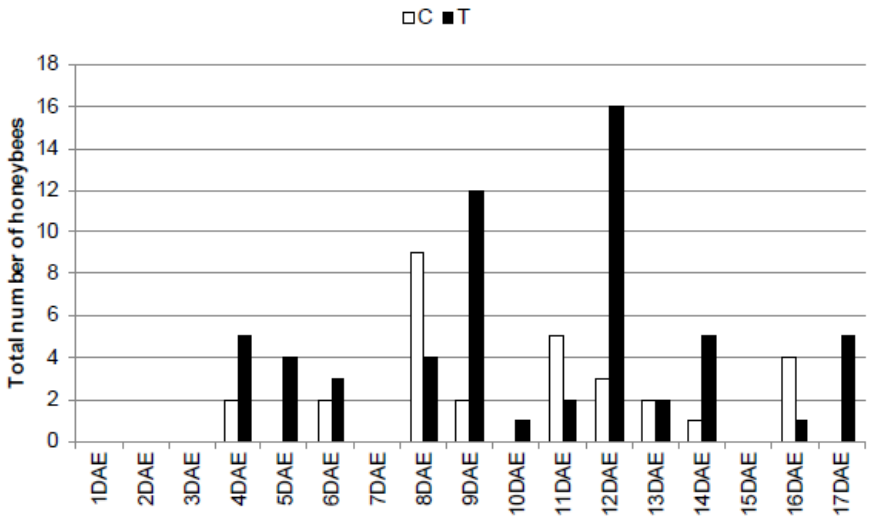


Figure B.9.5.1-15: Flight Intensity: Total number of honeybees observed in the five assessment areas (total area: 10 m^2) per assessment date from 1DAE to 17DAE. DAE: days after start of exposure

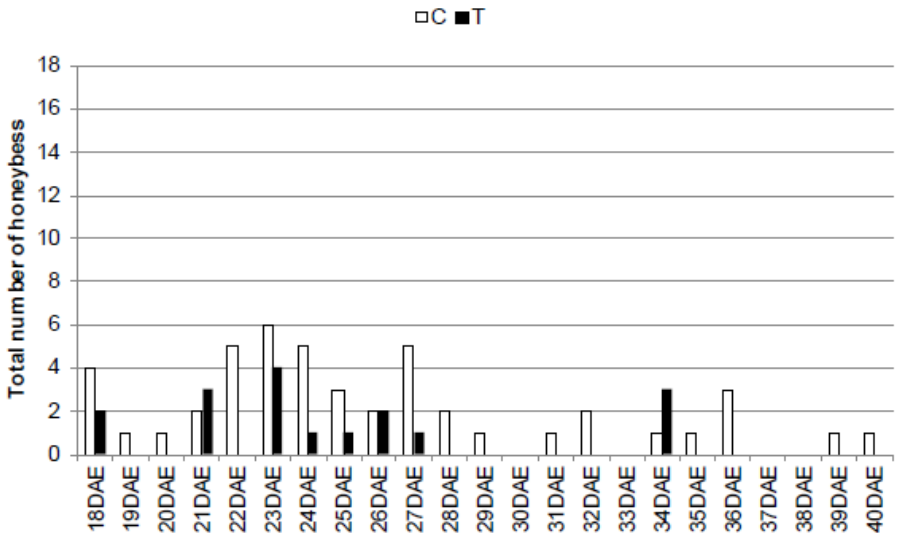


Figure B.9.5.1-16: Flight Intensity: Total number of honeybees observed in the five assessment areas (total area: 10 m^2) per assessment date from 18DAE to 40DAE. DAE: days after start of exposure

Behaviour of the bees

During the assessment period from 1DAE to 40DAE, small numbers of honeybees exhibiting abnormal behaviour were observed on 30 out of 40 days in both the test item treatment group and the control group. On the remaining days, only normal behaviour was recorded in both treatment groups. Overall, no notable differences in the abundance and frequency of the occurrence of abnormal behaviour were observed between the test item treatment group and the control. If abnormal behaviour was observed, it was only observed in a small number of honeybees on all assessment dates in both, in the test item treatment group and in the control group. No test-item related adverse effects on honeybee behaviour were observed.

Occurrence of guttation and percentage of plants displaying guttation

In the control group, guttation of sugar beet plants in the assessment areas was observed on 3 out of 40 assessment days. In the concurrently assessed off-crop area, guttation occurred on 25 out of 40 assessment days. In the test item treatment group, guttation of sugar beet plants in the assessment areas was observed on 5 out of 40 assessment days. In the concurrently assessed off-crop area, guttation occurred on 20 out of 40 assessment days. When guttation occurred in the in-crop assessment areas in the control group, the percentage of plants exhibiting guttation per assessment area varied from 2.9 %

to 57.1 %. In the test item treatment group, the percentage of plants exhibiting guttation per assessment area varied from 3.0 % to 82.1 %, when guttation was detected.

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

Condition of the colonies

Strength of the colonies

Throughout the entire observation period, the mean colony strength in the test item treatment group T was on the same level as or slightly higher than in the control group C. Thus, no test-item related adverse effects on colony strength were observed during the entire course of the study (see Figure B.9.5.1-17).

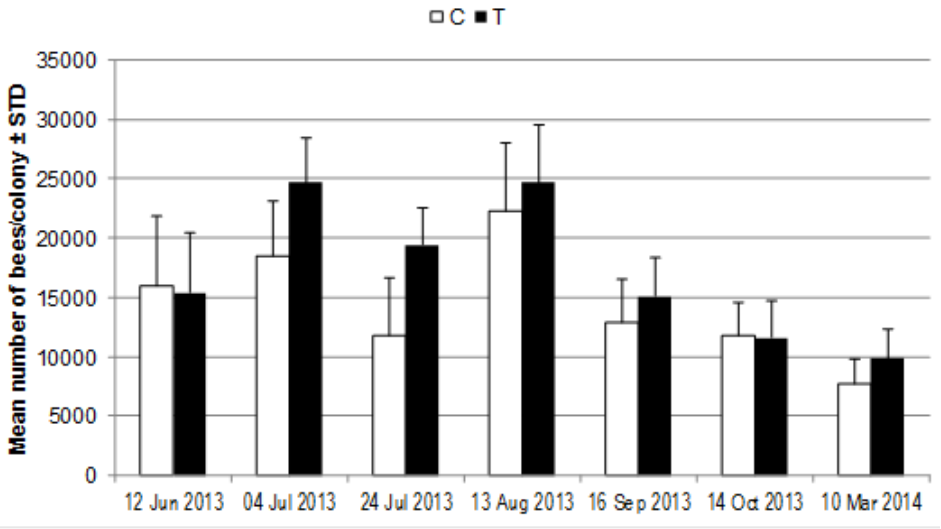
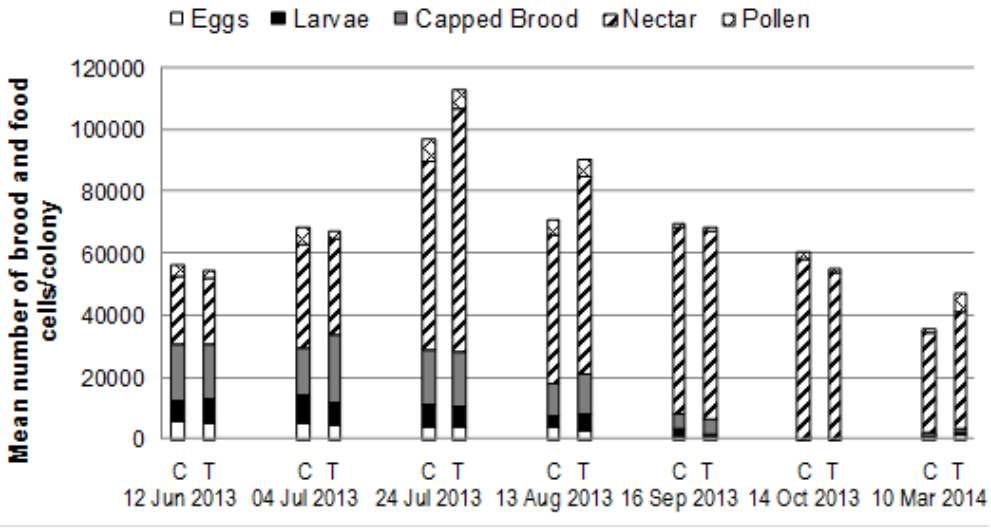


Figure B.9.5.1-17: Colony strength: Mean colony strength (mean number of bees per colony) in the treatment groups C and T

Brood stages and overwintering performance

In the colonies of the control group C and the test item treatment group T the natural and typical changes and fluctuations in the relative amount of the different pre-imaginal stages, i.e. egg stage, larval and pupal stage, occurred during the observation period. The overwintering period lasted from 14 October 2013 until 10 Mar 2014. After overwintering, all colonies of the test item treatment group and the control were viable and all were found to have resumed breeding activity (except colony Cc). Thus, no test item-related adverse effects were observed on colony vitality and brood development, including queen survival and overwintering performance (see Figure B.9.5.1-18).



FigureB.9.5.1-18: Brood Stages and Overwintering Performance: Mean number of cells covered with brood and food in the treatment groups C and T

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

Colony health
Evaluation of Varroa infestation in the colonies
Varroa mite occurrence in the colonies was assessed via a '*Varroa* board' beneath the hives. The infestation level of a colony was monitored by counting dead mites on the board. From the first assessment on 03 Sep 2013 (*Varroa* board was inserted on 13 Aug 2013) to 14 Oct 2013 only small numbers of mites were detected. Both the control and test item treatment colonies showed approximately the same low *Varroa* infestation levels during the course of the study and at the end of the honeybee season. No test item-related adverse effects were detected.

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

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

Residue analysis
The determined clothianidin residues in guttation liquid, as analysed in the samples collected on each day where guttation droplets were actually present on the sugar beet plants in the test item treatment group T, were within the range of 17-64, 2.9-12 and 3.1-11 µg/kg for parent clothianidin and its

metabolites TZNG and TZMU, respectively. The corresponding imidacloprid residues were within the range of 2.9-10, 1.2-4.2 and < LOQ-1.3 µg/kg for parent imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefine, respectively. Residues of beta-cyfluthrin in all guttation liquid samples were virtually inexistent (see Table B.9.5.1-12 below).

Table B.9.5.1-12: Range of residues determined in guttation liquid samples

Sample Name (Sample ID)	Days after start of exposure	Residues [µg/kg]						
		Clothianidin	TZNG	TZMU	Imida-cloprid	Imida-cloprid-5-hydroxy	Imida-cloprid-olefine	Beta-cyfluthrin
L13-00170-T-12DAE-GL-A	12	17	2.9	3.1	2.9	1.2	<LOQ	<LOQ* /<LOD
L13-00170-T-16DAE-GL-A	16	64	12	11	9.7	4.2	1.3	<LOQ* /<LOD
L13-00170-T-17DAE-GL-A	17	60	7.6	7.0	10	1.9	<LOQ	<LOQ* /<LOD

LOD/LOQ = 0.3 µg/L / 1 µg/L for guttation liquid samples (clothianidin, imidacloprid and metabolites)
Beta-Cyfluthrin: LOQ/LOD (Guttation liquid) = 10 µg/kg (10ppb);
** = Due to the low compound sensitivity in the matrix guttation liquid, the LOQ for beta-cyfluthrin was set to 10 µg/kg. An exact and significant LOD could not be determined. Nevertheless an observation of the corresponding measurements shows no countable peaks at the expected retention time. Therefore, it was sufficiently proven that residues of beta-cyfluthrin in all guttation liquid samples were <LOQ / <LOD and as such virtually inexistent.*

Conclusions

Overall, it can be concluded that the exposure of honeybee colonies to guttation liquid from sugar beet plants, grown from pills, commercially prepared with the insecticides clothianidin, imidacloprid and beta-cyfluthrin at a rate corresponding to nominally 0.6 mg clothianidin/pill + 0.3 mg imidacloprid/pill + 0.08 mg beta-cyfluthrin/pill during the first approximately 6 weeks after emergence, did neither cause acute, short-term nor long-term adverse effects on mortality, honeybee behaviour, colony strength, colony health and vitality, brood and food development and overwintering performance in the exposed colonies.

RMS Comments

The treatment in the present study consisted of sugar beet seeds treated with clothianidin, imidacloprid and beta-cyfluthrin. During review of the bee study protocols by EFSA[39], the question was raised whether an application of both imidacloprid and clothianidin to the same field would have any influence on the uptake of both substances by the plants and the measured residues in bee relevant matrices. It was decided at Pesticides Peer Review Meeting on the review of bee study protocols (April 2014) that the applicant should document (supported with data) whether the mixture of imidacloprid and clothianidin may result in a different root uptake for each individual substance. Data was provided and is discussed in section B.9.2.1 (1.3/5, Hammel & Vrbka 2014), and is considered acceptable to demonstrate that the limitation on the uptake of an individual active substance is not influenced by another active substance in the field.

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, overwintering of all colonies at the same post-treatment location, size of the study field > 2 ha. However, a total of 8 pairs of colonies were set-up (8 colonies at the control and treatment plot), which which might potentially be too low to achieve sufficient statistical power.

[39] European Food Safety Authority (2014). Outcome of the peer review of bee study protocols submitted by Bayer CropScience AG to assess the effects of clothianidin on bees. EFSA supporting publication 2014:EN-599.

It is noted that the duration of the observations for honeybee flight intensity in the field and honeybees visiting sugar beet plats is very short (only 1 minutes for each of the 5 assessment areas, so 5 minutes per day in total). RMS is of the opinion that this period could be too short to obtain correct information on the frequency at which bees visit the sugar beet crop. The fact that the number of honeybees observed to visit the sugar beet plants in the present study is much higher compared to the other study performed in sugar beet (see 1.6/4 Rexer, 2014a) supports this hypothesis.

Despite the limitations discussed above, the study is considered acceptable to be used in support of the risk assessment.

During Peer Review it was noted that the intended application rate for clothianidin in this study was 78 g a.s./ha, which is less than the maximum value for the proposed uses (see comment 5(34) in the Reporting Table). However, although the nominal application rate was 78 g a.s./ha, the actual application rate was higher (80 g a.s./ha). The loading per pill was 0.6615 mg/pill and thus higher than the maximum registered rate (0.6 mg/seed). The application rate of 80 g a.s./ha exceeds the registred rate in most countries. Only the registered rate in Spain, Belgium and Italy is higher than the application rate tested in this study. However, these rates (except the one registered in Spain) are exceeded by the application rate tested in the study by Rexer (2014a) (see study 1.6/4 above).

B.9.5.2. Exposure

Exposure from contaminated guttation water is considered a potentially relevant route of exposure for honeybees, bumblebees and solitary bees. The applicant submitted studies performed in winter cereals and sugar beet 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. As winter cereals are sown in autumn there are potentially two guttation periods to which honeybees could be exposed: one in autumn shortly after crop emergence and before overwintering and again in the spring after winter hibernation. In the cereal studies the same colonies were exposed to both guttation periods. Sugar beets are drilled in the spring and hence have one guttation period during that time. 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/L)	Reference
Winter wheat	86.4% Autumn 87.9% Spring	72.7% Autumn 64.4% Spring	0.5% Autumn 11.9% Spring	CTD_{autumn}: 1.0 – 13.0 CTD_{spring}: <LOQ – 0.39 TZNG: <LOQ – 0.59 TZMU: <LOQ – 0.32	1.6/1 Hofmann & Lueckmann, 2014
Winter barley	84.2% Autumn 80.7% Spring	46.6% Autumn 56.3% Spring	2.6% Autumn 16.0% Spring	CTD_{autumn}: 0.03 – 2.3 CTD_{spring}: <LOQ – 0.18 TZNG: <LOQ – 0.05 TZMU: <LOQ – 0.02	1.6/2 Hofmann, Garrido & Lueckmann, 2012
Winter barley	100% Autumn 89.4% Spring	73.1% Autumn 69.1% Spring	0.6% Autumn 14.8% Spring	CTD_{autumn}: <LOQ – 8.5 CTD_{spring}: <LOD – 0.15	1.6/3 Hofmann, Staffel & Aumeier, 2014
Sugar beet	14.3% Spring	Yes, but bees do not visit crop	0%	CTD: 0.15 – 0.33 TZNG: 0.035 – 0.057 TZMU: 0.036 – 0.053	1.6/4 Rexer, 2014a
Sugar beet	35% Spring	Yes, but bees do not visit crop	0%	CTD: 0.017 – 0.064 TZNG: 0.029 – 0.012 TZMU: 0.031 – 0.11	1.6/5 Rexer, 2014b

Notes: CTD = Clothianidin, TZNG and TZMU are metabolites of clothianidin.

In winter cereals guttation was observed in both treated and untreated crops and was a fairly common event in both the autumn and spring exposure periods. The frequency to which guttation occurred in cereals was similar between wheat and barley and was also generally independent of the year of study. Bees were similarly likely to be active on days where guttation occurred in winter cereals in autumn as they were in spring. However, far fewer bees (as a proportion of those observed at the study sites) were observed to be collecting guttation water in autumn compared to spring. This could be explained by the fact that in autumn the colonies are declining in size and preparing to overwinter and in spring colonies are active and increasing in size as egg laying recommences after the overwintering period. Thus, the autumn colonies have a lower demand for resources compared to those in spring.

Residue levels of clothianidin and its major plant metabolites (TZNG and TZMU) in guttation fluid produced by winter cereals were similar with an indication that residues in the spring are far lower than those observed in autumn. This could be explained by the fact that in the spring the cereal plants are older, larger and in a phase of rapid growth in contrast to the plants in the autumn about to enter winter. Consequently when residues are higher in autumn bees are far less likely to collect guttation water compared to the spring when residues are lower.

In contrast to the observations in winter cereals, guttation was far less common in sugar beet. Bees were active on days when guttation occurred but were only rarely observed to visit the fields sown with either treated or untreated seeds. Bees were not observed collecting guttation water from sugar beet plants at any time during these experiments. Compared to the studies in winter cereals, however, the observed area was smaller (10 m² in sugar beets vs. > 40 m² in cereals) and the observation time shorter (about 5 minutes/day in sugar beet vs. at least 35 minutes/day in cereals). This could potentially have biased the observation results in sugar beet, and makes comparison between the two crops difficult. Residue levels of clothianidin and its major plant metabolites (TZNG and TZMU) in guttation fluid produced by sugar beet plants in spring (i.e. shortly after emergence) were at least an order of magnitude lower than the residues found in guttation fluid produced by winter cereals in the autumn. Overall, the exposure of honeybees to clothianidin residues in guttation fluid from sugar beet seems lower than from winter cereals.

In conclusion, consumption of contaminated guttation fluid is a possible route of exposure for bees, especially for winter cereals. Exposure of honeybees to clothianidin residues in guttation fluid from sugar beet seems lower than for winter cereals, but cannot completely be ruled out based on the available data. Therefore, a risk assessment will be performed for both the use in winter cereals and in sugar beet.

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.

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 submitted by the applicant. Minimum, maximum, mean and 90th percentile measured residue values of clothianidin in guttation fluid from winter cereals are shown in Table B.9.5.3.1-1. As clothianidin residues were much higher in autumn (during the first weeks after emergence) compared to residues in spring, a separate risk assessment was performed for both seasons. In the original version of this Addendum, the highest available 90th percentile residue values (11.3 mg/L for autumn and 0.1 mg/L for spring) were used as PEC for the acute risk assessment. For the chronic risk assessment, the highest mean values were used (5.2 mg/L for autumn and 0.047 mg/L for spring).

Table B.9.5.3.1-1: Minimum, maximum, mean and 90th percentile concentration of clothianidin (mg/L), measured in guttation water from winter cereals, either in autumn or in spring.

Crop	Plant growth period	Number of samples	Clothianidin Residue (mg/L)				Reference
			min	max	Mean*	90th percentile*	
Winter wheat	Autumn	18	1.7	13.0	5.53	11.3	Hofmann & Lueckmann, 2014
	Spring	92	<LOQ	0.39	0.041	0.060	
Winter barley	Autumn	85	0.03	2.30	0.47	1.06	Hofmann, Garrido & Lueckmann, 2012
	Spring	90	<LOQ	0.18	0.047	0.10	
Winter barley	Autumn	221	<LOQ	8.511	0.566	1.065	Hofmann, Staffel & Aumeier, 2014
	Spring	233	<LOD	0.150	0.023	0.045	

*LOQ = 0.01 mg/L, LOD = 0.001 mg/L for guttation liquid samples; * for the calculation of the mean, median and 90th percentile values, concentrations reported as <LOD were assigned the value of the LOD (0.001 mg/L) as a conservative approach. Values reported as <LOQ were assigned the value of the LOQ (0.01 mg/L)*

The measured residues in guttation water from the studies in sugar beet are shown in Table B.9.5.3.1-2. As the number of samples was much lower compared to the studies in winter cereals, results from the individual samples are shown. In the original version of this Addendum, the highest measured residue value (0.327 mg/L) was used in the acute risk assessment, due to the limited number of samples. For the chronic risk assessment, the overall mean value (0.165 mg/L) was used.

Table B.9.5.3.1-2: Measured concentration of clothianidin (mg/L), measured in guttation water from sugar beet.

Sample Name (Sample ID)	Days after start of exposure	Clothianidin residues (mg/L)	Reference
L13-00171-T-14DAE-GL-A	14	0.222	Rexer, 2014a
L13-00171-T-15DAE-GL-A	15	0.327	
L13-00171-T-22DAE-GL-A	22	0.237	
L13-00171-T-26DAE-GL-A	26	0.153	
L13-00171-T-27DAE-GL-A	27	0.159	
L13-00171-T-29DAE-GL-A	29	0.248	
L13-00170-T-12DAE-GL-A	12	0.017	Rexer, 2014b
L13-00170-T-16DAE-GL-A	16	0.064	
L13-00170-T-17DAE-GL-A	17	0.060	
Overall mean		0.165	

Note: In the study reports, results are expressed as µg/kg. To be in line with the results from the studies in winter cereals, they were transformed 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)

During Peer Review, it was argued that there was not sufficient consideration of whether exposure represents a 90th percentile situation. During Pesticides Peer Review Meeting 145 it was agreed that the available dataset for both cereals and sugar beet is not sufficient for selecting the 90th percentile of exposure as suggested by the EFSA Guidance Document for bees (For sugar beet the number of field sites was less than 5; For cereals the number of field sites tested was higher than 5, but all sites were located in Germany and thus do not represent the whole area of use). 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, 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 not 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 in cereals in autumn. 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 for cereals in autumn. As the chronic endpoints for honeybee larvae and adult honeybees are expressed over 5 and 10 days, respectively, the TWA concentration to be used in the chronic assessment should be calculated over 5 and 10 days. The use of an overall mean concentration (calculated for the whole sampling period), as proposed in the orginal version of this Addendum, was not considered acceptable.

The concentration of clothianidin in guttation fluid produced by cereals in spring is lower compared to the concentration in autumn. However, no decline in clothianidin concentrations was observed over time. Therefore, for the updated risk assessment for guttation exposure in cereals in spring, the maximum residue values will be used for both the acute and chronic assessment. For sugar beet, there is also no indication that the clothianidin concentration in guttation fluid declines over time, based on the limited number of samples available (see Table B.9.5.3.1-2). Therefore, the maximum available residue values will be used in the updated risk assessment for both the acute and chronic risk.

The Tier 2 risk assessment was updated taking into account the outcome of Pesticides Peer Review Meeting 145, as discussed above. Table B.9.5.3.1-3 shows the maximum and TWA concentrations of

clothianidin in guttation fluid measured in the different studies in cereals and sugar beet. Values in bold are used in the risk assessment.

Table B.9.5.3.1-3: Maximum and mean (over 5 days, 10 days and the whole assessment period) concentrations of clothianidin (mg/L), measured in guttation water from winter cereals (either in autumn or in spring) and sugar beet.

Crop	Plant growth period	Clothianidin Residue (mg/L)				Reference
		Maximum	Mean over first 5 days*	Mean over first 10 days*	Overall mean*	
Winter wheat	Autumn	**13.0**	**5.84**	**5.53**	5.53	Hofmann & Lueckmann, 2014
	Spring	**0.39**	-	-	0.041	
Winter barley	Autumn	2.30	0.78	0.58	0.47	Hofmann, Garrido & Lueckmann, 2012
	Spring	0.18	-	-	0.047	
Winter barley	Autumn	8.511	0.954	0.744	0.566	Hofmann, Staffel & Aumeier, 2014
	Spring	0.150	-	-	0.023	
Sugar beet	-	**0.327**	-	-	0.165	Rexer, 2014a and 2014b

*LOQ = 0.01 mg/L, LOD = 0.001 mg/L for guttation liquid samples; * for the calculation of the mean values, concentrations reported as <LOD were assigned the value of the LOD (0.001 mg/L) as a conservative approach. Values reported as <LOQ were assigned the value of the LOQ (0.01 mg/L); concentrations in bold were used as PEC value in the risk assessment*

The calculated ETR values for both the use in winter cereals and in sugar beet are shown in Table B.9.5.3.1-4. The relevant toxicity endpoints are taken from Table B.9.1.3.1-3.

Table B.9.5.3.1-4: Tier 2 ETR calculations for acute adult oral, chronic adult oral and larval exposure through the consumption of clothianidin contaminated guttation water in winter cereals and sugar beet.

Acute adult oral exposure						
Crop	Season	W (µL/bee/day)	PEC (µg/µL)	$LD_{50,oral}$ (µg a.s/bee)	ETR	Trigger
Winter cereals	Autumn	11.4	0.0130	0.00379	39.1	0.2
	Spring	11.4	0.00039	0.00379	1.17	0.2
Sugar beet	-	11.4	0.000327	0.00379	0.98	0.2
Chronic adult exposure						
Crop	Season	W (µL/bee/day)	PEC (µg/µL)	LDD_{50} (µg a.s/bee/ day)	ETR	Trigger
Winter cereals	Autumn	11.4	0.00553	0.00138	45.7	0.03
	Spring	11.4	0.00039	0.00138	3.22	0.03
Sugar beet	-	11.4	0.000327	0.00138	2.70	0.03
Larval exposure						
Crop	Season	W (µL/bee/day)	PEC (µg/µL)	NOED (µg a.s./larva /development period)	ETR	Trigger
Winter cereals	Autumn	111	0.00584	0.00528	122.8	0.2
	Spring	111	0.00039	0.00528	8.19	0.2
Sugar beet	-	111	0.000327	0.00528	6.87	0.2

For winter cereals in autumn, the ETR values largely exceed the relevant trigger, due to the relatively high clothianidin residues measured in the first weeks after emergence of the cereals. In spring in cereals and in sugar beet, the ETR values also exceeded, but with a smaller difference. 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. Five 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 seed treatment in winter cereals (i.e. 50 g a.s./dt, corresponding to 100 g a.s./ha) and beet crops (i.e. 60g/U, corresponding to 90 g a.s./ha). Therefore, the available studies are representative for the currently registered uses. Table B.9.5.3.1-5 provides an overview of the different guttation studies available.

One study in winter cereals (Hofmann, Staffel & Aumeier, 2014) and both sugar beet studies (Rexer 2014a and 2014b) were performed with seeds treated with the maximum use rates of both clothianidin and imidacloprid in a single formulation. The levels of parent molecules present in guttation water of both substances together were similar to when they are used separately. Although formulations containing both imidacloprid and clothianidin are not currently registered in Europe, a combination of both active substances could be applied during seed treatment, and additionally the notifier has on-going registrations for formulations which contain a mixture of both clothianidin and imidacloprid. Thus, this situation represents a realistic exposure scenario. Based on the physiological properties which determine guttation, and on the observations in these studies, it can be demonstrated that the presence of one active substance does not influence the uptake and expression of the second active substance. Hence the results are also applicable to solo formulations. Further information on the fact that the presence of imidacloprid does not influence the uptake of clothianidin by plants and residues in bee relevant matrices is provided in section B.9.2.1 (1.3/5, Hammel & Vrbka 2014).

Peer Review it was noted that the intended application rate for clothianidin in the studies in surgar beet (Rexer 2014a and b) was 78 g a.s./ha, which is less than the maximum value for the proposed uses (see comment 5(34) in the Reporting Table). However, although the nominal application rate was 78 g a.s./ha, the actual application rate was higher in both studies (94 g a.s./ha and 80 g a.s./ha in Rexer 2014a and 2014b, respectively). The loading per pill was 0.6615 mg/pill and thus higher than the maximum registered rate (0.6 mg/seed). Due to some differences in the assumed seeding rate, the registered amount of clothianidin/ha in Spain is slightly higher than the actual application rates in the studies. For all other countries, the registered rate is exceeded in at least one of the trials.

All studies were conducted in Germany. Due to its climate, Germany offers ideal conditions for the formation of guttation fluid. The cool autumn and spring temperatures ensure that the fluid produced will be available for an extended period of time and not rapidly evaporate, maximizing the likelihood of exposure and representing reasonable worst case exposure conditions.

During Peer Review, it was argued that guttation depends on multiple factors including agronomic practices. The studies submitted were all conducted in Germany. It is stated that the data are worst case, however, this statement is not well documented (see comment 5(32) in the Reporting Table). In response to this comment, the applicant provided the following argumentation (*text in italic*):

Studies were set up under conditions (weather, soil temperature and soil moisture and humidity) that would promote guttation events as seen by the high frequency observed in the trials. The time of year was such that the persistence of the guttation fluid would also be maximized and this is also presented in the reports that the guttation fluid persistent up to the time of day for active bee flight. Observations showed bees to be visiting the crop albeit in low numbers which is due to the attractiveness of the crop. During the time where guttation was far more frequent (autumn) bees have a lower demand for water compared to spring (when guttation was less frequent) and temperatures were higher.
Although a hot dry environment would be the case where bees would have a higher water demand these are not conditions for the production or presence of guttation fluid. Consequently the conditions of the trials are considered to a reasonable worst case and bees were exposed and presence of test item in guttation fluid was confirmed analytically.

The argumentation above is considered acceptable, and RMS still considers the available guttation studies to provide reasonable worst case exposure conditions.

Table B.9.5.3.1-5: 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
Winter wheat	Seed treatment: CTD 375 g/L FS	CTD 100 g/ha Control	2 2	5 5	Pre-sowing (dust and guttation)	Autumn 2009 & Spring 2010	1.6/1 Hofmann & Lueckmann, 2014
Winter barley	Seed treatment: CTD 375 g/L FS	CTD 100 g/ha Control	2 2	5 5	Pre-sowing (dust and guttation)	autumn 2009 & Spring 2010	1.6/2 Hofmann, Garrido & Lueckmann, 2012
Winter barley	Seed treatment FS IMD+CTD 100+175 g/L	IMD+CTD 100+175 g/ha Control	5 5	5 5	Pre-sowing (dust and guttation)	Autumn 2011 & Spring 2012	1.6/3 Hofmann, Staffel & Aumeier, 2014
Sugar beet	Pill: CTD+IMD 0.6+0.3mg/pill	IMD+CTD 39+78 g/ha Control	1 1	8 8	Guttation from BBCH 12 (42 days)	Spring 2013	1.6/4 Rexer, 2014a
Sugar beet	Pill: CTD+IMD 0.6+0.3mg/pill	IMD+CTD 39+78 g/ha Control	1 1	8 8	Guttation from BBCH 12 (40 days)	Spring 2013	1.6/5 Rexer, 2014b

Notes: CTD = Clothianidin, IMD = Imidacloprid. Winter cereal seeds (control and treated) were additionally treated with a fluency agent 50 mL INTECO®/dt to reduce dust formation at drilling.

The studies in winter cereals were performed at a range of geographical locations (Northern, Central and Southern Germany) and over a period of years to ensure a wide range of natural and agricultural conditions. A total of nine clothianidin treated and nine untreated cereal fields were studied, which exceeds the minimum number of 5 sites that is recommended in the EFSA Guidance Document to obtain reliable results from field studies. Located at the edge of each winter cereal field were five honeybee colonies. These colonies were present at the edge of each field during sowing so were also exposed to dust generated by seed drilling equipment. Consequently a total of 30 colonies were exposed to guttation and dust drift in winter cereal crops (15 treated and 15 untreated). It is therefore considered that this is a comprehensive programme of research investigating the potential influence of guttation water from treated crops on honeybee colonies.

For sugar beets, results from only two study sites, located relatively close to each other in Southern Germany, are available. For the sugar beet studies, eight colonies were placed at the edge of each of the four fields (two treated and 2 untreated). Consequently a total of 32 colonies were exposed to guttation in sugar beet crops (16 treated and 16 untreated). While the number of study sites and geographical spread is limited, the sugar beet studies are still considered to provide a good indication of the potential influence of guttation water from treated crops on honeybee colonies.

As winter cereals are sown in autumn there are potentially two guttation periods to which honeybees could be exposed: one in autumn shortly after crop emergence and before overwintering and again in the spring after winter hibernation. In the cereal studies the same colonies were exposed to both guttation periods. Sugar beets are drilled in the spring and hence have one guttation period during that time.

At all test locations and for each crop guttation was observed. A summary of the frequency to which guttation was observed, the extent of bee exposure and the levels of residues encountered in guttation fluid is provided in Table B.9.5.2-1 in Section B.9.5.2. In winter cereals guttation was observed to be a fairly common event in both the autumn and spring exposure periods. However, far fewer bees were observed collecting guttation water in autumn compared to spring. Further, residues of clothianidin in guttation fluid were lower in spring compared to those in autumn. Consequently when residues are higher in autumn bees are far less likely to collect guttation water compared to the spring when residues are lower. In contrast to the observations winter cereals, guttation was far less common in sugar beet. Bees were active on days when guttation occurred but were only rarely observed to visit the fields sown with either treated or untreated seeds. Bees were not observed collecting guttation water from sugar beet plants at any time during these experiments. Overall, the exposure of honeybees to clothianidin residues in guttation fluid from sugar beet seems lower than from winter cereals.

At all test locations and for each crop the potential acute and chronic effects on honeybee colonies were monitored including mortality, behaviour, health status, colony strength and overwintering success. A summary of the effects due to exposure to guttation water from insecticide treated and control crops (no insecticide seed treatment) is shown in Table B.9.5.3.1-6.

Daily mortality levels of colonies located at the edge of winter cereal fields were generally observed to be at a low level. Occasional peaks of mortality were observed but these occurred at both treated and control sites and were of similar magnitude. There was a slight tendency for more frequent peaks at treated field sites than at control sites. However, these do not follow a systematic pattern related to guttation events or exposure and are most probably due to local weather conditions, especially in the studies conducted in autumn 2009 (Hofmann *et al.*, 2012 and Hofman & Leuckmann, 2014) where the weather was cold approaching winter.

Due to an error in the allocation of colonies at the initiation of the winter cereal studies initiated in 2009, a higher proportion of weaker colonies were assigned to the clothianidin sites than at the control sites so that the distribution of colonies was different across treatments with the control receiving the strongest ones. Due to this it was not possible to conclude on the overwintering success for colonies at the treated sites in Hofmann *et al.* (2012). However, in the study by Hofmann & Leuckmann (2014), which was performed in the harsh winter of 2009-2010 (the same year as the study by Hofmann *et al.* 2012), the overwintering rate was 86% and 89% in the colonies exposed at the control and treated sites, respectively. In a follow up study conducted in winter barley in 2011-2012 (Hoffmann *et al.*, 2014) where colonies were exposed to seed treated with both clothianidin and imidacloprid, again a harsh winter was experienced by the colonies. This lead to an overwintering rate of 57.8% and 67.9% in the colonies exposed at control and treated sites, respectively. The overall test conditions, even for the control colonies, were stringent in all studies with winter cereals. The colonies were placed in atypical locations (i.e. at the edge of a cereal field during autumn and spring) where there were no flowering crops within 3 km. Standard commercial beekeeping practice would not have chosen such tough locations for honeybee colonies.

In the studies where honeybee colonies were exposed to guttating sugar beet (Rexler, 2014a and b) mortality was generally low and consistent with no difference between the colonies located at the treated and control site. Bees were not observed to visit the fields (treated or control) to collect guttation water and presumably used other sources to meet their needs (e.g. water from the off-crop area – guttation, dew, rain). Overwintering success at these Southern Germany locations was 100% for all colonies irrespective of their study location.

Honeybee behaviour as well as other factors relating to colony wellbeing (colony strength, health status such as presence and level of Varroa, viruses and other pathogens) were unaffected by exposure to guttating winter cereal or sugar beet crops treated with clothianidin (and imidacloprid) as a seed treatment.

Table B.9.5.3.1-6: Observed effects on honeybee colonies after exposure to guttation fluid in the studies in winter cereals and sugar beet

Crop	Mortality Dead bees/colony/day				Behaviour	Colony strength & health status	Over-wintering success	Reference
Winter wheat	Trt	Season/ location	No. dead	Peak mort	No treatment related effects	No treatment related effects	CTD: 89% Con: 86%	1.6/1 Hofmann & Lueckmann, 2014
	CTD	A/N	<20	65				
		S/N	<20-40	60				
		A/S	<10	25				
		S/S	<10	-				
	Con	A/N	<20	80				
		S/N	<20-40	78				
		A/S	<10	25				
		S/S	<10-15	-				
Winter barley	Trt	Season/ location	No. dead	Peak mort	No treatment related effects	No treatment related effects	CTD: N/A Con: 80%	1.6/2 Hoffmann, Garrido & Lueckmann, 2012
	CTD	A/N	<20	60				
		S/N	<20-35	-				
		A/S	<20-40	100				
		S/S	<10-25	-				
	Con	A/N	<10-25	35				
		S/N	<10-20	56				
		A/S	<10-40	75				
		S/S	<10-25	-				
Winter barley	Autumn CTD+IMD: <20-55 Con: >20-45		Spring CTD+IMD: <20-40 Con: >20-35		No treatment related effects	No treatment related effects	CTD+IMD: 67.9% Con: 57.8%	1.6/3 Hoffmann, Staffel & Aumeier, 2014
Sugar beet	CTD+IMD: 16.6 Con: 12.9				No treatment related effects	No treatment related effects	CTD+IMD: 100% Con: 100%	1.6/4 Rexer, 2014a
Sugar beet	CTD+IMD: 22.4 Con: 21.5				No treatment related effects	No treatment related effects	CTD+IMD: 100% Con: 100%	1.6/5 Rexer, 2014b

Notes: Trt = treatment; CTD = Clothianidin; IMD = Imidacloprid, Con = control plots (no insecticide seed treatment). A/N = Autumn Northern Germany, S/N = Spring Northern Germany, A/S = Autumn Southern Germany, S/S = Spring Southern Germany.

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, even whith 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. This conclusion is supported by other studies from published literature, performed under both semi-

field and field conditions, on the impact and relevance of guttation events on the exposure of honeybees to neonicotinoid insecticides due to the cultivation of crops from treated seeds (Frommberger *et al.*[40], 2012 Joachimsmeier *et al.* [41], 2012a).

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[42]). Second, the treated crops (winter cereals and sugar beet) 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. Third, water collected for use by the colony can come from a variety of sources located close to the colony (generally within a few meters) and not just from guttation fluid. Dew and guttation fluid from off-crop vegetation can thus be an important (and likely more relevant) source of water. Especially off-crop grassland, which likely surrounds honeybee colonies, will provide more droplet/m² than the sown crop at an early stage. Fourth, as the plant grows the frequency of guttation events declines. Similarly, insecticide concentrations in guttation fluid tend to decline during spring. Overall, the exposure of honeybee colonies to clothianidin present in guttation fluid from sugar beets and winter cereals seems to be limited.

From the available field studies in winter cereals and sugar beets, the following can be concluded:

59. Guttation appears to occur more frequently in winter cereals than in sugar beet,
60. The conditions of the cereal study represented harsh environmental conditions which were not favourable for honeybee colonies irrespective of treatment,
61. Mortality, behaviour, as well as other factors relating to colony wellbeing (colony strength, health status such as presence and level of Varroa, viruses and other pathogens) were unaffected by exposure to guttating winter cereal or sugar beet crops treated with clothianidin as a seed treatment. At the winter cereal sites colonies were also exposed to dust generated by equipment at sowing.

During Peer Review, it was argued that in the field studies on guttation, the following wat noted (see comment 5(31) in the Reporting Table):

1. the frequency of guttation was high;
2. the bee activity was high during the occurrence of guttation;
3. bees were observed collecting guttation fluids, even if a low percentage (up to 16%);
4. high residue levels of clothianidin were detected in the guttation fluids;
5. the number of colonies per site was small (i.e.5), which may means that the studies have a low statistical power;
6. a trend of higher mortality than the control was also seen.

By considering the overwintering rates, the studies indicated no impact on honeybee colony of residues in guttation fluids. However, it was argued that a high acute risk cannot fully be excluded with these studies.

At Pesticides Peer Review Meeting 145, the higher tier risk assessment was discussed. A detailed discussion on each single study available investigating occurrence of guttation and effects on honeybees was not performed, but the available dataset was considered for drawing a conclusion. In this discussion, both the available studies for seed treatment in cereals and sugar beet (Bayer Crop

[40] Frommberger, M.; Pistorius, J.; Schier, A.; Joachimsmeier, I.; Schenke, D. (2012). Guttation and the risk for honey bee colonies (Apis mellifera L.): a worst case semi-field scenario in maize with special consideration of impact on bee brood and brood development. Hazards of pesticides to bees : 11th International Symposium of the ICP-BR Bee Protection Group ; Wageningen, (The Netherlands), November 2 - 4, 2011.

[41] Joachimsmeier, I.; Pistorius, J.; Heimbach, U.; Schenke, D.; Kirchner, W. (2012). Guttation and risk for honey bee colonies (Apis mellifera L.): Use of guttation drops by honey bees after migration of colonies - a field study. Hazards of pesticides to bees: 11th International Symposium of the ICP-BR Bee Protection Group; Wageningen, (The Netherlands), November 2 - 4, 2011.

[42] 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

Science, see Section B.9.5.1 of this Addendum) and for granular use in potato and maize (Sumitomo, see Section B.9.5.1 of the Addendum for the Sumitomo data) were considered together. This is considered justified as in the EFSA Conclusion for seed treatment and granular uses of clothianidin (2013)[43], 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 cereals and sugar beet 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. It was noted that, for cereals, if the three available studies would be pooled together, the statistical power might be higher.

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. 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).

Conclusion: Overall, the acute and chronic risk to honeybee colony development and survival, resulting from exposure to residues of clothianidin in guttation fluid produced by winter cereals and sugar beet plants at the currently registered maximum seed dressing rates, is considered acceptable.

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

[43] 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.

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.

In addition, the applicant provided the argumentation below (text in italic) to demonstrate that bumblebees and solitary bees collect only very low amounts of water, and thus that exposure to guttation fluid represents a negligible route of exposure for non-*Apis* bees. As the risk to non-*Apis* bees is already covered by the risk assessment for honeybees, the reliability of the papers referenced in this argumentation was not evaluated by the RMS.

Only honeybees (Genus: Apis) are known to collect water for thermoregulation and diluting food (Lindauer[44], 1955; Seeley[45], 1986, Roubik[46], 1989). Solitary bees obtain their water needs from dietary sources and the high level of metabolic water generated during flight. Michener[47] (1974), states that the only source of water for solitary bees is floral nectar and according to a review by Nicholson[48] (2009), it is not clear whether solitary bees drink water for their own needs as distinct from seeking dilute nectar. Indeed there are several other publications that conclude different bee species obtain their water needs from nectar and therefore do not take up water (Bertsch[49], 1984; Nicolson & Louw[50], 1982; Nicolson[51], 1998; Willmer[52], 1986 and Willmer[53], 1988).

For bumblebees it is expected that they do not collect drinking water (Nicolson 2009). Nevertheless marked individuals of Bombus terrestris were observed to collect water under hot and dry conditions, although the reason remains unclear (Ferry & Corbet[54], 1996). The authors state that it is unlikely that there is a water deficit for individual worker bees and such behaviour has never been reported for bumblebees before and thus the behaviour might be a consequence of unusual warm and dry weather conditions. There are also no further publications which record such behaviour by bumblebees again suggesting that this is not a common occurrence. Overall for individual bees there is the major problem of disposing of the excess water from their diet which is based on nectar leading to the need to maximize energy gain and minimize water load.

Consequently a risk assessment for non-Apis bees due to exposure to guttation fluid represents a negligible route of exposure so no risk assessment is required. Consequently the risk to non- Apis bees is acceptable.

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[44] Lindauer. M. (1955). The water economy and temperature regulation of the honeybee colony. Bee World 36. 62-72; 81-92; 105-111.

[45] Seeley. T.D. (1995). The Wisdom of the Hive: the Social Physiology of Honeybee Colonies. Cambridge. MA: Harvard University Press.

[46] Roubik. D.W. (1989). Ecology and natural history of tropical bees. Cambridge University Press. Cambridge

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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

The applicant submitted three studies in which the dust drift ground deposition was assessed in either winter wheat or winter barley. Further, two effect studies were submitted, that investigated the potential impact of dust drift from clothianidin treated winter barley or sugar beet seeds on honeybees. In both effect studies, the dust drift deposition was also assessed.

Dust drift during the sowing of treated cereal seeds

Report:	**1.7/1; Hofmann, S. & Lueckmann, J.; 2010a**
Title:	**Monitoring of dust drift deposits during and after sowing of winter barley (W-BAR) treated with Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 145.2 (60 + 70 + 7.2 + 8 g/L) or Clothianidin & Beta-Cyfluthrin FS 455 (375 + 80 g/L) on fields in Germany**
Report No.:	R09247-1
Document No.:	M-366273-01-1
Guideline(s):	91/414/EEC of July 15, 1991, SANCO/3029/99 Rev. 4, 2000-07-11
Guideline deviation(s):	not specified
GLP/GEP:	no

Objective

The objective of the study was to determine the residues of imidacloprid and clothianidin in dust drift deposits during and after sowing of winter barley treated with Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 145.2 (60 + 70 + 7.2 + 8 g/L) or Clothianidin & Beta-Cyfluthrin FS 455 (375 + 80 g/L) on fields in Germany.

Material and Methods

Test item

Two different winter barley (W-BAR) varieties (i.e. Lomerit and Highlight) were purchased untreated and commercially cleaned-up from a commercial seed distributor (Gut Peterhof, D-50127 Bergheim, Germany) and were thereafter seed-treated at Bayer CropScience's Seed Treatment Application Centre in D-40789 Monheim am Rhein, Germany (non-GLP):

62. Manta® Plus FS 145.2 (TOX08744-00) treated winter barley seeds, dressed with 1000mL product/100 kg seeds (= nominally 70 g imidacloprid/100 kg seeds); identification of treated seeds: TOX08780-00 (variety Lomerit); TOX08779-00 (variety Highlight)

And

63. Smaragd® forte FS 455 (TOX08741-00) treated winter barley seeds, dressed with 133mL product/100 kg seeds (= nominally 50 g clothianidin/100 kg seeds); identification of treated seeds: TOX08775-00 (variety Lomerit); TOX08774-00 (variety Highlight).

After seed-dressing, the seeds were subject to chemical analysis for the determination of the actual seed loading. Finally, the seed bags were unequivocally labelled and shipped via road transport to the respective study sites in Germany.

No measurements of the Heubach value (% dust) and not Heubach-as-value (considering the concentration of as in dust) were made for the treated seeds used in this study.

Study sites and sowing

The multiple site study was conducted at two different regions in Germany: one in Southern Germany in the federal state of Baden-Würtemberg in Renningen, southwest of Stuttgart at the experimental station Ihinger Hof of the University Hohenheim (in the following called Ihinger Hof) and the second in Northern Germany in the federal state of Lower Saxony near Celle northeast of Hannover (in the following called Celle) with two fields per location. The sizes of the test fields sown with Manta® Plus-treated W-BAR seeds at Ihinger Hof and Celle were 4.8 ha and 8.0 ha, respectively. The fields drilled with Smaragd® forte treated W-BAR seeds at Ihinger Hof and Celle were 3.9 ha and 7.0 ha, respectively. The variety of W-BAR sown at Ihinger Hof was 'Highlight' and the variety drilled at Celle was 'Lomerit'. The soil type for each of the study field is not reported.

A total of 200 kg seeds/ha were sown at both test locations resulting in nominal application rates of 140 g imidacloprid a.s./ha on fields drilled with Manta® Plus and 100 g clothianidin a.s./ha on fields drilled with Smaragd® forte. The seeds were drilled using two different pneumatic sowing machines:

64. Field 8 and 9: 4 m Accord Pneumatic DA Kreiselegge HR 4001
65. Field 14 and 15: 3 m Amazone AD-P 303 Special

Table B.9.6.1-1: Study field sites, winter barley varieties and sowing procedures

Sample no.	Field no.	Municipality, federal state	Field size* (ha)	W-BAR variety	Seed treatment	Sowing density (kg/ha)	Nominal appl. Rate (g a.s./ha)
1	8	71272 Renningen (Ihinger hof), BW	4.8	Highlight	Manta Plus	200	140[1]
2	9		3.9		Smaragd forte		100[2]
3	15	29223 Celle, LS	7.0	Lomerit	Smaragd forte		100[2]
4	14		8.0		Manta plus		140[1]

**sown area; [1] = imidacloprid; [2] = clothianidin; BW = Baden-Württemberg; LS = Lower Saxony*

Sampling method during sowing

Shortly before sowing the wind direction at the site was determined and ten Petri-dishes were placed in groups of two at distances of 1, 3 and 5 m from the downwind border of the field to give a total of 30 Petri-dishes per field. The actual placement of the Petri-dishes on the field edges followed the actual wind direction, in order to collect as much dust as possible. The actual situation per monitoring field, including the exact position of the sampling areas in relation to the rest of the field, the study plot dimensions (length & width of the sown area), any adaptations to the prevailing local conditions as well as the wind direction and wind speed during the sowing operation was documented in the raw data.

Each Petri-dish for sampling dust drift deposits (Ø 13.7 cm, 147.41 cm²) was filled with 70 to 80 ml of a 1:1 (v/v) glycerol/water mixture immediately before the start of the sowing. The Petri-dishes were arranged horizontally using metal racks approximately 1.5 to 2 cm above the soil or at the height of the ground vegetation surface, depending on the field boundary morphology. If necessary, the vegetation at the field border was removed to allow air to move freely across the open Petri-dishes. In order to allow any airborne dust to settle, the Petri-dishes remained open for 15 minutes following the cessation of sowing operations. The aqueous sampling medium of each Petri-dish was then individually transferred to a separate polyethylene flask. To ensure that all possible deposits of imidacloprid or respectively clothianidin from the inside of the Petri-dish were transferred to the corresponding polyethylene flask, each Petri-dish and its corresponding funnel was additionally rinsed with fresh tap water (≈ 20 mL) and the rinse was combined with the content of the respective Petri-dish within the corresponding polyethylene flask. After rinsing, each polyethylene flask was tightly closed. To avoid cross-contaminations the Petri-dishes were always approached from the downwind direction. Each polyethylene flask was labelled with the sampling date and an individual sample identification number consisting of the field number and the sampler number.

Sampling method after sowing

In order to monitor any potential dust drift during the 24h-period following sowing, a second set of ten Petri-dishes were placed in pairs at the approximate middle of each field side at a distance of 1 m to the field borders to give a total of 40 Petri-dishes per field. After 24 hours the sampling medium from each dish was individually transferred to a separate polyethylene flask following up the same workflow as described in the section above.

Weather conditions

When samples were collected during sowing operations, wind speed and wind direction were determined with the aid of an anemometer. Readings were recorded at the beginning of sowing operations at regular intervals during the sowing process, once sowing was completed and at appropriate times thereafter. Minimum and maximum air temperatures and precipitation were recorded (using a min-max thermometer and rain gauge, respectively) from the beginning of sowing until the end of the 24h post-sowing sampling period.

Residue analysis

Imidacloprid and clothianidin residues in the samples were subsequently determined by Bayer CropScience AG by High Performance Liquid Chromatography, coupled with Tandem Mass Spectrometry. Until shipment, the samples were stored at room temperature.

Results

Weather conditions

The weather conditions as measured for each of the field sites are shown in Table B.9.6.1-2 to B.9.6.1-5.

Table B.9.6.1-2: Weather conditions during sowing and the 24h sampling period at field 8 (treated with imidacloprid).

Date	Time (hh:mm)	Wind speed (m/s)	Wind direction	Σ Precipitation (mm)*	Temperature (°C)**
22.09.2009	15:00 (start of sowing)	0-2	SSO	0	Min. 12 Max. 22
	15:30	1-2	O-NO		
	16:00	1-2	N-NO		
	17:00	1-2	NO		
	18:15	0-1	N		
	19:00	0-1	N		
	19:25 (end of sowing)	0	-		
	20:00 (start of 24h sampling)	0-1	W	0	
	21:00	0	-		
	22:00	0	-		
23.09.2009	18:00	0-1	NW		
	19:00	0-1	NW		
	20:00 (end of 24h sampling)	0-1	NW		

**at the end of the respective sampling; **between beginning and end of the sampling*

Table B.9.6.1-3: Weather conditions during sowing and the 24h sampling period at field 9 (treated with clothianidin).

Date	Time (hh:mm)	Wind speed (m/s)	Wind direction	Σ Precipitation (mm)*	Temperature (°C)**
23.09.2009	10:00 (start of sowing)	0-1	SO	0	Min. 8 Max. 23
	11:00	0-1	NO		
	12:00	0-1	N		
	13:00	0-1	NO		
	14:00	0-1	SO		
	14:30 (end of sowing)	1-2	NW		
	15:15 (start of 24h sampling)	0-1	NW	0	
	16:15	0-1	NO		
	17:15	0-1	NW		
	18:15	0-1	NW		
24.09.2009	12:15	0-1	SO		
	13:15	0-1	NW		
	14:15 (end of 24h sampling)	1-2	N		

**at the end of the respective sampling; **between beginning and end of the sampling*

Table B.9.6.1-4: Weather conditions during sowing and the 24h sampling period at field 15 (treated with clothianidin).

Date	Time (hh:mm)	Wind speed (m/s)	Wind direction	Σ Precipitation (mm)*	Temperature (°C)**
28.09.2009	15:00 (start of sowing)	3-4	W	0	Min. 14 Max. 20
	16:00	0-1	W		
	17:00	3-4	W		
	18:00	1-2	NW		
	19:00	1-2	NW		
	20:00	0.1	NW		
	20:15 (end of sowing)	0-1	NW		
	21:00 (start of 24h sampling)	0-1	NW	5	
	22:00	1-2	W		
	23:00	0-1	NW		
	24:00	0-1	W		
29.09.2009	15:00	2-4	W		
	16:15 (end of 24h sampling)	1-3	W		

**at the end of the respective sampling; **between beginning and end of the sampling*

Table B.9.6.1-5: Weather conditions during sowing and the 24h sampling period at field 14 (treated with imidacloprid).

Date	Time (hh:mm)	Wind speed (m/s)	Wind direction	Σ Precipitation (mm)*	Temperature (°C)**
30.09.2009	12:25 (start of sowing)	2-3	W	< 0.5	Min. 12 Max. 23
	13:25	2-4	SW		
	14:25	3-4	SW		
	15:25	2-4	SW		
	16:25	2-3	SW		
	17:25	2-3	SW		
	17:45 (end of sowing)	0-1	SW		
	18:15 (start of 24h sampling)	0-1	SW	4.0	
	19:15	1-2	SW		
	20:15	2-4	SW		
	21:15	4-6	SW		
01.10.2009	15:15	2-3	NW		
	16:15	4-6	NW		
	17:15	4-7	NW		
	18:15 (end of 24h sampling)	2-3	NW		

**at the end of the respective sampling; **between beginning and end of the sampling*

Dust drift samples

A total number of 279 samples were collected from fields drilled with Manta® Plus or Smaragd® forte -treated seeds. One Petri-dish was inadvertently left closed. Of these 279 samples, 208 samples (74.5 %) were found to contain no quantifiable residues of imidacloprid or clothianidin, respectively (<LOQ); this included 194 samples (69.5% of all 279 samples) with no detectable residues (<LOD). A total of 63 samples (22.6 %) were found to contain residues of imidacloprid or clothianidin above the limit of quantification (LOQ). 55 of these samples were taken at the time of sowing, the remaining 8 were taken 24h after drilling was completed. The maximum observed residue level was 0.283 g a.s./ha (see Table B.9.6.1-1).

For mathematical processing, the data sets obtained with imidacloprid and clothianidin were combined and any residue value below the limit of detection (LOD = 0.004 g a.s./ha) was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification (LOQ = 0.014 g a.s./ha) was conservatively set to equal the LOQ. The calculated average residue values for samples collected during the sowing operation were 0.019 g a.s./ha for samples at a nominal distance of 1 m to the sowing border, 0.029 g a.s./ha for samples at a nominal distance from of 3 m and 0.020 g a.s./ha for samples at a nominal distance of 5 m. For the samples collected during a 24h-period after sowing, the average residue value was below the LOQ. The 90th percentile residue values during the sowing operation were 0.037 g a.s./ha, 0.031 g a.s./ha and 0.027 g a.s./ha for the nominal distance of 1 m, 3 m and 5 m, respectively. For the samples collected during a 24h-period after sowing, the 90th percentile residue value was below the LOD (see Table B.9.6.1-6).

Table B.9.6.1-6: Summary of residues (imidacloprid and clothianidin combined) at respective distances to the field borders

	During Sowing			24h-sampling	Total
Nominal distance (actual distance)°	**1m (1m)**	**3m (3m)**	**5m (4.5-5m)**	**1m (0.8-1m)**	
No. of samples analysed	40	40	40	159	279
No. of samples not recovered in the field	0	0	0	1	1
Residue level	**Number of samples with residue levels [n]**				
<LOQ	22	21	22	151	216
0.014-0.050 g a.s./ha	18	16	17	8	59
0.051-0.100 g a.s./ha	0	0	0	0	0
>0.100 g a.s./ha	0	3	1	0	4
Residue levels [g a.s./ha]					
Average**	0.019	0.029	0.020	<LOD	n.a.
90th percentile**	0.037	0.031	0.027	<LOD	
Maximum**	0.045	0.283	0.272	0.026	

LOD = 0.004 g a.s./ha (imidacloprid, clothianidin); LOQ = 0.014 g a.s./ha (imidacloprid, clothianidin); n.a. = not applicable

° In some cases the position of the Petri-dishes had to be adjusted from the intended distance due to the surrounding structures of the field.

** In one case due to an operator error the lid of one single Petri-dish was inadvertently not removed during the 24h-period after sowing; as such, no potentially dislodged residues could be trapped with this particular Petri-dish and consequently this sample was not considered for the mathematical processing.*

*** Calculated from the respective number of analysed samples, imidacloprid and clothianidin, combined; any residue value below the limit of detection was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification was conservatively set to equal the LOQ.*

Conclusion

The present study included 4 treatment groups, with two varieties of winter barley either treated with imidacloprid or clothianidin, sown at 4 different fields. Dust drift was monitored in Petri-dishes placed at several distances from the downwind border of the field during sowing until 15 minutes after sowing, and in Petri-dishes at 1m distance at each side of the field for 24h after sowing.

The 90th percentile calculated for the combined data set of all 4 fields was 0.037 g a.s./ha, 0.031 g a.s./ha, and 0.027 g a.s./ha for a distance of 1 m 3, and 5 m respectively. The 90th percentile for the 24 h samples was < LOD (<0.004 g a.s./ha). These results indicate that the dust drift deposits, produced during and after the sowing of Manta® Plus or Smaragd® forte - treated W-BAR seeds with pneumatic sowing machines, are limited.

RMS Comments

The present study has the same experimental set-up as study 1.7/2 (Hofmann & Lueckmann, 2010b), with only the sown crop being different (winter barley vs. winter wheat). The same comments apply to both studies.

Several experimental deviations are reported, which are discussed below. First, the wind direction was not constant during the sowing at the Ihinger Hof sites. As a concequence, it is likely that the Petri-dishes were not exposed the a 'worst case' dust drift scenario for the whole of the monitoring period. Second, petri-dishes were not always placed at the right distance from the field border due to natural or artificial obstacles. As there is no clear relationship between the distance to the field and the concentration of active substance measured in the Petri dishes, this is however not considered a problem. Third, the 24h sampling period was stopped prematurely (before the end of the 24h period) on field 15.

Even though imidacloprid and clothianidin were applied to separate fields, their measured concentrations in the Petri-dishes are combined to calculate on 90th percentile residue value for the whole study. The applicant argues that these studies are intended as a measure of dust drift from

commercial formulations, and are therefore not specifically related to imidacloprid or clothianidin. As this Addendum concerns clothianidin only, data for clothianidin should be available for the risk assessment. Results specifically for clothianidin dust drift are reported in the report of the analytical phase of the present study (report No. MR-09-153 / M-359032-01-1), and are summarized in Table B.9.6.1-7 below. As there is no clear relationship between the distance to the field and the measured concentration of the active substance, an average and 90th percentile value for all samples was calculated as well.

Table B.9.6.1-7: Summary of residues for clothianidin at respective distances to the field borders

	During Sowing				24h-sampling
Nominal distance (actual distance)°	**1m (1m)**	**3m (3m)**	**5m (4.5-5m)**	**Over all distances**	**1m (0.8-1m)**
No. of samples analysed	20	20	20	60	79
No. of samples not recovered in the field	0	0	0	0	1
Residue level	**Number of samples with residue levels [n]**				
<LOQ	10	10	11	31	71
0.014-0.050 g a.s./ha	10	7	8	25	8
0.051-0.100 g a.s./ha	0	0	0	0	0
>0.100 g a.s./ha	0	3	1	4	0
Residue levels [g a.s./ha]					
Average**	0.022	0.045	0.025	0.031	<LOQ
90th percentile**	0.042	0.213	0.025	0.042	0.019
Maximum**	0.045	0.283	0.272	0.283	0.026

LOD = 0.004 g a.s./ha (clothianidin); LOQ = 0.014 g a.s./ha (clothianidin)

° In some cases the position of the Petri-dishes had to be adjusted from the intended distance due to the surrounding structures of the field.

** In one case due to an operator error the lid of one single Petri-dish was inadvertently not removed during the 24h-period after sowing; as such, no potentially dislodged residues could be trapped with this particular Petri-dish and consequently this sample was not considered for the mathematical processing.*

*** Calculated from the respective number of analysed samples; any residue value below the limit of detection was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification was conservatively set to equal the LOQ.*

During Peer Review, it was argued that this study (together with the study by Hofmann (2014b) gives only limited information for evaluation of dust deposition (see comment 5(37) in the Reporting Table). It was considered that it cannot be decided whether these two studies reflect a best-case situation as no Heubach value (% dust) and no Heubach-as values (considering the concentration of as in dust) are available. Further, RMS was requested to give more experimental data about the studies: meteorological data and data on soil type. The information that was available from the study report on wind, temperature and precipitation was included in the study summary above for each trial site. The soil type of each site could not be included, as it is not reported in the study report. RMS was also requested to present the results from each of the trial sites separately instead of only showing an overall summary of the results. The results for the two study sites treated with clothianidin were obtained from the report of the analytical phase of the present study (report No. MR-09-153 / M-359032-01-1), and are summarized, for each study site separately, in Table B.9.6.1-8. The results for the two sites treated with imidacloprid are not included here.

Table B.9.6.1-8: Summary of residues for clothianidin, measured at each field separately, at respective distances to the field borders.

Field 9 – Ihinger hof					
	During Sowing				**24h-sampling**
Nominal distance (actual distance)°	**1m (1m)**	**3m (3m)**	**5m (4.5-5m)**	**Over all distances**	**1m (0.8-1m)**
No. of samples analysed	10	10	10	30	39
No. of samples not recovered in the field	0	0	0	0	1
Residue level	**Number of samples with residue levels [n]**				
<LOQ	10	10	10	30	39
0.014-0.050 g a.s./ha	0	0	0	0	0
0.051-0.100 g a.s./ha	0	0	0	0	0
>0.100 g a.s./ha	0	0	0	0	0
Residue levels [g a.s./ha]					
Average**	<LOQ	<LOQ	<LOQ	<LOQ	<LOD
90th percentile**	<LOQ	<LOD	<LOD	<LOD	<LOD
Maximum**	<LOQ	<LOQ	<LOQ	<LOQ	<LOD
Field 15 – Celle					
	During Sowing				**24h-sampling**
Nominal distance (actual distance)°	**1m (1m)**	**3m (3m)**	**5m (4.5-5m)**	**Over all distances**	**1m (0.8-1m)**
No. of samples analysed	10	10	10	30	40
No. of samples not recovered in the field	0	0	0	0	0
Residue level	**Number of samples with residue levels [n]**				
<LOQ	0	0	1	1	32
0.014-0.050 g a.s./ha	10	7	8	25	7
0.051-0.100 g a.s./ha	0	0	0	0	0
>0.100 g a.s./ha	0	3	1	4	0
Residue levels [g a.s./ha]					
Average**	0.031	0.086	0.044	0.054	<LOQ
90th percentile**	0.045	0.276	0.247	0.213	0.024
Maximum**	0.045	0.283	0.272	0.283	0.026

LOD = 0.004 g a.s./ha (clothianidin); LOQ = 0.014 g a.s./ha (clothianidin)

° In some cases the position of the Petri-dishes had to be adjusted from the intended distance due to the surrounding structures of the field.

** In one case due to an operator error the lid of one single Petri-dish was inadvertently not removed during the 24h-period after sowing; as such, no potentially dislodged residues could be trapped with this particular Petri-dish and consequently this sample was not considered for the mathematical processing.*

*** Calculated from the respective number of analysed samples; any residue value below the limit of detection was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification was conservatively set to equal the LOQ.*

During Peer Review it was noted that there was a highly increased deposition at 3 m and at 5 m (up to 0.28 g a.s./ha) compared to 1 m (0.03 – 0.05 g a.s./ha) (see comment 5(37) in the Reporting Table). The reason for this increase is unclear. It is noted that such high residue values for clothianidin were only measured on field 15, in 3 out of 10 samples at 3 m and in 1 out of 10 samples at 5 m. This could potentially be explained by a relatively high variability in both wind speed and wind direction during the trial at this field site, which could have resulted in some Petri dishes receiving an unexpectedly high amount of dust. In contrast to the maximum values, the average residues at 1, 3 and 5 m are similar, especially of the results from field 9 are also taken into account.

Overall, due to the limitations discussed above, the quantitative data obtained in these studies are not considered the be suitable as a 'worst case' for use in risk assessment. At Pesticides Peer Review Meeting 145, the experts agreed to this conclucion. No information on the Heubach value and the Heubach a.s. value is available, and this information is considered as essential by SANCO/10553/2012 to properly address dust drift deposition.

Report:	**1.7/2; Hofmann, S. & Lueckmann, J.; 2010b**
Title:	**Monitoring of dust drift deposits during and after sowing of winter wheat (W-WHT) treated with Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 145.2 (60 + 70 + 7.2 + 8 g/L) or Clothianidin & Beta-Cyfluthrin FS 455 (375 + 80 g/L) on fields in Germany**
Report No.:	R09247-2
Document No.:	M-366277-01-1
Guideline(s):	91/414/EEC of July 15, 1991, SANCO/3029/99 Rev. 4, 2000-07-11
Guideline deviation(s):	not specified
GLP/GEP:	no

Objective
The objective of the study was to determine the residues of imidacloprid and clothianidin in dust drift deposits during and after sowing of winter wheat treated with Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 145.2 (60 + 70 + 7.2 + 8 g/L) or Clothianidin & Beta-Cyfluthrin FS 455 (375 + 80 g/L) on fields in Germany.

Material and Methods
Test item
Two different winter wheat (W-WHT) varieties (i.e. Hermann and Manager) were purchased untreated and commercially cleaned-up from a commercial seed distributor (Gut Peterhof, D-50127 Bergheim, Germany) and were thereafter seed-treated at Bayer CropScience's Seed Treatment Application Centre in D-40789 Monheim am Rhein, Germany (non-GLP):

66. Manta® Plus FS 145.2 (TOX08744-00) treated winter wheat seeds, dressed with 1000mL product/100 kg seeds (= nominally 70 g imidacloprid/100 kg seeds); identification of treated seeds: TOX08781-00 (variety Manager); TOX08782-00 (variety Hermann)

And

67. Smaragd® forte FS 455 (TOX08741-00) treated winter wheat seeds, dressed with 133mL product/100 kg seeds (= nominally 50 g clothianidin/100 kg seeds); identification of treated seeds: TOX08776-00 (variety Manager); TOX08777-00 (variety Hermann)

After seed-dressing, the seeds were subject to chemical analysis for the determination of the actual seed loading. Finally, the seed bags were unequivocally labelled and shipped via road transport to the respective study sites in Germany.

No measurements of the Heubach value (% dust) and not Heubach-as-value (considering the concentration of as in dust) were made for the treated seeds used in this study.

Study sites and sowing
The multiple site study was conducted at two different regions in Germany: one in Southern Germany in the federal state of Baden-Würtemberg in Renningen, southwest of Stuttgart at the experimental station Ihinger Hof of the University Hohenheim (in the following called Ihinger Hof) and the second in Northern Germany in the federal state of Lower Saxony near Celle northeast of Hannover (in the following called Celle) with two fields per location. The sizes of the test fields sown with Manta® Plus-treated W-WHT seeds at Ihinger Hof and Celle were 6.0 ha and 16.21 ha, respectively. The fields drilled with Smaragd® forte treated W-WHT seeds at Ihinger Hof and Celle were 4.0 ha and 9.84 ha,

respectively. The variety of W-WHT sown at both study sites was 'Manager'. The soil type for each of the study field is not reported.

A total of 200 kg seeds/ha were sown at both test locations resulting in nominal application rates of 140 g imidacloprid a.s./ha on fields drilled with Manta® Plus and 100 g clothianidin a.s./ha on fields drilled with Smaragd® forte. The seeds were drilled using two different pneumatic sowing machines:

68. Field 11 and 12: 3 m John Deere 750 A, incl. harrow
69. Field 17 and 18: 6 m Horsch Pronto 6 DC, incl. harrow

Table B.9.6.1-9: Study field sites, winter wheat varieties and sowing procedures

Sample no.	Field no.	Municipality, federal state	Field size* (ha)	W-BAR variety	Seed treatment	Sowing density (kg/ha)	Nominal appl. Rate (g a.s./ha)
5	12	71272 Renningen (Ihinger hof), BW	4.0	Hermann	Manta Plus	200	140[1]
6	11		6.0		Smaragd forte		100[2]
7	17	29223 Celle, LS	16.2	Manager	Smaragd forte		100[2]
8	18		9.8		Manta plus		140[1]

**sown area; [1] = imidacloprid; [2] = clothianidin; BW = Baden-Württemberg; LS = Lower Saxony*

Sampling method during sowing

Shortly before sowing the wind direction at the site was determined and ten Petri-dishes were placed in groups of two at distances of 1, 3 and 5 m from the downwind border of the field to give a total of 30 Petri-dishes per field. The actual placement of the Petri-dishes on the field edges followed the actual wind direction, in order to collect as much dust as possible. The actual situation per monitoring field, including the exact position of the sampling areas in relation to the rest of the field, the study plot dimensions (length & width of the sown area), any adaptations to the prevailing local conditions as well as the wind direction and wind speed during the sowing operation was documented in the raw data.

Each Petri-dish for sampling dust drift deposits (Ø 13.7 cm, 147.41 cm²) was filled with 70 to 80 ml of a 1:1 (v/v) glycerol/water mixture immediately before the start of the sowing. The Petri-dishes were arranged horizontally using metal racks approximately 1.5 to 2 cm above the soil or at the height of the ground vegetation surface, depending on the field boundary morphology. If necessary, the vegetation at the field border was removed to allow air to move freely across the open Petri-dishes. In order to allow any airborne dust to settle, the Petri-dishes remained open for 15 minutes following the cessation of sowing operations. The aqueous sampling medium of each Petri-dish was then individually transferred to a separate polyethylene flask. To ensure that all possible deposits of imidacloprid or respectively clothianidin from the inside of the Petri-dish were transferred to the corresponding polyethylene flask, each Petri-dish and its corresponding funnel was additionally rinsed with fresh tap water (≈ 20 mL) and the rinse was combined with the content of the respective Petri-dish within the corresponding polyethylene flask. After rinsing, each polyethylene flask was tightly closed. To avoid cross-contaminations the Petri-dishes were always approached from the downwind direction. Each polyethylene flask was labelled with the sampling date and an individual sample identification number consisting of the field number and the sampler number.

Sampling method after sowing

In order to monitor any potential dust drift during the 24h-period following sowing, a second set of ten Petri-dishes were placed in pairs at the approximate middle of each field side at a distance of 1 m to the field borders to give a total of 40 Petri-dishes per field (where necessary the distance of 1 m had to be adjusted to the field boundary morphology). After 24 hours the sampling medium from each dish was individually transferred to a separate polyethylene flask following up the same workflow as described in the section above.

Weather conditions

When samples were collected during sowing operations, wind speed and wind direction were determined with the aid of an anemometer. Readings were recorded at the beginning of sowing operations at regular intervals during the sowing process, once sowing was completed and at appropriate times thereafter. Minimum and maximum air temperatures and precipitation were recorded (using a min-max thermometer and rain gauge, respectively) from the beginning of sowing until the end of the 24h post-sowing sampling period.

Residue analysis

Imidacloprid and clothianidin residues in the samples were subsequently determined by Bayer CropScience AG by High Performance Liquid Chromatography, coupled with Tandem Mass Spectrometry. Until shipment, the samples were stored at room temperature.

Results

Weather conditions

The weather conditions as measured for each of the field sites are shownin Table B.9.6.1-10 to B.9.6.1-13.

Table B.9.6.1-10: Weather conditions during sowing and the 24h sampling period at field 11 (treated with clothianidin).

Date	Time (hh:mm)	Wind speed (m/s)	Wind direction	Σ Precipitation (mm)*	Temperature (°C)**
06.10.2009	08:45 (start of sowing)	2-4	SW	0	Min. 12 Max. 27
	09:45	1-3	S		
	10:45	1-2	S		
	11:45	1-2	SW		
	12:45	1-2	S		
	13:45	1-3	S		
	14:45	0-1	S		
	15:45	1-3	S		
	16:45	2-4	SW		
	17:45 (end of sowing)	3-5	SW		
	18:00 (start of 24h sampling)	2-4	SW	0	
	19:00	2-4	S		
	20:00	2-4	S		
	21:00	2-5	S		
07.10.2009	15:00	1-2	SW		
	16:00	1-3	SW		
	17:00	0-2	S		
	18:00 (end of 24h sampling)	0-1	SO		

**at the end of the respective sampling; **between beginning and end of the sampling*

Table B.9.6.1-11: Weather conditions during sowing and the 24h sampling period at field 12 (treated with imidacloprid).

Date	Time (hh:mm)	Wind speed (m/s)	Wind direction	Σ Precipitation (mm)*	Temperature (°C)**
05.10.2009	09:00 (start of sowing)	2-4	SW	< 0.01	Min. 8 Max. 14
	10:00	1-3	S		
	11:00	1-2	SW		
	12:00	1-2	S		
	13:00	1-2	SO		
	14:00 (end of sowing)	1-3	S		
	14:15 (start of 24h sampling)	0-1	S	2.5	
	15:15	1-3	S		
	16:15	2-4	S		
	17:15	3-5	SW		
07.10.2009	20:00 (end of 24h sampling)°	2-4	SW		

**at the end of the respective sampling; **between beginning and end of the sampling; °24h-sampling had to be cancelled at 20:00 due to upcoming rain.*

Table B.9.6.1-12: Weather conditions during sowing and the 24h sampling period at field 17 (treated with clothianidin).

Date	Time (hh:mm)	Wind speed (m/s)	Wind direction	Σ Precipitation (mm)*	Temperature (°C)**
15.10.2009	14:00 (start of sowing)	3-5	NW	0	Min. 3.5 Max. 9
	15:00	4-5	N/NW		
	16:05	1-2	NW		
	17:00	1-2	NW		
	18:00 (end of sowing)	1-2	NW		
	18:45 (start of 24h sampling)	1-2	NW	8.5	
	19:45	1-2	NW		
	20:45	0-1	NW		
	21:45	0-1	NW		
16.10.2009	12:45	5-7	W		
	13:45	5-7	W		
	14:45	6-9	W/NW		
	15:45 (end of 24h sampling)	6-9	W/NW		

**at the end of the respective sampling; **between beginning and end of the sampling*

Table B.9.6.1-13: Weather conditions during sowing and the 24h sampling period at field 18 (treated with imidacloprid).

Date	Time (hh:mm)	Wind speed (m/s)	Wind direction	Σ Precipitation (mm)*	Temperature (°C)**
15.10.2009	18:20 (start of sowing)	1-2	NW	0	Min. 3.5 Max. 9
	19:20	1-2	NW		
	20:05 (end of sowing)	1-2	NW		
	20:45 (start of 24h sampling)	0-1	NW	8.5	
	21:45	0-1	NW		
	22:45	0-1	NW		
	23:40	0-1	NW		
16.10.2009	13:45	5-7	W		
	14:45	6-9	W/NW		
	15:45	6-9	W/NW		
	16:45 (end of 24h sampling)	3-5	W		

**at the end of the respective sampling; **between beginning and end of the sampling*

Dust drift samples

A total number of 280 samples were collected from fields drilled with Manta® Plus or Smaragd® forte -treated seeds. Of these 280 samples, 272 samples (97.1 %) were found to contain no quantifiable residues of imidacloprid or clothianidin, respectively (< LOQ); this included 228 samples (81.4% of all 280 samples) with no detectable residues (<LOD). A total of 8 samples (2.8 %) were found to contain residues of imidacloprid or clothianidin above the limit of quantification (LOQ). 5 of these samples were taken at the time of sowing, the remaining 3 were taken 24h after drilling was completed. The maximum observed residue level was 0.258 g a.s./ha (see Table B.9.6.1-14).

For mathematical processing, the data sets obtained with imidacloprid and clothianidin were combined and any residue value below the limit of detection (LOD = 0.004 g a.s./ha) was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification (LOQ = 0.014 g a.s./ha) was conservatively set to equal the LOQ. Both, the calculated average and 90th percentile residue values for all samples collected during the sowing operation at the nominal distances of 1 m, 3 m and 5 m were below LOQ. For the samples collected during a 24h-period after sowing, the average residue value was < LOQ and the 90th percentile residue value was < LOD (see Table B.9.6.1-14).

Table B.9.6.1-14: Summary of residues (imidacloprid and clothianidin combined) at respective distances to the field borders

	During Sowing			24h-sampling	Total
Nominal distance (actual distance)°	**1m (1-2m)**	**3m (3-4m)**	**5m (5-6m)**	**1m (-1, 0 or 1m)**	
No. of samples analysed	40	40	40	160	280
No. of samples not recovered in the field	0	0	0	0	0
Residue level	**Number of samples with residue levels [n]**				
<LOQ	39	37	39	157	272
0.014-0.050 g a.s./ha	1	3	0	3	7
0.051-0.100 g a.s./ha	0	0	0	0	0
>0.100 g a.s./ha	0	0	1	0	1
Residue levels [g a.s./ha]					
Average**	<LOQ	<LOQ	<LOQ	<LOQ	n.a.
90th percentile**	<LOQ	<LOQ	<LOQ	<LOQ	
Maximum**	0.034	0.030	0.258	0.027	

LOD = 0.004 g a.s./ha (imidacloprid, clothianidin); LOQ = 0.014 g a.s./ha (imidacloprid, clothianidin); n.a. = not applicable

° In some cases the position of the Petri-dishes had to be adjusted from the intended distance due to the surrounding structures of the field.

*** Calculated from the respective number of analysed samples, imidacloprid and clothianidin, combined; any residue value below the limit of detection was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification was conservatively set to equal the LOQ.*

Conclusion

The present study followed the same design as study 1.7/1 (Hofmann & Leuckmann, 2010a) but winter wheat was treated and sown instead of winter barley. There were 4 treatment groups, with two varieties of winter wheat either treated with imidacloprid or clothianidin, sown at 4 different fields. Dust drift was monitored in Petri-dishes placed at several distances from the downwind border of the field during sowing until 15 minutes after sowing, and in Petri-dishes at 1m distance at each side of the field for 24h after sowing.

The 90th percentile calculated for the combined data set of all 4 fields was < LOQ (<0.014 g a.s./ha) for all 3 distances (1 m, 3 m, and 5 m). The 90th percentile for the 24 h samples was < LOD (<0.004 g a.s./ha). These results indicate that the dust drift deposits, produced during and after the sowing of Manta® Plus or Smaragd® forte - treated W-WHT seeds with pneumatic sowing machines, is limited.

RMS Comments

The present study has the same experimental set-up as study 1.7/1 (Hofmann & Lueckmann, 2010a), with only the sown crop being different (winter barley vs. winter wheat). The same comments apply to both studies.

Several experimental deviations are reported, which are discussed below.First, the wind direction was not constant during the sowing at the Ihinger Hof sites. As a concequence, it is likely that the Petri-dishes were not exposed the a ‘worst case’ dust drift scenario for the whole of the monitoring period. Second, petri-dishes were not always placed at the right distance from the field border due to natural or artificial obstacles. As there is no clear relationship between the distance to the field and the concentration of active substance measured in the Petri dishes, this is however not considered a problem. Third, the 24h sampling period was stopped prematurely (before the end of the 24h period) on fields 17 and 12.

Even though imidacloprid and clothianidin were applied to separate fields, their measured concentrations in the Petri-dishes are combined to calculate on 90th percentile residue value for the whole study. The applicant argues that these studies are intended as a measure of dust drift from

commercial formulations, and are therefore not specifically related to imidacloprid or clothianidin. . As this Addendum concerns clothianidin only, data for clothianidin should be available for the risk assessment. Results specifically for clothianidin dust drift are reported in the report of the analytical phase of the present study (report No. MR-09-159 / M-358970-01-1), and are summarized in Table B.9.6.1-15 below. As there is no clear relationship between the distance to the field and the measured concentration of the active substance, an average and 90th percentile value for all samples was calculated as well.

Table B.9.6.1-15: Summary of residues for clothianidin at respective distances to the field borders

	During Sowing				**24h-sampling**
Nominal distance (actual distance)°	**1m (1 or -4 m)**	**3m (3 or -2 m)**	**5m (5 or 0m)**	**Over all distances**	**1m (0 or 1m)**
No. of samples analysed	20	20	20	60	80
No. of samples not recovered in the field	0	0	0	0	0
Residue level	**Number of samples with residue levels [n]**				
<LOQ	19	17	19	55	78
0.014-0.050 g a.s./ha	1	3	0	4	2
0.051-0.100 g a.s./ha	0	0	0	0	0
>0.100 g a.s./ha	0	0	1	1	0
Residue levels [g a.s./ha]					
Average**	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
90^{tho}%ile**	<LOQ	0.023	<LOQ	<LOQ	<LOQ
Maximum**	0.034	0.030	0.258	0.258	0.027

LOD = 0.004 g a.s./ha (clothianidin); LOQ = 0.014 g a.s./ha clothianidin)

° In some cases the position of the Petri-dishes had to be adjusted from the intended distance due to the surrounding structures of the field.

*** Calculated from the respective number of analysed samples, imidacloprid and clothianidin, combined; any residue value below the limit of detection was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification was conservatively set to equal the LOQ.*

During Peer Review, it was argued that this study (together with the study by Hofmann (2014a) gives only limited information for evaluation of dus deposition (see comment 5(37) in the Reporting Table). It was considered that it cannot be decided whether these two studies reflect a best-case situation as no Heubach value (% dust) and no Heubach-as values (considering the concentration of as in dust) are available. Further, RMS was requested to give more experimental data about the studies: meteorological data and data on soil type. The information that was available from the study report on wind, temperature and precipitation was included in the study summary above for each trial site. The soil type of each site could not be included, as it is not reported in the study report. RMS was also requested to present the results from each of the trial sites separately instead of only showing an overall summary of the results. The results for the two study sites treated with clothianidin were obtained from the report of the analytical phase of the present study (report No. MR-09-153 / M-359032-01-1), and are summarized, for each study site separately, in Table B.9.6.1-16. The results for the two sites treated with imidacloprid are not included here.

Table B.9.6.1-16: Summary of residues for clothianidin, measured at each field separately, at respective distances to the field borders.

Field 11 – Ihinger hof					
	During Sowing				**24h-sampling**
Nominal distance (actual distance)°	**1m (1m)**	**3m (3m)**	**5m (4.5-5m)**	**Over all distances**	**1m (0.8-1m)**
No. of samples analysed	10	10	10	30	40
No. of samples not recovered in the field	0	0	0	0	0
Residue level	**Number of samples with residue levels [n]**				
<LOQ	10	10	10	30	40
0.014-0.050 g a.s./ha	0	0	0	0	0
0.051-0.100 g a.s./ha	0	0	0	0	0
>0.100 g a.s./ha	0	0	0	0	0
Residue levels [g a.s./ha]					
Average**	<LOQ	<LOQ	<LOQ	<LOQ	<LOD
90th percentile**	<LOQ	<LOQ	<LOQ	<LOQ	<LOD
Maximum**	<LOQ	<LOQ	<LOQ	<LOQ	<LOD
Field 17 – Celle					
	During Sowing				**24h-sampling**
Nominal distance (actual distance)°	**1m (1m)**	**3m (3m)**	**5m (4.5-5m)**	**Over all distances**	**1m (0.8-1m)**
No. of samples analysed	10	10	10	30	40
No. of samples not recovered in the field	0	0	0	0	0
Residue level	**Number of samples with residue levels [n]**				
<LOQ	9	7	9	25	38
0.014-0.050 g a.s./ha	1	3	0	4	2
0.051-0.100 g a.s./ha	0	0	0	0	0
>0.100 g a.s./ha	0	0	1	1	0
Residue levels [g a.s./ha]					
Average**	<LOQ	<LOQ	0.030	0.017	<LOD
90th percentile**	0.032	0.029	0.234	0.029	<LOD
Maximum**	0.034	0.030	0.258	0.258	0.027

LOD = 0.004 g a.s./ha (clothianidin); LOQ = 0.014 g a.s./ha clothianidin)

° In some cases the position of the Petri-dishes had to be adjusted from the intended distance due to the surrounding structures of the field.

*** Calculated from the respective number of analysed samples, imidacloprid and clothianidin, combined; any residue value below the limit of detection was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification was conservatively set to equal the LOQ.*

During Peer Review it was noted that there was a highly increased deposition at 5 m (up to 0.258 g a.s./ha) compared to 1 m (0.03 – 0.05 g a.s./ha) (see comment 5(37) in the Reporting Table). The reason for this increase is unclear. It is noted that such high residue values for clothianidin were only measured on field 17, in 1 out of 10 samples. All other samples for that distance had residues <LOQ. In contrast to the maximum values, the average residues at 1, 3 and 5 m are similar, especially of the results from field 11 are also taken into account.

Overall, due to the limitations discussed above, the quantitative data obtained in these studies are not considered the be suitable as a 'worst case' for use in risk assessment. At Pesticides Peer Review Meeting 145, the experts agreed to this conclusion. No information on the Heubach value and the Heubach a.s. value is available, and this information is considered as essential by SANCO/10553/2012 to properly address dust drift deposition.

Report:	**1.7/3; Lueckmann, J.; 2014**
Title:	**Second amendment to final report - Investigation of dust drift deposits of clothianidin & imidacloprid treated winter barley seeds with pneumatic sowing machinery on fields in Germany in autumn 2011**
Report No.:	R11129
Document No.:	M-502885-03-1
Guideline(s):	BBA Drift Guideline Part VII, 2-1.1
Guideline deviation(s):	not specified
GLP/GEP:	yes

Objective
This study investigates the aerial and ground dust drift deposits of clothianidin & imidacloprid after sowing of treated winter barley seeds with pneumatic sowing machinery on three study fields in Germany in autum 2011.

Materials and method
Test item
Winter barley seeds dressed with Clothianidin + Imidacloprid FS 100 + 175 G at a nominal seed-treatment rate of 200 mL product/100 kg seeds (which corresponds to nominally 20 g clothianidin and 35 g imidacloprid/100 kg seeds).

Study sites and sowing
The study was conducted on three study fields in the district of Giessen (Hesse) in Germany on three commercial winter barley fields. The dimension of the drilled area on each individual study field was approximately 50 m x 200 m which corresponds to a treated area of approximately 1.0 ha. The target drilling rate was 200 kg/ha (actual 194.9 to 211.6 kg/ha). Each pneumatic sowing machine was filled on the farm site. Sowing of the dressed seeds was exclusively performed by typical commercial pneumatic sowing machinery, provided by the respective cooperating farmer. The following machinery was used:

70. Field 1: Lemken Compact Solitär 9, width: 3m; pneumatic
71. Field 2: Kuhn Venta AL 302, width: 3m; pneumatic
72. Field 3: Horsch Pronto 3DC, width: 3m; pneumatic

Sampling method
Shortly before sowing the wind direction was determined and two different sampling devices to measure aerial and ground dust drift deposits were set up at the downwind border on each study field or its boundary (depending on the actual field boundary morphology): Petri-dishes, horizontally arranged at a height of approximately 2 cm above the soil surface (to measure ground deposition) and vertically erected gauze-netting-samplers (effective sampling area: 2 m x 3.3 m, to measure aerial deposition). The sampling devices were set up rectangular to the prevailing wind direction. The drilling was only performed when the wind speed at the beginning of each row was between 2 and 5 m/s and the deviation to the prevailing wind direction was $\leq \pm 30°$. The border of the downwind study field side was described as “zero line”.

Samples of dressed seeds were taken at the time of bagging and from the used seed bags shortly before filling of the drilling machine for Heubach analysis by the Seed Growth Center of Bayer CropScience AG (non-GLP).

Two lines of 3 x 10 Petri-dishes were set-up in pairs of two along a line of 5 m at a distance of 3 and 1 m to the zero line. The space between each row of ten Petri-dishes was approximately 9.3 m. Additionally one line of three gauze-netting-samplers were set-up in a distance of 3 m to the zero line. Sampling devices were arranged in an alternating order around the center of the zero line where wind breaking structures were lacking, in order to exclude any deflection of the wind. Shortly before

beginning of the sowing the gauze-nettingsamplers were wetted with a 1:1 (v/v) glycerol/water mixture and the Petri-dishes were filled with 80 mL of a 1:1 (v/v) glycerol/water mixture. Soil samples for the analysis of residues, water content (non-GLP) and soil characterisation (non-GLP) were taken shortly before sowing.

Additionally, field fortification samples (0 µg, 1 µg, 100 µg clothianidin + imidacloprid/fortified gauze sample and 0 µg, 0.1 µg, 10 µg clothianidin + imidacloprid/fortified Petri-dish sample) were established just before the start of sowing in order to investigate the stability of the samples during transport and storage.

Thirty minutes after sowing of the respective study field, the aqueous solutions of the Petri-dishes and the gauze samples (five 50 x 50 cm squares were cut-out of each individual netting) were gathered and immediately transferred into separate polyethylene flasks.

Soil sampling

Shortly before starting the sowing operation, three soil samples per study plot were taken by using a soil corer from at least 20 locations randomly selected per sample on each study plot. The three pooled samples were used for soil characterisation, determination of the water content and for residue analysis (non-GLP).

Recording of meteorological conditions

Wind direction and wind speed was recorded with an anemometer at a fixed height of 2 m above the ground, during the sowing procedure of each drilling row, after the 30 minute interval after sowing and after sample collection was completed. The minimum and maximum temperature, precipitation, percentage cloud cover were measured between start of drilling and end of sample collection. Daily weather data over a period of ten days before drilling were gathered from a weather station placed near the corresponding study fields.

Residue analysis

Residues of clothianidin and imidacloprid in all Petri-dishes and gauze netting samples as well as all field fortification samples, filters used in the Heubach abrasion tests obtained from the seed samples taken shortly before drilling and in soil samples were analysed by laboratory of the Analytical Test Site (BCS-D-HS-RA, Bayer CropScience AG) (Schöning R., Report # MR-12/006). Chromatography and detection by MS/MS in Heubach filters, gauze nettings and Petri-dish solutions was done according to method MR-338/00 (clothianidin) and MR-06/144 (imidacloprid). Analysis in soil samples was done according to method MR-106/02 (clothianidin) and MR-106/03 (imidacloprid).

The Limits of Quantitation (LOQ) for clothianidin and imidacloprid for the gauze samples were 0.04 g a.s./ha, respectively. The corresponding Limits of Detection (LOD) were 0.01 g a.s./ha. For the Petri-dish samples the LOQs for clothianidin and imidacloprid were 0.07 g a.s./ha, respectively, the corresponding LODs were 0.02 g a.s./ha. For the soil samples the LOQs were 5 µg a.s./kg soil for clothianidin and imidacloprid, respectively, the corresponding LODs were 2 µg a.s./kg soil.

Results

Soil characterization and moisture (non-GLP)

The soil characteristics and water content is shown in Table B.9.6.1-17 for each of the study fields.

Table B.9.6.1-17: Soil characterization and water content

	Study field 1	**Study field 2**	**Study field 3**
Soil code [1)]	Lu	Lt2	Tu4
Type of soil	Silty loam	Slightly clayey loam	Coarse silty clay
Clay (< 0.002 mm)	17.2	32.4	25.6
Fine silt (0.002 – 0.006 mm)	5.7	5.4	5.2
Medium silt (0.006 – 0.020 mm)	21.6	18.2	19.9
Coarse silt (0.020 – 0.063 mm)	35.1	25.9	42.9

Fine sand (0.063 – 0.200 mm)	10.8	6.3	3.3
Medium sand (0.200 – 0.630 mm)	7.8	8.6	1.9
Coarse sand (0.630 – 2.000 mm)	1.8	3.2	1.2
Cation exchange capacity [meq/100g]	19.25	20.75	18.88
Lime content [% $CaCO_3$]	0.3	<0.3	0.3
Organic carbonate [% C]	1.63	1.73	1.56
Total carbonate [% C]	1.63	1.73	1.56
Total nitrogen [mg N/100g]	141	145	140
Maximum moisture capacity [%]	53.2	47.8	54.8
pH value (KCl 0.01 M)	6.2	6.8	7.0
Water content [%]	18.2	16.5	16.6

[1)]according to the classification of the Bodenkundliche Kartieranleitung KA5 (2005):
'main soil type': L = loam, U = silt, T = clay, S = sand
'soil type group': l = loamy, u = silty, t = clayey, s = sandy
Specification of 'soil type group': 2 = low, 3 = middle, 4 = srong

Weather conditions during sowing and sampling

Weather was always dry during and after sowing. Details about the cloudiness, temperature and precipitation during sowing for each study plot are shown in Table B.9.6.1-18.

Table B.9.6.1-18: cloudiness, temperature and precipitation recorded at each study plot during sowing.

Study field	Location	Cloudiness (%)	Temperature (°C)	Sum precipitation (mm)*
Study field 1	Fernwald-Albach	50-80	Min. 19.3 Max. 20.0	0.0
Study field 2	Hungen	0-5	Min. 22.4 Max. 25.5	0.0
Study field 3	Lützellinden	0	Min. 26.0 Max. 28.7	0.0

**between beginning of drilling and end of sampling*

For drilling at study field 1 the target wind direction was 265°. The measured mean wind direction was 280° (± 19°). The mean wind speed was 3.3 m/s (± 0.9 m/s). For study field 2 the target wind direction was 120°. The measured mean wind direction was 129° (± 33°). The mean wind speed was 2.4 m/s (± 0.9 m/s). The target wind direction for study field 3 was 140°. The measured mean wind direction was 128° (± 14°). The mean wind speed was 3.8 m/s (± 0.9 m/s).

Heubach dust values and analytical content of imidacloprid and clothianidin

The Heubach value determined shortly after the seed treatment process was 0.045 g/100 kg. Additional Heubach values were determined after sowing from samples taken shortly before sowing. These measurements resulted in Heubach values of 0.097 g/100 kg, 0.022 g/100 kg and 0.144 g/100 kg for study field 1, study field 2, and study field 3, respectively.

The filter from the Heubach-tests that were conducted after sowing were analysed for their content of clothianidin and imidacloprid residues. For clothianidin the mean residue content of the filters were 0.97 mg/100 kg seeds, 0.72 mg/100 kg seeds, and 0.74 mg/100 kg seeds for study field 1, study field 2, and study field 3, respectively. For imidacloprid the mean residue content of the filters were 1.05 mg/100 kg seeds, 0.80 mg/100 kg seeds, and 0.82 mg/100 kg seeds for study field 1, study field 2, and study field 3, respectively.

Residues of clothianidin and imidacloprid in Petri dishes and Gauze-netting samples

In 44 of the 60 Petri-dish samples from study field 1 the residue level of clothianidin was below the LOD and in 8 Petri-dish samples below the LOQ. Eight Petri-dish samples had residue values above the LOQ (range 0.08 – 1.7 g a.s/ha). In 41 of the 60 Petri-dish samples from study field 1 the residue level of imidacloprid was below the LOD and in 8 samples below the LOQ. Eleven samples had residue values above the LOQ (range 0.08 – 2.4 g a.s./ha) In all Petri-dish samples from study field 2

and study field 3 the residue level of clothianidin and imidacloprid was below the LOD. None of the 45 gauze samples from study field 1, 2 and 3 had residue levels above the LOQ (0.04 g a.s./ha) of clothianidin or imidacloprid.

For calculations, residue values below or equal to the LOD were set conservatively to the LOD (0.02 g a.s./ha in Petri-dish samples and 0.01 g a.s./ha in gauze netting samples). Residue values below the LOQ were conservatively set to the LOQ (0.07 g a.s./ha in Petri-dish samples and 0.04 g a.s./ha in gauze netting samples). If all residue values of one sample type of one study field were <LOD or < LOQ the mean value and the 90th percentile are reported as <LOD or <LOQ, respectively.

The average residue level of clothianidin found in the Petri-dishes placed at a distance of 1 m to the zero line was 0.10 g a.s./ha at study field 1 and <LOD at study field 2 and 3. At a distance of 3 m o the zero line the average residue level of clothianidin in the Petri-dishes was 0.05 g a.s./ha at study field 1 and <LOD at study field 2 and 3. For imidacloprid the average residue level in the Petri-dishes from study field 1 at 1 m distance to the zero line was 0.14 g a.s./ha and <LOD at study field 2 and 3. At a distance of 3 m to the zero line the average residue level of imidacloprid in the Petri-dishes was 0.07 g a.s./ha at study field 1 and <LOD at study field 2 and 3. The mean residue level of clothianidin and imidacloprid in the gauze netting was 0.040 g a.s./ha for all three study fields, as values >LOD and ≤LOQ were set to LOQ for calculation.

The results of the residue analysis of all samples are summarised in the Table B.9.6.1-19 below.

Table B.9.6.1-19: Summary of clothianidin and imidacloprid residues in Petri-dishes and gauze nettings

	Residue levels of clothianidin [g a.s./ha]								
	Study field 1			**Study field 2**			**Study field 3**		
	Petri-dish		**Gauze netting**	**Petri-dish**		**Gauze netting**	**Petri-dish**		**Gauze netting**
	1 m	**3 m**		**1 m**	**3 m**		**1 m**	**3 m**	
Mean *	0.10	0.05	0.02	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
90th percentile*	0.12	0.07	0.04	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
Max *	1.66	0.50	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
Min *	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
	Residue levels of imidacloprid [g a.s./ha]								
	Study field 1			**Study field 2**			**Study field 3**		
	Petri-dish		**Gauze netting**	**Petri-dish**		**Gauze netting**	**Petri-dish**		**Gauze netting**
	1 m	**3 m**		**1 m**	**3 m**		**1 m**	**3 m**	
Mean *	0.14	0.07	0.03	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
90th percentile*	0.20	0.11	0.04	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
Max *	2.41	0.75	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ
Min *	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ

LOD Petri-dish = 0.02 g a.s./ha; LOQ Petri-dish = 0.07 g a.s./ha;
LOD gauze netting = 0.01 g a.s./ha; LOQ gauze netting = 0.04 g a.s./ha;
** calculatd from the number of analysed samples per study field with rounded values: 30 Petri-dishes per distance, 15 gauze netting samples; residue values below the LOD were conservatively set to equal the LOD, residue values above the LOD and below or equal to the LOQ were conservatively set equal to the LOQ*

Conclusion

The highest residues in Petri-dish samples were found for field one, with a 90th percentile residue level of 0.12 a.s./ha for clothianidin. In field 2 and field 3 the 90th percentile residue level in the Petri-dish samples were <LOD (<0.02 g a.s./ha). The 90th percentile residue level in gauze samples from all three fields were <LOQ (<0.04 g a.s./ha).

RMS comments

Compared to studies 1.7/1 and 1.7/2 (Hofman & Lueckmann, 2010a and b), the present study has a more elaborate study protocol and less deviations of this protocol were reported. Consequently, the

results from the present study are considered to be more reliable and of higher scientific quality. Moreover, residue data for imidacloprid and clothianidin are reported separately.

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

During Peer Review, RMS was requested to include relevant meteorological data and data on soil type for the 3 study fields (see comment 5(38) in the Reporting Table). These data was added to the study summary above. Further, it was noted that is was not clear why study field 1 gives residue values of to 2.4 g a.s./ha and the studies 2 and 3 give no residues >LOQ.

RMS was also requested to discuss the Heubach values and the Heubach a.s. values from this study in the light of the data given in the draft Guidance Document on seed treatment (SANCO/10553/2014, January 2014), in order to ensure that these seed parameters from the study represent the agricultural practice in Europe. The reference Heubach values for cereals (values which are met by certified seed treatment facilities) which are included in this draft Guidance Document are 2 g dust/ha and 3.5 g dust/ha for a sowing rate of 180 kg/ha, for seeds treated with or without coating agent, respectively. Recalculated for a sowing rate of 200 kg/ha, which was the target sowing rate in the present study, these values correspond to 2.2 and 3.9 g dust/ha. The Heubach values measured before sowing were 0.097, 0.022 and 0.144 g dust/100 kg seeds for field 1, 2 and 3, respectively. Based on a sowing rate of 200 kg/ha, these values correspond to 0.194, 0.044 and 0.288 g dust/ha. The reference Heubach a.s. value for cereals reported in the draft Guidance Document is 0.20 g a.s. in dust/ha for a sowing rate of 180 kg/ha. Recalculated for a sowing rate of 200 kg/ha, this corresponds to 0.22 g a.s. in dust/ha. In the present study, the concentration of clothianidin in dust was measured to be 0.97, 0.42 and 0.74 mg a.s./100 kg, for field 1, 2 and 3, respectively. Based on a sowing rate of 200 kg/ha, these values correspond to 0.00194, 0.00084 and 0.00148 g a.s. in dust/ha. Both the Heubach value and Heubach a.s. value measured in the present study are thus considerably lower than the reference value for cereals reported in the draft Guidance Document.

Effect studies to assess the potential impact of dust drift on honeybees

Report:	**1.7/4; Lueckmann, J. & Staffel, J.; 2015**
Title:	**Final report - Assessment of potential impacts on honeybee colony development, their hibernation performance and concurrent monitoring of aerial dust drift during the sowing operation of Redigo Deter FS 300 G - Treated winter barley with typical commercial pneumatic sowing technology, directly adjacent to full-flowering *Phacelia tanacetifolia* in United Kingdom**
Report No.:	GLP 199
Document No.:	M-504538-03-1
Guideline(s):	The dust drift part of this study design follows the BBA Drift Guideline Part VII, 2-1.1 (1992). The analytical phase follows SANCO/825/00/rev.8.1. For the bee health part of the study there is not test guideline defined.
Guideline deviation(s):	not specified
GLP/GEP:	Yes

Objective
This study aimed to assess potential effects on honeybee colonies during and after air sowing operation of winter barley seeds, sown in June directly adjacent to full-flowering *Phacelia tanacetifolia*. The employed winter barley seeds were commercially treated with Redigo Deter FS 300 G (nominal rate: 50.0 g clothianidin/100 kg seeds). Moreover, dust drift deposits during the sowing operation of the treated winter barley seeds were concurrently monitored.

Material and Methods
Test and control item
The test item consisted of conventional winter barley seeds dressed with Redigo Deter FS 300 G (containing clothianidin and the fungicide prothioconazole; nominal treatment rate of 50.0 g clothianidin/100 kg seeds). The control item consisted of conventional winter barley seeds of the same variety that were not treated with clothianidin. They only received a standard fungicidal treatment (Prothioconazole FS 100 G, active substance prothioconazole).

The test and control items were seed-treated and bagged at the Seed Treatment Application Centre of Bayer CropScience AG in D-40789 Monheim am Rhein, Germany (non-GLP), by employing typical seed-treatment and bagging practices.

Study site and sowing
The study was conducted in the vicinity of Selby, North Yorkshire, United Kingdom, on four different study fields, each two control and treatment fields. To ensure exposure of the honeybees to the potential arising dust drift deposits, the winter barley sowing area was surrounded by flowering *Phacelia tanacetifolia*, a highly bee attractive crop (see Figure B.9.6.1-1). The dimension of the winter barley-sown area inside the *Phacelia tanacetifolia* fields on each study field was approximately 2.1 ha (effective 1.77 to 2.61 ha). The target sowing rate was 200 kg/ha for the control and 212 kg/ha on the treatment fields (due to the analysed degree of insecticide loading of 94.3 %, effective 227.59 to 228.93 kg/ha) which corresponded to nominally 100 g clothianidin/ha (effective 107.4 to 108.0 g clothianidin/ha). In order to keep driving distances with filled sowing machines constant, the air sowing machines were filled on previously designated filling points at an approximate distance of 1 km from the treatment fields. For the sowing of the treated winter barley seeds, two typical commercially available pneumatic sowing machines were used:

73. For the control fields: 6 m Horsch Pronto 6 DC
74. For the treatment fields: 3 m Horsch Express 3TD

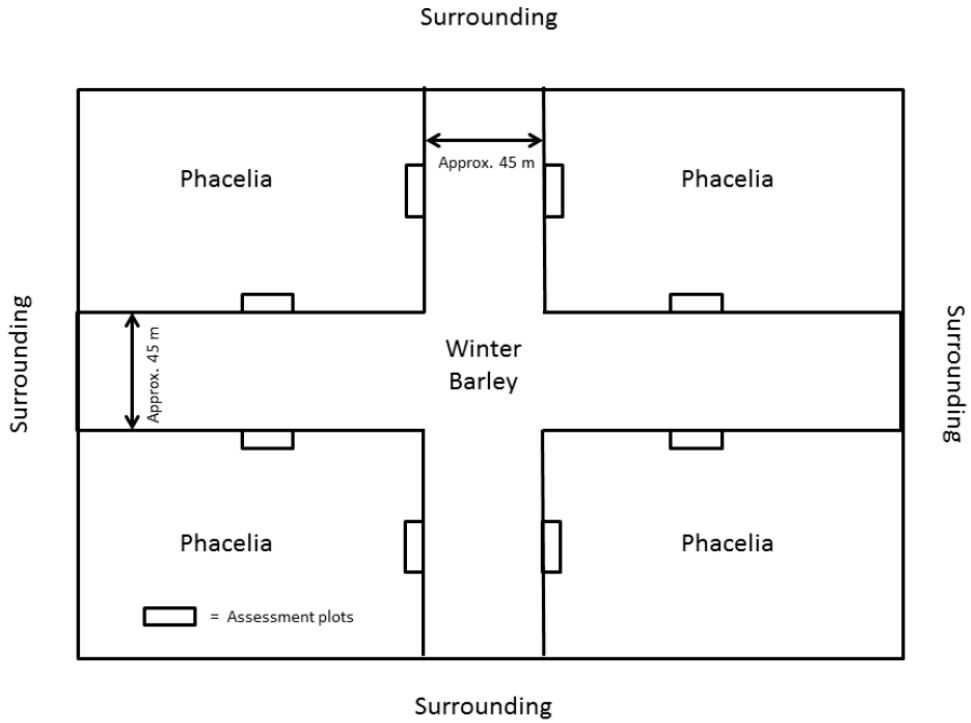

Figure B.9.6.1-1: Schematic design of the study fields

Set-up of honeybee hives

In total 32 honeybee colonies were monitored in the study, eight on each study field. The honeybee colonies were placed in the assessment plots on 12 June 2014, with a distance of approximately 3 m between the edge of the winter barley sowing area and the hive entrance. When a queen died or showed significant reduced egg laying capacity, it was replaced by another sister queen. The entrance of each hive was straightened in the direction to the *Phacelia* to correspond to the apicultural practice. After the exposure period the honeybees were relocated to a monitoring and hibernation site on 10 July 2014 in the region of York without intensive agricultural activities in the near vicinity.

Honeybee mortality and behaviour

The mortality of honeybees (e.g. workers, pupae, drones) was recorded at the study fields using dead bee traps. If there were ten or more dead bees in one colony after sowing, they were sampled for potential further residue analysis. Behavioural abnormalities of the honeybees at the entrance hole were recorded during the mortality assessments.

Population development and health assessment

Population strength and development (number of cells filled with eggs, larvae or capped brood) as well as food stores (i.e. pollen and nectar) were assessed every three weeks. At each assessment the percentage coverage of bees, sealed brood, open brood, eggs and food stores (pollen and nectar) on each side of each frame was recorded. This was judged by eye by an experienced assessor who carried out all of the colony assessments. The percentage coverage was given to the closest 5%. For analysis, these percentages were converted to total numbers per hive equivalents per hive. The quotient between honeybee numbers after and before hibernation was calculated as a value for hibernation success of honeybee colonies.

During the Field Phase and Bee Health Phase, bee colonies were kept according to Good Apicultural Practice and all typical apicultural measures were respected.

Dust drift sampling

Three days before the start of the sowing activities seed samples for Heubach analysis (non- GLP) and seed loading (non-GLP) were taken from five seed bags.

To measure aerial and ground dust drift deposits vertically erected gauze-netting-samplers were set up on each assessment plot at the treatment fields. The sowing was only performed when the wind speed at the beginning of the sowing was below 5 m/s.

A total of eight units of gauze-netting-samplers (each with an effective sampling area of approximately 2 m x 3.3 m) were set up at a distance of approximately 3 m from the zero line (edge of the winter barley field). Shortly before the beginning of the sowing the gauze-netting-samplers were wetted with a 1:1 (v/v) glycerol/water mixture. Soil samples for water content and soil characterisation were taken shortly before sowing.

Additionally, field fortification samples (0 µg, 1 µg, 100 µg imidacloprid and clothianidin fortified gauze sample) were established just before the start of sowing in order to investigate the stability of the samples during transport and storage.

30 minutes after the completion of sowing, the gauze samples (five 50 cm x 50 cm squares cut out of each individual netting unit) were gathered and immediately transferred into separate polyethylene wide mouth bottles.

Meteorological conditions

At the location of dust drift sampling during the sowing operation, wind direction and wind speed were recorded with an anemometer. The minimum and maximum temperatures and precipitation were recorded between the start of sowing and the end of sample collection. While the bee colonies were placed at the study fields daily weather data were obtained from the nearest weather station.

Residue analysis

Clothianidin residues in the gauze samples were determined by the Analytical Test Site Bayer CropScience AG.

Results

Honeybee mortality

In the control and treatment group, adult honeybee mortality was on the same, generally low level. Ten and eleven days after sowing the mortality was statistically significant higher in the treatment (mean mortality 30 bees/hive and 13 bees/hive, respectively) than in the control group. There were no apicultural or other treatments before or during these days. The weather data indicated a rain event on day 9 after sowing. Considering the size of the colonies with on average approximately 11,000 – 20,000 bees/hive, the observed mortalities did not affect the overall colony health.

The mortality of the worker bee brood was also on a very low level. On most days, only one or two larvae resp. pupae were found in the dead bee trap. Also the control mortality was during the most time of the Field Phase above the mortality of the test item treated group. Due to this and the very low numbers of dead brood in the dead bee traps, no statistic evaluation was conducted and it can be concluded, that there was no test item related effect, regarding to the worker bee brood mortality.

Honeybee colony development

At the pre-sowing assessment, the mean number of worker bees per colony was very similar in the control and test item group. Both groups increased their colony strength in a similar way towards the first and the second colony assessment after sowing, which resulted in similar numbers of adult worker bees, slightly higher in the control group. In the third colony assessment after sowing, the mean number of adult worker bees was higher in the test item treated group, as their number increased again whilst there was a minor decrease in the mean number of adult worker bees in the control group. Apart from a small increase in the control group in the fourth colony assessment the colonies started to reduce their size towards winter and showed colony strengths of approximately 15,000 to 18,000 adult worker bees before overwintering (hibernation). No statistically significant difference between control and test item treated colonies were detected in 2014.

However, in spring 2015 after overwintering the colonies of the test item group showed a statistically significant better overwintering performancethan those of the control group, regarding colony strength ($p = 0.019$). This is also described by the overwintering index, which is higher in the test item treated group (0.618) than in the control group (0.443).

Due to the good food supply at the study fields, the amount of brood increased in the period from the pre-sowing assessment towards the first assessment after sowing and remained at this level until the second assessment. From the second assessment on the colony strength decreased as bees started preparing for hibernation. The total brood amount was during the Field Phase and Bee Health Phase at approximately the same level in both groups, no statistical differences were detectable in 2014, whereas it was statistically significantly higher in the test item group ($p = 0.026$) after overwintering in April 2015.

During the Field Phase and Bee Health Phase, altogether five queens were replaced by another sister queen according to Good Apicultural Practice due to different. As the number of replaced queens was similar in the treatment and the control group it can be concluded that there is no hint for a test item related effect on the health of the queens.

Altogether, it can be concluded that the test item did not affect the honeybee health in any manner.

Varroa destructor infestation

Natural daily mite fall was recorded during all colony assessments 2014. It was on a generally low and equal level (no statistical significant differences) that did not influence the honeybee colonies in any manner. Although there was a statistically significant difference of *Varroa* infestation between the control group and the test item treated group at colony assessment 2, the *Varroa* infestation was on a generally low and equal level not influencing the honeybee colonies in any manner.

Soil characteristics and water content

The results of the soil characterisation and of the determination of the soil water content of the treatment fields are given in Table B.9.6.1-20.

Table B.9.6.1-20: Data of soil characterisation

	Study field T1	**Study field T2**
Soil code [1)]	SL4	sL2
Type of soil	Strong sandy loam	Low sandy loam
Clay (< 0.002 mm)	15.9	22.7
Silt (0.002 – 0. 63 mm)	14.9	49.5
Sand (0.063 – 2.00 mm)	69.2	27.8
Cation exchange capacity [meq/100g]	11.5	13.3
Lime content [% $CaCO_3$]	<0.1	15.8
Organic carbonate [% C]	1.57	1.61
pH value ($CaCl_2$)	5.93	7.47
Water content [%]	8.95	9.28

1) according to the classification of the Bodenkundliche Kartieranleitung KA5 (2005):
'main soil type': L = loam, U = silt, T = clay, S = sand; 'soil type group': l = loamy, u = silty, t= clayey, s = sandy; specification of 'soil type group': 2 = low, 3 = middle, 4 = strong

Meteorological data

Sowing at the treatment fields was only conducted when the wind speed shortly before start was below 5 m/s. Wind conditions were stable during sowing at the sowing on study field T1 (main wind direction east, mean wind speed approximately 2.5 m/s ranging from 1.9 and 3.0 m/s) and study field T2 (main wind direction Northeast, mean wind speed approximately 1.5 m/s ranging from 0.4 and 2.2 m/s).

Weather conditions during the time the honeybee colonies were located at the study fields and at the hibernation site were typical for this time of the year in this region. Thus, there were no weather caused unexpected positive or negative effects on the honeybee health.

Heubach values and seed loading

The Heubach value of samples taken at the time of bagging was 0.31 g/100 kg seeds with a content of 0.024 g clothianidin/100 kg seeds. Heubach value from seeds collected before sowing was 0.52 g/100 kg seeds with a content of 0.026 g clothianidin/100 kg seeds.

Residues

No residues were found in the control gauze samples. In the field spike samples, the mean recovery was 98% ± 4.7% at study field T1 and 99% ± 1.7% at study field T2. The Limit of Quantification (LOQ) of clothianidin residues in gauze netting samples was 1 µg clothianidin/L gauze extract, equivalent to 0.04 g a.s./ha. The Limit of Detection (LOD) was 0.1 µg clothianidin/L gauze extract, equivalent to 0.004 g a.s./ha.

The measured residues on the different assessment plots are shown in Table B.9.6.1-21. On study field T1, a clear wind-depending distribution of residues could be shown. Downwind assessment plots had distinctly higher residues (0.22 to 0.60 g a.s./ha, mean values) compared to those determined on the upwind assessment plots, which were all below the LOQ (<0.04 g a.s./ha). Due to changing wind conditions, the association of the assessment plots at study field T2 to upwind and downwind was not as clear as on study field T1. This was also demonstrated by relatively low residue levels also on the downwind assessment plots (0.05 to 0.15 g a.s./ha, mean values).

The overall mean and 90th percentile value for the measured residues in all downwind plots (plot A3, A5, A6 and A8) for field T1 are 0.37 g a.s./ha and 0.61 g a.s./ha, respectively. For field T2, the overall mean and 90th percentile value for the measured residues in all downwind plots (plot A3, A5, A6 and A8) are 0.10 g a.s./ha and 0.16 g a.s./ha, respectively.

Table B.9.6.1-21: Mean and range of residues of clothianidin on vertically erected gauze netting samples

Study field	Assessment plot	Mean residue level ± SD (g clothianidin/ha)	Range (minimum-maximum) (g clothianidin/ha)
T1	A1	<LOD	<LOD
	A2	<LOD	<LOD
	A3*	0.39 ± 0.05	0.32 – 0.44
	A4	<LOQ	<LOQ
	A5*	0.29 ± 0.05	0.22 – 0.37
	A6*	0.60 ± 0.03	0.55 – 0.63
	A7	0.04 ± 0.00	<LOQ – 0.04
	A8*	0.22 ± 0.03	0.17 – 0.25
T2	A1	0.02 ± 0.02	< LOD - <LOQ
	A2	<LOQ	<LOQ
	A3*	0.09 ± 0.01	0.07 – 0.10
	A4	<LOQ	<LOQ
	A5*	0.15 ± 0.01	0.14 – 0.16
	A6*	0.13 ± 0.02	0.11 – 0.16
	A7	<LOQ	<LOQ
	A8*	0.05 ± 0.00	0.05 – 0.06

*Notes: assessment plots indicated with an * were downwind during sowing, all other upwind; LOQ = 0.04 g a.s./ha; LOD = 0.004 g a.s./ha*

Additionally, a technical error occurred during the second sowing row in front of assessment plot A8, where the sowing machine did not sow during the passing of the gauze netting sampler in a distance of six to nine meter. This could have reduced the residue level on the gauze sampler and can be an explanation, beside of the changing wind conditions, why the mean residue level at assessment plot A8

(0.05 a.s./ha) was the lowest of all downwind assessment plots on both treatment fields.

Conclusion
To assess the potential effects of Redigo Deter FS 300 G on the colony development of honeybees (*Apis mellifera* L.), Redigo Deter FS 300 G–treated winter barley seeds (nominal treatment rate 50.0 g clothianidin/100 kg seeds) were sown during bee flight under field conditions in summer 2014. To increase the possible exposition of the bees, the winter barley was sown inside two fields of flowering *Phacelia tanacetifolia*, a highly bee attractive crop.

The dust drift measurements made during the sowing operation of clothianidin-treated winter barley seeds on the treatment fields (nominal treatment rate 50.0 g clothianidin/100 kg seeds) indicate that seed-treatment dust, abraded and released during the sowing operation with typical, commercial available pneumatic sowing equipment, resulted in a measurable off-crop exposure, which was distinctly higher at the downwind borders of the winter barley sowing areas as compared to the corresponding upwind borders. The maximum vertical dust deposition, as measured by vertically erected gauze-netting units, directly adjacent to the winter barley sowing areas, corresponded to a maximum drift rate of 0.63 g clothianidin/ha.

The sowing of Redigo Deter FS 300 G-treated winter barley seeds did not cause any negative effects on the survival of adult bees and bee pupae, foraging activity, behavior, on colony development, hibernation performance and colony strength as well as on the bee brood.

Thus this study demonstrated that Redigo Deter FS 300 G–treated winter barley seeds (nominal treatment rate 50.0 g clothianidin a.s./100 kg seeds), sown during bee flight close to a bee attractive crop, did not adversely affect honeybee colonies.

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, all colonies were transferred to the same post-treatment location. However, the field size of on of the control sites (C2, 1.77 ha) was just below the recommended minimum size of 2ha. This was considered to be a minor deviation and does not influence the validity of the study. A total of 16 pairs of colonies were set-up (8 colonies at each of the 2 treatment and control plots), which is considered to be enough to achieve sufficient statistical power.

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

Report:	**1.7/5; Lueckmann, J. & Staffel, J; 2014**
Title:	**Final report - Assessment of potential impacts on honeybee colony development, their hibernation performance and concurrent monitoring of aerial dust drift during the sowing operation of Poncho Beta Plus - Treated sugar beet pills with typical commercial vacuum-pneumatic sowing technology, directly adjacent to full-flowering *Phacelia tanacetifolia* in Germany**
Report No.:	195
Document No.:	M-504065-01-1
Guideline(s):	The dust drift part of this study design follows the BBA Drift Guideline Part VII, 2-1.1 (1992). The analytical phase follows SANCO/825/00/rev.8.1. For the bee health part of the study there is not test guideline defined.
Guideline deviation(s):	not specified
GLP/GEP:	yes

Objective

This study aimed to assess potential effects on honeybee colonies during and after vacuum-pneumatic sowing operation of coated sugar beet pills, sown directly adjacent to full-flowering *Phacelia tanacetifolia*. The employed sugar beet pills were commercially treated with Poncho Beta Plus (nominal rate: 0.60 mg clothianidin/pill, 0.08 mg beta-cyfluthrin/pill and 0.30 mg imidacloprid/pill). Moreover, dust drift deposits during the sowing operation of the treated sugar beet pills were concurrently monitored.

Material and Methods

Test and control item

The test item consisted of commercially prepared sugar beet pills, treated with Poncho Beta Plus, at a nominal rate of 0.60 mg clothianidin a.s./pill, 0.08 mg beta-cyfluthrin a.s./pill and 0.30 mg imidacloprid a.s./pill.

The sugar beet pills were seed-coated and bagged at KWS SAAT AG (D-37555 Einbeck, Germany) (non-GLP), by employing typical seed-treatment and bagging practises. The pills received a conventional seed treatment and were dressed in addition to Poncho Beta Plus also with the two standard fungicides Thiram 65 ZR and Hymexazol WP 70. The coated pills were bagged into 1 Unit (=100,000 pills) cardboxes, and were labelled with a unique label and the TOX-Number.

Maize seeds, dressed with only one standard fungicidal seed-treatment (Thiram SC 700, active substance: thiram), have been used for the control group. The control fields alse served as control fields in another study (GLP study No. 176), where maize was used as the crop of interest. Thus, in the control of the current study maize was sown. Control maize seed were dressed and bagged by the Seed Treatment Application Centre of Bayer CropScience AG in D-40789 Monheim am Rhein, Germany (non-GLP).

Study sites and sowing

The study was conducted in the vicinity of Nauen, Eastern Germany, on three study fields, two control and one treatment field. Originally, it was planned to use a second field for sowing of the test item. However, due to adverse soil conditions, the *Phacelia* plants on this study field was grown poor and patchy and did not meet the requirement of uniformly full flowering *Phacelia*, so that it could not be used.

Maize seeds were sown on the control fields and sugar beet pills were sown on the treatment field. To expose the honeybees to the potential arising dust drift deposits, the sugar beet and the control maize sowing areas were surrounded by flowering *Phacelia tanacetifolia*, a highly bee attractive crop (see figure B.9.6.1-2). The dimension of the sugar beet and the control maize-drilled areas inside the *Phacelia tanacetifolia* fields on each study field were approximately 2.6 ha. The target sowing rate

was 130,000 sugar beet pills and 100,000 maize seeds/ha (actual 137,708 sugar beet pills/ha and 103,189 to 101,368 maize seeds/ha). This corresponded to nominally 78.0 g clothianidin a.s./ha, 10.4 g beta-cyfluthrin a.s./ha and 39.0 g imidacloprid a.s./ha. In order to keep driving distances with filled sowing machines constant, the vacuum pneumatic sowing machines were filled on previously designated filling points at an approximate distance of 1 km from the study fields. For the sowing, a vacuum-pneumatic sowing machine (with deflector technology for the control fields and dismounted deflector technology for the treatment field, manufacturer: Amazone, Type: ED 452-K) were used.

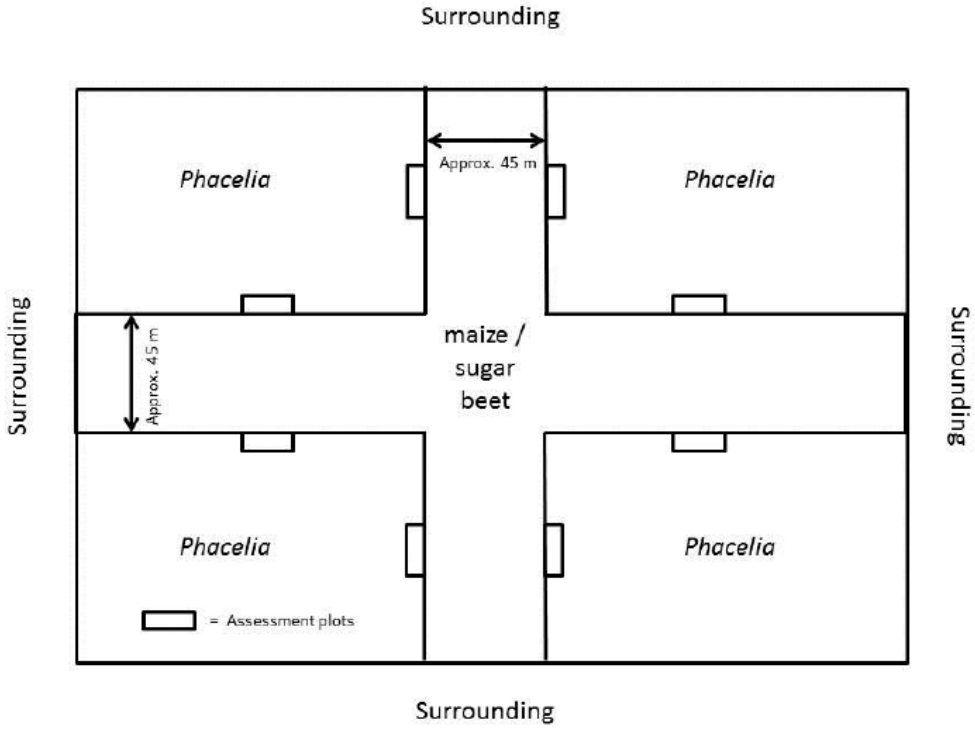

Figure B.9.6.1-2: Schematic design of the study fields

After the exposure the honeybees were relocated to three monitoring sites in a region of North-Rhine-Westphalia near Gummersbach, with no intensive agricultural activities in the near vicinity. The honeybee hives were set up on these three different locations to avoid potential impacts due to a high density of honeybee hives, like a lack of food due to food concurrence or *Varroa destructor* infestation. To avoid local factors influencing the results of this study, honeybee hives from each study field were relocated randomly to the monitoring sites (one third of the hives of each study field to each monitoring site).

Set-up of honeybee hives

In total 48 honeybee colonies were monitored in the study, 16 on each study field. The honeybee colonies were placed in the assessment plots on 27.06.2013 with a distance of approximately 3 m between the edge of the maize or sugar beet sowing area and the hive entrance. When a queen died or showed significant reduced egg laying capacity, it was replaced by another sister queen. The entrance of each hive was straightened in the direction to the *Phacelia* to correspond to the apicultural practise. They were relocated to the monitoring sites in the night of 23.07.2013 to 24.07.2013 (after the end of *Phacelia flowering*).

Honeybee mortality and behaviour

The mortality of honeybees (e.g. workers, pupae, drones) was recorded using dead bee traps while the honeybees were located at the study fields. If there were ten or more dead bees in one colony after sowing, they were placed in a sample bottle and labeled unmistakably for potential further residue analysis. Since there were no sampling periods with clearly increased bee mortality no analysis of bee

samples have been conducted. Behavioural abnormalities of the honeybees at the entrance hole were recorded during the mortality assessments.

Honeybee colony strength and health assessment

Population strength and development (number of cells filled with eggs, larvae or capped brood) as well as food stores (i.e. pollen and nectar) were assessed using the estimation method developed by the Bee Institute Liebefeld (Imdorf, Buehlmann et al. 1987). The precolony assessment was done shortly after colony setup, but before sowing, for the definition of the starting conditions of the colonies. Further colony assessments were done every three weeks until mid of October. In March 2014, the last colony assessment took place to evaluate the overwintering success of the honeybee hives.

Sampling method

To measure aerial dust drift deposits, vertically erected gauze samplers were set up on each assessment plot at the treatment field. The sowing started when the wind speed was below 5 m/s. Eight gauze samplers (each with an effective sampling area of 2 m x 3.3 m) were set up at a distance of approximately 3 m from the zero line on each assessment plot. Shortly before the beginning of the sowing the gauze samplers were wetted with a 1:1 (v/v) glycerol/water mixture. 30 minutes after the completion of sowing, the gauze samples (five 50 x 50 cm squares cut out of each gauze sampler) were gathered and immediately transferred into separate polyethylene flasks.

Additionally, field fortification samples (0 µg, 1 µg, 100 µg clothianidin/betacyfluthrin/ imidacloprid/methiocarb fortified gauze sample) were established just before the start of sowing of the test item in order to investigate the stability of the samples during transport and storage. Soil samples for water content analysis (non-GLP) and soil characterisation (non-GLP) were taken shortly before sowing on all study fields.

Meteorological conditions

At the location of dust drift sampling during the sowing operation, wind direction and wind speed were recorded with an anemometer. The minimum and maximum temperatures and precipitation were recorded between the start of sowing and the end of sample collection. While the bee colonies were placed at the study fields daily weather data were obtained from the nearest weather station.

Residue analysis

Residues of clothianidin, imidacloprid and beta-cyfluthrin in gauze samples as well as all field fortification samples were analysed by Bayer CropScience AG (Schöning R. & Ballmann C., Report: MR-14/074). Chromatography and detection by MS/MS in gauze was done according to the methods 00554/M001 (clothianidin), 00537/M002 (imidacloprid) and 00922 (beta-cyfluthrin).

The Limit of Quantitation (LOQ) of the gauze samples (0.25 m_2) was 0.04 g a.s./ha for all analytes. The Limit of Detection (LOD) was 0.004 g a.s./ha for both clothianidin and imidacloprid and 0.012 g a.s./ha for beta-cyfluthrin.

Results

Honeybee mortality

In control and treatment group, worker bee mortality was on the same, generally low level, mostly around five to ten dead bees per day in mean. A statistical significant difference between control and treatment worker bee mortality could be seen on some days before the application, so that a test item related effect can be excluded. After sowing, the mean worker bee mortality in the treatment group was never significantly higher than in the control group. In contrast, on two days the worker bee mortality in the control group was significantly higher than in the treatment group. However, no test item related effect regarding to the worker bee mortality could be detected during the whole Field Phase. The mortality of the bee brood was on a very low level (mean control group: 0.52 ± 1.92; mean treatment group: 0.28 ± 0.67). On most days, no brood was found in the dead bee traps.

Honeybee colony development

Honeybee colony strength showed a similar development in the control and treatment group. It slightly increased during the first three weeks after setup of the bee colonies on the study fields. Due to the excellent food supply, the amount of brood increased in the same period. This led to a strong increase of the colony strength from the first to the second colony assessment, both in control and treatment colonies. From the second colony assessment (mid of August), the colony strength decreased towards winter and stagnated on a stable level. During winter, all colonies lost worker bees and due to the normal reduction or even stop of the breeding activity, the number of worker bees decreased towards spring. In the whole Field Phase, the mean colony strength of the control and treatment group was on the same level, no statistical significant differences were detectable.

The mean amount of honeybee brood was at the pre-colony assessment in the treatment group statistically significantly higher than in the control group. This is probably due to a slightly faster adaption of queens of the treatment group to the new colony size after assembling the colonies prior to the pre-colony assessment. This is a random factor that cannot be excluded, even if sister queens are used in this study. Also in the first colony assessment it was higher, but not statistically significant anymore. However, this indicates that the test item had no adverse effect to honeybee brood. The honeybee brood increased even during sowing to the first colony assessment and decreased afterwards rapidly to a very low level at the fifth colony assessment. This is a normal development for honeybee colonies, which reduce their brood amount typically towards winter. With the beginning of the spring the honeybees started to breed again, approximately on the same level both in control and treatment group.

Varroa destructor infestation

While the infestation with *Varroa* mites was on approximately the same level in colonies of the control and the treatment group, there were significant differences between the three monitoring sites. Statistical analysis showed no significant differences between the locations Agger 1 and Agger 2, but between these two locations and the location Müller in some cases. After the second formic acid treatment, the number of dead *Varroa* mites was statistically significantly higher at the location Müller than at the location Agger 2. After the first oxalic acid treatment, the number was also higher than at both other locations, but not statistically significantly. In contrast to this, it was statistically significantly lower after the second oxalic treatment in winter. The main reason therefore is the reduced strength of the colonies at Müller compared to the colonies at Agger 1 and Agger 2.

Soil characteristics and water content

The results of the soil characterisation and of the determination of the soil water content of the treatment fields are given in Table B.9.6.1-22.

Table B.9.6.1-22: Data of soil characterisation

	Study field C1	**Study field C2**	**Study field T2**
Soil code [1)]	Su2	Sl2	Sl3
Type of soil	Low silty sand	Low sandy loeam	Middle loamy sand
Clay (< 0.002 mm)	4.7	7.4	9.5
Silt (0.002 – 0. 63 mm)	18.1	15.2	18.9
Sand (0.063 – 2.00 mm)	77.2	77.4	71.6
Cation exchange capacity [meq/100g]	22.8	24.0	26.6
Lime content [% $CaCO_3$]	1.7	3.4	9.4
Organic carbonate [% C]	2.23	2.73	2.52
pH value ($CaCl_2$)	7.59	7.67	7.67
Water content [%]	8.6	9.8	9.7

1) according to the classification of the Bodenkundliche Kartieranleitung KA5 (2005):
'main soil type': L = loam, U = silt, T = clay, S = sand; 'soil type group': l = loamy, u = silty, t= clayey, s = sandy; specification of 'soil type group': 2 = low, 3 = middle, 4 = strong

Meteorological data
Sowing at the treatment fields was conducted when the wind speed shortly before start was below 5 m/s. Wind conditions were stable during sowing on study field T2. The main wind direction was northeast and the mean wind speed amounted to be approximately 2 m/s ranging from 0.8 and 2.8 m/s.

A comparison of the data taken by the data logger and rain gauges between the study fields indicated similar weather conditions within the study site. Thus, all study fields can be considered as comparable regarding their weather conditions. During hibernation, no adverse weather conditions like long lasting, intensive rainfalls or extreme coldness occurred. Therefore, it can be assumed that the weather conditions did not affect the hibernation ability of the honeybee colonies.

Residues
The results of all field spiked fortification gauze samples showed that clothianidin, imidacloprid and beta-cyfluthrin were stable during storage and transport. Residues in control samples were always below the LOD.

No residues of clothianidin, imidacloprid and beta-cyfluthrin above the LOD (0.012 g a.s./ha for beta-cyfluthrin and 0.004 g a.s./ha for clothianidin and imidacloprid) were detected in any of the gauze samples obtained from the study field during sowing of the test item.

Conclusion
To assess the potential effects of Poncho Beta Plus on the colony development of honeybees (*Apis mellifera* L.), Poncho Beta Plus-treated sugar beet pills (0.60 mg clothianidin a.s./pill, 0.08 mg beta-cyfluthrin a.s./pill and 0.30 mg imidacloprid a.s./pill) were sown (138,500 sugar beet pills/ha) during bee flight in summer 2013. To increase the possible exposition of the bees, the sugar beet was sown inside a field of flowering *Phacelia tanacetifolia*, a highly bee attractive crop.

The application of Poncho Beta Plus did not cause any effects on the survival of adult bees and bee pupae, foraging activity, behaviour, colony development and colony strength as well as on the bee brood and the hibernation success.

The dust drift measurements made during the sowing operation of Poncho Beta Plus-treated sugar beet pills on the treatment field indicate that pill-treatment dust, abraded and released during the sowing operation with non-modified (not deflected) vacuum-pneumatic sowing equipment and dismounted chassis of the discharged air system, did not result in a measurable off-field exposure as all analysed samples were below their respective LOD (0.012 g a.s./ha for beta-cyfluthrin and 0.004 g a.s./ha for clothianidin and imidacloprid).

Thus this study demonstrated that Poncho Beta Plus – treated sugar beet pills (0.60 mg clothianidin a.s./pill, 0.08 mg beta-cyfluthrin a.s./pill and 0.30 mg imidacloprid a.s./pill), sown during bee flight did not adversely affect honeybee colonies.

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, overwintering of all colonies at the same post-treatment location, field size of the study fields >2ha. A total of 16 colonies were set-up on the treated field, and 32 at the control fields, which is considered to be enough to achieve sufficient statistical power.

It is noted that the application rate for clothianidin in the present study is 78 g a.s./ha. However, the maximum application rate currently authorized in the EU is 90 g a.s./ha. Consequently, the amount of active substance applied in this study, does not cover all currently registered uses.

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

B.9.6.2. Exposure

When seeds treated with clothianidin are sown, honeybees, bumblebees and solitary bees foraging in either the treated field, the field margin or the adjacent crop are potentially exposed to dust particles emitted during sowing. According to the EFSA Guidance Document on bees, exposure in the treated field could occur through dust particles deposited on weeds present in the field. However, as demonstrated in section B.9.3, weeds will not be present at sowing (due to seed bed preparation), and thus this route of exposure is not relevant for seed treatment uses. Further, in the EFSA Guidance Document it is also considered possible that bees are exposed to dust drift within the treated field, by flying through the cloud of dust that results from the drilling process, when foraging. Whilst this situation has occurred and may have contributed to incidents, the likelihood in terms of frequency of occurrence is unknown. Therefore, this route of exposure will not be considered in the present assessment. In conclusion, only exposure to dust drift in the field margin or in adjacent crops is considered relevant for seed treatment uses. As exposure in adjacent crops will always be lower than for the field margin, this risk is covered by the assessment for the field margin.

The applicant submitted studies measuring dust drift residues in the field margin in winter cereals and sugar beet. For winter cereals, dust drift was measured in 4 different studies (three in Germany and one in the United Kingdom), with measurements made on two or three field sites per study. In most studies, an application rate of 100 g a.s./ha was used (which covers the currently authorized uses), except in Lueckmann (2014) who applied only 40 g a.s./ha. A summary of the measured residue levels following dust drift is shown in Table B.9.6.2.1-1. These data indicate that seed treatment dust, abraded and released during the sowing operation with typical commercial available pneumatic sowing equipment, resulted in a measurable off-crop exposure in most cases. Measured residues are however highly variable, and strongly influenced by wind direction (distinctly higher residues at the downwind border of the sowing areas as compared to the upwind borders).

Table B.9.6.2.1-1: Summary of the measured clothianidin residue levels following dust drift in the available studies in winter cereals

Crop	Field site	Mean (g a.s./ha)	90th percentile (g a.s./ha)	Maximum (g a.s./ha)	Reference
Winter barley[1]	Ingerhof & Celle combined	0.031	0.042	0.283	1.7/1 Hofmann & Lueckmann, 2010a
Winter wheat[1]	Ingerhof & Celle combined	<LOQ	<LOQ	0.258	1.7/2 Hofmann & Lueckmann, 2010b
Winter barley[2]	1	0.10	0.12	1.66	1.7/3 Lueckmann, 2014
	2	<LOD	<LOD	<LOD	
	3	<LOD	<LOD	<LOD	
Winter barley[3]	T1	0.37	0.61	0.63	1.7/4 Lueckmann & Staffel, 2015
	T2	0.10	0.16	0.16	

[1]*values for both study fields and all distances to the field as a whole; for these studies LOS = 0.004 g a.s./ha, LOQ = 0.014 g a.s./ha. These values are only provided as information, as these studies are not considered suitable as a 'worst-case' for use in the risk assessment, as no Heubach values and Heubach a.s. values are available for the treated seeds used in these studies.*

[2]*values measured in Petri-dishes at a distance of 1m to the field (these values are the highest measured in this study); for this study LOD = 0.02 g a.s./ha, LOQ = 0.07 g a.s./ha*

[3]*Values calculated based on measurements made over all downwind assessment plots (these values are the highest measured in this study). For both fields, the downwind assessment plots were plots A3, A5, A6 and A8; for this study LOD = 0.004 g a.s./ha, LOQ = 0.04 g a.s./ha*

In the EFSA Conclusion on the risk assessment for bees for clothianidin (2013), reference is made to an additional study that measured the dust drift from mechanical and pneumatic sowing of clothianidin treated winter barley seeds (Nikolakis *et al.*, 2008, study 21 in the Study evaluation notes). In this study, the highest Petri dish 90th percentile values, considering ground deposition, was 0.033 g a.s./ha (pneumatic machine) and 0.029 g a.s./ha (mechanical machine). Considering atmospheric dust drift, the highest 90th percentile value was 0.212 g a.s./ha (pneumatic machine), with residues being detected

above the LOQ up to 5 m height and 30 m distance from the "zero-line". These results are generally in line with the findings of the studies submitted as confirmatory data.

It should be noted that information on dust drift is only available for winter barley and winter wheat, and not for other cereals for which application of clothianidin as seed treatment is registered. As one of the factors influencing dust abrasion is the crop (seed), extrapolation of data to other crops is highly uncertain. However, due to their similarity it is likely that extrapolation is possible for cereals. This is supported by the fact that for winter wheat similar low values of dust drift were obtained compared to winter barley. In the original version of this Addendum, it was suggested to use the highest available 90th percentile residue value (0.61 g a.s./ha, from the downwind side of the study fields in the study with winter barley) in the risk assessment as a reasonable 'worst case' for winter cereals in general. This was however not considered acceptable at Pesticides Peer Review Meeting 145 (see below).

At Pesticides Peer Review Meeting 145, the draft SANCO Guidance Document for seed treatment (SANCO/10553/2012, Version January 2014) was considered to be the appropriate Guidance Document to assess the risk from dust drift exposure. Values for dust deposition used in the EFSA Guidance Document for bees were derived from an earlier version of this SANCO Guidance Document. SANCO/10553/2012, Version January 2014, was updated based on more recent and additional data on dust drift, and was therefore considered the latest best available knowledge (in line with the article 21 of Regulation (EU) 1107/2009). In the EFSA Guidance Document, it is considered that the amount of active substance deposited in the field margin through dust drift is only a function of the amount of applied active substance in the field. However, more recent data (which is incorporated in SANCO/10553/2012, Version January 2014) has shown that this is not the case. The deposition of active substances with abraded dust in non-target areas is strongly dependent on the seed quality, more than on the application rate. The majority of the experts considered that SANCO/10553/2012, Version January 2014, should be used in the exposure assessment, while the minority considered that the EFSA Guidance Document should be used as it is a final version and published. Following the decision of Pesticides Peer Review Meeting 145, the exposure and risk assessment are updated according to SANCO/10553/2012 (Version January 2014).

At Pesticides Peer Review Meeting 145, it was noted that the studies by Leuckmann (2014) and Lueckmann & Staffel (2015) are acceptable. However, it was argued that the dust deposit values from the SANCO Guidance Document were derived from a large dataset. Individual studies with few varieties might not be sufficient to overrule the values reported in SANCO/10553/2012 (Version January 2014) as the amount of active substance deposits through dust drift is very much dependent on the quality of the seed dressing rather than the properties of the active substance. Therefore, according to SANCO/10553/2012 (Version January 2014), the studies by Lueckmann (2014) and Lueckmann & Staffel (2015) alone are not sufficient for estimating the exposure from dust deposition. These studies would however be useful to extend the dataset on dust deposition used to determine the values reported in the SANCO Guidance Document. Overall, it was agreed to use only the exposure values in the SANCO Guidance Document in the Tier 1 calculations. No value from the available studies was considered suitable to refine the assessment at Tier 2.

During Peer Review, it was noted that the EFSA Guidance Document for bees suggests to select the sowing machine at EU level that delivers 90th percentile based on ranking of dust emission and area of use (Appendix N page 191), in order to ensure that the machine used for experimental measurement cover the 90th percentile. It was argued that this exercise would be needed to conclude that the measured value of 0.61 g a.s./ha (which was used in the risk assessment in the orginal version of this Addendum) is worst-case for Europe (see comment 5(39) in the Reporting Table). At Pesticides Peer Review Meeting 145, it was noted that there is indeed no information as to whether the machinery used in all the studies covers the 90th percentile of exposure. It was however acknowledged that it is at present very difficult to perform such an assessment.

For sugar beet, the occurrence of dust drift was investigated in one study, performed at one field site in Germany (Leuckmann & Staffel, 2014b). All samples that were analysed in this study did not have

detectable clothianidin residues (measured residue below the LOD of 0.004 g a.s./ha). Thus, pill treatment dust, abraded and released during sowing of treated sugar beet pills with non-modified (not deflected) vacuum-pneumatic sowing equipment did not result in measurable off-field exposure. In the EFSA Conclusion on the risk assessment for bees for clothianidin (2013), one additional study on dust drift from clothianidin treated sugar beet pills is referenced (Lueckmann & Städtler, 2009, study 11 in the study evaluation notes). In this study, clothianidin residues above the LOQ were only detected in 3 out of 1390 samples, further indicating that dust drift produced after sowing of treated sugar beet pills is very limited. Further, Appendix C of the EFSA Guidance Document for bees states that for (sugar and fodder) beet dust drift is not a relevant exposure route. Sugar beet seeds are pelleted (pills), with the active ingredient not on the outside of the seed but closed in by an inert layer. As a consequence, much less dust containing the active substance is released during sowing compared to basic seed treatment, where the active ingredient is present on the outside surface of the seed.

At Pesticides Peer Review Meeting 145, it was however considered necessary to include the Tier 1 risk assessment based on deposition values from the SANCO Guidance Document for the use in sugar beet in the Addendum. Therefore, this assessment was added to Section B.9.6.3.

It is noted that during peer review of the EFSA Conclusion on the risk assessment for bees for clothianidin (2013), the experts questioned the data set on dust deposition available at that time. In particular, the seeds used in the field trials were considered to be of high quality, and hence not representative of standard EU situations. However, according to Commission Directive 2010/21/EU, the use of clothianidin as seed treatment is only permitted 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 of the seed, storage and transport can be minimised. Consequently, in the original version of this Addendum, RMS considered the use of "high quality seeds" a pre-requisite of the use of clothianidin, and hence considered that the studies performed with such seeds comply with the directive in the seed quality relevant for the use of clothianidin in sugar beet and cereals in Europe. The newly submitted studies, in which such "high quality treated seed" were used as well, were considered representative, and suitable for use in the risk assessment.

During Peer Review, it was however argued that it was not clear whether the available studies covered the best-case or worst-case situation in terms of dust drift (see comment 5(37), 5(38) and 5(42) in the Reporting Table). For the studies by Hofmann & Lueckmann (2014a and b), it was noted that the draft SANCO Guidance Document for seed treatment (SANCO/10553/2012, Version January 2014) gives 0.38 g/ha as a worst-case dust deposition for cereals which is 8 times higher than the maximum of measured in these studies. For the study by Lueckmann (2014), the Heubach and Heubach a.s. values measured in the study were compared to the reference values for cereals reported in the draft SANCO Guidance Document. The measured values were well below the reference values of the draft SANCO Guidance Document (for details, please refer to the study summary of study 1.7/3 in Section B.9.6.1). Overall, it was argued that the seed quality used for the trials seemed to be a lot better than qualities available on the market. Considering the maximum allowable dust amounts per 100 kg, e.g. 5 g in some countries, the chosen seed treatment quality in the studies here represents dust amounts far below the maximum allowed rates. It was noted that to ensure that no effects on bees occur, the seed treatment quality needs to be guaranteed in the first place. Then trials reflecting a bad seed treatment quality of the upper end of what may occur needs to be tested. It was considered that this was not the case here. At Pesticides Peer Review Meeting 145, this issue was further discussed. The experts agreed that the quality of seeds used in the available studies was not representative of the standard treated seeds on the market, and therefore the exposure could not be considered as worst-case.

B.9.6.3. Risk assessment

B.9.6.3.1. Risk assessment for honeybees

The risk assessment was performed following the EFSA Guidance Document on bees. As stated in section B.9.6.2, only exposure to dust drift in the field margin and adjacent crops is considered relevant. As exposure in the latter will be lower than in field margins, the risk assessment was only performed for field margins.

At Pesticides Peer Review Meeting 145, the draft SANCO Guidance Document for seed treatment (SANCO/10553/2012, Version January 2014) was considered to be the appropriate Guidance Document to calculate the dust drift exposure for the risk assessment. Values for dust deposition used in the EFSA Guidance Document for bees were derived from an earlier version of this SANCO Guidance Document. SANCO/10553/2012, Version January 2014, was updated based on more recent and additional data on dust drift, and was therefore considered the latest best available knowledge (in line with the article 21 of Regulation (EU) 1107/2009). In the EFSA Guidance Document, it is considered that the amount of active substance deposited in the field margin through dust drift is only a function of the amount of applied active substance in the field. However, more recent data (which is incorporated in SANCO/10553/2012, Version January 2014) has shown that this is not the case. The deposition of active substances with abraded dust in non-target areas is strongly dependent on the seed quality, more than on the application rate. The majority of the experts considered that SANCO/10553/2012, Version January 2014, should be used in the exposure assessment, while the minority considered that the EFSA Guidance Document should be used as it is a final version and published. Following the decision of Pesticides Peer Review Meeting 145, the exposure and risk assessment below are updated according to SANCO/10553/2012 (Version January 2014).

In section B.9.6.2 it was further demonstrated that exposure to dust drift from treated sugar beet seeds is negligible. Following the EFSA Conclusion on the risk assessment for bees (2013), it is therefore concluded that the risk following exposure to dust drift from treated sugar beet seeds is acceptable. Nevertheless, at Pesticides Peer Review Meeting 145, it was however considered necessary to include the Tier 1 risk assessment based on deposition values from SANCO/10553/2012 (Version January 2014) for the use in sugar beet in the Addendum. Therefore, this assessment was added below.

Tier 1 risk assessment

According to the EFSA Guidance Document, both the acute risk through contact exposure as the oral acute and chronic risk to adult bees and larvae should be assessed. Oral exposure to adults occurs when residues of clothianidin deposited on plants in the field margin are transported to nectar and pollen, which are then consumed by the bees and/or transported to the hive.

The level of exposure to clothianidin following dust drift deposits is calculated by using the draft SANCO Guidance Document for seed treatment (SANCO/10553/2012, Version January 2014). According to this Guidance Document, the emission of dust from treated seeds during seeding is variable, depending upon the care taken during all the steps; from the preparation of seeds before treatment, up to the type of driller used. For the calculations below, it is assumed that pneumatic machines are used, which are equipped with pertinent devices ensuring dust deflection to soil.

The exposure of non-target organisms in off-field areas will in general depend on the amount of dust abraded from treated seeds and emitted during sowing. However, not only the mere amount of dust will be determinant of exposure, but also the content of active substance (a.s.) in the abraded dust that will deposit off-field. The amount of dust that is easily abraded from treated seeds is measured in a standard test, of which the result is expressed by the **Heubach value** that refers to the amount of dust per sown area (e.g. **g dust/ha**). The **active substance content (in % a.s.)** in Heubach dust from treated seeds is deemed to be determined by the active substance dose applied per seed unit. However, based on the dataset available up to now, it is not possible to draw this general conclusion. No general,

constant relationships can be described between dust amounts (Heubach values) and the active substance contents (%) in dust from treated seeds on the one hand and the seed dressing or field application rates on the other hand. This has further significant implications for the assessment of the risk arising from the sowing of treated seeds, since the seed quality parameter 'Heubach value' (g dust/ha) and 'active substance content' (a.s. % in dust) do drive the deposition rates of active substances in the off-field, and not the application rate in-field.

To facilitate the risk assessment procedure regarding dust abraded from treated seeds, a proxy parameter is defined that best describes and aggregates the seed quality parameter described above in terms of emission of contaminated dust. This variable is the **amount of active substance released with Heubach dust per sown area ('Heubach a.s.' in g a.s./ha)** and can directly be employed in the determination of predicted environmental concentrations in non-target areas:

$$Heubach\ a.s.\left(g\ a.s.in\ dust/ha\right)=\frac{Heubach\ value\left(g\ dust/ha\right)\times a.s.\left(\%\ in\ dust\right)}{100}$$

Where a.s. = active substance
Heubach value = amount of abraded fine dust (<200 µm) in g per seed unit or sown area (ha)

In order to assess the risk for non-target organisms in off-field areas arising from the sowing of treated seeds, the seed quality parameters determinant for exposure should be known or correctly estimated. SANCO/10553/2012 (Version January 2014) defines 3 regulatory scenarios, depending on whether the seed quality parameters are known or should be estimated:

1. **Product specific** assessment: can be used when reliable and representative data regarding the relevant quality parameters of the product to be assessed are available
2. Assessment based on **reference values**: to be used when the quality parameters regarding the treated seeds to be assessed have to comply with legal requirements. The compliance with minimum data requirements is established through certification of the seed treatment facilities.
3. Assessment based on **worst case values**: to be used when no legal requirements apply and/or no quality data are available or certified regarding the treated seeds to be assessed.

For clothianidin, there are certain legal requirements with regard to seed quality. According to Commission Directive 2010/21/EU, the use of clothianidin as seed treatment is only permitted 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 of the seed, storage and transport can be minimised. Therefore, it is considered appropriate to perform an assessment based on reference values. Nevertheless, an assessment based on worst case values is also included below. For cereals, a relationship between the seed dressing rate and the content of active substance could be derived, and therefore also a product specific assessment was performed. For sugar beet, such a relationship could not be determined based on the available data. Therefore, no product specific assessment was performed for this use.

The deposition values presented in SANCO/10553/2012 (Version January 2014) were standardized for a certain amount of seeds/ha (see Table 10-2 in Section 10.5.2 of the Guidance Document). Therefore, in a first step, these values have to be corrected according to the seed units given in the GAP table. The correction factors to be used in the exposure calculations for the use in winter cereals and sugar beet are shown in Table B.9.6.3.1-1. Based on the corrected Heubach values and the content of a.s. in dust, the Heubach a.s. value was calculated for the lowest and highest application rate of clothianidin for the use in winter cereals and beet (see Table B.9.6.3.1-2).

Table B.9.6.3.1-1: Lowest and highest authorized application rate and seed units of clothianidin containing formulations for use as a seed treatment in winter cereals and beet, the seed units used in SANCO/10553/2012 (Version January 2014) and the correction factor to be applied in the exposure calculations.

Crop	According to GAP			Seed units used in SANCO dust GD	Correction factor
	Application rate		Seed units		
Winter cereals	Lowest	59 g a.s./ha (27 g a.s./100 kg seeds)	220 kg seed/ha	180 kg seed/ha	1.22
	Highest	100 g a.s./ha (50 g a.s./100 kg seeds)	200 kg seed/ha	180 kg seed/ha	1.11
Beet*	Lowest	10 g a.s./ha (10 g a.s./u)	100,000 seeds/ha	100,000 seeds/ha	1
	Highest	108 g a.s./ha (60 g a.s./u)	180,000 seeds/ha	100,000 seeds/ha	1.8

*Note: *1 unit = 100,000 seeds*

Table B.9.6.3.1-2: Heubach values, content of a.s. in dust and Heubach a.s. values for the lowest and highest authorized application rate for clothianidin in winter cereals and beet according to SANCO/10553/2012 (Version January 2014).

Crop	Application rate		Regulatory scenario	Heubach value from SANCO dust GD (g dust/ha)[1]	Corrected Heubach value (g dust/ha)	Content of a.s. in dust (% a.s. in dust)	Heubach a.s. value (g a.s. in dust/ha)
Winter cereals	Lowest	59 g a.s./ha (220 kg seeds/ha)	Product specific	- [2]	- [2]	6.32 [3]	0.15 [4]
			Reference value	2	2.44	10	0.24
			Worst case	3	3.67	25	0.92
	Highest	100 g a.s./ha (200 kg seeds/ha)	Product specific	- [2]	- [2]	11.7 [3]	0.26 [4]
			Reference value	2	2.22	10	0.22
			Worst case	3	3.33	25	0.83
Beet	Lowest	10 g a.s./ha (1 u/ha)	Reference value	0.05	0.05	2	0.001
			Worst case	0.1	0.05	10	0.01
	Highest	108 g a.s./ha (1.8 u/ha)	Reference value	0.05	0.09	2	0.0018
			Worst case	0.1	0.18	10	0.018

[1] Values from SANCO/10533/2012 (Version January 2014) for cereals are for 180 kg seeds/ha, and for sugar beet 100,000 seeds/ha (1 u/ha). For cereals, the value for seed treatment with sticker was used.
[2] No product specific Heubach values are available
[3] To calculate this value, it was considered that the content of a.s. in dust from cereals is equal to 13% of the seed dressing rate, expressed as g/180 kg (see Table 10-2 of SANCO/10533/2012, Version January 2014); A drilling rate of 220 kg seeds/ha corresponds to a dressing rate of 27 g a.s./100 kg seeds, or 48 g a.s./180 kg seeds; A drilling rate of 200 kg seeds/ha corresponds to 50 g a.s./100 kg seeds, or 90 g a.s./180 kg seeds.
[4] To calculate this value, the Heubach value for the reference scenario was used.

According to the draft SANCO Guidance Document for seed treatment, the deposition in non-target areas was shown to be related to the amount of active substance released during sowing, i.e. to the seed quality of the sown seeds as 'Heubach a.s.' values. The Predicted Environmental Concentation (PEC) for 2D dust ground deposition can be calculated using this relationship as expressed in the following equation:

$$PEC_{2D\,dust\,ground\,deposition}(g\,a.s./ha) = Heubach\,a.s.(g\,a.s./ha) \times crop\,specific\,deposition\,factor$$

where PEC = Predicted Environmental Concentration
a.s. = active substance
Heubach a.s. = g active substance in abraded Heubach dust per ha of sown area
Deposition factor = deposition factor determined in field studies with different crop types. This deposition factor was calculated by regression analyses.

For cereals, this crop specific deposition factor was determined to be 0.5. For sugar beet, the data available when the SANCO Guidance Document was drafted was not sufficient to determine a general deposition factor. A reference PEC_{2D} value of 0,02 g a.s./ha was derived from one study instead, which is a factor 20 higher than the heubach a.s. value for this scenario. Therefore, as a conservative approach, the same factor of 20 was used to calculate a PEC_{2D} value for the worst-case scenario. The calculated PEC2D values are shown in Table B.9.6.3.1-3.

According to SANCO/10553/2012 (Version January 2014), it has been shown that species living or foraging in 3-dimensional structures like hedgerows, trees or other crops are exposed to higher deposition rates of contaminated dust than species living on the ground. To address this issue, an extrapolation factor between 2-D and 3-D deposition was derived. Based on the experimental results from several studies in different crops, a factor of 13 has been determined. As foliar dwelling non-target arthropods like honeybees and other pollinators forage on 3-dimensional structures, the 3-D deposition should be taken into account to determine the realistic worst case exposure. The PEC3D values are shown in Table B.9.6.3.1-3, and were calculated using the following equation:

$$PEC_{3D\,dust\,deposition}(g\,a.s./ha) = PEC_{2D\,ground\,dust\,deposition}(g\,a.s./ha) \times 3D\ extrapolation\ factor$$

Where PEC = Predicted Environmental Concentration
a.s. = active substance
3D factor = extrapolation factor was determined in field studies with different crop types to be **13**. The 3D extrapolation factor describes the ratio between dust deposition in 3D structures (measured in gauze netting) and 2D structures (measured in Petri dishes).

Table B.9.6.3.1-3: Heubach a.s. values, PEC_{2D} and PEC_{3D} dust deposition values for the lowest and highest authorized application rate for clothianidin in winter cereals and beet according to SANCO/10553/2012 (Version January 2014).

Crop	Application rate		Regulatory scenario	Heubach a.s. value (g a.s. in dust/ha)	PEC 2D dust deposition (g a.s./ha)	PEC 3D dust deposition (g a.s./ha)
Winter cereals	Lowest	59 g a.s./ha (220 kg seeds/ha)	Product specific	0.15	0.075	1.00
			Reference value	0.24	0.122	1.59
			Worst case	0.92	0.458	5.96
	Highest	100 g a.s./ha (200 kg seeds/ha)	Product specific	0.26	0.130	1.69
			Reference value	0.22	0.110	1.44
			Worst case	0.83	0.417	5.42
Beet	Lowest	10 g a.s./ha (1 u/ha)	Reference value	0.001	0.020	0.26
			Worst case	0.01	0.200	2.60
	Highest	108 g a.s./ha (1.8 u/ha)	Reference value	0.0018	0.036	0.47
			Worst case	0.018	0.360	4.68

The PEC_{3D} values from Table B.9.6.3.1-3 are used to represent the exposure to residues from clothianidin through dust drift. For the oral and contact risk assessment, HQ and ETR values will be calculated based on the relevant equations from the EFSA Guidance Document.

According to the EFSA Guidance Document on bees, the hazard quotient (HQ) for contact exposure for the field margin from dust drift after sowing of treated seeds, is calculated by the following equation at first tier:

$$HQ = \frac{f_{dep} * AR}{LD_{50\ contact}}$$

Where AR = application rate in g a.s./ha
f_{dep} = fraction of the dose deposited on the type of plants that foragers visit (see Appendix X of the EFSA Guidance Document)
$LD_{50,contact}$ is expressed in µg a.s./bee

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

In the equation to calculate the HQ value above, the exposure is represented by $f_{dep} * AR$. For the present assessment, the exposure was calculated based on SANCO/10553/2012 (Version January 2014) as PEC_{3D}. Therfore, to calculate the HQ, *fdep * AR* will be replaced by the PEC_{3D}.

For oral exposure, Exposure Toxicity Ratios (ETR) for plants in the field margin are calculated with the equations below. The relevant shortcut values (and the methodology used to determine these values) are presented in Table J7 Appendix J of the EFSA Guidance Document. 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 = 3.7 (shortcut value for acute exposure to forager honeybees, taken from Table J7 in Appendix J of the Guidance Document)
E_f = 0.099 for cereals without deflector, 0.0099 for cereals with deflector (According to Appendix X of the Guidance Document)
$LD_{50,oral}$ 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 = 2.9 (shortcut value for chronic exposure to forager honeybees, taken from Table J7 in Appendix J of the Guidance Document)
E_f = 0.099 for cereals without deflector, 0.0099 for cereals with deflector (According to Appendix X of the Guidance Document)
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 = 2.2 (shortcut value for honeybee larvae, taken from Table J7 in Appendix J of the Guidance Document)
E_f = 0.099 for cereals without deflector, 0.0099 for cereals with deflector (According to Appendix X of the Guidance Document)
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.

In the equations to calculate the ETR values above, the exposure is represented by *AR* * E_f. For the present assessment, the exposure was however calculated based on SANCO/10553/2012 (Version January 2014) as PEC_{3D}. The PEC_{3D} represents the active substance residues deposited in the field margin throught dust drift. According to Appendix H of the EFSA Guidance Document for bees, this dust deposition value can be multiplied by 1/3 for the assessment of concentrations in nectar and pollen entering the hive, to account for dilution of the concentrations in the field marging because the average deposition is lower than in the downwind direction. Therefore, to calculate the ETR, *AR* * E_f will be replaced by 1/3 * PEC_{3D}.

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 was performed using the highest and lowest authorized 'maximum application rate' for winter cereals and beet (see Table B.9.6.3.1-1). The relevant toxicity endpoints are taken from Table B.9.1.3.1-3. As the PEC_{3D} was calculated assuming that pneumatic sowing machines equipped with pertinent devices ensuring dust deflection to soil are used, the risk assessment is only valid for situations where this equipment is used. The calculated Tier 1 HQ values for both winter cereals and beet are shown in Table B.9.6.3.1-4. The ETR values are shown in Table B.9.6.3.1-5 and Table B.9.6.3.1-6 for winter cereals and beet, respectively.

Table B.9.6.3.1-4: Tier 1 HQ calculations for acute adult contact exposure through dust drift for the lowest and highest authorized 'maximum application rate' of clothianidin in winter cereals and beet.

Crop	Application rate (g a.s./ha)		Regulatory scenario	PEC_{3D} (g a.s./ha)	$LD_{50,contact}$ (µg a.s./bee)	HQ	Trigger
Winter cereals	Lowest	59	Product specific	1.00	0.0275	36.5	14
			Reference value	1.59	0.0275	57.8	14
			Worst case	5.96	0.0275	216.7	14
	Highest	100	Product specific	1.69	0.0275	61.5	14
			Reference value	1.44	0.0275	52.5	14
			Worst case	5.42	0.0275	196.9	14
Beet	Lowest	10	Reference value	0.26	0.0275	9.45	14
			Worst case	2.60	0.0275	94.5	14
	Highest	108	Reference value	0.47	0.0275	17.1	14
			Worst case	4.68	0.0275	170.2	14

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

Table B.9.6.3.1-5: Tier 1 ETR calculations for acute adult oral, chronic adult oral and larval exposure from plants in the field margin for the lowest and highest authorized 'maximum application rate' of clothianidin in winter cereals.

Acute adult oral exposure									
Crop	Application rate (kg a.s./ha)		Regulatory scenario	1/3 * PEC_{3D} (kg a.s./ha)	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Winter cereals	Lowest	0.059	Product specific	0.00033	3.7	-	0.00379	0.33	0.2
			Reference value	0.00053	3.7	-	0.00379	0.52	0.2
			Worst case	0.00200	3.7	-	0.00379	1.95	0.2
	Highest	0.100	Product specific	0.00057	3.7	-	0.00379	0.55	0.2
			Reference value	0.00047	3.7	-	0.00379	0.45	0.2
			Worst case	0.00180	3.7	-	0.00379	1.75	0.2
Chronic adult oral exposure									
Crop	Application rate (kg a.s./ha)		Regulatory scenario	1/3 * PEC_{3D} (kg a.s./ha)	SV	twa	LDD_{50} (µg a.s./bee/ day)	ETR	Trigger
Winter cereals	Lowest	0.059	Product specific	0.00033	2.9	1	0.00138	0.70	0.03
			Reference value	0.00053	2.9	1	0.00138	1.12	0.03
			Worst case	0.00200	2.9	1	0.00138	4.20	0.03
	Highest	0.100	Product specific	0.00057	2.9	1	0.00138	1.19	0.03
			Reference value	0.00047	2.9	1	0.00138	0.98	0.03
			Worst case	0.00180	2.9	1	0.00138	3.78	0.03
Larval exposure									
Crop	Application rate (kg a.s./ha)		Regulatory scenario	1/3 * PEC_{3D} (kg a.s./ha)	SV	twa	NOED (µg a.s./larva /development period)	ETR	Trigger
Winter cereals	Lowest	0.059	Product specific	0.00033	2.2	1	0.00528	0.14	0.2
			Reference value	0.00053	2.2	1	0.00528	0.22	0.2
			Worst case	0.00200	2.2	1	0.00528	0.83	0.2
	Highest	0.100	Product specific	0.00057	2.2	1	0.00528	0.24	0.2
			Reference value	0.00047	2.2	1	0.00528	0.19	0.2
			Worst case	0.00180	2.2	1	0.00528	0.75	0.2

For winter cereals, most ETR values exceed the relevant trigger values, indicating a potential risk. Only for the chronic risk assessment for larval exposure, the ETR values are below the trigger for the product specific assessment for the lowest application rate (59 g a.s./ha) and for the assessment based on reference values for the highest application rate (100 g a.s./ha). As a potential risk is identified for all honeybee developmental stages, especially when worst-case deposition values are considered, further consideration is necessary.

For beet, the ETR values are below the relevant trigger values for the acute risk to adult honeybees and for the chronic risk to honeybee larvae for the assessment based on reference values, indicating an acceptable risk. However, if worst-case deposition values are considered, the ETR values exceed the trigger. For the chronic risk to adult honeybees, the ETR values exceed the relevant trigger regardless of the regulatory scenario. Further consideration is thus necessary.

Table B.9.6.3.1-6: Tier 1 ETR calculations for acute adult oral, chronic adult oral and larval exposure from plants in the field margin for the lowest and highest authorized 'maximum application rate' of clothianidin in beet.

Acute adult oral exposure									
Crop	Application rate (kg a.s./ha)		Regulatory scenario	1/3 * PEC_{3D} (kg a.s./ha)	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Beet	Lowest	0.010	Reference value	0.000087	3.7	-	0.00379	0.084	0.2
			Worst case	0.00087	3.7	-	0.00379	0.846	0.2
	Highest	0.108	Reference value	0.00016	3.7	-	0.00379	0.153	0.2
			Worst case	0.00156	3.7	-	0.00379	1.52	0.2
Chronic adult oral exposure									
Crop	Application rate (kg a.s./ha)		Regulatory scenario	1/3 * PEC_{3D} (kg a.s./ha)	SV	twa	LDD_{50} (µg a.s./bee/ day)	ETR	Trigger
Beet	Lowest	0.010	Reference value	0.000087	2.9	1	0.00138	0.182	0.03
			Worst case	0.00087	2.9	1	0.00138	1.821	0.03
	Highest	0.108	Reference value	0.00016	2.9	1	0.00138	0.329	0.03
			Worst case	0.00156	2.9	1	0.00138	3.278	0.03
Larval exposure									
Crop	Application rate (kg a.s./ha)		Regulatory scenario	1/3 * PEC_{3D} (kg a.s./ha)	SV	twa	NOED (µg a.s./larva /development period)	ETR	Trigger
Beet	Lowest	0.010	Reference value	0.000087	2.2	1	0.00528	0.036	0.2
			Worst case	0.00087	2.2	1	0.00528	0.361	0.2
	Highest	0.108	Reference value	0.00016	2.2	1	0.00528	0.065	0.2
			Worst case	0.00156	2.2	1	0.00528	0.650	0.2

Tier 2 risk assessment based on measured dust deposits

A number of dust drift studies in cereals is available, from which in the original version of this Addendum a reasonable worst case dust deposit value of 0.61 g a.s./ha was derived for winter cereals (highest available 90th percentile value from winter barley, see section B.9.6.2). Using this value, the HQ for contact exposure was refined. As this refined HQ still exceeded the trigger, no acceptable acute risk to honeybees could be demonstrated.

At Pesticides Peer Review Meeting 145, the draft SANCO Guidance Document for seed treatment (SANCO/10553/2012, Version January 2014) was considered to be the appropriate Guidance Document to assess the risk from dust drift exposure (see Section B.9.6.2). It was noted that the studies by Leuckmann (2014) and Lueckmann & Staffel (2015), which were used to derive the refined dust deposit value of 0.61 g a.s./ha for winter cereals, are acceptable. However, it was argued that the dust deposit values from the SANCO Guidance Document were derived from a large dataset. Individual studies with few varieties might not be sufficient to overrule the values reported in SANCO/10553/2012 (Version January 2014) as the amount of active substance deposits through dust drift is very much dependent on the quality of the seed dressing rather than the properties of the active substance. Therefore, according to SANCO/10553/2012 (Version January 2014), the studies by Lueckmann (2014) and Lueckmann & Staffel (2015) alone are not sufficient for estimating the exposure from dust deposition in cereals. These studies would however be useful to extend the dataset on dust deposition used to determine the values reported in the SANCO Guidance Document. Overall, no value from the available studies was considered suitable to refine the assessment at Tier 2.

To refine the risk assessment for oral exposure, residue levels in nectar and pollen in plants or crops exposed to dust drift are needed. However, such residue levels are not available, nor is an official guidance on how to measure them. Consequently, the tier 2 risk assessment for oral exposure

following dust drift cannot be performed. However, this will be further considered in the higher tier assessment.

Risk assessment based on higher tier studies

Further refinements to the risk assessment could be based on field effect studies. For the use in **winter cereals**, one study (Lueckmann & Staffel, 2015) on the effect on colony survival and development due to exposure to dust drift after sowing of treated winter barley seeds was submitted by the applicant. This study covers the maximum application rate for clothianidin (CTD) used as seed treatment in winter cereals (i.e. 50 g a.s./dt, corresponding to 100 g a.s./ha). Therefore, the available study is representative for the currently registered uses in winter cereals.

The study by Lueckmann & Staffel (2015) was conducted in the UK, and aimed to assess potential effects on honeybee colonies during and after air sowing of winter barley seeds. The study was performed on four different study fields, each two control and treatment fields. The test item consisted of conventional winter barley seeds dressed with Redigo Deter FS 300 G (containing clothianidin at a nominal treatment rate of 50.0 g/100 seeds, corresponding to an application of 100 g a.s./ha). On the control field, winter barley seeds of the same variety that were not treated with clothianidin were sown. To ensure exposure of the honeybees to potential dust drift deposits, the winter barley was sown in June, in an area was surrounded by full-flowering *Phacelia tanacetifolia*, a highly bee attractive crop. Further, the sowing operation took place when bees were actively foraging. For the sowing of the treated winter barley seeds, typical commercially available pneumatic sowing machines were used. Honeybee colonies were placed with a distance of approximately 3 m between the edge of the winter barley sowing area an the hive entrance. On each of the four study fields (2 treated and two untreated), eight colonies were placed, resulting in a total 32 colonies that were exposed to dust drift from winter cereals (16 treated and 16 untreated).

The EFSA Guidance Document on the risk assessment for bees suggests that at least 5 sites, which are representative of the crop and the use of the compound, are necessary to determine a reliable result from field studies for use in risk assessment. However, this study was only performed at two treated and two control sites, with a limited geographical spread. Nevertheless, due to the study design that leads to a worst case exposure (the treated field surrounded by a highly bee attractive crop, where bees were actively foraging during the sowing operation), this study is considered to provide a good indication of the potential influence of dust deposits on honeybee colonies.

Dust deposits were monitored during sowing in all wind direction around the sowing area. Measured residues indicate that seed treatment dust, abraded and released during the sowing operation with typical commercially available pneumatic sowing equipment, resulted in a measurable off-crop exposure in most cases. Details on the measured dust deposits are shown in Table B.9.6.1-1 in section B.9.6.1.

At both the control and treated sites, the potential acute and chronic effects on honeybee colonies were monitored including mortality, behaviour, health status, colony strength and overwintering success. A summary of the effects due to exposure to dust deposits insecticide treated and control crops (no insecticide seed treatment) is provided below.

Daily adult honeybee mortality levels was on the same, generally low level in both the treatment and the control group. Ten and eleven days after sowing the mortality was statistically significant higher in the treatment than in the control group (mean mortality 30 bees/hive and 13 bees/hive, respectively). There were no apicultural or other treatments before or during these days. The weather data indicated a rain event on day 9 after sowing. Considering the size of the colonies with on average approximately 11,000 – 20,000 bees/hive, the observed mortalities did not affect the overall colony health. The mortality of the worker bee brood was also on a very low level in both treatment and control.

Colony development was very similar between the control and test item group, with a similar initial mean number of worker bees and a similar increase in colony strength towards the first and second colony assessment after sowing. In the third colony assessment, the mean number of adult worker bees was higher in the test item treated group, as their number increased again whilst there was a minor decrease in the control group. Colonies in both groups similarly started to decrease in size towards winter and showed colony strengths of approximately 15,000 to 18,000 adult bees before overwintering. Overall, no statistically significant difference between control and test item treated colonies were detected in 2014. However, in spring 2015, after overwintering, the colonies of the test item group showed a statistically significant better overwintering performance than those of the control group regarding colony strength. The total brood amount was the whole study period at approximately the same level in both groups, no statistical differences were detectable. The level of *Varroa* infestation was also unaffected by exposure to dust deposits from treated winter barley seeds.

Overall, the sowing of Redigo Deter FS 300 G-treated winter barley seeds did not cause any negative effects on the survival of adult bees and bee pupae, foraging activity, behaviour, on colony development, hibernation performance and colony strength as well as on the bee brood.

When following the risk assessment scheme for exposure from dust drift to plants in the field margin as suggested by the EFSA Guidance Document on bees, an unacceptable acute and chronic risk is found for the use in winter cereals, even with calculations based on measured clothianidin dust deposits at tier two. Although measured deposits of clothianidin are high enough to theoretically pose an unacceptable risk to bees, acute and chronic colony level effects were not observed in the available field study. This could be explained by the conservative nature of the first tier risk assessment, which is focusing on a relatively narrow strip downwind at the edge of the treated field. However, bees present beyond this strip or foraging upwind during the sowing will be considerably less exposed.

At Pesticides Peer Review Meeting 145, the available higher tier effect study by Leuckmann & Staffel (2015) in winter barley was discussed. It was noted that in the original version of this Addendum, RMS concluded a low risk on the basis of the observations from this study, showing no acute and long-term effects. The biological observations were done on *phacelia* as adjacent crop. It was however noted that the statistical power of the study was not assessed, but it is likely to be low (i.e. 2 control and 2 treated fields each filed with 8 hives). The study was conducted in UK and it was considered not representative of other EU conditions. The meteorological conditions and the bee activity in the study should be compared with other EU situations for ensuring that it represents a worst-case. The RMS noted that the use of *phacelia*, being a highly attractive crop, was supposed to cover uncertainties regarding other factors influencing the exposure. One study with 2 sites was however not considered sufficient to address the exposure and effect Specific Protection Goals (SPG).

During Peer Review, it was argued that the seed quality used for the field effect studies seemed to be a lot better than qualities available on the market (see comment 5(42) in the Reporting Table). Considering the maximum allowable dust amounts per 100 kg, e.g. 5 g in some countries, the chosen seed treatment quality in the studies here represents dust amounts far below the maximum allowed rates. It was noted that to ensure that no effects on bees occur, the seed treatment quality needs to be guaranteed in the first place. Then trials reflecting a bad seed treatment quality of the upper end of what may occur needs to be tested. It was considered not to be the case here. At Pesticides Peer Review Meeting 145, this issue was further discussed. The experts agreed that the quality of seeds used in the available studies was not representative of the standard treated seeds on the market, and therefore the exposure could not be considered as worst-case.

Overall, the majority of the experts at Pesticides Peer Review Meeting 145 considered that the study by Lueckmann & Staffel (2015) alone, without further data, cannot be considered sufficient to draw a conclusion regarding the effects of dust drift depositions on bees. It was noted that with respect to the winter cereal uses, the study may represent a worst-case situation (sowing in the study done when flowering field margin were present). Nevertheless, the experts considered that the risk to honeybees from dust exposure for winter cereals should be further addressed.

In the EFSA Conclusion on the risk assessment for bees for clothianidin (2013)[55], a low risk to bees was concluded for the use in **beets** based on a low and infrequent dust deposition when pelleted beet seeds are sown. Nevertheless, a Tier 1 risk assessment was performed based on SANCO/10553/2012 (Version January 2014), which indicated a potential risk to honeybees for oral and contact exposure through dust drift when worst-case dust deposition values were assumed.

To further refine the risk assessment for beets, one study (Lueckmann & Staffel, 2014) on the effect on colony survival and development due to exposure to dust drift after sowing of treated sugar beet pills was submitted by the applicant. This study was conducted in Germany, following a similar experimental setup as the study by Leuckmann & Staffel (2015) in winter barley discussed above (e.g. sugar beet pills sown in an area surrounded by full-flowering *Phacelia tanacetifolia*). Further, to obtain a worst-case exposure, non modified (not deflected) vacuum-pneumatic sowing equipment was used. The results of this study in sugar beet showed no acute and chronic effects on honeybee colonies (including mortality, behaviour, health status, colony strength and overwintering success). Further, clothianidin deposits following dust drift were also measured by Lueckmann & Staffel (2014). All samples that were analysed did not have detectable clothianidin residues (measured residue below the LOD of 0.004 g a.s./ha). Thus, pill treatment dust, abraded and released during sowing of treated sugar beet pills with non-modified (not deflected) vacuum-pneumatic sowing equipment did not result in measurable off-field exposure. These results are in line with the dust drift studies in sugar beet considered for the EFSA Conclusion on the risk assessment for bees for clothianidin (2013).

At Pesticides Peer Review Meeting 145, the available higher tier effect study by Leuckmann & Staffel (2014) in sugar beet was discussed. As the study design was similar as for the UK study in winter cereals (Lueckmann & Staffel, 2015), the same conclusion is valid in this case: the statistical power was expected to be low (i.e. 2 control fields and 1 treated field each with 16 honeybee hives) and as it was conducted only in Germany it was considered not representative of other EU conditions. However, it was noted that the concentration of the active substance and the dust deposition is very low. The experts considered that the low exposure is sufficient as line of evidence to conclude a low risk to bees for exposure through dust drift from clothianidin treated sugar beet pills (and fodder bee/beet pills, assuming the same technology for seed pelleting and drilling).

Conclusions

Overall, the acute and chronic risk to honeybee colony development and survival, resulting from exposure to residues of clothianidin in dust deposits after sowing of treated beet seeds at the currently registered maximum seed dressing rates, is considered acceptable.

For winter cereals, the risk from both oral and contact exposure to dust drift was not acceptable at tier 1. The available higher tier data was not sufficient to conclude that the risk can be considered acceptable.

[55] 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.9.6.3.2. Risk assessment for bumblebees

The risk assessment was performed following the EFSA Guidance Document on bees. As stated in section B.9.6.2, only exposure to dust drift in the field margin and adjacent crops is considered relevant. As exposure in the latter will be lower than in field margins, the risk assessment was only performed for field margins.

At Pesticides Peer Review Meeting 145, it was agreed to use the draft SANCO Guidance Document for seed treatment (SANCO/10553/2012, Version January 2014) for the exposure assessment for dust drift. For the rationale behind this decision, please refer to Section B.9.6.3.2. Following this decision, the exposure and risk assessment below are updated according to SANCO/10553/2012 (Version January 2014).

In section B.9.6.2 it was further demonstrated that exposure to dust drift from treated sugar beet seeds is negligible. Following the EFSA Conclusion on the risk assessment for bees (2013), it is therefore concluded that the risk following exposure to dust drift from treated sugar beet seeds is acceptable. Nevertheless, at Pesticides Peer Review Meeting 145, it was however considered necessary to include the Tier 1 risk assessment based on deposition values from SANCO/10553/2012 (Version January 2014) for the use in sugar beet in the Addendum. Therefore, this assessment was added below.

Tier 1 risk assessment

According to the EFSA Guidance Document, both the acute risk through contact exposure as the oral acute and chronic risk to adult bumblebees and bumblebee larvae should be assessed. Oral exposure to adults occurs when residues of clothianidin deposited on plants in the field margin are transported to nectar and pollen, which are then consumed by the bees and/or transported to the hive.

The level of exposure to clothianidin following dust drift deposits was calculated by using the draft SANCO Guidance Document for seed treatment (SANCO/10553/2012, Version January 2014). For details on these calculations, and the assumptions made, reference is made to Section B.9.6.3.1. The PEC_{3D} dust deposition values, which are used in the risk assessment, are summarized in Table B.9.6.3.2-1.

Table B.9.6.3.2-1: PEC_{3D} dust deposition values for the lowest and highest authorized application rate for clothianidin in winter cereals and beet according to SANCO/10553/2012 (Version January 2014).

Crop	Application rate		Regulatory scenario	PEC 3D dust deposition (g a.s./ha)
Winter cereals	Lowest	59 g a.s./ha (220 kg seeds/ha)	Product specific	1.00
			Reference value	1.59
			Worst case	5.96
	Highest	100 g a.s./ha (200 kg seeds/ha)	Product specific	1.69
			Reference value	1.44
			Worst case	5.42
Beet	Lowest	10 g a.s./ha (1 u/ha)	Reference value	0.26
			Worst case	2.60
	Highest	108 g a.s./ha (1.8 u/ha)	Reference value	0.47
			Worst case	4.68

The PEC_{3D} values from Table B.9.6.3.2-1 are used to represent the exposure to residues from clothianidin through dust drift. For the oral and contact risk assessment, HQ and ETR values will be calculated based on the relevant equations from the EFSA Guidance Document.

According to the EFSA Guidance Document on bees, the hazard quotient (HQ) for contact exposure for the field margin from dust drift after sowing of treated seeds, is calculated by the following equation at first tier:

$$HQ = \frac{f_{dep} * AR}{LD_{50\ contact}}$$

Where AR = application rate in g a.s./ha
f_{dep} = fraction of the dose deposited on the type of plants that foragers visit (see Appendix X of the EFSA Guidance Document)
$LD_{50,contact}$ is expressed in µg a.s./bee

If HQ > 2.3, a potential risk is identified, and a higher tier risk assessment should be performed. If the HQ is below this trigger, the risk is acceptable.

In the equation to calculate the HQ value above, the exposure is represented by f_{dep} * *AR*. For the present assessment, the exposure was calculated based on SANCO/10553/2012 (Version January 2014) as PEC_{3D}. Therfore, to calculate the HQ, *fdep * AR* will be replaced by the PEC_{3D}.

For oral exposure, Exposure Toxicity Ratios (ETR) for plants in the field margin are calculated with the equations below. The relevant shortcut values (and the methodology used to determine these values) are presented in Table J7 Appendix J of the EFSA Guidance Document. 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 = 6.5 (shortcut value for acute exposure to adult bumblebees, taken from Table J7 in Appendix J of the Guidance Document)
E_f = 0.099 for cereals without deflector, 0.0099 for cereals with deflector (According to Appendix X of the Guidance Document)
$LD_{50,oral}$ 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 = 5.9 (shortcut value for chronic exposure to adult bumblebees, taken from Table J7 in Appendix J of the Guidance Document)
E_f = 0.099 for cereals without deflector, 0.0099 for cereals with deflector (According to Appendix X of the Guidance Document)
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 = 2.6 (shortcut value for bumblebee larvae, taken from Table J7 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 = 0.099 for cereals without deflector, 0.0099 for cereals with deflector (According to Appendix X of the Guidance Document)
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.

In the equations to calculate the ETR values above, the exposure is represented by *AR* * E_f. For the present assessment, the exposure was however calculated based on SANCO/10553/2012 (Version January 2014) as PEC_{3D}. The PEC_{3D} represents the active substance residues deposited in the field margin throught dust drift. According to Appendix H of the EFSA Guidance Document for bees, this dust deposition value can be multiplied by 1/3 for the assessment of concentrations in nectar and pollen entering the hive, to account for dilution of the concentrations in the field marging because the average deposition is lower than in the downwind direction. Therefore, to calculate the ETR, *AR* * E_f will be replaced by 1/3 * PEC_{3D}.

The first tier risk assessment was performed using the highest and lowest authorized 'maximum application rate' for winter cereals and beet (see Table B.9.6.3.2-1). 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. As the PEC_{3D} was calculated assuming that pneumatic sowing machines equipped with pertinent devices ensuring dust deflection to soil are used, the risk assessment is only valid for situations where this equipment is used. The calculated Tier 1 HQ values for both winter cereals and beet are shown in Table B.9.6.3.2-2. The ETR values are shown in Table B.9.6.3.2-3 and Table B.9.6.3.2-4 for winter cereals and beet, respectively.

Table B.9.6.3.2-2: Tier 1 HQ calculations for acute adult contact exposure through dust drift for the lowest and highest authorized 'maximum application rate' of clothianidin in winter cereals and beet.

Crop	Application rate (g a.s./ha)		Regulatory scenario	PEC_{3D} (g a.s./ha)	$LD_{50,contact}$ (µg a.s./bee)	HQ	Trigger
Winter cereals	Lowest	59	Product specific	1.00	0.1483	6.77	2.3
			Reference value	1.59	0.1483	10.71	2.3
			Worst case	5.96	0.1483	40.18	2.3
	Highest	100	Product specific	1.69	0.1483	11.40	2.3
			Reference value	1.44	0.1483	9.74	2.3
			Worst case	5.42	0.1483	36.53	2.3
Beet	Lowest	10	Reference value	0.26	0.1483	1.75	2.3
			Worst case	2.60	0.1483	17.53	2.3
	Highest	108	Reference value	0.47	0.1483	3.17	2.3
			Worst case	4.68	0.1483	31.56	2.3

For both the use in beet, the HQ value is below the relevant trigger for the lowest application rate if the assessment is based on reference values, which indicated that the risk is acceptable in this case. However, if worst case dust deposition values are considered, the HQ value exceeds the trigger. For the highest application rate for the use in beet and for both the lowest and highest application rate for

the use in winter cereals, the HQ values exceed the trigger, regardless of the regulatory scenario considered. Further consideration is thus necessary.

Table B.9.6.3.2-3: Tier 1 ETR calculations for acute adult oral and chronic adult oral exposure from plants in the field margin for the lowest and highest authorized 'maximum application rate' of clothianidin in winter cereals.

Acute adult oral exposure									
Crop	Application rate (kg a.s./ha)		Regulatory scenario	1/3 * PEC_{3D} (kg a.s./ha)	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Winter cereals	Lowest	0.059	Product specific	0.00033	6.5	-	0.00191	1.13	0.036
			Reference value	0.00053	6.5	-	0.00191	1.81	0.036
			Worst case	0.00200	6.5	-	0.00191	6.81	0.036
	Highest	0.100	Product specific	0.00057	6.5	-	0.00191	1.93	0.036
			Reference value	0.00047	6.5	-	0.00191	1.59	0.036
			Worst case	0.00180	6.5	-	0.00191	6.13	0.036
Chronic adult oral exposure									
Crop	Application rate (kg a.s./ha)		Regulatory scenario	1/3 * PEC_{3D} (kg a.s./ha)	SV	twa	LDD_{50} (µg a.s./bee/ day)	ETR	Trigger
Winter cereals	Lowest	0.059	Product specific	0.00033	5.9	1	0.000138	14.25	0.0048
			Reference value	0.00053	5.9	1	0.000138	22.80	0.0048
			Worst case	0.00200	5.9	1	0.000138	85.51	0.0048
	Highest	0.100	Product specific	0.00057	5.9	1	0.000138	24.23	0.0048
			Reference value	0.00047	5.9	1	0.000138	19.95	0.0048
			Worst case	0.00180	5.9	1	0.000138	76.95	0.0048

Table B.9.6.3.2-4: Tier 1 ETR calculations for acute adult oral and chronic adult oral exposure from plants in the field margin for the lowest and highest authorized 'maximum application rate' of clothianidin in beet.

Acute adult oral exposure									
Crop	Application rate (kg a.s./ha)		Regulatory scenario	1/3 * PEC_{3D} (kg a.s./ha)	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Beet	Lowest	0.010	Reference value	0.000087	6.5	-	0.00191	0.29	0.036
			Worst case	0.00087	6.5	-	0.00191	2.95	0.036
	Highest	0.108	Reference value	0.00016	6.5	-	0.00191	0.53	0.036
			Worst case	0.00156	6.5	-	0.00191	5.31	0.036
Chronic adult oral exposure									
Crop	Application rate (kg a.s./ha)		Regulatory scenario	1/3 * PEC_{3D} (kg a.s./ha)	SV	twa	LDD_{50} (µg a.s./bee/ day)	ETR	Trigger
Beet	Lowest	0.010	Reference value	0.000087	5.9	1	0.000138	3.71	0.0048
			Worst case	0.00087	5.9	1	0.000138	37.05	0.0048
	Highest	0.108	Reference value	0.00016	5.9	1	0.000138	6.70	0.0048
			Worst case	0.00156	5.9	1	0.000138	66.7	0.0048

For both the use in winter cereals and beet, all ETR values exceed the relevant trigger values, regardless of the regulatory scenario considered. A potential risk oral acute and chornic risk is thus identified for adult bumblebees. Consequently, further consideration is necessary.

Tier 2 risk assessment based on measured dust deposits

A number of dust drift studies in cereals is available, from which in the original version of this Addendum a reasonable worst case dust deposit value of 0.61 g a.s./ha was derived for winter cereals (highest available 90th percentile value from winter barley, see section B.9.6.2). Using this value, the

HQ for contact exposure can be refined. As this refined HQ still exceeded the trigger, no acceptable acute risk to honeybees could be demonstrated.

At Pesticides Peer Review Meeting 145, the draft SANCO Guidance Document for seed treatment (SANCO/10553/2012, Version January 2014) was considered to be the appropriate Guidance Document to assess the risk from dust drift exposure (see Section B.9.6.2). It was noted that the studies by Leuckmann (2014) and Lueckmann & Staffel (2015), which were used to derive the refined dust deposit value of 0.61 g a.s./ha for winter cereals, are acceptable. However, it was argued that the dust deposit values from the SANCO Guidance Document were derived from a large dataset. Individual studies with few varieties might not be sufficient to overrule the values reported in SANCO/10553/2012 (Version January 2014) as the amount of active substance deposits through dust drift is very much dependent on the quality of the seed dressing rather than the properties of the active substance. Therefore, according to SANCO/10553/2012 (Version January 2014), the studies by Lueckmann (2014) and Lueckmann & Staffel (2015) alone are not sufficient for estimating the exposure from dust deposition in cereals. These studies would however be useful to extend the dataset on dust deposition used to determine the values reported in the SANCO Guidance Document. Overall, no value from the available studies was considered suitable to refine the assessment at Tier 2.

To refine the risk assessment for oral exposure, residue levels in nectar and pollen in plants or crops exposed to dust drift are needed. However, such residue levels are not available, nor is an official guidance on how to measure them. Consequently, the tier 2 risk assessment for oral exposure following dust drift cannot be performed. However, this will be further considered in the higher tier assessment.

Risk assessment based on higher tier studies

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 exposure to dust drift after sowing of treated winter cereal and beet seeds. Nevertheless, other higher tier data is available.

For the **use in winter cereals**, an effect study that assesses the effect on honeybee colonies following the exposure to dust drift after sowing of treated winter barley seeds is available (Lueckmann & Staffel, 2015). In this study, winter barley was sown in June, in an area surrounded by full-flowering *Phacelia tanacetifolia*, a highly bee attractive crop. Further, the sowing operation took place when bees were actively foraging. As dust drift deposits containing measurable residues of clothianidin we found after sowing, honeybees were clearly exposed to dust drift. However, the mortality directly after sowing was not higher in the test item treated group compared to the control, indicating there is no acute risk for honeybees through contact exposure. As the acute contact toxicity of clothianidin to bumblebees is lower than for honeybees ($LD_{50,contact}$ of 148.3 ng/bee and 27.5 ng/bee, respectively), RMS assumed in the original version of this Addendum that no increased acute mortality is to be expected for bumblebees as well.

During Peer Review, it was argued that although the acute contact toxicity for bumble bees is lower than honeybees, it is not appropriate to conclude that no increased acute mortality is to be expected in field based on the field study by Lueckmann & Staffel (2015), because exposure estimates and trigger values are different. Further, it was stated that this study was explicitly designed to investigate the development of honeybee colonies. The colonies and populations of wild bumblebees do not have the same capacity to replace worker/bee losses compared to the super organism of a honeybee colony. The significant effect (mortality of workers) measured in this test will thus have a much higher impact on a population of wild bumblebees than on honeybees (see comment 5(40) and 5(47) in the Reporting Table). In response to these comments, the applicant submitted the following argumentation (*text in italic*):

The applicant agrees with the RMS that it is appropriate to conclude from the field study on the effects of dust exposure that no increase in acute mortality is to be expected and that the contact toxicity of

clothianidin is significantly lower towards bumblebees compared to honeybees. Regarding exposure the estimates for honeybees and bumblebees are equal as can be seen in the respective risk assessment tables for tier 1 (Table B.9.6.3.1-2 for honeybees and B.9.6.3.2-2 for bumblebees) and tier 2 (Table B.9.6.3.1-4 for honeybees and B.9.6.3.1-4 for bumblebees). Based on the EFSA GD the trigger value of 2.3 for bumblebees is 6 times more conservative than the trigger value of 14 for honeybees. Maintaining the same level of conservatism for both species is achieved when considering that the contact toxicity endpoint of LD_{50} = 0.1483 µg a.s./bumblebee is 5.4 times higher than the value of LD_{50} = 0.0275 µg a.s./honeybee, which balances out the difference in trigger values. Therefore, for honeybees and bumblebees the same level of conservatism is met and thus, the higher tier field study performed with honeybees (as presented in the higher tier risk assessment for bumblebees) is representative also for bumblebees.

Further, in the honeybee field study by Lueckmann & Staffel (2015), the application (drilling of dressed seeds) was performed in summer which represents a worst case exposure scenario for honeybee colonies when considering that the currently permitted use in cereals is restricted to autumn-sown cereals. In the case of bumblebee colonies exposure is limited in autumn since by this time of the year the annual colonies of bumble bees decrease anyway in the number of workers as bumble bee colonies do not overwinter and new colonies are founded each spring by new queens. Therefore bumble bee colonies do not store nectar as honeybee colonies do. Consequently it should possible to read across from honey bees as a worst case. As no adverse effects were noted in field study where honey bee colonies were exposed to dust drift the impact on bumblebees is considered to be acceptable.

At Pesticides Peer Review Meeting 145, the field effect study by Leuckmann & Staffel (2015) in winter barley was discussed. It was noted that the statistical power of the study was not assessed, but it is likely to be low (i.e. 2 control and 2 treated fields each filed with 8 hives). The study was conducted in UK and it was considered not representative of other EU conditions. The meteorological conditions and the bee activity in the study should be compared with other EU situations for ensuring that it represents a worst-case. The RMS noted that the use of *phacelia*, being a highly attractive crop, was supposed to cover uncertainties regarding other factors influencing the exposure. One study with 2 sites was however not considered sufficient to address the exposure and effect Specific Protection Goals (SPG). Further, it was agreed that the quality of seeds used in this study was a lot better than the qualities available on the market, and therefore the exposure could not be considered as a representative worst-case. As it was agreed that this study alone, without further data, could not be considered sufficient to draw a conclusion regarding the effect of dust drift depositions on honeybees, it also cannot be considered sufficient to support an extrapolation of the results to bumblebees.

Further, a field effect study which investigated the effects of residues in nectar and pollen of clothianidin treated (seed treatment) oilseed rape on bumblebee colony development was submitted by the applicant (Sterk & Peters, 2014; see section B.9.7.1, Study 1.8/9). This study is part of a large scale monitoring project on the effects of seed treatment of oilseed rape with clothianidin on honeybees, bumblebees and solitary bees. For this monitoring project, two study sites (treated site and control site) were selected in Northern Germany, each covering an area of about 65 km² and containing about 20 study fields sown with oilseed rape. Oilseed rape sown in the treated site were seed treated with clothianidin, while those sown in the control site were untreated. For the bumblebee study, six study locations were identified at each study site within a central area (3 km diameter) where bumblebee hives were set up. Of the six locations in each study site, three locations were situated at the edge of oilseed rape fields, and three location at about 400m distant from the oilseed rape fields. At each study location, 10 bumblebee colonies were placed, resulting in a total of 120 colonies that were exposed to nectar and pollen from oilseed rape (60 treated and 60 untreated). It could be argued that only one study is available and that the geographical spread of the study locations is limited. However, a high number of colonies was monitored, which should result in a sufficient statistical power. Overall, this bumblebee field study is considered to provide a good indication of the potential influence of nectar and pollen from plants in the field margin contaminated by dust drift.

The amount of clothianidin applied to the fields sown with oilseed rape in the treatment site varied with the clothianidin loading of the oilseed rape seeds and the amount of sown seeds. On average, treatment fields received 28.8 ± 10.0 g a.s./ha (see Russ et al., 2014; Study 1.8/5). As this value is much higher than the clothianidin residues deposited on plants in the field margin through dust drift (0.61 g a.s./ha in cereals, see Section B.9.6.2), it can be expected that residues in pollen and nectar from plants in the field margin exposed to dust drift will be considereably lower than those in pollen and nectar from the treated oilseed rape crop. Consequently, exposure of bumblebees to residues of clothianidin in the field study by Sterk & Peters (2014) can be considered worst case compared to exposure through nectar and pollen from plants contaminated through dust drift.

Sterk & Peters (2014) found no treatment related effects on the development of bumblebee hives (measured as evolution in number of workers, colony weight, brood size and number of new queens), neither during blossom in spring nor thereafter until the end of the season. The weather and the distance to the oilseed rape fields were the main influencing variables on the development of the bumblebee colonies. Based on these results it is reasonable to assume that, due to the lower exposure, no effect would be seen in studies with plants exposed to dust drift. Therefore, the acute and long-term risk to bumblebees following exposure to nectar and pollen from plants in the field margin contaminated through dust drift is considered acceptable.

During Peer Review, the extrapolation of the results from the large scale field study in oilseed rape to demonstrate an acceptable risk to bumblebees following exposure to dust drift was questioned (see comment 5(48) and 5(49)). For this extrapolation, it has been assumed that the residues measured in the oilseed rape fields are worst case compared to the residues in wild flowers in the field margin. It was argued that on the one hand this might be true, but on the other hand there is not sufficient information available about the amount of residue which has to be expected shortly after sowing in wild plants of the field margin. In the field study with oilseed rape clothianidin has had more than a half year to degrade. Thus, it was considered that in flowering plants of field margins shortly after sowing higher residue concentrations might be found. It was therefore argued that it is highly questionable whether these studies are suitable for a higher tier risk refinement for an application in cereals or sugar beet. In response to this comment, the applicant submitted the following argumentation (*text in italic*):

As explained in detail in comment 5(47) the exposure of bumble bees and solitary bees is limited in autumn, which is also true for the oral exposure to dust drift. When cereals are drilled in autumn, field margins are only little populated with flowering plants, if at all.
Furthermore, the size of field margins is small compared to the area covered by the mass flowering bee attractive crop, such as oil seed rape that was investigated in the large scale monitoring program in which bumble bees and solitary bees conducted over an exposure duration of several weeks and during a period of the year that is relevant for collection of nectar and pollen as well as for breeding activity of these species.
Whereas (if at all) individuals may forage in a field margin in autumn, the dose a bumble bee colony or solitary bees may encounter in spring and summer by foraging in a mass flowering crop is seen to be well covered by the information obtained in this large monitoring program. For solitary bees, autumn is not a time where they are active as adult and are not nesting. At this time of year solitary bees are at a development stage (e.g. larval and pupal stages) within hidden nesting places where exposure to dust drift is unlikely.

At Pesticides Peer Review Meeting 145, the large scale monitoring study in oilseed rape was discussed. For the solitary bee, *Osmia*, the experts noted that the pollen composition indicated that oilseed rape is not a relevant source of pollen. For Bumblebees, the range of pollen composition was very high (2-100%) with an average of 50%. It was argued that in this case it could be useful to only consider the results from hives with a large proportion of OSR pollen to obtain a worst-case exposure situation, but this would further reduce the power of the study. Based on the current evaluation of the data presented in the study report, extrapolation to other scenarios was considered not fully reliable because not worst-case.

It was noted that the study was performed in Germany. A similarity analysis between the study area and other oilseed rape growing areas in Europe was performed, but it seems that it does not cover the landscape composition (i.e. differences in field margin composition in oilseed rape areas other than DE may influence the proportion of pollen from different plant species entering into the hive, for example when more attractive plants are available in the filed margin). An in depth evaluation of the similarity analysis provided with the study would be appropriate to confirm this.

It was noted that the complexity of the study design and the number of analyses and observations performed and reported would require a peer review of all the original study reports. A full consideration of this study within the confirmatory data procedure was not feasible. The study will be evaluated more deeply under the review on the neonicotinoids (Ref. EFSA question number: EFSA-Q-2015-00771).

Overall, the experts considered that this study, for the time being, cannot be used to draw firm conclusions on possible extrapolation of the results to other scenarios (i.e. succeeding crops, field margin and treated crop other than OSR) for honeybees. Further consideration for bumblebees would be needed. However, for solitary bees the experts considered that the extrapolation to other crops or scenarios could not be reliably performed because likely the conditions in the study were not worst case for these species.

For the **use in beet**, a field effect study that assesses the effect on honeybee colonies following the exposure to dust drift after sowing of treated sugar beet pills is available (Lueckmann & Staffel, 2014). In that study, clothianidin deposits following dust drift were measured. All samples that were analysed did not have detectable clothianidin residues (measured residue below the LOD of 0.004 g a.s./ha). Thus, pill treatment dust, abraded and released during sowing of treated sugar beet pills with non-modified (not deflected) vacuum-pneumatic sowing equipment did not result in measurable off-field exposure. These results are in line with the dust drift studies in sugar beet considered for the EFSA Conclusion on the risk assessment for bees for clothianidin (2013). At Pesticides Peer Review Meeting 145, the experts considered that the low exposure is sufficient as line of evidence to conclude a low risk to bumblebees for exposure through dust drift from clothianidin treated sugar beet pills (and fodder beet/beet pills, assuming the same technology for seed pelleting and drilling).

Conclusions

Due to negligible exposure, the risk to bumblebees resulting from residues of clothianidin in dust deposits after sowing of treated beet seeds at the currently registered maximum seed dressing rates, is considered acceptable.

For winter cereals, the risk from both oral and contact exposure to dust drift was not acceptable at tier 1. The available higher tier data was not sufficient to conclude that the risk can be considered acceptable.

B.9.6.3.3. Risk assessment for solitary bees

The risk assessment was performed following the EFSA Guidance Document on bees. As stated in section B.9.6.2, only exposure to dust drift in the field margin and adjacent crops is considered relevant. As exposure in the latter will be lower than in field margins, the risk assessment was only performed for field margins.

At Pesticides Peer Review Meeting 145, it was agreed to use the draft SANCO Guidance Document for seed treatment (SANCO/10553/2012, Version January 2014) for the exposure assessment for dust drift. For the rationale behind this decision, please refer to Section B.9.6.3.2. Following this decision, the exposure and risk assessment below are updated according to SANCO/10553/2012 (Version January 2014).

In section B.9.6.2 it was further demonstrated that exposure to dust drift from treated sugar beet seeds is negligible. Following the EFSA Conclusion on the risk assessment for bees (2013), it is therefore concluded that the risk following exposure to dust drift from treated sugar beet seeds is acceptable. Nevertheless, at Pesticides Peer Review Meeting 145, it was however considered necessary to include the Tier 1 risk assessment based on deposition values from SANCO/10553/2012 (Version January 2014) for the use in sugar beet in the Addendum. Therefore, this assessment was added below.

Tier 1 risk assessment

According to the EFSA Guidance Document, both the acute risk through contact exposure as the oral acute and chronic risk to adult bumblebees and bumblebee larvae should be assessed. Oral exposure to adults occurs when residues of clothianidin deposited on plants in the field margin are transported to nectar and pollen, which are then consumed by the bees and/or transported to the hive.

The level of exposure to clothianidin following dust drift deposits was calculated by using the draft SANCO Guidance Document for seed treatment (SANCO/10553/2012, Version January 2014). For details on these calculations, and the assumptions made, reference is made to Section B.9.6.3.1. The PEC_{3D} dust deposition values, which are used in the risk assessment, are summarized in Table B.9.6.3.3-1.

Table B.9.6.3.3-1: PEC_{3D} dust deposition values for the lowest and highest authorized application rate for clothianidin in winter cereals and beet according to SANCO/10553/2012 (Version January 2014).

Crop	Application rate		Regulatory scenario	PEC 3D dust deposition (g a.s./ha)
Winter cereals	Lowest	59 g a.s./ha (220 kg seeds/ha)	Product specific	1.00
			Reference value	1.59
			Worst case	5.96
	Highest	100 g a.s./ha (200 kg seeds/ha)	Product specific	1.69
			Reference value	1.44
			Worst case	5.42
Beet	Lowest	10 g a.s./ha (1 u/ha)	Reference value	0.26
			Worst case	2.60
	Highest	108 g a.s./ha (1.8 u/ha)	Reference value	0.47
			Worst case	4.68

The PEC_{3D} values from Table B.9.6.3.3-1 are used to represent the exposure to residues from clothianidin through dust drift. For the oral and contact risk assessment, HQ and ETR values will be calculated based on the relevant equations from the EFSA Guidance Document.

According to the EFSA Guidance Document on bees, the hazard quotient (HQ) for contact exposure for the field margin from dust drift after sowing of treated seeds, is calculated by the following equation at first tier:

$$HQ = \frac{f_{dep} * AR}{LD_{50\ contact}}$$

Where AR = application rate in g a.s./ha
f_{dep} = fraction of the dose deposited on the type of plants that foragers visit (see Appendix X of the EFSA Guidance Document)
$LD_{50,contact}$ is expressed in µg a.s./bee

If HQ > 2.6, a potential risk is identified, and a higher tier risk assessment should be performed. If the HQ is below this trigger, the risk is acceptable.

In the equation to calculate the HQ value above, the exposure is represented by $f_{dep} * AR$. For the present assessment, the exposure was calculated based on SANCO/10553/2012 (Version January 2014) as PEC_{3D}. Therfore, to calculate the HQ, *fdep * AR* will be replaced by the PEC_{3D}.

For oral exposure, Exposure Toxicity Ratios (ETR) for plants in the field margin are calculated with the equations below. The relevant shortcut values (and the methodology used to determine these values) are presented in Table J7 Appendix J of the EFSA Guidance Document. 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 = 2.3 (shortcut value for exposure to adult solitary bees, taken from Table J7 in Appendix J of the Guidance Document)
E_f = 0.099 for cereals without deflector, 0.0099 for cereals with deflector (According to Appendix X of the Guidance Document)
$LD_{50,oral}$ 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 = 2.3 (shortcut value for exposure to adult solitary bees, taken from Table J7 in Appendix J of the Guidance Document)
E_f = 0.099 for cereals without deflector, 0.0099 for cereals with deflector (According to Appendix X of the Guidance Document)
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 = 30.8 (shortcut value for solitary bee larvae, taken from Table J7 in Appendix J of the Guidance Document)
E_f = 0.099 for cereals without deflector, 0.0099 for cereals with deflector (According to Appendix X of the Guidance Document)
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.

In the equations to calculate the ETR values above, the exposure is represented by *AR* * E_f. For the present assessment, the exposure was however calculated based on SANCO/10553/2012 (Version January 2014) as PEC_{3D}. The PEC_{3D} represents the active substance residues deposited in the field margin throught dust drift. According to Appendix H of the EFSA Guidance Document for bees, this dust deposition value can be multiplied by 1/3 for the assessment of concentrations in nectar and pollen entering the hive, to account for dilution of the concentrations in the field marging because the average deposition is lower than in the downwind direction. Therefore, to calculate the ETR, *AR* * E_f will be replaced by 1/3 * PEC_{3D}.

The first tier risk assessment was performed using the highest and lowest authorized 'maximum application rate' for winter cereals and beet (see Table B.9.6.3.3-1). 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. As the PEC_{3D} was calculated assuming that pneumatic sowing machines equipped with pertinent devices ensuring dust deflection to soil are used, the risk assessment is only valid for situations where this equipment is used. The calculated Tier 1 HQ values for both winter cereals and beet are shown in Table B.9.6.3.3-2. The ETR values are shown in Table B.9.6.3.3-3 and Table B.9.6.3.3-4 for winter cereals and beet, respectively.

Table B.9.6.3.3-2: Tier 1 HQ calculations for acute adult contact exposure through dust drift for the lowest and highest authorized 'maximum application rate' of clothianidin in winter cereals and beet.

Crop	Application rate (g a.s./ha)		Regulatory scenario	PEC_{3D} (g a.s./ha)	$LD_{50,contact}$ (µg a.s./bee)	HQ	Trigger
Winter cereals	Lowest	59	Product specific	1.00	0.00275	365.04	2.6
			Reference value	1.59	0.00275	577.78	2.6
			Worst case	5.96	0.00275	2166.67	2.6
	Highest	100	Product specific	1.69	0.00275	614.55	2.6
			Reference value	1.44	0.00275	525.25	2.6
			Worst case	5.42	0.00275	1969.70	2.6
Beet	Lowest	10	Reference value	0.26	0.00275	94.55	2.6
			Worst case	2.60	0.00275	945.45	2.6
	Highest	108	Reference value	0.47	0.00275	170.91	2.6
			Worst case	4.68	0.00275	1701.81	2.6

For both the use in winter cereals an beet, the HQ values for both the lowest and highest 'maximum application rate' exceed the trigger value, regardless of the regulatory scenario considered. Further consideration is thus needed.

Table B.9.6.3.3-3: Tier 1 ETR calculations for acute adult oral and chronic adult oral exposure from plants in the field margin for the lowest and highest authorized 'maximum application rate' of clothianidin in winter cereals.

Acute adult oral exposure									
Crop	Application rate (kg a.s./ha)		Regulatory scenario	1/3 * PEC_{3D} (kg a.s./ha)	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Winter cereals	Lowest	0.059	Product specific	0.00033	2.3	-	0.000379	2.02	0.04
			Reference value	0.00053	2.3	-	0.000379	3.24	0.04
			Worst case	0.00200	2.3	-	0.000379	12.14	0.04
	Highest	0.100	Product specific	0.00057	2.3	-	0.000379	3.44	0.04
			Reference value	0.00047	2.3	-	0.000379	2.83	0.04
			Worst case	0.00180	2.3	-	0.000379	10.92	0.04
Chronic adult oral exposure									
Crop	Application rate (kg a.s./ha)		Regulatory scenario	1/3 * PEC_{3D} (kg a.s./ha)	SV	twa	LDD_{50} (µg a.s./bee/ day)	ETR	Trigger
Winter cereals	Lowest	0.059	Product specific	0.00033	2.3	1	0.000138	5.56	0.0054
			Reference value	0.00053	2.3	1	0.000138	8.89	0.0054
			Worst case	0.00200	2.3	1	0.000138	33.33	0.0054
	Highest	0.100	Product specific	0.00057	2.3	1	0.000138	9.44	0.0054
			Reference value	0.00047	2.3	1	0.000138	7.78	0.0054
			Worst case	0.00180	2.3	1	0.000138	30.00	0.0054

Table B.9.6.3.3-4: Tier 1 ETR calculations for acute adult oral and chronic adult oral exposure from plants in the field margin for the lowest and highest authorized 'maximum application rate' of clothianidin in beet.

Acute adult oral exposure									
Crop	Application rate (kg a.s./ha)		Regulatory scenario	1/3 * PEC_{3D} (kg a.s./ha)	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Beet	Lowest	0.010	Reference value	0.000087	2.3	-	0.000379	0.53	0.04
			Worst case	0.00087	2.3	-	0.000379	5.26	0.04
	Highest	0.108	Reference value	0.00016	2.3	-	0.000379	0.95	0.04
			Worst case	0.00156	2.3	-	0.000379	9.46	0.04
Chronic adult oral exposure									
Crop	Application rate (kg a.s./ha)		Regulatory scenario	1/3 * PEC_{3D} (kg a.s./ha)	SV	twa	LDD_{50} (µg a.s./bee/ day)	ETR	Trigger
Beet	Lowest	0.010	Reference value	0.000087	2.3	1	0.000138	1.44	0.0054
			Worst case	0.00087	2.3	1	0.000138	14.44	0.0054
	Highest	0.108	Reference value	0.00016	2.3	1	0.000138	2.61	0.0054
			Worst case	0.00156	2.3	1	0.000138	26.00	0.0054

For both the use in winter cereals and beet, all ETR values exceed the relevant trigger values, regardless of the regulatory scenario considered. A potential risk oral acute and chornic risk is thus identified for adult solitary bees. Consequently, further consideration is necessary.

Tier 2 risk assessment based on measured dust deposits

A number of dust drift studies in cereals is available, from which in the original version of this Addendum a reasonable worst case dust deposit value of 0.61 g a.s./ha was derived for winter cereals (highest available 90th percentile value from winter barley, see section B.9.6.2). Using this value, the HQ for contact exposure can be refined. As this refined HQ still exceeded the trigger, no acceptable acute risk to honeybees could be demonstrated.

At Pesticides Peer Review Meeting 145, the draft SANCO Guidance Document for seed treatment (SANCO/10553/2012, Version January 2014) was considered to be the appropriate Guidance

Document to assess the risk from dust drift exposure (see Section B.9.6.2). It was noted that the studies by Leuckmann (2014) and Lueckmann & Staffel (2015), which were used to derive the refined dust deposit value of 0.61 g a.s./ha for winter cereals, are acceptable. However, it was argued that the dust deposit values from the SANCO Guidance Document were derived from a large dataset. Individual studies with few varieties might not be sufficient to overrule the values reported in SANCO/10553/2012 (Version January 2014) as the amount of active substance deposits through dust drift is very much dependent on the quality of the seed dressing rather than the properties of the active substance. Therefore, according to SANCO/10553/2012 (Version January 2014), the studies by Lueckmann (2014) and Lueckmann & Staffel (2015) alone are not sufficient for estimating the exposure from dust deposition in cereals. These studies would however be useful to extend the dataset on dust deposition used to determine the values reported in the SANCO Guidance Document. Overall, no value from the available studies was considered suitable to refine the assessment at Tier 2.

To refine the risk assessment for oral exposure, residue levels in nectar and pollen in plants or crops exposed to dust drift are needed. However, such residue levels are not available, nor is an official guidance on how to measure them. Consequently, the tier 2 risk assessment for oral exposure following dust drift cannot be performed. However, this will be further considered in the higher tier assessment.

Risk assessment based on higher tier studies

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 exposure to dust drift after sowing of treated winter cereal and beet seeds. Nevertheless, other higher tier data is available.

For the **use in winter cereals**, an effect study that assesses the effect on honeybee colonies following the exposure to dust drift after sowing of treated winter barley seeds is available (Lueckmann & Staffel, 2015). In this study, winter barley was sown in June, in an area surrounded by full-flowering *Phacelia tanacetifolia*, a highly bee attractive crop. Further, the sowing operation took place when bees were actively foraging. As dust drift deposits containing measurable residues of clothianidin we found after sowing, honeybees were clearly exposed to dust drift. However, the mortality directly after sowing was not higher in the test item treated group compared to the control, indicating there is no acute risk for honeybees through contact exposure. As there is no data available on the contact toxicity of clothianidin to solitary bees (due to the lack of agreed test methodology), it is difficult to extrapolate the results from this study with honeybees to solitary bees. Consequently, this part of the risk assessment could not be finalized.

At Pesticides Peer Review Meeting 145, the field effect study by Leuckmann & Staffel (2015) in winter barley was discussed. It was noted that the statistical power of the study was not assessed, but it is likely to be low (i.e. 2 control and 2 treated fields each filed with 8 hives). The study was conducted in UK and it was considered not representative of other EU conditions. The meteorological conditions and the bee activity in the study should be compared with other EU situations for ensuring that it represents a worst-case. The RMS noted that the use of *phacelia*, being a highly attractive crop, was supposed to cover uncertainties regarding other factors influencing the exposure. One study with 2 sites was however not considered sufficient to address the exposure and effect Specific Protection Goals (SPG). Further, it was agreed that the quality of seeds used in this study was a lot better than the qualities available on the market, and therefore the exposure could not be considered as a representative worst-case. As it was agreed that this study alone, without further data, could not be considered sufficient to draw a conclusion regarding the effect of dust drift depositions on honeybees, it also cannot be considered sufficient to support an extrapolation of the results to solitary bees, even if data on the acute toxicity of clothianidin to solitary bees would be available..

Further, a field effect study which investigated the effects of residues in nectar and pollen of clothianidin treated (seed treatment) oilseed rape on the development and reproduction of solitary bees was submitted by the applicant (Peters, 2015; see section B.9.7.1, Study 1.8/8). This study was conducted with the red mason bee *Osmia bicornis*. In Appendix Q of the EFSA Guidance Document

on bees, this species is proposed as test species in the risk assessment scheme for solitary bees. The study by Peters (2015) is part of a large scale monitoring project on the effects of seed treatment of oilseed rape with clothianidin on honeybees, bumblebees and solitary bees. For this monitoring project, two study sites (treated site and control site) were selected in Northern Germany, each covering an area of about 65 km² and containing about 20 study fields sown with oilseed rape. Oilseed rape sown in the treated site were seed treated with clothianidin, while those sown in the control site were untreated. For the solitary bee study, six study locations were identified at each study site where nesting shelters and solitary bee cocoons were set up. Of the six locations in each study site, three locations were situated at the edge of oilseed rape fields, and three location at about 100m distant from the oilseed rape fields. At each study location, three nesting shelters containing two or three nesting blocks (with 200 nesting holes) were placed. This resulted in 36 nesting shelters in total (18 treated and 18 untreated). Further, 1500 cocoons of red mason bees were set up at each test location. It could be argued that only one study is available and that the geographical spread of the study locations is limited. However, a high number of nesting mason bee females was monitored, which should result in a sufficient statistical power. Overall, this solitary bee field study is considered to provide a good indication of the potential influence of nectar and pollen from succeeding crops on solitary bees.

The amount of clothianidin applied to the fields sown with oilseed rape in the treatment site varied with the clothianidin loading of the oilseed rape seeds and the amount of sown seeds. On average, treatment fields received 28.8 ± 10.0 g a.s./ha (see Russ et al., 2014; Study 1.8/5). As this value is much higher than the clothianidin residues deposited on plants in the field margin through dust drift (0.61 g a.s./ha in cereals, see Section B.9.6.2), it can be expected that residues in pollen and nectar from plants in the field margin exposed to dust drift will be considereably lower than those in pollen and nectar from the treated oilseed rape crop. Consequently, exposure of solitary bees to residues of clothianidin in the field study by Peters (2015) can thus be considered worst case compared to exposure through nectar and pollen from plants contaminated through dust drift.

The results from Peters (2015) indicate that Elado dressed oilseed rape had no impact on the development of red mason bees neither on the nest building nor on the reproduction, neither during blossom in spring nor thereafter until autumn. Also in the Study Locations which were selected at the edge of oilseed rape fields no effects of Clothianidin were measurable although mason bees at these locations were more intensively exposed to Elado dressed oilseed rape. The weather and especially the sunshine was the main influencing variable on the nest building activity and reproduction of the mason bees. Based on these results it is reasonable to assume that, due to the lower exposure, no effect would be seen in studies with plants exposed to dust drift. Therefore, the acute and long-term risk to solitary bees following exposure to nectar and pollen from plants in the field margin contaminated to dust drift is considered acceptable.

During Peer Review, the extrapolation of the results from the large scale field study in oilseed rape to demonstrate an acceptable risk to solitary bees following exposure to dust drift was questioned (see comment 5(48) and 5(49)). For this extrapolation, it has been assumed that the residues measured in the oilseed rape fields are worst case compared to the residues in wild flowers in the field margin. It was argued that on the one hand this might be true, but on the other hand there is not sufficient information available about the amount of residue which has to be expected shortly after sowing in wild plants of the field margin. In the field study with oilseed rape clothianidin has had more than a half year to degrade. Thus, it was considered that in flowering plants of field margins shortly after sowing higher residue concentrations might be found. It was therefore argued that it is highly questionable whether these studies are suitable for a higher tier risk refinement for an application in cereals or sugar beet. In response to this comment, the applicant submitted the following argumentation (*text in italic*):

In the large scale field study in oilseed rape, the application was performed in summer which represents a worst case exposure scenario considering that the currently permitted use in cereals is restricted to autumn-sown cereals. For solitary bees, autumn is not a time where they are active as adult and are not nesting. At this time of year solitary bees are at a development stage (e.g. larval and

pupal stages) within hidden nesting places. Consequently, exposure (oral and contact) to dust drift is unlikely. When cereals are drilled in autumn, field margins are only little populated with flowering plants, if at all.
Furthermore, the size of field margins is small compared to the area covered by the mass flowering bee attractive crop, such as oilseed rape that was investigated in the large scale monitoring program in which bumblebees and solitary bees conducted over an exposure duration of several weeks and during a period of the year that is relevant for collection of nectar and pollen as well as for breeding activity of these species.
Whereas (if at all) individuals may forage in a field margin in autumn, the dose a bumble bee colony or solitary bees may encounter in spring and summer by foraging in a mass flowering crop is seen to be well covered by the information obtained in this large monitoring program. For solitary bees, autumn is not a time where they are active as adult and are not nesting. At this time of year solitary bees are at a development stage (e.g. larval and pupal stages) within hidden nesting places where exposure to dust drift is unlikely.

During Peer Review, it was also argued that a field study with one species of solitary bees is not considered sufficient addressing the risk to bees taking into account the high variability between the different species of solitary bees. In addition, it should be considered that the used solitary bees (mason bees) are food-generalists. Hence, the available field study might not cover the variability between species and the realistic exposure to solitary bees (see commenting 5(20) and 5(44) in the Reporting Table). In response to this comment, the applicant pointed out that the solitary bee species *Osmia bicornis* investigated in this study is the representative solitary bee species recommended by the EFSA Guidance Document on bees. Further, the applicant submitted a literature evaluation, which is summarized in Section B.9.7.1 (Exeler N., 2015; study 1.8/10). Based on this literature evaluation the applicant is of the opinion that only 2% of a regional bee species pool represents the dominant crop-visiting species. These pollinator species are generally common and polylectic, foraging on a range of different plant species. The applicant claims to be not aware of any information that refers to presence of food specialists in oilseed rape and therefore this exposure scenario appears unrealistic. As the life cycle for solitary bee species is overall comparable, the applicant considers that the field study is also representative for other species of solitary bees.

At Pesticides Peer Review Meeting 145, the large scale monitoring study in oilseed rape was discussed. For the solitary bee, *Osmia*, the experts noted that the pollen composition indicated that oilseed rape is not a relevant source of pollen. Therefore, the exposure in this study cannot be considered worst-case, and therefore extrapolation to other scenarios was considered not fully reliable.

It was noted that the study was performed in Germany. A similarity analysis between the study area and other oilseed rape growing areas in Europe was performed, but it seems that it does not cover the landscape composition (i.e. differences in field margin composition in oilseed rape areas other than DE may influence the proportion of pollen from different plant species entering into the hive, for example when more attractive plants are available in the filed margin). An in depth evaluation of the similarity analysis provided with the study would be appropriate to confirm this.

It was noted that the complexity of the study design and the number of analyses and observations performed and reported would require a peer review of all the original study reports. A full consideration of this study within the confirmatory data procedure was not feasible. The study will be evaluated more deeply under the review on the neonicotinoids (Ref. EFSA question number: EFSA-Q-2015-00771).

Overall, the experts considered that this study, for the time being, cannot be used to draw firm conclusions on possible extrapolation of the results to other scenarios (i.e. succeeding crops, field margin and treated crop other than OSR) for honeybees. Further consideration for bumblebees would be needed. However, for solitary bees the experts considered that the extrapolation to other crops or scenarios could not be reliably performed because likely the conditions in the study were not worst case for these species.

For the **use in beet**, a field effect study that assesses the effect on honeybee colonies following the exposure to dust drift after sowing of treated sugar beet pills is available (Lueckmann & Staffel, 2014). In that study, clothianidin deposits following dust drift were measured. All samples that were analysed did not have detectable clothianidin residues (measured residue below the LOD of 0.004 g a.s./ha). Thus, pill treatment dust, abraded and released during sowing of treated sugar beet pills with non-modified (not deflected) vacuum-pneumatic sowing equipment did not result in measurable off-field exposure. These results are in line with the dust drift studies in sugar beet considered for the EFSA Conclusion on the risk assessment for bees for clothianidin (2013). At Pesticides Peer Review Meeting 145, the experts considered that the low exposure is sufficient as line of evidence to conclude a low risk to solitary bees for exposure through dust drift from clothianidin treated sugar beet pills (and fodder beet/beet pills, assuming the same technology for seed pelleting and drilling).

Conclusions

Due to negligible exposure, the risk to solitary bees resulting from residues of clothianidin in dust deposits after sowing of treated sugar beet seeds at the currently registered maximum seed dressing rates, is considered acceptable.

For winter cereals, the risk from both oral and contact exposure to dust drift was not acceptable at tier 1. The available higher tier data was not sufficient to conclude that the risk can be considered acceptable.

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 report of a large scale monitoring study on the effects of clothianidin treated oilseed rape (seed treatment) on honeybees, bumblebees and solitary bees (the Red Mason Bee, *Osmia bicornis*) was submitted by the applicant. As this monitoring study was a large and multi-disciplinary project, the results have been reported in eight study reports, each covering a specific aspect, and a nineth project overview and summary report.

As the use of clothianidin as seed treatment for oilseed rape is currently not authorized, this study falls outside of the scope of the confirmatory data requirements in the strict sence. However, the results for honeybees, bumblebees and solitary bees provide information that could be used in support of the risk assessment for other routes of exposure than consumption of nectar and pollen from the treated crop.

At Pesticides Peer Review Meeting 145, it was noted that the complexity of the study design and the number of analyses and observations performed and reported would require a peer review of all the original study reports. A full consideration of this study within the confirmatory data procedure was not feasible. The study will be evaluated more deeply under the review on the neonicotinoids (Ref. EFSA question number: EFSA-Q-2015-00771). Overall, the experts considered that this study, for the time being, cannot be used to draw firm conclusions on possible extrapolation of the results to other scenarios (i.e. succeeding crops, field margin and treated crop other oilseed). The summaries of the studies that were part of the large scale monitoring study in oilseed rape are provided below for information only.

Report:	**1.8/1; Heimbach, F.; Russ, A.; 2014**
Title:	**Interim report - Large-scale monitoring of long-term effects of Elado (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: I project overview and summary**
Report No.:	B13055-0
Document No.:	M-503588-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	no

This Interim Report covers all final results and discussions except of wintering and emergence of mason bees in spring 2015. Subsequent data on these aspects will be included in the final version of this report which is due in April 2015.

Objectives

The purpose of the study was to monitor possible adverse side effects of Elado dressed Oilseed Rape (OSR) on honeybee and bumblebee colonies, and on solitary mason bees. The present report provides an overview of the different studies of the project, combines findings from different studies and summarizes important results.

Project organisation

The project "Large-scale Monitoring of Long-term Effects of Elado (10g Clothianidin & 2g Beta-Cyfluthrin/kg seed) Dressed Oilseed Rape on Pollinating Insects in Mecklenburg-Vorpommern, Germany" consists of four different pollinator studies performed in the Project Area at the same time: a honeybee monitoring study (Study ref. 1.8/7; Project Study No. P13081-1 "Effects on Honeybees (*Apis mellifera*)"), a mason bee monitoring study (Study ref. 1.8/8; Project Report No. B14013 "Short-

and Long-term Effects on Red Mason Bees (*Osmia bicornis*)"), a bumblebee monitoring study (Study ref. 1.8/9; Project Report No. B14014 "Effects on Large Earth Bumblebees (*Bombus terrestris*)"), and a residue analysis of nectar and pollen from foraging honeybees (Study ref. 1.8/6; Project Report No. B13081-2 "Residues of Clothianidin in Nectar and Pollen collected by Honeybees in Tunnel Tents").

The selection of the Project Area and the Study Sites took place in summer 2013 and are described in detail in Study 1.8/2 (Project Report No. B13055-1 (non-GLP) subtitled "Project Area and Study Fields Characterisation"). Study Fields were either drilled with Elado-dressed OSR seeds (Treatment Site) or with Clothianidin-free OSR seeds (Control Site). Before drilling in autumn 2013, soil samples were collected from all Study Fields for the analysis of Clothianidin residues and soil characterisation. The results of these studies are reported in Study 1.8/4 (Project Report No. B13055-2 (non-GLP) subtitled "Residues of Clothianidin in Soil before Drilling and "oil Characterisation"). Furthermore, Clothianidin loadings of the OSR seeds were analysed and the entire development of OSR from drilling to harvest was monitored. The corresponding results are reported under Study 1.8/5 (Project Report No. B13055-3 (non-GLP) subtitled "Seed Characterisation, Drilling and Growth of Oilseed Rape"). To ensure the comparability of Control and Treatment Sites a Site Similarity Analysis was performed and is reported under Study 1.8/3 (Report "Site Similarity Certification of Study Sites and its Relevance for other Rape Cultivation Sites in Europe") by the Spatial Business Integration GmbH, Darmstadt, Germany.

Summary of the results
Test item: Elado® (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed Oilseed Rape (OSR).

The Project Area was chosen to be representative for other OSR cultivation areas in Europe and comparison between the control and treatments site indicated that they are as similar as possible in terms of climate, geography, habitats, agriculture and further land-uses and, therefore, represent comparable landscapes for pollinating insects. The crop and PPP history of the study fields had no effect on the study design. The size of the study sites exceed effective collecting flight distances of honeybees, bumblebees and mason bees and, hence, guaranteed the exposure of the bee colonies and the solitary bees to about 1,800 ha OSR in total. The project area provided a suitable background for the large-scale monitoring of long-term effects of Elado® dressed OSR on pollinating insects.

This extensive bee monitoring study revealed an exposure of all three bee species to clothianidin at the treatment site, but this exposure had no detrimental effects on the development of hives, brood or nesting activities. Furthermore, the health of the bees, respectively infestation rates of diseases or parasites were not different at the treatment and control site.

The distance to OSR fields caused higher energy expenditures and resulted in reduced storage of food resources (less honey yield in honeybees), limited investment into the queen brood (bumblebees) or a higher parasite load due to longer absence from the nest site in mason bees. In addition, the development of the hives of honey and bumblebees and the nesting activity of mason bees was significantly affected by meteorological conditions.

During the exposure phase, all three bee species were in very good health conditions and showed very low infestation rates with parasites or diseases. Though, towards the end of the post exposure phase, honeybees suffered from severe varroosis and concomitant virus infections which was not related to the clothianidin treatment, but was caused by a high proportion of flumethrine resistant *Varroa* mites.

Residues of clothianidin were highest in samples from honeybees, which is not surprising, because they also collected the highest proportion of OSR pollen. Although the proportion of OSR pollen in samples from mason bees averaged only 11 to 18 %, they were also exposed to clothianidin as confirmed by the residue analysis of the collected pollen.

In conclusion, clothianidin treated OSR did not cause any detrimental effects on the development of hives and brood of honeybees and bumblebees or the nesting activity and reproduction of mason bees

neither during OSR blossom in spring nor thereafter until the end of the study. The pollen composition, infestation with diseases and parasite load was also not affected by the exposure to clothianidin treated OSR in any of the investigated bee species.

RMS Comments
This report provides an overwiew of the different studies performed within this large scale monitoring project, and describes the most important results. For details on the design and results from each specific study, reference is made to the respective study reports.

Report:	**1.8/2; Schimmer, M.; Russ, A.; 2014**
Title:	**Large-scale monitoring of long-term effects of Elado (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: II project area and study fields characterisation**
Report No.:	B13055-1
Document No.:	M-503370-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	no

This study is part of a large-scale monitoring project on the effects of seed treatment of Oilseed Rape with clothianidin on honeybees, bumblebees and solitary bees. An overview of the entire monitoring project is provided under Study 1.8/1 (Heimback & Russ, 2014).

Objective
The aims of this study were to describe the project area and provide a complete characterisation of the study fields to establish a geographical basis and reference frame for a consistent terminology among the upcoming studies of the project "Large-scale Monitoring of Long-term Effects of Elado® (10 g Clothianidin & 2 g Beta-Cyfluthrin / kg seed) Dressed Oilseed Rape on Pollinating Insects in Mecklenburg-Vorpommern, Germany".

Material and Methods
Test item: Elado® (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed Oilseed Rape (OSR).

Definition of Study Sites and Study Fields
The monitoring studies were conducted in the northeast of Germany, in Mecklenburg-West Pomerania (Mecklenburg-Vorpommern) because of the suitable landscape characteristics and agronomic infrastructure. The Project Area consists of two circular Study Sites, and is located aboute 30 km east of the state capital Schwerin. The Study Sites consisted of a control site with different OSR varieties without a clothianidin dressing and a treatment site with different OSR varieties with commercial dressings containing clothianidin. Both Study Sites are located next to each other, but are separated by a corridor of approximately 1 km in width. Each of the circular study sites covers an area of about 65 km^2 (9 km in diameter).

The selection of the Study Sites was based on a geo-based landscape analysis of landscape structures relevant for honeybees and bumblebees, such as arable land, hedges, grassland, forests, water bodies, and settlements to ensure a maximum spatial comparability between the sites. A detailed description of the site similarity analysis is given in study report 1.8/3 "Site Similarity Certification of Study Sites and its Relevance for other Rape Cultivation sites in Europe.

The Study Sites were defined to surround the prospective locations of the central bee hives and cover sufficient buffer area to account for the major foraging flying distances of the pollinating insects in

focus. Hence, in both Study Sites, a Core Area of 7 km in diameter is monitored in depth (Figure B.9.7.1-1).

The northern Study Site is the Control Site and comprises 19 OSR Study Fields. Of these, Clothianidin-free OSR varieties were cultivated on 17 Study Fields, but 2 small Study Fields at the outer edge of this area contained Clothianidin-dressed OSR (see Project Reports No B13055-3 "Seed Characterisation, Drilling and Growth of Oilseed Rape" and B13081-1 "Effects on Honeybees (*Apis mellifera*)" for further discussion). The southern Study Site, the Treatment Site, comprises 27 Study Fields which were drilled with Elado dressed seeds of different OSR varieties.

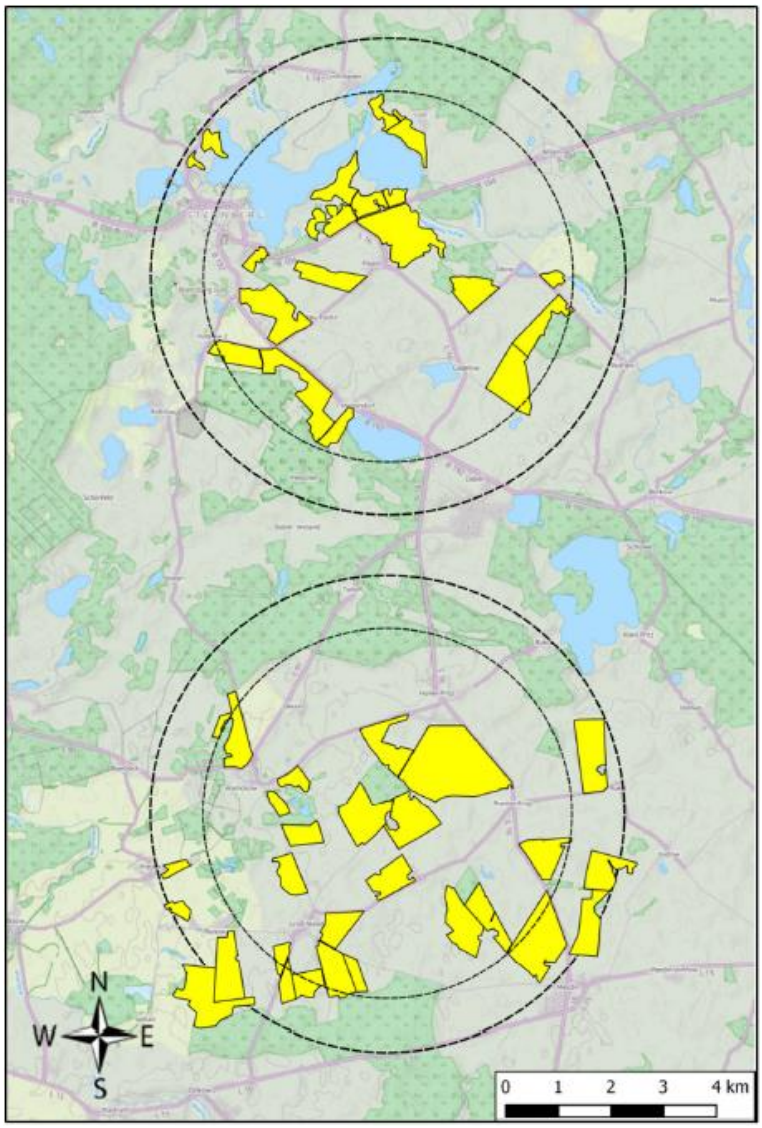

Figure B.9.7.1-1 Study Fields at the Control (North) and Treatment Site (South). Outer Circles depict the Marging of the Study Sites (9 km diameter), Inner Circles the Boundary of the Core Area (7 km diameter). Yellow polygones indicate the Study Fields.

Characterisation of the Study Sites

The characterisation of the study sites included a habitat mapping and an assessment of alternative forage plants for the different bee species. The study fields were comprehensively characterised and compared regarding their history of crops and application of plant protection products during the five years previous to the study.

Findings
Because the Study Sites are located next to each other they can be considered as similar as possible in climate, geography, habitats, natural preservation areas, agriculture and other land-uses.

In total, 46 Study Fields are located in both Study Sites, which encompassed 1871 ha of OSR crop of which 1766 ha were situated inside the boundary of the Study Sites. Of this area, OSR was cultivated on 641 ha of the Control Site and on 1125 ha of the Treatment Site. This corresponds to 10.1 and 17.7% of the area of the Control and Treatment Site, respectively. The median size of Study Fields was 32.8 ha at the Control Site and 35.6 ha at the Treatment Site.

The habitat mapping indicates that the habitat distribution at both Study Sites is quite diverse and although large cutting areas exist, the whole area is well structured by a diversity of small forest patches and groves of trees, hedges including shrubs, water bodies of different sizes and kettles.

During the exposure phase, OSR is a highly attractive forage plant for bees. Other crops at the Study Sites are not considered as food source, because grains and maize are wind-pollinated, while sugar beet is harvested before it develops florescence. The contribution of weeds at the arable land to the nourishment of bees is also negligible because they are controlled by herbicide application and were found to be in flower only towards the end of the exposure phase at the edge of the fields. Hence, alternative forage plants are mainly found at untreated habitats. However, the alternative forage plants at different habitats (hedges, kettles, edges of the forest and groves of trees, grassland, field margins, urban areas) were found to play a limited role as food resource, especially for honeybees which are faithful to certain blooms and change the food source only after depleting the former resource. The survey of alternative forage plants can however only be a rough estimate on the availability of other food sources for the bees in focus.

Conclusion
The selected project area is representative for Northern Germany and provides a suitable background for the large-scale monitoring of long-term effects of Elado® dressed OSR on pollinating insects.

Because of their similar structural elements and the similar agricultural background the Study Sites represent comparable landscapes for pollinating insects. The size of the study sites exceeds effective collecting flight distances of honeybees, bumblebees and mason bees and, hence, guarantees the exposure of the bee colonies and the solitary bees to about 1,800 ha OSR in total. No other crops in this area can be considered as food source during OSR blossom and alternative forage plants are only available on a small scale and limited amounts.

RMS Comments
The study report provides a comprehensive characterisation of the selected study sites. RMS agrees that the selected project area is representative of Northern Germany and provides a suitable background for the intended large-scale monitoring study.

Report:	**1.8/3; Born, K.; 2014**
Title:	**Large-scale monitoring of long-term effects of Elado (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: III site similarity certification of study sites and its relevance for other rape cultivation sites in Europe**
Report No.:	M-503372-01-1
Document No.:	M-503372-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	no

This study is part of a large-scale monitoring project on the effects of seed treatment of Oilseed Rape with clothianidin on honeybees, bumblebees and solitary bees. An overview of the entire monitoring project is provided under Study 1.8/1 (Heimback & Russ, 2014).

Objective
This Site Similarity Certification (SSC) aims at proving two study sites in a typical oilseed rape (OSR) growing region in Northern Germany to be similar with regard to their site conditions, in particular their landscapes and the forage provided for pollinating insects like bees. For these aspects, the SSC also investigates the relevance of the study sites for the state of Mecklenburg-Vorpommern and other European OSR cultivation sites.

Material and Methods
Test item: Elado® (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed Oilseed Rape

The study sites which are compared are located in the region of DEU-Ludwigslust-Parchim near Sternberg about 30 km east of Schwerin in the German federal state of Mecklenburg-Vorpommern.

The study sites C and T (control, treatment) are judged to be similar in their landscapes and thus the bees´ forage and their site conditions during the relevant period of OSR flowering and to be representative for DEU-Mecklenburg-Vorpommern (Tab. 1). Particularly the parameters land use and availability of OSR and alternative forage, climate, soil, OSR phenology and weather conditions (air temperature, precipitation, solar radiation) are compared. The analyses of the site characteristics were focused on the period of OSR flowering (BBCH 60 – 69) when the bees collect nectar and pollen.

Findings
The results indicate the relevance of the study sites C and T for the OSR cultivation sites in Europe by showing them to be covered by smaller OSR areas compared to the Study Sites C and T and offering areas with alternative forage to the bees.

Cultivation sites holding equal or larger OSR areas than 20.6 % of the total site area, as in the Study Site T, are unlikely to occur at large scale in Europe. Even sites with OSR areas larger than 16 %, as existent in study site C, are not found. The highest portions of OSR of the total site area are identified in north eastern Germany in Nordwestmecklenburg and Ostholstein with 15.8 % and 15.1 %, respectively.

In 44.0 % of the OSR cultivation regions in Europe crops like fruit trees and berries are cultivated, which can be used by bees as alternative forage during OSR blossom. In addition, plant species growing at the edges of forests, on natural grassland and pastures are available in 95.4 %, 63.4 % and 95.8 % of the European OSR cultivation sites which can serve bees for alternative forage as well.

The cultivation sites in this analysis are defined to be administrative units on NUTS 2 or NUTS 3 level which are characterized in terms of the areas of OSR cultivation and alternative forages, here fruit trees and berry plantations, forest edges, natural grassland and pastures. Although being different in

size and much larger than the study sites, the results presented for these cultivation sites provide an indication of the amount of OSR and other plants to which the bees are exposed to.

Conclusions
In summary, the analysis indicates that the study sites in Mecklenburg-Vorpommern, selected for the OSR insect pollinator monitoring project, most probably present the worst case in terms of high density of OSR and low availability of alternative bees` forage, in comparison to other European cultivation sites. This provides an indication of the relevance of the study sites C and T for the OSR cultivation sites in Europe.

Table B.9.7.1-1: Conclusion

Study Site C(ontrol) / Study Site T(reatment)	similar	Yes
Study Sites C, T / Mecklenburg-Vorpommern	representative	Yes
Study Sites C, T / Other European OSR cultivation sites	relevant	Yes

RMS Comments
The study is considered acceptable, and demonstrates that the control and treatment study site are similar with regard to their site conditions, in particular their landscapes and the forage provided for pollinating insects like bees.
The analysis of the relevance of the study sites for other OSR cultivation sites in Europe was not evaluated, as this falls out of the scope of the confirmatory data.

Report:	**1.8/4; Benito, M. M.; Russ, A.; Schimmer, M.; 2014**
Title:	**Large-scale monitoring of long-term effects of Elado (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: IV residues of clothianidin in soil before drilling and soil characterisation**
Report No.:	B13055-2
Document No.:	M-503397-01-1
Guideline(s):	No official test guideline is available for present type of study. The study was conducted under consideration of the EU Guidance on the Risk Assessment of Plant Protection Products on Bees (Apis mellifera, Bombus spp. and solitary bees) (EFSA 2013)
Guideline deviation(s):	not applicable
GLP/GEP:	no

This study is part of a large-scale monitoring project on the effects of seed treatment of Oilseed Rape with clothianidin on honeybees, bumblebees and solitary bees. An overview of the entire monitoring project is provided under Study 1.8/1 (Heimback & Russ, 2014).

Objective
The aim of this study was to describe the soils of the study fields in terms of composition and their physical characteristics. Furthermore, the soils were analysed for residues of the neonicotinoid clothianidin.

Material and Methods
A description of the study fields is provided in Study 1.8/2 (Schimmer & Russ, 2014). Each study field of the Core Area was divided into equally sized sampling plots of approximately 10 ha. Per plot ten sampling points were evenly distributed. At each previously determined sampling point, soil samples were collected in August 2013, before drilling of the OSR seeds. Samples were taken with a steel open hand samples by cutting out approximatel 10 by 5 cm. Samples were cleaned from coarse contamination (e.g. plant material and waste) and all samples of a plot were combined to a pooled plot sample and thoroughly mixed before analysis.

The samples were analysed by Eurofins Institute Jaeger regarding the pH, total organic carbon, water holding capacity, and the texture. Quantification of clothianidin residues was based on the multi-residue sample preparation technique QuEChERs and conducted by Eurofins Hamburg. A method validation for the determination of clothianidin in soil was part of this study.

Findings
The soils of the 35 study fields inside the core areas at the control and treatment sites have an average pH of 6.2 and, hence, are slightly acid to neutral. They have a low total organic carbon content of 0.9 % and, due to their high percentage of sandy components (67 %), have also a medium to low water holding capacity. Of the soils 98 % are classified as loamy sands. Despite marginal differences in the soil composition and physical characterization between the study sites they can be considered as equal because the variability within a study site is generally higher than between the sites.

The residue analysis did not reveal any relevant concentrations of clothianidin in the soils of the study fields before drilling of OSR seeds in August 2013. No residues of clothianidin were found in 82 % of the 134 soil samples (below limit of detection, LOD = 1.5 µg/kg). Small clothianidin peaks in chromatograms below the limit of quantification (LOQ = 5 µg/kg) were found for 24 soil samples, 6 from control fields and 18 of treatment fields. Hence, clothianidin residues were only found in four out of 17 control and six out of 17 treatment fields, but these were below the limit of quantification. All other analysed samples were below the limit of detection.

Conclusions
The analyses revealed comparable conditions of the soils of the control and treatment site. Clothianidin residues in soils of the study fields were < LOQ or even < LOD. Therefore, no confounding effects on the study results are to be expected neither from the soil characteristics nor from clothianidin residues.

RMS Comments
RMS agrees that no confounding effects on the study results are to be expected neither from the soil characteristics nor from clothianidin residues in soil (from previous applications).

Report:	**1.8/5; Russ, A.; Schimmer, M.; Benito, M.; 2014**
Title:	**Final report - Large-scale monitoring of long-term effects of Elado (10 g Clothianidin & 2 g Beta-Cyfluthrin / kg seed) Dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: V seed characterisation, Drilling and Growth of oilseed rape**
Report No.:	B13055-3
Document No.:	M-504076-01-1
Guideline(s):	No official test guideline is available for present type of study. The study was conducted under consideration of the EU Guidance on the Risk Assessment of Plant Protection Products on Bees (Apis mellifera, Bombus spp. and solitary bees) (EFSA 2013)
Guideline deviation(s):	not applicable
GLP/GEP:	no

This study is part of a large-scale monitoring project on the effects of seed treatment of Oilseed Rape with clothianidin on honeybees, bumblebees and solitary bees. An overview of the entire monitoring project is provided under Study 1.8/1 (Heimback & Russ, 2014).

Objective
The aim of this study was to provide a comprehensive description of all aspects relevant for the development of the OSR plants at the study fields during the harvest year 2014. Furthermore, the amounts of clothianidin loadings on OSR seeds were analysed and compared between the study fields.

Material and Methods
Test item: Elado® (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed Oilseed Rape (OSR).

A description of the study fields is provided in Study 1.8/2 (Schimmer & Russ, 2014).

Meteorological data from the harvest year 2014 were obtained from the nearby meteorological station of the German Weather Service in Goldberg and were compared to long-term averages. In August 2013, during the drilling of OSR at the study fields, seed samples were taken for characterisation and analysis of clothianidin loadings by Eurofins Hamburg. This analysis was based on the Liquid Chromatography Mass Spectrometry/Mass Spectrometry (LC-MS/MS). Recommendations for PPP spraying applications at the study fields were provided by the Julius-Kuehn-Institute, Braunschweig. Information regarding the variety of OSR seeds, drilling rate and date, as well as further agricultural activities on the study fields was reported by the farmers. The development of OSR plants was assessed seven times between November 2013 and May 2014 and categorized as BBCH stages.

Findings
During the harvest year 2014, weather conditions at the project area did not considerably deviate from the long-term average, although the winter was slightly warmer. The exposure phase was also comparable to the long-term average and was characterised by two slightly warmer and one marginally colder period.

In August 2013, seeds from 33 different OSR varieties were drilled to the study fields, 3.4 kg/ha on average (2.8 kg/ha for the control fields, 36 kg/ha for the treatment fields). The seeds had a mean thousand seed weight of 6.2 g. At the control site, exclusively single varieties were sowed at the study fields, whereas at the treatment site, more than one variety was sowed at five study fields. However, the OSR plants developed relatively homogenous at all study fields. Clothianidin loading among OSR seeds from treatment fields averaged 8.0 g/kg seeds. But traces of clothianidin were also found in seeds from the control site, which were contaminated during coating in commercial seed treatment facilities. However, the amount of clothianidin of 0.02 g/kg seed is considered too low to have any effects. During sowing of OSR seeds, clothianidin is transferred into the soils of the study fields. The

amount of clothianidin averages 28.8 ± 10.0 g/ha for treatment fields. It varies with the clothianidin loading of the OSR seeds and the amoint of sowed seeds.

On average, five insecticidal treatments were applied to control fields and four insecticidal sprayings to treatment fields between August 2013 and May 2014. The additional pyrethroid application was sprayed to most of the control fields because they lacked the insecticidal dressing of the OSR seeds. No neonicotinoids other than the treatment seed dressing were applied.

Conclusions
All study fields were treated according to Good Agricultural Practice. At control fields OSR without an Elado® dressing were cultivated, while Elado® coated OSR from established seed merchants were drilled to treatment fields. Farmers therefore treated control fields with additional insecticidal applications. The OSR plants developed well at all study fields and showed no significant differences between control and treatment fields.

RMS Comments
RMS agrees that OSR plants showed no significant differences in development between control and treatment fields.

Report:	**1.8/6; Persigehl, M.; 2014**
Title:	**Final report - Large-scale monitoring of long-term effects of Elado (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: VI residues of clothianidin in nectar and pollen collected by honeybees in tunnel tents**
Report No.:	B13081-2
Document No.:	M-504416-01-1
Guideline(s):	ENV/MC/CHEM(98)17 , Directive 2004/10/EC
Guideline deviation(s):	not applicable
GLP/GEP:	yes

This study is part of a large-scale monitoring project on the effects of seed treatment of Oilseed Rape with clothianidin on honeybees, bumblebees and solitary bees. An overview of the entire monitoring project is provided under Study 1.8/1 (Heimback & Russ, 2014).

Objective
The aim of this study was to provide reliable data of clothianidin residues in nectar and pollen of Elado® treated and untreated Oilseed Rape (OSR) collected by honeybees. The study represents a worst case scenario of the exposure of honeybees to clothianidin and its active metabolites TZNG and TZMU under outdoor conditions.

Material and Methods
Test item: Elado® (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed Oilseed Rape (OSR).

Study sites and sowing
A description of the study fields is provided in Study 1.8/2 (Schimmer & Russ, 2014). For the sampling of pollen and nectar, study fields were divided into sub-areas of approximately 10 ha. The main aim of subdividing the study fields to generate sub-areas was to achieve an appropriate number of samples related to the field size. In accordance with the given criteria, the study fields of the Control site were divided into 58 sub-areas and the 18 Study fields of the Treatment sites were divided into 96 sub-areas.

Study fields concerning the Treatment study site were drillend in autumn 2013 with OSR seeds dresses with Elado, while study fields of the Control study site were drilled in autumn 2013 with OSR seeds not dressed with Elado. Detailed information of drilled seeds (e.g. varieties, batch numbers, nominal and analysed dressing rates and details of drilling dates and sowing rates) is provided in Project Report No. B13055-3 (Study 1.8/5; Russ, Schimmer & Benito, 2014).

Pollen and nectar sampling

Between 21 April and 16 May 2014, tunnel tents were erected at the sub-areas (1 per sub-area, with an approximate area of 50 m²) and commercial small honeybee colonies (*Apis mellifera*) were enclosed to expose them exclusively to OSR at blossom. The commercial small colonies were approximately 30x30x30 cm in size and fitted with 6 combs. Each colony consisted of about 2500 bees, to guarantee an appropriate amount of honetbees to forage nectar and pollen. All colonies were well fed, healthy according to good honeybee keeping practice, free of obvious diseases, queen-right, and as similar as possible in orde to guarantee uniform bee material.

Nectar and pollen from OSR plants were collected via honeybees kept in the tunnel tents. To ensure that samples of nectar and pollen originated from OSR flowers of the Study field sub-area, honeybees were enclosed in the tunnel tents at least one dat before the sampling event. Each honeybee colony was used only once for a sampling event and was removed after successfully collecting pollen and nectar. During exposure in the tunnel tents, hives were equipped with a sliding door and a pollen trap at the entrance of the hive.

Nectar was sampled via the collection of returning honeybee workers, whose honey stomach was later on dissected in the laboratories of the test facility. To ensure the targeted amount of nectar about 400 honeybees were sampled per sampling event (about 200 bees for the main sample and the retain sample each).

For collection of pollen samples, pollen traps were attached in front of the entrance of the honeybee hive. The targeted minimum biomass per specimen was about 300 mg. In case it was not possible to collect a sufficient amount of pollen (<300 mg) within an acceptable time, a minimum of more than 100 mg pollen was achieved.

Residue analysis

Residue analyses of nectar and pollen were conducted by Eurofins Agroscience Services Chem GmbH (under the study no S14-03638 / BAY-1410 – Lindner & Giesau, 2014) based on the multi-residue-sample preparation technique QuEChERS and LC-MS/MS. This method was validated for the determination of clothianidin and its metabolites TZNG and TZMU in/on pollen and nectar within EAS Chem GmbH study no S13-04864 (BAY-1318). However, acceptable procedural recoveries determined within the study S14-03638 indicated an adequate performance of the method (mean recoveries within 70-110%; RSD<20%; n=4 per fortification level for pollen; n=2 for nectar). The LOQ was 1.0 µg/kg for each of the three analytes.

Findings

Neither clothianidin nor its metabolites TZNG and TZMU were present at 34 samples of nectar and pollen from the control site: residue concentrations of clothianidin in pollen and nectar were below the limit of detection (LOD = 0.3 µg/kg), except of three pollen samples with clothianidin residues of below the limit of quantification (LOQ = 1.0 µg/kg). The metabolites TZNG and TZMU were analysed as < LOD in all pollen and nectar samples, except of one pollen sample where TZNG was analysed as < LOQ.

At the treatment site, clothianidin was detected in pollen and nectar of all study fields. In 34 out of 39 pollen samples and 22 out of 39 nectar samples of the treatment site residue concentrations were slightly higher than the LOQ. Residues of TZNG in pollen and nectar were lower than the LOQ or

even lower than the LOD, whereas residues of TZMU were below the LOD in all samples. Maximum analysed concentrations of clothianidin were 3.6 µg/kg in nectar (T2-1) and 3.5 µg/kg in pollen (T9-3 and T12-1).

Repeated analysis of clothianidin concentrations in nectar and pollen during OSR blossom did not show a clear trend of concentrations over time because of a relatively high variability in space and time.

Statistical analysis of the results showed that there was no significant correlation between the clothianidin loading per seed and the residue concentrations in pollen and nectar. Similarly, ther was no significant correlation between clothianidin residues in pollen and nectar and the clothianidin loading (mg/ha) of the study fields.

Overall, this study constitutes a worst case scenario of honeybee exposure, since honeybees collected pollen and nectar only from the target plant OSR in tunnel tents.

Conclusions
The study provides reliable field data of clothianidin residues in pollen and nectar collected by honeybees on OSR fields in tunnel tents. Overall, clothianidin and its metabolites TZNG and TZMU could not be analysed in nectar and pollen at the control site. Clothianidin residues on treatment fields were on average[56] 1.3 µg/kg (median: 1.1 µg/kg) in nectar and 1.7 µg/kg (median 1.6 µg/kg) in pollen. Concentrations of the metabolites on treatment fields were below LOD except of some samples with concentrations < LOQ.

RMS Comments
The study is considered acceptable. RMS agrees that a worst-case exposure of honeybees was achieved by confining the honeybee colonies t.

[56] Average residue concentrations were calculated (using 0.0 µg/kg for residues "<LOD" and 0.65 µg/kg for concentrations "<LOQ" (mean of LOD and LOQ) to provide a conservative estimate regarding potential biological effects.

Report:	**1.8/7; Rolke, D.; Persigehl, M.; Gruenewald, B.; Blenau, W.; 2014**
Title:	**Large-scale monitoring of long-term effects of Elado (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: VII effects on honeybees (Apis mellifera)**
Report No.:	B13081-1
Document No.:	M-503572-01-1
Guideline(s):	none
Guideline deviation(s):	not applicable
GLP/GEP:	yes

This study is part of a large-scale monitoring project on the effects of seed treatment of Oilseed Rape with clothianidin on honeybees, bumblebees and solitary bees. An overview of the entire monitoring project is provided under Study 1.8/1 (Heimback & Russ, 2014).

Objective
This study investigates the potential side effects of clothianidin treated oilseed rape (OSR) on the development (adult bees and brood), honey production and health of honeybees (*Apis mellifera*). In addition, pollen, nectar and honey were sampled to determine the percentage of OSR pollen and to quantify clothianidin residues.

Material and Methods
Test item: Elado® (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed Oilseed Rape (OSR).
Test organism: honeybee (*Apis mellifera carnica*). Commercial honeybee colonies (10 combs) bred in a normal beekeeping practice, disease-free and queen-right.

Study sites
Two study sites have been selected in Mecklenburg-Vorpommern in Northern Germany at Sternberg about 30 km east of Schwerin: an untreated control site with different OSR varieties without a clothianidin dressing and a treatment site with different OSR varieties with commercial dressings containing the active substance clothianidin. Study fields in both the control and treatment site were drilled in autumn 2013. Each of the approximately circular study sites covered an area of about 65 km^2 (9 km in diameter). An inner core area of each study site of 7 km in diameter was investigated in depth. Because they were located next to each other they can be considered as similar as possible. A more detailed description is provided in Study 1.8/2 (Schimmer & Russ, 2014). Detailed information of drilled seeds (e.g. varieties, batch numbers, nominal and analysed dressing rates and details of drilling dates and sowing rates) is provided in Project Report No. B13055-3 (Study 1.8/5; Russ, Schimmer & Benito, 2014).

For this honeybee effect monitoring study, six locations were identified at each study site within a core area for the positioning of the honeybee hives: three locations at the edge of OSR fields, three locations about 400 m distant from OSR fields.

During the Post-Exposure Phase, the study continued at 4 Study Locations in Erlensee, Hesse in West-Central Germany, which were chose to be as close together and similar as possible. All Study Locations were withing the area of a former military airbae without any agricultural or horticultural activities. The immediate surrounding of the Study Locations consisted of grassland that is extensively grazed by sheep to keep the vegetation low.

Set-up of honeybee hives
At each study location, eight honeybee hives were established during the exposure phase, summing up to a total of 96 bee hives at the 12 study locations. All colonies originated from the same starting material. The hive entrances were south-facing. Below each hive entrance, an approaching mat facilitated the landing of bees. Seen from behind, the three leftmost hives were placed together at one

metal frame, followed by one single hive on an own metal frame and equipped with a bee have scale. To the right, the same arrangement was repeated for hives 4-8.

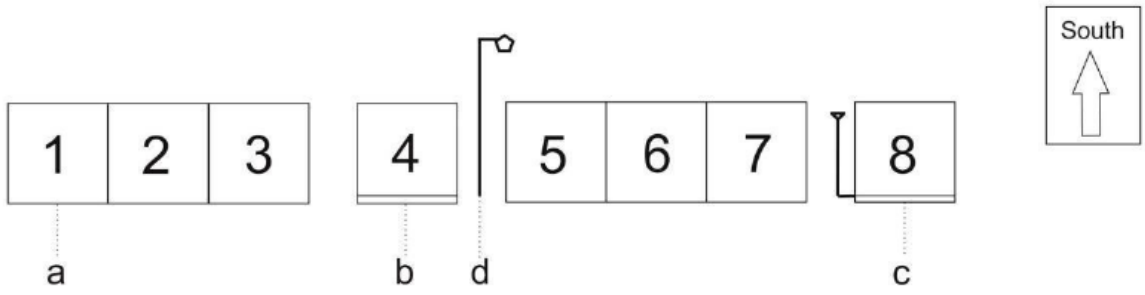

Figure B.9.7.1-2: Schematic Drawing of Arrangement of Honey Bee Hives at the Study Locations. a = Honey Bee Hive, b = Honey Bee Hive on a Balance, c = Honey Bee Hive on a Balance connected to a Rain Gauge, d = Anemometer

Test procedure

The study was divided into two parts: the Exposure Phase (in Mecklenburg-Vorpommern) and a subsequent Post Exposure Phase.

Exposure phase

The Exposure Phase lasted 28 days and started with the placement of the first honeybee hives at 22.04.2014 (DAP 0) at BBCH stage 63 to 65 (flowering) until 20.5.2014 at BBCH 74-79. All 96 honeybee hives were thus exposed to full flowering OSR crops (BBCH 65).

The effects of the clothianidin seed treatment were followed until the end of OSR blossom by recording the development of the colony size and brood, the weight of the hive, the amount of collected honey and the infection with different diseases and parasites

The assessment of colony development was done according to the Liebefeld method and by computed brood analysis. During the exposure phase, the interval between colony assessments was 7 ± 2 days. At every study location, two hives were placed on validated balances which continuously measured the weight of the hive and recorded local weather conditions. For estimation of the honeybee mortality during the Exposure Phase, flat plastic trays covered with metal grids were introduced into the bottom board of the hives. The numbers of dead bees on the grids were counted during each assessment. To assess infestation with *Varroa* mites during the Exposure Phase, naturally occurring fall of mites was recorded weekly. For this, the same flat plastic trays covered with metal grid as for honeybee mortality assessment were used. The number of dead mites in the trays was counted during each assessment. For the investigation of bee diseases (*Nosema* sp. and viruses), adult bees were sampled from the colony at two different time points during the Exposure Phase, and immediately deep frozen until analysis.

Analysis of pollen composition

During the Exposure Phase, pollen was sampled from returning honeybee workers using pollen traps that were introduced in the bottom board of the hives. Pollen samples were taken twice from all experimental colonies at two different time points during OSR blossom. In the laboratory of the test facility, the pollen samples were adequately prepared to determine the percentage of OSR pollen in the pollen loads.

Residue analysis of pollen, nectar and honey

In addition to analysis of pollen composition, pollen were sampled (using pollen traps as described above) for residue analysis. Further, nectar for residue analysis was sampled via honeybee workers whose honey stomach was later dissected in the laboratory of the test facility. Each colony was sampled twice, the first time between DAR +10 and DAP +16, and the second time between DAP +21

and DAP +24. Clothianidin residues were also measured in honey samples. These assessments were performed during the exposure phase in OSR.

Residue analysis of pollen, nectar and honey was conducted ar Eurofins Agroscience Services Chem GmbH under internal Study No. S13-04864 (BAY-1318). Specimens of pollen, nectar and honey were analysed using an analytical method based on the multi-residue sample preparation technique QuEChERS. The residue detection was realised with LC-MS/MS. The Limit of Quantification (LOQ) was 1.0 µg/kg and the Limit of Detection (LOD) 0.3 µg/kg.

Post exposure phase

After the field phase (Post Exposure Phase) the hives were placed in extensively used sandy grassland site in Erlensee, Germany. There were four Post Exposure Study Locations at the former airbase in Erlensee. At every location, two colonies from each Study Location were set up. The selection and order of placement was randomized.

At the Post Exposure Study Locations, the same parameters as during the Exposure Phase were assessed (except pollen, nectar and honey samples for residue analysis) until end of September 2014 (no overwintering assessment was performed).

Findings

Weather conditions

The weather conditions at the project area were comparable for both the treatment and control sites. Meteorological conditions were comparable to the long-term averages and no extremes occurred. There were only marginal differences between the individual Study Locations and between Control and Treatment Site.

Development of honeybee colonies

For each honeybee colony, the number of adult bees and brood cells was assessed at four time points during the Exposure Phase, and two times during the Post Exposure Phase. Due to heavy infestation with *Varroa* mites in assessment 7 (DAP +153 – DAP +155), the colony development was no longer influenced by the original study design. Therefore, the data of the last assessment were not included in the statistics but are reported along with the data of previous assessments in the Appendix. Since the aim of this Study was to examine possible effects of OSR dressing on honey bees during the Exposure Phase, the data comparison of experimental groups was continued according to their grouping during the Exposure Phase, even if the colonies were re-grouped during Post Exposure Phase.

During the Exposure Phase, all colonies developed very well with typical characteristics. In general, the same pattern of development could be observed in all colonies during the study period which followed a bell-curve. Although the colonies at the Treatment site appeared to have slightly less adult bees at the beginning of the exposure phase, this difference was not statistically significant and all Study Locations showed a similar variability in number of adult bees. Therefore, colonies had similar starting conditions and similarly populated colonies at the beginning of the test. The increase in numbers of adult bees was almost linear. Until the second assessment at DAP 11-13, the number of adult bees increased by approximately one third. The increase continued for all colonies until the number of adult bees peaked between the assessments 4 and 5. The development of the number of adult bees was similar between Control and Treatment Site. Furthermore, no statistically differences occurred between Study Locations situated at the edge of OSR fields and located in 400 m distance to OSR fields, which further indicates no effect from Clothiandin treated OSR. The development of the numbers of worker bees is rather affected by meterological conditions.

Table B.9.7.1-2: Mean numbers of adult honey bees in Study Sites at different time points during the Exposure Phase and Post Exposure Phase (C = Control Site; T = Treatment Site).

	Study Site	DAP	Assessment	Number of bees	
				Mean	Standard deviation
Exposure Phase	Control	4-7	1	12651.3	2554.5
	Treatment	4-7	1	11394.0	2319.9
	Control	11-13	2	18804.8	4496.7
	Treatment	11-13	2	13611.6	2909.1
	Control	18-22	3	21660.7	4867.2
	Treatment	19-22	3	20517.0	3086.4
	Control	24-27	4	27761.8	6273.5
	Treatment	24-28	4	25857.6	4248.3
Post Exposure Phase	Control	50-52	5	25652.7	7748.8
	Treatment	50-52	5	21978.5	7582.0
	Control	92-97	6	11582.2	2421.0
	Treatment	92-97	6	10758.9	2752.7

The numbers of worker and drone brood cells showed typical fluctuations which indicated optimal breeding conditions. The pattern of the fluctuations in the number of open and capped worker brood cells was similar between Control and Treatment Sites. At DAP + 4 – DAP +7 (1st Assessment) capped worker brood cell numbers ranged between 11313 ± 3563 in Study Location CC and 16713 ± 4083 in Study Location CA. The number of cells decreased slightly towards the second assessment and reached their maximum during the assessment 4 with an average of 16350 ± 3446 capped worker brood cells at the Control Site compared to 17081 ± 4524 at the Treatment Site. At DAP + 4 – DAP +7 (1st Assessment) open worker brood cell numbers ranged between 4625 ± 2118 in Study Location TD and 9075 ± 4091 in Study Location CC. At the peak of open brood cells, numbers averaged 12710 ± 3816 at the Control and 12115 ± 4685 at the Treatment Site, but decreased until the sixth assessment to 4579 ± 2428 and 4226 ± 2190 at the Control and Treatment Site, respectively. There were marginal higher numbers of closed and open worker brood cells at the Treatment Sites compared to the Control Sites, but this difference was not statistically different at any assessment. Furthermore, no statistical differences could be observed between Study Locations situated at the edge of OSR fields and located in 400 m distance to OSR fields and additionally, no relevant effect of weather conditions were observed.

At DAP + 4 – DAP +7 (1st Assessment) most of the colonies showed no capped drone brood cells. However, in all Study Locations, except CB, some capped drone brood cells were recorded being up to 98 ± 85 in Study Location CF. At DAP + 50 – DAP +52 (5th Assessment) the number of capped drone brood cells ranged between 674 ± 722 (Study Location TD) and 2052 ± 465 (Study Location TC). The variability within Study Locations was relatively high. Except in Locations TD and TE where the average number of capped drone brood cells was low, the variability between Study Locations was similar. At the 6th assessment, 28 colonies (10 in Control Site and 18 in Treatment Site) had already no capped drone brood anymore. Marginally lower numbers of capped drone brood cells at the Treatment Site differed significantly from the Control Site. In general, the high variability observed indicates that the number drone brood cells is not a good indicator and less relevant as compared to the number of adult bees or worker brood cells. However, Study Locations situated at the edge of OSR fields did not differ from those located in 400 m distance to OSR fields. Meteorological conditions did not influence the number of capped drone brood.

During the Exposure Phase, two colonies per Study Location were weighed continuously by bee hive scales. As only two out of 8 colonies were weighed, this subset of data cannot provide a comprehensive picture of all colonies. In fact, this data mainly provide information about the main trend in colony development in respect to foraging periods and colony growth. In general, all measured colonies showed the same pattern of development during the Exposure Phase. From Day After Placement (DAP) +3 to DAP +9 a continuous increase of 23.18 ± 2.93 % in colony weight (Control Site) and 22.91 ± 3.41 % (Treatment Site) took place. From DAP +10 until DAP + 20

(Control Site), respectively DAP +21 (Treatment Site) the weight of the measured colonies changed only marginally due to unfavourable weather conditions . From DAP +21 (Control Site), respectively DAP +22 (Treatment Site) until DAP +27 the colony weight increased again. At DAP +28, the weight of all colonies decreased slightly. Overall, from DAP +3 until DAP +26, colonies increased by 84.6 % - 89.1 % in weight. There were no main differences between colonies located at the same Study Location except in Locations CC and TA, where the weight of one colony increased slower than the other. Colonies of the Control and Treatment Site developed almost synchronously. An effect of the distance between colonies and OSR fields was not observed. Like during the Exposure Phase, all measured colonies showed the same pattern of development during the Post Exposure Phase.

At the end of the Exposure Phase, between 23.7 kg and 27.0 kg honey was extracted, that contained 62.0 % to 83.5 % of pollen originated from OSR. This indicates OSR as major nectar source during the exposure phase.. There was no obvious treatment effect on the amount of honey yield, but yields differed according to the distance to OSR fields (with statistically significant lower yields from colonies that were located 400m apart from OSR fields). This effect is consistent both for the Control and the Treatment Site.

Overall, the development of the honeybee colonies was not significantly negative affected by their position at clothianidin seed treated OSR fields. The colony development was slightly influenced by weather conditions. Additionally, the distance to OSR fields had a marginally significant effect on the colonies, with distant hives produced less amount of honey during the exposure phase.

Colony health

The health of the colonies was monitored throughout the Study. *Nosema sp.* played only a minor role in challenging honeybee health throughout this study. At the first sampling (beginning of exposure phase) *Nosema sp.* was present in seven samples out of four study locations. At the second and third sampling, only three hives of four study locations contained spores, respectively. In addition, the infection of the honeybees by viruses was very low during the exposure phase. Kashmir Bee Virus (KBV), Acute Bee Paralysis Virus (ABPV) and Chronic Bee Paralysis Virus (CBPV) were not detected in any of the samples. Deformed Wing Virus (DWV) was present in seven samples at the first and three samples at the second sampling. Samples from the half of the study locations (three at control and three at treatment site) were free of DWV. At the end of the post exposure phase, an increase in infection with *Varroa* related viruses (DWV and ABPV) was recorded.

During the exposure phase, the infestation of honeybee colonies by *Varroa* mites was assessed by recording the naturally occurring fall of mites. With rates of 0.00 – 0.47 fallen mites per day, the infestation was low and according to common rates for spring and early summer. During the second half of the Post exposure phase, the naturally occurring fall of mites increased exponentially. Numbers of phoretic mites per 500 bees were extraordinarily high, ranging between 13 – 35 mites. The obligatory *Varroa* treatment, here with the use of flumethrine, was not successful because of a high proportion of flumethrine resistant mites within all colonies of all study locations. This led to a heavy varroosis with the loss of 21 of the originally 96 colonies and severe damages in the remaining colonies. However, there were no significant differences between control and treatment site.

Honeybee mortality

The average rate of dead bees remained low throughout the Exposure Phase in all honeybee colonies and ranged between 0 and 1.43 dead bees per day. Despite a period of about one week (from 16.08.2014 to 22.08.2014) with a higher daily mortality rate (up to 5.9 dead worker bees), the average rate remained low after transportation to Erlensee. Overall, the rate of fallen bees per day varied considerably over time and also among the Study Locations. No differences in honeybee mortality between the Control and Treatment Site were detected.

Table B.9.7.1-3: Daily Fall of dead Bees per Study Location during the Exposure Phase, the Beginning of the Post Exposure Phase and during Flumethrine Treatment. Sample Sizes differ due to the Exclusion of Colonies (TB-4 due to Swarming, TC-7 due to Queen Loss).

	Study Location	CA	CB	CC	CD	CE	CF	TA	TB	TC	TD	TE	TF
Exposure Phase	Sample Size	8	8	8	8	8	8	8	8	8	8	8	8
	Minimum	0.0	0.0	0.0	0.0	0.0	0.0	0.06	0.0	0.05	0.0	0.0	0.11
	Median	**0.06**	**0.21**	**0.05**	**0.06**	**0.19**	**0.06**	**0.15**	**0.15**	**0.11**	**0.02**	**0.06**	**0.29**
	Maximum	0.22	0.35	0.16	0.31	0.44	0.12	0.35	0.41	0.21	0.14	0.12	0.79
Post Exposure Phase	Sample Size	8	8	8	8	8	8	8	7	7	8	8	8
	Minimum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Median	**0.0**	**0.11**	**0.0**	**0.14**	**0.04**	**0.04**	**0.0**	**0.0**	**0.14**	**0.04**	**0.0**	**0.0**
	Maximum	0.43	0.29	0.29	0.43	0.64	0.14	0.07	0.50	0.21	0.43	0.07	0.43
Flumetrhine Treatment	Sample Size	8	8	8	8	8	8	8	7	7	8	8	8
	Minimum	0.08	0.20	0.04	0.04	0.06	0.10	0.16	0.18	0.14	0.18	0.14	0.18
	Median	**0.74**	**0.38**	**0.19**	**0.19**	**0.42**	**0.43**	**0.38**	**0.37**	**0.22**	**0.50**	**0.28**	**0.38**
	Maximum	1.33	2.94	0.82	1.65	1.51	0.69	0.45	0.98	1.16	3.94	0.39	0.71

The number of lost colonies per Study Location showed some variation, but there were no differences between the four treatment groups.

Table B.9.7.1-3: Number of honeybee colonies with no bees left at DAP +155 in the different treatment groups

CA	CB	CC	CD	CE	CF	**Control**
2	2	2	2	0	3	**11**
TA	TB	TC	TD	TE	TF	**Treatment**
1	1	2	4	0	1	**9**
Edge		**10**	**Distant**		**10**	**20**

Pollen composition

The high attractiveness of OSR for the honeybees was supported by the palynological analysis of pollen pellets. The percentage of OSR pollen in the pollen pellets was very high, with a mean percentage of more than 71 % in all areas, and up to 91 % within the hives at locations at the edge of OSR fields of the treated area. There was considerable variation in pollen composition between Study Locations and between sampling dates, which are due to the local situation in the neighbourhood of Study Locations (differences in surrounding vegetation). Nevertheless, these results indicate OSR as the major pollen source during the exposure phase. Therefore it can be assumed that honeybees were highly exposed to pollen from clothianidin dressed OSR fields at the treatment site.

Residues of clothianidin in pollen, nectar and honey

Residues of clothianidin were below the limit of detection (LOD = 0.3 µg/kg) in most cases, never exceeding the limit of quantification (LOQ = 1.0 µg/kg) in pollen, nectar and honey that originated from the control site, which confirms that honeybees did not leave the control site to forage on OSR fields outside this area.

The concentration of clothianidin in pollen from the treatment site was generally low, with only one out of 48 samples with a concentration above LOQ (1.1 µg/kg) at the first sampling. At the second sampling, 23 out of 46 samples had measurable concentrations of up to 2.7 µg/kg. The overall mean of both samplings was 0.73 ± 0.49 µg/kg, which is below the LOQ. The concentration of both analysed metabolites of clothianidin, TZNG and TZMU, was below the limit of detection (< LOD) in all except one pollen samples.

Nectar samples gathered from dissected foragers contained average clothianidin concentrations below LOQ in samples of the treatment site. In 22 out of 96 samples, a concentration exceeding the LOQ was measured, with a maximum of 1.6 µg/kg. Both TZNG and TZMU were below LOD in all samples except one.

Honey samples contained on average higher concentrations of clothianidin than pollen and nectar that reflects the concentrating process in honey production by the bees. Again, the residue analysis found concentrations that averaged 1.4 ± 0.5 µg/kg. 37 out of 48 samples contained measureable concentrations of clothianidin up to 2.1 µg/kg. Both TZNG and TZMU were below LOQ or even LOD in all samples.

Conclusions
The study provides reliable field data on the development of honeybee colonies exposed to clothianidin dressed oilseed rape. The honeybees used oilseed rape as major source for pollen and nectar and thus were exposed to clothianidin in the treatment site. However, residues of clothianidin were relatively low in nectar and pollen samples and also concentrations in honey samples stayed short above the limit of quantification. Seed treatment of clothianidin on oilseed rape did not cause any detrimental effects on the composition of collected pollen and the development of adult bees or their brood, nor the honey production, pollen composition or infestation rates with diseases and *Varroa* mites, neither during blossom in spring nor thereafter until the end of the study in autumn.

RMS Comments
The study was performed following the recommendations from the EFSA Guidance Document. Overall, it is well designed, used large field sites and a high number of honeybee colonies. Further, colony development was monitored based on different parameters and using different methods.

At Pesticides Peer Review Meeting 145, it was noted that the complexity of the study design and the number of analyses and observations performed and reported would require a peer review of all the original study reports. A full consideration of this study within the confirmatory data procedure was not feasible. The study will be evaluated more deeply under the review on the neonicotinoids (Ref. EFSA question number: EFSA-Q-2015-00771).

Report:	**1.8/8; Peters, B.; 2015**
Title:	**Final report - Large-scale monitoring of long-term effects of Elado (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: VIII short- and long-term effects on red mason bees (Osmia bicornis)**
Report No.:	B14013
Document No.:	M-503583-02-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	yes

This study is part of a large-scale monitoring project on the effects of seed treatment of Oilseed Rape with clothianidin on honeybees, bumblebees and solitary bees. An overview of the entire monitoring project is provided under Study 1.8/1 (Heimback & Russ, 2014).

Objective
This study investigates the potential side effects of clothianidin treated oilseed rape (OSR) on the reproduction and nest building of Red Mason Bees (*Osmia bicornis*) including the emerging success of offspring. In addition, pollen was sampled from the brood cells to determine the percentage of OSR pollen collected by the females and to quantify clothianidin residues in the stored pollen.

Material and methods:

Test item: Elado® (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed Oilseed Rape (OSR).

Test organisms: Red Mason Bees (*Osmia bicornis*).

18.000 cocoons were provided by "bienenhotel.de, Drosselweg 9, 18057 Rostock, Germany". The cocoons of the mason bees were kept in a refrigerator at -2 °C to +4 °C, over the entire winter period of 2013/2014. One week before the cocoons were placed in cardboard boxes into the nesting shelters at all Study Locations, they were incubated for five days at 8 °C and two days at 11 °C by "bienenhotel.de". This process ensured a more equal emerging progress in the field. To allocate the mason bee cocoons, 750 cocoons were counted by hand and put in every cardboard box with a constant sex ratio of 6:5 (males : females) by the "bienenhotel.de". The differentiation between male and female cocoons is possible by size. After allocation of cocoons, cardboard boxes were placed at the Study Locations.

Study sites

Two study sites have been selected in Mecklenburg-Vorpommern in Northern Germany at Sternberg about 30 km east of Schwerin: an untreated control (OC) site with different OSR varieties without a clothianidin dressing and a treatment site (OT) with different OSR varieties with commercial dressings containing the active substance clothianidin. Study fields in both the control and treatment site were drilled in autumn 2013. Each of the approximately circular study sites covered an area of about 65 km^2 (9 km in diameter). An inner core area of each study site of 7 km in diameter was investigated in depth. Because they were located next to each other they can be considered as similar as possible. A more detailed description is provided in Study 1.8/2 (Schimmer & Russ, 2014). Detailed information of drilled seeds (e.g. varieties, batch numbers, nominal and analysed dressing rates and details of drilling dates and sowing rates) is provided in Project Report No. B13055-3 (Study 1.8/5; Russ, Schimmer & Benito, 2014).

For this mason bee effect monitoring study, six locations were identified (A-F) at each study site (OC or OT) within a central area for the positioning of the nesting shelters with the cocoons: three locations at the edge of OSR fields, three locations about 100 m distant from OSR fields.

Set-up of nesting shelters

At each study location, three nesting shelters were established, summing up to 36 nesting shelters at the 12 study locations. Nesting shelters were erected south-east facing, exposed to direct sunlight and to be protected against rain. In all cases, the nesting shelters were placed in front of a forest, a hedge or big shrubs to ensure a similar protection from wind.

Each nesting shelter consisted of a plastic tub mounted to two wooden stakes (2 m height) which were dug into the ground. Inside the plastic tub, nesting blocks were put on two wooden strips. To protect the nesting boxes from bird predation the opening of the plastic tubs was covered with chicken wire. To prevent damage caused by ant predation, glue rings were put on each wooden stick. A nesting blocks was composed of 20 medium-desnity fibreboards (MDF, 16 x 16 cm), each containing 10 parallel drilled nesting holes (8 mm diameter). Each nesting shelter comprised three nesting blocks except the central nesting shelter at each location which contained only two nesting blocks and two perforated cardboard boxes with 750 cocoons of red mason bees each.

Test procedure

The study period lasted for one year and is divided into two parts: the Field Phase and the consecutive Post Exposure Phase. The Field Phase started in April 2014 and ended in May 2014. The last investigations of the Post Exposure Phase were finished in April 2015.

Field phase

The field phase lasted five weeks and exposure started with the placement of the cardboard boxed with cocoons at the nesting shelters at the beginning of OSR full flowering (= Day after Placement, DAP 0, 21.04.2014, BBCH 63-65).

To assess the hatching success, empty cocoons were counted. For the assessment of the nest building and activity of the mason bees, nesting females were observed on a weekly basis after dusk, when they stayed in their nesting holes for the night. In parallel to the female observation the cell completion was assessed by counting the number of closed nesting holes per nesting block. At the late end of OSR blossom nesting shelters were covered with gauze to avoid further nest building activities (DAP 32, 23.05.2014, BBCH 74-79).

Pollen for composition analysis was sampled twice at every study location during OSR blossom. From each nesting block 10 subsamples were collected and combined to a pooled sample. The exact nesting boards and nesting holes from which the pollen subsamples were taken were recorded and the sampled holes marked to avoid sampling from the same cells twice. Pollen for residue analysis was sampled once at each location. At each Study Location, nesting block no. 3 was used to collect the main sample and nesting block no. 6 for the retain sample. The target amount of pollen was set to 200 mg per sample.

Post exposure phase

After completion of the Field Phase (DAP 35 26.05.2014) all nesting blocks were removed from the Study Locations and stored in an empty agricultural hall under dark conditions for 10 weeks. The assessment of the reproduction took place at early August 2014 at the time when mason bees should have been fully developed inside the cocoons. To assess the reproduction, the cocoons were harvested from the nesting blocks, counted and sorted by sex (indicated by the size of the cocoon). Undeveloped eggs and larvae inside of the cells were also counted as well as typical parasites. In October 2014, the remaining cocoons were harvested. Afterwards all cocoons were put in a refrigerator for wintering at a mean temperature of 1.6 to 6.7°C.

After wintering in spring (March) 2015 the harvested cocoons were set up for emergence for a duration of maximum 4 weeks. 10 days before the cocoons were taken out of the refrigerator, the incubating temperature was gradually raised to 12.5°C. For all 12 Study Locations, the emergence of mason bees was assessed separately. Cardboard boxes containing the cocoons were placed at room temperature in a large, black plastic box at which and emergence trap was fixed. The emerged male and female bees were counted every day. After 4 weeks, the number of closed cocoons was counted and closed cocoons were opened to check for undeveloped bees or parasites.

Analysis of pollen composition

In the laboratory of the test facility, the pollen samples were adequately prepared to identify the percentage of OSR pollen and other pollen grains using a microscope. The percentage of the most common forage plants was recorded.

Residue analysis of pollen

Residue analysis of pollen was conducted at Eurofins Agroscience Services Chem GmbH under internal Study No. S14-03798 (BAY-1411). Specimens of pollen were analysed using an analytical method based on the multi-residue sample preparation technique QuEChERS. The residue detection was realised with LC-MS/MS. The Limit of Quantification (LOQ) was 1.0 µg/kg and the Limit of Detection (LOD) 0.3 µg/kg.

Findings

Weather conditions

The weather conditions at the project area were comparable for both the treatment and control sites. Meteorological conditions differed only marginally between study sites and no extremes occurred.

Hatching success from deliverd cocoons and acceptance of the nesting shelters

On average, 91 % (range 87 - 94 %) of mason bees emerged at the study locations in the four weeks of the field phase. Similar numbers of bees emerged in the Control Site (91.6%) in comparison to the Treatment Site (90.0%). Mason bees accepted the provided nesting shelters and females started

immediately with nest building. The consistent hatching process at all Study Locations constituted an equal initial point for the study. Study location OCC had to be excluded from statistical evaluations since this location deviated extremely from all others in the second half of the field phase. The OSR plants at this location reached the highest height of 1.90 m which led to shading and, hence, climatic differences compared to the other study locations.

Nest building activity of female mason bees

At all study locations, female mason bees built linear nests into the nesting blocks. The number of nest building females increased consistently over the course of the Field Phase in all Study Locations. Two of the study locations at the edge of OSR fields (OCB at the control site and OTD at the treatment site) showed a delayed increase of nesting females, but were not distinctly different from the other Study Locations at the last assessment. Only the number of nesting females at study location OCC deviated from the pattern seen at the other study locations, but was regarded as outlier for the statistical analysis. Overall, considering natural fluctuations, a similar trend is shown for the four different location categories (Control and Treatment, on the edge of an OSR field or at a distance of 100m).

The numbers of completed nesting holes were relatively low until the fourth assessment but increased considerably at the end of the study period (on DAP 30/31). At the study locations OCB en OCC less completed nesting holes occurred, which could be attributed to the later emergence and nest building activities at these locations. At the treatment site and the distant locations significantly more completed nesting holes as well as parasites like *Ptinus* and *Cacoxenus* were found. In general, parasites (Mean Control 1.9 % (2.1 % by including OCC), Mean Treatment 2.3 %) occurred in very low numbers, indicating that the nesting shelters were well constructed and protected against parasitism. Even though more parasites occurred at the treatment site these results do not indicate a treatment effect since the occurrence of parasites depended very on climatic conditions and especially on natural occurring solitary bees in the surrounding area. Furthermore the highest amount of infested cells was found in the control site at study location OCF. The difference in the number of completed nesting holes has to be considered carefully since it is also affected by the intensity of nest building activities.

Reproduction rate at cocoon harvest

At all Study Locations mason bees produced offspring. In addition, undeveloped eggs and larvae occurred at al Study Locations in greater or lower numbers. In total, for each nesting female on average 7.48 new cocoons were produced at the Control Site and 8.61 at the Treatment Site. At the Control Site, 911 undeveloped eggs or larvae (4.9% of brood cells) wer found, which is slightly higher than the 2.5% brood cells (606 individuals) at the Treatment Site. By comparison of the four location categories, the proportion of the undeveloped individuals was slightly higher at the distant locations.

Emerging successs of offspring and actual sex ratio

In total 91.2% of offspring emerged from the wintered cocoons at the Control Site and 92.2% at the Treatment Site. While the distant fields at the Control Site showed slightly higher proportions of successful emergence, the edge fields in the Treatment Site yielded slightly higher emergence.

After 4 weeks of emergence, in total 1260 cocoons (6.9%) of the Control Site and 1459 (6.1%) of the Treatment Site remained closed. The content of the cocoons was differentiated between undeveloped bees and males and females still alive (which might have emerged during subsequent days after taking down of the emergence traps). In the Treatment Site a slightly higher percentage of living bees and a slightly lower percentage of undeveloped bees were observed as compared to the Control Site.

For the offspring of the Control Site a sex ratio of 71.6 males to 28.4 females was recorded, wherefore the sex ratio of offspring from the Treatment Site resulted in 68.2 males to 31.8 females.

Pollen composition

The amount of OSR pollen in the brood cells of mason bees averaged 18.0 % at the control site and 10.7 % at the treatment site with a range from 5.7 % to 41.2 % between the two sampling events. A

higher amount of pollen was collected from non-OSR plants. Rosaceae including the subfamily Pyreae represented the highest percentage of non-OSR pollen with 55 % and 45 % at the control and treatment site, respectively.

Residues of Clothianidin in Pollen

Pollen at the brood cells did not contain detectable residues of Clothianidin at the control site (all samples below limit of detection, LOD = 0.3 µg/kg), whereas residues in two locations at the treatment site were quantified as 1.1 and 1.7 µg/kg. At the other locations at the treatment site, clothianidin concentrations were below the limit of quantification (LOQ = 1.0 µg/kg).

Pollen samples for residues and samples for pollen composition analysis could not be collected on the same dates. Furthermore, the amount of OSR pollen varied considerably. Therefore, no reliable correlation between clothianidin residues in pollen at the brood cells and the amount of OSR pollen could be calculated.

Statistical evaluation

Considering data from all Study Locations, significantly more nesting females were found at the Treatment Site. However, this significant difference is no longer available when Study Location OCC is excluded from the data set. The distance to an OSR field did not affect the number of nesting females.

Climatic conditions affected the development and reproduction of mason bees to certain extents. The temperature correlated negatively with the number of offspring and positively with the number of completed nesting holes and infested cells. Wind speed influenced all reproduction endpoints negatively. The relative sun exposure duration was an important predictor for the number of nesting females and offspring but at the same time for the occurrence of pollen mites while *Cacoxenus* parasites were negatively influenced by the sunshine duration.

Table B.9.7.1-2: Statistical significances of the influence of different factors on nest building and reproduction as well as parasitism, excluding study location OCC

	Development				Parasites		
	Nesting Females	**Completed Nesting Holes**	**Male Cocoons**	**Female Cocoons**	***Ptinus*-Infested Cells**	***Cacoxenus*-Infested Cells**	**Pollen Mites-Infested Cells**
Intercept	0.83*** (0.18)	-7.73*** (0.57)	5.71*** (0.20)	5.34*** (0.17)	-10.04*** (2.65)	-1.09*** (0.00)	-0.74 (1.07)
Treatment	0.18 (0.09)	1.34*** (0.31)	-0.38 (0.27)	-0.27 (0.23)	9.77** (3.05)	2.15*** (0.00)	-0.90 (1.36)
Distant to OSR (100 m)	-0.10 (0.10)	0.88** (0.33)	-0.02 (0.15)	0.07 (0.13)	2.17* (1.03)	1.32*** (0.00)	0.82 (0.74)
Temperature Sum		0.04*** (0.01)	-0.51** (0.19)	-0.35* (0.16)	4.40** (1.46)	1.69*** (0.00)	-1.42 (0.96)
Humidity Sum			-0.42*** (0.11)	-0.22* (0.10)	1.14 (0.97)	0.62*** (0.00)	-1.14* (0.56)
Wind Speed Sum	-0.01 (0.01)	-0.07*** (0.02)	-0.33*** (0.09)	-0.13 (0.07)	-1.22 (0.80)	-0.25*** (0.00)	-0.58 (0.43)
Mean Sunshine Duration	1.14*** (0.22)	0.71 (0.73)	0.48*** (0.12)	0.23* (0.10)	0.63 (1.27)	-0.88*** (0.00)	1.17* (0.57)

Positive significant Negative significant

***p < 0.001, **p < 0.01, *p < 0.05

Significantly more males and females emerged from cocoons of the Treatment Site compared to the Control Site although percentages in the treatment are only 1% higher. Significantly higher numbers of undeveloped females and pupae were found in remaining cocoons of the Control Site. Between offspring cocoons from edge and distant fields no statistical differences could be measured for the emergence of adult bees.

Table B.9.7.1-3: Statistical significances of the influence of different factors on emerging success of offspring and the contents of remaining cocoons, excluding study location OCC

	Emerging success		Undeveloped individueals				Alive bees	
	Emerges males	Emerged females	Males	Females	Pupae	Prepupae	Males	Females
Intercept	4.11***	4.47***	3.69***	4.54***	4.28***	1.61***	1.25***	3.00***
	(0.14)	(0.17)	(0.11)	(0.07)	(0.08)	(0.32)	(0.38)	(0.16)
Treatment	0.41*	0.26	-0.21	-0.59***	-0.33**	-0.07	0.69	-0.20
	(0.18)	(0.21)	(0.15)	(0.11)	(0.12)	(0.41)	(0.44)	(0.21)
Distant to OSR (100 m)	-0.02	0.14	0.25	-0.70***	0.27**	0.85*	-0.41	-0.38
	(0.18)	(0.21)	(0.14)	(0.11)	(0.10)	(0.36)	(0.53)	(0.22)

Positive significant Negative significant

***p < 0.001, **p < 0.01, *p < 0.05

Discussion

In general, the set-up of the study was proved to be efficient and provided reliable results. The consistent hatching process at all Study Locations constituted an equal initial point for the study. Although study locations were selected to be as similar as possible in climate and vegetation structure, the number of nesting females was extraordinary low at one study location of the control site (OCC) in the second part of the study because the OSR plants even overgrew the nesting shelters of this study field. Nevertheless, the quality of all other locations proved to be very good for the aims of the study.

The relative sunshine exposure duration seemed to be the most important climatic factor for the development of mason bees since the number of nesting females as well as the number of offspring correlated significantly positive with the sunshine duration. Temperature, humidity and wind influenced the number of offspring (cocoons, undeveloped larvae and undeveloped eggs) negatively.

The pollen composition analysis indicated that mason bees preferred to collect pollen from the surrounding hedges and trees. Nevertheless, about 10 to 20 % of pollen was foraged in OSR and the analysed concentrations of clothianidin in pollen verified the exposure of mason bees. Furthermore, the results of the residue analysis showed that the control mason bees only foraged OSR pollen inside the control site, as no detectable residues were found in the respective pollen, whereas the OSR pollen in the brood cells in the treatment site contained low concentrations of clothianidin (< LOQ – 1.7 µg/kg).

Discussion and conclusions

The results indicate that Elado® dressed OSR had no impact on the development of red mason bees neither on the nest building nor on the reproduction. Also in the Study Locations which were selected at the edge of OSR fields no effects of Clothianidin were measurable although mason bees at these locations were more intensively exposed to Elado® dressed OSR. The weather and especially the sunshine was the main influencing variable on the nest building activity and reproduction of the mason bees.

In summary, clothianidin treated OSR did not cause any detrimental effects on the collection of OSR pollen, the nest building activity, the reproduction rate nor the infestation of parasites, neither during blossom in spring nor thereafter until autumn.

RMS Comments
As there are currently no agreed test protocols for field effect studies for bumblebees, it is difficult to assess the suitability of the present study for risk assessment purposes. However, the study was well designed and followed the recommendations from Appendix Q of the EFSA Guidance Document on bees: *Osmia bicornis* was used as test species, sufficiently large large field sites and a high number of bees was used. Further, nesting activity was monitored and emergence after overwintering was assessed.

At Pesticides Peer Review Meeting 145, it was noted that the complexity of the study design and the number of analyses and observations performed and reported would require a peer review of all the original study reports. A full consideration of this study within the confirmatory data procedure was not feasible. The study will be evaluated more deeply under the review on the neonicotinoids (Ref. EFSA question number: EFSA-Q-2015-00771).

Report:	**1.8/9; Sterk, G.; Peters, B.; 2014**
Title:	**Large-scale monitoring of long-term effects of Elado (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: IX effects on large earth bumblebees (*Bombus terrestris*)**
Report No.:	B14014
Document No.:	M-503580-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	yes

This study is part of a large-scale monitoring project on the effects of seed treatment of Oilseed Rape with clothianidin on honeybees, bumblebees and solitary bees. An overview of the entire monitoring project is provided under Study 1.8/1 (Heimback & Russ, 2014).

Objective
This study investigates the potential side effects of clothianidin treated oilseed rape (OSR) on the development and population size (number of workers, drones and new formed queens) of Large Earth Bumblebees (*Bombus terrestris*). In addition, pollen was sampled from returning bumblebee workers to determine the percentage of OSR pollen and to quantify clothianidin residues in the pollen pellets.

Material and Methods
Test item: Elado® (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed Oilseed Rape (OSR).
Test organisms: Large earth bumblebee (*Bombus terrestris dalmatinus*). Commercial bumblebee colonies, obtained from Koppert Biological Systems (Veilingweg 14, 2651 Berkel en Rodenrijs, The Netherlands), were used in this study.

Study sites
Two study sites have been selected in Mecklenburg-Vorpommern in Northern Germany at Sternberg about 30 km east of Schwerin: an untreated control site with different OSR varieties without a clothianidin dressing and a treatment site with different OSR varieties with commercial dressings containing the active substance clothianidin. Study fields in both the control and treatment site were drilled in autumn 2013. Each of the approximately circular study sites covered an area of about 65 km² (9 km in diameter). An inner core area of each study site of 7 km in diameter was investigated in depth. Because they were located next to each other, the control and treatment site can be considered as similar as possible. A more detailed description is provided in Study 1.8/2 (Schimmer & Russ,

2014). Detailed information of drilled seeds (e.g. varieties, batch numbers, nominal and analysed dressing rates and details of drilling dates and sowing rates) is provided in Project Report No. B13055-3 (Study 1.8/5; Russ, Schimmer & Benito, 2014).

For this bumblebee effect monitoring study, six locations were identified at each study site within a central area (3 km diameter) for the positioning of the bumblebee hives. The study locations were chosen to be as similar as possible in climate, geography, settlings, hedges, grassland, woodland, natural preservation areas, agriculture and other land-uses. Of the six locations in each study site, three locations were situated at the edge of OSR fields, and three locations at about 400 m distant from OSR fields.

Placement of hives

141 Commercial bumblebee colonies (with the bumblebee subspecies *Bombus terrestris dalmatinus*) were obtained from Koppert Biological Systems (The Netherlands) (120 colonies for the study, and 21 in excess as substitute in case of damage).

At each study location, three tripols (= multi-hives) composed of three single hives and one extra single hive were established at the beginning of OSR blossom (Day after Placement = DAP 0, 24.04.2014), summing up to 36 tripols with 108 hives and 12 single hives. All hives were south-facing to be protected against wind and rain. The tripols were used for the regular assessments and the single hive for collection of pollen pellets. To achieve a high comparability, every hive consisted of a mother queen from the same hibernation batch and 40 to 50 workers of roughly the same age, which were especially prepared for this study. Insulating wool was placed outside the hive on top, to make observations possible.

Test procedure

The study period lasted for two months and was divided into two parts: the Exposure Phase (in Mecklenburg-Vorpommern) and a subsequent Post Exposure Phase. The latter started in Mecklenburg-Vorpommern for one day and was continued in Belgium. The Exposure Phase started in April 2014 and ended with the removal of the hives from the fields in May 2014.

Exposure phase

The exposure phase lasted 22 days, from the beginning of OSR full flowering (BBCH 65) to the end of OSR blossom. All study locations were checked daily during the Exposure Phase to ensure that hives were unscathed. Twice a week, during the flight time of the bumblebees, the mother queen and the colony building rate were assessed. Therefore, the tripol was opened and the hives checked for the vitality of the original mother queen. The colony building rate was assessed by weighing the colonies and estimating the brood size and the number of workers according to categorisation systems.

Pollen for pollen composition analysis was sampled twice at every study location during OSR blossom from single hives (at DAP 4/6 and 15/17). To obtain the samples, returning bumblebee workers loaded with pollen were caught with a vacuum collector containing dry ice. During the first sampling event, approximately 20 bumblebees were collected and during the second event approximately 40 bumblebees. Pollen for residue analysis was sampled once at each location. At each study location, 11 to 21 bumblebee workers were sampled for the main sample, and additional 13 to 20 workers for the retain sample. The target amount of pollen was set to 200 mg. Before shipment, the pollen loads were picked from the legs of the bumblebees and put into tubes to get a contaminant-free pollen sample.

Post exposure phase

The hives reached the ‘turning point’ at the end of the OSR blooming and were subsequently removed from the study locations and transported to the accommodation of the study site (DAP 22), where the last assessment was conducted. The turning point is characterized by the cessation of the production of new workers and the first appearance of drones and young queens. Afterwards, they were transported to the Nature Park Lieteberg (Belgium), where weekly assessments (mother queen and colony building

rate) continued. The hives were placed randomised at one site on frames, so that the hives from the Control and Treatment Sites were no longer separated.

In June, at the end of the life time of the colonies, the hives were frozen and dissected in the laboratory. Queens, workers and drones were sorted and counted while undeveloped larvae inside of the cells were estimated.

Analysis of pollen composition
In the laboratory of the test facility, the pollen samples were adequately prepared to identify the percentage of OSR pollen and other pollen grains using a microscope. The percentage of the most common forage plants was recorded.

Residue analysis of pollen
Residue analysis of pollen was conducted at Eurofins Agroscience Services Chem GmbH under internal Study No. S14-03799 (BAY-1412). Specimens of pollen were analysed using an analytical method based on the multi-residue sample preparation technique QuEChERS. The residue detection was realised with LC-MS/MS. The Limit of Quantification (LOQ) was 1.0 µg/kg and the Limit of Detection (LOD) 0.3 µg/kg.

Findings
Weather conditions
The weather conditions at the project area were comparable for both, the treatment and control sites. Meteorological conditions differed only marginally between study sites and no extremes occurred.

Development of bumblebee colonies
With the exception of two out of 108 hives (CE-2-2 and CE-3-2) there was a very homogenous and continuous development in all the hives in each Study Site on all Study Locations. Hives CE-2-2 and CE-3-2 developed poorly and had no queen brood cells on DAP 43. Therefore statistical calculations were performed excluding these two hives. However, values including these two hives were always included in the comparison. The overall fast growth of bumblebee hives is an indication of the high nutrient value of the collected pollen and nectar.

No abnormalities in behaviour (e.g. apathy or lack of flight activity) were observed in any hive. On top of that, highly specialized behaviour like cooling the hives or guarding by young workers was often observed during the trial.

Colony building rate
During the exposure phase, the colony weight steadily increased at all hives and was very similar between the different locations. After transportation to Lieteberg (post exposure phase) the weight of the hives decreased slightly. This is especially because the bumblebees fed on the collected and stored nectar, due to a lack of alternative food sources during the transport and less accessible and intensive food supply during the post exposure phase in Belgium.

During the exposue phase, the brood size was steadily enlarging at all hives, except for the two hives at study location CE. The hives at the ege of an OSR field were growing slightly faster in size than those positioned distant from OSR fields during the first weeks. From then on the mean volumes of the colonies did not differ anymore between the location categories. During the post exposure phase, the sizes stayed relatively constant for all hives.

During the exposure phase, numbers of workers steadily increased at all hivesn with more than 130 workers per hive ath the peak of colony development at DAP 23. After transport to Lieteberg, the numbers of workers and drones dropped drastically. This is because the production of workers stopped at the end of the exposure phase after reaching the “Turning Point”.

The recorded parameters showed normal variations for field trials between study locations at the control and treatment site, respectively.

Turning point and reproduction endpoint

All hives reached the turning point (where predominant worker development shifts to exclusive development of drones and young queens) quite simultaneously, between DAP 19 and DAP 23. The number of hives that had reached the turning point was similar between Treatment and Control Site.

The numbers of new queens were in general rather high for commercial hives indicating a good food supply. The number of new queens was similar between hives at the edge of an OSR field at the Treatment and Control Site with 3431 and 3452 queens, respectively. Similarly, for hives distant from the treated and untreated OSR fields the total numbers were comparable with 2558 and 2611 new queens, respectively. The mean number of young queens was about 100 queens per hive.

Pollen composition

The composition of the pollen pellets varied between different study locations and sampling dates. Depending on the availability of alternative flowering plants, the amount of OSR pollen varied between 2.6 and 100 % (mean 43.9 %). Overall, the proportion of OSR amounted to 37.3 % at the control site and 50.5 % at the treatment site. The main alternative forage plants were *Salix* spp. and *Aesculus hippocastaneum* with up to 80.3 % and 53.1 %, respectively.

Residues of Clothianidin in Pollen

Pollen pellets collected by bumblebee workers at the control site did not contain any detectable clothianidin residues (all samples below limit of detection, LOD = 0.3 µg/kg). Only in three study locations in the treatment site the concentration of clothianidin was high enough to be quantified resulting to 1.0 µg/kg for study location TC and TD and 1.3 µg/kg for study location TF. At all other treatment locations, the concentration of clothianidin residues was below the limit of quantification (LOQ = 1.0 µg/kg). The concentrations of the two metabolites TZNG and TZMU in pollen pellets were below the limit of detection (LOD = 0.3 µg/kg) with the exception of study location TC where the value for TZNG was below the limit of quantification.

Unfortunately pollen samples for residues and samples for pollen composition analysis could not be collected on the same dates. Because OSR pollen content in pellets fluctuated between sampling days, it is not possible to relate clothianidin residue concentrations in pollen to OSR pollen contents.

Statistical evaluation

The development of the bumblebee hives was not significantly negatively affected by their position at the control or treatment site. The hive development and the number of workers were slightly influenced by weather conditions. Additionally, the distance to OSR fields had a marginally significant effect on the colonies, with distant hives having a smaller brood size and fewer numbers of young queens (Table B.9.7.1-3).

Table B.9.7.1-3: Statistical significances of the influence of different factors on the colony development and the reproduction endpoint.

Summary of Poisson GLMM results; hives CE-2-2 and CE-3-2 excluded from calculation; the factor levels control and edge field were set as reference level to calculate the differences to the factor levels treatment and distant field, respectively. Standard deviation in brackets

	Weight of the Hive	Numbers of Workers	Brood Size	Number of Young Queens	Number of Queen Brood Cells	Number of Workers	Number of Drones
Intercept	601.09*** (17.78)	56.35*** (5.97)	6.02*** (0.05)	5.25*** (1.07)	7.15*** (1.03)	5.42*** (1.47)	1.37 (1.75)
Treatment	9.09 (6.52)	-0.87 (6.31)	-0.09 (0.05)	-0.06 (0.38)	0.76* (0.36)	0.68 (0.51)	-0.36 (0.61)
Distant to OSR (400 m)	2.15 (5.72)	-8.03 (5.87)	-0.11* (0.04)	-0.37* (0.16)	0.06 (0.15)	0.30 (0.22)	0.05 (0.26)
Temperature (sum)	-3.44*** (0.38)	-0.91*** (0.09)	0.00*** (0.00)	0.16 (0.19)	0.22 (0.19)	0.13 (0.27)	0.01 (0.31)
Humidity (sum)	0.48* (0.21)	0.14*** (0.02)	0.00*** (0.00)	0.13 (0.11)	-0.11 (0.11)	-0.39** (0.15)	0.42* (0.18)
Wind Speed (sum)	-0.79 (1.54)	0.48* (0.24)	0.01** (0.00)	0.19* (0.08)	0.09 (0.08)	-0.11 (0.11)	-0.05 (0.13)
Precipitation (sum)	-3.17*** (0.57)	0.01 (0.14)	0.01*** (0.00)	-0.01 (0.03)	-0.07** (0.02)	-0.05 (0.03)	0.02 (0.04)

Positive significant Negative significant

***p < 0.001, **p < 0.01, *p < 0.05

Conclusions

In summary, clothianidin treated OSR did not cause any detrimental effects on the collection of OSR pollen, the development of the hives nor the formation of drones and new queens, neither during blossom in spring nor thereafter until the end of the season. The weather and the distance to the OSR fields were the main influencing variables on the development of the bumblebee colonies.

RMS Comments

As there are currently no agreed test protocols for field effect studies for bumblebees, it is difficult to assess the suitability of the present study for risk assessment purposes. However, the study was well designed, used large field sites and a high number of bumblebee colonies. Further, colony development was monitored based on different parameters (colony weight, brood size, number of workers and number of new queens).

At Pesticides Peer Review Meeting 145, it was noted that the complexity of the study design and the number of analyses and observations performed and reported would require a peer review of all the original study reports. A full consideration of this study within the confirmatory data procedure was not feasible. The study will be evaluated more deeply under the review on the neonicotinoids (Ref. EFSA question number: EFSA-Q-2015-00771).

Report:	**1.8/10; Exeler N.; 2015**
Title:	**Additional information regarding the presence of food generalist and specialist solitary bee species in agricultural landscapes**
Document No.:	M-537831-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	no

Objective
Following the comments received during Peer Review of this Addendum, a literature evaluation was performed to evaluate whether or not the mason bee (*Osmia bicornis*), which is tested in the field study performed by Peters (2015) (See study 1.8/8 in Section B.9.7.1), can be considered as representative for solitary bee species.

Literatue evaluation
Honeybees are reported to visit almost all major crops grown within Europe for which insect pollination is needed. Usually they are the most numerous visitors and therefore the best studied pollinators. In contrast bumblebees and solitary bees are often less abundant, thus observations and literature is less extensive (Corbet *et al.*, 1991).

Among wild bees, bumblebees are considered to be the most important group as crop pollinators due to their broad flower choice, specific behavior during flower visit (buzz pollination), active for long periods of the year, and high abundance. There are some examples of crops that are pollinated more effectively by wild bees than by honey bees. Red clover and field bean for example have a long flower tube and thus the nectar can only be reached by long-tongued bees such as several bumblebee species. Alfalfa is a crop that is mainly pollinated by solitary bees (*Megachile rotundata* and *Nomia melanderi*). For the pollination of orchards, the solitary bees *Osmia bicornis* and *O. cornuta* are often introduced commercially in addition to the honey bee as their potential to pollinate under unfavourable conditions (cloudy weather, cool temperatures) is higher.

The known examples of non-*Apis* pollinators (as described above) are species that are polylectic (food generalists), capable of using various plant species as pollen and nectar source.

This is confirmed by a recent literature evaluation of crop-visiting bee communities that revealed that only 2 % of a regional species pool represent the dominant crop-visiting species and account for approximately 80 % of all visits. Further analysis of the species pool in detail indicated that these **species are generally common**, whereas rare and threatened species hardly ever contribute to crop pollination (Kleijn *et al.*, 2015).

However, studies focusing on the diversity of non-*Apis* bees in crops are still relatively few in number. A monitoring study of bee diversity in organic and conventional cereal fields (Holzschuh *et al.*, 2007) revealed reduced bee diversity in conventionally farmed fields with a mean number of bee species of 2.1 bee species per field. Although the number of bee species in organic fields was higher, **in both types of growing system only polylectic species**, i.e. those foraging on a range of different plant species, were recorded. Higher bee diversity in organic fields is possibly due to a higher number and density of flowering weed species present in the fields. In contrast, in conventional fields the resource availability is typcially sparse.

Most species of solitary bees visit numerous plant species. In Germany approximately 550 wild bee species are present; and of these only 140 species are specialized/oligolectic (Kratochwil 2003). The distribution of a specialized/oligolectic bee species will be determined by the distribution of its favoured forage plant and additionally by the availability of suitable nesting sites. In agricultural landscapes the availability of semi-natural areas, which is important for nesting (Steffan-Dewenter *et al.*, 2002; Kremen *et al.*, 2004) is low (approx. 2 % Westphal *et al.*, 2003). These factors are known to

reduce the overall diversity of wild bees in agricultural areas and might explain the absence of specialists in conventional fields and even in organic fields.

The life cycle of oligolectic and polylectic solitary bee species is the same, each female is responsible for her own offspring (finding a suitable nesting site, foraging for pollen and nectar to provision the eggs). The only difference is the restriction to a certain pollen source for oligolectic bees. The fact that oligolectic bee species are underrepresented in agricultural landscapes and not present in non-*Apis* pollinator-crop-systems (see examples above: pollination systems of alfalfa, red clover etc.), makes the use of a polylectic solitary bee species in potential test systems for plant protection products more representative and realistic in particular with respect to potential exposure scenarios.

Based on the current knowledge it is concluded:

1. Only 2 % of a regional bee species pool represent the dominant crop-visiting species and account for approx. 80 % of all visits
2. These pollinator species are generally common and polylectic, foraging on a range of different plant species, while rare and threatened species hardly ever contribute to crop pollination
3. For weeds in the field a survey revealed a community of flower-visiting wild bees which only composed of polylectic species
4. The decision to use *Osmia bicornis* as a surrogate species is based on the fact that there is already some literature available on the life cycle and ecology as well as there is practical knowledge on management and rearing. The life cycle of oligolectic (food specialist) and polylectic (food generalist) solitary bee species is the same, each female is responsible for her own offspring. Consequently, conclusions drawn from studies conducted with food generalist solitary bee species can be representative for food specialists and is also more relevant to the bee species pool present in arable fields.

B.9.7.2. Exposure

Currently, the use of clothianidin as seed treatment is authorized in winter cereals and beets. In the original version of this Addendum, it was considered that no exposure through ingestion of contaminated nectar and pollen was to be expected for honeybees, bumblebees and solitary bees for these uses, as in an earlier version of Appendix D of the EFSA Guidance Document on bees winter cereals and beets were not considered attractive to bees for the consumption of pollen and nectar. During Peer Review, it was however noted that the revised version of Appendix D states that although cereals are not attractive for nectar and are generally considered low attractive to honeybees for pollen, pollen collection from cereals cannot be excluded at all due to controversial information found in literature (see comment 5(11) in the Reporting Table). In response to this comment, the applicant provided to following argumentation to demonstrate that the treated crop scenario is not relevant for cereals (*text in italic*):

Cereal crops are wind pollinated and hence are not intrinsically attractive to bees. There is no nectar reward or visual cue offered by such plants to pollinating insects. As such if pollen were collected it would be in small quantities which would be insignificant at the colony or population level, although could be relevant at the level of an individual. A cereal crop would not be subject to forager recruitment (e.g. by waggle dance) as there is no sugar or energy supply to fuel sustained foraging flights. For bumble and wild bees these typically use a much wider range of plants for food and we are not aware of any oligolectic wild bees which rely on cereals and grasses as a supply of food. Also it would be bad bee keeping practice to use cereals as a food source for honey bees.

At Pesticides Peer Review Meeting 145, the argumentation provided by the applicant was discussed. The conclusion of the EFSA Guidance Document on bees that pollen collection cannot be excluded for cereals is based on controversial data found in literature. It is stated in the EFSA Guidance Document that further data should be provided to exclude collection of pollen by honeybees, bumblebees and solitary bees. As no additional data was presented to support the argumentation of the applicant, the experts concluded that the EFSA Guidance Document is still the reference point for attractiveness to

cereals, and that therefore a risk assessment for the treated crop scenario in cereals should be included in this Addendum.

For the use in beets, it was argued during Peer Review that the revised version of Appendix D of the EFSA Guidance Document states that this crop is attractive for nectar collection and that pollen collection cannot be excluded (see comment 5(11) in the Reporting Table). In response to this comment, the applicant provided to following argumentation to demonstrate that the treated crop scenario is not relevant for beets (*text in italic*):

Sugar beet are a biennial plant which flowers only in the second season, hence in normal agricultural practice sugar beets are harvested before flowering and therefore an exposure to pollen and nectar for honeybees, bumblebees and solitary bees is not possible. Hence it is not to understand how they can be attractive and provide a food source which is relevant at population or colony level. Therefore, although the treated crop scenario cannot be excluded as a potential route of ANY exposure the low levels of attractiveness of the crop and low potential levels of food reward are considered to be negligible at the level of the colony or population.

At Pesticides Peer Review Meeting 145, the argumentation provided by the applicant was discussed. The experts agreed that as sugarbeet is a biannual crop, in normal agricultural practice the exposure to pollen and nectar is not relevant. However, when sugar beet is grown for seed production, exposure to pollen and nectar is possible in the second season. It was noted that the treated crop scenario as presented in the EFSA Guidance Document might not be suitable for biannual crops, as nectar and pollen are only produced in the second year. Therefore, this situation might rather be considered comparable to the succeeding crop scenario. The experts considered that a specific treated crop scenario should be developed for biannual crops. For the use under evaluation, it is not clear from the GAP table presented in Section A of this Addendum whether or not the registered uses also include the use in beets grown for seed production. Overall, it was considered that in most cases sugar beets will be grown following normal agricultural practice, and that thus the treated crop scenario is not relevant. At Member State level, where uses on beet are authorized, it should be further considered if clothianidin is used as seed treatment in beets grown for seed production or not, and whether or not the risk to bees from this use is acceptable.

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 winter cereals, the risk assessment was performed for this use. As no exposure is expected to nectar and pollen from beets as treated crops (see Section B.9.7.2), a risk assessment is not necessary, and the risk can be considered acceptable (at least for beets not grown for seed production).

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 product applied as seed treatment. 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 cereals 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 mg a.s./seed
SV = 0.012 (as cereals only produce pollen and forager honeybees do not consume any 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 the treated crop scenario for seed treatment)
$LD_{50,oral}$ 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 mg a.s./seed
SV = 0.012 (as cereals only produce pollen and forager honeybees do not consume any 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 the treated crop scenario for seed treatment)
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 mg a.s./seed
SV = 0.002 (as cereals only produce pollen and forager honeybees do not consume any 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 the treated crop scenario for seed treatment)
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.

According to the EFSA Guidance Document on bees, ETR values for the treated crop scenario for seed treatments should be calculated based on an application rate expressed both as mg a.s./seed and kg a.s./ha. The application rate expressed as kg a.s./ha is mentioned in the GAP table (see Table A-3). Both the highest and lowest authorized 'maximum application rate' for winter cereals will be considered in the first tier risk assessment. However, no information on the application rate per seed (mg a.s./seed) is available. At Pesticides Peer Review Meeting 145, it was therefore discussed which values for seed weight should be considered to calculate the application rate per seed. Some references on the weight of cereal kernels were provided by Member States, with an estimated weight range for 1000 seeds considering different cultivars from 21 to 61 g. As the worst case assumption could lead to high risk, the majority of the experts agreed that the risk assessment should be performed with both the best and worst case assumptions for seed weight (21 to 61 g/1000 seeds). The application rates considered in the risk assessment are shown in Table B.9.7.3.1-1.

Table B.9.7.3.1-1: Lowest and highest authorized 'maximum application rate' of clothianidin containing formulations for use as a seed treatment in winter cereals. Application rates are expressed per ha and per seed, considering a weight range for 1000 seeds from 21 to 61g.

	In g a.s./ha	In g a.s./100 kg seed	Thousand grain weight (g/100 seeds)	In mg a.s./seed
Lowest 'maximum application rate'	59	27	21	0.0057
			61	0.0165
Highest 'maximum application rate'	100	50	21	0.0105
			61	0.0305

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 for the application rate expressed in kg a.s./ha and in Table B.9.7.3.1-3 for the application rate expressed in mg a.s./seed.

Table B.9.7.3.1-2: Tier 1 ETR calculations for acute adult oral, chronic adult oral and larval exposure for the lowest and highest authorized 'maximum application rate' (expressed as kg a.s./ha) of clothianidin in winter cereals.

Acute adult oral exposure								
Crop	Application rate (kg a.s./ha)		E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Winter cereals	Lowest	0.059	1	0.012	-	0.00379	0.187	0.2
	Highest	0.100	1	0.012	-	0.00379	0.317	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
Winter cereals	Lowest	0.059	1	0.012	1	0.00138	0.513	0.03
	Highest	0.100	1	0.012	1	0.00138	0.870	0.03
Larval exposure								
Crop	Application rate (kg a.s./ha)		E_f	SV	twa	NOED (µg a.s./larva /development period)	ETR	Trigger
Winter cereals	Lowest	0.059	1	0.002	1	0.00528	0.022	0.2
	Highest	0.100	1	0.002	1	0.00528	0.038	0.2

Based on the application rate expressed as kg a.s./ha, the ETR for acute risk to adult honeybees is below the relevant trigger for the lowest 'maximum application rate', indicating an acceptable risk. For the highest 'maximum application rate', however, the ETR exceeds the trigger. The ETR for the chronic risk to adult honeybees exceeds the relevant trigger for all application rates considered, indicating a potential risk. Further consideration is thus necessary. Finally, for the risk to honeybee

larvae, the ETR is below the trigger for all application rates considered, indicating that the risk is acceptable.

Table B.9.7.3.1-3: Tier 1 ETR calculations for acute adult oral, chronic adult oral and larval exposure for the lowest and highest authorized 'maximum application rate' (expressed as mg a.s./seed) of clothianidin in winter cereals.

Acute adult oral exposure								
Crop	Application rate (mg a.s./seed)		E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Winter cereals	Lowest – best case	0.0057	1	0.012	-	0.00379	0.018	0.2
	Lowest – worst case	0.0165	1	0.012	-	0.00379	0.052	0.2
	Highest – best case	0.0105	1	0.012	-	0.00379	0.033	0.2
	Highest – worst case	0.0305	1	0.012	-	0.00379	0.097	0.2
Chronic adult oral exposure								
Crop	Application rate (mg a.s./seed)		E_f	SV	twa	LDD_{50} (µg a.s./bee/day)	ETR	Trigger
Winter cereals	Lowest – best case	0.0057	1	0.012	1	0.00138	0.049	0.03
	Lowest – worst case	0.0165	1	0.012	1	0.00138	0.143	0.03
	Highest – best case	0.0105	1	0.012	1	0.00138	0.091	0.03
	Highest – worst case	0.0305	1	0.012	1	0.00138	0.265	0.03
Larval exposure								
Crop	Application rate (mg a.s./seed)		E_f	SV	twa	NOED (µg a.s./larva /development period)	ETR	Trigger
Winter cereals	Lowest – best case	0.0057	1	0.002	1	0.00528	0.002	0.2
	Lowest – worst case	0.0165	1	0.002	1	0.00528	0.006	0.2
	Highest – best case	0.0105	1	0.002	1	0.00528	0.004	0.2
	Highest – worst case	0.0305	1	0.002	1	0.00528	0.012	0.2

Based on he application rate expressed as mg a.s./seed, the ETR for acute risk to adult honeybees and the chronic risk to honeybee larvae are below the relevant trigger, even for the worst-case assumption for the highest 'maximum application rate', indicating an acceptable risk. The ETR for chronic risk to adult honeybees however exceeds the relevant trigger for all application rates considered. 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. However, no data on clothianidin residues in pollen of winter cereals treated with clothianidin through seed treatment are available. Consequently, no tier 2 assessment could be performed.

Higher tier risk assessment

Further refinements to the risk assessment could be based on field effect studies. However, for the use in cereals, no studies investigating the effect of exposure through pollen contaminated with clothianidin through seed treatment are available. Field studies performed in other crops treated with clothianidin could potentially be used as a surrogate for studies in cereals, provided that it is demonstrated that exposure was higher compared to what is expected from cereals as treated crop.

A number of field studies is available, which were previously evelauted by EFSA for the EFSA Conclusion on the risk assessment for bees for clothianidin (2013). However, these studies were not considered acceptable for use in the risk assessment due to a number of different shortcomings. For more details on these studies, please refer the higher tier assessment for the risk to honeybees for succeeding crops (see Section B.9.2.3.1) and the EFSA Conclusion on clothianidin (2013).

A new field effect study which invastigated the effects of residues in nectar and pollen of clothianidin treated oilseed rape on honeybee colony development (Rolke et al. 2014; see Section B.9.7.1, Study 1.8/7) was submitted by the applicant. This field study is part of a large scale monitoring project on the effects of seed treatment of oilseed rape with clothianidin on honeybees, bumblebees and solitary bees. For this monitoring project, two study sites (treated site and control site) were selected in Northern Germany, each covering an area of about 65 km² and containing about 20 study fields sown with oilseed rape. Oilseed rape sown in the treated site were seed treated with clothianidin, while those sown in the control site were untreated. For the honeybee study, six study locations were identified at each study site within a core area (7 km diameter) where honeybee hives were set up. Of the six locations in each study site, three locations were situated at the edge of oilseed rape fields, and three location at about 400m distant from the oilseed rape fields. At each study location, 8 honeybee hives were placed, resulting in a total of 96 colonies that were exposed to nectar and pollen from oilseed rape (48 treated and 48 untreated). As oilseed rape is a highly bee attractive crop for both pollen and nectar, it might be reasonable to assume that the exposure in this study in oilseed rape will be worst-case compared to winter cereals, and thus that the results from this study could be extrapolated.

At Pesticides Peer Review Meeting 145, the large scale monitoring study in oilseed rape was discussed. It was noted that the study was performed in Germany. A similarity analysis between the study area and other oilseed rape growing areas in Europe was performed, but it seems that it does not cover the landscape composition (i.e. differences in field margin composition in oilseed rape areas other than Germany may influence the proportion of pollen from different plant species entering into the hive, for example when more attractive plants are available in the filed margin). An in depth evaluation of the similarity analysis provided with the study would be appropriate to confirm this.

It was noted that the complexity of the study design and the number of analyses and observations performed and reported would require a peer review of all the original study reports. A full consideration of this study within the confirmatory data procedure was not feasible. The study will be evaluated more deeply under the review on the neonicotinoids (Ref. EFSA question number: EFSA-Q-2015-00771).

Overall, the experts considered that this study, for the time being, cannot be used to draw firm conclusions on possible extrapolation of the results to other scenarios (i.e. succeeding crops, field margin and treated crop other than oilseed rape) for honeybees.

Conclusions

The risk to honeybees from seed treatment with clothianidin in beet is considered acceptable, based on the fact that there will be no exposure from the treated crop (as beets are harvested before flowering). This conclusion is however only valid for beets that are not grown for seed production.

For the use in winter cereals, the acute risk to adult honeybees and the chronic risk to honeybee larvae was acceptable at tier 1. For the chronic risk to adult honeybees, this was however not the case. No suitable data was available to perform a tier 2 or higher tier risk assessment.

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 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 winter cereals, the risk assessment was performed for this use. As no exposure is expected to nectar and pollen from beets as treated crops (see Section B.9.7.2), a risk assessment is not necessary, and the risk can be considered acceptable (at least for beets not grown for seed production).

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 product applied as seed treatment. 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 cereals 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.

According to the EFSA Guidance Document on bees, ETR values for the treated crop scenario for seed treatments should be calculated based on an application rate expressed both as mg a.s./seed and kg a.s./ha. The application rate expressed as kg a.s./ha is mentioned in the GAP table (see Table A-3). Both the highest and lowest authorized 'maximum application rate' for winter cereals will be considered in the first tier risk assessment. However, no information on the application rate per seed (mg a.s./seed) is available. At Pesticides Peer Review Meeting 145, it was therefore discussed which values for seed weight should be considered to calculate the application rate per seed. Some references on the weight of cereal kernels were provided by Member States, with an estimated weight range for 1000 seeds considering different cultivars from 21 to 61 g. As the worst case assumption could lead to high risk, the majority of the experts agreed that the risk assessment should be performed with both the best and worst case assumptions for seed weight (21 to 61 g/1000 seeds). The application rates considered in the risk assessment are shown in Table B.9.7.3.2-1.

Table B.9.7.3.2-1: Lowest and highest authorized 'maximum application rate' of clothianidin containing formulations for use as a seed treatment in winter cereals. Application rates are expressed per ha and per seed, considering a weight range for 1000 seeds from 21 to 61g.

	In g a.s./ha	In g a.s./100 kg seed	Thousand grain weight (g/100 seeds)	In mg a.s./seed
Lowest 'maximum application rate'	59	27	21	0.0057
			61	0.0165
Highest 'maximum application rate'	100	50	21	0.0105
			61	0.0305

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 calculated Tier 1 ETR values are shown in Table B.9.7.3.2-2 for the application rate expressed in kg a.s./ha and in Table B.9.7.3.2-3 for the application rate expressed in mg a.s./seed.

Table B.9.7.3.2-2: Tier 1 ETR calculations for acute adult oral and chronic adult oral exposure for the lowest and highest authorized 'maximum application rate' (expressed as kg a.s./ha) of clothianidin in winter cereals.

Acute adult oral exposure								
Crop	Application rate (kg a.s./ha)		E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Winter cereals	Lowest	0.059	1	0.030	-	0.00191	0.927	0.036
	Highest	0.100	1	0.030	-	0.00191	1.571	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
Winter cereals	Lowest	0.059	1	0.030	1	0.000138	12.826	0.0048
	Highest	0.100	1	0.030	1	0.000138	21.739	0.0048

Table B.9.7.3.2-3: Tier 1 ETR calculations for acute adult oral and chronic adult oral exposure for the lowest and highest authorized 'maximum application rate' (expressed as mg a.s./seed) of clothianidin in winter cereals.

Acute adult oral exposure								
Crop	Application rate (mg a.s./seed)		E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Winter cereals	Lowest – best case	0.0057	1	0.030	-	0.00191	0.089	0.036
	Lowest – worst case	0.0165	1	0.030	-	0.00191	0.259	0.036
	Highest – best case	0.0105	1	0.030	-	0.00191	0.165	0.036
	Highest – worst case	0.0305	1	0.030	-	0.00191	0.479	0.036
Chronic adult oral exposure								
Crop	Application rate (mg a.s./seed)		E_f	SV	twa	LDD_{50} (µg a.s./bee/day)	ETR	Trigger
Winter cereals	Lowest – best case	0.0057	1	0.030	1	0.000138	1.233	0.0048
	Lowest – worst case	0.0165	1	0.030	1	0.000138	3.580	0.0048
	Highest – best case	0.0105	1	0.030	1	0.000138	2.283	0.0048
	Highest – worst case	0.0305	1	0.030	1	0.000138	6.630	0.0048

Based on the application rate expressed as both kg a.s./ha and mg a.s./seed, the ETR for acute and chronic risk to adult bumblebees exceeds the relevant trigger, for all application rates considered. 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. However, no data on clothianidin residues in pollen of winter cereals treated with clothianidin through seed treatment are available. Consequently, no tier 2 assessment could be performed.

Higher tier risk assessment

Further refinements to the risk assessment could be based on field effect studies. However, for the use in cereals, no studies investigating the effect of exposure through pollen contaminated with clothianidin through seed treatment are available. Field studies performed in other crops treated with clothianidin could potentially be used as a surrogate for studies in cereals, provided that it is demonstrated that exposure was higher compared to what is expected from cereals as treated crop.

A field effect study which investigates the effects of residues in nectar and pollen of clothianidin treated (seed treatment) oilseed rape on bumblebee colonies is available (Sterk & Peter, 2014; see section B.9.7.1, Study 1.8/9). This study is part of a large scale monitoring project on the effects of seed treatment of oilseed rape with clothianidin on honeybees, bumblebees and solitary bees. For this monitoring project, two study sites (treated site and control site) were selected in Northern Germany,

each covering an area of about 65 km² and containing about 20 study fields sown with oilseed rape. Oilseed rape sown in the treated site were seed treated with clothianidin, while those sown in the control site were untreated. For the bumblebee study, six study locations were identified at each study site within a central area (3 km diameter) where bumblebee hives were set up. Of the six locations in each study site, three locations were situated at the edge of oilseed rape fields, and three location at about 400m distant from the oilseed rape fields. At each study location, 10 bumblebee colonies were placed, resulting in a total of 120 colonies that were exposed to nectar and pollen from oilseed rape (60 treated and 60 untreated). As oilseed rape is a highly bee attractive crop for both pollen and nectar, it might be reasonable to assume that the exposure in this study in oilseed rape will be worst-case compared to winter cereals, and thus that the results from this study could be extrapolated.

At Pesticides Peer Review Meeting 145, the large scale monitoring study in oilseed rape was discussed. For the solitary bee, *Osmia*, the experts noted that the pollen composition indicated that oilseed rape is not a relevant source of pollen. For Bumblebees, the range of pollen composition was very high (2-100%) with an average of 50%. It was argued that in this case it could be useful to only consider the results from hives with a large proportion of oilseed rape pollen to obtain a worst-case exposure situation, but this would further reduce the power of the study. Based on the current evaluation of the data presented in the study report, extrapolation to other scenarios was considered not fully reliable because not worst-case.

It was noted that the study was performed in Germany. A similarity analysis between the study area and other oilseed rape growing areas in Europe was performed, but it seems that it does not cover the landscape composition (i.e. differences in field margin composition in oilseed rape areas other than DE may influence the proportion of pollen from different plant species entering into the hive, for example when more attractive plants are available in the filed margin). An in depth evaluation of the similarity analysis provided with the study would be appropriate to confirm this.

It was noted that the complexity of the study design and the number of analyses and observations performed and reported would require a peer review of all the original study reports. A full consideration of this study within the confirmatory data procedure was not feasible. The study will be evaluated more deeply under the review on the neonicotinoids (Ref. EFSA question number: EFSA-Q-2015-00771).

Overall, the experts considered that this study, for the time being, cannot be used to draw firm conclusions on possible extrapolation of the results to other scenarios (i.e. succeeding crops, field margin and treated crop other than OSR) for honeybees. Further consideration for bumblebees would be needed. However, for solitary bees the experts considered that the extrapolation to other crops or scenarios could not be reliably performed because likely the conditions in the study were not worst case for these species.

Conclusions

The risk to bumblebees from seed treatment with clothianidin in beet is considered acceptable, based on the fact that there will be no exposure from the treated crop (as beets are harvested before flowering). This conclusion is however only valid for beets that are not grown for seed production.

For the use in winter cereals, the acute and chronic risk to adult bumblebees was not acceptable at tier 1. No suitable data was available to perform a tier 2 or higher tier risk assessment.

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 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 winter cereals, the risk assessment was performed for this use. As no exposure is expected to nectar and pollen from beets as treated crops (see Section B.9.7.2), a risk assessment is not necessary, and the risk can be considered acceptable (at least for beets not grown for seed production).

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 product applied as seed treatment. 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 cereals 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.

According to the EFSA Guidance Document on bees, ETR values for the treated crop scenario for seed treatments should be calculated based on an application rate expressed both as mg a.s./seed and kg a.s./ha. The application rate expressed as kg a.s./ha is mentioned in the GAP table (see Table A-3). Both the highest and lowest authorized 'maximum application rate' for winter cereals will be considered in the first tier risk assessment. However, no information on the application rate per seed (mg a.s./seed) is available. At Pesticides Peer Review Meeting 145, it was therefore discussed which values for seed weight should be considered to calculate the application rate per seed. Some references on the weight of cereal kernels were provided by Member States, with an estimated weight range for 1000 seeds considering different cultivars from 21 to 61 g. As the worst case assumption could lead to high risk, the majority of the experts agreed that the risk assessment should be performed with both the best and worst case assumptions for seed weight (21 to 61 g/1000 seeds). The application rates considered in the risk assessment are shown in Table B.9.7.3.3-1.

Table B.9.7.3.3-1: Lowest and highest authorized 'maximum application rate' of clothianidin containing formulations for use as a seed treatment in winter cereals. Application rates are expressed per ha and per seed, considering a weight range for 1000 seeds from 21 to 61g.

	In g a.s./ha	In g a.s./100 kg seed	Thousand grain weight (g/100 seeds)	In mg a.s./seed
Lowest 'maximum application rate'	59	27	21	0.0057
			61	0.0165
Highest 'maximum application rate'	100	50	21	0.0105
			61	0.0305

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 bumblebee larvae could not be performed. The calculated Tier 1 ETR values are shown in Table B.9.7.3.3-2 for the application rate expressed in kg a.s./ha and in Table B.9.7.3.3-3 for the application rate expressed in mg a.s./seed.

Table B.9.7.3.3-2: Tier 1 ETR calculations for acute adult oral and chronic adult oral exposure for the lowest and highest authorized 'maximum application rate' (expressed as kg a.s./ha) of clothianidin in winter cereals.

Acute adult oral exposure								
Crop	Application rate (kg a.s./ha)		E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Winter cereals	Lowest	0.059	1	0.010	-	0.000379	1.557	0.04
	Highest	0.100	1	0.010	-	0.000379	2.639	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
Winter cereals	Lowest	0.059	1	0.010	1	0.000138	4.275	0.0054
	Highest	0.100	1	0.010	1	0.000138	7.246	0.0054

Table B.9.7.3.3-3: Tier 1 ETR calculations for acute adult oral and chronic adult oral exposure for the lowest and highest authorized 'maximum application rate' (expressed as mg a.s./seed) of clothianidin in winter cereals.

Acute adult oral exposure								
Crop	Application rate (mg a.s./seed)		E_f	SV	twa	$LD_{50,oral}$ (µg a.s./bee)	ETR	Trigger
Winter cereals	Lowest – best case	0.0057	1	0.010	-	0.000379	0.150	0.04
	Lowest – worst case	0.0165	1	0.010	-	0.000379	0.435	0.04
	Highest – best case	0.0105	1	0.010	-	0.000379	0.277	0.04
	Highest – worst case	0.0305	1	0.010	-	0.000379	0.805	0.04
Chronic adult oral exposure								
Crop	Application rate (mg a.s./seed)		E_f	SV	twa	LDD_{50} (µg a.s./bee/day)	ETR	Trigger
Winter cereals	Lowest – best case	0.0057	1	0.010	1	0.000138	0.411	0.0054
	Lowest – worst case	0.0165	1	0.010	1	0.000138	1.193	0.0054
	Highest – best case	0.0105	1	0.010	1	0.000138	0.761	0.0054
	Highest – worst case	0.0305	1	0.010	1	0.000138	2.210	0.0054

Based on the application rate expressed as both kg a.s./ha and mg a.s./seed, the ETR for acute and chronic risk to adult solitary bees exceeds the relevant trigger, for all application rates considered. 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. However, no data on clothianidin residues in pollen of winter cereals treated with clothianidin through seed treatment are available. Consequently, no tier 2 assessment could be performed.

Higher tier risk assessment

Further refinements to the risk assessment could be based on field effect studies. However, for the use in cereals, no studies investigating the effect of exposure through pollen contaminated with clothianidin through seed treatment are available. Field studies performed in other crops treated with clothianidin could potentially be used as a surrogate for studies in cereals, provided that it is demonstrated that exposure was higher compared to what is expected from cereals as treated crop.

A field effect study which investigated the effects of residues in nectar and pollen of clothianidin treated (seed treatment) oilseed rape on the development and reproduction of solitary bees is available (Peters, 2015; see Section B.9.7.1, Study 1.8/8). The field study (Peters, 2015) was conducted with the red mason bee *Osmia bicornis*. In Appendix Q of the EFSA Guidance Document on bees, this species is proposed as test species in the risk assessment scheme for solitary bees. The study by Peters (2015) is part of a large scale monitoring project on the effects of seed treatment of oilseed rape with clothianidin on honeybees, bumblebees and solitary bees. For this monitoring project, two study sites (treated site and control site) were selected in Northern Germany, each covering an area of about 65 km² and containing about 20 study fields sown with oilseed rape. Oilseed rape sown in the treated site were seed treated with clothianidin, while those sown in the control site were untreated. For the solitary bee study, six study locations were identified at each study site where nesting shelters and solitary bee cocoons were set up. Of the six locations in each study site, three locations were situated at the edge of oilseed rape fields, and three location at about 100m distant from the oilseed rape fields. At each study location, three nesting shelters containing each two or three nesting blocks (with 200 nesting holes) were placed. This resulted in 36 nesting shelters in total (18 treated and 18 untreated). Further, 1500 cocoons of red mason bees were set up at each test location. As oilseed rape is a highly bee attractive crop for both pollen and nectar, it might be reasonable to assume that the exposure in

this study in oilseed rape will be worst-case compared to winter cereals, and thus that the results from this study could be extrapolated.

At Pesticides Peer Review Meeting 145, the large scale monitoring study in oilseed rape was discussed. For the solitary bee, *Osmia*, the experts noted that the pollen composition indicated that oilseed rape is not a relevant source of pollen. For Bumblebees, the range of pollen composition was very high (2-100%) with an average of 50%. It was argued that in this case it could be useful to only consider the results from hives with a large proportion of oilseed rape pollen to obtain a worst-case exposure situation, but this would further reduce the power of the study. Based on the current evaluation of the data presented in the study report, extrapolation to other scenarios was considered not fully reliable because not worst-case.

It was noted that the study was performed in Germany. A similarity analysis between the study area and other oilseed rape growing areas in Europe was performed, but it seems that it does not cover the landscape composition (i.e. differences in field margin composition in oilseed rape areas other than DE may influence the proportion of pollen from different plant species entering into the hive, for example when more attractive plants are available in the filed margin). An in depth evaluation of the similarity analysis provided with the study would be appropriate to confirm this.

It was noted that the complexity of the study design and the number of analyses and observations performed and reported would require a peer review of all the original study reports. A full consideration of this study within the confirmatory data procedure was not feasible. The study will be evaluated more deeply under the review on the neonicotinoids (Ref. EFSA question number: EFSA-Q-2015-00771).

Overall, the experts considered that this study, for the time being, cannot be used to draw firm conclusions on possible extrapolation of the results to other scenarios (i.e. succeeding crops, field margin and treated crop other than OSR) for honeybees. Further consideration for bumblebees would be needed. However, for solitary bees the experts considered that the extrapolation to other crops or scenarios could not be reliably performed because likely the conditions in the study were not worst case for these species.

Conclusions

The risk to solitary bees from seed treatment with clothianidin in beet is considered acceptable, based on the fact that there will be no exposure from the treated crop (as beets are harvested before flowering). This conclusion is however only valid for beets that are not grown for seed production.

For the use in winter cereals, the acute and chronic risk to adult solitary bees was not acceptable at tier 1. No suitable data was available to perform a tier 2 or higher tier risk assessment.

C. OVERALL CONCLUSIONS

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 seed treatments through the consumption of contaminated nectar and pollen from succeeding crops was identified at tier 1 and 2. Higher Tier field effect studies with treated primary crops (in which residues in pollen and nectar exceeded those measured in the succeeding crop studies) could potentially be used to refine the risk assessment. However, the available large scale monitoring study performed in oilseed rape requires further in depth evaluation (which will be performed within EFSA-Q-2015-00771). For the time being, this study cannot be used to extrapolate the results to other scenarios. Consequently, no acceptable risk to could be concluded.

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 to bumblebees and solitary bee following exposure to dust drift from treated sugar beet seeds is acceptable, due to the negligible exposure to dust from treated sugar beet pills. For the use in winter cereals, a potential risk was identified at tier one. For both bumblebees and solitary bees, the available higher tier data was not sufficient to conclude that the risk is acceptable.

The risk to bumblebees and solitary bees following exposure to nectar and pollen from the treated crop is considered acceptable for the use in beets. As (sugar) beets are harvested before flowering, there will be no exposure to bees from contaminated nectar and pollen. This conclusion is however only valid for beets that are not grown for seed production. At Member State level, where uses on beet are authorized, it should be further considered if clothianidin is used as seed treatment in beets grown for seed production or not, and whether or not the risk to bees from this use is acceptable.

For the use in winter cereals, the acute and chronic risk to adult bumblebees and solitary bees from exposure to pollen from the treated crop was not acceptable at tier 1. No suitable data was available to perform a tier 2 or higher tier risk assessment. Higher tier effect studies could potentially be used to refine the risk assessment for winter cereals. However, the available large scale monitoring study performed in oilseed rape requires further in depth evaluation (which will be performed within EFSA-Q-2015-00771). For the time being, this study cannot be used to extrapolate the results to other crops. Consequently, no acceptable risk to could be concluded.

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 for chronic adult exposure. Higher Tier field effect studies with treated primary crops (in which residues in pollen and nectar exceeded those measured in the succeeding crop studies) could potentially be used to refine the risk assessment. However, the available large scale monitoring study performed in oilseed rape requires further in depth evaluation (which will be performed within EFSA-Q-2015-00771). For the time being, this study cannot be used to extrapolate the results to other scenarios. Consequently, no acceptable risk to could be concluded.

The **potential uptake via roots of flowering weeds** was not assessed. However, based on the evaluation of data extracted from the untreated control plots of efficacy trials on herbicidal active ingredients, together with all other data available, the exposure of honeybees and non-*Apis* bees through nectar and pollen of flowering weeds is considered negligible. 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 an argumentation provided by the applicant, the 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.

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. 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 winter cereals and sugar beets, together with all other available studies investigating the effects from guttation exposure, the risk was considered acceptable.

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** is considered acceptable for the use in beet, as for sugar beet pills (and for fodder beet/beet, assuming the same technology for seed pelleting and drilling), exposure to bees through dust drift is negligibly low. For the use in winter cereals, a potential risk was identified at tier one and two. The available higher tier effect study submitted by the applicant, was not considered sufficient to demonstrate an acceptable risk. The risk to bees from dust exposure for winter cereals should be further addressed.

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** is considered acceptable for the use in beets. As (sugar) beets are harvested before flowering, there will be no exposure to bees from contaminated nectar and pollen. This conclusion is however only valid for beets that are not grown for seed production. At Member State level, where uses on beet are authorized, it should be further considered if clothianidin is used as seed treatment in beets grown for seed production or not, and whether or not the risk to bees from this use is acceptable.

For the use in winter cereals, a risk assessment was performed as exposure to clothianidin contaminated pollen could not completely be excluded. For winter cereals as treated crop, the acute risk to adult honeybees and the chronic risk to honeybee larvae was acceptable at tier 1. For the chronic risk to adult honeybees, this was however not the case. No suitable data was available to perform a tier 2 or higher tier risk assessment. Higher tier effect studies could potentially be used to refine the risk assessment for winter cereals. However, the available large scale monitoring study performed in oilseed rape requires further in depth evaluation (which will be performed within EFSA-Q-2015-00771). For the time being, this study cannot be used to extrapolate the results to other crops. Consequently, no acceptable risk to could be concluded.

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:

- Field effect studies that investigate the acute and long-term risk to honeybees foraging in nectar and pollen in succeeding crops
- Further exposure and effect studies that investigate exposure to dust drift 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 winter cereals.

- Exposure and effect studies that investigate the exposure to clothianidin contaminated pollen from winter cereals treated with clothianidin through seed treatment, and the long-term risk to colony survival and development from such exposure for the registered use in winter cereals.

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)
- Field effect studies that investigate the acute and long-term risk to bumblebees and solitary bees foraging in nectar and pollen in succeeding crops
- Exposure and effect studies that investigate exposure to dust drift and the acute and long-term risk to bumblebee colonies and solitary bees.
- Exposure and effect studies that investigate the exposure to clothianidin contaminated pollen from winter cereals treated with clothianidin through seed treatment, and the acute and long-term risk to bumblebees and solitary bees foraging in pollen in winter cereals.

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

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protection claimed Y/N	Justification if data protection is claimed	Owner
1.2/1	Harkin, S.	2014	Clothianidin: Acut contact an oral toxicity to bumblebee (Bombus terrestris) The Food and Environment Research Agency, York, United Kingdom Sumitomo Chemical Company, Report No.: B2AK1000, Document Number: M-504345-01-1 Date: 2014-12-04 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Sumitomo Chemical Company
1.2/2	Pfeiffer, S.	2014	Clothianidin + imidacloprid FS 275 (100+175 g/L): Acute contact toxicity to the bumblebee, Bombus terrestris L. under laboratory conditions Eurofins Agroscience Services, EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S13-05151, Document Number: M-494283-01-1 Date: 2014-05-05 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience
1.2/3	Schmitzer, S.	2014	Effects of clothianidin + imidacloprid FS 275 (100+175) G (acute contact and oral) on honeybees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 89691035, Document Number: M-501653-01-1 Date: 2014-11-10 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience

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1.2/4	Pfeiffer, S.	2014	Clothianidin + fluopicolide + fluoxastrobin FS 510 (300+120+90 g/L) - Acute contact toxicity to the bumblebee, Bombus terrestris L. under laboratory conditions Eurofins Agroscience Services, EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S13-05150, Document Number: M-494271-01-1 Date: 2014-05-02 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience
1.2/5	Schmitzer, S.	2010	Effects of clothianidin + fluopicolide + fluoxastrobin FS 510 (300+120+90) G (acute contact and oral) on honeybees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 53631035, Document Number: M-367011-01-1 Date: 2010-04-15 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience
1.2/6	Pfeiffer, S.	2014	Clothianidin + prothioconazole FS 300 (250+50 g/L) - Acute contact toxicity to the bumblebee, Bombus terrestris L. under laboratory conditions Eurofins Agroscience Services, EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S13-05152, Edition Number: M-494300-01-1 Date: 2014-05-05 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience

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1.2/7	Schmitzer, S.	2014	Effects of clothianidin + prothioconazole FS 300 (250+50) G (acute contact and oral) on honeybees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 89681035, Document Number: M-501142-01-1 Date: 2014-11-03 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience
1.3/1	Jarratt, N.	2014	Determination of clothianidin residues in bee relevant matrices, collected in a succeeding crop scenario with natural aged clothianidin residues - Field phase conducted with phacelia and maize in the UK (Goole, East Yorkshire) The Food and Environment Research Agency, York, United Kingdom Bayer CropScience, Report No.: B2BN2000, Document Number: M-504590-01-1 Date: 2014-12-05 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience

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1.3/2	Jarratt, N.	2014	Determination of clothianidin residues in bee relevant matrices, collected in a succeeding crop scenario with natural aged clothianidin residues - Field phase conducted with phacelia and maize in the UK (Thorney, Cambridgeshire) The Food and Environment Research Agency, York, United Kingdom Bayer CropScience, Report No.: B2BN3000, Document Number: M-504595-01-1 Date: 2014-12-04 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience
1.3/3	Jarratt, N.	2014	Determination of clothianidin residues in bee relevant matrices, collected in a succeeding crop scenario with natural aged clothianidin residues - Field phase conducted with phacelia and maize in the UK (Sawtry, Cambridgeshire) The Food and Environment Research Agency, York, United Kingdom Bayer CropScience, Report No.: B2BN4000, Document Number: M-504601-01-1 Date: 2014-12-04 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience

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1.3/4	Xu, T.; Dyer, Daniel	2014	Clothianidin plant bioavailability and soil accumulation study - Clothianidin (TI-435) Valent USA Corporation Dublin Laboratory, Dublin, CA, USA Bayer CropScience, Report No.: METIY004, Document Number: M-498438-01-1 Date: 2014-10-01 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience
1.3/5	Hammel, K.; Vrbka, L.	2014	Calculation of plateau concentrations in soil for imidacloprid and clothianidin Bayer CropScience, Report No.: EnSa-14-1318, Document Number: M-503458-01-1 Date: 2014-11-28 GLP/GEP: n.a., unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience

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1.3/6	Ythier, E.	2014	Determination of the residues of clothianidin in bee relevant matrices collected from succeeding crops following application of clothianidin FS 600B G via soil incorporation to plateau concentration and sowing of clothianidin-treated winter barley seeds. Field phase conducted in southern France SynTech Research France SAS, La Chapelle de Guinchay, France Bayer CropScience, Report No.: 7SRFR13C4, Report includes Trial Nos.: P672134725 SRFR13-001-7IC4 SRFR13-002-7IC4 SRFR13-003-7IC4 Document Number: M-504814-01-1 Date: 2014-12-09 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience
1.3/7	Striffler, B.; Ballhaus	2014	Residues of clothianidin in nectar and pollen of flowering rotational crops in Western Germany tier3 solutions GmbH, Leverkusen, Germany Bayer CropScience, Report No.: P13068-1, Document Number: M-504884-01-1 Date: 2014-12-10 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience

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1.4/1	Garside, C. M.; Miles, M.; Kriszan, M.	2014	Statement - Evaluation of the occurrence of flowering weeds in agricultural crops: Cereals, sugar beet and potatoes Bayer CropScience, Report No.: M-505126-01-1, Document Number: M-505126-01-1 Date: 2014-12-10 GLP/GEP: n.a., unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience
1.5/1	Nauen, R.	2013	Statement - Information on the occurrence or possible occurrence of the development of resistance of the plant protection product Janus Forte (for submission in Europe) Bayer CropScience Bayer CropScience, Report No.: M-453965-01-1, Document Number: M-453965-01-1 Date: 2013-05-20 GLP/GEP: n.a., unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience
1.6/1	Hofmann, S.; Lueckmann, J.	2014	Field study to monitor potential effects on honeybees from exposure to guttation fluid of winter wheat (W-WHT), seed-treated either with an imidacloprid or a clothianidin combi-product RifCon GmbH, Heidelberg, Germany Bayer CropScience, Report No.: R09247-4, Document Number: M-498939-01-1 Date: 2014-07-14 GLP/GEP: no, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience

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1.6/2	Hofmann, S.; Garrido, C.; Lueckmann, J.	2012	Field study to monitor potential effects on honeybees from exposure to guttation fluid of winter barley (W-BAR), seed-treated either with an imidacloprid or a clothianidin combi-product RifCon GmbH, Heidelberg, Germany Bayer CropScience, Report No.: R09247-3, Document Number: M-498922-01-1 Date: 2012-10-17 GLP/GEP: no, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience
1.6/3	Hofmann, S.; Staffel, J.; Aumeier, P.	2014	Field study to monitor potential effects on honeybees from exposure to guttation fluid of winter barley (W-BAR), seed-treated with the insecticidal seed-treatment product clothianidin + imidacloprid FS 100 + 175 G in Germany in 2011/2012 RIFCON GmbH, Hirschberg, Germany Bayer CropScience, Report No.: R11130, Document Number: M-501261-01-1 Date: 2014-11-04 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience

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1.6/4	Rexer, H. U.	2014	A long-term field study to monitor potential effects on the honeybee (Apis mellifera L.) from exposure to guttation fluid of sugar beets, seed-treated with the insecticides clothianidin + imidacloprid + beta-cyfluthrin in Southern Germany in 2013 and 2014 Eurofins Agrosciences Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S13-00171, Report includes Trial Nos.: S13-00171-00171-L3 S13-00171-01 S13-00171-L1 S13-00171-L2 Document Number: M-500724-01-1 Date: 2014-09-30 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience

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1.6/5	Rexer, H. U.	2014	A long-term field study to monitor potential effects on the honeybee (Apis mellifera L.) from exposure to guttation fluid of sugar beets, seed-treated with the insecticides clothianidin + imidacloprid + beta-cyfluthrin in Southern Germany in 2013 and 2014 Eurofins Agrosciences Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S13-00170, Report includes Trial Nos.: S13-00170-00170-L3 S13-00170-01 S13-00170-L1 S13-00170-L2 Document Number: M-500734-01-1 Date: 2014-09-30 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience
1.7/1	Hofmann, S.; Lueckmann, J.	2010	Monitoring of dust drift deposits during and after sowing of winter barley (W-BAR) treated with Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 145.2 (60 + 70 + 7.2 + 8 g/L) or Clothianidin & Beta-Cyfluthrin FS 455 (375 + 80 g/L) on fields in Germany RifCon GmbH, Heidelberg, Germany Bayer CropScience, Report No.: R09247-1, Document Number: M-366273-01-1 Date: 2010-03-09 GLP/GEP: no, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience

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1.7/2	Hofmann, S.; Lueckmann, J.	2010	Monitoring of dust drift deposits during and after sowing of winter wheat (W-WHT) treated with Triadimenol & Imidacloprid & Fuberidazol & Imazalil FS 145.2 (60 + 70 + 7.2 + 8 g/L) or Clothianidin & Beta-Cyfluthrin FS 455 (375 + 80 g/L) on fields in Germany RifCon GmbH, Heidelberg, Germany Bayer CropScience, Report No.: R09247-2, Document Number: M-366277-01-1 Date: 2010-03-09 GLP/GEP: no, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience
1.7/3	Lueckmann, J.	2014	Second amendment to final report - Investigation of dust drift deposits of clothianidin & imidacloprid treated winter barley seeds with pneumatic sowing machinery on fields in Germany in autumn 2011 RifCon GmbH, Heidelberg, Germany Bayer CropScience, Report No.: R11129, Document Number: M-502885-03-1 Date: 2014-11-20, Amended: 2014-12-05 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience

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1.7/4	Lueckmann, J.; Staffel, J.	2015	Final report - Assessment of potential impacts on honeybee colony development, their hibernation performance and concurrent monitoring of aerial dust drift during the sowing operation of Redigo Deter FS 300 G - Treated winter barley with typical commercial pneumatic sowing technology, directly adjacent to full-flowering Phacelia tanacetifolia in United Kingdom RIFCon GmbH, Hirschberg, Germany Bayer CropScience, Report No.: GLP 199, R1440008, Document Number: M-504538-03-1 Date: 2015-02-19 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience
1.7/5	Staffel, J.; Lueckmann, J.	2014	Final report - Assessment of potential impacts on honeybee colony development, their hibernation performance and concurrent monitoring of aerial dust drift during the sowing operation of Poncho Beta Plus - Treated sugar beet pills with typical commercial vacuum-pneumatic sowing technology, directly adjacent to full-flowering Phacelia tanacetifolia in Germany RIFCon GmbH, Hirschberg, Germany Bayer CropScience, Report No.: 195, Document Number: M-504065-01-1 Date: 2014-11-28 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience

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1.8/1	Heimbach, F.; Russ, A.	2014	Interim report - Large-scale monitoring of long-term effects of Elado (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: I project overview and summary tier3 solutions GmbH, Leverkusen, Germany Bayer CropScience, Report No.: B13055-0, Document Number: M-503588-01-1 Date: 2014-11-28 GLP/GEP: no, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience
1.8/2	Schimmer, M.; Russ, A.	2014	Large-scale monitoring of long-term effects of Elado (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: II project area and study fields characterisation tier3 solutions GmbH, Leverkusen, Germany Bayer CropScience, Report No.: B13055-1, Document Number: M-503370-01-1 Date: 2014-11-18 GLP/GEP: no, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience

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1.8/3	Born, K.	2014	Large-scale monitoring of long-term effects of Elado (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: III site similarity certification of study sites and its elevance for other rape cultivation sites in Europe Spatial Business Integration BCS, Report No.: M-503372-01-1, Document Number: M-503372-01-1 Date: 2014-09-23 GLP/GEP: no, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	BCS
1.8/4	Benito, M. M.; Russ, A.; Schimmer, M.	2014	Large-scale monitoring of long-term effects of Elado (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: IV residues of clothianidin in soil before drilling and soil characterisation tier3 solutions GmbH, Leverkusen, Germany Bayer CropScience, Report No.: B13055-2, Document Number: M-503397-01-1 Date: 2014-11-18 GLP/GEP: no, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience

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1.8/5	Russ, A.; Schimmer, M.; Benito, M.	2014	Final report - Large-scale monitoring of long-term effects of elado (10 g Clothianidin & 2 g Beta-Cyfluthrin / kg seed) Dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: V seed characterisation, Drilling and Growth of oilseed rape tier3 solutions GmbH, Leverkusen, Germany Bayer CropScience, Report No.: B13055-3, Document Number: M-504076-01-1 Date: 2014-11-18 GLP/GEP: no, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience
1.8/6	Persigehl, M.	2014	Final report - Large-scale monitoring of long-term effects of Elado (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: VI residues of clothianidin in nectar and pollen collected by honeybees in tunnel tent tier3 solutions GmbH, Leverkusen, Germany Bayer CropScience, Report No.: B13081-2, Document Number: M-504416-01-1 Date: 2014-11-26 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience

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1.8/7	Rolke, D.; Persigehl, M.; Gruenewald, B.; Blenau, W.	2014	Large-scale monitoring of long-term effects of Elado (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: VII effects on honeybees (Apis mellifera) tier3 solutions GmbH, Leverkusen, Germany Bayer CropScience, Report No.: B13081-1, Document Number: M-503572-01-1 Date: 2014-11-28 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience
1.8/8	Peters, B.	2015	Final report - Large-scale monitoring of long-term effects of Elado (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: VIII short- and long-term effects on red mason bees (Osmia bicornis) tier3 solutions GmbH, Leverkusen, Germany Bayer CropScience, Report No.: B14013, Document Number: M-503583-02-1 Date: 2014-11-26 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience

Annex point / reference number	**Author(s)**	**Year**	**Title** **Source *(where different from company)*** **Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not**	**Data protection claimed Y/N**	**Justification if data protection is claimed**	**Owner**
1.8/9	Sterk, G.; Peters, B.	2014	Large-scale monitoring of long-term effects of Elado (10 g clothianidin & 2 g beta-cyfluthrin / kg seed) dressed oilseed rape on pollinating insects in Mecklenburg-Vorpommern, Germany: IX effects on large earth bumblebees (Bombus terrestris) tier3 solutions GmbH, Leverkusen, Germany Bayer CropScience, Report No.: B14014, Document Number: M-503580-01-1 Date: 2014-11-26 GLP/GEP: yes, unpublished	Yes	New data submitted to fulfil the data requirements according to 485/2013	Bayer CropScience