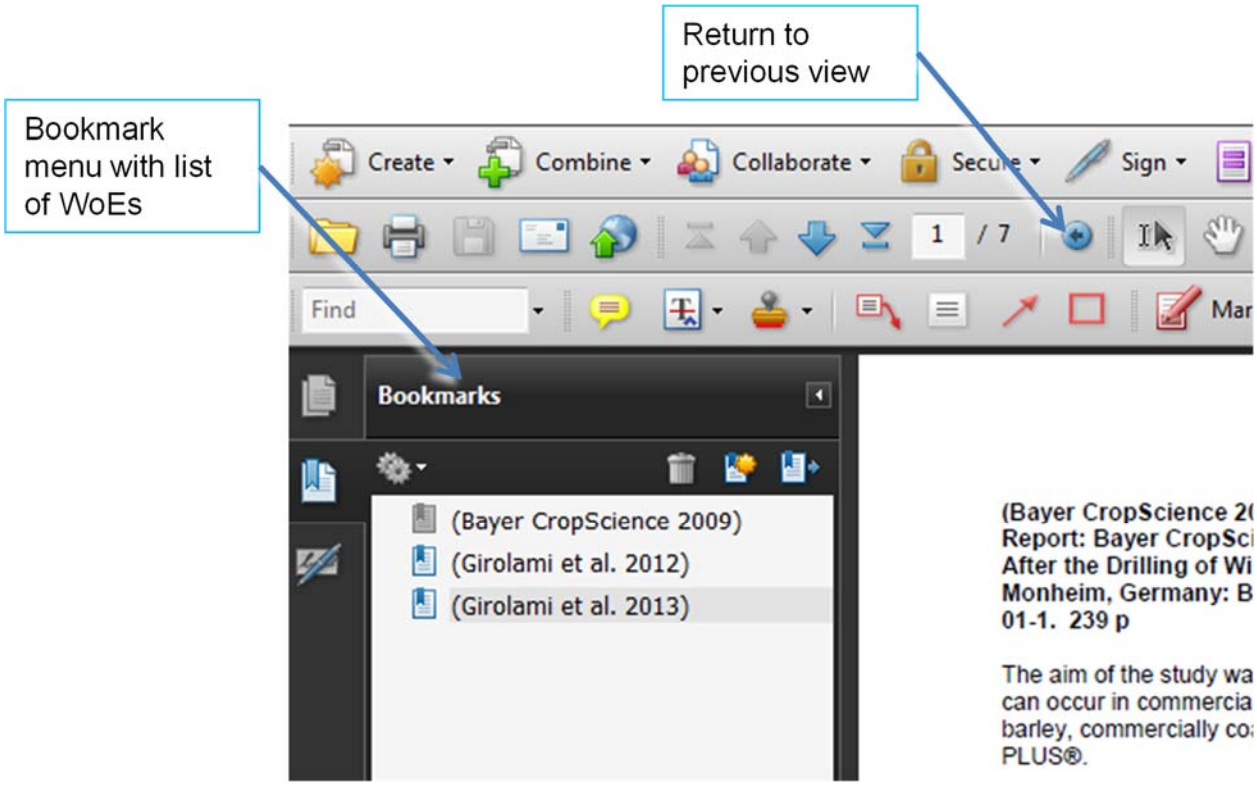
Supplemental information

Supplemental information for Stephenson & Solomon. 2017. Weight of evidence assessment of higher tier studies on the toxicity and risks of neonicotinoids in honeybees. 2. Imidacloprid.

The following are the QWoE evaluations for the studies used in the analysis. This SI is a complete description of the WoE analysis of the papers and reports used in the paper. This is an Adobe PDF file and can be fully searched with the Adobe PDF search function.

To navigate to the QWoEs, open the bookmarks panel and click on the heading or bookmark for the paper/report of interest (see screen-shot below). To return to your previous location, used the “back button” as shown.



Each major heading is followed by an enlarged copy of the relevant figure from the paper. This figure is followed by one or more figures that include a key to all the data points on the main figure. These a listed by number and study and response number is shown in a table on the right of the figure. To navigate to a specific study and response, click on the relevant cell in the table and this will take you to page on which that response is evaluated. To return to the previous view, click on the button shown in the above diagram.

Colony-level toxicity studies with IMI in honeybees

(*A figure is not included in this section*)

(Bayer CropScience 2013)

Report: Bayer CropScience. 2013. Pilot Study: Honey Bee Brood and Colony Level Effects Following Imidacloprid Intake via Treated Artificial Diet in a Field Study in North Carolina. Research Triangle Park, NC: Bayer CropScience (Unpublished Report). Report M-442868-02-1. 219 p

Thirty colonies (chosen based on colony condition assessments) with all brood stages and laying queen were randomly assigned to treatments. Treatments (6) included two control groups (T1 and T2) fed either unamended sugar syrup (1000 mL) or a pollen patty (300 g) twice a week, respectively and two treatment groups (T3 and T4) fed either syrup sugar amended with 50 µg IMI/L or a pollen patty amended with 50 µg IMI/kg (low dose), respectively, and the higher dose included two treatment groups (T5 and T6) fed either syrup sugar amended with 200 µg IMI/L or a pollen patty amended with 200 µg IMI/kg. Treatments 1, 3, and 5 were fed artificial nectar (1000 mL, twice weekly; initiated June 25, 2012, ended August 7, 2012) and allowed to forage freely for pollen. Treatment groups 2, 4, and 6 were fed artificial pollen patties (300 g, twice weekly) but were prevented from foraging for natural pollen by placing pollen traps at the entrance to the hive; they could forage on natural nectar. Consumption of artificial nectar ranged from 8660 to 12,000 mL over the 6-wk period and the pollen fed group were fed 3,600 g in the 6-wk period. Consumption rates of pollen ranged from 621.5 to 1716.1 g over 6 wk period with the low dose treatment consuming 1,301.1 g and the high dose group consuming 821.4 g. Nectar fed colonies had 7500-7800 bees and pollen fed colonies had 4700-7200 bees.

The residue concentrations in the bee bread and honey collected from T1 was contaminated in week 5 because IMI concentrations of 6.3 and 7.3 µg/kg were measured. The contamination in the control treatment was attributed to hive robbing of bee bread and honey. The residues in the prepared sugar syrup in weeks 1 and 5 ranged from no detection (an anomaly) to 55.1 µg/L for the low treatment and from 114-253 µg/L for the high treatment; stored bee bread, honey and brood food had no IMI residues, however in week 5 only honey had residues of 92.8 µg/kg. In the low treatment pollen-fed colonies the IMI residues in weeks 1 and 5 ranged from 35-51.9 µg/kg in the pollen patties; stored bee bread, honey and brood food had no IMI residues; trapped pollen had 9.1 µg IMI/kg in week 5. In the high treatment pollen-fed colonies the average residues ranged from 147-175 µg/kg in the pollen patties. No IMI residues were measured in bee bread, stored honey, or trapped pollen.

Responses 1-5: Quality methods		**Score**
Experimental design and hypotheses	The purpose of this pilot study was to determine **feasibility** of investigating the potential for colony-level effects on honey bees (*Apis mellifera* L.) during and after forced consumption of artificial diets fortified with imidacloprid at pre-determined levels. The pollen patty was made from 500 mL water, 500 mg of sugar and 700 mg of Mega Bee powder. Source and concentration of IMI was verified prior to start of experiments. The field study was a site near Prospect Hill NC in a mostly forested area. Selection criteria for hives were met. A colony had 10 frames, 7 occupied and 3 for expansion. There were 5 colonies in each of the 6 treatments. Treatments were separated by 125 ft and the control from the high by 293 feet. Each hive was equipped with a dead bee trap at the entrance. Nectar and pollen patties were place to optimize access. Formulation (amounts and methods) for artificial syrup and pol-	**3**

	len patties described. HOBO recorded relative humidity and temperature over the study period. Colony Condition Assessments (CCAs) included mortality in the dead bee traps, IPM board for sampling *Varroa* and *Nosema*, frame by frame assessments percentage of each matrix, bee population as an estimate of frame coverage and the same for eggs and larvae (open brood), honey, pollen, capped brood, mites, viruses and beetles. Each side of a frame was analysed separately for a total of 20 observations. The total area of a frame was 17,200 cm^2 so the observed percentage of each matrix was converted to this area ratio. Bee population was estimated by assuming a density of 125 bees per 100 cm^2 (x1.25). CCAs were completed bi-weekly except for the final week of feeding. CCAs were made in prior to the start of feeding and once per week on weeks 6, 7, and 8. Residues were measured on sugar syrup and pollen patty samples collected 1 and 5 wks after the start of feeding. Pre-feeding and week 5 post-feeding residues in prepared syrup and pollen were measured. Pollen from pollen traps was measured for IMI residues on 1 and 5 wks and during the CCA events in September and October. During week 5, samples of bee bread, honey and brood food were also collected. Analytical methods cited (Mullin et al. 2010) but reference not provided. No description of statistical methods was included and the differences or lack of differences among treatments had no p values associated with the observations (**major weakness**).	
Use of GLP and QA/QC.	Non-GLP but in the spirit of GPL	**3**
Exposure concentrations	Three treatments = 0, 50, 200 µg/L; nominal exposure concentrations were measured analytically in the different matrices.	**4**
Transparency of data.	Raw data provided in tables in appendices. No statistical analyses accompanied summary data	**4**
Number of samples and replication.	Repeated measures over time. Replicate hives per treatment was 5.	**1**
Overall evaluation of methods	Computed mean of above	**3.00**
No description of statistical methods was included and the differences or lack of differences among treatments had no p values associated with the observations (major weakness).	**0.5**	**1.50**

Response 1. Effect of exposure to honey bees of low and high IMI concentrations in amended syrup and pollen patties – bee mortality		**Score**
Statistical significance of responses	Variability between replicate treatments was high and mortality was highest in the first two weeks of the exposure (Figure 1). There was no apparent impact on bee mortality attributable to the IMI treatments although the dead bee counts were higher in the syrup diets because pollen traps attached to the pollen colonies to prevent the intake of natural pollen, hindered the bees ability to exclude dead bees from the hives.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**

Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Effect of exposure to honey bees of low and high IMI concentrations in amended syrup and pollen patties – colony condition: pollen stores in nectar hives (T1, T3, T5) and pollen hives (T2, T4, T6)		**Score**
Statistical significance of responses	Variability between and within treatments is unknown (Figures 2 and 3). On average pollen stores in nectar hives were nearly 2x higher in the control group (T1) than those in the IMI treatments (T3 and T5) before and after the start of feeding. On average pollen stores in pollen hives (T2, T4, T6) were comparable at the start of the study with a decrease noted immediately post exposure in the low and control treatments which were approximately 1/3 of the high treatment levels. By the end of the summer, the pollen stores were comparable among treatments. When pollen traps were removed to allow natural pollen to be collected, the pollen stores increased in both the control and low treatments.	**0**
Concentration- or dose-response	There was a dose-dependent reduction in stored pollen in the artificial nectar fed groups. **LOAEC 50 µg/L (unbounded)**	**1**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	The decrease in stored pollen could ultimately impact colony health and strength.	**3**
Mechanism and or mode of action of effect explained	Pollen patty consumption was higher in the untreated and low rate than in the high rate early in the study, but comparable in all three at the end of the study (Figure 4).	**0**
Overall evaluation of relevance (computed)		**1.00**
Overall evaluation of relevance (SEJ).		

Response 3. Effect of exposure to honey bees of low and high IMI concentrations in amended syrup and pollen patties – colony condition: honey stores in nectar hives (T1, T3, T5) and pollen hives (T2, T4, T6)		**Score**
Statistical significance of responses	For the nectar-fed hives, honey stores increased during the study, while pollen-fed hives decreased until these groups were provided with artificial syrup in August to prevent starvation (Figures 5 and 6). The honey storage in pollen fed hives was half that in the pollen-fed hives. The honey stores in the nectar-fed hives were highest for the highest treatment but decreased at the end of the study to levels below that of the low treatment.	**0**
Concentration- or dose-response	There was no dose-dependent reduction in stored honey in the artificial nectar fed groups. **LOAEC 50 µg/L (unbounded)** reductions observed for both the low and high treatments.	**1**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	The decrease in stored honey could ultimately impact colony health and strength.	**3**
Mechanism and or mode of action of effect explained	Consumption rates were comparable between the control and low treatments and they consumed all nectar provided (Figure 7); bees in the high treatment stopped storing honey even with nectar and space in hive available. No mechanism was suggested or investigated.	**0**

Overall evaluation of relevance (computed)	**1.00**
Overall evaluation of relevance (SEJ).	

Response 4. Effect of exposure to honey bees of low and high IMI concentrations in amended syrup and pollen patties – colony strength: bee populations in nectar hives (T1, T3, T5) and pollen hives (T2, T4, T6)		**Score**
Statistical significance of responses	There was a net decrease in mean colony strength from CCA-1 to CCA-8 and the average number of bees in a colony decreased over the study period in a dose-dependent manner (Figure 8) in the nectar-fed treatments. In the pollen-fed treatments (Figure 9) the decrease occurred in all treatments. **LOAEC 50 µg/L (unbounded).**	**0**
Concentration- or dose-response	There was a dose-dependent reduction in the size of the bee population in the artificial nectar fed groups for both the low and high treatments.	**1**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	The decrease in colony strength could influence the ability of the colony to overwinter. The reduction in all treatments in the pollen-fed groups suggests that even when the pollen traps were removed and natural pollen could then be collected, the decrease could not be reversed.	**3**
Mechanism and or mode of action of effect explained	No mechanism was investigated or provided.	**0**
Overall evaluation of relevance (computed)		**1.00**

Response 5. Effect of exposure to honey bees of low and high IMI concentrations in amended syrup and pollen patties – colony strength: brood development in nectar hives (T1, T3, T5) and pollen hives (T2, T4, T6)		**Score**
Statistical significance of responses	Brood development was comparable for the untreated and low IMI groups; however, brood production ceased in the high nectar-fed hives following exposure with some recovery occurring in the fall (Figure 10). **LOAEC 200 µg/L; NOAEC= 50 µg/kg**. For the pollen-fed treatments (Figure 11) the differences became apparent at the end of the study when the total brood had decreased for both IMI treatments to over half of that in the control.	**0**
Concentration- or dose-response	There was a dose-dependent reduction in the brood development in the artificial nectar fed groups relative to that for the controls; however, there was no such difference apparent in the pollen-fed hives.	**1**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	The decrease in brood development could result in a decrease in colony strength that ultimately could influence the ability of the colony to overwinter. The reduction in all treatments in the pollen-fed groups suggests that even when the brood development would not recover in the affected hives prior to overwintering.	**3**
Mechanism and or mode of action of effect explained	No mechanism was investigated or provided; however, the authors suggest that the reduction in brood production might be attributed to pollen foraging and not direct toxicity.	**1**
Overall evaluation of relevance (computed)		**1.25**

Narrative
Unrealistically high exposure concentrations fed directly to honey bees in amended syrup (artificial nectar) and in amended pollen patties. The lowest LOAEC (unbounded) was 50 µg/L or µg/kg.

Expert Judgement		
QA	Yes	September 28, 2015
SEJ	Yes	October 1, 2015

(Bayer CropScience 2014g)
Report: Bayer CropScience. 2014. Honey Bee Colony Feeding Study, Evaluating the Effects of Imidacloprid-Fortified Artificial Nectar Diet on Long Term Colony Health in a Field Study in North Carolina: Colony Condition Assessment Data & Statistic. Research Triangle Park, NC: Bayer CropScience (Unpublished Interim Report). Report M-478404-02-1. 36 p

A colony-feeding study was conducted with honey bee colonies (*Apis mellifera* L.) in a field setting with free-foraging colonies exposed through sucrose solution dosed with different concentrations of imidacloprid. The purpose of this study was to evaluate the potential of imidacloprid exposure to result in adverse effects on the long-term health of honey bee colonies. At treatment levels of 50 ppb and above, numerous endpoints were repeatedly affected, with pollen stores and capped brood initially being affected. The no observable adverse effect level (NOAEL) for this study is 25 ppb (based on nominal exposure).

Responses 1-6: Quality methods		**Score**
Experimental design and hypotheses	**Hypothesis:** Ingestion of nectar contaminated with imidacloprid over a prolonged period (42 d) will adversely affect honeybee colony health. Total of 84 hives divided into two groups after a colony condition assessment was completed (CCAs - criteria for selection included all stages of brood, a laying queen and stored pollen and nectar). Hives blocked across site according to colony strength based on total coverage of brood (A strongest; L weakest). Exposure in the 42-d feeding trial (twice per week for 6 weeks) was dietary using imidacloprid-fortified artificial nectar. There were seven treatments (two untreated controls and five exposure concentrations – 12.5, 25, 50, 100, 200 ppb). Location of hives was in rural North Carolina in an area that lacked extensive acreages of crops and was characterized by low pesticide usage and at a time of low floral nectar availability. Chronic exposure was via ingestion of spiked artificial nectar (42 days) and the study lasted 10 months. Measurement endpoints for assessing colony health included: presence of various life stages, frame area covered by adult bees, open brood, capped brood, eggs, stored pollen and honey. Data were collected three times prior to exposure, once during exposure, once directly after exposure, and once in September and in October 2013 and the following March 2014 (over-wintering). Colony health (CCA) was assessed 3x prior to exposure (non-GLP) i.e., May, June; feeding started at the end of June; assessments continued in July, Aug, Sept, October and the following March (2013). Two applications of miticide Apiguard® (active ingredient – thymol) occurred in the fall which could confound results. The infestation level with *Varroa* mites was measured by sampling nurse bees to obtain mite counts before and after feeding (CCA – 3, 5) and in March 2014 (CCA - 8th) (overwintering). Hive weights were recorded each month from June to October 2013 and the following March 2014. Stored bee bread and bee-collected nectar were collected from uncapped cells and the concentration of imidacloprid determined. Hive matrices were collected from all (CCA – 4th) or part of the hives to monitor residues in nectar and pollen on May 30, July 18, August 14, and March 22, 2014 Pollen was collected from monitoring hives (used to collect pollen from local flora to assess potential	**4**

	contamination from outside sources) on June 28, July 3, 12, 19, August 2, and October 17, 2013, corresponding to Weeks 1, 2, 3, 5, and 16, respectively. The concentration of imidacloprid was determined in the feeding solution from monitoring hives after the start of feeding on weeks 1, 2, 5; pre-feeding samples were also collected and analysed. The stability of the test material was evaluated. **Weakness:** • There is no explanation why the hives in the two highest treatments were moved after the 5th CCA. (**minor**). • Statistical tools applied were appropriate and transparent but no estimates of the variability associated with each endpoint were provided (**minor**). • Although residue data were collected, none were included and exposure concentrations were not measured (verified) (**major weakness**); therefore, the NOAEL of 25 ppb was based on nominal values. • For the data presented in Table 2 only total numbers are given with no indication of the variability within or among treatments.	
Use of GLP and QA/QC.	Some GLP was used but no QA/QC discussed	**3**
Exposure concentrations	No measured values were reported so nominal exposure concentrations were not verified; samples of nectar were collected and allegedly analyzed and stability assessed, no data reported. Concentrations tested 5 treatments plus 2 controls (nominal not measured).	**0**
Transparency of data.	Only summary data were presented.	**2**
Number of samples and replication.	There were multiple exposure concentrations ≥5 and two controls for a total of 7 treatments. There were 84 hives in total with 8 assessment periods (3 prior and 5 post application); 84 hives in total; therefore, there were 12 hives per treatment if the number of hives were distributed evenly over the treatments; this is unclear. The number of replicate hives sampled per sampling event (i.e., CCAs) is unclear.	**4**
Overall evaluation of methods	Computed mean of above.	**2.60**
Although residue data were collected, none were included and exposure concentrations were not measured (verified) (**major weakness**); therefore the NOAEL of 25 ppb was based on nominal values.	**0.5**	**1.80**

Response 1-5. Effects on colony health (i.e., multiple endpoints including frame area with adult bees, open brood, eggs, stored pollen and honey)		**Score**
Statistical significance of responses	Significant differences were present for a number of endpoints at the three higher (unrealistic) exposure concentrations of 50, 100, 200 ppb (Table 1); however, there was no consistent pattern or trend to the two significant differences at concentrations below 50 ppb	**0**
Concentration- or dose-response	Well-defined dose-response curves for pollen stores and capped brood but based on nominal exposure values not measured	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	A significant decrease in the number of pupating honeybees (capped brood) could potentially indicate compromised hive health if the trend was consistent for all CCAs. This was not the case for the two lowest exposure concentrations. Clearly, the pattern was present at the three higher exposure concentrations of 50,100 and 200 ppb and hive health was compromised; this inference was supported by results for other endpoints as well (e.g., pollen stores, number of adult bees, hive	**0**

	weights for each month post application; number of eggs, number of open brood cells).	
Mechanism and or mode of action of effect explained	No mechanism was suggested or investigated.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 6. Effects on colony health (pupating capped brood)		**Score**
Statistical significance of responses	The endpoint of capped brood at the 6th CCA was significantly different in the 12.5 and 25 ppb treatments with P values of 0.02 and 0.03, respectively (Table 1). This was considered to be a statistical anomaly because 1) there were 6 other measurement endpoints x 5 CCAs = 30 comparisons at these lower exposure rates that showed no significant difference; 2) the response at CCA6 was not monotonic (only 3 treatments); and, there were no significant differences at these concentrations in subsequent assessments (e.g., CCA 7 and 8).	**0**
Concentration- or dose-response	Well-defined dose-response curves for capped brood (and pollen stores) and but based on nominal exposure values not measured	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	A significant decrease in the number of pupating honeybees (capped brood) could potentially indicate compromised hive health if the trend was consistent for all CCAs. This was not the case for the two lowest exposure concentrations. Clearly, the pattern was present at the three higher exposure concentrations of 50,100 and 200 ppb and hive health was compromised; this inference was supported by results for other endpoints as well (e.g., pollen stores, number of adult bees, hive weights for each month post application; number of eggs, number of open brood cells)	**0**
Mechanism and or mode of action of effect explained	No mechanism was suggested or investigated.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Expert Judgement		
QA	Yes	September 28, 2015
SEJ	Yes	October 1, 2015

(Bayer CropScience 2014h)
Report: Bayer CropScience. 2014. Honey Bee Brood and Colony Level Effects Following Imidacloprid Intake Via Treated Artificial Diet in a Field Study in North Carolina. Research Triangle Park, NC: Bayer CropScience (Unpublished Report). Report M-501299-01-1. 360 p

A multi-concentration (12.5, 25, 50, 100 and 200 µg/L) field study was conducted to assess the effects of IMI on long-term survival and colony health of honey bees (*Apis mellifera* L.) exposed via ingestion of a spiked sucrose solution. The chemically-spiked sucrose solution was placed directly into the hives twice weekly for an exposure period of 42 d. During that time and after overwintering, the condition of the colony was monitored (CCAs). Endpoints measured included adult survival, nectar and pollen stores, colony development (brood cells capped and uncapped, eggs, larvae development), colony strength, hive weight, overwintering performance etc.

Responses 1-8: Quality methods		**Score**
Experimental design and hypotheses	**Hypothesis:** Ingestion of nectar contaminated with IMI over a prolonged period (42 d) will adversely affect honeybee colony health. The objectives were to determine the potential long-term effects on honey bee colony performance during and after dietary intake of imidacloprid-amended sucrose solution. Total of 84 hives of 7000 to 8000 bees at time of exposure were divided into two groups after a colony condition assessment was completed (CCAs - criteria for selection included all stages of brood, a laying queen and stored pollen and nectar) after the second CCA on May 30, 2013. Hives were blocked across site per colony strength based on total coverage of brood (A strongest; L weakest). There were 12 apiary sites (hives A to L) and the hives were randomly allocated to the sites after the second CCA. There were 8 hives per apiary or assessment site (7 for biological assessment and one for sample collection) arranged in a semi-circle. In total, there were 6 treatment groups with 12 hives (replicates) per test treatment group. There were two control groups, for a total of 24 replicates. Monitoring hives were established at each apiary site to collect pollen for residue analyses. Exposure in the 42-d feeding trial (twice per week for 6 weeks) was dietary using imidacloprid-fortified artificial nectar; exposure started on June 26, 2013. There were seven treatments (two untreated controls and five exposure concentrations – 12.5, 25, 50, 100, 200 µg/L). Location of hives was in rural North Carolina in an area that lacked extensive acreages of crops and was characterized by low pesticide usage and at a time of low floral nectar availability. **Measurement endpoints** for assessing colony health included: presence of various life stages, frame area covered by adult bees, open brood, capped brood, eggs, stored pollen and honey. Data were collected three times prior to exposure, once during exposure, once directly after exposure, and once in September and in October 2013 and the following March 2014 (over-wintering). Colony health (CCA) was assessed 3-x prior to exposure (non-GLP) i.e., May, June; feeding started at the end of June; assessments continued in July, Aug, Sept, October and the following March (2013).	**4**

	Two applications of miticide Apiguard® (active ingredient – thymol) occurred in the fall which could confound results. The infestation level with *Varroa* mites was measured by sampling nurse bees to obtain mite counts before and after feeding (CCA – 3 and 5) and in March 2014 (CCA – 8, overwintering). Hive weights were recorded each month from June to October 2013 and the following March (2014). Stored bee bread and bee-collected nectar were collected from uncapped cells and the concentration of imidacloprid determined. Hive matrices were collected from all (CCA – 4) or part of the hives to monitor residues in nectar and pollen on May 30 (week -4), July 18 (CCA4, week 3), August 14 (CCA5, week 7), and March 22, 2014 (CCA8). Pollen was collected from monitoring hives (used to collect pollen from local flora to assess potential contamination from outside sources) on June 28, July 3, 12, 19, August 2, October 17, 2013 corresponding to Weeks 1, 2, 3, 5, and 16, respectively. The concentration of imidacloprid was determined in the feeding solution from monitoring hives after the start of feeding on weeks 1, 2, 5; pre-feeding samples were also collected and analyzed. The stability of the test material was evaluated. Use of realistic exposures; only the lowest concentration of 12.5 µg/L was considered environmentally realistic according to literature data. Stored bee bread and bee-collected nectar stores were collected from uncapped cells and residues of imidacloprid were measured. Statistical tools applied were appropriate and were transparent.	
Use of GLP and QA/QC	GLP was used but no QA/QC reported, except for the analytical section (Appendix 12); GLP certificates were included	**3**
Exposure concentrations	Concentrations in the dosing solution were measured in the five treatments and the controls. Although no measured values were reported in the main text, these were listed in Appendix 12, Section 5.5. Measured concentrations were within 10% of nominal; hence data were reported and analyzed based on nominal values. Samples of nectar and pollen were collected and analyzed and stability assessed, data were reported in the Appendix 12, Sections 4, & 5.1 to 5.10. Concentrations tested were 5 treatments plus 2 controls	**4**
Transparency of data	Summary and raw data were presented. No numerical estimates of the variability associated with each endpoint were provided. (Error bars were provided on some graphs).	**3**
Number of samples and replication.	There were multiple exposure concentrations ≥5 and two controls for a total of 7 treatments (2 controls). There were 84 hives in total with 8 assessment periods (3 prior and 5 post application).	**4**
Overall evaluation of methods	Computed mean of above	**3.60**
Score for expert judgment on quality of the study	**1**	**3.60**

Response 1. Colony condition (mean number of bees per hive) in bees exposed to IMI in artificial nectar at concentrations from 12.5 to 200 µg/L for 42 d.		**Score**
Statistical significance of responses	There were no significant effects at 12 and 25 µg/L ($p < 0.05$, Williams test, Table 8) **NOAEC = 25 µg/L**. At concentrations ≥50 µg/L. there were significant decreases ($p < 0.05$, Williams test, Table 8) **LOAEC = 50 µg/L.**	**0**

Concentration- or dose-response	Concentration responses was not characterized but none was evident below the LOAEC (50 µg/L).	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	No relevant effects below the LOAEC (50 µg/L)	**0**
Mechanism and or mode of action of effect explained	None proposed but IMI is toxic to insects and the effects observed above the NOAEC are plausible.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Colony condition (mean number of egg cells per hive) in bees exposed to IMI in artificial nectar at concentrations from 12.5 to 200 µg/L for 42 d.		**Score**
Statistical significance of responses	There were no significant effects at 12 and 25 µg/L ($p < 0.05$, Williams test, Table 8) **NOAEC = 25 µg/L**. At concentrations ≥50 µg/L only one significant decrease (CCA8) in the five measurements was observed ($p < 0.01$, Williams test, Table 8) **LOAEC = 50 µg/L.**	**0**
Concentration- or dose-response	Concentration responses was not characterized but none was evident below the LOAEC (50 µg/L).	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	No relevant effects below the LOAEC (50 µg/L)	**0**
Mechanism and or mode of action of effect explained	None proposed but this is an apical endpoint and response is consistent with direct effects on bees above the NOAEC.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 3. Colony condition (mean number of larval in open cells per hive) in bees exposed to IMI in artificial nectar at concentrations from 12.5 to 200 µg/L for 42 d.		**Score**
Statistical significance of responses	There were no significant effects at 12, 25, and 50 µg/L ($p < 0.05$, Williams test, Table 8) **NOAEC = 50 µg/L**. At concentrations ≥100 µg/L. significant decreases were observed ($p < 0.05$, Williams test, Table 8) **LOAEC = 100 µg/L.**	**0**
Concentration- or dose-response	Concentration responses was not characterized but none was evident below the LOAEC (100 µg/L).	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	No relevant effects below the LOAEC (100 µg/L)	**0**
Mechanism and or mode of action of effect explained	None proposed but this is an apical endpoint and response is consistent with direct effects on bees above the NOAEC.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 4. Colony condition (mean number of pupae in capped cells per hive) in bees exposed to IMI in artificial nectar at concentrations from 12.5 to 200 µg/L for 42 d.		**Score**
Statistical significance of responses	Results were inconsistent with only one statistically significant difference for one endpoint on one occasion at the nominal exposure concentration of 12.5 (p=0.02, William's test) and 25 µg/L (p=0.03, William's test) (Table 8 – capped 6); otherwise, significant differences occurred at environmentally unrealistic concentrations ≥50 µg/L. For example, capped 4 and capped 5 showed significant decreases at 50 to 200 ppb (p<0.01) and capped 8 at 50 ppb (p<0.05 (Table 8).	**0**
Concentration- or dose-response	Although the authors state that there is no dose response among the 12.5, 25 and 50 ppb treatment levels; there is a well-defined dose response observed in the two higher treatments Figure 7. That said the dose-response occurs at unrealistically high exposure concentrations.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	No relevant effects consistently below the LOAEC (50 µg/L)	**0**
Mechanism and or mode of action of effect explained	None proposed but this is an apical endpoint and response is consistent with direct effects on bees above the NOAEC.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 5. Food stores (nectar; honey cells per hive) in bees exposed to IMI in artificial nectar at concentrations from 12.5 to 200 µg/L for 42 d.		**Score**
Statistical significance of responses	There were no significant effects at any exposure (p <0.05, Williams and Dunnett's test, Table 8) at the first two measurements times. There were no significant effects at 12 and 25 µg/L at the last three sampling times (p <0.05, Williams test, Table 8) **NOAEC = 25 µg/L**. At concentrations ≥50 µg/L. There were significant decreases at the last three sampling times (p <0.05, Williams test, Table 8) **LOAEC = 50 µg/L.**	**0**
Concentration- or dose-response	Concentration responses was not characterized but none was evident below the LOAEC (50 µg/L).	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	No relevant effects below the LOAEC (50 µg/L)	**0**
Mechanism and or mode of action of effect explained	None proposed but this is an apical endpoint and response is consistent with direct effects on bees above the NOAEC.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 6. Food stores (number of pollen cells per hive) in bees exposed to IMI in artificial nectar at concentrations from 12.5 to 200 µg/L for 42 d.		**Score**
Statistical significance of responses	There were no significant effects at 12 and 25 µg/L (p <0.05, Williams and Dunnett's test, Table 8) **NOAEC = 25 µg/L**. At concentrations ≥50 µg/L. there were three significant decreases in the five measurements (p <0.05, Williams test, Table 8) **LOAEC = 50 µg/L.**	**0**
Concentration- or dose-response	Concentration responses was not characterized but none was evident below the LOAEC (50 µg/L).	**0**

Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	No relevant effects below the LOAEC (50 µg/L)	**0**
Mechanism and or mode of action of effect explained	None proposed but this is an apical endpoint and response is consistent with direct effects on bees above the NOAEC.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 7. Colony health (infections with *Varroa* mites) in bees exposed to IMI in artificial nectar at concentrations from 12.5 to 200 µg/L for 42 d.		**Score**
Statistical significance of responses	Statistical analyses were not conducted but, from data in Fig. 11 Table 31 to 36 in Appendix A4, no significance was apparent and for the larger exposures n was too small for statistical tests. Assumed to be non-significant at all exposures, **NOAEC = 200 µg/L**	**0**
Concentration- or dose-response	Concentration responses were not characterized but none was evident (Fig. 11).	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 8. Colony health (weights of hives) in bees exposed to IMI in artificial nectar at concentrations from 12.5 to 200 µg/L for 42 d.		**Score**
Statistical significance of responses	There were no significant effects at 12 and 25 µg/L ($p < 0.05$, Williams test, Table 8) **NOAEC = 25 µg/L**. At concentrations ≥50 µg/L. there were significant decreases ($p \leq 0.01$, Williams test, Table 8) **LOAEC = 50 µg/L.**	**0**
Concentration- or dose-response	Concentration response was not characterized but none was evident below the LOAEC (50 µg/L). Authors reported a concentration-response trend (Figure 13) in hive weights at the higher exposure concentrations except for the august weights (especially for the 50 to 200 ppb)	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	No relevant effects below the LOAEC (50 µg/L)	**0**
Mechanism and or mode of action of effect explained	None proposed but this is an apical endpoint and response is consistent with direct effects on bees above the NOAEC.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Expert Judgement		
QA	Yes	September 29, 2015
SEJ	Yes	September 30, 2015

Narrative
Results indicated that the LOAEC was 50 µg/L and the NOAEC was 25 µg/L. Pollen stores were reduced at ≥50 µg/L; brood development was adversely affected at ≥50 µg/L; hive weights were significantly reduced at ≥50 µg/L; and overwintering performance was adversely affected at 100 and 200 µg/L.

Assuming that bees were only exposed to IMI via the sugar syrup the following doses equivalent to the NOAEC and LOAEC for the above responses were estimated for adult bees. Because there were essentially no consistent adverse effects at realistic exposure concentrations, responses were not scored.

Source	Matrix	Conc. IMI µg/L	Dose ng/bee	Life-stage	mg of food/d
NOAEC conc.	Sugar syrup	25.0	7.3	Adult	292
LOAEC conc.	Sugar syrup	50.0	14.6	Adult	292

Data from three studies were used to assess toxicity of IMI to honeybees exposed via artificial diets formulated with IMI-amended syrup or pollen patties. The mean quality of these studies was on average higher than the mean quality of the seed-dressing studies primarily because the experimental designs of these studies entailed larger sample sizes, multiple concentrations, better control of potentially confounding factors, and the use of appropriate statistical procedures to analyse the data. The QoM of the IMI-feeding studies was 2.48 ± SE of 0.23. One meta-analysis[1] was excluded because raw data were not provided and studies with anomalous results were excluded.

Only one of the studies[2] (Bayer CropScience 2013) reported responses that were scored as effect concentrations. Although there was no apparent adverse effect on honeybee mortality because of honeybees being exposed to both IMI-amended syrup and pollen patties, the variability was high both within and among treatments which had unrealistically high IMI concentrations (e.g., 0, 50 and 200 µg/kg in both the syrup and pollen or in combination – 50/200, 200/50 µg/kg). The highest IMI residues in bee bread and honey were measured to be 6.3 and 7.3 µg/kg, respectively and the mean concentrations were substantially lower. The control samples had IMI residues as well which were thought to be attributable to hive-robbing of bee bread and honey which confounded the interpretation of the effects. Although the variability was high, there was a dose-dependent reduction in stored pollen in the treatments fed nectar with 50 and 200 µg IMI/L. There was also a dose-dependent impact on the population in both the nectar-fed and pollen-fed hives because of the impact on brood development, which was greater in the

[1] Cresswell JE. 2011. A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees. *Ecotoxicology* 20:149-157.
[2] Bayer CropScience. 2013. Pilot Study: Honey Bee Brood and Colony Level Effects Following Imidacloprid Intake Via Treated Artificial Diet in a Field Study in North Carolina. Research Triangle Park, NC: Bayer CropScience (Unpublished Report). No. M-442868-02-1. 219 p.

nectar-fed hives versus the pollen-fed hives. The extrapolation from feeding studies where honeybees are exposed to much higher levels of a chemical than they would normally encounter in the field is questionable and a better study[3] was available to characterize toxicity.

[3] Bayer CropScience. 2014. Honey Bee Brood and Colony Level Effects Following Imidacloprid Intake Via Treated Artificial Diet in a Field Study in North Carolina. Research Triangle Park, NC: Bayer CropScience (Unpublished Report). No. M-501299-01-1. 360 p.

(Wu-Smart and Spivak 2016)

Paper: Wu-Smart J, Spivak M. 2016. Sub-lethal effects of dietary neonicotinoid insecticide exposure on honey bee queen fecundity and colony development. *Scientific Reports* 6:32108.

Summary or comment: This paper reports the results of a colony-level toxicity test of the effects of IMI on honeybees. The authors used colonies of 1000, 3000, and 7000 bees to assess effects of feeding syrup amended with IMI to provide nominal exposures concentrations of 0, 10, 20, 50, and 100 µg/kg (ppb).

Responses 1-9: Quality methods		**Score**
Experimental design and hypotheses	The study was conducted at the University of Minnesota campus in Saint Paul, MN. Worker bees and sister queens were removed from healthy field colonies located in Chaska, MN to set up the experimental observation hives located at the University of Minnesota campus in Saint Paul, MN (approximately 64 km away). Field colonies, requeened with queen cells each time, were used to stock observation hives multiple times each summer between May and August yielding three or four replicate studies each year over three years (2012–2014). One-, two-, and five-frame observation hives were established with wooden Langstroth-type deep frames and undrawn plastic foundation, and for each replicate, were given a laying queen and roughly 1500, 3000, or 7000 workers estimated by weight of worker bees (0.23, 0.45, and 0.91 kg respectively). Colonies were provided with 2 to 4 g of pollen supplement and sugar syrup (1:1 w/w assumed) for 1-2 d before treatment. Smaller colonies (1500- and 3000-bees) were placed in glass-walled observation hives. The larger colonies (7000-bees) were placed in Ulster observation hives containing a bottom box holding 4 standard frames and a division board feeder where treatment syrup was provided. The early morning of observation days, the fifth frame, containing the queen, was placed in an upper section made of clear Plexiglass and separated from frames below by a queen excluder. All observation hives were housed in sheds maintained at constant temperature and relative humidity (23–25°C and 70%). Additionally, all hives contained an entrance leading to the outside allowing bees to freely forage around some agricultural fields and urban residential neighbourhoods. Weather data were not reported even though the study spanned three years (*weakness*). The number of experimental colonies ranged from 8–20 per treatment and totalled 79 colonies over three years. Queen absconding events, where the entire colony left the hive, occurred in four smaller colonies (1500- and 3000-bees) and one 7000-bee colony. There was no apparent relationship to treatment but these colonies were removed from the analysis. Exposure was only for three weeks and, because the colonies were terminated at the end of the exposure, it was not possible to assess delayed responses or overwintering success (*weakness*). Given the ca 5- to 6-week lifespan of adult workers, it would have been difficult to measure changes in populations in the 3-week duration of the study (***major weakness***). A six-week study period would have been more appropriate. Exposures: After the pre-treatment period and when egg laying was confirmed, each colony was randomly assigned an IMI treatment (nominal concentrations of 0, 10, 20, 50, and 100 ppb in 50% sucrose syrup (presumed to be w/w). Colonies were given proportional amounts of sucrose	**1**

	solution containing imidacloprid: 80, 160 and 320 mL for 1500-, 3000-, and 7000-bee colonies, respectively. Syrup was replenished every other day for 3 weeks. The authors reported that, in most cases, the syrup was fully consumed in the first 24 h of exposure, so bees were required to freely forage on other resources. That the bees were not provided with enough syrup to sustain the hive is a *weakness* in that any IMI accumulated in the hive was diluted by uncontaminated nectar from outside sources. Thus, the actual exposures of the bees are unknown but are likely less than those in the amended syrup. This makes the data unusable for characterising toxicity (***major weakness***). Stock solutions of imidacloprid (100 ppm) were prepared every two weeks using 99.5 ± 0.5% technical grade imidacloprid purchased from a commercial source dissolved with agitation in 50% sucrose overnight. Treatment solutions were prepared every week. Analysis: Three-mL samples of treated syrup were collected over the 3-week exposure period; (n = 18 in total; 3-6 per treatment level and colony size). The exact method of collection was not reported but the samples were frozen at -80℃. How they were transported to the analytical facility and the use of travel and storage spikes was not reported (*weakness*). Samples of bees and nectar/honey from the hives were also analysed. Samples were analysed for residues of imidacloprid and metabolites by the US Environmental Protection Agency Analytical Chemistry Branch (ACB) in Washington DC and the USDA Agricultural Marketing Service, National Science Laboratory (AMS-NSL) in Gastonia NC using the QuEChERS pesticide extraction method. Extraction methods were described and ^{2}H-IMI internal standard was used. The samples were then analysed with LC-MS/MS. LODs for IMI, IMI=, and IMI-OH were 1, 10 and 25 µg/kg, respectively. Recovery and its variance were not reported. Several endpoints were measured in the study. The methods used are enumerated below: Survival: Adult worker bees were enumerated after the end of the 21-d exposures (d-23). Each colony was anaesthetized using carbon dioxide and placed into a −20°C freezer to kill the bees. Final adult worker population was determined by using the average weight of 10-subsets of ten individual bees to estimate the total number of adult workers from the total weight of the worker bee population. Behaviour of the queens: These were measured during two 15-min. observations made every day for 1500- and 3000-bee colonies, and every other day for 7000-bee colonies to minimize disturbance. Queen observations from the morning (7–11 am) and afternoon (12–4 pm) were averaged to account for any changes in hive activity due to weather and outside temperature. Activity was monitored by tracing the queen's travel path with a felt-tipped pen onto acetate sheets placed over each observation hive. The distance travelled (cm) by the queen was then quantified using a digital plan measure tool (Scale Master Pro model 6025). Immobility in queens, or the time spent "resting" was also measured and defined as when the queens were not moving or grooming themselves and did not include when queens were being groomed or fed by nurse bees. Whether the same people did the enumerations during the three years and whether they were blinded as to treatment was not reported (*potential weakness*).	

	Laying of eggs: This was measured as part of the observations on the behaviour of the queen (above). Data were reported as number of eggs laid per 15 min. Foraging activity of adult worker bees: This was measured by recording the number of workers entering and exiting the entrance of each 3000- and 7000-bee colony during one-minute observations twice a day. Observations made on foraging behaviour in 1500-bee colonies were limited and were removed from the analysis of data. Hygienic behaviour of workers: This behaviour was measured only in 7000-bee colonies pre- and post-imidacloprid treatment. Hygienic behaviour was measured using a freeze-killed brood assay, in which a 3-inch (7.6 cm) polyvinyl chloride tube was gently pushed into a section of comb containing a large area of sealed pupal cells (taken from non-experimental field colonies). The number of empty cells was counted and recorded before pouring 400 ml of liquid nitrogen to freeze-kill roughly 160 pupae. The frame was then temporarily put into 7000-bee colonies and the proportion of pupae completely or partially removed from the cells was quantified after 24 h to assess hygienic behaviour. Hygienic behaviour was used as a measure of worker activity inside the hive, and is defined as the ability of worker bees to detect and remove diseased and mite-infested brood thereby limiting within-colony transmission of pathogens and parasites. Food stores: After three weeks of imidacloprid exposure each colony was anaesthetized using carbon dioxide and placed into a −20°C freezer. Food stores, or the number of cells completely or partially filled with nectar or honey and pollen, was also quantified. Brood pattern: After killing the colony (as above), brood production was assessed by counting all frame cells containing eggs, larvae or pupae. Brood pattern was assessed by placing a parallelogram (containing 100 cells) over 3-4 areas of sealed pupae within the colony and quantifying the average proportion of empty cells not containing pupae for each colony (Fig. 3B). It is assumed that the values were averaged for each colony. Unused cells: Unused cells were enumerated at the end of the exposure period but would be negatively correlated to the number of used cells which were assessed separately. This response was excluded from the QWoE analysis. Statistics: Statistical methods were described and appropriate (p 9). Although study year was treated as a random variable, it is not clear how potential differences from one year to the next influenced the analytical results (*weakness*). P values and F values were reported.	
Use of GLP and QA/QC.	This was not a GLP study and QA was not reported. The protocol was well described. Analyses of samples for residues was conducted at USEPA and USDA laboratories so this portion of the work was assumed to have QC.	**2**
Exposure concentrations	Exposure concentrations in syrup were measured between three and six times (for various treatments) over the three-week exposure period (SI Table S2). Dates of sampling were not reported. At the end of the three-week exposure period, stored nectar and worker bees were analysed. The mean exposure concentrations were 6.4 ± 4.4, 32.9 ± 2.1, 57.7 ± 6.1 and 94.2 ± 5.2 ppb. Toxic metabolites (IMI= and IMI-OH) were not detected in the syrup. Although not stated, it was assumed that concentrations are in µg/kg. Concentrations in the control were <LOD. The value for variance was not labelled but the value could not be replicated from some of the raw data in SI Table S2.	**4**

Transparency of data.	All raw data were not provided. Some raw data were provided in tables. Mean data and variance were provided in graphs	**3**
Number of samples and replication.	The study was carried out at one site only but repeated over a period of three years. From the data provided, it is not clear how many replicates were used in each year but the authors state three to four replicates per years. It is presumed that the data from the three years were combined for analysis but it is not clear if year-to-year differences were accounted for first (*weakness*).	**3**
Overall evaluation of methods	Computed mean of above	**2.60**
Score for expert judgment on quality of the study. The provision of insufficient syrup (and IMI) to fully sustain the colony resulted in too much uncertainty to allow the effect data to be used for assessing risks. The short observation period also reduced the likelihood that adverse effects on survival could have been observed. Two **major weaknesses**.	**0.25**	**0.65**

Response 1. Survival of worker bees in colonies of different population size exposed to IMI at measured concentrations of 6.4, 32.9, 57.7, and 94.2 µg/kg for three weeks.		**Score**
Statistical significance of responses	There was no statistically significant difference in number of adult worker bees in any treatment at the end of the exposure period within each colony size. (Fig S3, p = 0.241).	**0**
Concentration- or dose-response	There was no apparent concentration response	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since there were no significant adverse effects, there was no relevance to apical endpoints.	**0**
Mechanism and or mode of action of effect explained	It is surprising that there was no effect on survival at the higher doses.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Behaviour of queens in colonies of different population size during exposure to IMI at concentrations of 6.4, 32.9, 57.7, and 94.2 µg/kg for three weeks.		**Score**
Statistical significance of responses	Queens in untreated colonies traversed greater distances per observation compared to queens in treated colonies of the same size (p = 0.0012, text p 3). Queens in untreated colonies were also more active, as determined by the proportion of time spent immobile per observation (average ± SE of 28% ± 0.04, 34% ± 0.03, and 13% ± 0.02), compared to queens in 32.9, 57.7, and 94.2 µg/kg treated colonies (63% ± 0.1, 58% ± 0.06, and 37% ± 0.05) in 1500-, 3000-, and 7000- colonies, respectively. There were significant differences in queen immobility among treatments in 1500- and 3000-bee colonies (p <0.0001, text p 3) but **not** among treated 7000-bee colonies (p >0.73, text p 3 and Figure S1B).	**4**
Concentration- or dose-response	Distance travelled showed a concentration-dependent decrease in the 1500- and 3000-bee colonies (Fig S1A) but this was not characterized. Queen immobility showed concentration-dependent increases in the 1500- and 3000-bee colony size but not in the 7000-bee colonies (Fig S1B). This response was not characterized.	**1**
Relevance of effect to apical endpoints	Although immobility might have affected number of eggs laid by the queen, there were no effects on number of adult worker bees at the end of the study.	**4**

(survival and/or reproduction, and fitness)		
Mechanism and or mode of action of effect explained	Analysis of queens revealed concentrations of IMI less than the LOD (1 ng/g) and it is unlikely that queens were directly exposed to IMI as they do not feed on pollen and nectar. Worker nurse bees attending to queens, or retinue bees, were observed feeding and grooming queens in all colonies throughout the experiment, indicating it is unlikely that changes in behavior resulted from poor queen attendance. Trophallaxis was proposed but could not be demonstrated given that analytical attempts to confirm exposure indicated none.	**0**
Overall evaluation of relevance (computed)		**2.25**
Overall evaluation of relevance (SEJ).		

Response 3. Egg laying by queens in colonies of different population size during exposure to IMI at concentrations of 6.4, 32.9, 57.7, and 94.2 µg/kg for three weeks.		**Score**
Statistical significance of responses	There were statistically significant ($p < 0.05$) reductions in numbers of eggs laid in the 1500- and 3000-bee colony size but not in the 7000-bee colonies (Table 1).	**3**
Concentration- or dose-response	There was no consistent concentration-response observed in the 1500-, 3000-, 7000-bee colonies but this was not characterized (Table 1)	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Although number of eggs laid by the queen was decreased in the small colonies, there were no adverse effects on apical endpoints demonstrated within the study periods for colonies with realistic bee numbers (i.e., realistic colony size).	**1**
Mechanism and or mode of action of effect explained	Analysis of queens revealed concentrations of IMI less than the LOD (1 ng/g) and it is unlikely that queens were directly exposed to IMI as they do not feed on pollen and nectar. Worker nurse bees attending to queens, or retinue bees, were observed feeding and grooming queens in all colonies throughout the experiment, indicating it is unlikely that reduced egg-laying was the result of poor queen attendance. Although no plausible mechanism was proposed or supported by analysis of IMI in the bees, the authors attributed the observed adverse effects to "some physiological effect from exposure to IMI or metabolites" that was not supported analytically.	**0**
Overall evaluation of relevance (computed)		**1.00**
Overall evaluation of relevance (SEJ).		

Response 4. Foraging activity of adult worker bees in colonies of population size of 3000 and 7000 bees during exposure to IMI at concentrations of 6.4, 32.9, 57.7, and 94.2 µg/kg for three weeks.		**Score**
Statistical significance of responses	There were significantly ($p < 0.0001$, Fig S2A & B) more workers from control colonies observed entering and exiting the colony as compared to colonies treated with IMI. Foraging activity was not assessed in the 1500-bee colonies.	**4**
Concentration- or dose-response	Differences among treatments in foraging activity in the 3000-bee colonies showed concentration-dependence (Fig S2A) while in the 7000-bee colonies showed no concentration-dependence (Fig S2B). Concentration-responses were not characterized.	**1**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Differences in foraging activity were not reflected in stores of nectar but might have resulted in less collection of pollen in the 1500- and 3000-bee colonies where a concentration-response was also observed.	**1**

Mechanism and or mode of action of effect explained	A specific mechanism was not proposed nor tested experimentally. The lack of concentration-response in the 7000-bee colonies suggests that there might have been a confounding variable that was not considered.	**0**
Overall evaluation of relevance (computed)		**1.50**
Overall evaluation of relevance (SEJ).		

Response 5. Hygienic behaviour of adult worker bees in colonies of 7000 bees after exposure to IMI at concentrations of 6.4, 32.9, 57.7, and 94.2 µg/kg for three weeks.		**Score**
Statistical significance of responses	Significantly ($p < 0.05$, Fig 2) lower hygienic behavior were observed in the 57.7, and 94.2 µg/kg treatments only.	**4**
Concentration- or dose-response	There was no concentration response observed and this was not characterized.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Hygienic behaviors were reduced from pre-exposure 84% to 63% post exposure and 96% to 73% in 57.7 and 94.2 µg/kg treatments, respectively so the differences were not large. There were no effects on survival of adult workers.	**3**
Mechanism and or mode of action of effect explained	No mechanisms were investigated but effects on behavior could be because of sublethal effects on bees.	**0**
Overall evaluation of relevance (computed)		**1.75**
Overall evaluation of relevance (SEJ).		

Response 6. Nectar stores in colonies of different population size after exposure to IMI at concentrations of 6.4, 32.9, 57.7, and 94.2 µg/kg for three weeks.		**Score**
Statistical significance of responses	There were no statistical differences among any of the colonies in the number of cells containing stored nectar and honey ($p = 0.16$, text p 4).	**0**
Concentration- or dose-response	There was no apparent concentration response.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since there were no significant effects, there was no relevance to apical endpoints.	**0**
Mechanism and or mode of action of effect explained	Since there were no significant effects, there was no mechanism proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 7. Pollen stores in colonies of different population size after exposure to IMI at concentrations of 6.4, 32.9, 57.7, and 94.2 µg/kg for three weeks.		**Score**
Statistical significance of responses	Untreated colonies had significantly more cells containing stored pollen ($p < 0.0001$, text p 4) than treated colonies, except in 1500-bee colonies exposed at 6.4 µg/kg. The average number of cells containing pollen in treated colonies was 61–71% (1500-bee colonies), 94–138% (3000-bee colonies) and 125–161% (7000-bee colonies) lower than pollen stores found in untreated colonies at the same population size (Table 1).	**4**
Concentration- or dose-response	There was no apparent concentration response (Table 1) and none was characterized.	**0**
Relevance of effect to apical endpoints	There were no effects on number of adult worker bees, larvae and pupae at the end of the study, suggesting that the smaller amounts of pollen did not adversely affect apical endpoints.	**0**

(survival and/or reproduction, and fitness)		
Mechanism and or mode of action of effect explained	No mechanisms were specifically investigated but effects on behavior could have affected foraging.	**0**
Overall evaluation of relevance (computed)		**1.00**
Overall evaluation of relevance (SEJ).		

Response 7. Brood pattern in colonies of different population size after exposure to IMI at concentrations of 6.4, 32.9, 57.7, and 94.2 µg/kg for three weeks.		**Score**
Statistical significance of responses	The proportion of cells not containing pupae (empty) in a brood area of 100 cells was only significantly increased in the colonies with 1500 bees that were exposed to 94.2 µg/kg IMI ($p < 0.05$, Fig 3C). For the colonies with 3000 and 7000 bees, there were significant increases in all treatments in the 3000-bee colonies but only in the two highest treatments of the 7000-bee colonies (e.g., 57.7, and 94.2 µg/kg; $p < 0.0001$, Fig 3C). Results indicated significantly worse brood pattern (more empty cells), particularly at higher treatments (57.7, and 94.2 µg/kg), compared to untreated colonies	**4**
Concentration- or dose-response	Concentration-response was not characterized. There was no obvious or consistent concentration-response in the colonies with 1500 bees. However, a concentration response was observed but not characterized in the colonies of 3000 and 7000 bees.	**1**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	There were no effects on number of adult worker bees at the end of the study but adverse effects on apical endpoints could be expected in a study of longer duration.	**3**
Mechanism and or mode of action of effect explained	No mechanisms were investigated but effects on behavior could have affected foraging. Mechanisms considered included presence of brood disease (not assessed); a failing queen (not observed); poor brood care (not observed); and/or pollen stores (significantly reduced).	**2**
Overall evaluation of relevance (computed)		**1.25**
Overall evaluation of relevance (SEJ).		

Response 8. Number of cells containing larvae in colonies of different population size after exposure to IMI at concentrations of 6.4, 32.9, 57.7, and 94.2 µg/kg for three weeks.		**Score**
Statistical significance of responses	IMI-treated colonies of 1500 bees showed an inconsistent number of larvae and a decrease in the number of pupae as dose increased, but differences were not statistically significant ($p \geq 0.05$). The number of larvae in 3000-bee colonies was only significantly reduced between control and 94.2 µg/kg treatments ($p < 0.05$, Table 1). There were no significant differences in the number of larvae in any exposure concentration in the 7000-bee colonies ($p > 0.05$, Table 1).	**2**
Concentration- or dose-response	A consistent concentration-response was not observed, nor characterized.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	There were no demonstrable, consistent adverse effects to apical endpoints at the end of the study suggesting relevance was low.	**1**

Mechanism and or mode of action of effect explained	The authors proposed that preferential cannibalism of young larvae to feed larger older larvae resulted from the pollen deficit (p 6) but this was not verified or quantified or investigated.	**2**
Overall evaluation of relevance (computed)		**1.25**
Overall evaluation of relevance (SEJ).		

Response 9. Number of cells containing pupae in colonies of different population size after exposure to IMI at concentrations of 6.4, 32.9, 57.7, and 94.2 µg/kg for three weeks.		**Score**
Statistical significance of responses	IMI-treated colonies of 1500-bees showed no significant decreases in number of pupae ($p > 0.05$, Table 1) from the controls. In the 3000-bee colonies, there were significant differences between number of pupae in the control and treated colonies ($p < 0.05$, Table 1) but the differences between exposed colonies were inconsistent. In the 7000-bee colonies, the only significant decrease in the number of pupae from the control was in the 57.7 µg/kg ($p < 0.05$, Table 1)	**2**
Concentration- or dose-response	A concentration-response was only observed (but not characterized) in number of pupae in 1500-bee colonies (Table 1).	**1**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	There were no effects on number of adult worker bees at the end of the study but adverse effects on apical endpoints could be expected in a study of longer duration.	**2**
Mechanism and or mode of action of effect explained	No mechanisms were specifically investigated. The authors proposed that the overall effects on brood production and pattern in this study were likely caused by a combination of factors, including effects on queen behavior, direct toxicity from contaminated food, reduced brood care and lack of pollen, but it is unclear which factors had the greatest impact on brood development.	**2**
Overall evaluation of relevance (computed)		**1.75**
Overall evaluation of relevance (SEJ).		

Notes for narrative

Overall, the authors reported that colony size affected the severity of the responses observed in the study. However, their largest colonies were only 7000-bees and more realistic larger colonies were not included in the study. Colonies larger than 7000 bees would be expected to have greater resiliency and redundancy. These data are thus not directly extrapolatable to larger hives used in commercial production of honeybees. The study was also not suitable for the characterization of toxicity values at the level of the colony. The duration of the observations (3 weeks) was too short in relation to the lifespan of worker bees (5-6 weeks) so effects might have been missed. The exposures were compromised because the bees were not provided with sufficient syrup to satisfy their needs. Thus, the exact exposures from the treated syrup were diluted by nectar collected outside the hive and dose per bee cannot be calculated. Thus, these data cannot be used to extrapolate to risks from exposures to IMI in bee-relevant matrices in the field. However, the concentration of IMI in the honey from 7000-bee hives exposed to 32.9 µg IMI/kg syrup was about 25 µg/kg which is close to the NOAEC derived from the toxicity tests above. The overall paucity of adverse effects in these hives at this and greater concentrations is consistent with the toxicity endpoint used in the QWoE.

SEJ	Yes	July 27, 2017
QA	Yes	July 27, 2017

Risks to honeybees from exposures resulting from uptake and translocation of IMI from seed treatments

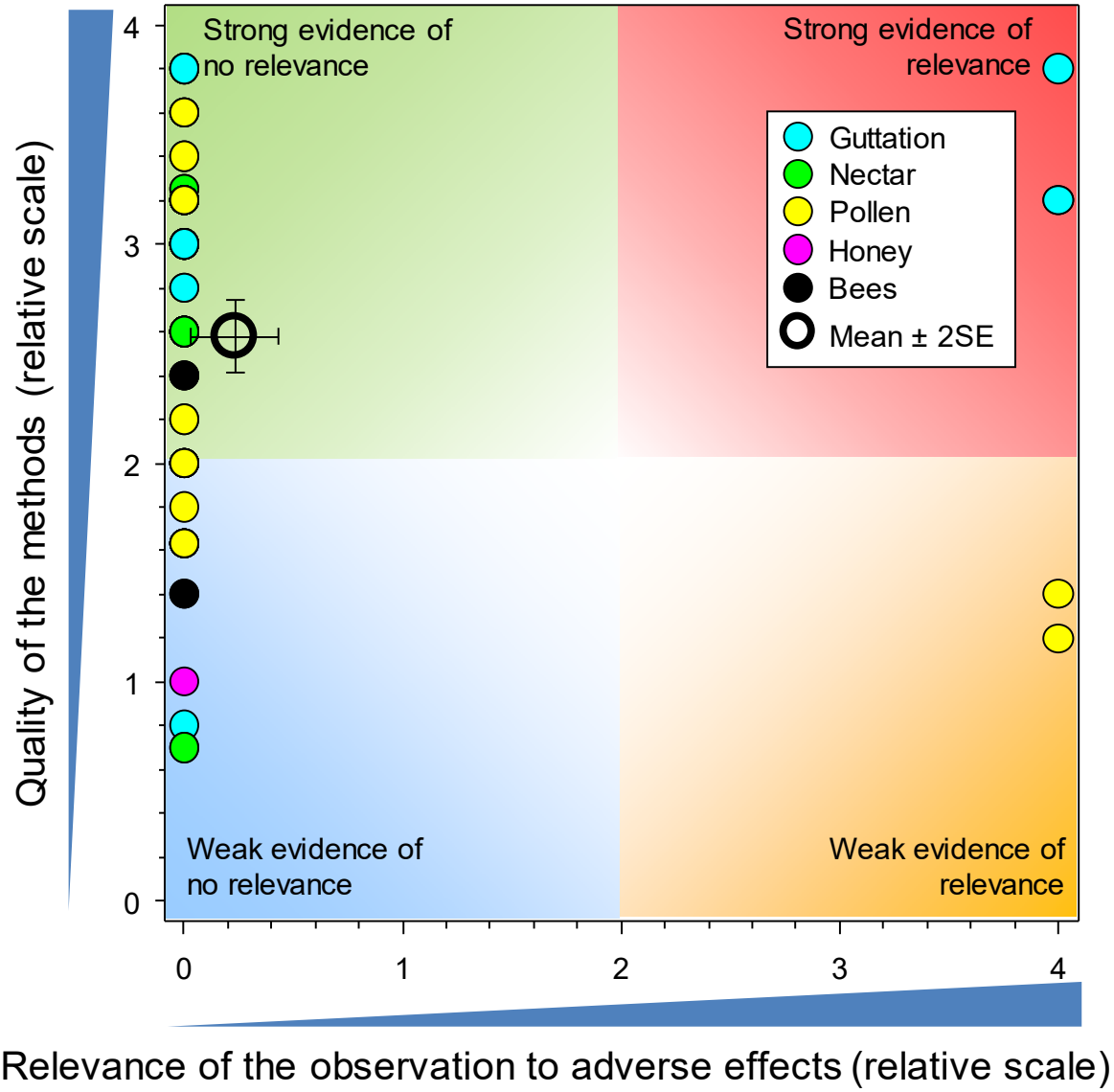
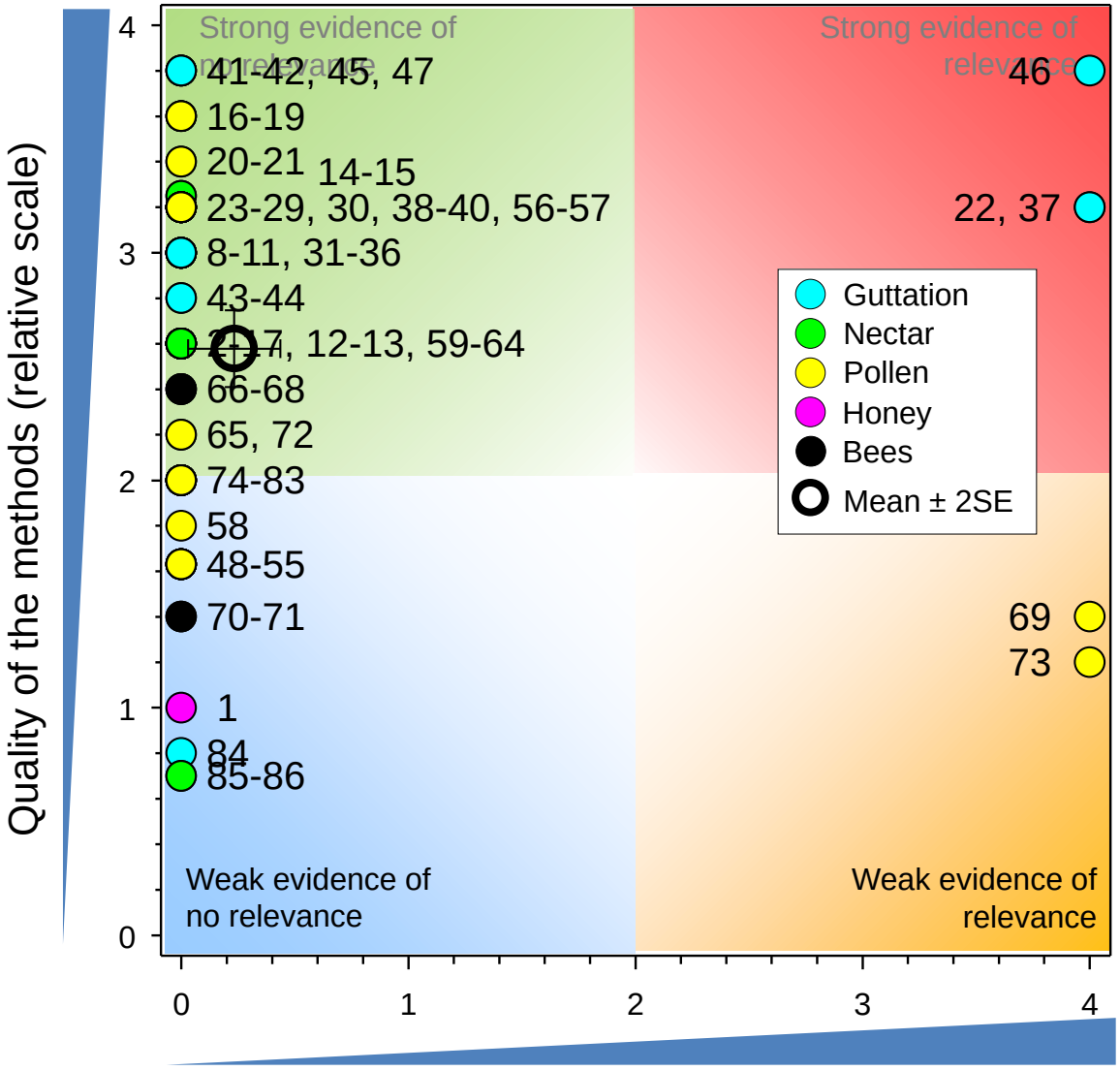


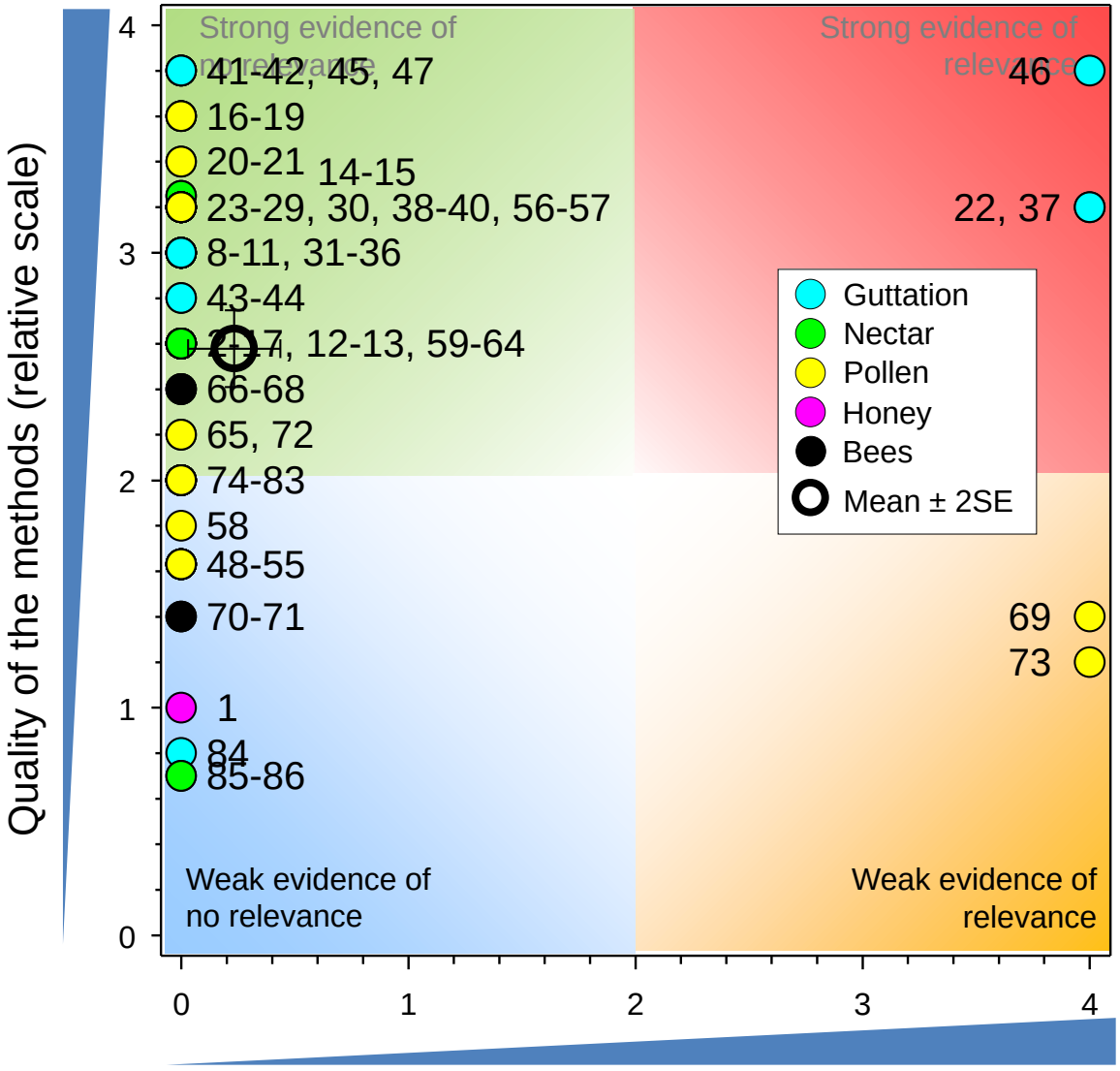
Figure 2. Strengths and relevance of exposure values from controlled field studies with IMI treated seeds and mixed treatments (where application methods were not defined). Number of responses assessed = 86. Symbols may obscure others, see SI for all responses. There were no points obscured by the legend in the upper right quadrant.

Hazards of exposures of bees to IMI via seed and mixed treatments (SI)



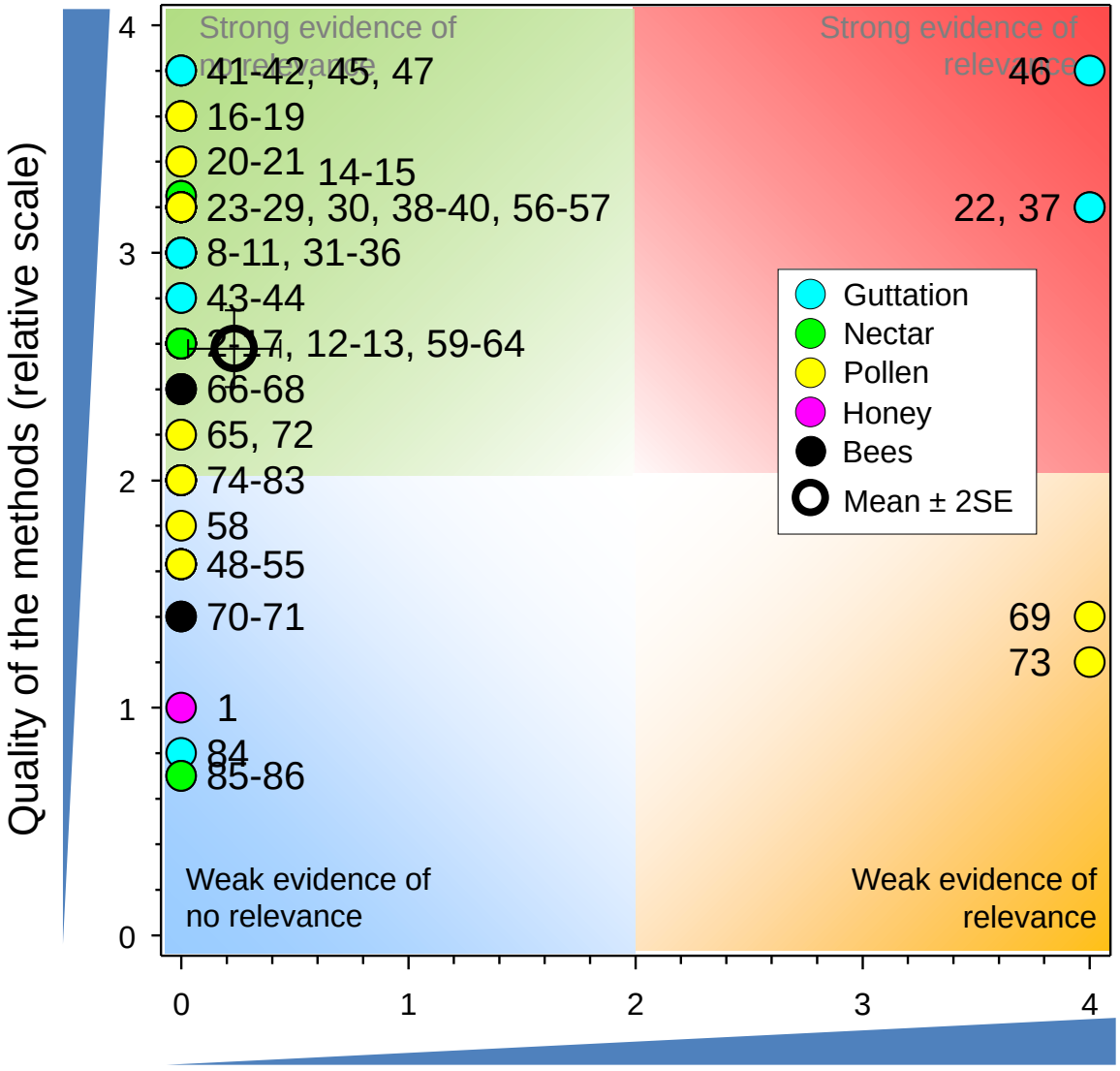
1 = (Bargańska et al. 2013) Resp 1
2 = (Bayer Corporation 2002) Resp 1
3 = (Bayer Corporation 2002) Resp 2
4 = (Bayer Corporation 2002) Resp 3
5 = (Bayer CropScience 1999b) Resp 1
6 = (Bayer CropScience 1999c) Resp 1
7 = (Bayer CropScience 1999c) Resp 2
8 = (Bayer CropScience 1999d) Resp 1
9 = (Bayer CropScience 1999d) Resp 2
10 = (Bayer CropScience 1999e) Resp 1
11 = (Bayer CropScience 1999e) Resp 2
12 = (Bayer CropScience 1999a) Resp 1
13 = (Bayer CropScience 1999a) Resp 2
14 = (Bayer CropScience AG 2010) Resp 1
15 = (Bayer CropScience AG 2010) Resp 2
16 = (Bayer CropScience 2011a) Resp 1
17 = (Bayer CropScience 2011a) Resp 2
18 = (Bayer CropScience 2011b) Resp 1
19 = (Bayer CropScience 2011b) Resp 2
20 = (Bayer CropScience 2012a) Resp 1
21 = (Bayer CropScience 2012a) Resp 2
22 = (Bayer CropScience 2012b) Resp 1
23 = (Bayer CropScience 2012b) Resp 2

Hazards of exposures of bees to IMI via seed and mixed treatments (SI)



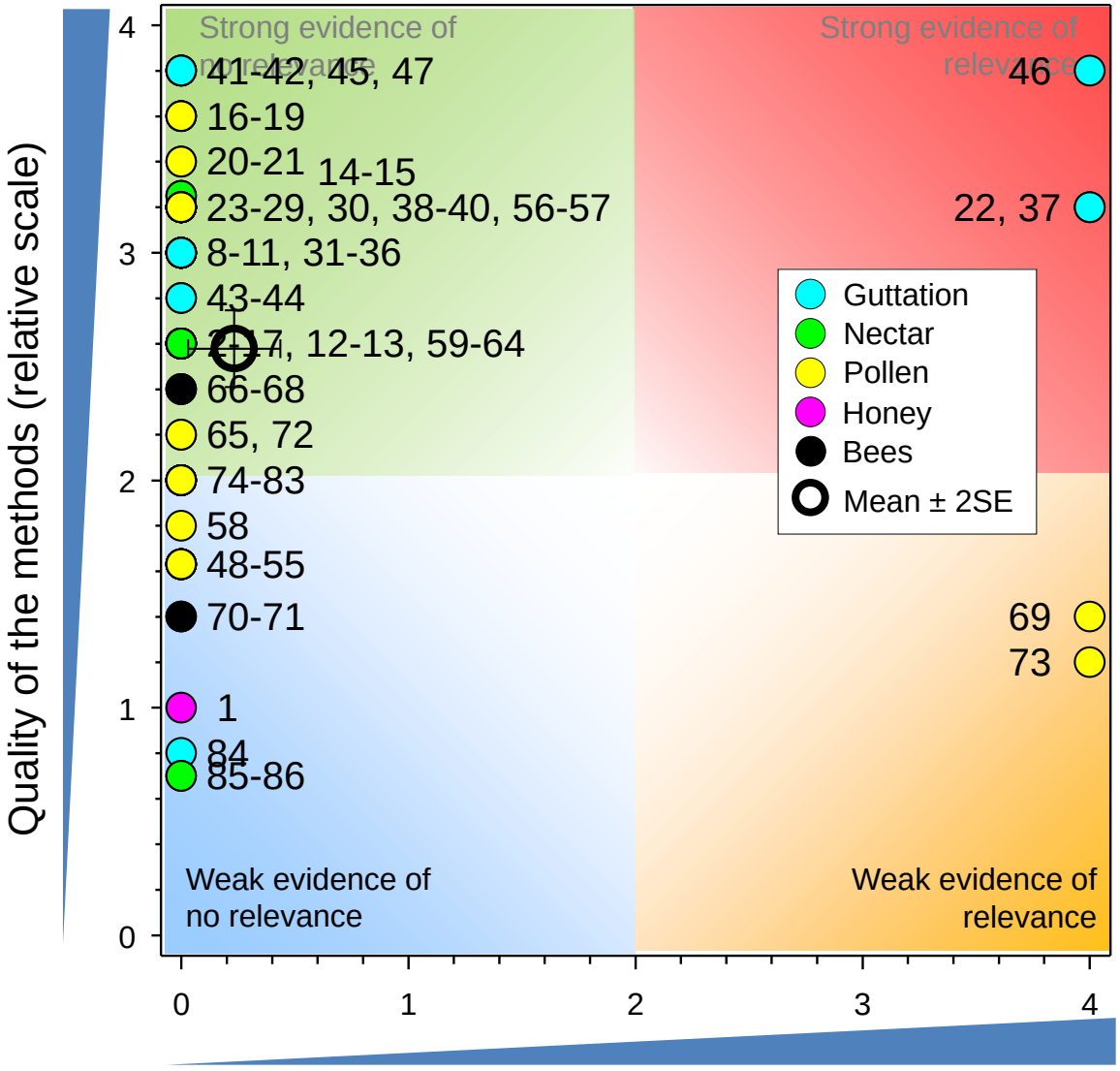
24 = (Bayer CropScience 2012b) Resp 3
25 = (Bayer CropScience 2012b) Resp 4
26 = (Bayer CropScience 2014i) Resp 1
27 = (Bayer CropScience 2014i) Resp 2
28 = (Bayer CropScience 2014j) Resp 1
29 = (Bayer CropScience 2014j) Resp 2
30 = (Bayer CropScience 2014j) Resp 3
31 = (Bayer CropScience 2014k) Resp 1
32 = (Bayer CropScience 2014k) Resp 2
33 = (Bayer CropScience 2014k) Resp 3
34 = (Bayer CropScience 2014k) Resp 4
35 = (Bayer CropScience 2014k) Resp 5
36 = (Bayer CropScience 2014k) Resp 6
37 = (Bayer CropScience AG 2014) Resp 1
38 = (Bayer CropScience AG 2014) Resp 2
39 = (Bayer CropScience AG 2014) Resp 3
40 = (Bayer CropScience AG 2014) Resp 4
41 = (Bayer CropScience 2014a) Resp 4
42 = (Bayer CropScience 2014a) Resp 5
43 = (Bayer CropScience 2014b) Resp 1
44 = (Bayer CropScience 2014c) Resp 1
45 = (Bayer CropScience 2014d) Resp 1
46 = (Bayer CropScience 2014d) Resp 2

Hazards of exposures of bees to IMI via seed and mixed treatments (SI)



47 = (Bayer CropScience 2014d) Resp 3
48 = (Bayer CropScience AG 2015a) Resp 1
49 = (Bayer CropScience AG 2015a) Resp 2
50 = (Bayer CropScience AG 2015a) Resp 3
51 = (Bayer CropScience AG 2015a) Resp 4
52 = (Bayer CropScience 2015a) Resp 1
53 = (Bayer CropScience 2015a) Resp 2
54 = (Bayer CropScience 2015a) Resp 3
55 = (Bayer CropScience 2015a) Resp 4
56 = (Bayer CropScience 2015b) Resp 1
57 = (Bayer CropScience 2015b) Resp 2
58 = (Bernal et al. 2010) Resp 1
59 = (Botias et al. 2015) Resp 1
60 = (Botias et al. 2015) Resp 2
61 = (Botias et al. 2015) Resp 3
62 = (Botias et al. 2015) Resp 4
63 = (Botias et al. 2015) Resp 5
64 = (Botias et al. 2015) Resp 6
65 = (Chauzat et al. 2006) Resp 1
66 = (Chauzat et al. 2011) Resp 1
67 = (Chauzat et al. 2011) Resp 2
68 = (Chauzat et al. 2011) Resp 3
69 = (Kasiotis et al. 2014) Resp 1

Hazards of exposures of bees to IMI via seed and mixed treatments (SI)



70 = (Kasiotis et al. 2014) Resp 2
71 = (Kasiotis et al. 2014) Resp 3
72 = (Laurent and Rathahao 2003) Resp 1
73 = (Mullin et al. 2010) Resp 1
74 = (Pohorecka et al. 2012b) Resp 1
75 = (Pohorecka et al. 2012b) Resp 2
76 = (Pohorecka et al. 2012b) Resp 3
77 = (Pohorecka et al. 2012b) Resp 4
78 = (Pohorecka et al. 2012b) Resp 5
79 = (Pohorecka et al. 2012b) Resp 6
80 = (Pohorecka et al. 2012b) Resp 7
81 = (Pohorecka et al. 2012b) Resp 8
82 = (Pohorecka et al. 2012b) Resp 9
83 = (Pohorecka et al. 2012b) Resp 10
84 = (Reetz et al. 2011) Resp 1
85 = (Stewart et al. 2014) Resp 1
86 = (Stewart et al. 2014) Resp 2

(Bargańska et al. 2013)
Paper: Bargańska Ż, Ślebioda M, Namieśnik J. 2013. Pesticide residues levels in honey from apiaries located of Northern Poland. *Food Control* 31:196-201.

This paper reported the concentrations of 30 pesticide residues found in honey samples collected in northern region of Poland (Pomerania) in the year 2010. Only the data for IMI are assessed here.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	Forty-five honey samples of different origins were obtained from beekeepers of Pomerania (Poland) in the summer of 2010. The samples were supplied to the laboratory by the Regional Beekeepers Association in Gdansk and were packaged in glass containers. Information of collection of the samples and procedures to avoid contamination were not reported (weakness). All samples were kept at -10°C until analysis (less than the appropriate temperature of -20±4°C. No controls (field blanks), field, travel, and storage spikes were reported (**major weakness**). Samples of honey were homogenized and ca. 1 g was weighed into and extracted with 10 mL of acetonitrile,10 mL water and 50 µL of internal standard solution (triphenyl phosphate). Isotopically labelled internal standards for IMI were not used (weakness). The method of extraction and analysis was an adaptation of QuEChERS and was well described. Standards were obtained from a commercial source but purity was not reported. Concentrations of metabolites were not measured. Analysis was by LC/MS/MS. The analytical method was validated using a standardized method[4]. For IMI the LOD was 1.28 µg/kg, the LOQ was 3.84 µg/kg and the recovery varied between 90.3 and 95% (Author Table 2). Statistical analysis was not conducted but, curiously, the maximum values were reported with “expanded uncertainty”, the calculation of which was not reported.	**2**
Use of GLP and QA/QC	This was not a GLP study. There was no QA and minimal QC.	**2**
Transparency of data	Raw data were not provided. Mean values were reported in a Table (3), only maximum and minimum values and frequency of detection were provided.	**2**
Number of samples and replication	There was no replication but 45 sites were sampled.	**1**
Duration of study sufficient to observe trends	The duration of sampling was not reported but was assumed to be appropriate for the design of the study.	**3**

[4] SANCO/2007/3131. (2007). Method validation and quality control procedures for pesticide residues analysis in food and feed. < http://ec.europa.eu/food/plant/protection/resources/qualcontrol_en.pdf >.

Overall evaluation of methods	Computed mean of above		**2.00**
Score for expert judgment on quality the study. One major weakness identified.		**0.5**	**1.00**

Relevance

IMI was detected in 5 of the 45 samples of honey (11% and close to the 90th centile). Concentrations of IMI ranged from less than the LOQ to the maximum values reported below.

Hazard quotients for exposures to TMX in honey bees exposed via honey hives in Poland (data from Table 3).

Source	Matrix	Conc. IMI µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
Hives from Poland	Honey (max)	15.2	4.4	7.3	Adult	292

Where toxicity values were exceeded by exposure values, relevance is shown with yellow highlight in the Table. Green cells are not relevant.

Response 1: Relevance to adverse effects			**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in honey collected across Poland resulted in exposures that were all less than the NOAED		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert judgment		
QA	Yes	September 26, 2015
SEJ	Yes	October 1, 2015

(Bayer Corporation 2002)
Report: Bayer Corporation. 2002. Imidacloprid (Admire®) Residue Levels Following In-furrow Application in Potato Fields in Prince Edward Island and New Brunswick. Stilwell, Kansas: Bayer Corporation, (Unpublished Report). Report M-061850-01-1. 120 p

The objectives of this study were to determine concentrations of IMI plus IMI-OH and IMI= (IMI was applied in-furrow to potatoes one and two years previously) in various matrices in clover fields planted on previously-treated soils. The matrices were: 1) soil, clover leaves, and clover flowers, and wild flowers; 2) pollen and nectar collected from honey bees foraging in clover fields; and 3) uncapped honey collected from the hives placed in the clover fields. Only the concentrations in nectar, pollen, and uncapped honey were assessed.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	The study was conducted in Prince Edward Island, Canada and only the fields where nectar and pollen were collected are described. The field component of study was poorly described (weakness). Four fields of potatoes were treated in-furrow in spring of 1999 with IMI (Admire®) at a rate of 850 ml formulation/hectare (applied material not analyzed – potential weakness). The following year (not clear if this is correct), the fields were planted with a mixture of red clover (*Trifolium pretense*), alsike clover (*Trifolium hybridum*), and timothy grass. On July 18, 2001, eight hives of bees were placed at the edge of each field. The biological collections were done in July-September of 2001. A composite sample of 160 soil-cores was collected from each field for residue analysis. Bees were collected with a "bug-vac" in the field (two fields) or at the entrance to the hive (two fields), frozen on dry-ice and moved the same day to a laboratory for long-term storage in a freezer at -20 ± 5°C. Foraging bees were samples on the control site. The actual period and duration of collection was not clearly reported (weakness). Pollen and nectar were collected after thawing the frozen bees to room temperature. Working in a cold room, the contents of the nectar in the honey-stomach was removed with a syringe and fine needle (100 bees). The target amount of sample was 2-4 g (min. 1 g). Samples were pooled from each site. After collection, the samples were frozen and stored at -20 ± 5°C. Pollen was removed from the bees (200 bees) using sterile forceps and probes and pooled for each site. The target amount of sample was 4–5 g (min. 2 g). Unripe honey (equivalent of 1-2 frames of uncapped honey) was collected from the hives on Aug 22 and Sep 14 2001. The uncapped honey was extracted by cutting the comb into sections, crushing them and straining into a collection bowl. A 45-g sample of honey was decanted and stored at -20 ± 5°C. All samples were shipped on dry ice to Enviro-Test for analysis at the end of October 2001. Controls served as field blanks. Analyses were conducted at Enviro-Test Laboratories. Analytical standards were 98.4% pure (IMI), 99.3% pure (IMI-OH) and 98.6% pure (IMI=). Analysis was by LC/MS/MS. The analytical method was completely described. Recovery of IMI from soil was 90±22%. In phase recoveries for IMI, IMI-OH, and IMI= from pollen were 96%, 94% and 74% resp., n=2. In honey (nectar not tested) verification recoveries were 101±9.3%, 85±19%, and 91±22%, resp., n=6. The LOD for soil was 2 µg/kg.	**2**

	Statistical analyses were not report.	
Use of GLP and QA/QC	The field-component of the study was not GLP and there was no QC or QA. The analysis was conducted under full GLP with QA.	**2**
Transparency of data	All raw data were provided. Variance was not reported.	**4**
Number of samples and replication	There were four sites and one replicate per site for nectar and pollen and two for uncapped honey.	**2**
Duration of study sufficient to observe trends	The duration of the study (58 d) was suitable for the design but the pooling of samples did not allow trends to be established.	**3**
Overall evaluation of methods	Computed mean of above	**2.6**
Score for expert judgment on quality the study.	**1**	**2.60**

Relevance

Concentrations of IMI in the soil ranged 14 to 25 µg/kg with a mean of 20 µg/kg (Fig. 9) in the four treated fields. No residues were detected in soil in the control field. No residues were detected in samples of clover flowers (Figure 11) from the treated fields or control where nectar, pollen and honey were sampled. Given the LOD of the method of 2 µg/kg, and no detections in nectar and pollen, there were no relevant exposures.

Hazard quotients for exposures to ΣIMD in honey bees via pollen, nectar, and honey from clover (data from Figs 14, 15 & 16).

Source	Matrix	Conc. IMI µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
Clover grown in soil prev. treated with IMI	Pollen from bees	0	0.0	7.3	Larva	124
Clover grown in soil prev. treated with IMI	Nectar from foraging bees	0	0.0	7.3	Adult	292
Clover grown in soil prev. treated with IMI	Uncapped honey	0	0.0	7.3	Adult	292

Where toxicity values were exceeded by exposure values, relevance is shown with yellow highlight in the Table. Green cells are not relevant.

Response 1: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in nectar resulted in exposures that were all less than the NOAED.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 2: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in pollen resulted in exposures that were all less than the NOAED.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 3: Relevance to adverse effects			Score
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in honey resulted in exposures that were all less than the NOAED.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert judgment		
QA	Yes	September 14, 2015
SEJ	Yes	October 1, 2015

(Bayer CropScience 1999b)
Report: Bayer CropScience. 1999. Residues of Imidacloprid and Imidacloprid Metabolites in Nectar, Blossoms, Pollen and Honey Bees Sampled from a Summer Rape Field in Sweden and Effects of These Residues on Foraging Honeybees. Leverkusen, Germany: Bayer CropScience (Unpublished Report). Report M-006811-01-1. 28 p

Response 1: Quality methods		**Score**
Experimental design and hypotheses	The study took place in the vicinity of Borlunda-Skelinge, South of Eslov in Sweden. The study was well described. The study took place in 1998 with seeding on April 28, 1998 and the plots were not treated with IMI previously. The plot was seeded with rape seeds (Maskot) treated with a mixture of beta-cyfluthrin and IMI with analyzed concentrations of 78.3 g/L and 428.2 g/L respectively. The seeds were treated with 2.5 L of formulation per 100 kg. Seeds were planted at a rate of 5 kg seed/ha. The treated seed was analyzed for IMI (1026 g/dt imidacloprid). In addition to the test plot, a control with no prior treatment or treated seed was prepared. Soil samples were only taken to characterize the soil properties, IMI was not analyzed for (*potential weakness*). Details of weather and other relevant conditions during sampling were recorded. Biological sampling was conducted between July 2-6, 1998. Bees were obtained from a local source and contained approx. 5000 bees per hive. Mesh tunnels were installed on the field to confine bees to the treated and control plants during sampling. Bees were sampled directly from the flowers and frozen in the field on dry ice and then stored in a freezer at -19 to -20°C. Honeybulbs were removed from surrounding tissues and treatments were pooled and then stored on dry ice. After all honeybees were processed, samples were stored at -20°C until shipped to BCS on dry ice and then retained at -20°C until analysis. Pollen collected by the bees was removed but the sample was too small for analysis. To collect nectar from the flowers, they were covered with plastic bags 24 h before sampling (to prevent collection by bees) and then the nectar was collected directly from flowers by 5-µL Life-stage. Samples were pooled and stored on dry ice in the field. At the end of each sampling day, samples were transferred into a freezer (-20°C) before being shipped to BCS for analysis as above. Field blanks were prepared to check for contamination of nectar. Analysis was by LC/MS/MS and was fully described (Appendix 1). Analytical standards of IMI, IMI-OH and IMI= were used but purity was not reported. Isotopically labelled internal standards were not used (*potential weakness*). The LOQ for IMI and metabolites was 10 µg/L. Recovery was not reported (*potential weakness*). Control samples were <LOD. Statistical analyses were not reported.	**2**
Use of GLP and QA/QC	This was a full GLP study with QA. QC was not reported.	**3**
Transparency of data	All raw data were provided	**4**
Number of samples and replication	There was one replicate plot and a single site.	**1**

Duration of study sufficient to observe trends	Sampling was for a 2-d period during peak flowering. This period was considered shorter than ideal.		**3**
Overall evaluation of methods	Computed mean of above		**2.60**
Score for expert judgment on quality the study.		**1**	**2.60**

Relevance

IMI Exposure Bayer CropScience-1999, M-006811-01-1 (data from Table 1)
Hazard quotients for exposures to ΣIMD in honey bees via nectar in in summer oil-seed rape.

Source	Matrix	Conc. ΣIMD µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
Oil-seed rape nectar (bees)	Nectar from bees	10.0	2.92	7.3	Adult	292
Oil-seed rape nectar (flowers)	Hand-collected nectar	10.0	2.92	7.3	Adult	292

Where toxicity values were exceeded by exposure values, relevance is shown with yellow highlight in the Table. Green cells are not relevant.

Response 1: Relevance to adverse effects			**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI resulted in exposures that were all less than the NOAED from both sources of nectar.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert judgment		
QA	Yes	September 14, 2015
SEJ	Yes	October 1, 2015

(Bayer CropScience 1999c)
Report: Bayer CropScience. 1999. Residues of Imidacloprid and Imidacloprid Metabolites in Nectar, Blossoms, Pollen and Honey Bees Sampled from a French Summer Rape Field and Effects of These Residues on Foraging Honeybees. Leverkusen, Germany: Bayer CropScience (Unpublished Report). Report M-006815-01-1. 27 p

Response 1: Quality methods		**Score**
Experimental design and hypotheses	The study took place in the vicinity of Conches between la Neuve Lyre and la Vieille Lyre in Northern France. The study was well described. The study took place in 1998 with seeding on March 19, 1998 and the plots were not treated with IMI previously. The plot was seeded with rape seeds (Lisonne) treated with a mixture of beta-cyfluthrin and IMI with analyzed concentrations of 78.3 g/L and 428.2 g/L respectively. The seeds were treated with 2.5 L of formulation per 100 kg. Seeds were planted at a rate of 5 kg seed/ha. The treated seed was analyzed for IMI (1045 g/dt Imidacloprid). In addition to the test plot, a control with no prior treatment or treated seed was prepared. Soil samples were only taken to characterize the soil properties, IMI was not analyzed for (*potential weakness*). Details of weather and other relevant conditions during sampling were recorded. Biological sampling was conducted between June 15 and 18, 1998. Bees were obtained from a local source and contained approx. 5000 bees per hive. Mesh tunnels were installed on the field to confine bees to the treated and control plants during sampling. Bees were sampled directly from the flowers and frozen in the field on dry ice and then stored in a freezer at -20°C. Honeybulbs were removed from surrounding tissues and treatments were pooled and then stored on dry ice. After all honeybees were processed, samples were stored at -20°C until shipped to BCS on dry ice and then retained at -20°C until analysis. Pollen collected by the bees was removed, pooled, and stored at -20°C until shipped to BCS on dry ice and then retained at -20°C until analysis. To collect nectar from the flowers, they were covered with plastic bags 24 h before sampling (to prevent collection by bees) and then the nectar was collected directly from flowers by 5-µL Life-stage. Samples were pooled and stored on dry ice in the field. At the end of each sampling day, samples were transferred into a freezer (-20°C) before being shipped to BCS for analysis as above. Field blanks were prepared to check for contamination of nectar. Analysis was by LC/MS/MS and was fully described (Appendix 1). Analytical standards of IMI, IMI-OH and IMI= were used but purity was not reported. Isotopically labelled internal standards were not used (*potential weakness*). The LOQ for IMI and metabolites was 10 µg/L. Recovery was not reported (*potential weakness*). Control samples were <LOD. Statistical analyses were not reported.	**2**
Use of GLP and QA/QC	This was a full GLP study with QA. QC was not reported.	**3**
Transparency of data	All raw data were provided	**4**

Number of samples and replication	There was one replicate plot and a single site.	**1**
Duration of study sufficient to observe trends	Sampling was for a 2-d period during peak flowering. This period was considered shorter than ideal.	**3**
Overall evaluation of methods	Computed mean of above	**2.60**
Score for expert judgment on quality the study.	**1**	**2.60**

Relevance

Hazard quotients for exposures to ΣIMD in honey bees via nectar and pollen in in summer oilseed rape (from Table 1).

Source	Matrix	Conc. ΣIMD µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
Oil-seed rape nectar (bees)	Nectar from bees	10.0	2.92	7.3	Adult	292
Oil-seed rape nectar (flowers)	Hand-collected nectar	10.0	2.92	7.3	Adult	292
Oil-seed rape Pollen (bees)	Pollen from plants	10.0	1.24	7.3	Larva	124

Where toxicity values were exceeded by exposure values, relevance is shown with yellow highlight in the Table. Green cells are not relevant.

Response 1: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in both sources of nectar resulted in exposures that were all less than the NOAED.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 2: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in pollen resulted in exposures that were all less than the NOAED.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Expert judgment		
QA	Yes	September 14, 2015
SEJ	Yes	October 1, 2015

(Bayer CropScience 1999d, e)
Reports: Bayer Crop Science. 1999. Residue Levels of Imidacloprid and Imidacloprid Metabolites in Nectar, Blossoms and Pollen of Sunflowers Cultivated on Soils with Different Imidacloprid Residue Levels and Effects of these Residues on Foraging Honeybees: Test Location: Farmland "Laacher Hof' - 1999. Leverkusen, Germany: Bayer CropScience (Unpublished Report). Report M-016827-01-1. 44 p

And

Bayer CropScience. 1999. Residue Levels of Imidacloprid and Imidacloprid Metabolites in Nectar, Blossoms and Pollen of Sunflowers Cultivated on Soils with Different Imidacloprid Residue Levels and Effects of these Residues on Foraging Honeybees: Test Location: Farmland "Höfchen" - 1999. Leverkusen, Germany: Bayer CropScience (Unpublished Report). Report M-016820-01-1. 41 p

The two studies in this WoE were very similar in design but were carried out at two different sites. For this reason they were combined. These studies were on sunflower grown in soils previously treated with IMI and sunflower seeds treated with IMI and grown in soil that had not previously been treated with IMI. Only the concentrations in nectar and pollen were assessed.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	The study took place at Bayer experimental farms at Höfchen and Laacher Hof. The studies were well described. The study took place in 1999 with seeding on May 10 and 12 resp. Two plots at Höfchen and three at Laacher Hof had previously been treated with IMI and were seeded with untreated sunflower seeds of the Fleury var. One plot at each site that had not been treated previously with IMI was seeded with sunflower seeds treated with 150 g Gaucho WS 70/150,000 seeds (by analysis the content of IMI was 72.5% a.s.) The nominal rate was 105 g/150,000 seeds (analytical finding was 89.3 g/150,00 seeds). The drilling rate was 75,000 seeds/ha (or 1,800 seed per four 240 m^2 study plots). The seeds were also treated with a standard combined fungicide (carbendazim, metalaxyl and Cu-oxyquinolat). Previous pesticide use on the plots was described in detail. In addition to the test plots, a control with no prior treatment or treated seed was prepared. At time of seeding, soil samples (20 cores of 5 cm dia. and 30 cm long) were taken in each plot. Soils in all plots were subsampled at 0-20 and 20-30 cm and analyzed for residues of IMI, IMI-OH and IMI=. Sampling for pollen and nectar from bees was deemed to be inefficient so fresh nectar and pollen was collected from combs in hives installed in tunnels on the fields to study effects of the treatments on honey bees. Pollen was sampled only once from the combs but was collected on two other days directly from the flower heads. Samples were frozen in the field on dry ice and then stored in a freezer at -20°C. Male and female flowers were sampled and leaves of the sunflowers were sampled for analysis. Travel and storage spikes were not needed and samples from control plots served as field blanks. It is not clear if the samples were pooled or not but judging from the weights of the samples reported in Section 3 in Appendix III of the respective reports, they were pooled.	**2**

	Analysis was by LC/MS/MS and was fully described. Analytical standards of IMI, IMI-OH and IMI= were ≥ 98% pure. Isotopically labelled internal standards were not used (potential weakness). The LOQ for IMI in soil was 6 µg/kg and the LOD was 2 µg/kg. Recover of IMI from spiked soil was >92%. For biological samples, the LOD for IMI and IMI-OH were 1.5 µg/kg and 3 µg/kg for IMI=. The LOQs were 5 µg/kg for IMI and IMI-OH and 10 µg/kg for IMI=. Recovery from biological samples was not reported (*potential weakness*). There were no measurable residues in the soil in either of the control plots (LOD = 2 µg/kg). Measured residues of IMI in previously treated soils from Laacher Hof were 15.7, 14.3 and 12.7 µg/kg in the three test plots. At Höfchen, concentrations in the soil from previously treated plots were 18 and <LOQ (6 µg/kg). No residues of IMI were detected in the soil from the plots where treated seed was sown on previously untreated soil. Statistical analyses were not reported.	
Use of GLP and QA/QC	This was a full GLP study with QA. QC was not reported.	**3**
Transparency of data	All raw data were provided	**4**
Number of samples and replication	There were three replicate plots for the pretreated soil and one replicate for the treated seed in the Laacher Hof study and two replicate plots for the pretreated soil and one replicate for the treated seed in the Höfchen study.	**2**
Duration of study sufficient to observe trends	Sampling was for a 6-d period during peak flowering. This is considered appropriate for the design of the study.	**4**
Overall evaluation of methods	Computed mean of above	**3.00**
Score for expert judgment on quality the study.	**1**	**3.00**

Relevance

No residues of IMI or its metabolites were detected in nectar and pollen (or any other matrices) from controls or the previously treated plots where residues were detected in the soil. These data showed no relevant exposure. For the sunflowers grown from treated seeds at Laacher Hof, no residues were detected in honey or pollen but IMI was measured at the LOQ (5 µg/kg) in tissues of leaf. IMI-OH was also detected in leaf tissue at a concentration <LOQ. At Höfchen, the concentration of IMI in leaf tissue was 7 µg/kg (and 6 ug/kg at Laacher Hof) but that of the IMI-OH was <LOQ (5 µg/kg). In these cases, because IMI was detected in one tissue of the plant, it was assumed that IMI was present at half the LOD (0.75 µg/kg for IMI & IMI-OH and 1.5 µg/kg for IMI=) in nectar and pollen.

Hazard quotients for exposures to ΣIMD in honey bees via nectar and pollen in sunflower (from Table 3).

Source	Matrix	Conc. ΣIMD µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
Treated seed, Laacher Hof	Nectar from hive	3.0	0.88	7.3	Adult	292
Treated seed, Laacher Hof	Pollen from plants	3.0	0.37	7.3	Larva	124

Hazard quotients for exposures to ΣIMD in honey bees via nectar and pollen in sunflower (from Table 3).

Source	Matrix	Conc. ΣIMD µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
Treated seed, Laacher Hof	Pollen from hive	3.0	0.37	7.3	Larva	124
Treated seed Höfchen	Nectar from hive	3.0	0.88	7.3	Adult	292
Treated seed Höfchen	Pollen from plants	3.0	0.37	7.3	Larva	124
Treated seed Höfchen	Pollen from hive	3.0	0.37	7.3	Larva	124

Where no residues were detected, it was assumed that IMI, IMI-OH, and IMI= were at 0.5 LOD (0.15).

Where toxicity values were exceeded by exposure values, relevance is shown with yellow highlight in the Table. Green cells are not relevant.

Response 1: Relevance to adverse effects from exposure to pollen			**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of ΣIMD in pollen resulted in exposures that were all less than the NOAED on all occasions		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 2: Relevance to adverse effects from exposure to nectar			**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of ΣIMD in nectar resulted in exposures that were all less than the NOAED on all occasions		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert judgment		
QA	Yes	August 26, 2015
SEJ	Yes	October 1, 2015

(Bayer CropScience 1999a)
Report: Bayer CropScience. 1999. Residues of Imidacloprid and Imidacloprid Metabolites in Nectar, Blossoms, Pollen and Honey Bees Sampled from a British Summer Rape Field and Effects of These Residues on Foraging Honeybees. Leverkusen, Germany: Bayer CropScience (Unpublished Report). Report M-040023-01-1. 29 p

Response 1: Quality methods		**Score**
Experimental design and hypotheses	The study took place at the Bayer UK experimental Elm Farm. The studies were well described. The study took place in 1998 with seeding on March 20, 1998 and the plots were not treated with IMI previously. The plot was seeded with rape seeds (Lisonne) treated with a mixture of beta-cyfluthrin and IMI with analyzed concentrations of 78.3 g/L and 428.2 g/L respectively. The seeds were treated with 2.5 L of formulation per 100 kg. Seeds were planted at a rate of 5 kg seed/ha. The treated seed was analyzed for IMI (1019 g/dt Imidacloprid). In addition to the test plot, a control with no prior treatment or treated seed was prepared. Soil samples were only taken to characterize the soil properties, IMI was not analyzed for (*potential weakness*). Details of weather and other relevant conditions during sampling were recorded. Biological sampling was conducted between June 22 - 24, 1998. Bees were obtained from a local source and contained approx. 5000 bees per hive. Mesh tunnels were installed on the field to confine bees to the treated and control plants during sampling. Bees were sampled directly from the flowers and frozen in the field on dry ice and then stored in a freezer at -19 to -20°C. Honeybulbs were removed from surrounding tissues and treatments were pooled and then stored on dry ice. After all honeybees were processed, samples were stored at -20°C until shipped to BCS on dry ice and then retained at -20°C until analysis. Pollen collected by the bees was removed, pooled, and stored at -20°C until shipped to BCS on dry ice and then retained at -20°C until analysis. To collect nectar from the flowers, they were covered with plastic bags 24 h before sampling (to prevent collection by bees) and then the nectar was collected directly from flowers by 5-µL Life-stage. Samples were pooled and stored on dry ice in the field. At the end of each sampling day, samples were transferred into a freezer (-20°C) before being shipped to BCS for analysis as above. Field blanks were prepared to check for contamination of nectar. Analysis was by LC/MS/MS and was fully described (Appendix 1). Analytical standards of IMI, IMI-OH and IMI= were used but purity was not reported. Isotopically labelled internal standards were not used (*potential weakness*). The LOQ for IMI and metabolites was 10 ug/L. Recovery was not reported (*potential weakness*). Control samples were <LOD. Statistical analyses were not reported.	**2**
Use of GLP and QA/QC	This was a full GLP study with QA. QC was not reported.	**3**
Transparency of data	All raw data were provided	**4**
Number of samples and replication	There was one replicate plot and a single site.	**1**

Duration of study sufficient to observe trends	Sampling was for a 2-d period during peak flowering. This period was considered shorter than ideal.	**3**
Overall evaluation of methods	Computed mean of above	**2.60**
Score for expert judgment on quality the study.	**1**	**2.60**

Relevance

Hazard quotients for exposures to ΣIMD in honey bees via nectar and pollen in in summer oil-seed rape (from Table 1).

Source	Matrix	Conc. ΣIMD µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
Oil-seed rape nectar (bees)	Nectar from bees	10.0	2.92	7.3	Adult	292
Oil-seed rape nectar (flowers)	Hand-collected nectar	10.0	2.92	7.3	Adult	292
Oil-seed rape Pollen (bees)	Pollen from plants	10.0	1.24	7.3	Larva	124

Where toxicity values were exceeded by exposure values, relevance is shown with yellow highlight in the Table. Green cells are not relevant.

Response 1: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in both sources of nectar resulted in exposures that were all less than the NOAED	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 1: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in pollen resulted in exposures that were all less than the NOAED	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Expert judgment		
QA	Yes	September 14, 2015
SEJ	Yes	October 1, 2015

(Bayer CropScience AG 2010)

Bayer CropScience AG. 2010. Determination of Residues of Clothianidin and Imidacloprid and their Metabolites in Melon Following an Application of Clothianidin & Imidacloprid WS 56.25 + 18.75 as Seed Treatment. Niefern-Öschelbronn, Germany: Bayer CropScience AG (Unpublished Report). Report M-361798-01-1. 195 p

Comments on paper. This study was conducted on melons grown in Spain from seeds treated with CTD and IMI. Only analyses of bee-relevant matrices (pollen and nectar) were included in the WoE.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	The field component of this study was conducted in the Valencia region of Spain. Melon-seeds (*Cucumis melo*, Honey Moon var) were treated with a mixture of CTD and IMI at measured concentrations of 1.026 and 0.34 mg a.s., resp. per seed Table 2). Control seeds were the same var. The experimental phase of the study was initiated on Apr 22, 2008 and completed Aug 25, 2008. Melon plants were initially grown in the greenhouse and then transplanted into the field (May 29, 2008). The plants were grown in mesh tunnels (5 x 50 m) after transplanting and the density was 30,000 plants/ha. Temperature, humidity, and rainfall were recorded at a nearby weather station (non-GLP) and temperature and humidity were recorded in the mesh tunnels. One beehive was used per test plot and a colony-quality at least 5000 honeybees and free of disease. Source of the honeybees was not reported. Pollen was collected from the combs in the hives five times over the exposure period from 23 Jul to 20 Aug. The samples were a pool of three locations on the comb. Samples of pollen were collected in the same way on the same days. Samples for analysis were kept on blue ice during collection and then stored at <-18°C until analysis except for a period of three d during transport when the temperature reached -12.6°C. Samples were analyzed at BCS in Monheim using a standard method. Isotopically labelled internal standards were used. Purity of the internal standards for IMI, MIN-OH, and IMI= were not reported. The LOD was 1 µg/kg. For IMI-OH, recovery (RSD) was 98%(9.6) from pollen and 97%(13.2) from nectar. For IMI=, recovery (RSD) was 72%(12.7) from pollen and 88%(10.6) from nectar. (Tables 6-8). Soils were not analyzed (weakness). No detectable residues of IMI, or its metabolites were present in the nectar and pollen.	**3**
Use of GLP and QA/QC	The study was conducted under GLP and the analytical phase has full QA and QC.	**4**
Transparency of data	All raw data were provided.	**4**
Number of samples and replication	The study was conducted at one site with three replicates of the treatment group and one of the control (potential weakness). Five samples were taken over the period of the study (increased score).	**2**

Duration of study sufficient to observe trends	The duration of collection of nectar and pollen encompassed the flowering period and was appropriate for the design of the study.	**4**
Overall evaluation of methods	Computed mean of above	**3.25**
Score for expert judgment on quality the study.	**1**	**3.25**

Relevance

Hazard quotients for exposures to IMI in melon from Spain treated with IMI and CTD (p. 6-8)

Source of matrix	Sample	IMI in µg/kg or L	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg diet/d
Mellon	Pollen	0	0.00	7.3	Larva	124
Mellon	Nectar	0	0.00	7.3	Adult	292

Response 1: Relevance to adverse effects		Score
Comparison of exposure value to toxicity value	The concentrations of IMI measured in pollen resulted in exposures that were less than the NOAED for bees.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 2: Relevance to adverse effects		Score
Comparison of exposure value to toxicity value	The concentrations of IMI measured in nectar resulted in exposures that were less than the NOAED for bees.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Expert judgment		
QA	Yes	January 26, 2016
SEJ	Yes	February 5, 2016

(Bayer CropScience 2011a, b)
Reports: Bayer CropScience. 2011. Determination of Residue Levels of Imidacloprid, Imidacloprid-Monohydroxy and Imidacloprid-Olefine in Bee-Relevant Matrices of Winter Rape in a Cereal Succeeding Crop Scenario at Bayer CropScience AG Experimental Farm "Laacher Hof", Germany. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-406075-01-1. 117 p

and

Bayer CropScience. 2011. Determination of Residue Levels of Imidacloprid, Imidacloprid-Monohydroxy and Imidacloprid-Olefine in Bee-Relevant Matrices of Winter Rape in a Cereal Succeeding Crop Scenario at Bayer CropScience AG Experimental Farm "Höfchen", Germany. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-406083-01-1. 116 p

As the methods use in these two reports are virtually identical, the reports were treated as a single study on two sites for the purposed of WoE assessment. These studies examined the exposure of honeybees to IMI and its metabolites IMI-OH and IMI= by potential uptake of soil residues of imidacloprid into nectar and pollen of winter oil-seed rape (OSR) in a cereal succeeding crop scenario. Fields were treated with IMI to establish a long-term soil plateau concentration and were then planted with winter wheat seeds treated with IMI. In the late summer of the following year, the winter wheat was harvested and winter OSR was planted. Pollen and nectar for the OSR analyzed to determine exposure concentration in the following spring.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	Two tests plots (treated and control) were set up at the Bayer Experimental Farms "Laacher Hof", North Rhine-Westphalia, close to Monheim (Site 1) and "Höfchen", North Rhine-Westphalia, close to Burscheid (Site 2). To establish a long-term soil plateau concentration, the treatment plots were sprayed on 2007 10 18&19 with Manta Plus FS 145.2 (Baytan U® + Gaucho®) at a rate of 126 g IMI a.s./ha and cultivated to a depth of 20 cm. Immediately after this, winter wheat seeds (Dekan) dressed with Manta Plus FS 145.2 to a measured rate of 70.75 g a.s. imidacloprid/100 kg seeds (101% nominal). IMI-free wheat seeds were used in the control. Concentrations in the soils (15 pooled samples) were measured immediately after spraying and were 45.7 and 34 µg a.s./kg dry soil, respectively. Residues in soil in the control plots were >LOD. After harvesting the winter wheat in July & August 2008, respectively, concentrations in soil (15 pooled samples) were measured again and were 18.8 and 15.2 µg a.s./kg dry soil, respectively. In July & Aug 2008, the wheat was harvested and about 20 d later winter OSR (Adriana) with an IMI-free seed coating (Elado®; 400 g CTD a.s./L plus 80 g beta-cyfluthrin a.s./L, plus 700 g thiram a.s./L). On 2009-04-16 & 13, one gauze tunnel (approximately 50 m² surface area) was set up on the treatment and the control plots. A week later, one hive with about 3000 bees (*Apis mellifera carnica*) was placed in the tunnel on the test plot and on the control	**4**

	plot. On three and four different days (for Sites 1 and 2, respectively) during the flowering period of winter OSR (April to May 2009), nectar- and pollen foraging honeybees were manually collected inside the tunnels, killed by freezing on dry ice and stored deep frozen (-17 to -21°C). Afterwards, the frozen honeybees were thawed to room temperature worked up by separating pollen loads from the legs of the pollen forager bees and by extracting bee-collected nectar from the honey bulbs of the nectar forager bees with a syringe. Controls were analyzed and showed no detectable residues. Analyses were conducted at the Bayer Crop Science facility in Monheim using standard methods. Analysis was HPLC-MS-MS. ^{13}C and ^{2}H-labelled internal standards of IMI, IMI-OH and IMI= were used. The LOQ for all three products in pollen was 1 µg/kg and the LOD was estimated as 0.3 µg/kg. Recovery ranged from 73 to 106% and the RSD from 5.3 to 16.7% (<20%). For nectar, recovery ranged from 71 to 102% and the RSD from 3.1 to 10.2% (<20%). CTD was not analyzed for.	
Use of GLP and QA/QC	Full GLP study with QA and some QC.	**4**
Transparency of data	All data were provided.	**4**
Number of samples and replication	Two sites were used. Three replicate samples of nectar and pollen were collected on site 1 and four on site 2 at different times.	**2**
Duration of study sufficient to observe trends	The duration of the sampling (between late April and early May was appropriate to the design of the study.	**4**
Overall evaluation of methods	Computed mean of above	**3.6**
Score for expert judgment on quality the study.	**1**	**3.60**

Relevance

Hazard quotients for exposures to ΣIMD in honey bees via pollen and nectar in oil-seed rape (data from Section 5, pg. 10).

Source	Matrix	Conc. ΣIMD µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
OSR Site 1	Pollen max	2.3	0.285	7.3	Larva	124
OSR Site 2	Pollen max	0.6	0.074	7.3	Larva	124
OSR Site 1	Nectar max (<LOD)	0.45	0.131	7.3	Adult	292
OSR Site 2	Nectar max (<LOD)	0.45	0.131	7.3	Adult	292

Where no residues were detected, it was assumed that IMI, IMI-OH, and IMI= were at 0.5 LOD (0.15).

Where toxicity values were exceeded by exposure values, relevance is shown with yellow highlight in the Table. Green cells are not relevant.

Response 1: Relevance to adverse effects			Score
Comparison of exposure value exceeds toxicity value	The concentrations of ΣIMD in nectar resulted in exposures that were all less than the NOAED at both sites.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 2: Relevance to adverse effects			Score
Comparison of exposure value exceeds toxicity value	The concentrations of ΣIMD in pollen resulted in exposures that were all less than the NOAED at both sites.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert judgment		
QA	Yes	September 15, 2015
SEJ	Yes	October 1, 2015

(Bayer CropScience 2012a)
Report: Bayer CropScience. 2012. Imidacloprid - Determination of Residues of Imidacloprid in Pollen, Extrafloral Nectar Fluids and Nectar of Cotton Plants Grown from Imidacloprid-Treated Seeds in Two Cotton Growing Areas in Greece 2011. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-424399-01-1. 81 p

The field study was conducted in Greece, one trial in Giannitsa in the vicinity of Pella (Trial 1) and a second trial in Glafki in the vicinity of Larissa (Trial 2). The purpose of the study was the determination of residues of IMI in pollen, nectar and extra floral nectar in cotton plants grown from seeds treated with IMI.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	Cotton seeds (variety Flora and Carmen from Turkey) were pre-treated with IMI (Gaucho 350 FS®). Seeds (Flora, measured loading of 484.67 g a.s./100 kg seed; 88.8 g a.s./ha) were planted on 2011 05 12 at Pella and on 2011 05 13 at Larissa (Carmen, measured loading of 555.07 g a.s./100kg seed;109.2 g a.s./ha). The sizes of the plots on which the cotton was grown were 1.04 and 1.0 ha, respectively. Previous crops were cotton, maize and wheat and IMI had not been used on either site in the previous 2 y. Several insecticides were applied during the trial period, including abamectin, pymetrozine, lufenoron, deltamethrin, and Bt. Plants were irrigated and cultivated during the trial as per normal agricultural practices. Sampling of nectar and pollen began on 2011 08 01 and was completed on 2011 08 19. Three samples were taken at BBCH stage 63, 65 and 69 (81, 88 and 98 days after sowing) from at least 12 locations on each sampling day. Samples of nectar and extrafloral nectary fluids were collected with capillary tubes using micropipettes and a pooled sample of ≥400 µL was obtained. Pollen was sampled by cutting the anthers from the flowers with scissors and a pooled sample of anthers containing ≥400 mg was collected from at least 12 different locations across the field. Samples were frozen within 6 h of collection and stored at --18°C. Control samples from the field were analyzed but raw data were not reported. Analyses were conducted at Eurofins ADME BIOANALYSES in France. Analysis was by LC-MS-MS and ^{13}C and ^{2}H-labelled internal standards were used. Laboratory recovery was 92% for pollen and 86% for nectar. RSD was <20%. The LOQ was 1 µg/kg. Only IMI was analyzed for (potential weakness). Statistical analyses were not reported.	**3**
Use of GLP and QA/QC	The study was full GLP with QA and QC on the analysis.	**4**
Transparency of data	All raw data were provided	**4**
Number of samples and replication	The trial was conducted at two sites. Samples were taken three times during the study at different times, starting on 2011 08 01 to 2011 08 19.	**2**
Duration of study sufficient to observe trends	Duration of the sampling was suitable for the study design.	**4**

Overall evaluation of methods	Computed mean of above		**3.4**
Score for expert judgment on quality the study.		**1**	**3.40**

Relevance

Hazard quotients for exposures to IMI in honey bees via pollen, nectar and extrafloral nectary fluids in cotton (data from Appendix A04 Table 2)

Source	Matrix	Conc. IMI µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
Cotton	Anther max	5	0.62	7.3	Larva	124
Cotton	Nectar max	4	1.17	7.3	Adult	292
Cotton	Extra floral nectar fluid max	5	1.46	7.3	Adult	292

Where toxicity values were exceeded by exposure values, relevance is shown with yellow highlight in the Table. Green cells are not relevant.

Response 1: Relevance to adverse effects			**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in nectar resulted in exposures that were all less than the NOAED.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 2: Relevance to adverse effects			**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in pollen resulted in exposures that were all less than the NOAED.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert judgment		
QA	Yes	September 17, 2015
SEJ	Yes	October 1, 2015

(Bayer CropScience 2012b)

Report: Bayer CropScience. Report: Bayer CropScience. 2012. Final Report (Non-GLP) Field Study to Monitor Potential Effects on Honey Bees from Exposure to Guttation Fluid of Winter Barley (W-BAR), Seed-Treated Either with an Imidacloprid or a Clothianidin Combi-Product. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-498922-01-1. 260 p

This field study was conducted at locations in northern and southern Germany to determine if two varieties of winter barley seeds treated with either IMI or clothianidin would have adverse effects on honeybees exposed via the guttation exposure pathway. Concentrations of IMI were measured in guttation fluid and this component of the study is addressed in the WoE.

Response 1-4: Quality methods		**Score**
Experimental design and hypotheses	The field study was conducted at locations in northern (Celle, Lower Saxony) and southern (Ihinger Hof, Baden-Württemberg) Germany and the methods were well described. Field sizes varied among treatments; the size of the control fields were 2.30 ha (Field 7 in the south) and 3.75 ha (Field 13 in the north). The size of the IMI-treated fields was 4.85 ha (Field 8 in the south) and 8.00 ha (Field 14 in the north) (Table 5). Study sites and soils characterized for TOC, WHC, pH, particle-size distribution, soil type), experimental design clearly explained. The varieties of winter barley differed between the regions in the north and south, Lomerit (northern var.) and Highlight (southern var.). The crops were sown in the autumn of 2009 and the study extended over the winter to the spring of 2010 and ended with winter oil-seed flowering in the respective regions. Winter barley (W-BAR) seeds (var. Lomerit and Highlight, respectively) treated with IMI (triadimenol & imidacloprid & fuberidazole & imazalil FS 60 + 70 + 7.2 + 8). At each location, there were two treatment groups (one field with fungicide- and IMI-treated W-BAR seeds (target loading rate was 70 g a.s./100 kg seed; analysed seed loading was 60.01 and 68.82 g a.s./100 kg for Lomerit and Highlight, resp. (Table 2) Seeding rate of seed was 200 kg/ha) and a field sown with fungicide-treated (EfA®) seeds that served as an experimental control). All seeds were treated with an abrasion inhibitor (INTECO®; 50 mL) to minimize dust. Monitoring began on September 15 (south) and September 27 (north) and ceased on October 31, 2009. The monitoring period in the spring extended from March 23 and 25, 2010 to April 23 and 25, 2010 for Ihinger Hof and Celle, respectively. An assessment area was established at each study plot and was divided into defined in-crop and off-crop zones and additional areas were segregated for sampling of guttation fluid from plants in each subarea (n = 5 subareas; n = 3 each) When guttation was observed in the morning, samples of fluid were collected from plants in segregated areas (n=5 subareas; n=3 each with a volume of 1 mL) and, within 10 h of collection was deep frozen (-20°C) for later analysis. If water was observed in the leaf axils samples were collected and processed as for guttation fluids. No guttation fluid was collected from plants in the control treatment (*weakness*). Samples were shipped on dry ice.	**3**

	Analysis was undertaken at BCS (Attachment 9.1) using standard methods. The reference standards were >96% pure for IMI, IMI= and IMI-OH; purity of the isotopically labelled internal standards was not reported (*weakness*). Analysis was by LC-MS/MS. LOQ for IMI, IMI= and IMI-OH was 10 µg/L and the LOD 1 µg/L. Recovery(RSD) for IMI, IMI= and IMI-OH was 96%(7), 97%(4.8), and 97%(6.4), resp. Residues in guttation fluid were generally greater in the autumn than the spring. There was no statistical analysis of data. Centiles were calculated from the raw data.	
Use of GLP and QA/QC	GLP was followed for analytical work but it was a non-GLP study; signatures present. QA/QC complete with deviations reported.	**3**
Transparency of data	All raw data were provided	**4**
Number of samples and replication	Two treatments each with two sites for a total of 4 fields of different sizes. There were five hives per treatment, each with defined assessment areas associated with each colony.	**2**
Duration of study sufficient to observe trends	The exposures were measured over a period of 28 days, appropriate for the study design.	**4**
Overall evaluation of methods	Computed mean of above	**3.2**
Score for expert judgment on quality the study.	**1**	**3.20**

Relevance

Hazard quotients for mean concentration of IMI in guttation fluid from winter barley grown for seeds treated with IMI in two locations in Germany (attachment 9.1 Table 10)

Source of sample	Value	IMI in µg/L	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg water/d
Ihinger Hof autumn	Median (n=64)	413	3	7.3	Adult	8
Ihinger Hof autumn	90th centile (n=64)	5856	47	7.3	Adult	8
Ihinger Hof spring	Median (n=36)	51	0.4	7.3	Adult	8
Ihinger Hof spring	90th centile (n=26)	76	0.6	7.3	Adult	8
Celle autumn	Median (n=30)	805	6	7.3	Adult	8
Celle autumn	90th centile (n=30)	3285	26	7.3	Adult	8
Celle spring	Median (n=40)	48	0.4	7.3	Adult	8
Celle spring	90th centile (n=40)	40	0.3	7.3	Adult	8

Residues below the LOD or LOQ are reported as <LOD or <LOQ and summed into the Total Residue value as 1/2 the LOD or the LOQ. Where toxicity values were exceeded by exposure values, relevance is shown with orange or yellow highlight in the Table. Green cells indicate no relevant exposure.

Response 1: Relevance to adverse effects		**Score**
Comparison of exposure value to toxicity value	The 90th centile concentrations of IMI measured in guttation fluid at Ihinger Hof in the autumn resulted in exposures that were greater than the NOAED for honeybees.	**4**
Overall evaluation relevance	Computed mean of above score(s)	**4.00**

Score for expert judgment on relevance of the results.	**1**	**4.00**

Response 2: Relevance to adverse effects		**Score**
Comparison of exposure value to toxicity value	The median and 90th centile concentrations of IMI measured in guttation fluid at Ihinger Hof in the spring resulted in exposures that were less than the NOAED for honeybees.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 3: Relevance to adverse effects		**Score**
Comparison of exposure value to toxicity value	The 90th centile concentrations of IMI measured in guttation fluid at Celle in the autumn resulted in exposures that were greater than the LOAED for honeybees.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 4: Relevance to adverse effects		**Score**
Comparison of exposure value to toxicity value	The median and 90th centile concentrations of IMI measured in guttation fluid at Celle in the spring resulted in exposures that were less than the NOAED for honeybees.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Expert Judgement		
QA	Yes	November 16, 2015
SEJ	Yes	Monday, February 15, 2016

(Bayer CropScience 2014i)
Report: Bayer CropScience. 2014. Determination of the Residues of Imidacloprid and its Metabolites Imidacloprid-5-Hydroxy and Imidacloprid-Olefinin Bee Relevant Matrices Collected in a Succeeding Crop Scenario with Natural Aged Residues of Imidacloprid. Field Phase Conducted with Winter Oil Seed Rape in Northern France. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-504810-01-1. 119 p

This study was undertaken to quantify imidacloprid (IMI) and its active metabolites IMI- 5-hydroxy (IMI-OH) and IMI-olefin (IMI=) in matrices relevant to bees (pollen and nectar) collected from a succeeding crop of oil seed rape (OSR) following normal agricultural use of IMI (Gaucho®) for seed treatment of winter wheat and winter barley in 2012 and 2013, respectively.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	The hypothesis that was tested was that exposures from repeated use of IMI would not result in buildup of residues in soil such that exposures would exceed toxicity values in pollen and nectar. The study protocol was detailed. The one study site was in the area of Ribeaucourt in N France. IMI (Gaucho®) had been used as a seed dressing on winter wheat and winter barley in 2012 and 2013, respectively but analyses of the seed and the formulation were not conducted. However, analyses of soil for residues were conducted (based on the upper 15 cm). Concentration in soil at the beginning of bloom period of the OSR was 45 µg/kg dry soil. Soil was not analyzed for IMI-OH and IMI= (potential weakness). In the previous year winter OSR had been sown by the farmer. No IMI was applied. Prior to bloom, three bee-proof tunnels (10 m long x 5 m wide x 3 m high) were placed on the OSR plot. Shortly after blooming started (BBCH 62-64), a single honeybee colony was placed into each tunnel but the bees were excluded from access to the crop in the tunnel until the days of sampling. Pollen and nectar was collected three times (April 9-10, 2014; April 14-15, 2014; and April 17-18, 2014) from bees confined to the crop. A pollen trap was used to collect pollen from forager bees returning to the hive and bees were collected for removal of nectar from the honey-stomach. All samples were pooled to obtain the desired amount for analysis. Because of the design of the study, the possibility of contamination from other sources of IMI was minimal. After collection, samples were frozen at ≤-18°C (using dry ice or freezer) and maintained in that condition until analysis. Use of field blanks was not reported control samples were not from the test location (weakness). Analysis was conducted in the Bayer laboratories and was by HPLC coupled with electrospray and tandem mass spectrometry (MS/MS) detection. ^{13}C internal standards for IMI, IMI-OH and IMI= were used. LOD and LOQ were clearly described and latter were 0.6 µg/kg for pollen, 0.3 µg/kg for nectar, and 5.0 µg/kg for soil. LOQs for IMI-OH and IMI= for nectar and pollen were 1.0 µg/kg. The recoveries for all matrices were 81—116% and RSD values were <20% (Page 63). Statistical analyses of the residue data were not reported.	**3**

Use of GLP and QA/QC	The study was GLP with full QA and some QC. Components that were exceptions to GLP were described and did not affect the quality the study.	**4**
Transparency of data	All raw data were provided in the report. Ranges were provided but means, medians, and centiles were not.	**4**
Number of samples and replication	There was one site and one sample (consisting of 30 pooled subsamples; i.e., soil cores from 10 locations per tunnel) for soil analysis. For samples of matrices relevant to bees (pollen and nectar), there were three replicates per site with three samples taken over time.	**1**
Duration of study sufficient to observe trends	Soil samples to characterize concentrations in the soil were analyzed only once just before the exposure measurements (appropriate). Pollen and nectar were sampled on three occasions over a week in the period of bloom (potential weakness in terms of probabilistic analysis). For the design of the study, these times were considered appropriate.	**4**
Overall evaluation of methods Computed mean of above		**3.20**
Score for expert judgment on quality the study	**1**	**3.20**

Relevance

Hazard quotients for exposures to ΣIMD in pollen and nectar from oil-seed rape in honey bees (data from Tables 13 and 14)

Source	Matrix	Max Conc. ΣIMD µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
OSR	Pollen	1.6	0.20	7.3	Larva	124
OSR	Nectar	1	0.29	7.3	Adult	292

Where no residues were detected it was assumed that IMI-OH and IMI= were at 0.5 LOD (0.15 µg/kg). Where toxicity values were exceeded by exposure values, relevance is shown with yellow highlight in the Table. Green cells are not relevant.

Response 1: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in pollen resulted in exposures that were all less than the NOAED.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 1: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in nectar resulted in exposures that were all less than the NOAED.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Expert judgment		
QA	Yes	September 21, 2015
SEJ	Yes	October 2, 2015

(Bayer CropScience 2014j)
Report: Bayer CropScience. 2014. Determination of the Residues of Imidacloprid and its Metabolites Imidacloprid-5-Hydroxy and Imidacloprid-Olefin in Bee Relevant Matrices Collected in a Succeeding Crop Scenario with Natural Aged Residues of Imidacloprid. Field Phase Conducted with *Phacelia* and Maize in Northern France. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-504836-01-1. 160 p

This study was undertaken to quantify imidacloprid (IMI) and its active metabolites IMI- 5-hydroxy (IMI-OH) and IMI-olefin (IMI=) in matrices relevant to bees (pollen, nectar, and guttation fluid) collected from succeeding crops of phacelia (*Phacelia tanacetifolia*) and maize (*Zea mays*) following application of IMI (Gaucho®) for seed treatment of winter wheat (2014) Imprimo® for sugarbeet (2013) Gaucho® for winter barley in 2012 and winter wheat (2011 and 2009). No treatments were made in 2010 and 2008.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	The hypothesis that was tested was that exposures from repeated use of IMI would not result in buildup such that exposures would exceed toxicity values in pollen, nectar, and guttation fluid. The study protocol was detailed. The two study sites were in the area of Auxy in N France and were adjacent. IMI had been used as a seed dressing on various crops from 2008 to 2014 but analyses of the seed and the formulation were not conducted. However, analyses of soil for residues were conducted (based on the upper 15 cm of pooled samples, one from the phacelia plot and one each from the three maize subplots). Samples of soil were taken after the guttation study but before the pollen study in maize and before the pollen and nectar study in phacelia (potential weakness). Concentration in soil in the phacelia plot was 52 µg/kg dry soil and in the maize subplots ranged from 41–59 µg/kg dry soil. Soil was not analyzed for IMI-OH and IMI= (potential weakness). In the spring of 2014, untreated phacelia and maize seeds were sown. After successful germination, three bee-proof tunnels (10 m long x 5 m wide x 3 m high) were placed on the phacelia plot. Shortly after blooming started (% bloom not provided), a single honeybee colony was placed into each tunnel. The plot of maize was divided into three smaller sub plots, each similar in size for sampling of guttation fluid and pollen. For phacelia, pollen and nectar was collected three times (from June 30 to July 17) from bees confined to the crop. A pollen trap was used to collect pollen from forager bees returning to the hive and bees were collected for removal of nectar from the honey-stomach. Pollen was manually collected (from July 18 to 20) from tassels of maize (dried overnight) and guttation fluid was manually collected (from May 6 to June 11) from enough plants to provide a large enough sample for analysis. All samples were pooled to obtain the desired amount for analysis. Because of the design of the study, the possibility of contamination from other sources of IMI was minimal. After collection, samples were frozen at ≤-18°C (using dry ice or freezer) and maintained in that condition until analysis. Use of controls (field blanks) was not reported (weakness).	**3**

	Analysis was conducted in the Bayer laboratories and was by HPLC coupled with electrospray and tandem mass spectrometry (MS/MS) detection. ^{13}C internal standards for IMI, IMI-OH and IMI= were used. LOD and LOQ were clearly described and latter were 0.6 µg/kg for pollen, 0.3 µg/kg for nectar and 1.0 µg/L for guttation liquid and 5.0 µg/kg for soil. LOQs for IMI-OH and IMI= for all matrices were 1.0 µg/kg. The recoveries were 73-106% and RSD values were <20% (p. 96). Statistical analysis of the residues was not reported (a potential weakness).	
Use of GLP and QA/QC	The study was GLP with full QA and some QC. Components that were exceptions to GLP were described and did not affect the quality the study.	**4**
Transparency of data	All raw data were provided in the report; however, the data were not clearly presented in terms of the analytical values which were coded (Appendix 2, Tables 13–17) and not provided in the Tables of analyzed specimens in the main report. Ranges were provided but means, medians, and centiles were not.	**3**
Number of samples and replication	For soil samples, there were two sites and three replicate samples for one site and one for the other. For samples of matrices relevant to bees (pollen and nectar, there were three replicates per site. For the guttation fluid there were 14 samples of three replicates taken over a period of about one month.	**2**
Duration of study sufficient to observe trends	Pollen and nectar from phacelia were sampled on three occasions over a two-week period, and pollen from corn was collected on three occasions over 3 days in the period of bloom (potential weakness in terms of probabilistic analysis). Guttation fluid was sampled 14 times over a period of about a month from Early-May to Early-June. For the design of the study, these times were considered appropriate.	**4**
Overall evaluation of methods Computed mean of above		**3.20**
Score for expert judgment on quality the study.	**1**	**3.20**

Relevance

Hazard quotients for exposures to ΣIMD in honey bees via pollen, nectar, and guttation water from Phacelia and maize (data from Appendix 2, Tables 19 and 20).

Source	Matrix	Max Conc. ΣIMD µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
Phacelia	Pollen	1.5	0.19	7.3	Larva	124
Phacelia	Nectar	0.7	0.20	7.3	Adult	292
Maize	Pollen	1.21	0.15	7.3	Larva	124
Maize	Guttation	5.1	0.04	7.3	Adult	8

Where residues were <LOQ or <LOD, it was assumed that IMI-OH and IMI= were at 0.5 LOQ or 0.5 LOD. Where toxicity values were exceeded by exposure values, relevance is shown with yellow highlight in the Table. Green cells are not relevant.

Response 1: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of ΣIMD in nectar from phacelia resulted in exposures that were all less than the NOAED.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**

Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 2: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of ΣIMD in pollen from phacelia and corn resulted in exposures that were all less than the NOAED.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 3: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of ΣIMD in guttation fluid from corn resulted in exposures that were all less than the NOAED.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Expert judgment		
QA	Yes	September 23, 2015
SEJ	Yes	October 2, 2015

(Bayer CropScience 2014k)
Paper or report: Bayer CropScience. 2014. Determination of the Residues of Imidacloprid in Bee Relevant Matrices Collected from Succeeding Crops Following Application of Imidacloprid FS 600E G via Soil Incorporation to Plateau Concentration and Sowing of Imidacloprid-Treated Winter Barley Seeds. Field Phase Conducted in Southern France. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-504842-01-1. 240 p

This study was undertaken to quantify imidacloprid (IMI) and its active metabolites IMI- 5-hydroxy (IMI-OH) and IMI-olefin (IMI=) in matrices relevant to bees (pollen, nectar, and guttation fluid) collected from succeeding crops of phacelia (*Phacelia tanacetifolia*), mustard (*Sinapis arvensis*) and maize (*Zea mays*) following application of IMI FS 600E G via soil incorporation to achieve a reasonable worst-case plateau concentration and sowing of IMI-treated seeds of winter barley in the previous year. Two concentrations of IMI were used. For the low treatment, 87.3 g a.s./ha was applied to bare soil in September 2013 and then IMI-treated seeds of barley were sown in the October at a rate equivalent to 85.8 g a.s./ha. For the high treatment, the 154 g a.s./ha was applied to bare soil in Sep 2013 and then treated seeds of barley were sown in the October at a rate equivalent to 118.5 g a.s./ha.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	The hypothesis that was tested was that exposures from repeated use of IMI would not result in buildup such that exposures would exceed toxicity values in pollen, nectar, and guttation fluid. The study protocol was detailed. The two study sites were in the area of Nîmes in France and were adjacent. Two concentrations of IMI were used. For the low treatment, 87.3 g a.s./ha was applied to bare soil in September 2013 and then treated seeds of barley were sown in the October at a rate equivalent to 85.8 g a.s./ha. For the high treatment, the 154 g a.s./ha was applied to bare soil in September 2013 and then treated seeds of winter barley (Henriette) were sown in the October at a rate equivalent to 118.5 g a.s./ha. Analyses of soil for residues were based on the upper 15 cm. Concentrations in soil during bloom of the experimental period in the high treatment were 25–93 and in the low treatment were 34–82 µg/kg dry soil. The formulation and the seeds were analyzed to confirm application rate (Appendix 2 certificates of analysis). The mode of application was described. Use rates were described (analyzed). In the following spring (2014), the barley crop was removed and the untreated succeeding crops (mustard, phacelia and maize) sown. Three bee-proof tunnels (10 m long x 5 m wide x 3 m high) were placed on the phacelia and the mustard plot after successful germination. Shortly after blooming started (% bloom not provided), a single honeybee colony was placed into each tunnel for phacelia and of mustard. The plot of maize was divided into three smaller sub plots, each similar in size for sampling of guttation fluid and pollen.	**3**

	For mustard, pollen and nectar was collected three times (from July 10 to July 26) from bees confined to the crop. For phacelia, pollen and nectar were only collected once at the beginning of blooming (September 10 and October 23 for the high and low treatments, respectively in a separate study; p 23 & 110-111). A pollen trap was used to collect pollen from forager bees returning to the hive and bees were collected for removal of nectar from the honey-stomach. Pollen was manually collected (from July 17 to 28) from tassels of maize (dried overnight) and guttation fluid was manually collected (from May 13 to June 18) from enough plants to provide a large enough sample for analysis. All samples were pooled to obtain the desired amount for analysis. Because of the design of the study, the possibility of contamination from other sources of IMI was minimal. After collection, samples were frozen at ≤-18°C (using dry ice or freezer) and maintained in that condition until analysis. Control samples were not collected from the site (weakness). Analysis was conducted in the Bayer laboratories using HPLC coupled with electrospray and tandem mass spectrometry (MS/MS) detection. ^{13}C internal standards for IMI, IMI-OH and IMI= were used. LOD and LOQ were clearly described and latter were 0.6 µg/kg for pollen, 0.3 µg/kg for nectar and 1.0 µg/L for guttation liquid and 5.0 µg/kg for soil. LOQs for IMI-OH and IMI= for all matrices were 1.0 µg/kg. The recoveries were 80—109% and RSD values were below 20% (p. 121).	
Use of GLP and QA/QC	The study was GLP with full QA and some QC. Components that were exceptions to GLP were described and did not affect the quality the study.	**4**
Transparency of data	All raw data were provided in the report; however, the data were not clearly presented in terms of the analytical values which were coded (Tables 2-4; p134-135, Tables 16-23; p 152-157). Ranges were provided but means, medians, and centiles were not.	**3**
Number of samples and replication	For soil samples, there were two sites and three replicate samples per site. For samples of matrices relevant to bees (pollen and nectar, there were three replicates per site. For the guttation fluid there were 16 samples taken over a period of about one month.	**2**
Duration of study sufficient to observe trends	Soil samples to characterize the plateau concentrations in the soil were analyzed only once prior to the exposure measurements (appropriate). Mustard pollen and nectar were sampled on three occasions over a week (for mustard) or three days (pollen only for corn) in the period of bloom (potential weakness in terms of probabilistic analysis). Phacelia pollen and nectar were sampled on only one occasion, after re-drilling (potential weakness). Guttation fluid was sampled 16 times over a period of about a month from mid-May to mid-June. For the design of the study, these times were considered appropriate.	**3**
Overall evaluation of methods Computed mean of above		**3.00**

Score for expert judgment on quality the study.	**1**	**3.00**

Relevance

Hazard quotients for exposures to ΣIMD in honey bees exposed pollen, nectar, and guttation fluid from maize, mustard, and phacelia (data from pages 37-38)

Source	Matrix	Max Conc. ΣIMD µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
Mustard H	Pollen	6.4	0.79	7.3	Larva	124
Mustard L		6.8	0.84	7.3	Larva	124
Mustard H	Nectar	0.8	0.23	7.3	Adult	292
Mustard L		4.9	1.43	7.3	Adult	292
Phacelia H	Pollen	2.3	0.29	7.3	Larva	124
Phacelia L		0.9	0.11	7.3	Larva	124
Phacelia H	Nectar	1.3	0.38	7.3	Adult	292
Phacelia L		0.45	0.13	7.3	Adult	292
Maize H	Pollen	1.58	0.20	7.3	Larva	124
Maize L		1.85	0.23	7.3	Larva	124
Maize H	Guttation fluid	48	0.38	7.3	Adult	8
Maize L		99	0.79	7.3	Adult	8

Where residues were <LOQ or <LOD, it was assumed that IMI-OH and IMI= were at 0.5 LOQ or 0.5 LOD.

Where toxicity values were exceeded by exposure values, relevance is shown with yellow highlight in the Table. Green cells are not relevant.

Response 1: Relevance to adverse effects			**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in pollen collected from mustard resulted in exposures that were all less than the NOAED		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 2: Relevance to adverse effects			**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in nectar collected from mustard resulted in exposures that were all less than the NOAED		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 3: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in pollen collected from phacelia resulted in exposures that were all less than the NOAED	**0**

Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 4: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in nectar collected from phacelia resulted in exposures that were all less than the NOAED	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 5: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in pollen collected from maize resulted in exposures that were all less than the NOAED	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 6: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in guttation fluid collected from maize resulted in exposures that were all less than the NOAED	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Expert judgment		
QA	Yes	September 23, 2015
SEJ	Yes	October 2, 2015

(Bayer CropScience AG 2014)
Report: Bayer CropScience. 2014. Final Report (Non-GLP) Field Study to Monitor Potential Effects on Honey Bees from Exposure to Guttation Fluid of Winter Wheat (W-WHT), Seed-Treated Either with an Imidacloprid or a Clothianidin Combi-Product. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-498939-01-1. 242 p

A field study was conducted to determine if two varieties of winter wheat (var. Manager and Herrmann, resp.) seeds (W-WHT) treated with either IMI or clothianidin would have adverse effects on honeybees exposed via the guttation exposure pathway. Concentrations of IMI were measured in guttation fluid and this component of the study is addressed in the WoE.

Response 1-4: Quality methods		**Score**
Experimental design and hypotheses	This field study was conducted at locations in northern (Celle, Lower Saxony) and Southern) Germany (Ihinger Hof, Baden-Württemberg to determine if two varieties of winter wheat (var. Manager and Herrmann, resp.) seeds (W-WHT) treated with either Imidacloprid (IMI) (triadimenol & imidacloprid & fuberidazol and imazalil FS 60+70+7.2+8) or CTD (clothianidin & beta-cyfluthrin FS 375+80). The IMI plot in Ihinger Hof was 6 ha and in Celle it was 16.2 ha. At each location, there were two treatment groups (one field was sown with fungicide- and IMI-treated seeds (seed loading rate 70 g a.s./100 kg seed; analysed seed loading 89 and 107% of nominal for Manager and Hermann varieties, respectively; application rate 200 kg seeds/ha) One field (10 ha at Ihinger Hof and 6.3 ha in Celle) sown with fungicide-treated (EfA®) seeds that served as an experimental control) (Table 5). The crops were sown in the fall of 2009 and study extended over the winter to the spring of 2010 and ended with winter oil-seed flowering in the respective regions. Therefore, there were four field plots in total. All seeds were treated with an abrasion inhibitor (INTECO®) to minimize dust. When guttation was observed in the morning, samples of fluid were collected from plants in segregated areas (n=5 subareas; n=3 each with a volume of 1 mL) and, within 10 h of collection was deep frozen (-20°C) for later analysis. If water was observed in the leaf axils samples were collected and processed as for guttation fluids. The monitoring period began with seedling emergence and lasted for 3 (control) and 8 (CTD) days in the south until the end of October 2009. In the north, no monitoring was possible in autumn 2009 due to late seedling emergence (see Table 6). No guttation fluid was collected from plants in the control treatment (*weakness*). Samples were shipped on dry ice. Analysis was undertaken at BCS (Attachment 9.1) using standard methods. The reference standards were >96% pure for IMI, IMI= and IMI-OH; purity of the isotopically labelled internal standards was not reported (*weakness*). Analysis was by LC-MS/MS. LOQ for IMI, IMI= and IMI-OH was 10 µg/L and the LOD 1 µg/L. Recovery for IMI, IMI= and IMI-OH was 96%(7), 97%(4.8), and 97%(6.4), resp. Residues in guttation fluid were generally greater in the autumn than the spring. There was no statistical analysis of data. Centiles were calculated from the raw data.	**3**
Use of GLP and QA/QC	GLP was followed for analytical work but it was a non-GLP study; signatures present. QA/QC complete with deviations reported.	**3**
Transparency of data	All raw data were provided	**4**

Number of samples and replication	Two treatments each with two sites for a total of 4 fields of different sizes. There were five hives per treatment, each with defined assessment areas associated with each colony.	**2**
Duration of study sufficient to observe trends	Duration of exposure was only 3 and 9 days for the control and CTD treatments in the south and 0 days in the north. The spring duration of exposure was 4 weeks. Appropriate for the objectives of the study.	**4**
Overall evaluation of methods	Computed mean of above	**3.2**
Score for expert judgment on quality the study.	**1**	**3.20**

Relevance

Hazard quotients for mean concentration of IMI in guttation fluid from winter wheat grown for seeds treated with IMI in two locations in Germany (attachment 9.1 Table 10)

Source of sample	Value	IMI in µg/L	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg water/d
Ihinger Hof autumn	Median (n=11)	4070	33	7.3	Adult	8
Ihinger Hof autumn	90th centile (n=11)	6790	54	7.3	Adult	8
Ihinger Hof spring	Median (n=42)	26	0.2	7.3	Adult	8
Ihinger Hof spring	90th centile (n=42)	56	0.4	7.3	Adult	8
Celle autumn	Median (n=20)	51	0	7.3	Adult	8
Celle autumn	90th centile (n=20)	126	1	7.3	Adult	8
Celle spring	Median (n=36)	46	0.4	7.3	Adult	8
Celle spring	90th centile (n=36)	106	0.8	7.3	Adult	8

Residues below the LOD or LOQ are reported as <LOD or <LOQ and summed into the Total Residue value as 1/2 the LOD or the LOQ. Where toxicity values were exceeded by exposure values, relevance is shown with orange or yellow highlight in the Table. Green cells indicate no relevant exposure.

Response 1: Relevance to adverse effects		**Score**
Comparison of exposure value to toxicity value	The median and 90th centile concentrations of IMI measured in guttation fluid at Ihinger Hof in the autumn resulted in exposures that were greater than the LOAED for honeybees.	**4**
Overall evaluation relevance	Computed mean of above score(s)	**4.00**
Score for expert judgment on relevance of the results.	**1**	**4.00**

Response 2: Relevance to adverse effects		**Score**
Comparison of exposure value to toxicity value	The median and 90th centile concentrations of IMI measured in guttation fluid at Ihinger Hof in the spring resulted in exposures that were less than the NOAED for honeybees.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 3: Relevance to adverse effects			Score
Comparison of exposure value to toxicity value	The median and 90th centile concentrations of IMI measured in guttation fluid at Celle in the autumn resulted in exposures that were greater than the NOAED for honeybees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 4: Relevance to adverse effects			Score
Comparison of exposure value to toxicity value	The median and 90th centile concentrations of IMI measured in guttation fluid at Celle in the spring resulted in exposures that were less than the NOAED for honeybees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert Judgement		
QA	Yes	November 16, 2015
SEJ	Yes	Monday, February 15, 2016

(Bayer CropScience 2014a)
Report: Bayer CropScience. 2014. Determination of the Residues of Imidacloprid and its Metabolites 5-Hydroxy Imidacloprid and Imidacloprid Olefin in Bee Relevant Matrices Collected from Treated Cotton During Two Successive Years and in White Clover Planted after Treated Cotton. Research Triangle Park, NC: Bayer CropScience (Unpublished Report). Report M-501306-01-1. 1432 p

The purpose of this study was to determine the concentration of ΣIMD (IMI, IMI=, and IMI-OH) in pollen and nectar (and leaves, soil, and blossoms) from cotton plants grown from seed treated with Gaucho 600 Flowable® and sprayed with Admire Pro Systemic Protectant® for two successive years. In addition, concentrations if IMI in nectar and pollen from white clover grown soils where cotton plants were treated with IMI in the previous year. Only the data on nectar and pollen were analyzed in this WoE.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	All studies were conducted a research farm near Fisk, MO. All agronomic practices and treatments with IMI followed the agricultural norm. Cotton seeds were pima cotton (PHY 805 RF Pima). Source of the seeds of white clover was not reported. Two controls plots were planted with untreated cotton seeds in the first year. In year 2, one control was planted with untreated cotton seed and the other was planted with untreated clover. Controls received no spray applications. Two treated plots received seeds treated with Gaucho® at a rate of 0.375 mg a.s. per seed, equivalent to 53 g a.s./ha in year 1. The same plots were sprayed with Admire Pro® with five sprays 5-8 d apart from BBCH 19–60. All applications were made as foliar sprays (approximately 100 L/ha) from ground-based sprayers with a total rate of 390-400 g a.s./ha. For the plot planted with cotton in year 2, the above applications were repeated in year 2, whereas for the plot planted with white clover in the second year, the above applications were made only in year-1. Concentrations of a.s. in the formulations were verified by analysis. Analyses of IMI in soils were conducted but are not assessed here. Sampling of cotton began 13 to 15 days after the last application of IMI each year. There were five sampling intervals per year targeted for growth stages of BBCH 60, 61, 65, 67, and 69. Five composite samples of cotton blossoms for pollen and nectar processing, and cotton leaves were collected from the treated plots at each sampling interval. Two composite samples of cotton blossoms for pollen and were collected from the control plots at the same sampling intervals. Extrafloral nectar from the sub- and inner-bracteal nectaries was collected using a micropipette and placed into a 2-mL glass collection vial. Nectar from the floral nectary was removed and placed in a separate 2-mL vial. Pollen was removed from the cotton blossoms either by vacuum aspiration with filter tips or by tapping the pollen from the blossom. For clover (year-2), sample collection was targeted for growth stages of BBCH 61, 63, 65, and 67. At the start of blooming, two mesh tunnels were placed in each of the control plots and five on the test plot. Healthy and queen-right honey bees, *Apis mellifera* (purchased from local sources), were used to collect the clover pollen and nectar samples. One bee colony housed in a standard 10-frame Langstroth hive was placed in each tunnel 3-4 d prior to collection of samples. The colonies were equipped with pollen traps. Colonies and tunnels were removed after	**4**

	the end of pollen and nectar sampling. The bee colonies were in good condition, with no visible signs of infection or infestation, throughout their use in the study. Hive-deposited nectar (from uncapped cells) and pollen (from pollen traps attached to the hive) were collected after 3-4 d of foraging. All available pollen and nectar was collected. All samples were protected from sunlight and placed in coolers with ice packs after collection and were frozen within four h of collection or field processing. All samples were placed in frozen storage. Samples were shipped frozen and remained so until analysis. Transit stability samples were prepared to monitor the stability of the analytes during sample collection, transit, and storage. Concentrations in pollen and nectar from control fields varied between plots and years ranged from the <LOD to as large as 28 µg/kg (section 4.2), indicating likely contamination from spray drift. This contamination did not affect the results of the study. All samples were processed and analyzed at Bayer CropScience in RTP, NC. A standard and well-described method was used to analyze samples with LC-MS-MS (Appendix 4). ^{13}C and ^{2}H-labelled internal standards were used in the analysis of purity ranging from 90.7 to 100%; non-labelled standards ranged from 96.7 to 98.8% pure. Samples were analyzed within 5-11 d of extraction. The LOQs for IMI, IMI-OH, and IMI= in nectar and pollen were 1 µg/kg. The LODs were 0.4 and 0.03 µg/kg for IMI in pollen and nectar; 0.5 and 0.7 µg/kg for IMI-OH and 0.3 and 0.6 for IMI=, respectively. The overall mean values of the recoveries for each matrix were within the acceptable range of 73% to 120% (Appendix 4C Sect 2), and the RSDs were from 3 to 17% (<20%). Recovery studies for the storage conditions show no significant loss in storage (Section 3.4). Statistical analyses of the concentration data were reported and various centiles were calculated (Section 3.6.2).		
Use of GLP and QA/QC	The field and sampling phases of this study and the statistical analyses were not conducted under GLP. All analyses were conducted under GLP with full QA and QC. All data provided in the study were QAed		**4**
Transparency of data	All raw data were provided		**4**
Number of samples and replication	There were three trials with each with two controls and two treated plots. Samples of nectar and pollen were collected five times in each year. Samples from clover were collected four times.		**3**
Duration of study sufficient to observe trends	Cotton was sampled at BBCH 60, 61, 65, 67, and 69. Clover was sampled at BBCH 61, 63, 65, and 67. The sampling was appropriate to the design of the study.		**4**
Overall evaluation of methods	Computed mean of above		**3.80**
Score for expert judgment on quality the study.		**1**	**3.80**

Relevance

Hazard quotients for exposures to ΣIMD in honey bees via nectar and pollen in cotton and clover planted in cotton fields previously planted with treated cotton (Sections 3.6.3 to 3.6.6).

Source	Matrix	Conc. ΣIMD µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
Treated cotton 2012	Pollen highest median conc.(n=37)	2.4	0.30	7.3	Larva	124
Treated cotton 2013	Pollen highest median conc.(n=25)	3.2	0.40	7.3	Larva	124
Treated cotton 2012	Pollen highest 90th centile conc.(n=50)	11	1.36	7.3	Larva	124
Treated cotton 2013	Pollen highest 90th centile conc.(n=25)	20	2.48	7.3	Larva	124
Treated cotton 2012	EF nectar highest median conc.(n=38)	4.2	1.23	7.3	Adult	292
Treated cotton 2013	EF nectar highest median conc.(n=25)	11	3.21	7.3	Adult	292
Treated cotton 2012	EF nectar highest 90th centile conc.(n=45)	13	3.80	7.3	Adult	292
Treated cotton 2013	EF nectar highest 90th centile conc.(n=25)	21	6.13	7.3	Adult	292
Treated cotton 2012	F nectar highest median conc.(n=50)	8.6	2.51	7.3	Adult	292
Treated cotton 2013	F nectar highest median conc.(n=25)	12	3.50	7.3	Adult	292
Treated cotton 2012	F nectar highest 90th centile conc.(n=50)	14	4.09	7.3	Adult	292
Treated cotton 2013	F nectar highest 90th centile conc.(n=25)	30	8.76	7.3	Adult	292
Clover 2013	Pollen highest median conc. (n=13)	1.8	0.22	7.3	Larva	124
Clover 2013	Pollen highest 90th centile conc. (n=13)	3.7	0.46	7.3	Larva	124
Clover 2013	Nectar highest median conc.(n=14)	1.6	0.47	7.3	Adult	292

Hazard quotients for exposures to ΣIMD in honey bees via nectar and pollen in cotton and clover planted in cotton fields previously planted with treated cotton (Sections 3.6.3 to 3.6.6).

Source	Matrix	Conc. ΣIMD µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
Clover 2013	Nectar highest 90th centile conc.(n=14)	2.3	0.67	7.3	Adult	292

Where toxicity values were exceeded by exposure values, relevance is shown with yellow highlight in the Table. Green cells are not relevant.

Response 1: Relevance to adverse effects			Score
Comparison of exposure value exceeds toxicity value	The 90th centile concentration of ΣIMD in pollen from treated cotton resulted in an exposure that was less than the NOAED.		0
Overall evaluation relevance	Computed mean of above score(s)		0.00
Score for expert judgment on relevance of the results.		1	0.00

Response 2: Relevance to adverse effects			Score
Comparison of exposure value exceeds toxicity value	The 90th centile concentration of ΣIMD in EF nectar from treated cotton resulted in an exposure that was less than the NOAED.		0
Overall evaluation relevance	Computed mean of above score(s)		0.00
Score for expert judgment on relevance of the results.		1	0.00

Response 3: Relevance to adverse effects			Score
Comparison of exposure value exceeds toxicity value	The 90th centile concentration of ΣIMD in F nectar from treated cotton resulted in an exposure that was >NOAED but less than the LOAED in one of two years only.		2
Overall evaluation relevance	Computed mean of above score(s)		2.00
Score for expert judgment on relevance of the results. Only one of two years.		0.5	1.00

Response 4: Relevance to adverse effects			Score
Comparison of exposure value exceeds toxicity value	The 90th centile concentration of ΣIMD in pollen from clover grown in a previously treated field resulted in an exposure that was less than the NOAED.		0
Overall evaluation relevance	Computed mean of above score(s)		0.00
Score for expert judgment on relevance of the results.		1	0.00

Response 5: Relevance to adverse effects			Score
Comparison of exposure value exceeds toxicity value	The 90th centile concentration of ΣIMD in nectar from clover grown in a previously treated field resulted in an exposure that was less than the NOAED.		0
Overall evaluation relevance	Computed mean of above score(s)		0.00
Score for expert judgment on relevance of the results.		1	0.00

Expert judgment		
QA	Yes	September 26, 2015
SEJ	Yes	October 2, 2015

(Bayer CropScience 2014c, b)
Reports: Bayer CropScience. 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. Monheim, Germany: Bayer CropScience (Unpublished Report). No. M-500724-01-1. 242 p.

and

Bayer CropScience. 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. Monheim, Germany: Bayer CropScience (Unpublished Report). No. M-500734-01-1. 237 p.

These two studies were carried out by the same laboratory using almost identical methods; however, they were carried out at two sites, one in Pforzheim, Zip code; 75177 in Baden-Württemberg (Site-1), and the other in Gäufelden-Öschelbronn, Zip code; 71126, also in Baden-Württemberg (Site 2). Because of the similarities in the methods, they are assessed together as a single study with two sites. The studies were primarily carried out to assess the effects of guttation fluids from sugar beet treated with a seed dressing on honey bees. Only the exposures via guttation fluid are assessed in this WoE.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	The previous history of the sites was provided and they were either planted with winter wheat, summer barley, and were not treated with neonicotinoids in the previous year. No analyses of the soils were conducted (potential weakness). Treated and control sites were 5.5 and 4.7 km apart, for Site 1 and Site 2, respectively. The treated seeds were of the variety “Sabrina”. Each seed “pill” contained a nominal amount of 0.3 mg IMI, 0.6, mg CTD, and 0.08 mg beta-cyfluthrin and two fungicides, Hymexazol + TMTD. Seed pills were analyzed and content was confirmed to be within 10% of nominal (Table 1). Seeding took place on May 15 and 18, 2013 for Site 1 and May 15, 2013 for Site 2 at a rate of 130,000 seeds/ha with target application rates of 78 g CTD, 39 g IMI, and 10.4 g beta-cyfluthrin/ha. Actual application rates for Site 1 were 93.97 g/ha CTD (120% of nominal), 42.55 g/ha IMI (109% of nominal), and 11.4 g/ha beta-cyfluthrin (113% of nominal); and for Site 2 were 79.68 g/ha CTD (102% of nominal), 36.08 g/ha IMI (93% of nominal), and 9.98 g/ha beta-cyfluthrin (96% of nominal). The plants and surrounding non-crop areas were checked for guttation twice a day between sunrise and 12:00 in the first 21 d after the start of exposure and once a day between the same times up to 42 days after the start of exposure for Site 1 and40 days for Site 2. In Site-1, guttation fluid was collected on seven occasions (7, 14, 15, 22, 26, 27, and 29 d after start of exposure), however only six samples were analyzed. In Site-2 guttation fluid was collected on three occasions (12, 16, and 17 d after start of exposure) in capillary tubes and then transferred to microtubes. Samples were pooled. Samples were stored at ≤-16°C after collection and shipped in dry ice then stored at ≤-18°C. Storage	**3**

	spikes and blanks not needed but control plots (=field blanks) were not analyzed (potential weakness). Analysis of guttation fluid was by standard methods using HPLC MS/MS. ^{2}H and/or ^{13}C-labelled internal standards were used in the analysis. The LOQ for CTD, its metabolites TZNG and TZMU and IMI, IMI-OH, and IMI= was 1 µg/kg. The LOD for these substances as 0.1 µg/kg. The LOQ for beta-cyfluthrin was 10 µg/kg and it was never detected. This insecticide is not systemic and would not be expected to translocate into guttation fluid. Analyses were not reported for the control plots (weakness). Guttation in sugar beet was observed infrequently. In controls at Site-1, it was observed on 1 of 42 d and 11 of 42 d in the test group. In the off-crop areas it was observed on 22 and 26 d of 42 d. In controls at Site-2 it was observed on 3 of 40 d and 5 of 40 d in the test group. In the off-crop areas at Site-2, it was observed on 25 and 20 d of 40 d. Number of plants showing guttation was variable (Tables 31 and 32 in respective reports for Sites 1 and 2).	
Use of GLP and QA/QC	Full GLP with QA and QC	**4**
Transparency of data	All raw data were provided. Means and ranges were provided as well.	**4**
Number of samples and replication	There were two sites, each with a treatment and a control. Samples of guttation fluid were taken seven times during the study at Site-1 (only 6 were analyzed) and three times at Site-2.	**2**
Duration of study sufficient to observe trends	The duration of the study was 42 d at Site-1 and 40 d at Site-2. This is considered suitable for the design of the study.	**4**
Overall evaluation of methods	Computed mean of above	**3.4**
Score for expert judgment on quality the study.	**1**	**3.40**

Relevance

Hazard quotients for exposures to ΣIMD in honey bees via guttation fluid from sugar beet (from Table 1 of Appendix 14)

Days after emergence	Value	ΣIMD in µg/L	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg water/d
Site 1, 14	Pooled	50.7	0.41	7.3	Adult	8
Site 1, 15	Pooled	55.9	0.45	7.3	Adult	8
Site 1, 22	Pooled	52.5	0.42	7.3	Adult	8
Site 1, 26	Pooled	30.0	0.24	7.3	Adult	8
Site 1, 27	Pooled	34.8	0.28	7.3	Adult	8
Site 1, 29	Pooled	74.8	0.60	7.3	Adult	8
Site 2, 12	Pooled	4.6	0.04	7.3	Adult	8
Site 2, 16	Pooled	15.2	0.12	7.3	Adult	8
Site 2, 17	Pooled	12.4	0.10	7.3	Adult	8

Where toxicity values were exceeded by exposure values, relevance is shown with yellow highlight in the Table. Green cells are not relevant.

Response 1: Relevance to adverse effects from exposure to IMI in guttation fluid			Score
Comparison of exposure value exceeds toxicity value	The concentrations of ΣIMD in guttation fluid resulted in exposures that were all less than the NOAED on all occasions		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert judgment		
QA	Yes	August 25, 2015
SEJ	Yes	October 2, 2015

(Bayer CropScience 2014d)
Report: Bayer CropScience. 2014. Field Study to Monitor Potential Effects on Honey Bees From Exposure to Guttation Fluid of Winter Barley (W-BAR), Seed-Treated with the Insecticidal Seed-Treatment Product Clothianidin + Imidacloprid FS 100 + 175 G in Germany in 2011/2012. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-501261-01-1. 412 p

This study was undertaken to assess the potential effects of exposure via guttation fluid from barley, grown from seeds of winter barley treated with a seed-treatment product clothianidin (CTD) + imidacloprid (IMI) FS 100 + 175 G. The major part of the study was focused on effects; the only part of the study evaluated here is exposures to IMI in guttation water.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	The hypothesis that was tested was that exposures from use of IMI and (CTD) as a seed dressing in winter barley would not result in buildup such that exposures would exceed toxicity values in guttation fluid. The study protocol was detailed. Five sets of two study fields each were located in Hesse, Germany. Within each set, control and treatment fields were from 0.7 to 2.18 km apart. Prior use of IMI in these fields was not reported and pre-planting analyses of soil from the fields was not reported (potential weakness). All fields were seeded with winter barley (Campanile). Control seeds were treated with Baytan UFB® fungicide and Inteco (an anti-dust additive) only while treated seeds were dressed with Clothianidin + Imidacloprid FS 100 + 175 G; Baytan UFB®; and Inteco. Treatment solution was analyzed to confirm treatment rate (171.3 a.s. g/L). Guttation fluid was collected only when significant guttation was observed early in the morning on the treatment plots. Up to three samples with a volume of 0.6–1 mL were collected using glass Pasteur pipettes. Samples were collected in the early morning before bee flight activity started, between Sept. and Oct. 2011 and Mar. to Apr. 2012. Samples were stored in a cooler but transferred within 10 h a deep freezer (≤-18 °C). Guttation fluid was collected on each treatment plot every second day, if possible. No guttation fluid was collected on control plots (potential weakness). In this study about 70 to 95% of the guttation events occurred between the beginning of foraging and 13h00 in the autumn and spring, respectively. No field blanks or control samples were collected or analyzed (weakness). Soil samples were collected for characterization; however, IMI levels were not measured in the soil. Analysis was conducted in the Bayer laboratories and was by HPLC coupled with electrospray and tandem mass spectrometry (MS/MS) detection. ^{13}C & ^{2}H internal standards were used for IMI. Concentrations of the active metabolites, IMI-OH and IMI= were not analyzed for (potential weakness). For guttation fluid, the LOD and LOQ were clearly described and these were 1 and 10 µg/L, respectively. The mean recoveries were good; 94-102% and the mean RSD value was good 4.7 (<20%). Statistical analysis of residue data was not reported.	**3**
Use of GLP and QA/QC	The study was GLP with full QA/QC. Components that were exceptions to GLP were described and did not affect the quality the study.	**4**

Transparency of data	All raw data were provided in the report. The data were clearly presented in terms of the analytical values (Attachments, Table 6, 7 (New), 8-15. Ranges were provided as well but not means, medians, and centiles. Medians and centiles were calculated from the raw data, assuming that the values <LOD were zero	**4**
Number of samples and replication	For the guttation fluid there were 16–17 samples of three replicates (sometimes two and sometimes six) taken over the five treatment sites in the autumn and in the spring.	**4**
Duration of study sufficient to observe trends	Guttation fluid was sampled from 2011 09 28 to 2011 10 27 and again from 2012 03 16 to 2012 04 17. For the design of the study, these times were considered appropriate.	**4**
Overall evaluation of methods Computed mean of above		**3.80**
Score for expert judgment on quality the study.	**1**	**3.80**

Relevance

Hazard quotients for exposures to IMI in honey bees via guttation fluid from winter barley (data from Attachment 1 Tables 6 to 15)

Season	Value	IMI in µg/L	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg water/d
Autumn	Median	359	3	7.3	Adult	8
Autumn	90th centile	1442	12	7.3	Adult	8
Autumn	99th centile	5853	47	7.3	Adult	8
Spring	Median	7	0.06	7.3	Adult	8
Spring	90th centile	26	0.21	7.3	Adult	8
Spring	99th centile	61	0.49	7.3	Adult	8

Where toxicity values were exceeded by exposure values, relevance is shown with yellow and orange highlight in the Table. Green cells are not relevant.

Response 1: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The median concentration of IMI in guttation fluid collected in autumn resulted in exposures that were all less than the NOAED	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 2: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The 90th centile concentration of IMI in guttation fluid collected in autumn resulted in exposures that were >LOAED	**4**
Overall evaluation relevance	Computed mean of above score(s)	**4.00**
Score for expert judgment on relevance of the results.	**1**	**4.00**

Response 3: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The 99th centile concentration of IMI in guttation fluid collected in spring resulted in exposures that were all less than the NOAED	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Expert judgment		
QA	Yes	September 21, 2015
SEJ	Yes	October 2, 2015

(Bayer CropScience AG 2015a) & (Bayer CropScience 2015d)
Bayer CropScience. 2015. Determination of Residues of Imidacloprid and Clothianidin in Flowers, Leaves, Soil, Nectar and Pollen of Cotton after Seed Treatment with Gaucho® FS (Imidacloprid 600 FS) or Poncho® (Clothianidin 600 FS), or Foliar Application with Provado® 200 SC (Imidacloprid 200 SC) in a Semi-Field Study in Brazil. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-525732-01-1. 449 p

and

Bayer CropScience AG. 2015. Determination of Residues of Imidacloprid and Clothianidin in Flowers, Leaves, Soil, Nectar and Pollen of Cotton after Seed Treatment with Gaucho® FS (Imidacloprid 600 FS) or Poncho® (Clothianidin 600 FS), or Foliar Application with Provado® 200 SC (Imidacloprid 200 SC) in a Semi-Field Study in Brazil. Niefern-Oeschelbronn, Germany: Bayer CropScience AG (Unpublished Report). Report M-512861-01-1. 286 p

Comments on paper. The study was conducted to determine the concentrations of IMI or CTD and their metabolites in pollen and nectar collected forager bees, nectar and pollen from combs, pollen from pollen traps, soil, leaves, and flowers of cotton, following either seed treatment with Gaucho® FS (IMI) or Poncho® (CTD), or sequential foliar applications with Provado® 200 SC (IMI), under use conditions in Brazil. There were two very similar studies carried out in different regions. These were assessed separately. This assessment is only for residues of IMI in pollen and nectar that resulted from treatment of seeds.

Response 1-4: Quality methods		**Score**
Experimental design and hypotheses	The trial was conducted in the region of Lucas do Rio Verde, Province of Mato Grosso in Brazil. The test crop was cotton (*Gossypium* sp. var. FM 975 WS). Details of the field site were provided (Table 7). Cotton was seeded on 2014-04-04 at a rate of 15 kg seed/ha (Table 2). Agronomic practices were according to local farmer practice for cotton (Table 3). The history pesticide use was provided (Table 3). Weather conditions during seeding and treatment were recorded (Table 9). Weather conditions during the study were recorded (Appendix A1 Table 9). The test seed were treated with Gaucho® FS (Imidacloprid 600 FS) at a measured rate of 375 g IMI/100 kg seeds (Table 1). Control seed received no treatments. Honeybees (*Apis mellifera* L. Hymenoptera, Apidae (africanized)) were obtained from Ademar Antonio Buchner (Fazenda Pinesso, Nova Ubirata, Mato Grosso) and contained 10 frames with approximately 5000 bees per colony. Bees were free of disease and parasites. Sampling by honeybees, was conducted in tunnels (42 m x 6 m) covered with mesh to contain the bees. One honeybee colony was used per tunnel. A water supply for the bees was provided near the bee hive in the tunnel. Hives were placed in the tunnels on BBCH stage 62-63 = day zero (0DAS).	

	Forager bees were sampled five times from 1DAS to 12DAS for the preparation of pollen loads and nectar from honey stomachs for subsequent residue analysis. Forager bees were collected at the entrance to the hive using a bee vac. Directly after sampling, the forager bee samples and placed in a portable freezer and stored deep frozen until processing. For preparation of pollen and nectar, bees were taken from the freezer and pollen loads (if any) removed and frozen. The honeybulb was removed from the abdomen and frozen without separation. Pollen and nectar from combs were collected five times from 1DAS to 12DAS. One sample of pollen and one of nectar per colony was taken per sampling (if available. Nectar was collected from combs using a spoon. Pollen was extracted from combs with a pollen extractor. All samples were placed in a portable freezer directly after sampling and stored deep frozen until residue analysis. Pollen could not be collected from pollen traps as it was not attractive to bees. Samples of flowers were collected in field. Pollen was collected directly from the anthers with a pipette and a vacuum pump. Samples were stored in a portable freezer. This was done four times on 1DAS, 2DAS, 4DAS and 7DAS. Soil was sampled for residue analysis. Soil cores (5 cm width) were taken with soil corers to a depth of 20 cm from 12 different locations per plot, pooled, and an aliquot was taken for analysis. Soil samples were stored on blue ice (sampling before sowing) or dry ice (sampling after harvest) during transport and were subsequently stored deep frozen until residue analysis. Treated and untreated samples were kept separately to avoid contamination and other precautions were taken to prevent contamination. All samples for residue analysis were shipped deep frozen from Eurofins (Brazil) to BCS for analysis. The samples were transported by air cargo covered with dry ice. Storage and shipment conditions for residue samples were recorded by a max-min thermometer (Appendix A4, Tables 24&25). Analysis of was performed by HPLC/MS/MS at BCS in Monheim using standard methods and were well described. Reference standards were >97% pure. Internal standards were isotopically labelled and were >99% pure. For IMI, IMI-OH and IMI=, LOQs were 1 µg/kg in nectar and pollen (Recoveries for IMI(RSD) IMI-OH and IMI= from nectar were 100%(4.8), 86%(10.4) and 88%(11.3). resp. From pollen, recoveries were 97%(9), 90%(7.9), and 81%(13.5). resp. For soil, the LOD was 5 µg/kg, recovery was 84%(14) (Report M-512861-01-1; Table 7-32). All but one of the samples of test soil had a detectable amount of IMI (max = 33 µg/kg d.w.) and one of the two samples from the control had a concentration of 1.3 µg/kg. Report M-512861-01-1; Table 49). Two of the five samples of nectar from control bees had concentrations of IMI >LOD. The max value was 11 µg/kg. For pollen collected from anthers in the control, one of five samples had concentrations of IMI >LOD, max = 1.8 µg/kg (***major weakness***) – data in Report M-512861-01-1; Tables 33 & 37. This is likely contamination or labelling errors as	

	there were no residues of IMI in the flowers from control plants (Report M-512861-01-1; Table 42). No statistical analyses were conducted. Maximum concentrations were calculated from the raw data in Report M-512861-01-1.		
Use of GLP and QA/QC	This was a GLP study with full QA/QC. Deviations from the study plan were listed (Section 7) but did not compromise the result of the study. Lack of collection of pollen from cotton by bees did reduce the number of samples and the power of the test.		**4**
Transparency of data	All raw data were provided in Tables.		**4**
Number of samples and replication	There was one site and one treatment with IMI, replicated three times. The control was unreplicated and shared between the other treatments.		**1**
Duration of study sufficient to observe trends	The durations of sampling were sufficient for the objectives of the study.		**4**
Overall evaluation of methods	Computed mean of above		**3.25**
Score for expert judgment on quality the study. Contamination of the nectar from the bees in the control and the control soil was a **major weakness.**		**0.5**	**1.63**

Relevance

Hazard quotients for mean concentration of IMI in pollen and nectar from cotton grown in Brazil site 2 (Tables 33-48)

Source of matrix	Sample	IMI in µg/kg or L	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg diet/d
Cotton	Nectar from comb max (n=6)	2.4	0.30	7.3	Larva	126
Cotton	Nectar from bees max (n=15)	23	2.90	7.3	Larva	126
Cotton	Pollen from comb max (n=8)	2.9	0.85	7.3	Adult	292
Cotton	Pollen from anthers max (n=12)	10.1	2.95	7.3	Adult	292

Values <LOQ were treated as zero. Where toxicity values were exceeded by exposure values, relevance is shown with orange or yellow highlight in the Table. Green cells indicate no relevant exposure.

Response 1: Relevance to adverse effects			**Score**
Comparison of exposure value to toxicity value	The concentrations of IMI measured in nectar sampled from the hive but collected by honey bees foraging on cotton grown from treated seed resulted in exposures that were less than the NOAED for honeybees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 2: Relevance to adverse effects			**Score**
Comparison of exposure value to toxicity value	The concentrations of IMI measured in nectar collected by honey bees foraging on cotton grown from treated seed resulted in exposures that were less than the NOAED for honeybees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 3: Relevance to adverse effects			Score
Comparison of exposure value to toxicity value	The concentrations of IMI measured in pollen sampled from the hive but collected by honey bees foraging on cotton grown from treated seed resulted in exposures that were less than the NOAED for honeybees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 4: Relevance to adverse effects			Score
Comparison of exposure value to toxicity value	The concentrations of IMI measured in pollen from flowers of cotton grown from treated seed resulted in exposures that were less than the NOAED for honeybees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert judgment		
QA	Yes	January 29, 2016
SEJ	Yes	February 5, 2016

(Bayer CropScience 2015a) & (Bayer CropScience AG 2015c)
Paper or report: Bayer CropScience. 2015. Determination of Residues of Imidacloprid and Clothianidin in Flowers, Leaves, Soil, Nectar and Pollen of Cotton after Seed Treatment with Gaucho® FS (Imidacloprid 600 FS) or Poncho® (Clothianidin 600 FS), or Foliar Application with Provado® 200 SC (Imidacloprid 200 SC) in a Semi-Field Study in Brazil. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-525745-01-1. 403 p

and

Bayer CropScience AG. 2015. Determination of Residues of Imidacloprid and Clothianidin in Flowers, Leaves, Soil, Nectar and Pollen of Cotton after Seed Treatment with Gaucho® FS (Imidacloprid 600 FS) or Poncho® (Clothianidin 600 FS), or Foliar Application with Provado® 200 SC (Imidacloprid 200 SC) in a Semi-Field Study in Brazil. Niefern-Oeschelbronn, Germany: Bayer CropScience AG (Unpublished Report). Report M-519517-01-1. 242 p

Comments on paper. The study was conducted to determine the concentrations of IMI or CTD and their metabolites in pollen and nectar collected forager bees, nectar and pollen from combs, pollen from pollen traps, soil, leaves, and flowers of cotton, following either seed treatment with Gaucho® FS (IMI) or Poncho® (CTD), or sequential foliar applications with Provado® 200 SC (IMI), under use conditions in Brazil. There were two very similar studies carried out in different regions. These were assessed separately. This assessment is only for residues of IMI in pollen and nectar that resulted from treatment of seeds.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	The trial was conducted in the region of Luis Eduardo Magalhães, Province of Bahia in Brazil. The test crop was cotton (*Gossypium* sp. var. FB 966 LL). Details of the field site were provided (Table 7). Cotton was seeded on 2014-03-19 at a rate of 15 kg seed/ha (Table 10). Agronomic practices were according to local farmer practice for cotton (Table 8). The history pesticide use (none) was provided (Table 9). Weather conditions during seeding and treatment were recorded (Appendix A2, Tables 16-20). Weather conditions during the study were recorded (Appendix A1 Table 15). The test seed were treated with Gaucho® FS (Imidacloprid 600 FS) at a measured rate of 601 g IMI/100 kg seeds (Table 1). Control seed received no treatments. Honeybees (*Apis mellifera* L. Hymenoptera, Apidae (africanized)) were obtained from Gilmario dos Santos Mendes, Fazenda Olhos D'Agua Ibotirama, Bahia and contained 10 frames with approximately 5000 bees per colony. Bees were free of disease and parasites. Sampling by honeybees, was conducted in tunnels (42 m x 6 m) covered with mesh to contain the bees. One honeybee colony was used per tunnel. A water supply for the bees was provided near the bee hive in the tunnel. Hives were placed in the tunnels on BBCH stage 62-63 day zero (0DAS).	

	Forager bees were sampled five times from 1DAS to 12DAS for the preparation of pollen loads and nectar from honey stomachs for subsequent residue analysis. Directly after sampling, the forager bee samples and placed in a portable freezer and stored deep frozen until processing. For preparation of pollen and nectar, bees were taken from the freezer and pollen loads (if any) removed and frozen. The honeybulb was removed from the abdomen and frozen without separation. Pollen and nectar from combs were collected five times from 1DAS to 12DAS. One sample of pollen and one of nectar per colony was taken per sampling (if available. Nectar was collected from combs using a spoon. Pollen was extracted from combs with a pollen extractor. All samples were placed in a portable freezer directly after sampling and stored deep frozen until residue analysis. Pollen could not be collected from pollen traps as it was not attractive to bees. Samples of flowers were collected in field. Pollen was collected directly from the anthers with a pipette and a vacuum pump. Samples were stored in a portable freezer. Soil was sampled for residue analysis. Soil cores (5 cm width) were taken with soil corers to a depth of 20 cm from 12 different locations per plot, pooled, and an aliquot was taken for analysis. Soil samples were stored on blue ice (sampling before sowing) or dry ice (sampling after harvest) during transport and were subsequently stored deep frozen until residue analysis. Treated and untreated samples were kept separately to avoid contamination and other precautions were taken to prevent contamination. All samples for residue analysis were shipped deep frozen from Eurofins (Brazil) to BCS for analysis. The samples were transported by air cargo covered with dry ice. Storage and shipment conditions for residue samples were recorded by a max-min thermometer (Appendix A4, Tables 30&31). Analysis of was performed by HPLC/MS/MS at BCS in Monheim using standard methods and were well described. Reference standards were >96% pure. Internal standards were isotopically labelled and were >99% pure. For IMI, IMI-OH and IMI=, LOQs were 1 µg/kg in nectar and pollen (Recoveries for IMI(RSD) IMI-OH and IMI= from nectar were 98%(2.9), 92%(3.8) and 86%(13.3). resp. From pollen, recoveries were 107%(7.8), 95%(7), and 81%(13.7). resp. For soil, the LOD was 5 µg/kg, recovery was 100%(6.2) (Report M-519517-01-1; Table 7-32 & 49). One of the samples of test soil had a detectable amount of IMI (10 µg/kg d.w. Report M-519517-01-1; Table 49). Four of the five samples of nectar from control bees had concentrations of IMI >LOD. The max value was 7.1 µg/kg. For pollen collected from anthers in the control, three of five samples had concentrations of IMI >LOD, max = 3.1 µg/kg (*major weakness*) – data in Report M-519517-01-1; Tables 33 & 37. This is likely contamination or labelling errors as there were no residues of IMI in the flowers from control plants (Report M-519517-01-1; Tables 42).	

	No statistical analyses were conducted. Maximum concentrations were calculated from the raw data in Report M-519517-01-1.		
Use of GLP and QA/QC	This was a GLP study with full QA/QC. Deviations from the study plan were listed (Section 7) but did not compromise the result of the study. Lack of collection of pollen from cotton by bees did reduce the number of samples and the power of the study.		**4**
Transparency of data	All raw data were provided in Tables.		**4**
Number of samples and replication	There was one site and one treatment with CTD, replicated three times. The control was unreplicated and shared between the other treatments.		**1**
Duration of study sufficient to observe trends	The durations of sampling were sufficient for the objectives of the study.		**4**
Overall evaluation of methods	Computed mean of above		**3.25**
Score for expert judgment on quality the study. Contamination of the nectar from the bees in the control was contaminated – major weakness**.**		**0.5**	**1.63**

Relevance

Hazard quotients for mean concentration of IMI in pollen and nectar from cotton grown in Brazil site 1 (Tables 33-48)

Source of matrix	Sample	IMI in µg/kg or L	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg diet/d
Cotton	Nectar from comb max (n=15)	0	0.00	7.3	Larva	126
Cotton	Nectar from bees max (n=15)	28	3.53	7.3	Larva	126
Cotton	Pollen from comb max (n=15)	0	0.00	7.3	Adult	292
Cotton	Pollen from anthers max (n=12)	2.4	0.70	7.3	Adult	292

Values <LOQ were treated as zero. Where toxicity values were exceeded by exposure values, relevance is shown with orange or yellow highlight in the Table. Green cells indicate no relevant exposure.

Response 1: Relevance to adverse effects		**Score**
Comparison of exposure value to toxicity value	The concentrations of IMI measured in nectar sampled from the hive but collected by honey bees foraging on cotton grown from treated seed resulted in exposures that were less than the NOAED for honeybees.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 2: Relevance to adverse effects		**Score**
Comparison of exposure value to toxicity value	The concentrations of IMI measured in nectar collected by honey bees foraging on cotton grown from treated seed resulted in exposures that were less than the NOAED for honeybees.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 3: Relevance to adverse effects			Score
Comparison of exposure value to toxicity value	The concentrations of IMI measured in pollen sampled from the hive but collected by honey bees foraging on cotton grown from treated seed resulted in exposures that were less than the NOAED for honeybees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 4: Relevance to adverse effects			Score
Comparison of exposure value to toxicity value	The concentrations of IMI measured in pollen from flowers of cotton grown from treated seed resulted in exposures that were less than the NOAED for honeybees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert judgment		
QA	Yes	January 29, 2016
SEJ	Yes	February 5, 2016

(Bayer CropScience 2015b) & (Bayer CropScience AG 2015b)
Paper or report: Bayer CropScience. 2015. Determination of Residues of Imidacloprid and Clothianidin in Flowers, Leaves, Soil, Nectar and Pollen of Soybean after Seed Treatment with Gaucho® FS (Imidacloprid 600 FS) or Poncho® (Clothianidin 600 FS), or Foliar Application with Connect® Imidacloprid & Beta-Cyfluthrin 112.5 SC) in a Semi-Field Study in Brazil. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-525757-01-1. 405 p

and

Bayer CropScience AG. 2015. Determination of Residues of Imidacloprid and Clothianidin in Flowers, Leaves, Soil, Nectar and Pollen of Soybean after Seed Treatment with Gaucho® FS (Imidacloprid 600 FS) or Poncho® (Clothianidin 600 FS), or Foliar Application with Connect® (Imidacloprid & Beta-Cyfluthrin 112.5 SC) in a Semi-Field Study in Brazil. Niefern-Oeschelbronn, Germany: Bayer CropScience AG (Unpublished Report). Report M-515291-01-1. 241 p

Comments on paper. The study was conducted to determine the concentrations of IMI or CTD and their metabolites in pollen and nectar collected by forager bees, nectar and pollen from combs, pollen from pollen traps, soil, leaves, and flowers of cotton, following either seed treatment with soybean (*Glycine max*.), following either a seed treatment with Gaucho® FS (IMI) or Poncho® (clothianidin), or sequential foliar applications with Connect® 112.5 SC (IMI + beta-cyfluthrin, 100 + 12.5 SC), under use conditions in Brazil. This assessment is only for residues of IMI in pollen and nectar that resulted from treatment of seeds.

Response 1-2: Quality methods		**Score**
Experimental design and hypotheses	The trial was conducted in the region of region of Itapeva, Province of São Paulo, Brazil. The test crop was soybean (*Glycine max*); variety BMX Potencia RR. Details of the field site were provided (Table 6). Seeds were sown on 2013-12-06 at a rate of 90 kg seed/ha (Table 9). Agronomic practices were according to local farmer practice for cotton (Table 7). The history pesticide use was provided (Table 8). Weather conditions during seeding and treatment were recorded (Table 9). Weather conditions during the study were recorded (Appendix A1 Table 14). The test seed was treated with (Gaucho® FS, Imidacloprid 600 FS) at a measured rate of 124.5 g IMI/100 kg seeds (Table 1). Seeds were sown on 2013-12-06. Control seed received no treatments. Honeybees (*Apis mellifera* L. Hymenoptera, Apidae (Africanized)) were obtained from Carmo Henrique Contieri, Rua Floriano Peixoto, 266 – Capão Bonito, São Paulo, Brazil and contained 6 frames with approximately 8000 bees per colony. Bees were free of disease and parasites. Sampling by honeybees, was conducted in tunnels (45 m x 6 m) covered with mesh to contain the bees. One honeybee colony was used per tunnel. A water supply for the bees was provided near the bee hive in the tunnel. Hives were placed in the tunnels on BBCH stage 64 2014-02-04 = day zero (0DAS). Provision of water for the bees in the tunnels was not reported.	**3**

	Forager bees were sampled five times from 1DAS to 10DAS for the preparation of pollen loads and nectar from honey stomachs for subsequent residue analysis. Directly after sampling, the forager bee samples and placed in a portable freezer and stored deep frozen until processing. For preparation of pollen and nectar, bees were taken from the freezer and pollen loads (if any) removed and frozen. There were insufficient amounts of pollen for analysis (*weakness*). The honey-bulb was removed from the abdomen and frozen without separation. Pollen and nectar from combs were collected five times from 1DAS to 10DAS. One sample of pollen and one of nectar per colony was taken per sampling (if available, nectar was collected from combs using a spoon. Pollen was extracted from combs with a pollen extractor. All samples were placed in a portable freezer directly after sampling and stored deep frozen until residue analysis. Soil was sampled for residue analysis. Soil cores (5 cm width) were taken with soil corers to a depth of 20 cm from 5 different locations per plot, pooled, and an aliquot was taken for analysis. Soil samples were stored frozen during transport and were subsequently stored deep frozen until residue analysis. Treated and untreated samples were kept separately to avoid contamination and other precautions were taken to prevent contamination. All samples for residue analysis were shipped deep frozen from Eurofins (Brazil) to BCS for analysis. The samples were transported by air cargo covered with dry ice. Storage and shipment conditions for residue samples were recorded by a max-min thermometer (Appendix A4, Table 26 & 27). Analysis of was performed by HPLC/MS/MS at BCS in Monheim using standard methods and were well described. Reference standards were >96% pure. Internal standards were isotopically labelled and were >99% pure. For IMI, IMI-OH, and IMI=, LOQs were 1 µg/kg (Report M-M-515291-01-1; Table 37). Recoveries for IMI(RSD), TZNG, and TZMU from nectar were 98%(4.4), 92%(4.2) and 74%(5.6), resp. (Report M- M-515291-01-1; Tables 9-11). From pollen, recoveries were 80%(12.9), 89%(15.9), and 79%(20.7). resp. For soil, the LOQ was 5 µg/kg and recovery was 96%(5.9) (Report M-515291-01-1; Tables 51 & 33). The two samples of soil from the control plot did not have detectable amounts of IMI. (Report M- M-515291-01-1; Table 51). The test plots had three samples >LOD (max = 21 µg/kg d.w.) (Report M- M-515291-01-1; Table 51). None of the control samples of nectar or pollen has detectable concentration of IMI or metabolites (Report M- M-515291-01-1; Table 35, 39) No statistical analyses were conducted. Maximum concentrations were calculated from the raw data in Report M-515291-01-1.	
Use of GLP and QA/QC	This was a GLP study with full QA/QC. Deviations from the study plan were listed (Section 7) but did not compromise the result of the study.	**4**
Transparency of data	All raw data were provided in Tables.	**4**

Number of samples and replication	There was one site and one treatment with CTD, replicated three times. The control was unreplicated and shared between the other treatments.	**1**
Duration of study sufficient to observe trends	The durations of sampling were sufficient for the objectives of the study.	**4**
Overall evaluation of methods	Computed mean of above	**3.2**
Score for expert judgment on quality the study.	**1**	**3.20**

Relevance

Hazard quotients for mean concentration of IMI in pollen and nectar from soybean grown from treated seed in Brazil (Tables 36, 40)

Source of matrix	Sample	ΣIMD in µg/kg or L	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg diet/d
Soybean	Nectar from bees max (n=30)	0	0.00	7.3	Adult	292
Soybean	Pollen from comb max (n=15)	3.6	0.45	7.3	Larva	126

Values <LOQ were treated as zero. Where toxicity values were exceeded by exposure values, relevance is shown with orange or yellow highlight in the Table. Green cells indicate no relevant exposure.

Response 1: Relevance to adverse effects		**Score**
Comparison of exposure value to toxicity value	The concentrations of IMI measured in nectar collected by honeybees foraging on soybean grown from treated seed resulted in exposures that were less than the NOAED for honeybees.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 2: Relevance to adverse effects		**Score**
Comparison of exposure value to toxicity value	The concentrations of IMI measured in samples of pollen from the hive but collected by honeybees foraging on soybean grown from treated seed resulted in exposures that were less than the NOAED for honeybees.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Expert judgment		
QA	Yes	January 29, 2016
SEJ	Yes	February 5, 2016

(Bernal et al. 2010)
Paper: Bernal J, Garrido-Bailón E, Del Nozal MJ, González-Porto AV, Martín-Hernández R, Diego JC, Jiménez JJ, Bernal JL, Higes M. 2010. Overview of pesticide residues in stored pollen and their potential effect on bee colony (*Apis mellifera*) losses in Spain. *Journal of Economic Entomology* 103:1964-1971

The objective of this study was to evaluate residues pesticides in stored pollen from honeybee colonies and their possible impact on losses of honeybees in Spain. The study was initiated in 2006 and continued in 2007. Causal linkages to loss of bees could not be made so this study was included in the field-exposure WoEs.

Response 1: Quality methods			**Score**
Experimental design and hypotheses	Samples were taken from 1,021 representative apiaries from 17 different regions in Spain. Sites were randomly selected (method not described) and sampled in 2006 and 2007. In these two years, 448 and 397 apiaries were sampled in spring and 92 and 84 in autumn, resp. About 33% were from migratory (pollination services) colonies. The average number of beehives per apiary was 367. More than 110 g of stored pollen was randomly and aseptically extracted from the pollen cells of combs located in the brood chamber (number of hives not stated) and was stored at -20°C in the dark until analysis. Of the sample, 100 g was used for residue analysis and 10 g for palynological analysis to identify associated flora. The pollen samples were collected prior to treatment of the hives with control agents for parasites and diseases. Controls and field-blanks were not reported (weakness) Analysis of IMI was conducted with a published method[5] using LC/MS/MS. Extraction of the sample was described in detail. Purity of standards was not reported and it appears that isotopically labeled internal standards were not used. Percent recovery from the referenced method was 81 to 84% with a RSD of 10-15% and LOD reported in this study was 0.4 µg/kg. Mean values and their confidence intervals) were calculated using Statgraphics Centurion XV from Statpoint Technologies, Inc.		**2**
Use of GLP and QA/QC	Not a GLP study and use of QA and QC was not reported. However, the methods were described in sufficient detail.		**2**
Transparency of data	Raw data were not provided. Summary data with mean, median max. and min. values were provided in Tables.		**0**
Number of samples and replication	A large number (1,021) of sites were sampled but only some four times in the two-year period. IMI was not detected in any samples over the two years even though pollen from maize was identified in the samples.		**2**
Duration of study sufficient to observe trends	Only four sampling periods but more detects of pesticides other than IMI in the spring (Table 1) than the autumn (Table 2) consistent with use of these pesticides in protection of crops). Detects were related to periods of pesticide use. But no long term trends could be characterized.		**3**
Overall evaluation of methods	Computed mean of above		**1.80**
Score for expert judgment on quality the study.		**1**	**1.80**

[5] Bonmatin J M, Moineau I, Charvet R, Fleche C, Colin M E, Bengsch E R. 2003. A LC/APCI-MS/MS method for analysis of imidacloprid in soils, in plants, and in pollens. Analytical Chemistry 75:2027-2033.

Response 1: Relevance to adverse effects			Score
Comparison of exposure value exceeds toxicity value	IMI was not detected in any of the samples. No relevance.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert judgment		
QA	Yes	September 23, 2015
SEJ	Yes	October 13, 2015

(Botias et al. 2015)
Paper or report: Botias C, David A, Horwood J, Abdul-Sada A, Nicholls E, Hill E, Goulson D. 2015. Neonicotinoid residues in wildflowers, a potential route of chronic exposure for bees. Environmental Science & Technology 49:12731-12740

Comments on paper. This study reported concentrations of neonicotinoids in soil, pollen, and nectar sampled from fields planted with winter oilseed rape (OSR) in spring 2013 and soil, and nectar and pollen from wildflowers in the field margins from fields planted with OSR and winter wheat (WW). Pollen collected from forager honeybees was also sampled. Analytes were IMI, thiamethoxam (TMX), clothianadin (CTD) and thiacloprid. Only the results for IMI were included in this WoE.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	This study was conducted at five farms in East Sussex, U.K in 2013. Seven fields of winter-OSR (sown late August 2012) and five fields of WW (sown late September 2012) fields were selected at random (methods nor reported) from five farms in East Sussex, South-East England, U.K. The selected fields had varying cropping history and pesticide use (data provided in Tables S1a-S1g) but followed normal farming practices in the region. In 2013 the seeds of OSR were all treated with Cruiser® seed dressing in 2012 (280 g/L TMX, 8 g/L fludioxonil, and 32.2 g/L metalaxyl-M). The WW seeds were treated with Redigo Deter® (50 g/L prothioconazole and 250 g/L CTD). The seeds used in these fields were not analyzed, although "representative" seeds used in the region were (*potential weakness*). The areas of the fields and the climatic conditions were not reported (*potential weakness*). Soil samples were taken in the fields and the margins and the presence of residues of CTD, TMX, and IMI were confirmed by analysis (data not discussed in this WoE). To obtain pollen samples, flowers of oilseed rape (≈1,000 flowers) were gathered and stored on ice in coolers in the field and then frozen immediately at -80°C until further handling. Flowers were thawed and dried in an incubator at 37 °C for 24 hours to facilitate pollen release from the anthers. After drying, flowers were brushed over food strainers to separate pollen from anthers and sifted through multiple sieves of decreasing mesh size (mesh sizes from 250 to 45 µm. Collection of nectar from flowers was performed in situ through capillary action into glass Life-stage and, once filled; both ends were sealed with putty. Samples were pooled for analysis to obtain the volume needed for analysis. To collect pollen from foraging honeybees, one hive was placed in the vicinity of OSR fields at each farm at the beginning of the flowering period (May 2013) and remained at the same sites until the end of August 2013. The hives were equipped with pollen traps during four consecutive d at the beginning of June 2013 and for four d in mid-August 2013 to collect pollen loads from the returning honey bee foragers during the OSR blooming period, and also when no OSR was in flower. After 4 d, the traps were removed and the pollen loads were stored on ice and then at −80°C in the laboratory until analysis. Pollen loads within each sample were sorted by eye according to color, texture, size, and shape as indicators of different pollen types. All pollen types were separately weighed to calculate their relative abundance within the samples. A representative sample of loads from each pollen type was mounted,	**1**

	and pollen grains were identified under a microscope following standard methods and using reference specimens and published reference collections. Pollen and nectar samples were stored at -80°C prior to pesticide extraction and analysis. Analyses were performed within 10 months after collection. Transport and storage blanks and spikes were not reported but the storage conditions appear to be appropriate and this was not identified as a weakness. Analysis was well described in the paper and the SI. Samples were extracted using the QuEChERS method and analysis was by Ultra-high-performance liquid chromatography and tandem mass spectrometry (UHPLC−MS/MS). IMI was 0.5 µg/kg. For pollen, it was 0.48 µg/kg. (SI Table S5a). Recovery (SD) from nectar for IMI was 91%(11). Recovery (SD) from pollen for IMI was 92%(18). (SI Table S5b). Statistical methods were described but some of the data were incorrectly analyzed. The data were shown to not be normally distributed but arithmetic means and SDs were presented. The only useful data provided was the median (*weakness*). Upper centiles were not provided. Transformation of data between the MDL and MQL would have biased the arithmetic means. Data for pollen and nectar from OSR and were taken from Table 2 as were data for pollen collected by bees. The data for pollen from wildflowers in field margins were taken from SI Tables S2a – S2j and for nectar from Tables S3a-S3b. Medians and centiles were calculated from the raw data. Each measure was treated as independent.	
Use of GLP and QA/QC	This was not a GLP study and the QC was limited to the analysis.	**2**
Transparency of data	Raw data for concentrations of neonicotinoids in pollen and nectar from the field margins were provided but not for OSR or pollen collected by bees. Data were presented as summary values in graphs and Tables.	**2**
Number of samples and replication	There were five sites with seven reps for pollen and nectar of OSR and 5 for WW. For the samples of pollen and nectar from wildflowers in margins, there were 5 reps for OSR and WW.	**4**
Duration of study sufficient to observe trends	The period of flowering for the wild plants was not reported but it was assumed that the sampling of wildflowers occurred over the flowering period.	**4**
Overall evaluation of methods	Computed mean of above	**2.6**
Score for expert judgment on quality the study.	**1**	**2.60**

Relevance

Hazard quotients for exposures to IMI in pollen from wildflowers in field margins of OSR and WW in the UK. (Table 2, SI Tables S2a – S2j & S3a-S3b)

Source of matrix	Sample	IMI in µg/kg or L	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg diet/d
OSR 2013 UK	Pollen collected from plants max (n=21)	0	0.00	7.3	Larva	124

Hazard quotients for exposures to IMI in pollen from wildflowers in field margins of OSR and WW in the UK. (Table 2, SI Tables S2a – S2j & S3a-S3b)

Source of matrix	Sample	IMI in µg/kg or L	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg diet/d
OSR 2013 UK	Pollen collected from plants 50th centile (n=21)	0	0.00	7.3	Larva	124
OSR 2013 UK Jun	Pollen collected by bees max (n=34)	25.5	3.16	7.3	Larva	124
OSR 2013 UK Jun	Pollen collected by bees 50th centile (n=34)	0	0.00	7.3	Larva	124
OSR 2013 UK Aug	Pollen collected by bees max (n=46)	2.5	0.31	7.3	Larva	124
OSR 2013 UK Aug	Pollen collected by bees 50th centile (n=46)	0	0.00	7.3	Larva	124
OSR 2013 UK	Nectar collected from plants max (n=13)	0	0.00	7.3	Adult	292
OSR 2013 UK	Nectar collected from plants 50th centile (n=13)	0	0.00	7.3	Adult	292
Wild flowers from margins of OSR and WW 2013 UK	Pollen collected from plants 90th centile (n=95)	0	0.00	7.3	Larva	124
Wild flowers from margins of OSR and WW 2013 UK	Pollen collected from plants 50th centile (n=95)	0	0.00	7.3	Larva	124
Wild flowers from margins of OSR and WW 2013 UK	Nectar collected from plants 90th centile (n=28)	0	0.00	7.3	Adult	292
Wild flowers from margins of OSR and WW 2013 UK	Nectar collected from plants 50th centile (n=28)	0	0.00	7.3	Adult	292

Response 1: Relevance to adverse effects			**Score**
Comparison of exposure value to toxicity value	The maximum concentration of IMI measured in pollen collected from OSR plants resulted in exposures that were less than the NOAED for bees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 2: Relevance to adverse effects			**Score**
Comparison of exposure value to toxicity value	The maximum concentration of IMI measured in pollen collected from foraging bees near OSR plants in June resulted in exposures that were less than the NOAED for bees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 3: Relevance to adverse effects			Score
Comparison of exposure value to toxicity value	The maximum concentration of IMI measured in pollen collected from foraging bees near OSR plants in Aug resulted in exposures that were less than the NOAED for bees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 4: Relevance to adverse effects			Score
Comparison of exposure value to toxicity value	The maximum concentration of IMI measured in nectar collected from OSR flowers resulted in exposures that were less than the NOAED for bees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 5: Relevance to adverse effects			Score
Comparison of exposure value to toxicity value	The maximum concentration of IMI measured in pollen collected from wild-flowers in the margins of OSR and WW fields resulted in exposures that were less than the NOAED for bees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 6: Relevance to adverse effects			Score
Comparison of exposure value to toxicity value	The maximum concentration of IMI measured in nectar collected from wild-flowers in the margins of OSR and WW fields resulted in exposures that were less than the NOAED for bees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert judgment		
QA	Yes	January 26, 2016
SEJ	Yes	February 5, 2016

(Chauzat et al. 2006)
Paper: Chauzat M-P, Faucon J-P, Martel A-C, Lachaize J, Cougoule N, Aubert M. 2006. A survey of pesticide residues in pollen loads collected by honey bees in France. Journal of Economic Entomology 99:253-262.

The purpose of this paper was to characterize potential exposures to IMI in pollen collected from foraging bees in five regions of France (Eure, Yonne, Indre, Gers, and Gard). Concentrations of many pesticides were measured; only data for IMI were included in the assessment. The non-toxic metabolite of IMI, 6-chloronicotinic acid, was also analyzed for but was not included in the assessment.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	Apiaries were in five sites in France. In each site, apiaries were chosen depending on their environment (not clear what this means). At each apiary, five colonies were randomly selected and sampled four times and were visited four times a year. Two additional colonies were fitted with pollen traps and beekeepers asked to initiate collection of pollen one-week before each sampling event. Pollen was collected in paper envelops by the beekeepers every 2 or 3 d, depending on the season and immediately frozen in the beekeeper's freezer (temperature not stated). After collection, samples were transported in a cool-box and stored in the laboratory at -20°C until analysis. Controls (field-blanks) were not reported (weakness). Duplicate analyses were conducted at two laboratories: Agence Française de Sécurité Sanitaire des Aliments (AFSSA) laboratory, Sophia-Antipolis, France, and Groupement Interrégional sur les Recherches des Produits Agropharmaceutiques (GIRPA) laboratory, Angers, France. References to the method were not provided. Extraction was described and analysis was by LC/MS/MS. Standards were not described and percent recovery was not reported (weakness). The LOD for IMI was reported as 0.2 µg/kg and the LOQ was 1 µg/kg. IMI was detected in 40 samples of pollen in a total of 81 samples (49.4%). Frequency of detection was greater in spring than in other seasons. Methods of statistical analysis were based on frequency of detection. SAS for Windows version 8 was used and Fisher's exact probability and chi-square tests were performed to identify areal and temporal significance of detections. Threshold of significance was p = 0.05.	**2**
Use of GLP and QA/QC	This was not a GLP and data on QA and QC were not provided. The methods of collection of samples were incompletely described in terms of instructions to the bee-keepers and the analytical method were not described in detail but were carried out at established laboratories.	**1**
Transparency of data	Raw data were not provided. Frequency of detection, mean, maximum, and minimum values were provided but no indication of variance. The means were arithmetic means; the arithmetic median of the LOD and the maximum value analyzed. This mean value is unusable for hazard assessment. For IMI, 40 of 81 samples analyzed were >LOD (0.2 µg/kg). Upper centiles could not be calculated.	**1**
Number of samples and replication	There were five sites in each of five regions. The number of replicate hives for pollen was two at each site and pollen was pooled for analysis.	**3**

Duration of study sufficient to observe trends	The study started in Oct-Nov 2002 and was completed in Oct 2003. Samples were taken four times during that period. Duration is appropriate for the design of the study.		**4**
Overall evaluation of methods	Computed mean of above		**2.2**
Score for expert judgment on quality the study.		**1**	**2.20**

Relevance

Hazard quotients for exposures to IMI in honey bees via pollen from multiple sources (data from Table 2).

Source	Matrix	Max Conc. IMI µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
Pollen traps on hives in France	Pollen from hives, max	5.7	0.7	7.3	Larva	124

Where toxicity values were exceeded by exposure values, relevance is shown with yellow highlight in the Table. Green cells are not relevant.

Response 1: Relevance to adverse effects			**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in pollen resulted in exposures that were all less than the NOAED.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert judgment		
QA	Yes	September 26, 2015
SEJ	Yes	October 13, 2015

(Chauzat et al. 2011)
Paper: Chauzat M-P, Martel A-C, Cougoule N, Porta P, Lachaize J, Zeggane S, Aubert M, Carpentier P, Faucon J-P. 2011. An assessment of honeybee colony matrices, *Apis mellifera* (Hymenoptera: Apidae) to monitor pesticide presence in continental France. *Environmental Toxicology and Chemistry* 30:103-111.

This study was a continuation of a previous study[6] and was conducted at the same locations in France (Eure, Yonne, Indre, Gers, and Gard) but it was not clear if these were the same sites as in the earlier study. Concentrations of many pesticides were measured in bees, pollen loads, honey and beeswax; only data for IMI were included in the assessment. The non-toxic metabolite of IMI, 6-chloronicotinic acid, was also analyzed for but was not included in the assessment.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	A total of 24 apiaries were selected at the five locations. All were in agricultural areas (sunflower, canola, chestnut, and local mixed-flower) with the exception of one site in Gard (Mediterranean forest and scrub). The number of hives surveyed was kept constant by replacing any dead colony by another randomly selected in the same apiary. Samples were transported in a clean, insulated cooler and stored at -18°C until analysis. A total of 120 colonies were monitored. Colonies were visited four times per year; at the end of winter (March–April, visit A), before summer (May–June, visit B), during summer (July–August, visit C), and before winter (October–November, visit D). Adult bees were sampled at all visits; honey at visits B, C, and D; and beeswax at visit D only. Adult bees (ca. 500 individuals, foragers) were collected directly from outer frames by shaking individuals down into a paper envelope. Samples were pooled across the site. Honey was collected directly from hives. Uncapped cells were preferably chosen at sites B and C to measure concentrations in fresh honey. Mature honey from capped cells was chosen at visit D to measure pesticides in winter reserves. As for bees, samples were pooled across hives in the apiary, here analyses were assumed to represent IMI in the bees (body dose). IMI was not analyzed for in beeswax. At each apiary, two extra colonies were selected for the collection of pollen loads with pollen traps. Beekeepers asked to initiate collection of pollen one-wk before each sampling event. Pollen was collected every 2 or 3 d, depending on the season and immediately frozen in the beekeeper's freezer (temperature not stated but assumed to be as in the previous study (-20°C). Pollen samples were pooled by apiary for analysis. Use of controls (field-blanks) were not reported (weakness) Analyses were conducted at the Groupement Interrégional sur les Recherches des Produits Agropharmaceutiques (GIRPA) laboratory, Angers, France. References to the methods were not provided. Extraction was described in detail and analysis was by LC/MS/MS. Standards were provided by Cluzeau Info Labo but purity was not described for individual compounds. Percent recovery was between 70 and 120%. The LOD and LOQ. Although some analyses were conducted with an in internal standard, the analytes were not identified (weakness). Blank and spikes matrix was used for QA of the analyses in the laboratory but source of the matrix was not identified. LODs and LOQs	**2**

[6] Chauzat M-P, Faucon J-P, Martel A-C, Lachaize J, Cougoule N, Aubert M. 2006. A survey of pesticide residues in pollen loads collected by honey bees in France. *Journal of Economic Entomology* 99:253-262

	for IMI in bees, pollen, and honey were 0.3 & 1.0; 0.2 & 1.0; and 0.3 & 1.0 µg/kg, resp. Statistical analyses were conducted but not clearly described. From Fig. 2 and the text, the chi-square test was used to test for significant temporal differences in frequency of detections.	
Use of GLP and QA/QC	This was not a GLP but data on QA were provided. The methods of collection of samples were incompletely described in terms of instructions to the bee-keepers and the analytical method were not described in detail but were carried out at established laboratories.	**2**
Transparency of data	Raw data were not provided. Frequency of detection, mean, maximum, and minimum values were provided but no indication of variance. As in the previous study, concentrations tended to be greater in pollen and honey the spring samples. The means were arithmetic means; the arithmetic median of the LOD and the maximum value analyzed. This mean value is unusable for hazard assessment. For IMI, 21 of 187 samples of bees analyzed were >LOD (0.3 µg/kg). Of 185 pollen samples analyzed 75 were >LOD (0.2 µg/kg). Of 239 analyses of honey, 52 were > LOD (0.3 µg/kg). Upper centiles could not be calculated.	**1**
Number of samples and replication	There were five sites in each of four regions and four in the remaining region. The number of replicate hives for pollen was two at each apiary and pollen was pooled for analysis. There were five hives for collection of honey and bees at each apiary and samples were pooled in the site.	**3**
Duration of study sufficient to observe trends	The study started in Oct 2002 and was completed in Sep 2005. Samples were taken four times each year during that period. Duration is appropriate for the design of the study.	**4**
Overall evaluation of methods	Computed mean of above	**2.40**
Score for expert judgment on quality the study.	**1**	**2.40**

Relevance

Hazard quotients for exposures to IMI in honey bees via pollen from and honey from hives and concentrations measured in bees (data from Tables 2&3).

Source	Matrix	Conc. IMI µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
Pollen traps on hives in France	Pollen from hives, max	5.7	0.7	7.3	Larva	124
Honey from hives in France	Honey from hives	1.8	0.5	7.3	Adult	292
Bees collected from hives in France	Conc. In bee, max	11	1.1	7.3	Adult	NA

Where toxicity values were exceeded by exposure values, relevance is shown with yellow highlight in the Table. Green cells are not relevant.

Response 1: Relevance to adverse effects		**Score**
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in pollen from hives across France resulted in exposures that were all less than the NOAED	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 2: Relevance to adverse effects			Score
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in honey from hives across France resulted in exposures that were all less than the NOAED		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 3: Relevance to adverse effects			Score
Comparison of exposure value exceeds toxicity value	The concentrations of IMI in bees from hives across France resulted in exposures that were all less than the NOAED		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert judgment		
QA	Yes	September 26, 2015
SEJ	Yes	October 13, 2015

(Kasiotis et al. 2014)
Paper or report: Kasiotis KM, Anagnostopoulos C, Anastasiadou P, Machera K. 2014. Pesticide residues in honeybees, honey and bee pollen by LC-MS/MS screening: reported death incidents in honeybees. Science of the Total Environment 485-486:633-642.

The aim of this paper was to investigate deaths of bees in several areas of Greece. A method of analysis was developed and some samples were analyzed. As the samples were not taken at random and focused on incidents they are not representative of typical exposures. However, the data were included in the WoE analysis with the caveat. Only data for IMI were assessed.

Response 1: Quality methods			**Score**
Experimental design and hypotheses	The method of analysis was well described but sample collection was poorly described. Control samples of honeybees, bee pollen and honey were obtained from beehives used for organic apiculture. The investigated samples were collected by individuals or public authorities who observed incidents of bee death. Bees were collected very near or at the entrance of the hives; pollen was collected from the hives. No description of the collection of honey was provided (weakness). Other than cooling the samples with ice or dry-ice, no details of the collection procedures or avoidance of contamination were provided and control samples (field blanks) were not collected at the same time as the test samples (**major weakness**). After arrival at the laboratory, the samples were stored at −20°C until analysis. Isotopically labelled standards were not used (potential weakness). The analytical methods were clearly described and used a Triple Quad LC/MS for quantitation. Certified standards were obtained from a commercial supplier and were >90% pure; however, data for individual compounds were not provided. LOD and LOQ for IMI were 0.4 and 1.5 µg/kg, resp. Recovery for IMI ranged from 73 to 84% and RSDs from 8 to 15% (Table 3). Individual analytical data for the samples were provided in SI Table S12. Statistical analysis was not reported.		**2**
Use of GLP and QA/QC	Not a GLP study. Protocol provided but lacking some detail. Some QC on the analysis but no QA on field samples.		**1**
Transparency of data	Raw data were provided in SI.		**4**
Number of samples and replication	Several sites (>10) were sampled and some included multiple samples (from 1 to 5).		**3**
Duration of study sufficient to observe trends	The study began in May 2011 and ended in July 2013, duration sufficient for the objectives of the study.		**4**
Overall evaluation of methods	Computed mean of above		**2.8**
Score for expert judgment on quality the study (one major weakness).		**0.5**	**1.40**

Relevance
Of 14 samples of pollen analyzed from hives with putative poisoning, two contained IMI >LOD. Of the 44 sample of bees analyzed, two samples contained IMI >LOD. None of the 13 samples of honey contained IMI >LOD.

Hazard quotients for exposures to IMI in honey bees via pollen from and honey from hives and concentrations measured in bees. All samples from hives with suspected poisoning (from SI Table S12).

Source	Matrix	Conc. IMI µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
Greece 2011-2013	Pollen from hive, median	0	0.0	7.3	Larva	124
Greece 2011-2013	Pollen from hive, 95th centile	73	9.1	7.3	Larva	124
Greece 2011-2013	Honey from hive, median	0	0.0	7.3	Adult	292
Greece 2011-2013	Honey from hive, 95th centile	0	0.0	7.3	Adult	292
Greece 2011-2013	Conc. in bee, median	0	0.0	7.3	Adult	292
Greece 2011-2013	Conc. in bee, 95th centile	0	0.0	7.3	Adult	292

Response 1: Relevance to adverse effects from exposure to IMI in pollen.			**Score**
Comparison of exposure value exceeds toxicity value	The 95th centile concentrations of IMI in pollen was greater than the NOAED		**4**
Overall evaluation relevance	Computed mean of above score(s)		**4.00**
Score for expert judgment on relevance of the results.		**1**	**4.00**

Response 2: Relevance to adverse effects to IMI in honey.			**Score**
Comparison of exposure value exceeds toxicity value	The 95th centile concentrations of IMI in honey resulted in exposures that were all less than the NOAED		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 3: Relevance to adverse effects from exposure to IMI in pollen.			**Score**
Comparison of exposure value exceeds toxicity value	The 95th centile concentrations of IMI in bees resulted in exposures that were all less than the NOAED		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert judgment	Yes	October 1, 2015
QA	Yes	October 20, 2015

(Laurent and Rathahao 2003)
Paper or report: Laurent FM, Rathahao E. 2003. Distribution of [^{14}C] imidacloprid in sunflowers (*Helianthus annuus* L.) following seed treatment. *Journal of Agricultural and Food Chemistry* 51:8005-8010.

This paper was focused on measuring movement of ^{14}C-IMI and metabolites during the vegetative growth and blossoming of sunflower plants grown outdoors from seeds treated with IMI. Only the concentrations of IMI, IMI-OH, and IMI= in pollen were assessed in this WoE.

Response 1: Quality methods			**Score**
Experimental design and hypotheses	The protocol was well described. Uniformly ^{14}C-labelled IMI of purity >97% (Radio-HPLC) was used to follow the distribution of IMI treated sunflower (cv Albena RM) seed that has been dressed with Gaucho 70 WS® and Quinolate Pro FL® by Bayer Crop Science. Seeds contained 1 mg of IMI. The ^{14}C-IMI (5 or 7.5 µCi) was dissolved in acetone and applied in equal treatments on each face of the seed and the acetone then allowed to evaporate to dryness. Seeds were planted in pots (for the studies in growth cabinets) and after germination transplanted to outdoor lysimeters spaced as in normal agricultural practice. It is not clear how the complete transfer of the IMI was assured during transplanting (potential weakness). Samples were taken from plants at various stages and those for pollen were collected from the plants grown outdoors. Before blossoming, flower heads were enclosed in insect-proof paper bags. During blossoming, pollen was collected by sweeping up with a brush every second day until two-thirds of the florets were in blossom. At this point the flower heads were cut up for further analyses. Total radioactivity in pollen was analyzed by combustion in an oxidizer. The resulting $^{14}CO_2$ was trapped in a Carbosorb-Permafluor scintillation mixture and radioactivity quantified by liquid scintillation counting. Pollen was extracted with water/acetonitrile (80:20, v/v) with total recovery of 95%. Extracts and washing solutions were combined and concentrated to dryness on a rotary evaporator and then analyzed by radio-HPLC. IMI and metabolites of IMI were identified in other plant-tissues but only IMI was found in the pollen. The LOQ for IMI in pollen was 0.5 µg/kg. Statistical significance was reported for some of the data but the methods used were not provided.		**3**
Use of GLP and QA/QC	The study was not GLP and there was no QA or QC. Because of the use of labelled-IMI, QC is less relevant.		**3**
Transparency of data	Raw data were not provided. Summary data in the form of means and SDs were provided graphically (Fig. 4).		**2**
Number of samples and replication	There was only one site and five replicates for the collection of pollen. Samples appear to have been pooled over time.		**1**
Duration of study sufficient to observe trends	Duration of the study was sufficient for the objectives but trends were not characterized		**2**
Overall evaluation of methods	Computed mean of above		**2.20**
Score for expert judgment on quality the study.		**1**	**2.20**

Relevance

Hazard quotients for exposures to IMI in honey bees via pollen from sunflowers (data from Figure 4).

Source	Matrix	Conc. IMI µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
Sunflower, treated seed	Pollen from flowers mean	13	1.6	7.3	Larva	124
Sunflower, treated seed	Pollen from flowers max	36	4.5	7.3	Larva	124

Where toxicity values were exceeded by exposure values, relevance is shown with yellow highlight in the Table. Green cells are not relevant.

Response 1: Relevance to adverse effects			**Score**
Comparison of exposure value exceeds toxicity value	The maximum concentrations of IMI in pollen resulted in exposures that were all less than the NOAED		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert judgment		
QA	Yes	September 26, 2015
SEJ	Yes	October 13, 2015

(Mullin et al. 2010)
Paper: Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, Vanengelsdorp D, Pettis JS. 2010. High levels of miticides and agrochemicals in North American apiaries: Implications for honey bee health. PLoS ONE 5:e9754.

This study was not experimental. Samples of pollen, honey, and comb wax were analyzed for residues of insecticides. Only data for IMI, IMI-OH, IMI= are included in this assessment.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	Wax from honeycomb, pollen, and bees were collected from across N. America in 2007 and 2008. Samples were not collected at random (potential weakness) and included samples taken in relation to a study on colony collapse disorder. Various individuals were involved in collection of the samples and procedures varied. Samples were maintained at low temperatures (mostly -20°C) after collection. Control (field blanks) spikes were not reported. Given the variation in the collection procedures, lack of field spikes and blanks is a **major weakness.** Pesticide residue analysis was well described and was conducted by the USDA-AMS-NSL laboratory in Gastonia, NC. A modified QuEChERS method[7] was used that was adapted for smaller 3 g samples of material. Isotopically labelled standards were not reported to be used (potential weakness). Internal standards were of the "highest purity" available but this was not stated. Analysis was by LC/MS/MS. The LOD for IMI in wax and pollen was 2 µg/kg and that for IMI-OH and IMI= in pollen was 25 µg/kg. Concentrations of IMI-OH and IMI= were reported in Table 2 & 4.	**2**
Use of GLP and QA/QC	This was not a GLP study but the protocol was described; however, the descriptions were general and procedures could not be linked to individual samples. There was limited QC.	**2**
Transparency of data	Raw data were not provided in the paper or in the SI. Mean, SDs, and some centiles were provided. It is not clear how the centiles were calculated although it was stated that the concentrations <LOD were assumed to be zero. It is not clear how the authors arrived at some of their values. For example, in Table 1, residues of IMI were reported detected in only 2 of 208 samples of wax. These two values were 2.4 and 13.6 µg/kg and the other 206 samples were presumably <LOD (2 µg/kg). The median should be 0, not 8 µg/kg and the 95th centile should also have been 0, not 13 as listed in Table 1. Only one sample of 350 pollen samples had detectable (LOD = 25 µg/kg) concentrations of IMI= (554 µg/kg) and IMI-OH (152 µg/kg) and these were large compared to the maximum value for IMI (206 µg/kg). This sample may be an outlier or contaminated but this was not tested.	**1**
Number of samples and replication	It is not clear if the samples were replicated and how many were obtained from a particular site; however, the number of samples was >200, so a score of 4 was assigned.	**4**
Duration of study sufficient to observe trends	The span of the sampling for the study was 2 y but trend analysis was not conducted. Period was appropriate to the objectives of the study.	**3**

[7] Lehotay SJ, Mastovska K, Lightfield AR. 2005. Use of buffering and other means to improve results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables. J AOAC Int 88:615–629.

Overall evaluation of methods	Computed mean of above		**2.4**
Score for expert judgment on quality the study. One major weakness identified.		**0.5**	**1.20**

Relevance

Hazard quotients for exposures to ΣIMD in honey bees via wax, pollen, and residues in adult bees (data from Tables 1, 2 & 4).

Source	Matrix	Conc. IMI µg/kg	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg of food or water/d
N. America max (Fod=2/208)	Wax	13.6	0.00			
N. America max (Fod=10/350)	Pollen from hives	206	26	7.3	Larva	124
N. America ΣIMD max (Fod=10/350)	Pollen from hives	912	113	7.3	Larva	124
N. America ΣIMD max (Fod=0/140)	Bees	0.0	0.0		Adult	NA

Where toxicity values were exceeded by exposure values, relevance is shown with yellow highlight in the Table. Green cells are not relevant. Fod = Frequency of detection.

Response 1: Relevance to adverse effects			**Score**
Comparison of exposure value exceeds toxicity value	The maximum concentration of ΣIMD in pollen resulted in an exposure >LOAED		**4**
Overall evaluation relevance	Computed mean of above score(s)		**4.00**
Score for expert judgment on relevance of the results.		**1**	**4.00**

IMI was detected in two of 208 samples of wax at a concentration of 14 µg/kg but the proportion of exposure from was via the oral route was not known and hazard could not be calculated.

Expert judgment		
QA	Yes	September 24, 2015
SEJ	Yes	October 13, 2015

(Pohorecka et al. 2012b)
Paper or report: Pohorecka K, Skubida P, Miszczak A, Semkiw P, Sikorski P, Zagibajło K, Teper D, Kołtowski Z, Skubida M, Zdańska D, Bober A. 2012. Residues of neonicotinoid insecticides in bee collected plant materials from oilseed rape crops and their effect on bee colonies. Journal of Apicultural Science 56:116-134

Comments on paper. The aim of the study was to measure the concentration of neonicotinoid insecticides (including IMI, clothianidin & thiamethoxam) in nectar, pollen, honey, bee bread. This WoE is focused only on IMI. Other neonicotinoids were used as foliar sprays and are not included in the characterization of exposure.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	The study was conducted in Poland at an unreported location. In **2010**, two winter oilseed rape fields (wOSR) (field A - 41 ha - and field - B 35 ha) were treated with TMX (CRUISER OSR 322 FS) and IMI (CHINOOK PLUS 500 FS) as seed dressing, resp. In **2012**, three separate spring OSR fields (field C - 29 ha, field D - 21 ha and field E - 17 ha) were planted with OSR seed treated with TMX (CRUISER OSR 322 FS), CTD (MODESTO 480 FS), and IMI (CHINOOK PLUS 500 FS). Distances between the fields were not reported (*weakness*). All the crops were also treated with herbicides, fungicides and insecticides from other chemical classes as required by good agricultural practice. The detailed description of the products used was provided (Table 1). The blooming period of wOSR was 31 Apr to 21 May 2010. The sOSR bloomed from the 14 Jun to 2 Jul 2012. Seed was not analyzed for amount of a.s. and the rate of treatment was not reported (*weakness*). Bees used in the study were assessed for health and disease status. Ten colonies of honeybees (*Apis mellifera carnica, Apis mellifera caucasica*) were placed near each oilseed rape field, throughout the flowering period (ca. 3 weeks). In each group, an additional five hives equipped with pollen traps were designated only for collection of pollen loads. Two control groups of hives (one in 2010 and one in 2012) were in an area where no rape was grown. During flowering of rape samples of nectar were collected from rape flowers protected from insects by 16 m^2 mesh tunnels. A min of six samples were collected during the flowering period. Nectar flow from combs (100 g) was taken 7 and 14 d after the colonies were placed on the fields. Honey (100 g) was harvested separately for each colony (from honey chambers only) about one week after the blooming period. During the 3-week period of blooming, all the pollen loads that the bees had collected within a 3 to 4-day period were taken separately from the pollen traps of each of the five colonies. Samples of bee bread (app. 10×10 cm pieces of combs) were taken once, after the blooming period. About 100 of bee workers were taken from brood chamber one time, after the oilseed rape blooming period. Samples of pollen were taken for identification of source pollen. Bees were also collected for residue analysis but were not included in this WoE. All collected samples were frozen and stored at a low temperature of about -20°C.	**3**

	The residue analysis was described in detail. The standard for IMI were from commercial sources and were >93.3% (typically>99%) pure. Internal isotopically-labelled standards were not used (*weakness*). Extractions followed the QuEChERS method. Analysis was by LC-MS/MS. And the analysis followed the guideline of SANCO/825/00 rev 8.1. Mean recovery efficiencies for all collected matrices were from 70-110% and the relative standard deviation was less than 20%. LOQs (Table 2) for IMI in nectar and honey was 1 µg/kg. In pollen and bee-bread LOQs for IMI was 3 µg/kg. No residues of any seed treatments were detected in nectar, honey or pollen from the controls of wOSR (Table 6). Statistical analyses were carried out using Statistica 8 software. Means and medians were calculated but other centiles were not (*potential weakness*). Pollen from *Brassica napus* in the pollen collected by bees during flowering of rape was large in most samples, up to 84% (Table 3).	
Use of GLP and QA/QC	Not a GLP study but a reasonably well described protocol but no QC.	**1**
Transparency of data	Raw data were not provided. Means and other summary data were provided in Tables.	**1**
Number of samples and replication	Over both years there were five sites and only a single field for each product (IMI in 2012). Samples in fields were pseudoreplicated.	**1**
Duration of study sufficient to observe trends	The duration of the study was sufficient for the objectives of the study.	**4**
Overall evaluation of methods	Computed mean of above	**2**
Score for expert judgment on quality the study.	**1**	**2.00**

Relevance

Hazard quotient for maximum concentration of IMI in pollen and nectar from OSR (Tables 6 and 7)

Source of matrix	Sample	IMI in µg/kg or L	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg diet/d
Nectar collected from flowers of wOSR treated with IMI	Mean conc. (n=6)	0	0.00	7.3	Adult	292
Nectar collected from the hive of wOSR treated with IMI	Mean conc. (n=10)	0.6	0.18	7.3	Adult	292
Honey collected from the hive of wOSR treated with IMI	Mean conc. (n=10)	0.8	0.23	7.3	Adult	292
Pollen loads collected from the hive of wOSR treated with IMI	Mean conc. (n=20)	0	0.00	7.3	Larva	126
Bee-bread collected from the hive of wOSR treated with IMI	Mean conc. (n=10)	0	0.00	7.3	Larva	126
Nectar collected from flowers of sOSR treated with IMI	Mean conc. (n=7)	0	0.00	7.3	Adult	292

Hazard quotient for maximum concentration of IMI in pollen and nectar from OSR (Tables 6 and 7)

Source of matrix	Sample	IMI in µg/kg or L	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg diet/d
Nectar collected from the hive of sOSR treated with IMI	Mean conc. (n=20)	0.4	0.12	7.3	Adult	292
Honey collected from the hive of sOSR treated with IMI	Mean conc. (n=10)	0	0.00	7.3	Adult	292
Pollen loads collected from the hive of sOSR treated with IMI	Mean conc. (n=15)	0	0.00	7.3	Larva	126
Bee-bread collected from the hive of sOSR treated with IMI	Mean conc. (n=10)	0	0.00	7.3	Larva	126

Response 1: Relevance to adverse effects			**Score**
Comparison of exposure value to toxicity value	The concentrations of IMI measured in nectar collected from flowers of wOSR grown from seeds treated with IMI resulted in exposures that were less than the NOAED for bees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 2: Relevance to adverse effects			**Score**
Comparison of exposure value to toxicity value	The concentrations of IMI measured in nectar collected from the hives exposed to wOSR grown from seeds treated with IMI resulted in exposures that were less than the NOAED for bees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 3: Relevance to adverse effects			**Score**
Comparison of exposure value to toxicity value	The concentrations of IMI measured in honey collected from the hives exposed to wOSR grown from seeds treated with IMI resulted in exposures that were less than the NOAED for bees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 4: Relevance to adverse effects			**Score**
Comparison of exposure value to toxicity value	The concentrations of IMI measured in pollen loads collected from the hives exposed wOSR grown from seeds treated with IMI resulted in exposures that were less than the NOAED for bees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 5: Relevance to adverse effects		**Score**
Comparison of exposure value to toxicity value	The concentrations of IMI measured in bee-bread collected from the hives exposed to wOSR grown from seeds treated with IMI resulted in exposures that were less than the NOAED for bees.	**0**

Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 6: Relevance to adverse effects		**Score**
Comparison of exposure value to toxicity value	The concentrations of IMI measured in nectar collected from flowers of sOSR grown from seeds treated with IMI resulted in exposures that were less than the NOAED for bees.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 7: Relevance to adverse effects		**Score**
Comparison of exposure value to toxicity value	The concentrations of IMI measured in nectar collected from the hives exposed to sOSR grown from seeds treated with IMI resulted in exposures that were less than the NOAED for bees.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 8: Relevance to adverse effects		**Score**
Comparison of exposure value to toxicity value	The concentrations of IMI measured in honey collected from the hives exposed to sOSR grown from seeds treated with IMI resulted in exposures that were less than the NOAED for bees.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 9: Relevance to adverse effects		**Score**
Comparison of exposure value to toxicity value	The concentrations of IMI measured in pollen loads collected from the hives exposed sOSR grown from seeds treated with IMI resulted in exposures that were less than the NOAED for bees.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Response 10: Relevance to adverse effects		**Score**
Comparison of exposure value to toxicity value	The concentrations of IMI measured in bee-bread collected from the hives exposed to sOSR grown from seeds treated with IMI resulted in exposures that were less than the NOAED for bees.	**0**
Overall evaluation relevance	Computed mean of above score(s)	**0.00**
Score for expert judgment on relevance of the results.	**1**	**0.00**

Expert judgment		
QA	Yes	January 28, 2016
SEJ	Yes	February 5, 2016

(Reetz et al. 2011)
Paper or report: Reetz JE, Zühlke S, Spiteller M, Wallner K. 2011. Neonicotinoid insecticides translocated in guttated droplets of seed-treated maize and wheat: a threat to honeybees? Apidologie 42:596-606.

Comments on paper. This was a small-scale experimental study carried out in the field.

Response 1: Quality methods triticale seeds (IMI)			**Score**
Experimental design and hypotheses	The study was carried out in the experimental area "Heidfeldhof" of the Institute of Plant Breeding (University of Hohenheim). The size of the plots was not reported. Seeds of triticale were treated with Gasur® (1.75 mg IMI/seed) and the fungicide Efa®. The surrounding triticale was treated only with the fungicide Landor®. Concentrations on the seeds were not measured (*weakness*). Seeds were planted on 07 October 2008 into a field where broad bean had been planted. Guttation fluid was collected from multiple plants in each plot from Paril 7 to June 2, 2009 (Fig. 2) when guttation occurred. Pasteur pipettes were used to collect the drops which were then transferred to glass vials. Up to 3 mL was collected. The sampling interval was 2 d when the weather conditions were suitable. Procedures to prevent cross-contamination were followed. The samples were stored in the dark at 0–8°C but no transport and storage spikes and blanks were prepared (*major weakness*). Analysis was well described. The purity of the standards (from Sigma-Aldrich – presumed acceptable) was not reported but IMI-d4 was used as an internal standard. Compounds were identified and quantified by LCHR-MS (LTQ-Orbitrap mass-spectrometer). The LOD was reported as 5 µg/kg for IMI but recovery was not reported (*weakness*). No residues of IMI were detected in the surrounding triticale, treated only with the fungicide. Concentrations of IMI measured in guttation fluid remained relative constant (Fig. 2).		**1**
Use of GLP and QA/QC	The study was not conducted under GLP and there was no QA or QC reported. The protocol was incomplete.		**1**
Transparency of data	No raw data were provided. Data were presented in graphs.		**1**
Number of samples and replication	There was only one site per test and only one replicate. Multiple samples were taken over time.		**1**
Duration of study sufficient to observe trends	Guttation fluid was collected over a representative period.		**4**
Overall evaluation of methods	Computed mean of above		**1.6**
Score for expert judgment on quality the study. Lack of transport and storage spikes is a major weakness as the samples were not held at ≤-18°C		**0.5**	**0.80**

Relevance

Hazard quotient for maximum concentration of IMI in guttation fluid from maize in Germany (Fig 2)

Source of matrix	Sample	IMI in µg/kg or L	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg diet/d
Maize	Guttation fluid (max)	13	0.1	7.3	Adult	8

Response 1: Relevance to adverse effects			Score
Comparison of exposure value to toxicity value	The concentrations of IMI measured in pollen resulted in exposures that were less than the NOAED for bees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert judgment		
QA	Yes	January 28, 2016
SEJ	Yes	February 5, 2016

(Stewart et al. 2014)
Paper or report: Stewart SD, Lorenz GM, Catchot AL, Gore J, Cook D, Skinner J, Mueller TC, Johnson DR, Zawislak J, Barber J. 2014. Potential exposure of pollinators to neonicotinoid insecticides from the use of insecticide seed treatments in the Mid-Southern United States. Environmental Science & Technology 48:9762-9769

Comments on paper. This study was based on samples collected from small plot evaluations of seed treatments and from commercial fields in agricultural production areas in Arkansas, Mississippi, and Tennessee. Samples of soil, pollen, nectar, honeybees, and wildflowers were analyzed for concentrations of IMI, clothianidin and thiamethoxam and their metabolites. Unfortunately, bee-relevant nectar and pollen were not collected from the wildflowers but the whole flower was analyzed instead. These latter data are not usable in the WoE and only the data for IMI from corn and cotton pollen are included in this WoE.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	This study was conducted in 2012. The general locations of the field sites were reported (Table S3) but the precise locations of the field sites were not. Plot length varied from 9.1 to 13.7 m and there were no foliar applications of neonicotinoid insecticide prior to sampling. How far they were from other fields was not reported. The corn variety was DeKalb DKC66-96. Seeds were treated with: no insecticide, TMX (0.25 and 0.5 a.s. mg/seed, as Cruiser 5F®. CTD at 0.25 and 1.25 mg a.s./seed as Poncho 250®. Seeds of cotton were Phytogen 375 WRF and Stoneville 5458 B2RF. Treatments were untreated seed and seed treated with TMX (0.375 mg a.s/seed, as Cruiser Avicta®, and IMI (0.375 mg a.s./seed as Aeris®, Bayer CropScience). Concentrations in the seed were not verified by analysis (*potential weakness*) but soil samples did show the presence of residues (Table 3). Pollen from corn was collected by placing 25 tassels from each plot in a brown paper sack and transporting it to the laboratory. In the laboratory, tassels were beaten on the sides of an 18.9 L plastic bucket for several seconds. The pollen and anthers were then brushed into a sieve (6 mm; large mesh (*potential weakness*)) placed over a disposable paper plate. Sieving with the aid of a brush separated anthers and most other debris from the pollen. Pollen was then stored in microcentrifuge tubes. Corn pollen was composited by treatment across replicates within each of the four locations. Cotton pollen was collected from five tests. Pollen was collected from ten or more flowers from the center two rows of each plot. Cotton flowers were placed in small grocery bags and then transported to an air-conditioned work area. In the laboratory, pollen was separated from the flower by scrapping the pollen sacs with a small pair of scissors onto disposable paper plates. Pollen was then transferred into microcentrifuge tubes for storage. At three of the five locations, nectar was collected from the same treatments of cotton as pollen. Nectar was collected from the nectaries located on the pedicel of flowers with a 3-ml syringe with a 25-gauge, blunt needle. New syringes were used for each sample. Nectar from the extra-floral nectaries.	**2**

	Plots were irrigated just prior to collection to increase nectar production; however, this may have diluted the concentrations in the nectar (*weakness*). Care was taken to prevent cross-contamination. Pollen and nectar from cotton were composited by treatment across replicates within each of the five locations. Precautions were taken to prevent cross-contamination of samples. Foraging honey bees and the pollen they carried were collected from commercial apiaries. A total of 15 apiaries with four hives per apiary were sampled. Apiaries were located, on average, approximately 180 m from agricultural fields (crops not specified; *weakness*). Samples were collected at two different times, normal planting (Apr − May) and flowering (Jun 15 − Sep 9). A minimum of ten bees usually ≥30 were collected from each hive. In some cases, too few foraging bees were carrying pollen to collect four samples per apiary. Bee pollen samples from each apiary were composited by date, but even then, there were several cases where sample mass did not allow for analysis. All samples collected in this study were transported either on coolant packs or dry ice to a laboratory for processing, after which they were stored at -4 to -15°C. Shipment of samples was done in ice chests containing dry ice or coolant packs. Samples were stored from 6 - 12 months before being analyzed but the storage temperature was not reported. Reported temperatures were <-18°C and transport and storage blanks were not reported (*major weakness*) Samples were analyzed at the USDA AMS Science and Technology Laboratory Approval and Testing Division of the National Science Laboratories' Gastonia Lab in Gastonia, NC. The samples were extracted using the QuEChERS method, and analyzed by LC/MS/MS). Quantification was performed using external calibration standards prepared from certified standard reference material of unreported purity (*potential weakness*). Internal isotopically-labelled standards were not reported used. The LODs for CTD, IMI, and TMX were 1 µg/kg; LOQs were not reported (*weakness*). Percent recovery was not reported (*weakness*). CTD was detected at trace levels in pollen from plots of untreated corn seed and seed treated with TMX. CTD and TMX were detected at trace levels in the untreated plots of corn (Table 2). Statistical analysis was not conducted so medians and centiles were not available (*weakness*).	
Use of GLP and QA/QC	This study was not conducted under GLP and there was no QA or QC reported. A protocol was provided.	**1**
Transparency of data	Raw data were not provided. Only summary data were provided in Tables. It is not clear how many replicates there were. Percentages presented in Table 2 were not consistent with the number sites and pooling of replicates.	**1**
Number of samples and replication	For corn, there were four sites and four replicates (pooling of samples reduced the replicates to 1). For cotton there were five sites but not all were sampled for nectar.	**2**
Duration of study sufficient to observe trends	It appears that only one sampling was undertaken during pollen shed and nectar secretion.	**1**
Overall evaluation of methods	Computed mean of above	**1.4**

Score for expert judgment on quality the study. The lack of appropriate storage and transport spikes, the lack of information on conditions of long-term storage, and the use of transport temperatures >-18°C are a **major weakness**.	**0.5**	**0.70**

Relevance

Hazard quotients for mean concentration of IMI in pollen and nectar in the USA (Table 2 & p 9765)

Source of matrix	Sample	IMI in µg/kg or L	Dose ng/bee	Chronic NOAED ng/bee/d	Life-stage	mg diet/d
All crops	Pollen from forager bees max (n=24)	0	0.00	7.3	Larva	126
Cotton	Nectar max (n=15)	2.9	0.85	7.3	Adult	292

Values <LOQ were treated as zero. Where toxicity values were exceeded by exposure values, relevance is shown with orange or yellow highlight in the Table. Green cells indicate no relevant exposure.

Response 1: Relevance to adverse effects			**Score**
Comparison of exposure value to toxicity value	The concentrations of CTD measured in pollen from all crops resulted in exposures that were less than the NOAED for bees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Response 2: Relevance to adverse effects			**Score**
Comparison of exposure value to toxicity value	The concentrations of CTD measured in nectar of cotton grown from treated seed resulted in exposures that were less than the NOAED for bees.		**0**
Overall evaluation relevance	Computed mean of above score(s)		**0.00**
Score for expert judgment on relevance of the results.		**1**	**0.00**

Expert judgment		
QA	Yes	January 28, 2016
SEJ	Yes	February 5, 2016

Risks to honeybees resulting from exposure to dusts containing IMI

(Bayer CropScience 2009)

Report: Bayer CropScience. 2009. Monitoring of Dust Drift Deposits During and After the Drilling of Winter Barley, Dressed with MANTA PLUS® In Germany. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-342603-01-1. 239 p

The aim of the study was to measure the residues of IMI in dust drift deposits which can occur in commercial agricultural practice during and after the drilling of winter barley, commercially coated with the insecticidal seed treatment product MANTA PLUS®.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	Winter barley dressed with MANTA PLUS® (a.s. fuberidazole & imazalil & triadimenol & IMI, nominally 7.2 & 8.0 & 60.0 & 70.0 g a.s./L). Dressing rate was 500 mL MANTA PLUS®/100 kg seeds. The 20 commercial test fields were located in Germany. One was in Bavaria, two in Hesse, twelve in North Rhine-Westphalia and five in Schleswig-Holstein. The sizes of the fields ranged from 2–13.2 ha. Eight different winter barely varieties were drilled September 16-27, 2008 with seeding rates between 101–160 kg seeds/ha resulting in nominal application rates between 35.4 and 56.0 g IMI a.s./ha. Nine fields were drilled with pneumatic and eleven fields with mechanical drilling machines. Overall seven different pneumatic and nine different mechanical drilling machines were used. The sampling during seeding was conducted by placing 10 petri dishes in pairs in each of three rows 1, 3, and 5 m from the downwind edge of the field (n=30). Actual distances ranged from 0–2 m; 3–3.9 m; and 5–11 m. The dishes contained 70 to 80 mL of a 1:1 (v/v) mixture of glycerol and water and were placed horizontally on metal racks 1.5–2 cm above the soil or at the height of the surface vegetation, depending on the morphology of the field boundary. Fifteen min after drilling has ceased, the contents of the dishes was rinsed quantitatively into polyethylene flasks. For the post-seeding samples, 10 dishes were placed in pairs 1 m apart in a row 1 m from the edge of the field. This was done on all four sides of the field (n=40). After 24 h, the samples were collected as above. Samples were stored at room temperature until delivered by automobile to the Bayer CropScience AG, Institute for Human Safety – Residue Analysis. After delivery, the sample were refrigerated at an unstated temperature and were analyzed within 21 d of receipt. The use of field blanks, and/or controls was not reported and the storage temperature of the samples was room temperature. Storage spikes were not reported (**major weakness**).	**4**

	Residues of IMI were analyzed using a standard protocol with LC-MS-MS. ^{13}C, ^{2}H-labelled IMI was used as an internal standard. The LOQ for IMI was 0.020 µg a.s./Petri-dish, corresponding to 0.014 g a.s./ha. Recovery ranged from ranged from 94 to 110% and an RSD of 3.4% (n=81). During analysis, 71 samples were identified as having excessive concentrations (method of identification not reported (potential weakness). Visual observation revealed fragments of coated seeds and husks. Seed and husk fragments were not considered as relevant pathways of exposures for honeybees and these samples were excluded from the analysis. For the mathematical analysis of the data, values below the LOQ were set to the LOQ. The 90th centile was estimated from the raw data. Statistical analysis of the data was not reported.		
Use of GLP and QA/QC	The field phase was non-GLP but was well described. The analysis was a full GLP study with QC.		**3**
Transparency of data	All raw data were provided (see Section 6 for analytical results)		**4**
Number of samples and replication	There were 20 sites with 30 replicates per site during seeding and 40 replicates per site for the post seeding samples.		**4**
Duration of study sufficient to observe trends	Duration was considered appropriate for the objectives of the study.		**4**
Overall evaluation of methods	Computed mean of above		**3.80**
Score for expert judgment on quality the study.		**0.5**	**1.90**

Relevance
Raw data were in Section 6, Table 4. Some samples were lost in handling in the field so there were 1,394 samples analyzed. Seventy-one of these samples were excluded because of visual contamination with particles that would be too large to be picked up by a bee. Of the remaining 1,323 samples, 1,257 (95.0%) contained no quantifiable residues. The following relevant observations can be made.

- Sixty-six samples (5.0%) contained residues of IMI above the LOQ (0.014 g a.s./ha).
- Of the 66 samples containing quantifiable residues of IMI, 65 were taken at the time of sowing and one during the 24 h post-sowing period.
- Of the 66 samples containing quantifiable residues of IMI, 63 were associated with pneumatic sowing and 3 with mechanical sowing.
- The maximum measured residue was equivalent to 0.111 g a.s./ha, collected in one sample 1-m from the edge of the field during sowing. This was one of 180 samples at that distance from the field and represented the 99.4th centile of all samples.

Since the deposition amounts cannot be compared to oral toxicity values for IMI, these data were not included in the WoE graphic; however, the maximum measured deposition rate was 0.1 g IMI/ha and 95% of the samples were below the LOD (0.014 g IMI/ha).

Expert judgment		

QA	Yes	September 30, 2015
SEJ	Yes	October 1, 2015

(Girolami et al. 2012)
Report: Girolami V, Marzaro M, Vivan L, Mazzon L, Greatti M, Giorio C, Marton D, Tapparo A. 2012. Fatal powdering of bees in flight with particulates of neonicotinoids seed coating and humidity implication. *Journal of Applied Entomology* 136:17-26

This paper was a study on exposures of bees to dust resulting from seeding treated-corn seeds with a pneumatic drilling machine in 2009 and 2010. Commercially sourced seeds were treated with CTD and IMI. The study reported mortality of exposed bees kept under different conditions of humidity in 2009 and 2010. Concentrations of IMI on bees collected near a sugar-dispenser, at the hive, or in flight were measured in 2009. Only these exposure analyses are included in the WoE.

Response 1: Quality methods		**Score**
Experimental design and hypotheses	This study was conducted in Italy at the experimental farm of the University of Padova Agricultural Experimental Farm located in Legnaro. The plot was 50 by 70 m. Four hives of bees were obtained from a local source. Bees were trained to fly to a sugar-dispenser located on the other side of the field-plot so that they would fly through any dust released from the drilling machine. In 2009 hybrid corn seeds (Pioneer X1180D 964890 were treated with IMI (Gaucho 350FS® Bayer Cropscience AG) at a rate of 0.5 mg/seed. The seeds were reported to release dust at a rate of <3 g per 100 kg seeds but no raw data were provided and the treatment of the seeds was not confirmed by analysis (weakness). A Monosem NG Plus drilling machine was used for all the sowing operations. The waste-air pipe was situated on the right-hand side at a height of 1.8 m and an angle of 45° to the horizontal. A deflector for directing air towards the soil was not fitted (weakness as this does represent current good agricultural practice). Bees for exposure analysis were collected as follows: Thirty min after sowing coated seeds, live bees were collected at the sugar dispenser, placed in cages and taken to the laboratory where they were exposed to high humidity (≈ 100%) and subsequently died. Dead bees were also collected in front of the hive 3 h and 24 h after drilling. A similar protocol was followed for bees exposed to dust from drilling seeds treated with IMI. Here dead bees found at the sugar-dispenser were collect 30 min after seeding. Forty-five min after seeding, live bees were captured at the sugar dispenser, placed in cages and taken to the laboratory where they were exposed to high humidity (≈ 100%) and subsequently died. Dead bees were collected at the hive at 3 and 4 h after sowing and live bees were collected in a fine net 24 h after sowing. No field, travel, or storage blanks or spikes were reported (**major weakness**). Samples of bees were held at 2°C for “a few days” and then dried for 2 h in an oven at 100°C. Analyses were conducted after grinding the dead bees and extracting the analytes in methanol (**major weakness** as it is not possible to differentiate analyte on the surface of the bees from what was inside the bees). Samples were pooled. Analyses were conducted by liquid chromatography fitted with a UV-Vis diode array detector set to measure absorbance at 269 nm. Laboratory standards	**2**

	were >99.7% pure and the presumed LOD determined from an unspecified number of bees was either 100 or 500–1000 ng/bee with a recovery of 75–105% (weakness as the LOD was >LC50 for IMI and CTD). Statistical analyses of concentrations in bees were not conducted.		
Use of GLP and QA/QC	The study was not conducted under GLP and there was only limited QC of the analytical method. The description of the study was reasonably complete.		**1**
Transparency of data	Raw data were not provided. Data were presented in tabular form (Author Table 3) and were from pooled samples so variance could not be determined.		**1**
Number of samples and replication	There was one site and a single replicate collection and five time intervals for IMI.		**2**
Duration of study sufficient to observe trends	In terms of the design of the study, the duration was sufficient.		**4**
Overall evaluation of methods	Computed mean of above		**2.00**
Score for expert judgment on quality the study. Two identified		**0.25**	**0.50**

Relevance

Even including the 4 live bees captured 24 h after seeding with IMI-treated seed; all reported topical+body-dose exposures were greater than the LD50 for IMI or for CTD (Author Table 3). The relevance of the exposure studies cannot be determined as it is not possible to compare the amount of insecticide in and on the bees with an LD50 based on dose based on oral consumption. Total exposure to CTD and IMI are shown in the following Table.

Amount of neonicotinoid insecticide in and on bees exposed to dust from treated corn seed.

Analyte	Collection site	Time after drilling	No. of bees analysed	Quantity of insecticide in and on bees (ng/bee)
IMI	Sugar-dispenser	30 min	4	3661
	Sugar-dispenser	45 min	8	442
	Hive	3 h	8	500
	Hive	4 h	8	53
	Non-woven net	24 h	4	29

Response 1: Relevance to adverse effects			**Score**
Comparison of exposure value exceeds toxicity value	It was not possible to separate dose in body from the topical dose so a comparison to oral toxicity was not possible. Not included in the WoE assessment.		**NA**
Overall evaluation relevance	Computed mean of above score(s)		**NA**
Score for expert judgment on relevance of the results.		**1**	**NA**

Expert judgment		
QA	Yes	August 28, 2015
SEJ	Yes	October 1, 2015

(Girolami et al. 2013)
Report: Girolami V, Marzaro M, Vivan L, Mazzon L, Giorio C, Marton D, Tapparo A. 2013. Aerial powdering of bees inside mobile cages and the extent of neonicotinoid cloud surrounding corn drillers. *Journal of Applied Entomology* 137:35-44.

This study was very similar to an earlier paper[8]. This paper was a study on exposures of bees to dust resulting from seeding treated-corn seeds with a pneumatic drilling machine in 2011. Commercially sourced seeds were treated with CTD and IMI. The study reported mortality of exposed bees held in small cages in various locations in the plume of dust from the drilling machine. Concentrations of IMI were measured in bees from the exposure cages. Data for concentrations of CTD were reported in another study[9].

Response 1: Quality methods		**Score**
Experimental design and hypotheses	This study was conducted in Italy at the experimental farm of the University of Padova Agricultural Experimental Farm located in Legnaro. The plot was 50 by 70 m. Twelve hives of bees were obtained from a local source. Hybrid corn seeds, Pioneer X1180D 964890, were treated with CDT (Poncho® Bayer CropScience AG) at a rate of 1.25 mg per seed; and Pioneer PR32G44 were treated with IMI (Gaucho 350FS® Bayer CropScience AG) at a rate of 0.5 mg/seed. The seeds were reported to release dust at a rate of <3 g per 100 kg seeds but no raw data were provided and the treatment of the seeds was not confirmed by analysis (weakness). A Monosem NG Plus drilling machine was used for all the sowing operations. In one set of exposures, the waste-air pipe was situated on the right-hand side at a height of 1.8 m and an angle of 45° to the horizontal. In another set of exposures, a double deflector for directing air towards the soil was fitted. Bees for exposure analysis were collected from a single hive and transferred to a mesh cage (20 x 20 x 20 cm) where they were fed on honey. Bees were kept for one day only and unused bees released at the end of the day. For the exposures, one bee was transferred to smaller cages with a metal frame (5 x 5 x 5 cm) and covered with mesh (1.1 mm). To assess exposures in these single bees, the smaller cages were attached (ever 40 cm) to an aluminum bar 4 m long (n=10). The bar was supported at each end by a vertical pole of 2.5 m. The bar was carried by two people at a fast walking pace (6–8 km/h) past the side of the stationary drilling machine. The process was repeated for the right and left side and for the unmodified and modified driller. After exposure, the caged bees were immediately placed in a refrigerator at 2–4°C for 15 min to immobilize them. It was not clearly stated how the bees were exposed but it is assumed that the bar was moved through parallel to the drill, exposing ten bees at a time. The height of the sampler was not stated (weakness) and it is not clear how the ten bees per bar were exposed at five different distances under four different conditions (= 10 x 5 x 4 = 200 bees) when Author-Table 1 states that only 80	**1**

[8] Girolami V, Marzaro M, Vivan L, Mazzon L, Greatti M, Giorio C, Marton D, Tapparo A. 2012. Fatal powdering of bees in flight with particulates of neonicotinoids seed coating and humidity implication. *Journal of Applied Entomology* 136:17-26.
[9] Tapparo A, Marton D, Giorio C, Zanella A, Solda L, Marzaro M, Vivan L, Girolami V. 2012. Assessment of the environmental exposure of honeybees to the particulate matter containing neonicotinoid insecticides coming from corn coated seeds. *Environmental Science & Technology* 46:2592–2599.

	bees were used in Trial-7. Bees were individually analyzed to determine the content of IMI after freeze-drying (at -80°C) and extraction (**major weakness** as it is not possible to differentiate analyte on the surface of the bees from what was inside the bees). No field, travel, or storage blanks were reported conditions of storage were not specified other than "The samples of bees, pooled for analysis, were stored at +2°C for few days." (**major weakness**). A published method of analysis with LC and a DAD was used[9]. Freeze-dried bees were ground to a powder and extracted in MeOH with sonication for 30 min. Analysis was conducted with a UHPLC fitted with a DAD. Laboratory standards were >99.7% pure and the LOD was reported as 10 ng/bee (weakness as the LOD was >LC50 (7.3 ng/bee) for IMI and CTD) and a recovery of 78-104%. Statistical analyses of concentrations in bees were not conducted. Mean values were calculated in Author-Table-4 but variance was not. It appears that the mean values in the table are based on the detected values only.	
Use of GLP and QA/QC	The study was not conducted under GLP and there was only limited QC of the analytical method.	**1**
Transparency of data	The data on exposures (Author-Table 4) was not clearly presented. It is not clear if the concentrations reported at the various distances from the drill were from one bee or a pool of bees. The text is confusing in that the data in Author-Table 4 is ascribed to CTD (Page 40, R Col bottom).	**1**
Number of samples and replication	There one site and only a single replicate	**0**
Duration of study sufficient to observe trends	In view of the variance observed, the study should have been repeated several times.	**2**
Overall evaluation of methods	Computed mean of above	**1.00**
Score for expert judgment on quality the study.	**0.25**	**0.25**

Relevance

The relevance of the exposure study cannot be determined as it is not possible to compare the amount of IMI in and on the bees with an LD50 based on an oral exposure. Exposures to IMI are shown in the following Table.

Amount of IMI in and on bees exposed to dust from IMI-treated corn seed at various distances from the drill.

Driller equipment	Sampling side	IMI on and in bees (ng/bee)					
		Distance from drilling machine (m)					Mean
		1	2.25	4.5	6.75	9	
Unmodified	Right	4,786	457	142	523	199	1,221
	Left	<LOD	410	110	98	33	162
Modified (dual pipe deflectors)	Right	2,372	424	134	1778	500	1,042
	Left	<LOD	<LOD	<LOD	25	<LOD	25

Response 1: Relevance to adverse effects			Score
Comparison of exposure value exceeds toxicity value	Since exposures in and on the bees were not determined, it was not possible to compare the exposures to oral toxicity values.		**NA**
Overall evaluation relevance	Computed mean of above score(s)		**NA**
Score for expert judgment on relevance of the results.		**1**	**NA**

Expert judgment		
QA	Yes	August 28, 2015
SEJ	Yes	October 1, 2015

Effects of IMI on honeybees from use as a seed-treatment

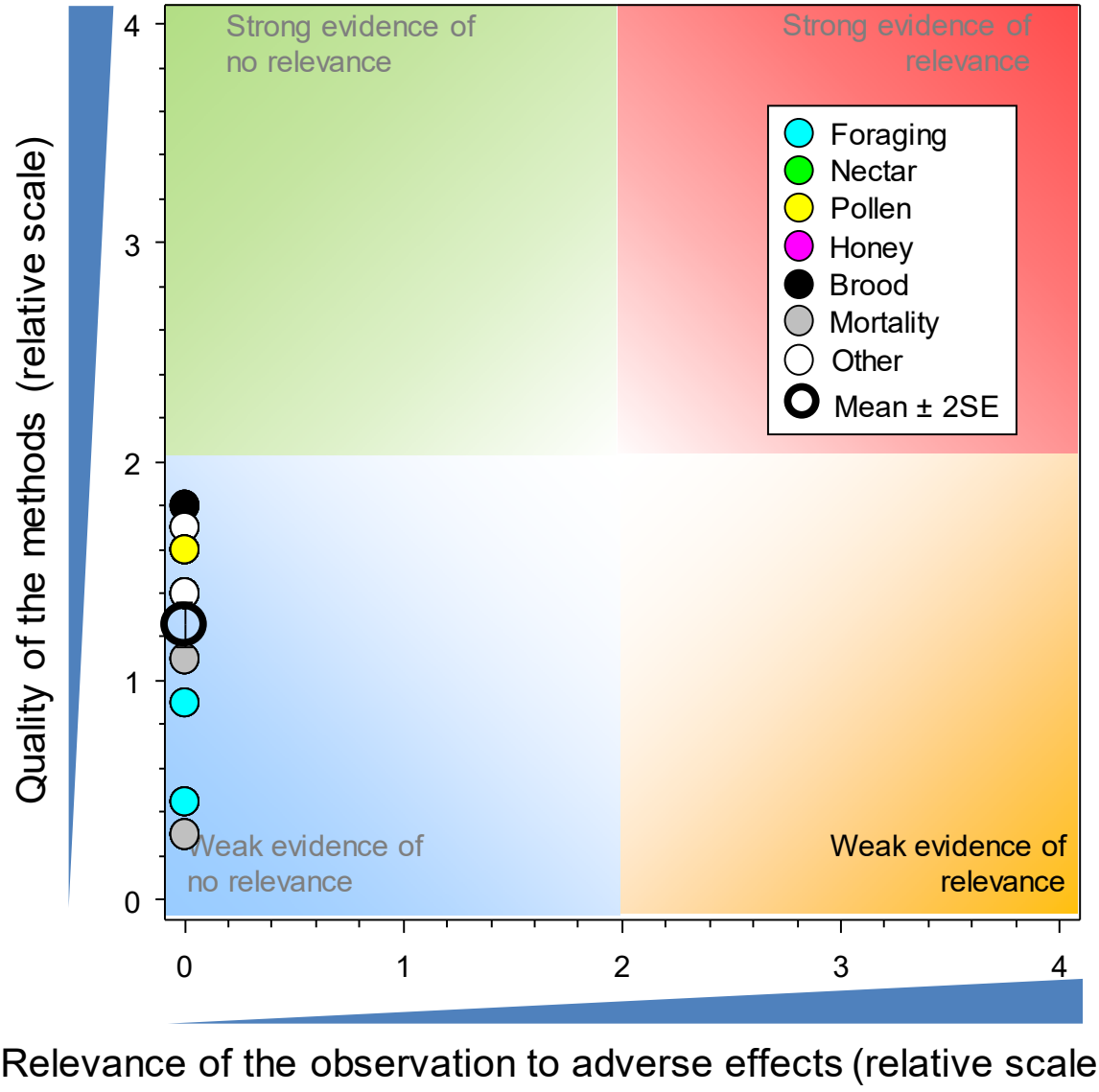
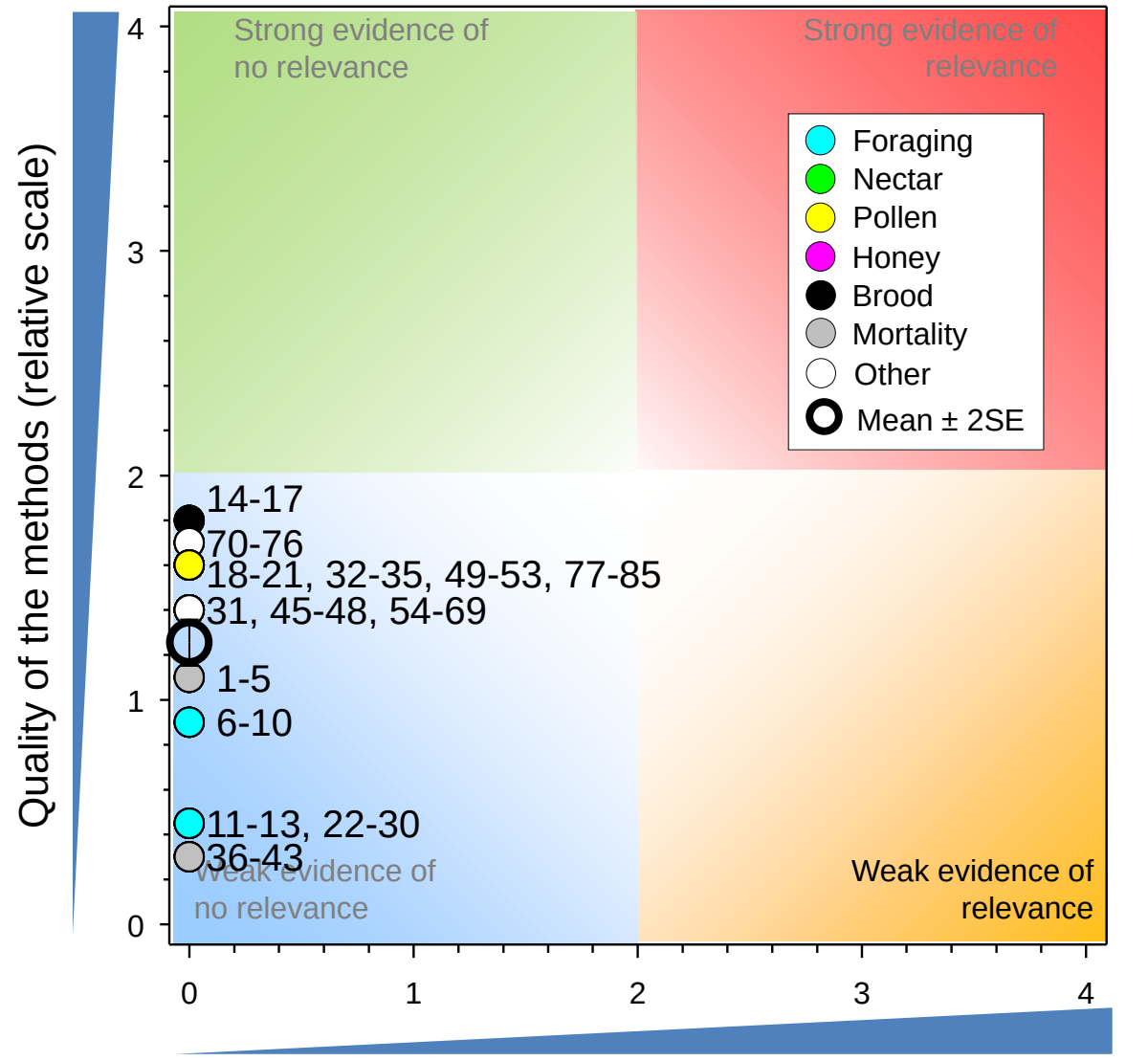


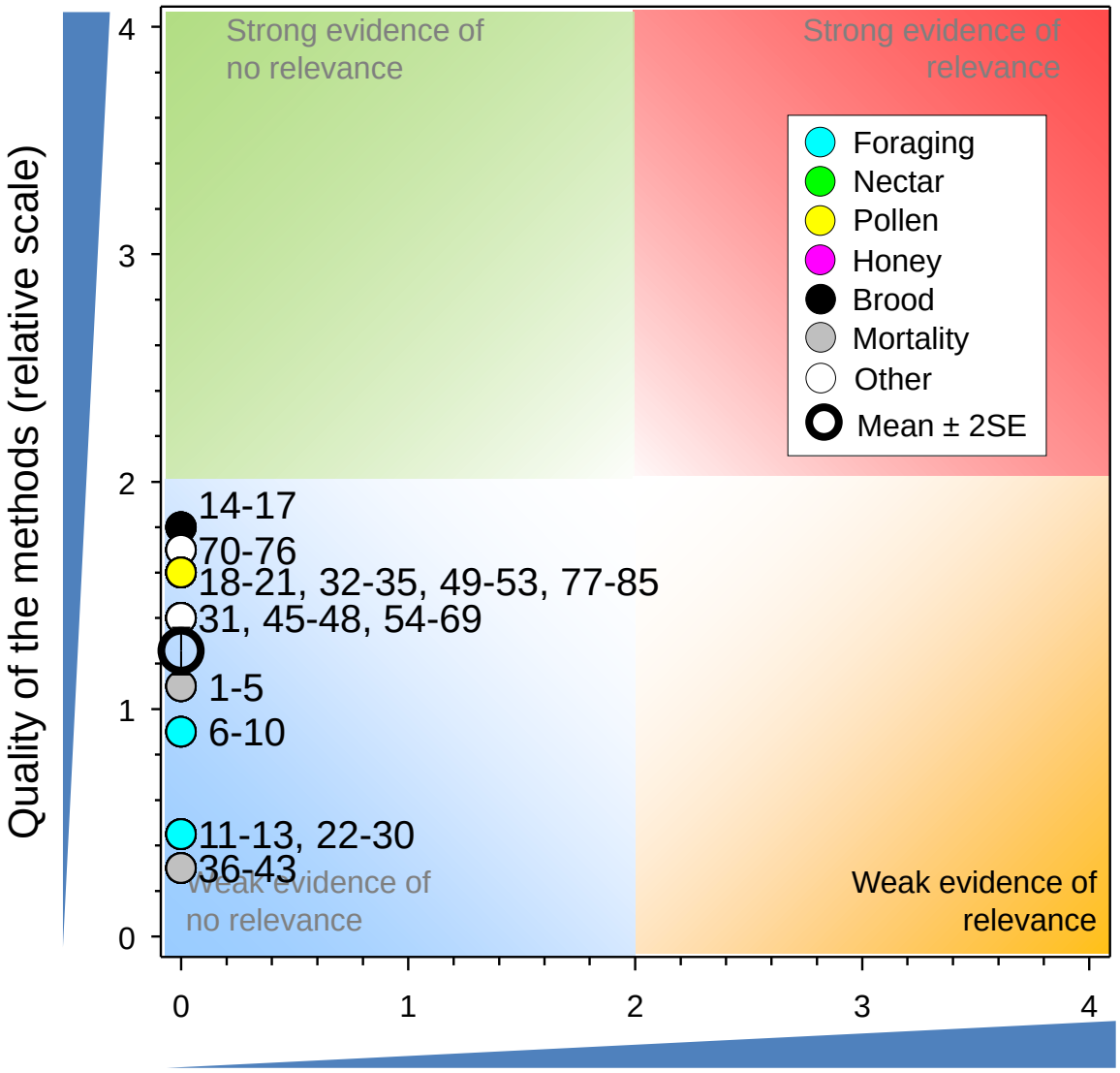
Figure 3. Strength and relevance of effects in honeybees exposed in controlled field studies via crops treated with IMI as seed dressings. Number of responses assessed = 85. Symbols may obscure others, see SI for all responses. There were no points in the upper right quadrant.

Field-effects studies on honeybees exposed IMI via seed treatments (SI)



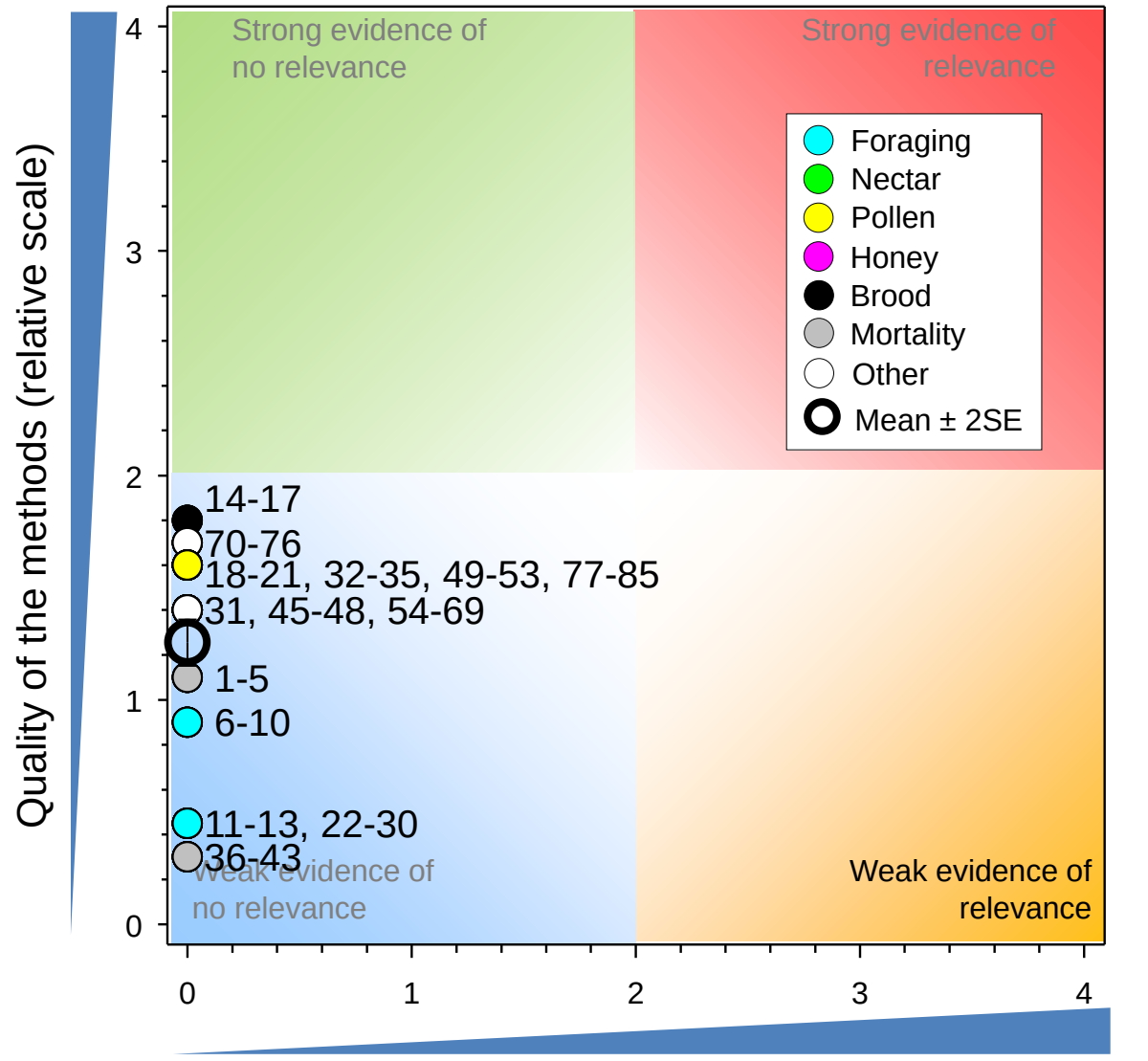
1 = (Bayer CropScience 1998) Resp 1
2 = (Bayer CropScience 1998) Resp 2
3 = (Bayer CropScience 1998) Resp 3
4 = (Bayer CropScience 1998) Resp 4
5 = (Bayer CropScience 1998) Resp 5
6 = (Bayer CropScience 1999b) Resp 1
7 = (Bayer CropScience 1999b) Resp 2
8 = (Bayer CropScience 1999b) Resp 3
9 = (Bayer CropScience 1999b) Resp 4
10 = (Bayer CropScience 1999b) Resp 5
11 = (Bayer CropScience 1999c) Resp 1
12 = (Bayer CropScience 1999c) Resp 2
13 = (Bayer CropScience 1999c) Resp 3
14 = (Bayer CropScience 1999d) Resp 1
15 = (Bayer CropScience 1999d) Resp 2
16 = (Bayer CropScience 1999d) Resp 3
17 = (Bayer CropScience 1999d) Resp 4
18 = (Bayer CropScience 1999e) Resp 1
19 = (Bayer CropScience 1999e) Resp 2
20 = (Bayer CropScience 1999e) Resp 3
21 = (Bayer CropScience 1999e) Resp 4
22 = (Bayer CropScience 1999a) Resp 1

Field-effects studies on honeybees exposed IMI via seed treatments (SI)



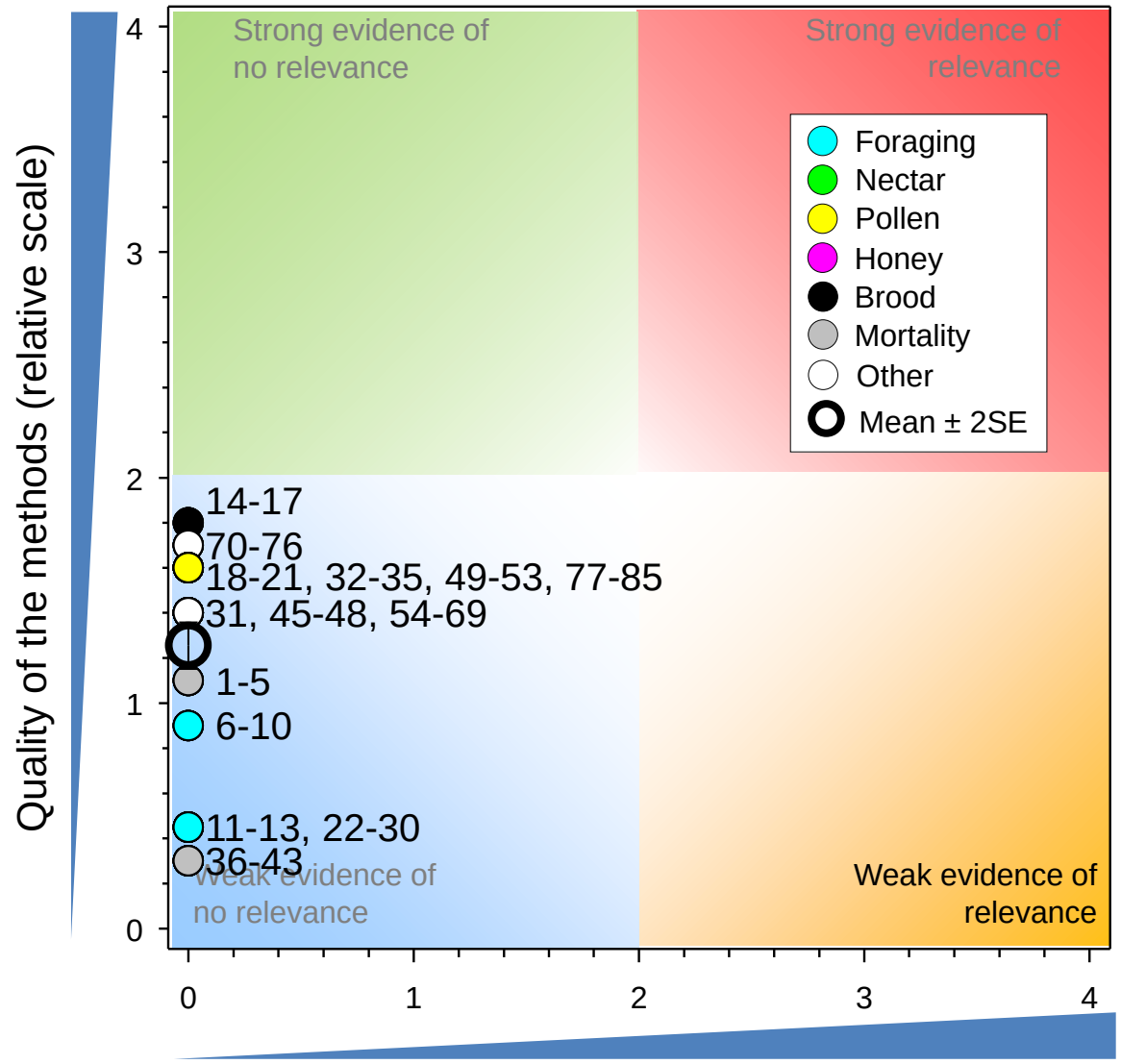
23 = (Bayer CropScience 1999a) Resp 2
24 = (Bayer CropScience 1999a) Resp 3
25 = (Bayer CropScience 1999a) Resp 4
26 = (Bayer CropScience 1999a) Resp 5
27 = (Bayer CropScience 2002) Resp 1
28 = (Bayer CropScience 2002) Resp 2
29 = (Bayer CropScience 2002) Resp 3
30 = (Bayer CropScience 2002) Resp 4
31 = (Bayer CropScience 2002) Resp 5
32 = (Bayer CropScience 2007) Resp 1
33 = (Bayer CropScience 2007) Resp 2
34 = (Bayer CropScience 2007) Resp 3
35 = (Bayer CropScience 2007) Resp 4
36 = (Bayer CropScience 2012c) Resp 1
37 = (Bayer CropScience 2012c) Resp 2
38 = (Bayer CropScience 2012c) Resp 3
39 = (Bayer CropScience 2012c) Resp 4
40 = (Bayer CropScience 2012c) Resp 5
41 = (Bayer CropScience 2012c) Resp 6
42 = (Bayer CropScience 2012c) Resp 7
43 = (Bayer CropScience 2012c) Resp 8
44 = (Bayer CropScience 2012b) Resp 1

Field-effects studies on honeybees exposed IMI via seed treatments (SI)



45 = (Bayer CropScience 2012b) Resp 2
46 = (Bayer CropScience 2012b) Resp 3
47 = (Bayer CropScience 2012b) Resp 4
48 = (Bayer CropScience 2012b) Resp 5
49 = (Bayer CropScience 2014f) Resp 1
50 = (Bayer CropScience 2014f) Resp 2
51 = (Bayer CropScience 2014f) Resp 3
52 = (Bayer CropScience 2014f) Resp 4
53 = (Bayer CropScience 2014f) Resp 5
54 = (Bayer CropScience 2014b) Resp 1
55 = (Bayer CropScience 2014b) Resp 2
56 = (Bayer CropScience 2014b) Resp 3
57 = (Bayer CropScience 2014b) Resp 4
58 = (Bayer CropScience 2014b) Resp 5
59 = (Bayer CropScience 2014b) Resp 6
60 = (Bayer CropScience 2014b) Resp 7
61 = (Bayer CropScience 2014b) Resp 8
62 = (Bayer CropScience 2014c) Resp 1
63 = (Bayer CropScience 2014c) Resp 2
64 = (Bayer CropScience 2014c) Resp 3
65 = (Bayer CropScience 2014c) Resp 4
66 = (Bayer CropScience 2014c) Resp 5

Field-effects studies on honeybees exposed IMI via seed treatments (SI)



67 = (Bayer CropScience 2014c) Resp 6
68 = (Bayer CropScience 2014c) Resp 7
69 = (Bayer CropScience 2014c) Resp 8
70 = (Bayer CropScience 2014d) Resp 1
71 = (Bayer CropScience 2014d) Resp 2
72 = (Bayer CropScience 2014d) Resp 3
73 = (Bayer CropScience 2014d) Resp 4
74 = (Bayer CropScience 2014d) Resp 5
75 = (Bayer CropScience 2014d) Resp 6
76 = (Bayer CropScience 2014d) Resp 7
77 = (Bayer CropScience 2014e) Resp 1
78 = (Bayer CropScience 2014e) Resp 2
79 = (Bayer CropScience 2014e) Resp 3
80 = (Bayer CropScience 2014e) Resp 4
81 = (Bayer CropScience 2014e) Resp 5
82 = (Bayer CropScience 2014e) Resp 6
83 = (Bayer CropScience 2014e) Resp 7
84 = (Bayer CropScience 2014e) Resp 8
85 = (Bayer CropScience 2014e) Resp 9

(Bayer CropScience 1998)

Report: Bayer CropScience. 1998. The impact of Gaucho 70 WS seed treated sunflower seeds on honey bees. Leverkusen, Germany: Bayer CropScience (Unpublished Report). Report M-006826-01-2. 24 p

Sunflower seeds (Fleury) were dressed with Gaucho 70 WS (0.7 mg a.i./seed) + Tutan 500 FS (thiram – fungicide) and Degranil (63.3 g) and sown (89,000 seeds and application rate of 59 g a.i. per ha) on May 8, 1998 near Metternich, Germany. The treated plot was located in Metternich and the control plot in Hausweiler, both in the Eurskirchen district, Germany. Four honey bee colonies were placed into the 1.25 ha field 75 days later when the plants were in flower. The control field near Hausweiler, Germany was sown with seeds dressed with 63.3 g of Tutan 500 FS + Degranil only on a similar-sized plot 4 km away. The objective was to determine if IMI residues in nectar of sunflowers would adversely affect honeybees. The endpoints measured were honey bee mortality, foraging intensity, pollen stores, colony weights and the number of bees returning to the hive from the treated field, bee behaviour, and residues of IMI and metabolites, in honey bladders or bees. They concluded that by the time sunflowers appear, there are no relevant residues of IMI in the nectar that could adversely affect bees.

Responses 1-5: Quality methods		**Score**
Experimental design and hypotheses	**Hypothesis:** No hypothesis was explicitly stated. There were only four honeybee colonies established on each field. The number of emerged plants on the treated and control fields was 56,167 and 45,250, respectively. Soil characteristics for both fields were provided; the control fields had soils of poorer quality than those in the treated field. The application rate was 59 g a.i./ha. **Measurement endpoints** included changes in hive weight, number of foraging visits to flowers, number of bees entering and leaving the hive (BeeSCAN), number of bees with pollen, mortality, residues in bees and honey stomachs. Hive weights were measured before the bees were introduced to the plots and on days 4 and 10 of the 11-day trial. Foraging was assessed daily by counting the number of bees on 6 x 100 flowering sunflower discs at six spots in the field. Number of bees returning to the hive was recorded with a device e.g., Bee SCAN. Note: the device in the control field was faulty so data are only the treated field. The number of bees carrying pollen to the nest was measured in one minutes using a landing board. Mortality was assessed using dead bee traps and a grid (2x3 m) on the ground in front of the hive. Residues measured in 100 foraging bees collected from sunflower discs in both fields on August 5, 1998. Honey bladder was dissected and measured separately. Non-GLP was used but no QA/QC discussed. Analytical methods described in detail in appendix HPLC-MS/MS including extraction procedures. LODs (0.01 mg/kg) were included in the analytical reports. No residues in any matrix was detected. **Weakness:** • No statistical analyses described or provided (**major weakness)** • There were no residues measured in any matrix (bee or nectar in bee bladder); below detection limit of 0.01 mg/kg	**3**
Use of GLP and QA/QC.	Non-GLP was used and no QA/QC discussed. Analytical methods described in detail in appendix HPLC-MS/MS including extraction procedures; no % recoveries reported (**minor weakness**)	**2**

Exposure concentrations	One application rate of 59 g a.i./ha; no residues in any matrix were determined (LOD= 0.01 mg/kg)	**4**
Transparency of data.	Summary data were presented in tables and figures only	**1**
Number of samples and replication.	Four colonies per field with multiple measurements on 2 or more occasions.	**1**
Overall evaluation of methods	Computed mean of above	**2.20**
One major weaknesses: no statistics described and no residues in any matrix so no exposure	**0.5**	**1.10**

Response 1. Change in weights of hives placed into fields with sunflowers growing from IMI-dressed seed		**Score**
Statistical significance of responses	There was no significant change in hive weights from July 27 to August 6, 1998 in either the treated plot or the control plot. No statistical analyses so no P values were included.	**0**
Concentration- or dose-response	Only one treatment concentration of 59 a.i. IMI/ha. There were no significant effects and a concentration-response was not observed	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Effects on foraging activity of honey bees in hives placed into fields with sunflowers growing from IMI-dressed seed		**Score**
Statistical significance of responses	The average foraging activity (n=9 occasions) in the control field was 57.2 bees whereas in the treated field it was nearly 3x that (142.8 bees); No statistical analyses so there was no P value and the difference was attributed to the better-quality flowers in the treated plot. There was no significant adverse effect.	**0**
Concentration- or dose-response	Only one treatment concentration of 59 a.i. IMI/ha. There were no significant adverse effects and a concentration-response was not observed	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant adverse response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 3. Effects on number of honey bees returning to hives placed into fields with sunflowers growing from IMI-dressed seed	**Score**

Statistical significance of responses	Data are available only for the IMI treatment because of equipment failure and therefore, there was no distinguishable significant adverse effect.	**0**
Concentration- or dose-response	Only one treatment concentration of 59 a.i. IMI/ha. There were no significant adverse effects and a concentration-response was not observed	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant adverse response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 4. Effects on the number of honey bees returning with pollen to the hives placed into fields with sunflowers growing from IMI-dressed seed		**Score**
Statistical significance of responses	No statistical analyses so no P values. The average number of bees returning to the control hives carrying pollen was 20.0 bees and for the treated field there were on average 24.2 bees. The origin of the pollen could not be identified but it was assumed to be sunflower because of the absence of wildflowers in the area.	**0**
Concentration- or dose-response	Only one treatment concentration of 59 a.i. IMI/ha. There were no significant adverse effects and a concentration-response was not observed	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant adverse response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 5. Effects on honeybee mortality in hives placed into fields with sunflowers growing from IMI-dressed seed		**Score**
Statistical significance of responses	The total number of dead bees in the control field was 147 and in the treated field it was 201. A t test indicated that there was no significant difference (Mann-Whitney Rank Analyses; P=0.471) but that the data were not normally distributed	**0**
Concentration- or dose-response	Only one treatment concentration of 59 a.i. IMI/ha. There were no significant adverse effects and a concentration-response was not observed	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant adverse response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**

Overall evaluation of relevance (computed)	**0.00**
Overall evaluation of relevance (SEJ).	

Narrative

No adverse effects data available in this study as a result of all residue data for nectar in honey bladder and bees themselves were below the detection limit of 0.01 mg/kg.

Expert Judgement		
QA	YES	August 2015
SEJ	Yes	October 2

(Bayer CropScience 1999b)

Report: Bayer CropScience. 1999. Residues of Imidacloprid and Imidacloprid Metabolites in Nectar, Blossoms, Pollen and Honey Bees Sampled from a Summer Rape Field in Sweden and Effects of These Residues on Foraging Honeybees. Leverkusen, Germany: Bayer CropScience (Unpublished Report). Report M-006811-01-1. 28 p

Field plots (1 for each treatment) in a field planted (April 28, 1998) with seed-dressed rape and untreated rape seed were established in near Borlunda-Skelinge, South of Eslov in Sweden to investigate exposure of honey bees to a mixture of IMI (Poncho FS 500) and a synthetic pyrethroid (beta-cyfluthrin) for potential effects. Residues (IMI, olefin- and hydroxy-metabolites) were measured in samples of honey bees (collected before and after exposure), nectar sampled by bees, nectar sampled from flowers, blossoms, and pollen collected by bees (insufficient pollen to measure). Effects on bee mortality, foraging and flight intensities, and behaviour were assessed. Only the effects data for foraging and flight activities were included in the report. All the analytical data included in Appendix. All residues measured in all matrices were below the detection limit of 0.01 mg/kg. Because there was no apparent exposure to IMI, it is not surprising that there were no adverse biological effects observed.

Responses 1-5: Quality methods		**Score**
Experimental design and hypotheses	Two plots (8x17 m) for the treated and control plots in one field (**major weakness** – no true replication) were each sown with dressed and undressed summer rape seed (var. Maskot), respectively at a rate of 5 kg/ha (68 g seed per 136 m^2; 2.5L/dt Poncho FS 500 (IMI=428.2 a.i. g/l)); plots were separated by a 2-m buffer strip (Figure 1). Application rates were confirmed and method of application and maintenance procedures provided. Source of bees provided; calibration of machinery described; previous crop was sugar beet. Hives (~5,000 bees/hive) were installed (July 1, 1998) at the time of flowering in tunnels (4x4 m and 2 m high); one hive per tunnel (**major weakness – insufficient replicates**) for each treatment. Samples of bees were collected for residue determination prior to being released into the tunnel. Bees were released to forage within the tunnels and the number of bees leaving and returning or foraging on flowers per unit time was recorded (i.e., no behaviour or mortality raw data). The exposure period was 5 days (July 2-6, 1998; hives were removed on July 7, 1998). Weather conditions over the 6-d study period were monitored. For residue assessment, honey bees were sampled prior to exposure and directly from flowers on which they were foraging- honey bulbs were dissected and the nectar collected by bees (200) analysed; nectar was also collected directly from rape flowers outside of the caged area and analysed; field soils were characterized; bees were measured before and after exposure. Analytical methods (HPLC/MS/MS) were described and field blanks and controls used. Measurement endpoint data were collected on days 1, 2, and 4 post-exposure during 5 minutes daily or on flowers in an area of 1 m^2. Non-quantitative behavioural observations include exaggerated motility, discoordinated movements (i.e., trembling, shaking, and apathy) and number of dead bees.	**1**
Use of GLP and QA/QC.	GLP study with signatures and certificate. The LOQ was <0.01 mg/kg no % recoveries reported (**minor weakness**)	**3**

Exposure concentrations	Non-detections in all matrices which suggests, bees were not exposed to concentrations of concern	**4**
Transparency of data.	Raw data were provided for three of five endpoints only; no data for mortality, behaviour.	**1**
Number of samples and replication.	One field, two plots, one hive in each plot = inadequate replication; samples were collected over time for repeated measures.	**0**
Overall evaluation of methods	Computed mean of above	**1.80**
A major weakness was the lack of power attributable to too few replicates.	**0.5**	**0.90**

Response 1. Effects Honey bee mortality		**Score**
Statistical significance of responses	No statistical analyses. Mortality data not included, only a statement to say there were no adverse effects observed for this endpoint.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Effects Honey bee behavior (trembling, apathy, shaking etc.)		**Score**
Statistical significance of responses	No statistical analyses and no data included, only a statement saying there were no adverse effects observed for this endpoint.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 3-4. Effects honey bee flight intensity (# bees leaving and returning to the hive)		**Score**
Statistical significance of responses	No statistical analyses. Three observations from days 1, 2 and 4. Data indicated that there were no adverse effects observed for this endpoint.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**

Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 5. Effects honey bee foraging		**Score**
Statistical significance of responses	No statistical analyses of data. Data indicated that there were no adverse effects observed for this endpoint.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Narrative

All residues measured in all matrices were below the detection limit of 0.01 mg/kg. Because there was no apparent exposure to IMI, it is not surprising that there were no adverse biological effects observed.

Expert Judgement		
QA	Yes	24 September 2015
SEJ	Yes	Oct 2, 2015

(Bayer CropScience 1999c)

Report: Bayer CropScience. 1999. Residues of Imidacloprid and Imidacloprid Metabolites in Nectar, Blossoms, Pollen and Honey Bees Sampled from a French Summer Rape Field and Effects of These Residues on Foraging Honeybees. Leverkusen, Germany: Bayer CropScience (Unpublished Report). Report M-006815-01-1. 27 p

Field plots (1 in each treatment) in a field planted (March 19, 1998) with seed-dressed rape and untreated rape seed were established in near Conches between la Neuve Lyre and la Vieille Lyre in Northern France to investigate exposure of honey bees to a mixture of IMI (Poncho FS 500) and a synthetic pyrethroid (beta-cyfluthrin) for potential effects. Residues (IMI, olefin- and hydroxy-metabolites) were measured in samples of honey bees (collected before and after exposure), nectar sampled by bees, nectar sampled from flowers, blossoms, and pollen collected by bees (insufficient pollen from blank samples to measure). Effect on behaviour of bees was assessed. No effects data were included in the report. All the analytical data included in Appendix. All residues measured in all matrices were below the detection limit of 0.01 mg/kg. Because there was no apparent exposure to IMI, it is not surprising that there were no adverse biological effects observed.

Responses 1-3: Quality methods		**Score**
Experimental design and hypotheses	There were two rows of 4 rape planted plots each on the trial site, however there was only 1 control plot and 1 treatment plot (**major weakness** – no true replication) (Fig. 1). Each plot was 4x20 m with 1 m space between adjacent plots and 8 m space between the two plot rows. Seeds of a variety of summer rape (Lisonne TGW) for the treatment plot were coated with IMI and Talcum blue. The plots were sown with dressed and undressed rape seed, respectively, at a rate of 5 kg/ha for both the control and the treated plots but the treated seeds were dressed with 2.5 L/ha of Poncho FS 500 (IMI = 428.2 a.i. g/L). Site history was not provided but source of bees was identified; calibration of machinery described. Hives (~5,000 bees/hive) were installed the evening of June 16 and removed June 18, 1998) at the time of flowering; they were placed into in tunnels (4x4 m and 2 m high); one hive per tunnel (**major weakness – insufficient replicates**) for each treatment. Samples of bees were collected over the two-day period for residue determination and prior to being released into the tunnel. Bees were released to forage within the tunnels and the number of bees leaving and returning or foraging on flowers per unit time was recorded (i.e., no behaviour or mortality raw data). The exposure period was 2 days (June 17-18, 1998; hives were removed on June 18, 1998). Weather conditions over the 3-d study period were monitored. For residue assessment, honey bees were sampled prior to exposure and directly from flowers on which they were foraging- honey bulbs were dissected and the nectar collected by bees (200) analysed; nectar was also collected directly from protected rape flowers outside of the caged area and analysed; field soils were characterized; bees were measured before and after exposure. Analytical methods (HPLC/MS/MS) were described and field blanks and controls used. Observational data were collected over the two days but no systematic collection was undertaken (methods not described – **major weakness**	**1**

	for effects data). Non-quantitative behavioural observations include exaggerated and dis-coordinated movements (i.e., trembling, shaking, and apathy), apathy, failure to fly, and suspicious mortality.	
Use of GLP and QA/QC.	GLP study with signatures and certificate. The LOQ was <0.01 mg/kg no % recoveries reported (**minor weakness**)	**3**
Exposure concentrations	Non-detections in all matrices which suggests, bees were not exposed to concentrations of concern	**4**
Transparency of data.	Raw data were provided for exposure concentrations only; no data for mortality, behaviour.	**1**
Number of samples and replication.	One field, two plots, one hive in each plot = inadequate replication; samples were collected over time for repeated measures.	**0**
Overall evaluation of methods	Computed mean of above	**1.80**
One field, two plots, one hive per plot – insufficient data (replicates); description of methods inadequate; no systematic collection of effects data	**0.25**	**0.45**

Response 1. Effects Honey bee mortality		**Score**
Statistical significance of responses	No statistical analyses. Mortality data not included, only a statement to say there were no adverse effects observed for this endpoint.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Effects Honey bee behavior (trembling, apathy, shaking etc.)		**Score**
Statistical significance of responses	No statistical analyses and no data included, only a statement saying there were no adverse effects observed for this endpoint.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 3. Effects honey bee flight failure		**Score**
Statistical significance of responses	No statistical analyses and no data were provided. Observational summary indicated that there were no adverse effects observed for this endpoint.	**0**

Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or re-production, and fit-ness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Narrative
All residues measured in all matrices were below the detection limit of 0.01 mg/kg. Because there was no apparent exposure to IMI, it is not surprising that there were no adverse biological effects observed. Honey bees were used to collected samples (nectar) to determine exposure pathways to honey bees for IMI residues. There was insufficient nectar and pollen collected by bees from the control plot so no control samples could be analyzed. The calculated treatment exposures indicated concentrations in nectar and pollen ranging between 1.24 and 2.92 ng/bee which were below the NOAED of 7.3 ng/bee/day.

Expert Judgement		
QA	Yes	25 September 2015
SEJ	Yes	October 2, 2015

(Bayer CropScience 1999d)
Report: Bayer CropScience. 1999. Residue Levels of Imidacloprid and Imidacloprid Metabolites in Nectar, Blossoms and Pollen of Sunflowers Cultivated on Soils with Different Imidacloprid Residue Levels and Effects of These Residues on Foraging Honeybees: Test Location: farmland "Hofchen" - 1999. Leverkusen, Germany: Bayer CropScience (Unpublished Report). Report M-016820-01-1. 41 p

Two studies (Bayer CropScience, 1999, M-016827-01-1 and M016820-01-1) in the exposure WoE were very similar in design but were carried out at two different sites. For this reason, they were combined. For the effects-WoE, the studies were scored separately. These studies were on sunflowers grown in soils previously treated with IMI and sunflower seeds treated with IMI and grown in soil that had not previously been treated with IMI. Only the concentrations in nectar and pollen were assessed.

Responses 1-4 Quality methods		**Score**
Experimental design and hypotheses	This study took place at Bayer experimental farms at Höfchen. The study was well described. The study comprising four field plots took place in 1999; seeding of plots occurred on May 10, 1999. Three plots at Höfchen were seeded with IMI-free sunflower seed (Fleury variety) and one of these plots had IMI-free soils (control) and the other two contained soils to which IMI had been applied previously. The fourth plot had soils free of IMI residues and was seeded with sunflower seed dressed with 150 g Gaucho WS 70/150,000 seeds (by analysis the content of IMI was 72.5% a.s.). The nominal rate was 105 g/150,000 seeds (analytical finding was 89.3 g/150,000 seeds). The drilling rate was 75,000 seeds/ha (or 1,800 seed per four 240 m^2 study plots). The seeds were also treated with a standard combined fungicide (carbendazim, metalaxyl and Cu-oxyquinolat). Previous pesticide use on the plots was described in detail. In addition to the three test plots, the control plot with no prior treatment or treated seed was prepared. At time of seeding, soil samples (20 cores of 5 cm dia. and 30 cm long) were taken in each plot. Soils in all plots were subsampled at 0-20 and 20-30 cm and analyzed for residues of IMI, IMI-OH and IMI= and their properties characterized. One bee colony with 3 combs (2-3K bees) was placed on July 26, 1999 into tunnels established on each field plot. The hives were removed from the tunnels on August 5, 1999. On days 2 and 3, ~100 bees foraging on the sunflowers with the tunnels were collected and the nectar from honeybulbs and pollen from pollen pockets collected. 1 g of honeybees was analyzed for residues. Additionally, on days 2, 4, and 8 sunflower nectar and pollen was collected directly from the combs and during additional pollen sampling (25 July to 30 July), pollen was collected directly from the flowers and analyzed for residues. Flowers and leaves from the sunflowers were collected and analyzed for residues. The effects on bees entailed monitoring foraging intensity (i.e., the number of bees on 100 flower heads on days 0, 1, 2, 3, 7, and 8 post-exposure); behavior and dis-coordinated movements; and mortality (number of bees on linen sheets and around the cage margins). No statistical analyses of data and no p values provided (**major weakness**)	**3**
Use of GLP and QA/QC.	GLP study with appropriate QA/QC; signatures; certification; origin of seed (dressed and undressed) was described. Analysis was by LC/MS/MS and was fully described. Analytical standards of IMI, IMI-OH and IMI= were ≥ 98% pure. Isotopically labelled internal standards were	**4**

	not used (potential weakness). The LOQ for IMI in soil was 6 µg/kg and the LOD was 2 µg/kg. Recover of IMI from spiked soil was >92%. For biological samples, the LOD for IMI and IMI-OH were 1.5 µg/kg and 3 µg/kg for IMI=. The LOQs were 5 µg/kg for IMI and IMI-OH and 10 µg/kg for IMI=. Recovery from biological samples was not reported (*potential weakness*).		
Exposure concentrations	IMI concentrations in soils There were no measurable residues in the soil in the control plots (LOD = 2 µg/kg). Concentrations in the soil from previously treated plots were 17.8 and <LOQ (6 µg/kg) for the two plots having IMI-free seed with IMI-residue soils. No residues of IMI were detected in the soil from the plots where treated seed was sown on previously untreated soil.		**4**
Transparency of data.	All raw data and summary data provided in the main document and the study protocol was provided; no statistical analyses of data provided (**major weakness**)		**4**
Number of samples and replication.	Four field plots including controls; no replicate hives per plot		**3**
Overall evaluation of methods	Computed mean of above		**3.60**
No statistical analyses of effects data		**0.5**	**1.80**

Response 1. Effects on changes in hive weight		**Score**
Statistical significance of responses	Hive weights decreased over the study period for all treatments (no statistical p values reported); however, the decreases (between 3.0 and 5.9%) were similar for all treatments.	**0**
Concentration- or dose-response	A concentration-response was neither observed nor characterized by the authors.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Effects on foraging activity		**Score**
Statistical significance of responses	Analyses was based on 6 observational periods and expressed as total number of foraging honey bees per 50 flower heads. The control averaged was 100 bees and the range for the treated plots was between 82 and 130. No difference in foraging activity was concluded.	**0**
Concentration- or dose-response	A concentration-response was neither observed nor characterized by the authors.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**

Overall evaluation of relevance (computed)	**0.00**
Overall evaluation of relevance (SEJ).	

Response 3. Effects on honeybee mortality		**Score**
Statistical significance of responses	Most of the mortality was observed at the "tent" margin; however, the total number of dead bees in the control plots was 17 and the range for the treated plots was 17-18 (Table 2). No difference among treatments in bee mortality was concluded.	**0**
Concentration- or dose-response	A concentration-response was neither observed nor characterized by the authors.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 4. Effects on population strength and brood status		**Score**
Statistical significance of responses	A semi-quantitative summary (Table 2) indicated that there was no significant effect on population strength, food stores, or brood development.	**0**
Concentration- or dose-response	A concentration-response was neither observed nor characterized by the authors.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Narrative
At Höfchen, the concentration of IMI in leaf tissue was 7 µg/kg (and 6 µg/kg at Laacher Hof) but that of the IMI-OH was <LOQ (5 µg/kg). In these cases, because IMI was detected in one. There were no significant effects attributable to IMI.

Expert Judgement		
QA	Yes	October 16, 2015
SEJ	Yes	October 15, 2015

(Bayer CropScience 1999e)

Report: Bayer CropScience. 1999. Residue Levels of Imidacloprid and Imidacloprid Metabolites in Nectar, Blossoms and Pollen of Sunflowers Cultivated on Soils with Different Imidacloprid Residue Levels and Effects of These Residues on Foraging Honeybees: Test Location: farmland "Laacher Hof" - 1999. Leverkusen, Germany: Bayer CropScience (Unpublished Report). Report M-016827-01-1. 44 p

The GLP study had 5 treatments (control and 4 test plots). It took place in experimental farmfield (Laacher Hof) plots 3 km south of Monheim, Germany. The fields were planted (drilled 0.58 U/ha = 2,105 see per four 240 m^2 plots) on May 12, 1999 with sunflower (variety Fleury) dressed with either 150 g/U Gaucho WS 70 (a.i., IMI 72.5%) plus a fungicide treatment (Carbendazim, Metalaxyl and Cu-Oxyquinolat) or only the fungicide for the control treatment. The objective of the study was to determine residues in soil, nectar, flowers (male and female – 20 g each), leaves, pollen sampled from hives and bees, and honeybees exposed to the sunflowers in tunnels and to assess any behavioural changes to the foraging or feeding bees. A final assessment of colony strength and brood status on day 8 after the first exposure (for test variant 1998 (2x) on day 13) was conducted. No residues were measured in any of the samples, except traces of IMI (0.006 mg/kg) in the leaves of plants from Gaucho-dressed seed and there were no significant effects on honeybee behaviour or colony strength or brood status. Biological endpoints included: foraging intensity (6 x on days 0, 1, 2, 3, 7, and 8 post-exposure) which was the number of bees foraging on randomly selected 100 sunflower heads on each plot), behavioural observations (motility, dis-coordinated movements – trembling & shaking – or apathy), and mortality – dead bees on linen traps in front of entrance to hives and in tunnels to hives.

Responses 1-4: Quality methods		**Score**
Experimental design and hypotheses	Treatments included a control and four field plots. Existing IMI residues might have been present from previous years on the treated plots. The fields were planted (drilled) on May 12, 1999 sunflower (variety Fleury) dressed with either 150g/150,000 seed Gaucho WS 70 (a.i., 72.5% IMI) plus a fungicide treatment (Carbendazim, Metalaxyl and Cu-Oxyguinolat) or only the fungicide for the control treatment. Objective focused on exposure more than effects; however, behavioural observations were systematically collected from caged bee colonies (2-3,000 honey bees/colony) from July 26– August 3, 1999 (8-d period) during the flowering of sunflowers (in variant 98(2x), the hive colony was installed on 21 July 1999 due to earlier flowering. Detailed history of plots included. Nectar was collected directly from plants during flowering and from the bees' honeybulbs. Honey bees were collected while foraging on days 2 and 3 following installation of the colony (for variant 98 (2x) sampling was done on days 6 and 8 after hive instalment) in tunnels (one hive with 3 combs) and used to extract nectar, pollen, and to measure residues. Pollen was collected from combs on days 2, 4, and 8 following installation of the colony. Additional pollen samples were collected in study plots between 25 and 29 July directly from the sunflower heads by shaking pollen out. Hives were removed on August 5, 1999. Male and female flowers (20g each) were collected at peak flowering. Leaves were collected on July 23, 1999. Description of analytical chemistry was acceptable. Weather data were collected and soils characterized. Descriptions of biological assessment methods were included. Hive weights decreased over the study period for all treatments (no statistical p values reported)	**3**

Use of GLP and QA/QC.	GLP study with certificates; all analytical work followed GLP and was fully described. Deviations were reported. LOQs and non-detection limits for IMI were provided for soil (0.006 mg/kg and 0.002 mg/kg, respectively) and biological material (0.005 mg/kg and 0.0015); LOQs for hydroxyl-IMI and olefin-IMI were 0.005 and 0.01 mg/kg, respectively; with non-detection limits of 0.0015 and 0.003, respectively. History of pesticide use on plots was provided.		**4**
Exposure concentrations	The application rates were verified. The soil residues were verified on study plots from soil samples collected the day of drilling (20 x 5-cm diameter cores collected to a depth of 30 cm from two diagonal transects in each plot. IMI residues in surface (0-30) soil prior to drilling were 0 for the control and one of the four treated fields, and 12.7, 14.3, and 15.7 µg/kg for the 3 remaining fields. Control nectar, pollen, honeybees, flowers, leaves were free of residues of IMI. Plant leaves for the IMI-dressed seeds had residues (0.006 mg/kg) for IMI. Traces of the metabolites were also found in the leaves of IMI-dressed seeds. Analytical methods described or cited and QA was appropriate (fortified soil samples with recovery rates of 95.7%; however, no recovery rates reported for biological material).		**4**
Transparency of data.	Summary data available in the main document but no details provided on colony strength or brood development and no statistical analyses were applied to the data (**major weakness).**		**2**
Number of samples and replication.	Five field plots including controls no replicate hives per plot		**3**
Overall evaluation of methods	Computed mean of above		**3.20**
No statistical analyses of data.		**0.5**	**1.60**

Response 1. Effects on changes in hive weight		**Score**
Statistical significance of responses	Hive weights decreased over the study period for all treatments (no statistical p values reported); however, the decreases (between 6.2 and 7.2%) were similar for all treatments	**0**
Concentration- or dose-response	A concentration-response was neither observed nor characterized by the authors.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Effects on foraging activity		**Score**
Statistical significance of responses	Analyses was based on 6 observational periods and expressed as total number of foraging honey bees per plot. The control total number was 143 bees and the range for the treated plots was between 61 and 252. No difference in foraging activity was concluded.	**0**
Concentration- or dose-response	A concentration-response was neither observed nor characterized by the authors.	**0**
Relevance of effect to apical endpoints	Since no significant response was observed, there was no relevance to the effects.	**0**

(survival and/or reproduction, and fitness)		
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 3. Effects on honeybee mortality		**Score**
Statistical significance of responses	Most of the mortality was observed at the "tent" margin; however, the total number of dead bees in the control plots was 11 and the range for the treated plots was 20-141. The site-specific peak mortality of variant 1998 (2x) (141) was attributed to an earlier exhausting of the sunflowers. No difference among treatments in bee mortality was concluded.	**0**
Concentration- or dose-response	A concentration-response was neither observed nor characterized by the authors.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 4. Effects on population strength and brood status		**Score**
Statistical significance of responses	A semi-quantitative summary (Table 2) indicated that there was no significant effect on population strength, food stores, or brood development.	**0**
Concentration- or dose-response	A concentration-response was neither observed nor characterized by the authors.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Expert Judgement		
QA	Yes	25 September 2015
SEJ	Yes	October 2, 2015

(Bayer CropScience 1999a)
Report: Bayer CropScience. 1999. Residues of Imidacloprid and Imidacloprid Metabolites in Nectar, Blossoms, Pollen and Honey Bees Sampled from a British Summer Rape Field and Effects of These Residues on Foraging Honeybees. Leverkusen, Germany: Bayer CropScience (Unpublished Report). Report M-040023-01-1. 29 p

Field plots (2 in each of 3 treatment) in a field planted (March 20, 1998) with seed-dressed rape and untreated rape seed were established on the Bayer Experimental Elm Farm in the UK to investigate exposure of honey bees to a mixture of IMI (Poncho FS 500) and a synthetic pyrethroid (beta-cyfluthrin) for potential effects. The third treatment was an experimental product with IMI. Residues (IMI, olefin- and hydroxy-metabolites) were measured in samples of honey bees (collected before and after exposure), nectar sampled by bees, nectar sampled from flowers, blossoms, and pollen collected by bees. Effects on bee mortality, foraging and flight intensities, and behaviour were assessed. Only the effects data for foraging and flight activities were included. All the analytical data included in Appendix. All residues measured in all matrices were below the detection limit of 0.01 mg/kg. Because there was no apparent exposure to IMI, it is not surprising that there were no adverse biological effects observed.

Responses 1-5: Quality methods		**Score**
Experimental design and hypotheses	There were two rows of 3 rape planted plots each on the trial site, however only one row was sampled (the other row served only as a reserve plot) (Fig. 1). Each plot was 4x15 m with 1 m space between adjacent plots. Seeds of a variety of rape (Lisonne TGW) were coated with IMI and Talcum blue. The plots were sown with dressed and undressed rape seed, respectively, at a rate of 5 kg/ha (= 30 g per 60 m^2 plot) for both the control and the treated plots but the treated seeds were dressed with 2.5 L/ha of Poncho FS 500. Site history was provided and source of bees was identified; calibration of machinery described; previous crop was grass. Hives (~5,000 bees/hive) were installed the evening of June 23 and removed June 25, 1998) at the time of flowering; they were placed into in tunnels (4x4 m and 2 m high); one hive per tunnel (**major weakness – insufficient replicates**) for each treatment. Samples of bees were collected for residue determination prior to placing beehives on the plots and for two-days after being released into the tunnel. Bees were released to forage within the tunnels and the number of bees leaving and returning or foraging on flowers per unit time was recorded (i.e., no behaviour or mortality raw data). The exposure period was 2 days (June 23-24, 1998. Weather conditions over the 3 d study period were monitored. For residue assessment, honey bees were sampled prior to exposure and directly from flowers on which they were foraging- honey bulbs were dissected and the nectar collected by bees (200) analysed; nectar was also collected directly from protected rape flowers (10-20 plants) outside of the caged area and analysed; field soils were characterized; bees were measured before and after exposure. Analytical methods (HPLC/MS/MS) were described and field blanks and controls used. Observational data were collected over the two days but no systematic collection was undertaken (methods not described – **major weakness** for effects data). Non-quantitative behavioural observations include exaggerated and dis-coordinated movements (i.e., trembling, shaking, and apathy), and failure to fly. Measurement endpoints included bee mortality (observational, no data), flight activity (# bees leaving and returning to	**1**

	hive), forging intensity (#bees which foraged on flowering rape during checks; 6 time periods).		
Use of GLP and QA/QC.	GLP study with signatures and certificate. The LOQ was <0.01 mg/kg, no % recoveries reported (**minor weakness**)		**3**
Exposure concentrations	Non-detections in all matrices which suggests, bees were not exposed to concentrations of concern		**4**
Transparency of data.	Raw data were provided for three of five endpoints only; no data for mortality, behaviour.		**1**
Number of samples and replication.	One field, two plots, one hive in each plot = inadequate replication; samples were collected over time for repeated measures.		**0**
Overall evaluation of methods	Computed mean of above		**1.80**
One field, two plots but only one in each treatment sampled, one hive per plot – insufficient data (replicates); no exposure data because all measured residues below detection limit		**0.25**	**0.45**

Response 1. Effects on honeybee mortality		**Score**
Statistical significance of responses	No statistical analyses. Mortality data not included, only a statement to say there were no adverse effects observed for this endpoint.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Effects on honeybee behavior (trembling, apathy, shaking etc.)		**Score**
Statistical significance of responses	No statistical analyses and no data included, only a statement saying there were no adverse effects observed for this endpoint.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 3. Effects on the number of bees leaving the hive		**Score**
Statistical significance of responses	No statistical analyses were provided. Data collected were variable and indicated that there were no adverse effects observed for this endpoint.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**

Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 4. Effects on honeybee foraging intensity		**Score**
Statistical significance of responses	No statistical analyses were provided. Data collected were variable and indicated that there were no adverse effects observed for this endpoint.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 5. Effects on the number of bees returning to the hive		**Score**
Statistical significance of responses	No statistical analyses were provided. Data collected were variable and indicated that there were no adverse effects observed for this endpoint.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Narrative
All residues (in honey bees before and after exposure, nectar from honey bulbs of bees before and after exposure, nectar sampled from flowers, in rape blossoms, and pollen collected by bees) measured in all matrices were below the detection limit of 0.01 mg/kg. Because there was no apparent exposure to IMI, it is not surprising that there were no adverse biological effects observed. Honey bees were used to collected samples (nectar) to determine exposure pathways honey bees for IMI residues.

Expert Judgement		
QA	Yes	25 September 2015
SEJ	Yes	October 2, 2015

(Bayer CropScience 2002)
Report: Bayer CropScience. 2002. Field Test: Side Effects of Oil-Seed Rape grown from Seeds Dressed with Imidacloprid and Beta-Cyfluthrin FS 500 on the Honey Bee (*Apis mellifera* L.). Leverkusen, Germany: Bayer CropScience (Unpublished Report). Report M-066846-01-1. 76 p

Oil-seed rape seed were dressed with 1051.17 g and 187.31 g a.i., respectively of Imidacloprid & beta-cyfluthrin (a synthetic pyrethroid) FS 500/100 kg seeds and Thiram (fungicide). Two fields: treated and untreated; the latter served as the experimental control included plots and were treated only with Thiram. The fields were located in northern Germany, near Hetendorf (Niedersachsen) and the distance between the treated and control groups was 4 km.

Responses 1-5: Quality methods		**Score**
Experimental design and hypotheses	The objective of the study was to determine the effects of oil-seed rape treated with the above product on honey bee (*Apis mellifera* L.) under field conditions following standard EPPO guidelines (1992) and SETAC guidelines (1995). One test field (23,432 m^2) was planted with oil-seed rape (*Brassica napus* L., var. Lirajet) dressed with 1051.17 g a.i. IMI/100 kg seeds and sown with 7 kg seeds. A second field (25,919 m^2) sown with 8.4 kg of undressed seeds served as the control treatment. There were 6 (normally developed, queen right) hives (4 colony groups each with 3 hives, 2 colony groups per field). Hives were placed in two groups of 3 next to each field prior to the full flowering stage April 27, 2000 (D0). Endpoints measured included: mortality (measured daily between d 1 and 14), flight intensity (up to 3 times per day, between d 1 and 14), colony condition and development of bee brood (4x on d 5, 11, 18, 47), and weight of test colonies (daily between d0 and d18). Residues were measured in pollen, nectar and honey collected from test colonies and nectar collected from blossoms. After end of flowering 12 May 2000, on June 27, 2000, all bee hives were moved to 40 km away from the test fields and monitored until the end of the bee season. Endpoints for the exposed group were compared to a comparable set of endpoints collected from the control. Only hives with certificates of health were used to ensure hives had similar strength at the start of the test. Each colony contained 2 brood bodies with 6-8 brood frames and 1 honey body with at least 8 frames. Dead be traps were placed at the hive entrance and dead bees were monitored on linen sheets that were in front of the hive and placed in three locations (5 m^2 each) in each field to monitor bee mortality. The second colony group with three hives was used for pollen, nectar and honey collection. Mortality of bees in traps (colony 1) and on the linen sheets was monitored daily after the first bloom for 14 d. Flight intensity was monitored in the field for 14 days starting the day after the hives were placed into the fields. Observations were made at multiple locations for periods of 1 minute. Condition or quality colony was measured every 6 days starting 5 days after the hives were set up. Colony strength (number of combs covered with bees), presence of healthy queen (eggs, queen cells), pollen and nectar storage areas, area containing eggs, larvae, pupae. Observations were made on bee behaviour. Product concentrations and loading rates were verified analytically. Source of bees identified. IMI concentrations were measured in honey, nectar, and pollen. Meteorology metrics and phenology of crop plants and others were reported. Analytical method provided as well as LODs	**3**

	and LOQs for IMI and metabolites. All residues were below the limits of detection 0.0015 mg/kg for IMI and hydroxy-metabolite and 0.003mg/kg for olefin-metabolite (40 samples) or LOQs (2 samples). No statistical analyses of data or p values presented (**major weakness**).		
Use of GLP and QA/QC.	GLP study with QA section and deviations reported; certificate provided		**4**
Exposure concentrations	Only one exposure application rate in combination with a second insecticide (Beta-Cyfluthrin) and a fungicide (Thiram). All residues below detection limits.		**3**
Transparency of data.	Data in appendices. Summary data in text		**3**
Number of samples and replication.	3 colonies for some endpoints; multiple measures over time usually a 14 d period but only one treatment field.		**1**
Overall evaluation of methods	Computed mean of above		**2.80**
There were no statistical analyses of data		**0.5**	**1.40**

Response 1. Effect of IMI and B-Cyfluthrin FS 500 on bee mortality		**Score**
Statistical significance of responses	No p values reported and no statistical analyses of data. Average number of dead bees during the evaluation (d 1-14) in the treated group was 8.3 bees/colony and day and 12 dead bees/colony and day in the control treatment (Tables 11 & 12).	**0**
Concentration- or dose-response	Only one concentration rate applied and no residues reported for nectar, bee bread or pollen.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Effect of IMI and Beta-Cyfluthrin FS 500 on honeybee flight intensity		**Score**
Statistical significance of responses	No p values reported and no statistical analyses of data. Average flight intensity was 3.3 bees/m^2/min in the treated group and 2.3 bees/m2/min for the control (Tables 13 & 14).	**0**
Concentration- or dose-response	Only one concentration rate applied and no residues reported for nectar, bee bread or pollen.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 3. Effect of IMI and Beta-Cyfluthrin FS 500 on honeybee brood development (comb area containing bees, eggs, larvae, pupae, food)	**Score**

Statistical significance of responses	No p values reported and no statistical analyses of data. Values for these parameters appeared comparable between the hives in the control and treatment groups (Tables 6 and 7)	**0**
Concentration- or dose-response	Only one concentration rate applied and no residues reported for nectar, bee bread or pollen.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 4. Effect of IMI and Beta-Cyfluthrin FS 500 on colony weight		**Score**
Statistical significance of responses	No p values reported and no statistical analyses of data. Colony weights were comparable between the two treatment groups (n=3) (Table 8, Figures 3 and 4).	**0**
Concentration- or dose-response	Only one concentration rate applied and no residues reported for nectar, bee bread or pollen.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 5. Effect of IMI and Beta-cyfluthrin FS 500 on honeybee behaviour		**Score**
Statistical significance of responses	No p values reported and no data. Honey bees in the control and treated groups did not exhibit any abnormal behaviours; intensive pollen and nectar collection behaviour was observed and was comparable between treatments	**0**
Concentration- or dose-response	Only one concentration rate applied and no residues reported for nectar, bee bread or pollen.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Narrative
There were no effects on any endpoints and no residues reported in nectar, pollen, bee bread or honey samples.

Expert Judgement		
QA	Yes	September 28, 2015
SEJ	Yes	October 2, 2015

(Bayer CropScience 2007)

Report: Bayer CropScience. 2007. Residue Levels of Imidacloprid and Imidacloprid Metabolites in Nectar, Blossoms and Pollen of Summer Rape Cultivated on Soils with Different Imidacloprid Residue Levels and Effects of These Residues on Foraging Honeybees: Test Location: farmland "Laacher Hof' - 1999. Report Amendment No. 1 13 April 2007. Leverkusen, Germany: Bayer CropScience (Unpublished Report). Report M-016828-02-1. 45 p

The GLP study had 5 treatments (control and 4 test plots). It took place in experimental farmfield (Laacher Hof) plots 3 km south of Monheim, Germany. The fields were planted (drilled 3.25 kg/ha) on May 12, 1999 with summer rape (variety Lisonne) dressed with either 25 ml/kg Poncho FS 500 (a.i., 79.7 g/L beta-Cyfluthrin and 427.4 g/L IMI) plus a fungicide (TMTD) or only the fungicide for the control treatment. The objective of the study was to determine residues in soil, nectar, flowers (male and female – 20 g each), leaves, pollen sampled from hives and bees, and honeybees exposed to the rape in tunnels and to assess any behavioural changes to the foraging or feeding bees. A final assessment of colony strength and brood status on September 3 was conducted. No residues were measured in any of the samples and there were no significant effects on honeybee behaviour or colony strength or brood status. Biological endpoints included: foraging intensity (6 x on days 2, 3, 4, 5, 6, and 9 post-exposure) which was the number of bees foraging during 1 minutes in a 1 m^2 area on each plot), behavioural observations (motility, dis-coordinated movements – trembling & shaking – or apathy), and mortality – dead bees on linen traps in front of entrance to hives and in tunnels to hives.

Responses 1-4: Quality methods		**Score**
Experimental design and hypotheses	Treatments included a control and four field plots. Existing IMI residues might have been present from previous years on the treated plots. The fields were planted (drilled) on May 12, 1999 with summer rape (variety Lisonne) dressed with either 25 ml/kg Poncho FS 500 (a.i., 79.7 g/L beta-Cyfluthrin and 427.4 g/L IMI) plus a fungicide (TMTD) or only the fungicide for the control treatment. Objective focused on exposure more than effects; however, behavioural observations were systematically collected from caged bee colonies (2-3,000 honey bees/colony) from July 10-20, 1999 (10-d period) during the flowering of summer rape. Detailed history of plots included. Nectar was collected directly from plants during flowering and from the bees' honeybulbs. Honey bees were collected while foraging on days 7 and 9 following installation of the colony on July 10 in tunnels (one hive with 3 combs) and used to extract nectar, pollen, and to measure residues. Hives were removed on July 20, 1999 at which time pollen was collected from the combs and pollen pockets of 100 bees in each tunnel. Male and female flowers (20 g each) were collected at peak flowering. Leaves were collected on July 13, 1999. Description of analytical chemistry was acceptable. Weather data were collected and soils characterized. Descriptions of biological assessment methods were included. Hive weights decreased over the study period for all treatments (no statistical p values reported)	**3**
Use of GLP and QA/QC.	GLP study with certificates; all analytical work followed GLP and was fully described. Deviations were reported. Report was amended in 2007 to correct three errors. LOQs and non-detection limits for IMI were provided for soil (0.006 mg/kg and 0.002 mg/kg, respectively) and biological material (0.005 mg/kg and 0.0015); LOQs for hydroxyl-IMI and olefin-IMI were 0.005 and 0.01 mg/kg, respectively; with non-detection limits of 0.0015 and 0.003, respectively. History of pesticide use on plots was provided.	**4**

Exposure concentrations	The application rates were verified. The soil residues were verified on study plots from soil samples collected the day of drilling (20 x 5-cm diameter cores collected to a depth of 30 cm from two diagonal transects in each plot. IMI residues in surface (0-30) soil prior to drilling were 0 for the control and one of the four treated fields, and 12.7, 14.3, and 15.7 µg/kg for the 3 remaining fields. Control nectar, pollen, honeybees, flowers, leaves were free of residues of IMI. Plant leaves for the treated plots had residues between the LOD and LOQs for IMI. Traces of the metabolites were found in some samples. Analytical methods described or cited and QA was appropriate (fortified soil samples with recovery rates of 95.7%; however, no recovery rates reported for biological material). Although the study lasted for longer the biological observations were made over a 10-d period and then at the end of the study on September 3, 2015.		**4**
Transparency of data.	Summary data available in the main document but no details provided on colony strength or brood development and no statistical analyses were applied to the data (**major weakness).**		**2**
Number of samples and replication.	Five field plots including controls no replicate hives per plot		**3**
Overall evaluation of methods	Computed mean of above		**3.20**
No statistical analyses of data were included; no P values.		**0.5**	**1.60**

Response 1. Effects on changes in hive weight		**Score**
Statistical significance of responses	Hive weights decreased over the study period for all treatments (no statistical p values reported); however, the decreases (between 2.9 and 4.2%) were similar for all treatments and judged to not be statistically significant.	**0**
Concentration- or dose-response	A concentration-response was neither observed nor characterized by the authors.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Effects on foraging activity		**Score**
Statistical significance of responses	Analyses was based on 6 observational periods and expressed as total number of foraging honey bees per plot. The control total number was 122 bees and the range for the treated plots was between 82 and 134. No difference in foraging activity was concluded.	**0**
Concentration- or dose-response	A concentration-response was neither observed nor characterized by the authors.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**

Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 3. Effects on honeybee mortality		**Score**
Statistical significance of responses	Most of the mortality was observed at the "tent" margin; however, the total number of dead bees in the control plots was 11 and the range for the treated plots was 9-11. No difference among treatments in bee mortality was concluded.	**0**
Concentration- or dose-response	A concentration-response was neither observed nor characterized by the authors.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 4. Effects on population strength and brood status		**Score**
Statistical significance of responses	A semi-quantitative summary (Table 2) indicated that there was no significant effect on population strength, food stores, or brood development.	**0**
Concentration- or dose-response	A concentration-response was neither observed nor characterized by the authors.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Expert Judgement		
QA	Yes	25 September 2015
SEJ	Yes	October 2, 2015

(Bayer CropScience 2012c)

Report: Bayer CropScience. 2012. Assessment of Exposure of Honey Bees (*Apis mellifera*) to Imidacloprid in Controlled Feeding Study, Interim Report. Research Triangle Park, NC: Bayer CropScience (Unpublished Report). Report M-442872-01-1. 20 p

No final report was available. Field trials were conducted in Missoula, Montana to determine the effects of feeding IMI to honey bees in artificial syrup and pollen over a 6 wk period. There were 5 treatments with colonies randomly assigned to each treatment (9 colonies per treatment for a total of 45 colonies). There was one field site and the treatments were clustered at different locations (5) on the site with a minimum distance of 100 m between treatments. Exposure period was 6 weeks (July 13 to August 24, 2012). Measurements were made either daily or at the beginning (before and after dosing), middle, and end of the study.

Responses 1-8: Quality methods			**Score**
Experimental design and hypotheses	Experimental design was a two factor, crossed experiment. The colonies were fed IMI amended syrup (50 or 200 µg/L) or amended pollen patties (50 or 200 µg/kg) so the treatment combinations were control 0 pollen/0 syrup (blue), 50/50 (red), 200/50 (white), 50/200 (grey), and 200/200 (yellow) (Figure 1). Confirmatory chemical analyses of doses were outstanding (**major weakness**). Syrup was fed to hives at a rate of 2000 mL per week; pollen at a rate of 500 g per week. Measurement endpoints include: number of bees in dead bee traps, area of nectar, pollen, eggs, larvae, capped brood, frames of bees, and total hive weight. Colony viability, bee population size and total colony weight was made at the end of the growing season just prior to overwintering. Qualitative description of results. Analytical measurements were not available (**major weakness**) and no statistical analysis of the results was undertaken.		**1**
Use of GLP and QA/QC.	Non-GLP but in the spirit of GLP		**0**
Exposure concentrations	Nominal only, no measured results available.		**0**
Transparency of data.	Only summary data available (means and SEs), no statistical analyses, no analytical data available		**1**
Number of samples and replication.	1 site, 5 locations on site.		**1**
Overall evaluation of methods	Computed mean of above		**0.60**
Analytical measurements were not available (**major weakness**) and no statistical analysis of the results was undertaken.		**0.5**	**0.30**

Response 1. Effects on overwintering viability of hives at the end of the growing season (November 23, 2012)		**Score**
Statistical significance of responses	All 9 control (0/0 treatment) hives and all 9 high dose colonies (200/200) were viable but two colonies in the high dose were weak and likely not to survive. The 50/200 treatment had seven viable colonies. The other two treatments had eight viable colonies. No statistical values or comparison made	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**

Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Effects on frame counts and colony weights of hives at the end of the growing season (November 23, 2012)		**Score**
Statistical significance of responses	Colonies exposed to the high syrup concentration of 200 µg/L were the most impacted; the lack of statistical analyses prevents identification of adverse effects that are statistically significant (Figures 2-5).	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 3. Effects on area of stored nectar and pollen for hives exposed to IMI treatments.		**Score**
Statistical significance of responses	Colonies exposed to the high syrup concentration of 200 µg/L consistently ranked among the lowest for area of stored nectar and pollen (Figure 11 and 13); the lack of statistical analyses prevents identification of adverse effects that are statistically significant	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 4. Effects on amount of pollen and syrup consumed by bees of hives exposed to IMI treatments		**Score**
Statistical significance of responses	Honey bees of colonies exposed to the high syrup concentration of 200 µg/L were the most impacted in terms of pollen substitute consumed (Figure 10); For nectar (Figure 12), the amount consumed appeared to be unaffected, with the colonies consuming most, if not all, of the syrup	**0**

	provided each week; the lack of statistical analyses prevents identification of adverse effects that are statistically significant. The control treatment consumed the most pollen and syrup consumption was comparable among treatments and standardized to 2000 mL per week.	
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 5-7. Effects on frame area of egg, larvae and pupae development for hives at the end of the growing season (November 23, 2012)		**Score**
Statistical significance of responses	The frame area with eggs (Fig. 7), larvae (Fig. 8), and pupae (Fig. 9) differed among treatments before dosing and appeared to be comparable for the post dose; lack of statistical analyses precludes identification of significant differences among treatments.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 8. Effects on cumulative number of dead bees in traps of hives exposed to IMI treatments.		**Score**
Statistical significance of responses	The number of dead bees in traps (Fig. 6) increase at the same rate over time regardless of treatment. The lack of statistical analyses precludes identification of significant differences among treatments.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Narrative

Essentially the data are unusable without the statistical analyses and chemical analyses. Qualitative differences cannot be quantified. The report is an interim report with results to date graphed. Tried to secure final report but unavailable as such.

Expert Judgement		
QA	Yes	September 28, 2015
SEJ	Yes	October 2, 2015

(Bayer CropScience 2012b)
Report: Bayer CropScience. 2012. Final Report (Non-GLP) Field Study to Monitor Potential Effects on Honey Bees from Exposure to Guttation Fluid of Winter Barley (W-BAR), Seed-Treated Either with an Imidacloprid or a Clothianidin Combi-Product. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-498922-01-1. 260 p

A field study was conducted at locations in northern (Celle, Lower Saxony) and southern (Baden-Wűrttemberg) Germany to determine if two varieties of winter barley (var. Lomerit and Highlight, respectively) seeds (W-WHT) treated with either Imidacloprid (IMI) (Triadimenol & Imidacloprid & Fuberidazol and Imazalil FS 60+70+7+8) or clothianidin (clothianidin & Beta-Cyfluthrin FS 375+80) would have an adverse effect on honey bees exposed via the guttation exposure pathway. The crops were sown in the fall of 2009 and the study extended over the winter to the spring of 2010 and ended with winter oil-seed flowering in the respective regions. At each location, there were two treatment groups (one field with Imidacloprid-treated W-BAR seeds (analysed seed loading 85.7 and 98.3% of nominal (70 g IMI a.s./100 kg seeds) for Lomerit and Highlight, respectively) and one with clothianidin-treated seeds) and a field sown with fungicide-treated (EfA®) seeds that served as an experimental control. Therefore, there were six field plots in total. All seeds were treated with an abrasion inhibitor (INTECO®) to minimize dust. The hives were set up in the IMI-treated fields on September 15 and 27 to October 31, 2009 and the monitoring period in the spring extended from March 23 and 25, 2010 to April 23 and 25, 2010. Fields were planted at a nominal rate of 200 kg seeds/ha. Field sizes varied among treatments and varieties with the control fields 2.30 and 3.75 ha for Highlight (southern) and Lomerit (northern) varieties, respectively. Those for Imidacloprid were 4.85 and 8.0 ha, respectively, and for clothianidin 3.32 and 7.02 ha, respectively.

At each of the six study fields, five honey bee colonies were placed into the herbaceous off-crop zone in fields either 7 (south) or 3 (north) days before seeds were sown. All of them were within 0.5m of the W-Bar crop. Seeds were sown on the September 22, 2009 in the south and September 28, 2009 in the north. An assessment area in front of each bee hive was divided into two in-crop zones (Zones 0 and 1) and an off-crop zone (0.5 m to each side of the hives, 2 m width in a U shape). 1 m^2 assessment areas were established to monitor guttation. Dead bee traps were established in front of each hive and monitored daily for dead honey bees. Samples of guttation fluid were collected when present and samples pooled to measure the potential exposure residues. The monitoring period began with seedling emergence and lasted until the end of October 2009. Each day the crops were monitored (35 minutes) for guttation and if the presence correlated with honey bee activity, the following data collected: the number of honey bees resting or walking on the ground or on the crop plants. Monitoring continued for 28 days the following spring. Guttation production and honeybee flight activity was monitored daily for 35 min at each plot, morning and evening. Colony strength and development followed the Liebefeld method three times in the fall September 24, October 15 and October 27 in southern Germany and September 25, October 19 and October 29 in northern Germany. Four colony heath assessments also occurred in the spring of 2010, once in March, twice in April and once in May 2010.Measurement endpoints included honeybee mortality, long-term colony development, and overwintering performance of bees in the two treatment groups relative to those in the controls. Additional endpoints included assessing the timing of exudation of guttation fluid by W-BAR and flight activity of honey bees. When these events were correlated, bee activity (e.g., bee visits) was monitored in areas adjacent to the colonies. Guttation exudates were collected and neonicotinoids were measured along with the number of dead bees in traps installed at the entrance to the trap. Colonies overwintered in their field locations. Hives were treated with oxalic acid to control mite infestation.

Results of the study were summarized.

- Colonies were established using selection criteria that would ensure a hive population capable of overwintering; however, the random allocation to treatments of the hives without consideration of colony strength led to a bias in the initial strengths of the colonies among treatments.
- Honeybee mortality over the fall was comparable among treatments and the increased mortality observed in all fields was weather related.
- Behavioural observations were also comparable among treatments. During the spring in 2010 symptoms observed were comparable; no symptoms were observed for bees in the control hives.
- Overwintering performance was comparable between the IMI (89%) and control (80%) treatments but significantly reduced in the fields associated with clothianidin treatments. Two colonies either failed to over winter or over wintered poorly in the IMI group. Varroa mite infestations were implicated in the poor or failure to over winter.
- Colony health especially in the hives in the northern plots was compromised by Varroa mite loads and climatic considerations.
- The maximum concentrations of clothianidin in guttation fluid was 2.3 mg a.s./L and for its two metabolites the maximum measured concentrations were 0.05 and 0.02 mg/L for TZNG and TZMU, respectively. The corresponding maximum Imidacloprid concentrations were 15 and for its metabolites 0.64 and 0.05 mg/L for Imidacloprid-5hydroxy and Imidacloprid-olefin, respectively.
- The frequency of guttation was >60% with no corresponding correlation with bee activity. Guttation in plants of the off-crop zone was less that for the barley in the in-crop zone.

Responses 1-5: Quality methods		**Score**
Experimental design and hypotheses	Hypothesis: Exposure of honey bees to residues of Imidacloprid and clothianidin in guttation fluid of treated winter barley would adversely affect survival of adult bees, brood and colony strength and development, and overwintering performance. Objectives: • to evaluate and compare the colony development and the overwintering performance of exposed honey bee colonies in the different treatment groups relative to the control group; • to determine if honeybee activity correlated with guttation fluid production; and • to measure residues of the parent compounds and metabolites in guttation fluid The study consisted of three treatments (control, Imidacloprid, clothianidin) at each of two locations (6 fields in total). At each of the six field sites each treatment had a total of 5 hives and 5 assessment areas. The assessment area was established at each study plot and was divided into defined In-crop and Off-crop zones and additional areas were segregated for sampling of guttation fluid from plants in each subarea (n=5 subareas; n=3 each with a volume of 1 mL). Dead traps were in front of the hives to monitor dead bees and were checked daily as well as an area 0.5 m2 in front of the dead traps. Monitoring started with seedling emergence and ended at the end of October (4 weeks in the south and three weeks in the north) and extended from March 23 or 25 to April 23 or 25, the following spring. Guttation production and honeybee flight activity was monitored daily for 35 min at each plot, morning and evening. Colony strength and colony heath assessments occurred in the fall of 2009 and spring of 2010.	**2**

	Analytical method was state of the art, fully described, with reported detection limits and recoveries. Purity of compound and origin provided. Neonicotinoids were measured in guttation fluid and in bees in the dead bee traps when n≥10 individuals. Study sites and soils characterized, experimental design clearly explained, replication adequate. Published methods modified; standardized methods not available. Duration of exposure and assessment events provided. The exposure duration was relatively short (<30 days) but considered to be chronic for the ingestion exposure. Measures to control *Varroa* mites were well documented and deviations reported. Plot soils were characterized TOC, WHC, pH, particle-size distribution, soil type). Overwintering indices were generally poor. **Weakness:** Duration of exposure was 4 weeks. All effects were assessed using nominal exposure concentrations. Actual dose to honey bees via guttation fluid was calculated based on the fluid concentration and the time bees spent collecting the material. Other pesticides were applied to the crop during the study. This is not expected to change the results of the study. No guttation fluid was collected from plants in the control treatment (**major weakness)**. Other factors unduly seemed to influence colony overwintering performance; adult mortality; colony strength (weather – cold wet, pests and disease). Variability was high within and among treatments. Poor description of statistical procedures used to analyse data; P values were calculated for proportion of plants displaying guttation (**major weakness**).	
Use of GLP and QA/QC.	The spirit of GLP was followed but it was a non-GLP study; GLP was followed for analytical work; signatures present. QA/QC complete with deviations reported; no reference substance required.	**3**
Exposure concentrations	Concentrations measured in guttation fluid; application to seeds confirmed and seeding rates reported; concentrations in plants and soils were not measured	**3**
Transparency of data.	Full access to summary data but some raw data missing	**4**
Number of samples and replication.	Three treatments each with 2 sites for a total of 6 fields of different sizes. There were 5 hives per treatment, each with defined assessment areas associated with each colony.	**2**
Overall evaluation of methods	Computed mean of above	**2.80**
No guttation fluid was collected from plants in the control treatment (major weakness); Poor description of statistical analyses and no reported P values	**0.5**	**1.40**

Response 1. Effects on honey bee mortality (dead bees in trap & on soil surface)		**Score**
Statistical significance of responses	There were no significant effects on mortality that were consistently different from the control treatments. Assumptions were not tested, methods poorly described, p-values not reported; figures and tables generally had associated measures of variation (although unsure if SD or SEs were plotted). Mortality in the late fall was attributed to the cold weather and was evident in all treatments including the control hives. Therefor, there appeared to be no discernable significant effect on mortality attributable to IMI.	**0**

Concentration- or dose-response	There were no significant effects and a concentration-response was not observed	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	No mechanism or mode of action was proposed; mortality was attributed to weather events.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Effects on honeybee behavior (disturbed coordination, cramps, vomiting, inability to fly)		**Score**
Statistical significance of responses	Behavioural observations were comparable among treatments (i.e., IMI vs CTD); however, there was no statistical comparison made with the control treatment and no symptoms were observed for bees in the control hives.	**0**
Concentration- or dose-response	There were no statistically significant effects identified and a concentration-response was not observed	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	No mechanism was suggested or investigated	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 3. Effects on honeybee brood development (number of bees, worker brood cells, drone brood cells)		**Score**
Statistical significance of responses	Assumptions were not tested, methods poorly described, p-values not reported; figures and tables generally had associated measures of variation (although unsure if SD or SEs were plotted).	**0**
Concentration- or dose-response	There was no concentration response relationship. Exposure of honey bees was via ingestion of guttation fluid. Linkage between concentration in guttation fluid and what the bees actually ingested is poor; however, because there was no pattern or trend of significant adverse effects and impacts to colony health were influence by other factors (weather, viruses, mite loading etc.), causality is questionable. Maximum concentration could be used as unbounded LOAEC.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 4. Effects on overwintering performance of colonies.		Score
Statistical significance of responses	Overwintering performance was comparable between the IMI (89%) and control (80%) treatments. *Varroa* mite infestations were implicated in the poor or failure to over winter. No statistical analyses or P values reported	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	No mechanism was suggested or investigated	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 5. Effects on flight activity		Score
Statistical significance of responses	Assumptions were not tested, methods poorly described, p-values not reported; figures and tables generally had associated measures of variation (although unsure if SD or SEs were plotted). Effects could not be discerned from this study because there was no comparison with the controls. Bees observed in the assessment areas were on plants and on soil in the in-crop and off-crop zones and 10.6% (334/3148 bees) were observed to actively ingest guttation fluid. More bees in the spring, ingested guttation fluid (301/1,881 bees).	**0**
Concentration- or dose-response	No significant concentration- or dose-response relationships were discerned	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Flight activity monitoring established that bees visited the study fields; about 16 % of bees took up guttation fluid in the spring and a much lower proportion took up guttation fluid in the fall. The maximum concentrations of IMI were 15 and 0.1 mg a.s./L in the fall and spring, respectively in guttation fluid collected from the crop in the treated fields. The metabolite (Imidacloprid-5-hydroxy or Imidacloprid-olefin) concentrations ranged between <LOD to 0.64 mg/L or <LOD to 0.05 mg/L, respectively.	**0**
Mechanism and or mode of action of effect explained	No mechanism was suggested or investigated.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Narrative
Conclusions:
Over-all, guttation fluid from winter barley grown from seed treated with either Imidacloprid or clothianidin and ingested by honey bees did not adversely affect honey bee colonies. The maximum exposure concentration for Imidacloprid was 15 mg/L which could be inferred as an unbounded NOAEC and that for clothianidin was 2.3 mg/L

Expert Judgement		
QA	Yes	September 28, 2015
SEJ	Yes	October 2, 2015

(Bayer CropScience 2014f)
Report: Bayer CropScience. 2014. Final Report (Non-GLP) Field Study to Monitor Potential Effects on Honey Bees from Exposure to Guttation Fluid of Winter Wheat (W-WHT), Seed-Treated Either with an Imidacloprid or a Clothianidin Combi-Product. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-498939-01-1. 242 p

A field study was conducted at locations in northern (Celle, Lower Saxony) and southern (Baden-Wűrttemberg) Germany to determine if two varieties of winter wheat (var. Manager and Herrmann, respectively) seeds (W-WHT) treated with either Imidacloprid (IMI) (Triadimenol & Imidacloprid & Fuberidazol and Imazalil FS 60+70+7.2+8) or clothianidin (clothianidin & Beta-Cyfluthrin FS 375+80) would have an adverse effect on honey bees exposed via the guttation exposure pathway. There were six fields in total (3 at each location): 2 were IMI-treated and 2 were experimental controls and 2 were treated with clothianidin.

Colony health especially in the hives in the northern plots was compromised by *Varroa* mite loads. All hives tested positive for deformed wing virus (DWV) and about half for *Nosema apis* and for some other viruses as well.

Conclusions:
Over all, guttation fluid from winter wheat grown from seed treated with neonicotinoids and ingested by honey bees did not adversely affect honey bee colonies.

Responses 1-5: Quality methods		**Score**
Experimental design and hypotheses	**Hypothesis:** Exposure of honey bees to residues of Imidacloprid and clothianidin in guttation fluid would adversely affect survival of adult bees, brood and colony strength and development and overwintering performance. **Objectives:** 1. to evaluate and compare the colony development and the overwintering performance of exposed honey bee colonies in the different treatment groups relative to the control group; 2. to determine if honeybee activity correlated with guttation; 3. to measure residues of the neonicotinoids in guttation fluid The crops were sown in the fall of 2009 and study extended over the winter to the spring of 2010 and ended with winter oil-seed flowering in the respective regions. At each location, there were two treatment groups (one field with Imidacloprid-treated W-WHT seeds (analysed seed loading 89 and 107.1% of nominal (70 g IMI a.s./100kg seeds) and one with clothianidin-treated seeds) and a field sown with fungicide-treated (EfA®) seeds that served as an experimental control. Therefore, there were six field plots in total. All seeds were treated with an abrasion inhibitor (INTECO®) to minimize dust. The initial exposure period was from October 2 to November 2, 2009 and the monitoring period in the spring extended from March 23 and 26 to April 23 and 26, 2010, for each variety (Hermann and Manager, respectively). Fields were planted at a nominal rate of 200 kg seeds/ha with the Hermann variety on October 5-6, 2009 and with the Manager variety on October 15, 2009. Field sizes varied among treatments and varieties with the control fields 11 and 6.3 ha for Hermann and Manager respectively. Those for Imidacloprid were 6 and 16.2 ha, respectively, and for clothianidin 4 and 9.8 ha, respectively. Hives were set up in both treated and control fields on either October 2 and 8, 2009, for each variety (Hermann and Manager), respectively.	**2**

	The study consisted of three treatments (control, Imidacloprid, clothianidin) at each of two locations (6 fields in total). At each of the six study fields, five honey bee colonies were placed 3 (control Hermann), 4 (treated Hermann) and 7 (Manager) days before seeds were sown. All were within 0.5 m of the W-WHT crop. At each of the six field sites each treatment had a total of 5 hives and 5 assessment areas. An assessment area in front of each bee hive was divided into two in-crop zones (Zones 0 and 1) and an off-crop zone (0.5 m to each side of the hives, 2 m width in a U shape). 1-m^2 assessment areas were established to monitor guttation. Samples of guttation fluid were collected from plants in segregated areas (n=5 subareas; n=3 each with a volume of 1 mL). Dead bee traps were located in front of the hives to monitor dead bees and were checked daily as well as an area 0.5 m^2 in front of the dead traps. The monitoring period began with seedling emergence and lasted for 4 consecutive weeks until the end of October 2009. Each day the crops were monitored (35 minutes) for guttation and if the presence correlated with honey bee activity, the following data collected: the number of honey bees resting or walking on the ground or on the crop plants. Monitoring continued for 28 days in the following spring. Guttation production and honeybee flight activity was monitored daily for 35 min at each plot, morning and evening. Colony strength and development followed the Liebefeld method twice in the fall October 6 and 7 in southern and northern Germany, respectively and October 27 and 28, respectively. Four colony heath assessments also occurred in the spring of 2010, once in March, twice in April and once in May 2010. Measurement endpoints included honeybee mortality, long-term colony development, and overwintering performance of bees in the two treatment groups relative to those in the controls. Additional endpoints included assessing the timing of exudation of guttation fluid by W-WHT and flight activity of honey bees. When these events were correlated, bee activity (e.g., bee visits) was monitored in areas adjacent to the colonies. Guttation exudates were collected and neonicotinoids were measured along with the number of dead bees in traps installed at the entrance to the trap. Colonies overwintered in their field locations. Hives were treated with oxalic acid to control mite infestation. Analytical method was state of the art, fully described, with reported detection limits and recoveries. Purity of compound and origin provided. Neonicotinoids were measured in guttation fluid and in bees in the dead bee traps when n≥10 individuals. No statistical analyses of data; P values were presented only for the proportion of plants displaying guttation fluid included (**major weakness).** Study sites and soils characterized, experimental design clearly explained, replication adequate. Published methods modified; standardized methods not available. Duration of exposure and assessment events provided. The exposure duration was relatively short (<30 days). Measures to control *Varroa* mites were well documented and deviations reported. Plot soils were characterized TOC, WHC, pH, particle-size distribution, soil type). Overwintering indices were generally poor. **Weakness:** Duration of exposure was 4 weeks. All effects were assessed using nominal exposure concentrations. Actual dose to honey bees via guttation fluid was calculated based on the fluid concentration and the time bees spent collecting the material. Other pesticides were applied to the crop during the study. This is not expected to change the results of the study. No guttation fluid was collected from plants in the control treatment (**major weakness**). Other	

	factors unduly seemed to influence colony overwintering performance; adult mortality; colony strength (weather – cold wet, pests and disease, colony death or die off for no apparent reason – i.e., development was normal but did not survive the winter); this was true for all three treatments including the controls. Variability was high within and among treatments		
Use of GLP and QA/QC.	GLP was followed for analytical work but it was a non-GLP study; signatures present. QA/QC complete with deviations reported; no reference substance required.		**3**
Exposure concentrations	Concentrations measured in guttation fluid; application to seeds confirmed and seeding rates reported; concentrations in plants and soils were not measured		**3**
Transparency of data.	Full access		**4**
Number of samples and replication.	3 treatments each with 2 sites for a total of 6 fields of different sizes. There were 5 hives per treatment, each with defined assessment areas associated with each colony.		**4**
Overall evaluation of methods			**3.20**
No guttation fluid was collected from plants in the control treatment (major weakness) and there were no statistical analyses (P values) to support interpretation of effects.		**0.5**	**1.60**

Response 1. Effects on bee mortality (dead bees in trap & on soil surface)		**Score**
Statistical significance of responses	Assumptions were not tested, methods poorly described, p-values not reported; figures and tables generally had associated measures of variation (although unsure if SD or SEs were plotted). Mortality in the late fall was attributed to the cold weather and was evident in all treatments including the control hives. Therefor, there appeared to be no discernable significant effect on mortality attributable to IMI. Mortality was greater in the fields located in the north than in the south (total number of dead bees) because of the colder temperatures in the north.	**0**
Concentration- or dose-response	There was no concentration response relationship.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Exposure of honey bees was via ingestion of guttation fluid. Linkage between concentration in guttation fluid and what the bees actually ingested is poor; however, because there was no pattern or trend of significant adverse effects and impacts to colony health were influenced by other factors (weather, viruses, mite loading etc.), causality is questionable.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Effect on overwintering performance of colony		**Score**
Statistical significance of responses	Assumptions were not tested, methods poorly described, p-values not reported; figures and tables generally had associated measures of variation (although unsure if SD or SEs were plotted). Two hives in the Imidacloprid treatment did not have sufficient numbers at the last assessment in October to over winter so these colonies were discarded. Of the 8 hives in the Imidacloprid treatment group that were viable prior to over wintering; only six overwintered and developed normally in the	

	spring. The overwintering performance was similar for the control treatments.	
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 3. Effects on honeybee behaviour		**Score**
Statistical significance of responses	Assumptions were not tested, methods poorly described, p-values not reported; figures and tables generally had associated measures of variation (although unsure if SD or SEs were plotted). In the northern fields (Celle) with both neonicotinoids, no monitoring was done because of late seedling emergence; however, cramps, disturbed coordination and vomiting were observed in bees associated with the southern fields (Baden-Wűrttemberg)	**0**
Concentration- or dose-response	A concentration-response was not observed	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	The relevance or significance of these behavioural observations was not linked to apical endpoints	**0**
Mechanism and or mode of action of effect explained	No mechanism was suggested or investigated.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 4. Effects on colony strength – brood development		**Score**
Statistical significance of responses	Assumptions were not tested, methods poorly described, p-values not reported; figures and tables generally had associated measures of variation (although unsure if SD or SEs were plotted). Colony development as reflected by number of bees, sealed brood, unsealed brood for the different assessments (Figure 23) was comparable to that in the controls and variability was high for assessments 5 and 6 within treatments.	**0**
Concentration- or dose-response	There was no concentration- or dose-response observed	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Two hives in the IMI treatments had insufficient bees to overwinter so they were discarded in the autumn. In the spring 6 of the 7 hives that over wintered developed normally whereas one hive had no brood and the queen was found dead. Three control hives were lost in over the winter and 7 hives developed normally in the spring.	**0**
Mechanism and or mode of action of effect explained	Colony health especially in the hives in the northern plots was compromised by *Varroa* mite loads. All hives tested positive for deformed wing virus (DWV) and about half for *Nosema apis* and for some other viruses as well. No mechanism was suggested or investigated.	**0**

Overall evaluation of relevance (computed)	**0.00**
Overall evaluation of relevance (SEJ).	

Response 5. Effects on flight activity		**Score**
Statistical significance of responses	Assumptions were not tested, methods poorly described, p-values not reported; figures and tables generally had associated measures of variation (although unsure if SD or SEs were plotted). Effects could not be discerned from this study because there was no comparison with the controls. Bees observed in the assessment areas were on plants and on soil in the in-crop and off-crop zones and 10.5% (343/3276 bees) were observed to actively ingest guttation fluid. More bees in the spring, ingested guttation fluid (341/2872 bees).	**0**
Concentration- or dose-response	No significant concentration- or dose-response relationships were discerned	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Flight activity monitoring established that bees visited the study fields; about 11.9 % of bees took up guttation fluid in the spring and a much lower proportion took up guttation fluid in the fall. The maximum concentrations of IMI were 6.9 and 0.19 mg a.s./L in the fall and spring, respectively in guttation fluid collected from the crop in the treated fields. The metabolite (Imidacloprid-5-hydroxy or Imidacloprid-olefin) concentrations ranged between <LOD to 0.61 mg/L or <LOD to 0.12 mg/L, respectively.	**0**
Mechanism and or mode of action of effect explained	No mechanism was suggested or investigated.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Narrative

Over all, guttation fluid from winter wheat grown from seed treated with neonicotinoids and ingested by honey bees did not adversely affect honey bee colonies.

The maximum exposure concentration for Imidacloprid was 6.9 mg/L which could be inferred as an unbounded NOAEC.

Expert Judgement		
QA	Yes	September 28, 2015
SEJ	Yes	October 2, 2015

(Bayer CropScience 2014c)
Reports: Bayer CropScience. 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. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-500724-01-1. 242 p

and

(Bayer CropScience 2014b)
Bayer CropScience. 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. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-500734-01-1. 237 p

The two studies in this WoE were very similar in design but were carried out at two different sites. For this reason, they were combined. These are studies of the effects of a neonicotinoid mixture and therefore can only be used if there are no effects. There were no effects.

Two study sites: Study 1 near Neulingen-Bauschlott (control field) and Pforzheim (treated field); and Study 2 near Gäufelden-Talfingen (control field) and Gäufelden-Öschelbronn (treated field), in south-western Germany (DL) each comprising two treatments – a control (2.47 and 2.76 ha fields, for studies 1 and 2, respectively) and a treated field (3.28 and 2.37 ha, for studies 1 and 2, respectively) – with known histories of chemical applications and use were used to study the effects of a neonic mixture on bees exposed via guttation fluid produced by sugar beet plants grown from seed treated with the neonic mixture and standard fungicides.

The commercially prepared sugar beet pills (Sabrina) were treated with a nominal rate of 0.60 mg chlothianidin a.s./pill, 0.08 mg betacyfluthrin a.s./pill and 0.30 mg Imidacloprid a.s./pill. The sugar beet pills were seed-coated with a conventional seed treatment and were dressed with the neonic mixture and two fungicides TMTD and Hymexazol WP 70. The target rate of seed application was 130,000 sugar beet pills with corresponding application rates of 78, 39, and 10.4 g of clothianidin/h, Imidacloprid/ha and beta-cyfluthrin/ha, respectively. The seeds were sown on May 15, 2013 (and continued on May 18 for treated field in Study 1); hives were introduced on the evening of June 12 and 13, 2013, for studies 1 and 2, respectively so day 0 was June 13 and 14, 2013, for studies 1 and 2, respectively. The exposure period was 0-42 d and 0-40 d in duration for studies 1 and 2, respectively, after which the hives for Study 2 were relocated to an interim monitoring site (July 24, 2013) and for both studies to a final monitoring site (July 26, 2013). There were 8 colonies per field (i.e., 8 per treatment and 8 per control) for a total of 16 hives per study.

Multiple endpoints were measured including honeybee mortality (dead-bee traps and linen sheets) and any abnormal behaviour (aggressiveness, intensive cleaning, clustering, cramping, trembling, locomotion problems, inactive, hanging) at the entrance to the hives. Flight intensity was assessed at least once a day during flight activity over the 42 and 40-day period (for studies 1 and 2, respectively) in five marked areas per field (1 minute observations of 5 x 2 meters squared areas). Also measured were the number of bees flying over the crop in the observation areas, the number of honey bees on sugar beet plants and found on the soil in the assessment areas, the number of honeybees taking up guttation fluid in the assessment area. Colony health assessments were conducted once before setup of the colonies and regularly thereafter until

the following spring to monitor over wintering performance. Additionally, hives were monitored for pests and viruses and the honey and nectar for AFB. Residues of guttation fluid from sugar beet plants were measured in the treated group only (**weakness**). Frequency of guttation relative to honey bee flight was assessed twice daily

Responses 1-8: Quality methods		**Score**
Experimental design and hypotheses	**Hypothesis:** Exposure of bees via guttation fluid from sugar beet plants that had been grown from seeds chemically-treated with a mixture of clothianidin, Imidacloprid, and betacyfluthrin will adversely affect survival of honey bees and honeybee colony health. For each study a total of 16 hives (pre-screened with selection criteria and free of Varroa mites and Nosema disease) were divided among the two treatments with 8 hives per treatment and placed into the field on the evening of 12 and 13 June 2013, for studies 1 and 2, respectively. Relocation occurred on July 24 (for study 2) and 26, 2013 (for studies 1 and 2) so the exposure period was 42 and 40 days for studies 1 and 2, respectively. Pre-exposure monitoring of bee mortality over a five-day period and daily post-exposure from day 0 to 42 for Study 1 and 0 to 40 for Study 2. Monitoring (relocated hives) occurred over an additional 3-month period; over-wintering performance was measured in March the following spring. **Measurement endpoints**: the number of dead bees was assessed daily from day 1 to 42 or 40 (for studies 1 and 2) using dead bee traps and linen strips. Flight intensity and behavior as well as the number of bees visiting sugar beet plants and the occurrence and proportion of guttation on the plants was assessed twice daily; assessing colony health included colony strength (number of bees, presence of queens, pollen, honey and nectar storage areas, number of eggs, larvae and capped cells per comb; total number of bees; total area containing the single brood stages etc.). Hives were assessed for health (virus, mite infestations and diseases). Seven colony assessments occurred on days -2, 20, 42, 68, 96, 124, and 273 for Study 1 and on days -2, 20, 40, 60, 94, 122 and 269 for Study 2 with bee-keeper checks in between. Overwintering performance was assessed on March 2014. GLP was followed, analytical certificates were included. Meteorological data were collected. Deviations were reported. **Weakness:** • No exposure concentrations were measured in field-collected samples (**major weakness**) • No statistical analyses or P values (**major weakness**).	**3**
Use of GLP and QA/QC.	GLP was used and QA/QC discussed; audited results included; Standard test protocol was followed.	**4**
Exposure concentrations	Residues measured in guttation fluid only but no bees were observed to drink or carry this fluid; stability was assessed and reported. Only one application rate in this study and the application of Imidacloprid was as one component in a mixture of three neonicotinoids.	**1**
Transparency of data.	Summary and raw data were presented	**4**
Number of samples and replication.	There was one control and one treated site each with 8 hives for a total of 16 hives per study. There were seven colony assessments conducted and each hive was assessed with assessment areas clearly defined.	**2**

Overall evaluation of methods	Statistical comparisons not described (methods or results) and no P values reported; exposure was to neonicotinoid mixture so causality elusive.	**2.80**
No exposure concentrations were measured in field-collected samples (major weakness). No statistical analyses or P values (major weakness)	**0.5**	**1.40**

Response 1. Effects on honeybee mortality (# dead bees in traps and on linens)		**Score**
Statistical significance of responses	There was no significant difference in adult bee mortality for bees exposed to the treated field relative to the bees in the control field; however, there were no P values presented. The mean daily mortality, was 12.9 and 16.6 (for Study 1) and 13.1 and 14.1 (for Study 2), dead bees/colony/day for the control group and treatment group, respectively.	**0**
Concentration- or dose-response	Only one treatment. There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed for the mixture	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Effects on flight intensity (mean number of foraging bees)		**Score**
Statistical significance of responses	The flight intensity and number of honey bees visiting sugar beet plants were unaffected by neonicotinoids; no P values were included. Food storage was comparable between the hives in the treated field and the control field	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects	**0**
Mechanism and or mode of action of effect explained	No mechanism was suggested or investigated.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 3. Effects on foraging frequency		**Score**
Statistical significance of responses	The number of plants with guttation fluid differed between the two treatment groups but was not thought to be treatment related; foraging activity was comparable between the control and the treatment groups but no P values were presented	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed	**0**
Relevance of effect to apical endpoints	Since no significant response was observed, there was no relevance to the effects	**0**

(survival and/or reproduction, and fitness)		
Mechanism and or mode of action of effect explained	No mechanism was suggested or investigated.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 4. Effects on honeybee behaviour		**Score**
Statistical significance of responses	Although abnormal behaviours were observed, the number and frequency of observations did not differ between the two treatment groups.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects	**0**
Mechanism and or mode of action of effect explained	No mechanism was suggested or investigated.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 5-7. Effects on colony strength (brood development; overwintering performance, queen development)		**Score**
Statistical significance of responses	Colony strength was comparable for hives in both treatments. The overwintering period lasted from October 2013 until 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 in Study 2). Colony health was unaffected by the neonicotinoid treatment and over wintering performance was unaffected.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects	**0**
Mechanism and or mode of action of effect explained	No mechanism was suggested or investigated.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 8. Effects on honeybee health (disease)		**Score**
Statistical significance of responses	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. Mite infestation and diseases were low in both the control hives and the treated hives.	**0**
Concentration- or dose-response	There was only one treatment group exposed to a neonicotinoid mixture; there was no concentration or dose-response described or quantified	**0**

Relevance of effect to apical endpoints (survival and/or re-production, and fit-ness)	Since no significant response was observed, there was no relevance to the effects	**0**
Mechanism and or mode of action of effect explained	No mechanism was suggested or investigated.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Narrative

There were no measurable adverse effects for this mixture of neonicotinoids and no measurable exposure concentrations. Residues measured in guttation fluid produce by sugar beets grown from treated seeds were in the following ranges: For Study 1: 153-327 µg/kg for CTD and its two metabolites TZNG (35-57 µg/kg) and TZMU (36-53 µg/kg); 18-61 for IMI and its two metabolites (Imidacloprid-5-hydroxy (6.9-16 µg/kg) and IMI-olefine (1.9-4.0 µg/kg)). For Study 2: 17-64 µg/kg for CTD and its two metabolites TZNG (2.9-12 µg/kg) and TZMU (3.1-11 µg/kg); 2.9-10 for IMI and its two metabolites (Imidacloprid-5-hydroxy (1.2-4.2 µg/kg) and IMI-olefine (<LOQ-1.3 µg/kg)). Beta-cyfluthrin was not found in guttation fluid.

Expert Judgement		
QA	Yes	September 24, 2015
SEJ	Yes	October 2, 2015

(Bayer CropScience 2014d)
Paper: Bayer CropScience. 2014. Field Study to Monitor Potential Effects on Honey Bees from Exposure to Guttation Fluid of Winter Barley (W-BAR), Seed-Treated with the Insecticidal Seed-Treatment Product Clothianidin + Imidacloprid FS 100 + 175 G in Germany in 2011/2012. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-501261-01-1. 412 p

A field study was conducted to determine if winter barley (var. Campanile) seeds (W-BAR) treated with a mixture of CTD + IMI (FS 100 + 175 G) sown pneumatically in the fall would have an adverse effect on honey bees exposed via the guttation exposure pathway over a 7-month period, from seedling emergence in the fall (2011) to the beginning of winter oil-seed rape (W-OSR) flowering in the spring (2012).

Responses 1-7: Quality methods		**Score**
Experimental design and hypotheses	**Hypothesis:** Exposure of honey bees to realistic concentrations (i.e., field application rates) of imidacloprid (IMI) and clothianidin (CTD) via residues in guttation would adversely affect survival of adult bees, brood and colony strength and development, overwintering performance, behaviour, queen survival, and colony vitality and health. The methods were fully described (exposure durations, exposure, sampling and manipulation dates) and where possible methods cited; test species were identified and described; the experimental design was rigorous with adequate sample size. Information on the origin of honey bees and handling was provided. Measures to control *Varroa* mites were well documented and deviations reported. Honeybee colonies were established adjacent to the fields where the treated seeds were planted. There were two treatments – one treated with the neonicotinoid mixture (CTD and IMI; 99.9 and 171.3 a.i. g/L, respectively) plus the fungicide Baytan® and INTECO® to reduced dust abrasion and one control treated with the fungicide and INTECO® only; each treatment comprised 4 field sites and 5 study plots. Seeds were sown between Sept. 19 and 26, 2011. In total, 50 hives, rated according to their bee-potential were allocated across treatments, ensuring that the distribution was relatively even in terms of the quality of hives. Hives were installed at study plots on 13 Sept. 2011. Plot soils were characterized (TOC, WHC, pH, particle-size distribution, soil type). Electrical conductivity was not measured. Monitoring started with seedling emergence between Sept. 28 and Oct. 4, 2011 and lasted 32 d in the fall and for 35 d in the following spring of 2012 (March 15 to April 17, 2012). Hives were relocated for overwintering in hangers on 28 Oct. 2011. Hives were relocated on 14 March 2012 back to the study sites and, after the CCS-6th assessment (17 April 2012) they were relocated and placed into urban garden scenario (area relative free of agrochemicals) and monitored for colony health 21 d later (7th assessment). Assessment of colony strength and development followed the [10]Liebefeld method starting 2-3 d after the colonies were set up and every 3 wks thereafter to the end of October in 2011 and again in spring 2012.	**4**

[10] Imdorf A., Buehlmann G., Gerig L., Kilchenmann V., Wille H. (1987): Überprüfung der Schätzmethode zur Ermittlung der Brutfläche und der Anzahl Arbeiterinnen in freifliegenden Bienenvölkern. - Apidologie 18, 137-146.

	Other pesticides were applied to the control and treated crop during the study period in 2011 (glyphosate, isoproturon, and pendimethalin – rates and timing were provided but potential effects of herbicides not considered). This was judged to not affect the results of the study. Measurement endpoints included mortality of bees, long-term colony development, overwintering performance of bees in the two treatment groups, and health of the colonies. These were related to the assessment endpoints. Dead-bee traps were in front of the hives to monitor dead bees and were checked daily as well as an area 0.5 m^2 in front of the traps. Production of guttation-fluid and honeybee flight activity was monitored daily for 35 min at each plot (one observer/2 study plots), morning and evening. For this assessment, each study plot was divided into defined In-crop and Off-crop zones and additional areas were segregated for sampling of guttation fluid from plants in each subarea (n = 5 sub-areas; n = 3 each with a volume of 1 mL). When these events were correlated with activity of bees (e.g., bee visits) was monitored in areas adjacent to the colonies. Guttation exudates were collected and neonicotinoids were measured along with the number of dead bees in traps installed at the entrance to the hives. The analytical method for guttation fluid was state of the art, fully described, with reported detection limits and recoveries. Purity of compound and origin provided. The protocol required that analyses of dead bees would be conducted if ≥10 individuals were found in the dead-bee traps. These analyses were not conducted because the number of dead bees was not >10 at any sampling event (potential weakness in the protocol). All effects were assessed using nominal exposure concentrations. Actual dose to honey bees via guttation fluid was calculated based on concentration in guttation fluid and the time bees spent collecting the material (weakness). Actual exposures in bees were not measured. Statistical analyse were conducted and the metrics were described. That said for many data, summary statistics were presented but no statistical comparisons were made (e.g., frequency or proportion of guttation in plants in the different zones and seasons and coincidence with bee flight activity). Justification for excluding colony strength data for control plots C1 and C5 was weak (intra-treatment variability). Analyses were done both ways and the results do not influence the conclusions of this study.	
Use of GLP and QA/QC.	GLP was followed; signatures present. QA/QC complete with deviations reported; no reference substance required. Published methods were modified as no standardized methods not available	**4**
Exposure concentrations	Concentrations measured in guttation fluid; application to seeds confirmed and seeding rates reported; concentrations in plants were not measured. Guttation fluid was not collected in the control fields and was not analysed for residues (**major weakness**).	**2**
Transparency of data.	All raw data were available. Assumptions were tested, methods described, p-values reported; Figures and Tables generally had associated measures of variation (although unsure if SD or SEs were plotted).	**4**

Number of samples and replication.	Two treatments each with four sites each with five study plots each with five honeybee colonies (one set); the study plots were located in two areas (three and two sets of colonies); 50 hives distributed across two treatments. Replication was adequate for the design of the study.	**3**
Overall evaluation of methods	Computed mean of above	**3.40**
Guttation fluid was not collected in the control fields and was not analyzed for residues (major weakness)	**0.5**	**1.70**

Response 1. Mortality of honey bees exposed to CTD and IMI via guttation fluid from winter barley grown from seed.		**Score**
Statistical significance of responses	There was no significant effect on mortality of bees in the fall (p = 0.11, Mann-Whitney U-test; Table A130) or in the spring of the following year (p = 0.72, Mann-Whitney U-test; Table A131)	**0**
Concentration- or dose-response	As there were no significant effects and concentrations in dead bees could not be measured, a concentration response, if any could not be characterized.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Colony strength (number of worker bees) exposed to CTD and IMI via guttation fluid from winter barley grown from seed.		**Score**
Statistical significance of responses	There were significantly **more** worker bees in the treatment colonies than the control colonies on final (7^{th}) assessment (p = 0.03, t-test; Figure 10)	**4**
Concentration- or dose-response	There were only two treatments; a concentration response was not characterized in the study.	**4**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	The greater number of workers is not an adverse effect.	**0**
Mechanism and or mode of action of effect explained	No mechanism was suggested or investigated.	**1**
Overall evaluation of relevance (computed)		**2.25**
Overall evaluation of relevance (SEJ). The response was non-adverse and score was reduced to zero by expert judgement.		**0**

Response 3. Total number of bees in hives exposed to CTD and IMI via guttation fluid from winter barley grown from seed.		**Score**
Statistical significance of responses	There were significantly **more** bees in the treatment colonies than the control colonies on final (7^{th}) assessment (p = 0.03, t-test; Figure 10)	**4**
Concentration- or dose-response	As only two concentrations there was a concentration response was evident but was not characterized in the study. The r^2 would be 1.	**4**

Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	The greater number of workers is not an adverse effect.	**0**
Mechanism and or mode of action of effect explained	No mechanism was suggested or investigated.	**1**
Overall evaluation of relevance (computed)		**2.25**
Overall evaluation of relevance (SEJ). The response was non-adverse and score was reduced to zero by expert judgement.		**0**

Response 4. Overwintering index of honey bees exposed to CTD and IMI via guttation fluid from winter barley grown from seed.		**Score**
Statistical significance of responses	The overwintering index for the control and IMI groups were not statistically different (p = 0.06, t-test, test page 77). When hives with lower colony prior to overwintering were excluded from the analyses, the comparison indicated that p = 0.43 t-test (page 77).	**0**
Concentration- or dose-response	As there were no significant effects, a concentration response, if any could not be characterized.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 5. Development of colonies of honey bees exposed to CTD and IMI via guttation fluid from winter barley grown from seed.		**Score**
Statistical significance of responses	These were tested by Mann-Whitney U test. There were no adverse effects. Number of brood cells (sum of eggs, worker bee larvae and worker bee pupae) was **significantly greater or enhanced** in the treated colonies relative to the control on the 5th, 6th, and 7th assessment (p = 0.03, 0.05, 0.02, respectively; page 80).	**4**
Concentration- or dose-response	There were only two treatments; a concentration response was not characterized in the study.	**4**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	The greater number of brood cells is not an adverse effect.	**0**
Mechanism and or mode of action of effect explained	No mechanism was suggested or investigated.	**1**
Overall evaluation of relevance (computed).		**2.25**
Overall evaluation of relevance (SEJ). The response was non-adverse and score was reduced to zero by expert judgement.		**0**

Response 6. Incidence of *Varroa* mites in colonies of honey bees exposed to CTD and IMI via guttation fluid from winter barley grown from seed.	**Score**

Statistical significance of responses	The incidence of *Varroa* mites (Table A 148) was variable between and within treatments. Differences were not statistically tested and the variability indicates no significant differences between the control and the IMI-treated hives (Tables A 151 and 152).	**0**
Concentration- or dose-response	As there were no significant effects concentration-response is not applicable.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed).		**0.00**
Overall evaluation of relevance (SEJ).		

Response 7. Behavior of bees in colonies of honey bees exposed to CTD and IMI via guttation fluid from winter barley grown from seed.		**Score**
Statistical significance of responses	There was no apparent effect on behavior of bees (Table 23); however, the number of bees affected was small for both treatments. The effect was assumed to be non-significant.	**0**
Concentration- or dose-response	As there were no significant effects concentration-response is not applicable.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed).		**0.00**
Overall evaluation of relevance (SEJ).		

Narrative

The foraging of bees and presence of guttation exudates were correlated in the morning but not in the evening and significantly more bees visited the study plots in the spring compared to the fall; however, a relatively small proportion (<5%, Table A 146) were observed to ingest dew and guttation fluids. The neonicotinoid residues in guttation decreased as the plant matured with maximum concentrations of imidacloprid and clothianidin of 6.65 and 8.51 mg/L, respectively, with a maximum single total value of 11.78 mg/L for both in October 2011. This is essentially the NOEC for the exposures via guttation fluids.

Expert Judgement		
QA	Yes	September 28, 2015
SEJ	Yes	July 31, 2015

(Bayer CropScience 2014e)
Paper: Bayer CropScience. 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. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-504065-01-1. 204 p

Summary or comment: The study site near Nauen, Eastern Germany (DL) comprising three treatments – a control treatment with two fields (two x 2.6 ha planted areas in 8-ha fields) and a treatment field of the same size – was conducted to examine the influence of a neonic mixture (Poncho Beta Plus) on multiple endpoints associated with behaviour and health of honey bees. Each study field was divided into eight (8) assessment plots (two on each arm of the planted cross) with two bee hives per plot. In each of the control fields, two strips approximately 45-m wide within a study field were sown (drilled) in a crosswise manner with corn (var. Amadeo) treated with Thiram SC700 (fungicide) and the treated fields were drilled with Poncho Beta Plus-treated sugar beet pills (var. Sabrina KWS). The commercially prepared sugar beet pills were treated with a nominal rate of 0.60 mg clothianidin a.s./pill, 0.08 mg betacyfluthrin a.s./pill and 0.30 mg Imidacloprid a.s./pill. The sugar beet pills were seed-coated with a conventional seed treatment and were dressed with Poncho Beta Plus and two fungicides Thiram 65 ZR and Hymexazol WP 70. The target rate of seed application was 130,000 sugar beet pills and 100,000 maize sees /ha; the actual seed application rate was 137,708 and 103,189 and 101,368 seed/ha for the treated and controls, respectively, which corresponds to nominally 78 g of clothianidin a.s./ha, 10.4 beta-cyfluthrin a.s./ha, and 39.0 g Imidacloprid a.s./ha. The exposure period was 26-27 d in duration after which the hives were relocated to a monitoring site. 48 hives were monitored in the study, 16 on each study area.

Adjacent to the assessment plots growing either corn or sugar beets were fields of *Phacelia tanacetifolia* known to attract bees (5.4 ha area in the 8-ha control and treatment fields). After the exposure period of 26-27 d, the honey bee hives were relocated to a monitoring site with minimal agricultural activity. The relocation involved locating the colonies such that one third of the hives from each field was randomly allocated to each monitoring site. The relocated hives were monitored for multiple endpoints to assess potential adverse effects attributable to exposure to Imidacloprid in dust. Multiple endpoints were measured including honeybee mortality (workers, pupae, drones) and any abnormal behaviour at the entrance to the hives. Colony health assessments were conducted shortly after set up and before sowing and then every three wks post planting. 30 min after sowing, dust drift samples were collected (using gauze samplers set up ~3 m from the zero line on each assessment plot) along with fortified (0, 1, 100 µg) field samples to determine residues. Water and soil samples were also collected for analyses. Analytical methods were described.

Responses 1-9: Quality methods		**Score**
Experimental design and hypotheses	**Hypothesis:** Exposure of bees during flight to Poncho Beta Plus containing a mixture of clothianidin, Imidacloprid, and beta-cyfluthrin during planting will adversely affect survival of honey bees and honeybee colony health. Total of 48 hives were divided among the three treatments with 16 hives per treatment and placed into the field on 27 June 2013. Relocation occurred on July 23-24, 2013 so the exposure period was either 26 or 27 days.	**4**

	Measurement endpoints for assessing colony health included: percent of bees foraging on pollen and nectar of *Phacelia* (confirmation of potential exposure); behavior (intensive cleaning, restlessness, coordination, intoxication); adult, drone, and worker bee mortality, pupae and larvae mortality in dead bee traps; colony strength and development (number of worker brood cells (open or capped), number of cells filled with eggs, larvae or capped brood, as well as food stores – pollen and nectar), adult bees in front of hive. Data were collected 6 times post-exposure, at 21 day intervals with the penultimate assessment in the fall on 15-16 October 2013 and the last in the spring (March) of 2014 to measure overwintering performance. Statistical procedures applied were described, assumptions tested; and procedures were appropriate. Analytical methods were described and appropriate. Detection limits presented in appendices. **Weakness:** • Three hives were removed from the study because of failure to develop; all were associated with one of the control treatments – this did not affect the study results • No exposure concentrations were measured in field-collected samples Because the Imidacloprid was one component in a mixture of three neonicotinoids, it was not possible to attribute effects to the compound (**major weakness**). However, there were no effects so at best, the highest measured concentration could be used as NOAEC. Because all potential exposure concentrations measured were below the limit of detection (0.004 g a.s./ha), and there were no adverse effects to survival and colony strength and development, the conclusion was that Poncho Beta Plus applied to sugar beet seeds which were sown when bees were actively flying and foraging did not adversely affect honeybee colonies. This is not a true measure of exposure and effect – no exposure = no effect.	
Use of GLP and QA/QC.	GLP was used and QA/QC discussed; audited results included	**4**
Exposure concentrations	No measured values above the detection limits were reported; so there is no measure of exposure; concentrations on seeds were verified; no concentrations were reported for samples of nectar and pollen (**major weakness**); stability was assessed and reported. Only one application rate in this study and the application of imidacloprid was as one component in a mixture of three neonicotinoids.	**2**
Transparency of data.	Summary and raw data were presented	**4**
Number of samples and replication.	There were two controls and one treated site each with 16 hives for a total of 48 hives. There were six assessment periods conducted and each hive was assessed with assessment areas clearly defined.	**2**
Overall evaluation of methods	Computed mean of above	**3.20**
Score for expert judgment. Data for mixtures can be used to assess impacts, if there are no adverse effects; it is not a true measure of exposure due to possible interactions. There were no measurable exposure concentrations; all samples were below the detection limit (**major weakness)**.	**0.5**	**1.60**

Response 1-3. Effects on honeybee mortality (workers, pupae, drones)	**Score**

Statistical significance of responses	There were significant differences in mortality prior to exposure P<0.05 and 0.01 (unpaired t-test); however, the mortality in the treatment group did not exceed that for the control (Tables A17-23) and there was no apparent pattern to the sporadic differences. When mortality was evaluated over the season, there were no significant differences from that of the control.	**0**
Concentration- or dose-response	There were no consistent significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since sporadic significant responses were observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 4. Effects on honeybee behaviour		**Score**
Statistical significance of responses	No behavioural abnormalities of the honey bees were observed during the daily mortality check or during the colony assessments.	**0**
Concentration- or dose-response	There were no consistent significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant responses were observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 5-6. Effects on colony strength (# worker bees, brood)		**Score**
Statistical significance of responses	Colony strength developed normally and similarly for both the control and the exposed group which exhibited a typical pattern of numbers waning toward the late autumn and recovering in the spring (Figures 12-13).	**0**
Concentration- or dose-response	There were no consistent significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant responses were observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant responses were observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 7+. Effects on colony development (number of bees, pupae, larvae, eggs, drones, drone pupae, larvae and eggs)		Score
Statistical significance of responses	Total brood development (Table A 39) was comparable in both treatments (Figure 13) and normal development occurred. There were no significant differences between the control and the treated group.	**0**
Concentration- or dose-response	There were no consistent significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant responses were observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant responses were observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 8. Effects on overwintering		Score
Statistical significance of responses	The honey bees started to breed again at approximately the same level in the spring and overwintering performance of the colonies was comparable among treatments.	**0**
Concentration- or dose-response	There were no consistent significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant responses were observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant responses were observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 9. Effects on colony development (pollen and honey storage)		Score
Statistical significance of responses	There were no consistent significant differences between treatments with regard to foraging activity, intensity, and estimates of pollen and honey stores.	**0**
Concentration- or dose-response	There were no consistent significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant responses were observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant responses were observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Narrative
Although a couple of sporadic significant differences were identified, overall there were no discernable significant differences, trends or patterns reported for the rate of application of this product. Because all potential exposure concentrations measured were below the limit of detection (0.004 g IMI a.s./ha), and there were no adverse effects to survival and colony strength and development, the conclusion was that Poncho Beta Plus (a mixture of neonicotinoids), applied to sugar beet seeds which were sown when bees were actively flying and foraging, did not adversely affect honeybee colonies. Mite infestations were significant different among sites until after the second oxalic acid application.

Expert Judgement		
QA	Yes	September 29, 2015
SEJ	Yes	September 30, 2015

Effects of IMI on honeybees resulting exposures to dust and carryover residues

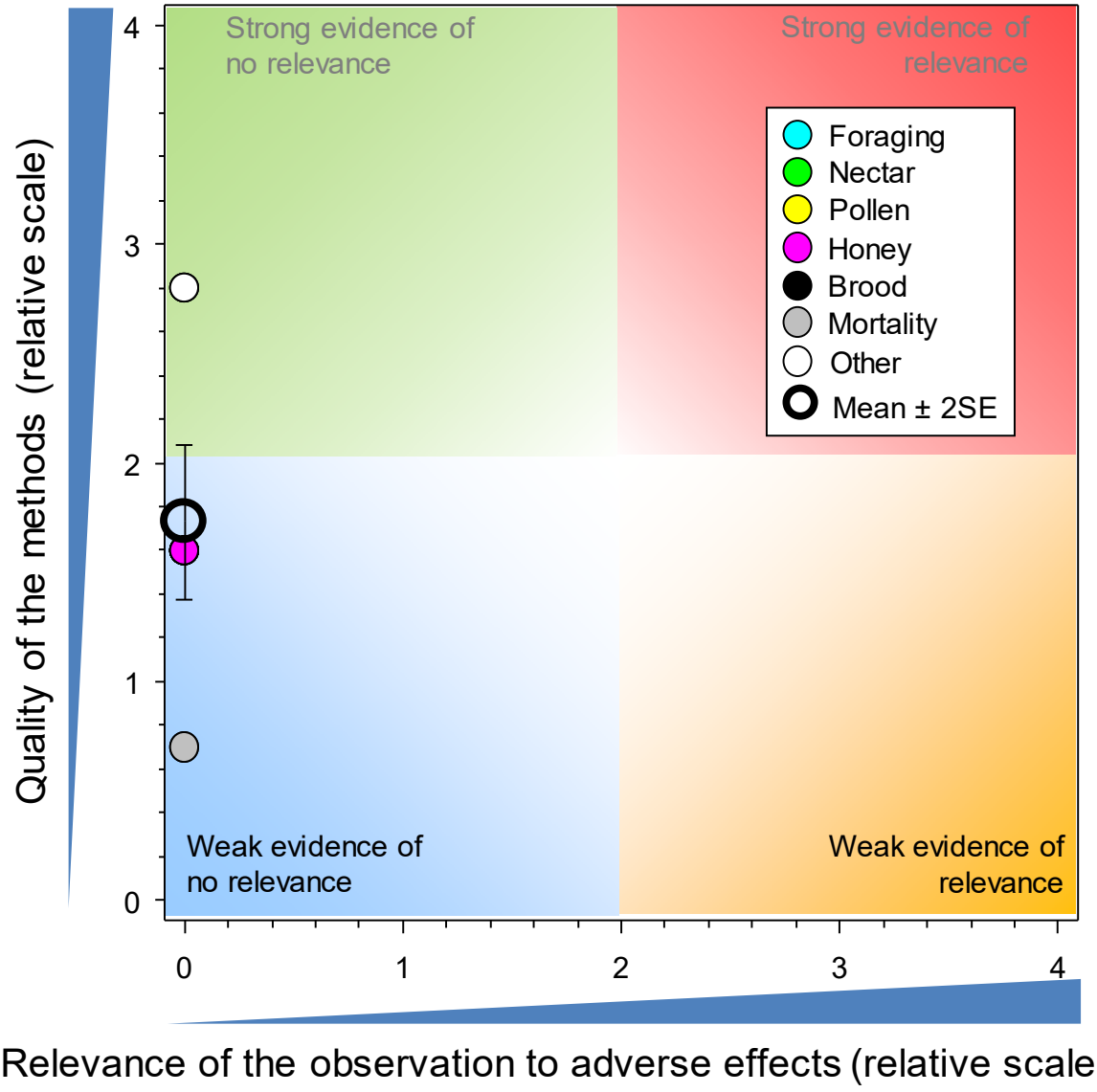
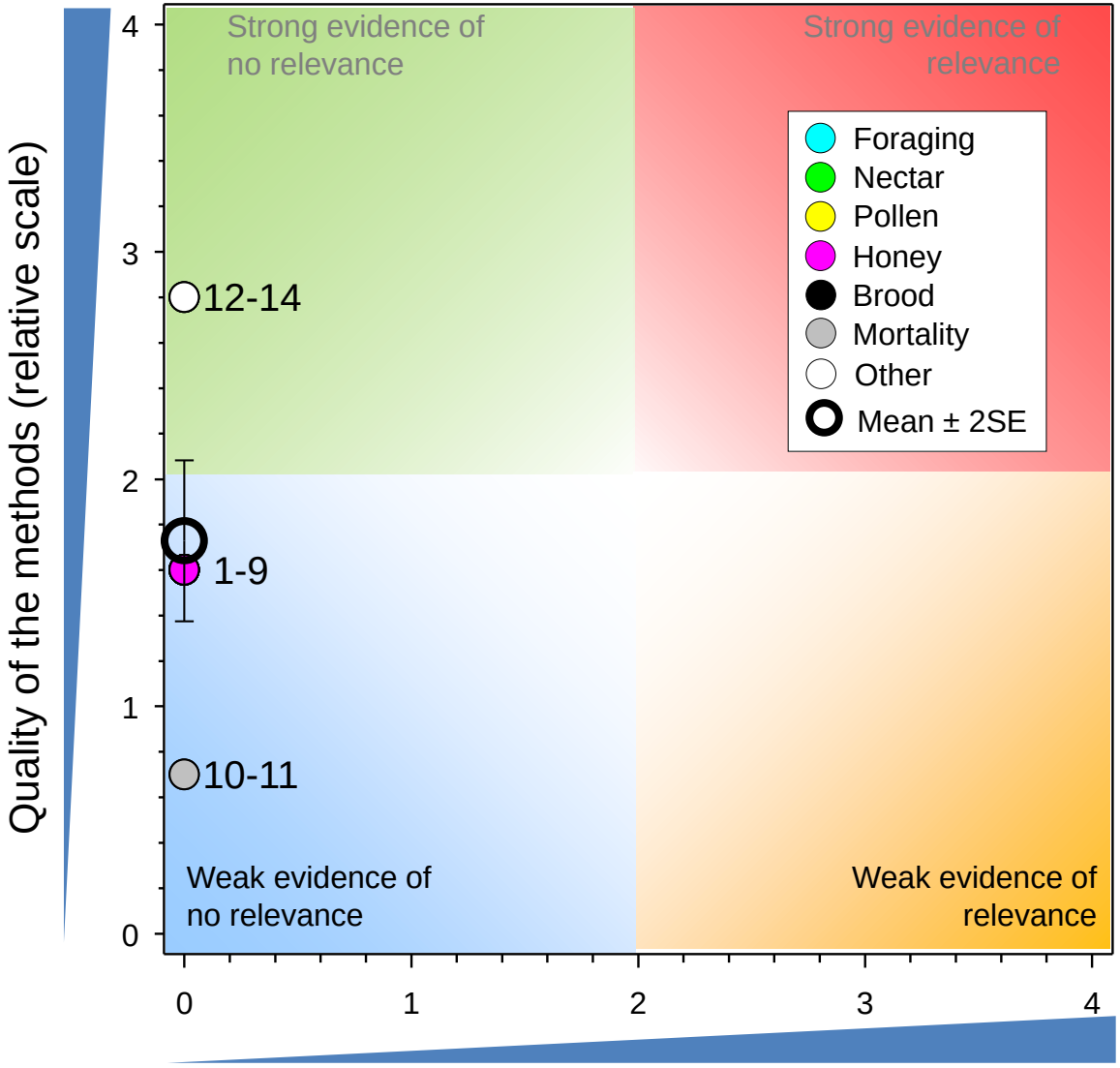


Figure 4. Strengths and relevance of effects observed in honeybees exposed in controlled field studies to IMI via dust and carryover of residues. Number of responses assessed = 14. Symbols may obscure others, see SI for all responses. There were no points in the upper right quadrant.

Field-effects studies on honeybees exposed to IMI via dust and residues (SI)



1 = (Bayer CropScience 2015c) Resp 1
2 = (Bayer CropScience 2015c) Resp 2
3 = (Bayer CropScience 2015c) Resp 3
4 = (Bayer CropScience 2015c) Resp 4
5 = (Bayer CropScience 2015c) Resp 5
6 = (Bayer CropScience 2015c) Resp 6
7 = (Bayer CropScience 2015c) Resp 7
8 = (Bayer CropScience 2015c) Resp 8
9 = (Bayer CropScience 2015c) Resp 9
10 = (Schnier et al. 2003) Resp 1
11 = (Schnier et al. 2003) Resp 2
12 = (Chauzat et al. 2009) Resp 1
13 = (Chauzat et al. 2009) Resp 2
14 = (Chauzat et al. 2009) Resp 3

(Bayer CropScience 2015c)
Report: Bayer CropScience. 2015. Assessment of Potential Impacts on Honeybee Colony Development, their Hibernation Performance and Concurrent Monitoring of Aerial Dust Drift During the Sowing Operation of Imidacloprid FS 350A G - Treated Winter Barley with Typical Commercial Pneumatic Sowing Technology, Directly Adjacent to Full-Flowering *Phacelia tanacetifolia* in United Kingdom. Monheim, Germany: Bayer CropScience (Unpublished Report). Report M-504522-02-1. 270 p

The study site in Selby, North Yorkshire (UK) comprised two treatments – one control and one treated treatment. Each treatment comprising 2 x 6-ha fields (2); each field was divided into eight (8) assessment plots with one bee hive per plot. Two strips approximately 45-m wide within a study field were sown with IMI-treated (or dressed) winter Barley seed (imidacloprid FS 350A G and a fungicide Prothioconazole FS 300). The control plots were sown with winter barley seed treated only with the fungicide. Fields were sown in a crosswise manner (~ 2 ha). Adjacent to the plots growing winter barley were fields of *Phacelia tanacetifolia* (blue or purple tansy) known to attract bees. After the exposure period of 16-17 d the honey bee hives were relocated to a monitoring site with minimal agricultural activity. The relocation involved locating the colonies such that one hive from each field was randomly allocated to form a triangle. The relocated hives were monitored for multiple endpoints to assess potential adverse effects attributable to exposure to imidacloprid in dust.

Responses 1-9: Quality methods		**Score**
Experimental design and hypotheses	**Objectives:** the objective was to determine if residues of imidacloprid in dust drift resulted from pneumatic seeding and to determine if honey bees exposed to residues of imidacloprid in dust during seeding were adversely affected acutely or chronically. The study site in north Yorkshire (UK) comprised two treatments – one control and one treated treatment. Each treatment comprising 2 6-ha fields (2), eight (8) assessment plots with one bee hive per plot. Hives were introduced at the time of flowering (June 12, 2014). Two strips approximately 45 m wide within a study field were sown with IMI-dressed winter Barley seed (imidacloprid FS 350A G and a fungicide prothioconazole FS 300). The control plots were sown with only the fungicide. Target sowing rates were 200 kg seed/ha. Adjacent to the plots growing winter barley were fields of *Phacelia tanacetifolia* known to attract bees. After the exposure period of 16-17 d the honey bee hives were relocated to a monitoring site with minimal agricultural activity. The relocation involved locating the colonies such that one hive from each field was randomly allocated to form a triangle. GLP study; seed treatment was confirmed analytically; seed loading was analysed and confirmed and rates adjusted to achieve the desired nominal concentration of 140 g imidacloprid/ha. Barley variety was Cassata and this was used in both treatments. Source of hives provided and hives included sister queens, densities of 10-15K bees and 15K brood cells. Seeds were sown in June while bees were flying. Bees were collected the same day that sowing occurred and then the following morning during bee flight. The pollen is purple and the proportion of bees with purple pollen was used to verify exposure. Additionally, the relative proportion of pollen foraging and nectar foraging bees was determined. Dust	**4**

	samplers were installed to collect samples of dust drift. Field fortified samples were included. Bee health monitoring looked at development and hibernation performance. One hive per assessment plot for a total of 32 hives placed on June 12, 2014. Behavioural observations at the entrance to hives were recorded. Dead bee traps were used to monitor mortality. These were checked and emptied daily. Hives were relocated on July 10, 2014 to the monitoring station. **Assessment endpoints included:** quality the colony – number of combs covered with bees; presence of a healthy queen – eggs and queen cells; % pollen storage and nectar storage; eggs, larvae and pupae, capped and uncapped; hive equivalents for total number of worker bees, drones, eggs, larvae, pupae, pollen, nectar etc.; hibernation performance. Statistical assumptions and procedures described. Analytical methods and procedures were described with detection limits provided. Spike field samples and estimates of recoveries included. Results indicated that imidacloprid residues were captured on the dust samples and that the concentration was correlated with wind direction and strength. The maximum drift rate was 0.32 g a.s./ha. The exposure period was approximately 16-17 days. There were no behavioural anomalies. Bees returning to the hive were foraging on pollen or nectar of *Phacelia* therefore exposure to potentially contaminated pollen and nectar was demonstrated. Mortality of adult bees was low and not related to imidacloprid. Similarly, worker bee brood (e.g., pupae, larvae) exhibited low mortality and was not related to exposure to imidacloprid. There were no significant differences between treatments with regard to brood development. Hibernation performance and health of queens were unaffected. *Varroa* mite populations were monitored. **Weakness** No analytical control sample for dust drift was collected and analysed. This weakness is not considered to have influence the results of the study significantly. The only measured concentrations were for verification of application rates, dust and soil. No nectar, guttation or pollen levels reported (**major weakness**).	
Use of GLP and QA/QC.	GLP field study and GLP analytical chemistry.	**3**
Exposure concentrations	Application rates provided and confirmed analytically. One application rate. Imidacloprid residues were measured in dust.	**2**
Transparency of data.	Details provided and deviations included	**4**
Number of samples and replication.	N=8; 1 hive per assessment area with 8 areas per study plot; two study sites per treatment; two study treatments (control and treated fields).	**3**
Overall evaluation of methods	Computed mean of above	**3.20**
Major weakness in that residues were not measured in nectar, guttation fluid, or pollen.	**0.5**	**1.60**

Response 1-3. Effects on honeybee mortality (workers, brood, drones)		Score
Statistical significance of responses	There were isolated statistical significant differences but no pattern or trend (Figures 11-12). For the most part the statistical analyses indicated no significant differences, variation was large. The sporadic significant differences were not attributed to the exposure of honeybees to imidacloprid in dust.	**0**
Concentration- or dose-response	There were no consistent significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since significant responses were random, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 4. Effects on honeybee behaviour		Score
Statistical significance of responses	No behavioral abnormalities of the honey bees were observed during the daily mortality check or during the colony assessments.	**0**
Concentration- or dose-response	There were no consistent significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant responses were observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 5-6. Effects on colony strength (# worker bees, brood)		Score
Statistical significance of responses	Colony strength developed normally and similarly for both the control and the exposed group which exhibited a typical pattern of numbers waning toward the late autumn and recovering in the spring. There were no significant differences in number of worker bees or brood (Figures 13-14).	**0**
Concentration- or dose-response	There were no consistent significant effects (Tables A38 to 57) and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant responses were observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant responses were observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 7+. Effects on colony development (number of bees, pupae, larvae, eggs, drones, drone pupae, larvae and eggs)		Score
Statistical significance of responses	Total brood development (Table A 57) was comparable in both treatments (Figure 14) and normal development occurred. There were no significant differences between the control and the treated group.	**0**
Concentration- or dose-response	There were no consistent significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant responses were observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant responses were observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 8. Effects on overwintering		Score
Statistical significance of responses	The honey bees started to breed again at approximately the same level in the spring and overwintering performance of the colonies was comparable among treatments (hibernation index for control and IMI treatments were 0.443 and 0.516, respectively, marginally better in IMI treatment).	**0**
Concentration- or dose-response	There were no consistent significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant responses were observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant responses were observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 9. Effects on colony development (pollen and honey storage)		Score
Statistical significance of responses	There were no consistent significant differences between treatments with regard to foraging activity, intensity, and estimates of pollen and honey stores.	**0**
Concentration- or dose-response	There were no consistent significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant responses were observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant responses were observed, no mechanism or mode of action was proposed	**0**

Overall evaluation of relevance (computed)	**0.00**
Overall evaluation of relevance (SEJ).	

Expert Judgment		
QA	Yes	September 29, 2015
SEJ	Yes	October 3, 2015

(Schnier et al. 2003)
Paper: Schnier HF, Wenig G, Laubert F, Simon V, Schmuck R. 2003. Honey bee safety of imidacloprid corn seed treatment. *Bulletin of Insectology* 56:73-76.

Colony losses in the Italian region of Friuli were high in 2000 and the dust from IMI-coated seeds during planting was implicated in these colony losses. Therefore, in 2001 two studies (field study and caged bee study) were conducted in parallel to investigate the amount of abraded seed dressing particles that result from pneumatic drilling and to determine if these particles with IMI could adversely affect honey bees. The results of these studies indicated that IMI-dust was not the cause of the colony collapses in 2000 and that the amount of dust generated (~4%) in worse-case scenario was insufficient to adversely affect honey bees. The abrasion rate was 4% for the seeds dressed with the standard formulation. Those dressed with the adjuvants had less abrasion (~2%).

Responses 1-2: Quality methods			**Score**
Experimental design and hypotheses	The field study had two fields and two components. One experimental plot was planted with corn seeds dressed with 143 g Gaucho FS 350 per unit (1 unit =50,000 corn seeds). This was the same seed used in Italy in 2000. Two other treatments were also investigated with Gaucho FS 350 which had novel adjuvants designed to decrease seed abrasion and thus reduce dust. The area planted was 8,000 m^2 and the drilling rate resulted in an application rate of 95 g IMI/ha. The earlier rate in Italy was approximately 75 g IMI/ha. Dust emitted from the drilling machine during operation was filtered and quantified. The effects of abraded Gaucho FS 350 on honey bees were measured using caged bees in replicates of 4. For the caged study, flowering rape plants were sprayed with Confidor SL 200 (17% IMI by weight) in 200L water per ha while the bees were foraging. The test deposition rates were 0.6, 1.2, 2, 4, 9 and 14 g IMI/ha. The reference control was sprayed with methyl-parathion, an equally toxic insecticide. The effect measurement endpoints include honeybee mortality and the number of bees foraging on plants before and after the flowers were sprayed with different concentrations of IMI. The exposure period and monitoring period was acute <6 d, 2 before and 3 (acute) after the flowers were sprayed. Although the methods were either described or cited, there was no description of either the statistics used or analytical chemistry methods. All data were presented as nominal values in terms of applications rates (**major weakness**).		**2**
Use of GLP and QA/QC.	No reference to the study being GLP and no QA described although the experimental positive control was methyl parathion.		**0**
Exposure concentrations	Multiple exposure concentrations were used for the caged bee study but there was no concentration-response relationship described. .		**0**
Transparency of data.	There was a lack of transparency and only summary data discussed.		**1**
Number of samples and replication.	Two fields, caged study done in replicates of 4.		**4**
Overall evaluation of methods	Computed mean of above		**1.40**
One major weakness (no measured concentrations)		**0.5**	**0.70**

Response 1. The effects of multiple exposure concentrations of IMI applied as foliar spray on plants where caged bees foraged: foraging intensity.	**Score**

Statistical significance of responses	There were no significant effects and a concentration-response was not observed for nominal application rates of 0.6, 1.2, 2, 4, 9 and 14 g IMI/ha. There was a significant decrease in foraging in the reference treatment. No p values were presented.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. The effects of multiple exposure concentrations of IMI applied as foliar spray on plants where caged bees foraged: bee mortality.		**Score**
Statistical significance of responses	There were no significant effects and a concentration-response was not observed for nominal application rates of 0.6, 1.2, 2, 4, 9 and 14 g IMI/ha. There was a significant increase in bee mortality in the reference treatment. No p values were presented.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Expert Judgement		
QA	Yes	September 28, 2015
SEJ	Yes	October 3, 2015

(Chauzat et al. 2009)
Paper: Chauzat M-P, Carpentier P, Martel A-C, Bougeard S, Cougoule N, Porta P, Lachaize J, Madec F, Aubert M, Faucon J-P. 2009. Influence of pesticide residues on honey bee (Hymenoptera: Apidae) colony health in France. Environmental Entomology 38:514-523.

A 3-y field survey in France from 2002 to 2005 investigated the imidacloprid and 6-chloronicotinic acid residues (as well as other pesticide residues) in honey, pollen and beeswax the relationship of these residues with colony health as measured by colony mortality and adult and brood abundance. There were four sampling periods per year and the number of pesticide residues detected per sampling period and per apiary ranged from 0 to 9. No pesticide residues were detected for 12.7% of the sampling periods. When present, the residues were usually found in pollen loads, honey and honeybee matrices and pairs of active ingredients were present in the honey bees or the pollen loads but not the beeswax or honey. No statistical relationships between the presence of pesticide residues and the abundance of brood, the number of adults, or colony mortality.

Responses 1-3: Quality methods		**Score**
Experimental design and hypotheses	There were five sites in different regions of France in areas associated with sunflower, canola, chestnut, and mixed flower honey. Five apiaries in each site and 5 bee colonies per apiary. 125 colonies randomly selected in five apiaries from five regions were monitored for mortality, population level, health status, management systems and pesticide residues over 3 years (2002-2005). The number of hives was kept constant by replacement of dead colonies by another one randomly selected from the same apiary. Sampling was done four times per year: at the end of the winter (March-April; visit A), before summer (May-June; B), during summer (July-August; C) and before winter (Oct-Nov; D). Mortality in front of the hives and flight activity were recorded. Full clinical examination included population evaluation and symptoms of diseases. Population metrics included number of interframes occupied by adult bees and the number occupied by open or capped brood. Bees were sampled at all visits, honey at visits B, C, and D, beeswax only at D. Pollen loads were sampled from traps. Multiple pesticide residues were measured; methods provided; LODs, LOQs provided. IMI residues and metabolites were determined using LC/MS/MS. Detection limits were method (pesticide) and matrix specific and provided. Endpoint metrics included the relationships between pesticide residues in various matrices and mortality, abundance of brood, number of adults, or colony mortality. Statistical procedures were adequately described. Relied on linear and fixed-effects multiple regression procedures to describe relationships between responses and residues. Effects relied on correlation with consideration of factors other than pesticides.	**4**
Use of GLP and QA/QC.	Non-GLP study but detection and quantitation limits were provided. Methods cited but no QA provided.	**1**
Exposure concentrations	Residues in pollen, honeybees and honey were measured. IMI residues were present in 57.3, 29.7 and 26.2 % of the positive samples for pollen loads, honey and honey bees. Mean residue concentrations for IMI in these matrices were 0.9, 0.7 and 1.2 µg/kg, respectively (Table 5).	**4**
Transparency of data.	Only summary data were presented.	**1**

Number of samples and replication.	N=125; 3 years; 5 regions, 5 apiaries per region, 5 colonies per apiary; multiple measures (four sampling event per year – A, B, C, D) Note: One of the original apiaries selected was removed because the apiarist left the program during the study; N=120.	**4**
Overall evaluation of methods	Computed mean of above	**2.80**
Score for expert judgment on quality of the study	**1**	**2.80**

Response 1. Effects of pesticide residues on colony mortality.		**Score**
Statistical significance of responses	Colony mortality was not correlated with IMI residues or pesticide residues; no P values presented. Winter mortality was 7.3, 9.2, and 5.8%, respectively for 2003, 2004, 2005. Seasonal mortality was 6.7, 10.8 and 7.6 %, respectively for 2003, 2004 and 2005. No statistical relationships were found among honey bee populations, colony mortality and the presence of pesticide residues.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Effects of pesticide residues on adult or brood abundance.		**Score**
Statistical significance of responses	There was no significant effect of individual pesticide residues on the abundance of broods whether the matrices were considered individually or pooled. Mixed effects models did not identify any significant effects.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 3. Effects of pesticide residues on adult abundance.		**Score**
Statistical significance of responses	There was no significant effect of individual pesticide residues on the abundance of adults whether the matrices were considered individually or pooled. Mixed effects models did not identify any significant effects.	**0**
Concentration- or dose-response	There were no significant effects and a concentration-response was not observed.	**0**
Relevance of effect to apical endpoints	Since no significant response was observed, there was no relevance to the effects.	**0**

(survival and/or re-production, and fit-ness)		
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Narrative
Pesticide residues were rarely found in honey with the exceptions of imidacloprid and coumaphos which were found in 29.7 and 8.5% of honey samples, respectively. IMI residues were on average 0.7 µg/kg in honey.

Expert Judgement		
QA	Yes	August 28, 2015
SEJ	Yes	October 3, 2015

Ecoepidemiology studies on the effects of IMI on honeybees

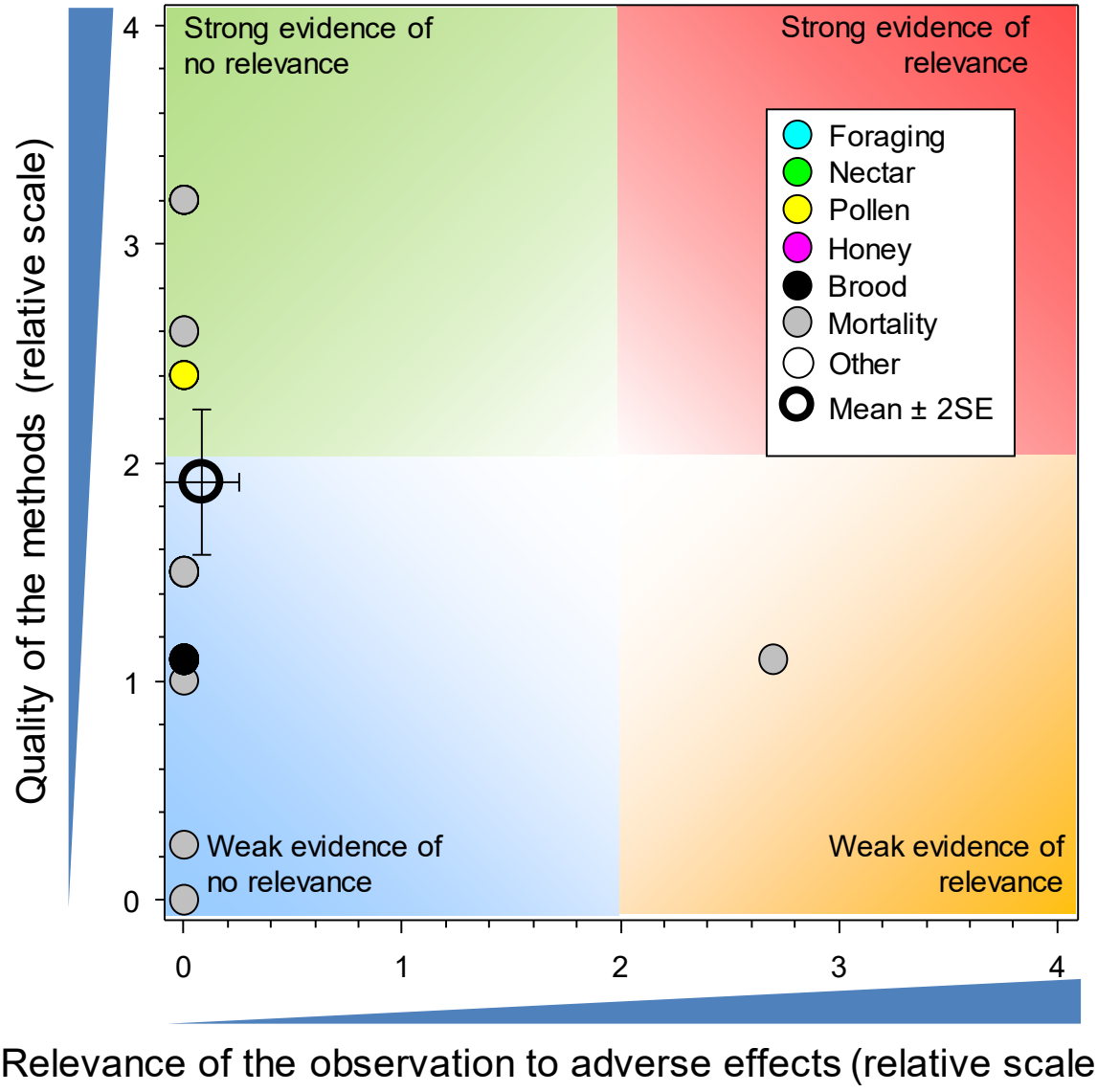


Figure 5. Strengths and relevance of ecoepidemiology, higher-tier observational studies conducted on IMI. Number of responses assessed = 32. Symbols may obscure others, see SI for all responses. There were no points in the upper right quadrant.

	detected in control sites in ON or MN. No metabolites were detected in ON or MN samples for the treated site (potential weakness). Detectable residue if IMI were reported for the MN test site on d 7 and 14 (7.6 and 4.4 µg/kg) but not at the ON site. The same pattern was observed for residues in nectar (Table 14). Statistical analysis was by ANOVA and Fisher's protected LSD and Tukey's Studentized range test (HSD) using Statistix®.		
Use of GLP and QA/QC.	This was not a GLP study but was conducted in the spirit of GLP. Analytical portion of the study was conducted under GLP with QA and QC.		**3**
Exposure concentrations	Exposures were measured in bee-relevant matrices and the geographic distribution of treated crops was characterized.		**4**
Transparency of data.	All raw data were provided as well as summary values in Tables.		**4**
Number of samples and replication.	The study was conducted at two locations each with one test site for treatment with CTD and IMI. There was one control site in each location.		**1**
Overall evaluation of methods	Computed mean of above		**3.0**
Score for expert judgment on quality of the study. One major weakness identified.		**0.5**	**1.5**

Response 1. Sealed honeybee brood		**Score**
Statistical significance of responses	There were no significant differences between amount of sealed brood in hives from control and treated sites in ON or MN (P >0.05; Table 5).	**0**
Concentration- or dose-response	Only one concentration was tested, concentration-response could not be characterized	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since there was no effect, there was no relevance.	**0**
Overall evaluation of relevance (computed)		**0.0**
Overall evaluation of relevance (SEJ).		

Response 2. Yield of honey		**Score**
Statistical significance of responses	There were no significant differences between yield of honey in hives from control and treated sites in ON or MN (P >0.05; Table 9).	**0**
Concentration- or dose-response	Only one concentration was tested; concentration-response could not be characterized.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since there was no effect, there was no relevance.	**0**
Overall evaluation of relevance (computed)		**0.0**
Overall evaluation of relevance (SEJ).		

Response 3. Collection of pollen		**Score**
Statistical significance of responses	There were no significant differences between number of bees collecting pollen in hives from control and treated sites in ON or MN (P >0.05; Table 6).	**0**
Concentration- or dose-response	Only one concentration was tested, concentration-response could not be characterized	**0**

Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since there was no effect, there was no relevance.	**0**
Overall evaluation of relevance (computed)		**0.0**
Overall evaluation of relevance (SEJ).		

Response 4. Collection of nectar		**Score**
Statistical significance of responses	There were no significant differences between number of bees collecting nectar in hives from control and treated sites in ON and MN (P >0.05; Table 6)	**0**
Concentration- or dose-response	Only one concentration was tested, concentration-response could not be characterized	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since there was no effect, there was no relevance.	**0**
Overall evaluation of relevance (computed)		**0.0**
Overall evaluation of relevance (SEJ).		

Response 5. Number of forager bees		**Score**
Statistical significance of responses	There were no significant differences between the number of foraging honey bees observed at control and treated sites in ON or MN (P >0.05; Table 7).	**0**
Concentration- or dose-response	Only one concentration was tested; concentration-response could not be characterized	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since there was no effect, there was no relevance.	**0**
Overall evaluation of relevance (computed)		**0.0**
Overall evaluation of relevance (SEJ).		

Response 6. Mortality of bees		**Score**
Statistical significance of responses	There were no significant differences between the mean number of dead bees in hives from control and treated sites in ON or MN (P >0.05; Table 8).	**0**
Concentration- or dose-response	Only one concentration was tested, concentration-response could not be characterized	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since there was no effect, there was no relevance.	**0**
Overall evaluation of relevance (computed)		**0.0**
Overall evaluation of relevance (SEJ).		

Expert Judgement		
QA	Yes	September 30, 2015
SEJ	Yes	October 16, 2015

(Bayer SA 2000)

Report: Bayer SA. 2000. Field Evaluation in Argentina of Possible Risk for Honey Bees from the Product 'Gaucho' on Sunflowers. Buenos Aires, Argentina: Bayer SA, (Unpublished Report). Report M-090720-02-1. 122 p

The aim of this study was a qualitative and quantitative evaluation of the development of bees and beehives exposed to flowering sunflower from seeds treated with IMI (Gaucho®). Responses measured were colony weight, frame covered (by honey, nectar, pollen, brood, and bee activity (bees with field activity and bees carrying pollen into the hives) and mortality.

Responses 1-7: Quality methods		**Score**
Experimental design and hypotheses	The experimental methods were described in great detail. There were two sites, one test and one control. Sites were located on the Estancia "La Catalina" and were 7.7 km apart. Each plot was 22.4 ha in size and was surrounded by natural grassland and soybeans. Sunflower seeds (*Helianthus annuus*). Variety/hybrid: Trade hybrid DEKASOL 3915 G3) were treated with Gaucho 60 FS®. The concentration of the formulation was verified by analysis. Seeds were treated with IMI and with fungicides (captan and metalaxyl) as per normal agricultural practice and mechanically seeded at 60,000 per ha in early December 1999. The treatment rate was 0.25 mg IMI/seed (Table 11). Pest management included the use of herbicides and insecticides applied to control and treated plants as per normal agricultural practices (Table 3). None of the insecticides was systemic. Pest management in the adjacent fields was described (Table 4–7) and herbicides and non-systemic insecticides were used. No spraying on the test fields took place when bees were present. Beehives were obtained from a local producer. Hives were held in a sunflower-free area prior to and after exposure and were treated *Varroa* mite and *Nosema*, after exposure (starting of Feb. 21, 2000) using recommended products (Table 8). Eight hives of ca 20,000 bees were placed in the centre of the control and treated plots. Hives were transferred to the sites on Feb 2, 2000 (d 0) at 5% bloom of the sunflowers and held until Feb 19, 2000 at 80% bloom completed. Hives were moved back to the sunflower-free site on Feb 19 (d 17) and held there. Hives were inspected and weighed on days 1, 12, 20, 41, and 48. Hives were inspected and samples taken on d 2, 12, 41 and d 216 (after overwintering). Hives were weighed to a sensitivity of 50 g. Coverage of frames was estimated from digital photographs. Field activity of bees in flowers, collecting pollen, and mortality in front of the hives was measured daily (section III.12). Samples of pollen, honey, and wax were collected and subsamples analysed in two different laboratories using documented methods. On d-12, pollen was composed of ca 25% sunflower in the controls and 30% in the test hives (Chart 8). Samples of soil and sunflower heads were also taken for analysis. The methods of analysis were not described in detail but they were developed in the Bayer Laboratories in Germany. There were no detections of IMI in soil (LOD=6 µg/kg) or any of the bee-relevant matrices	**3**

	(LOD=1.5 µg IMI/kg, 3 µg IMI=/kg, and 5 µg IMI-OH/kg), data from[11]. Analytical recovery was not reported (weakness). Statistical analyses were described in a separate annex not included in the report. There were no significant differences in weight, and coverage of frames on the first day of exposure (p 33).	
Use of GLP and QA/QC.	This was conducted in the spirit of GLP with a detailed protocol and SOPs.	**3**
Exposure concentrations	Measurements of exposures and treated seeds were made. Concentrations in control seed were <LOD.	**4**
Transparency of data.	Raw data were provided.	**4**
Number of samples and replication.	There were two sites and eight pseudoreplicated hives per site.	**2**
Overall evaluation of methods	Computed mean of above	**3.2**
Score for expert judgment on quality of the study	**1**	**3.2**

Response 1. Weight of the hives		**Score**
Statistical significance of responses	Hives from the treated site were significantly heavier than those in the control treatment on d 12 (P = 0.0008, p 32). There was no significant difference at d 41 (P >0.05, p 32)	**4**
Concentration- or dose-response	Since there was no concentration gradient, a concentration-response could not be measured.	**NA**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	No relevance as the increase in weight was not adverse	**0**
Overall evaluation of relevance (computed)		**2.0**
Overall evaluation of relevance (SEJ). As the response was not adverse, an SEJ of zero was assigned.		**0**

Response 2. Area of frame occupied by honey and nectar		**Score**
Statistical significance of responses	Hives from the treated site had significantly more cells with honey and nectar (P <0.05, p 34) than the control on d-12	**4**
Concentration- or dose-response	Since there was no concentration gradient, a concentration-response could not be measured.	**NA**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	No relevance as the increase in weight was not adverse	**0**
Overall evaluation of relevance (computed)		**2.0**
Overall evaluation of relevance (SEJ). As the response was not adverse, an SEJ of zero was assigned.		**0**

Response 3. Area of frame occupied by pollen		**Score**
Statistical significance of responses	Hives from the treated site had significantly more cells with pollen (P = 0.02, p 34) than the controls on d 12.	**4**

[11] Stadler T, Gines DM, Buteler M. 2003. Long-term toxicity assessment of imidacloprid to evaluate side effects on honey bees exposed to treated sunflower in Argentina. *Bulletin of Insectology* 56:77-81.

Concentration- or dose-response	Since there was no concentration gradient, a concentration-response could not be measured.	**NA**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	No relevance as the increase in weight was not adverse	**0**
Overall evaluation of relevance (computed)		**2.0**
Overall evaluation of relevance (SEJ). As the response was not adverse, an SEJ of zero was assigned.		**0**

Response 4. Area of frame occupied by worker brood		**Score**
Statistical significance of responses	Hives from the treated site had significantly more cells with brood (sealed, P = 0.004, and non-sealed, P = 0.002, p 34) than the control site on d 12.	**4**
Concentration- or dose-response	Since there was no concentration gradient, a concentration-response could not be measured.	**NA**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	No relevance as the increase in weight was not adverse	**0**
Overall evaluation of relevance (computed)		**2.0**
Overall evaluation of relevance (SEJ). As the response was not adverse, an SEJ of zero was assigned.		**0**

Response 5. Foraging activity		**Score**
Statistical significance of responses	Between d-1 and d-12, the mean daily number of bees foraging on sunflower was higher in the treated sunflower site (P = 0.032, p 40).	**4**
Concentration- or dose-response	Since there was no concentration gradient, a concentration-response could not be measured.	**NA**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	No relevance as the increase in weight was not adverse	**0**
Overall evaluation of relevance (computed)		**2.0**
Overall evaluation of relevance (SEJ). As the response was not adverse, an SEJ of zero was assigned.		**0**

Response 6. Bees with pollen		**Score**
Statistical significance of responses	The number of bees carrying pollen to the hive between days 3 and 17 was not significantly different (P = 0.12, p 42) between treated and controls.	**0**
Concentration- or dose-response	Since there was no concentration gradient, a concentration-response could not be measured.	**NA**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	No relevance as the increase in weight was not adverse	**0**
Overall evaluation of relevance (computed)		**0.0**
Overall evaluation of relevance (SEJ).		

Response 7. Mortality	**Score**

Statistical significance of responses	Although the number of dead bees collected per day in the test and control sites was greater on the day after transfer, the mean daily number of dead bees in front of hives between d 3 and 17 was not significantly different (P = 0.15, p 43) between treated and controls.	**0**
Concentration- or dose-response	Since there was no concentration gradient, a concentration-response could not be measured.	**NA**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	No relevance as the increase in weight was not adverse	**0**
Overall evaluation of relevance (computed)		**0.0**
Overall evaluation of relevance (SEJ).		**0**

Expert Judgement		
QA	Yes	October 1, 2015
SEJ	Yes	October 16, 2015

(Chauzat et al. 2010)
Chauzat M-P, Martel A-C, Blanchard P, Clément M-C, Schurr F, Lair C, Ribière M, Wallner K, Rosenkranz P, Faucon J-P. 2010. A case report of a honey bee colony poisoning incident in France. *Journal of Apicultural Research* 49:113-115.

Because incidents of honeybee poisonings had occurred in the Rhine Valley of Germany in April 2008, apiculturists in France were concerned that the colony losses might be attributable to neonicotinoid uses in agriculture. This WoE_{EPI} is based on a three-page summary of residue analyses of samples collected haphazardly and is basically unusable.

Responses 1: Quality methods		**Score**
Experimental design and hypotheses	Four apiaries across (20 km) the German border around Colmar had 60, 50, 34 and 10 colonies, respectively. Another 120 colonies were located near Rhinau a few meters across from the German border. Observations of mortality ranging from 12 to 100% were reported along with aggressive honeybee behaviour and sudden depopulation. Samples of dead honey bees were collected in May and June at each site by beekeepers (no methods described – ***major weakness***) and samples sent (unfrozen) to AFSS laboratory (Sophia Antipolis) for residue analyses for IMI, and other neonicotinoids by LC-MS-MS Cleanup and extraction was done but not described. LOQ was 0.05 ng/bee. They also examined bees for ABPV, IAPV, and CBPV viruses, *Nosema cerenae* and *Acarapis woodi* using cited methods. In July, samples of bee bread were collected from the same hives and sent to the same laboratory for residue analyses and palynological analyses. The samples were sent to Germany for further analysis using published methods by Anastassiades et al. 2003[12] Only 1 of 274 colonies sampled had measured CTD concentrations and only 2 samples had residues in bee bread (see below). No samples had ABPV, IAPV or *A. woodi*. However, pathogen levels were such that colony health was compromised and likely contributed to the decline of some colonies. No statistical procedures were applied to the data **(*major weakness*)**. Although IMI was analysed for, no residues were detected (text p. 112)	**1**
Use of GLP and QA/QC.	Non-GLP study and no methods for GLP described. Analytical methods cited.	**1**
Exposure concentrations	No detections of IMI.	**1**
Transparency of data.	No raw data available and no measure of the variability of metrics provided.	**0**
Number of samples and replication.	Sample sizes in Table 1 suggest that there was one sample of honeybees and one of bee-bread per hive for a total of five sites = 10 samples.	**2**
Overall evaluation of methods	Computed mean of above	**1.0**

[12] Anastassiades, M; Lehotay, S J; Stajnbaher, D; Schenck, F J. (2003) Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. Journal of AOAC International 86: 412-431

Score for expert judgement: No statistical analyses of data provided and no method description on how samples were collected for processed.	**0.25**	**0.25**

Response 1. Mortality of honeybees associated with residues of IMI.		**Score**
Statistical significance of responses	No statistical analyses of data and with no detects of IMI a causal relationship could not be established.	**0**
Concentration- or dose-response	There were no residues detected, co concentration response could be characterized.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since there were no exposures measured, there was no causal relationship.	**0**
Overall evaluation of relevance (computed)		**0.0**
Overall evaluation of relevance (SEJ).		**0**

Expert Judgement		
QA	Yes	February 14, 2016
SEJ	Yes	Wednesday, February 17, 2016

(CRA INA 2006)

Report: CRA INA. 2006. Monitoring of Depopulation and Mortality Events of Bees in Beehives with Different Agricultural Destinations in the Region Emilia Romagna Final Report 2006. Milan, Italy: CRA - Istituto Nazionale di Apicoltura. Report M-428630-01-2. 47 p

The objective of the study was to investigate the effects of the use of IMI as a seed dressing on maize on bees in the Emilia Romagna region of Italy. This was a continuation of a previous study in 2005.

Responses 1-3: Quality methods		**Score**
Experimental design and hypotheses	The study was conducted in 2006 in three areas with different crop-types. The sites were separated (≥2.5 km) and one area had maize as the predominant crop (A), one mixed crops (B), and the other without maize (C). There were three sites in each location and each consisted of seven or eight hives. Information on the apiaries was collected from beekeepers. This included possible confounders such as diseases. Hives were visited once per week and dead bees (dead bee trap fitted to the hive) collected for chemical and pathological analysis. Pollen was collected to determine sources[13]. After collection, bees were stored at -24°C but were at ambient temperature until collection (weakness). Dead bees were counted. Samples for analysis for IMI (and other pesticides) were pooled at each site for analysis (potential weakness). The "strength" of the hives was assessed using the "six-part method", which refers to area one sixth of surface of a comb side covered by bees/bee-brood. Production of honey was also measured. Samples of bees were processed at the Istituto Zooprofilattico Sperimentale delle Venezie di Padova (IZS) by grinding in liquid nitrogen and shipped to Bayer CropScience AG in Monheim for analysis of IMI. A sub-sample was also analysed for IMI locally using a published method[14]. The extraction of the bees was described but the methods of analysis, recovery, and LODs were not included in the report (**major weakness**). Statistical analyses were not reported and trends and differences were apparently assessed from the raw data.	**1**
Use of GLP and QA/QC.	This was not a GLP study and there was no general QA or QC. The methods used were described in a satisfactory manner.	**2**
Exposure concentrations	Concentrations of IMI (and other pesticides) were measured in bees. No residues of IMI were detected but other pesticides were (chlorpyrifos, dimethoate, methyl parathion). The mapping, distribution and location of maize in the areas was well documented (Figs 2-19) but the actual use of IMI seed treatments was not reported (weakness).	**4**
Transparency of data.	Raw data were provided in Tables and graphs but the variance was not reported.	**1**
Number of samples and replication.	There were three treatment locations each with three replicates.	**3**

[13] Louveaux J., Maurizio M., Vorwohl G, 1978. Methods of melissopalynology. Bee World, 139-157

[14] M. Rancan, A.G. Sabatini, G. Achilli, G.C. Galletti. 2006. Determination of Imidacloprid and metabolites by liquid chromatography with an electrochemical detector and post column photochemical reactor, Analitica Chimica Acta, 555: 20-24

Overall evaluation of methods	Computed mean of above		**2.2**
Score for expert judgment on quality of the study. One major weakness was identified.		**0.5**	**1.1**

Response 1. Mortality of bees.		**Score**
Statistical significance of responses	No statistical tests were conducted but a greater rate of mortality was observed in the maize treatment (Fig. 29). This was assumed to be statistically significant.	**4**
Concentration- or dose-response	This was not assessed and no IMI was detected in the dead bees. Assumed there was no concentration-response relationship.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Death of the bees is an apical response but lack of exposure to pesticides suggests no causal relationship to IMI.	**0**
Overall evaluation of relevance (computed)		**1.3**
Overall evaluation of relevance (SEJ). As there were no residues of IMI detected and confounders, such as the presence of other pesticides and bee viruses an SEJ of zero was assigned.		**0**

Response 2. Strengths of the hive and dimensions of brood.		**Score**
Statistical significance of responses	No statistical tests were conducted but large differences were not observed (Fig. 30 & 31). This was assumed to not be statistically significant.	**0**
Concentration- or dose-response	This was not assessed and no IMI was detected in the dead bees. Assumed there was no C-R.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Quality hives is an apical endpoint but as there no significant effect, there were no relevant effects.	**0**
Overall evaluation of relevance (computed)		**0.0**
Overall evaluation of relevance (SEJ).		**0**

Response 3. Production of honey.		**Score**
Statistical significance of responses	No statistical tests were conducted but large differences were not observed except where swarming had occurred (p 11). This was not assumed to be statistically significant.	**0**
Concentration- or dose-response	This was not assessed and no IMI was detected in the dead bees. Assumed there was no C-R.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Production of honey is an apical endpoint but as there no significant effect, there were no relevant effects.	**0**
Overall evaluation of relevance (computed)		**0.0**
Overall evaluation of relevance (SEJ).		**0**

Expert Judgement		
QA	Yes	October 1, 2015
SEJ	Yes	October 16, 2015

(CRA ISZVe 2005)

Report: CRA ISZVe. 2005. Monitoring About Possible Events of Decline of Bee Populations and Mortality in Different-Cultivated Areas in the Region Veneto Report 2005. Legnaro, Italy: Centro Regionale per l'Apicoltura, Istituto Zooprofilattico Sperimentale delle Venezie. Report M-428632-01-2. 35 p

The objective of the study was to investigate the effects of the use of IMI as a seed dressing on maize on bees in the Veneto region of Italy.

Responses 1-3: Quality methods		**Score**
Experimental design and hypotheses	The study was conducted in 2004-2005 in three areas with different crop-types. The sites were separated (≥2.5 km) and one area had maize as the predominant crop (A), one mixed crops (B), and the other without maize (C). There were three sites in each location and each consisted of eight hives. Information on the apiaries was collected from beekeepers. This included possible confounders such as diseases, weather, and pesticides other than IMI. Hives were visited once per week and dead bees (dead bee trap fitted to the hive) collected for chemical and pathological analysis. Pollen was collected to determine sources[15] but these were not quantified. After collection, bees were stored at -20°C but were at ambient temperature until collection (weakness). Dead bees were counted. Samples for analysis for IMI (and other pesticides) were pooled at each site for analysis (potential weakness). The "strength" of the hives was assessed using the "six-part method", which refers to area one sixth of surface of a comb side covered by bees/bee-brood. Samples of bees were processed at the Istituto Zooprofilattico Sperimentale delle Venezie di Padova (IZS) by grinding in liquid nitrogen and were apparently analyzed locally using a published method[16]. The extraction of the bees was well described but the methods of analysis, recovery, and LODs were not included in the report (**major weakness**). Statistical analyses were not reported and trends and differences were apparently assessed from the raw data.	**1**
Use of GLP and QA/QC.	This was not a GLP study and there was no general QA or QC. The methods used were described in a satisfactory manner.	**2**
Exposure concentrations	Concentrations of IMI (and other pesticides) were measured in bees. Residues of IMI were detected in all treatment areas but data on concentration were not provided. In addition, thiamethoxam and procimidone were detected in unspecified amounts. The mapping, distribution and location of maize in the areas was well documented (Figs 18-29) but the actual locations of the use of IMI seed treatments was not reported (weakness).	**4**
Transparency of data.	Raw data were provided in Tables and graphs but the variance was not reported.	**1**

[15] Louveaux J., Maurizio M., Vorwohl G, 1978. Methods of melissopalynology. Bee World, 139-157

[16] M. Rancan, A.G. Sabatini, G. Achilli, G.C. Galletti. 2006. Determination of Imidacloprid and metabolites by liquid chromatography with an electrochemical detector and post column photochemical reactor, Analitica Chimica Acta, 555: 20-24

Number of samples and replication.	There were three treatment locations each with three replicates.		**3**
Overall evaluation of methods	Computed mean of above		**2.2**
Score for expert judgment on quality of the study. One major weakness was identified.		**0.5**	**1.1**

Response 1. Mortality of bees.		**Score**
Statistical significance of responses	No statistical tests were conducted but a greater rate of mortality was observed in the maize treatment (Fig. 5 vs Fig. 8 & 11). This was assumed to be statistically significant. The authors suggested that use of IMI in fruit-trees may also be a source of exposure and mortality.	**4**
Concentration- or dose-response	This was not assessed and although IMI was detected in some of the dead bees; the timing was difficult to ascertain and exposures were not quantified. Assumed there was no concentration-response relationship.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Death of the bees is an apical response.	**4**
Overall evaluation of relevance (computed)		**2.7**
Overall evaluation of relevance (SEJ).		**0**

Response 2. Strengths of the hive and dimensions of brood.		**Score**
Statistical significance of responses	No statistical tests were conducted but large differences were not observed (Fig. 3 vs Fig. 7, 8 & 11). This was assumed to not be statistically significant.	**0**
Concentration- or dose-response	This was not assessed and did not appear to be related to IMI detected in the dead bees. Assumed there was no concentration-response relationship.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Quality hives is an apical endpoint but, as the effects were not significant, there was no relevance.	**0**
Overall evaluation of relevance (computed)		**0.0**
Overall evaluation of relevance (SEJ).		**0**

Response 3. Production of honey.		**Score**
Statistical significance of responses	No statistical tests were conducted but there were large variations within treatments (p 4-5). This was assumed to not be statistically significant.	**0**
Concentration- or dose-response	This was not assessed and it was assumed there was no concentration response relationship.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Production of honey is an apical endpoint but, as the effects were not judged to be significant, there was no relevance.	**0**
Overall evaluation of relevance (computed)		**0.0**
Overall evaluation of relevance (SEJ).		**0**

Expert Judgement		
QA	Yes	October 1, 2015
SEJ	Yes	October 16, 2015

(CRA ISZVe 2006)

Report: CRA ISZVe. 2006. Monitoring of Depopulation and Mortality Events of Bees in Beehives with Different Agricultural Destinations in the Region Veneto Report 2006. Legnaro, Italy: Centro Regionale per l'Apicoltura, Istituto Zooprofilattico Sperimentale delle Venezie. Report M-428631-01-2. 47 p

The objective of the study was to investigate the effects of the use of IMI as a seed dressing on maize on bees in the Veneto region of Italy.

Responses 1-2: Quality methods		**Score**
Experimental design and hypotheses	The study was conducted in 2005-2006 in three areas with different crop-types in Italy. The sites were separated (≥2.5 km) and one area had maize as the predominant crop, one mixed crops, and the other without maize. There were three sites in each location and each consisted of eight hives. Information on the apiaries was collected from beekeepers. This included possible confounders such as diseases. In 2005 and 2006, there were some changes in the bee-keeping practices and crop-types. Because of this, some sites were reclassified (weakness). Hives were visited once per week and dead bees (dead bee trap fitted to the hive) collected for chemical and pathological analysis. Pollen was collected to determine sources[17]. After collection, bees were stored at -20°C but were at ambient temperature until collection (weakness). Dead bees were counted. Samples for analysis for IMI (and other pesticides) were pooled at each site for analysis (potential weakness). The "strength" of the hives was assessed using the "six-part method", which refers to area one sixth of surface of a comb side covered by bees/bee-brood. Samples of bees were processed at the Istituto Zooprofilattico Sperimentale delle Venezie di Padova (IZS) by grinding in liquid nitrogen and shipped to Bayer CropScience AG in Monheim for analysis. The extraction of the bees was well described but the methods of analysis, recovery, and LODs were not included in the report (**major weakness**). Statistical analyses were not reported and trends and differences were apparently assessed from the raw data.	**1**
Use of GLP and QA/QC.	This was not a GLP study and there was no general QA or QC. The methods used were described in a satisfactory manner.	**2**
Exposure concentrations	Concentrations of IMI (and other pesticides) were measured in bees. No residues of IMI were detected but other pesticides were (fenitrothion, chlorpyrifos, procimidone, and fipronil). The mapping and location of maize in the areas was well documented (Figs 14-23) but the actual use of IMI seed treatments was not reported (weakness).	**4**
Transparency of data.	Raw data were provided in Tables and graphs but the variance was not reported. Data on production of honey (p 14) were likely erroneous and were excluded from this analysis.	**1**
Number of samples and replication.	There were three treatment locations each with three replicates.	**3**

[17] Louveaux J., Maurizio M., Vorwohl G, 1978. Methods of melissopalynology. Bee World, 139-157

Overall evaluation of methods	Computed mean of above	**2.2**
Score for expert judgment on quality of the study. One major weakness was identified.	**0.5**	**1.1**

Response 1. Mortality of bees.		**Score**
Statistical significance of responses	No statistical tests were conducted but a greater rate of mortality was observed in the maize treatment (Figure 13). This was assumed to be statistically significant.	**4**
Concentration- or dose-response	This was not assessed and no IMI was detected in the dead bees. Assumed there was no C-R.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Death of the bees is an apical response but lack of exposure to pesticides suggests no causal relationship to IMI.	**4**
Overall evaluation of relevance (computed)		**2.7**
Overall evaluation of relevance (SEJ). As there were no residues of IMI detected and as confounders, such as a drought in the area were present, an SEJ of zero was assigned.		**0**

Response 2. Strengths of the hive and dimensions of brood.		**Score**
Statistical significance of responses	No statistical tests were conducted but large differences were not observed (Figure 11). This was assumed to not be statistically significant.	**0**
Concentration- or dose-response	This was not assessed and no IMI was detected in the dead bees. Assumed there was no C-R.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Quality hives is an apical endpoint but, as the effects were not significant, there was no relevance.	**0**
Overall evaluation of relevance (computed)		**0.0**
Overall evaluation of relevance (SEJ).		**0**

Expert Judgement		
QA	Yes	October 2, 2015
SEJ	Yes	October 16, 2015

(Garrido-Bailón et al. 2010)

Paper: Garrido-Bailón E, Martín-Hernández R, Bernal J, Bernal JL, Martínez-Salvador A, Barrios L, Meana A, Higes M. 2010. Short communication: The detection of Israeli Acute Paralysis virus (IAPV), fipronil and imidacloprid in professional apiaries are not related with massive honey bee colony loss in Spain. *Spanish Journal of Agricultural Research* 8:658-661.

This study investigated the relationship between factors related to honeybee colony losses such as Israeli Acute Paralysis virus (IAPV) and certain pesticides, including IMI. Only the data for IMI were included in this WoE.

Responses 1: Quality methods		**Score**
Experimental design and hypotheses	This study was described only briefly. A total of 100 samples from different professional apiaries located in 33 different Spanish provinces were collected. Whether selection was random or not was not reported (weakness). The case definition for IAPV was the unusual presence of few adult bees from the hives with no or little build-up of dead bees in or in front of the hive as compared with highly productive hives in the same region and time of the year. For each sample, adult hive worker bees (n > 200) were sampled to determine the disease status, and stored pollen (>100 g) was collected from the brood chamber combs. The samples were collected by the veterinary service technicians and included a report about the health-status of the colonies at the time of sampling. Samples were collected in the spring (n = 50) and autumn (n = 50) of 2006. Of the samples, 55 were from diseased colonies (n = 28 spring samples, n = 27 autumn samples), and the other 45 from asymptomatic colonies (n = 22 spring samples, n = 23 autumn samples). Identification of IAPV was conducted using a published method[18]. Confounding considerations other than fipronil were not included in the study (**major weakness**) Analysis of pollen was referenced to other papers[19]. From the Higes et al 2008 reference, pollen was stored at -20°C in dark until analysed. Extraction was fully described. Analysis of residues in the extracts was carried out by gas chromatography with MS detection in combination with a matrix-matched calibration. Standards were not described; percent recovery was not reported and the LOD for IMI was not reported (weakness). Associations between IAPV and pesticide were tested with Chi-square procedures.	**1**
Use of GLP and QA/QC.	Not a GLP study and QC was not reported.	**1**
Exposure concentrations	Exposures in pollen were measured. IMI was not detected in any sample.	**4**

[18] Maori E., Tanne E., Sela I. 2007. Reciprocal sequence exchange between non-retro viruses and hosts leading to the appearance of new host phenotypes. Virology 362: 342-349

[19] Higes M, Martín-Hernández R, Botías C, Bailón EG, González-Porto AV, Barrios L, del Nozal MJ, Bernal JL, Jiménez JJ, Palencia PG, Meana A. 2008. How natural infection by *Nosema ceranae* causes honeybee colony collapse. Environmental Microbiology 10:2659-2669; and Jiménez JJ, Bernal JL, Del Nozal MJ, Martín MT, Mayo R. 2007. Comparative study of sample preparation procedures to determine fipronil in pollen by gas chromatography with mass spectrometric and electron capture detection. J Chromatogr A 1146, 8-16.

Transparency of data.	Raw data were not provided. Only summary data in a Table	**1**
Number of samples and replication.	There were 100 sites but only a single sample (replicate) was taken at each.	**3**
Overall evaluation of methods	Computed mean of above	**2.0**
Score for expert judgment on quality of the study. Other confounding factors were not considered – major weakness.	**0.5**	**1.0**

Response 1. Relationship between IAPV and exposure to IMI via pollen.		**Score**
Statistical significance of responses	No relationship was identified as no IMI was detected in the samples of pollen. There was no significant relationship between depopulation and another insecticide, fipronil, or IAPV (Chi-square p >0.05, Table 1).	**0**
Concentration- or dose-response	NA	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	As there was no association, IMI not relevant in this relationship.	**0**
Overall evaluation of relevance (computed)		**0.0**
Overall evaluation of relevance (SEJ).		

Expert Judgement		
QA	Yes	October 2, 2015
SEJ	Yes	October 16, 2015

(Nguyen et al. 2009)

Paper: Nguyen BK, Saegerman C, Pirard C, Mignon J, Widart J, Thirionet B, Verheggen FJ, Berkvens D, De Pauw E, Haubruge E. 2009. Does imidacloprid seed-treated maize have an impact on honey bee mortality? *Journal of Economic Entomology* 102:616-623.

The objective of this study was to investigate the potential impact of maize grown from IMI-treated seeds on mortality of honeybees in Belgium.

Responses 1-2: Quality methods		**Score**
Experimental design and hypotheses	The methods used in the study were generally well described. In each of 16 apiaries, three hives were randomly selected and assessed and sampled every 2 months between March 2004 and March 2005. Beekeepers were asked to follow their usual apicultural methods but to not move the three hives. Geographic information was collected in 2004 on the maize fields within a radius of 3,000 m of the test apiaries were located and all crops flowering at the same time as maize were catalogued. Each field was characterized by its surface area and the maximum and minimum distance to the apiary. All maize fields treated with IMI were noted. The mortality rate in an apiary was defined as the number of dead colonies (no live bees) divided by the total number of colonies in the apiary and was expressed as %. Pollen in the hives was identified to ensure that bees were collecting the maize pollen. Confounders and other stressors were considered and relationships for these tested. These included number of hives per apiary where there was a significant correlation between the number of colonies per apiary and mortality rate (r_s = 0.68; df = 14; P = 0.0035, Fig. 2). Other insecticides and miticides (e.g., rotenone) and products for pest management in hives and well as in products for crops were detected in the hives but correlations with mortality were not characterized. Mortality at the 16 sites ranged from 0 to 84%. Samples of honey from each colony was collected from randomly selected capped cells on honey super frames by using disposable plastic spatulas and were stored in brown glass jars. Randomly selected samples of food-free beeswax (25 cm^2) were collected from honey super frames of each colony by using disposable spatulas and placed in sealable freezer bags. Twenty honey bees from each colony also were collected. Ten workers were collected in the hives and 10 at the entrance. All samples were stored at -20°C before residue analysis. Field blanks and spikes were not reported and the time to analysis was not reported (weakness). Extraction of the samples was described but the standards were not mentioned in this paper. Samples were analysed by a published method[20] using CG-MS-MS and HPLC-MS-MS and was well described. The LOD for IMI was 0.05 µg/kg. IMI= and IMI-OH were not measured (potential weakness). IMI was detected in honey in 8% of the hives at concentrations >LOD and <LOQ. IMI was not detected in bees or in wax.	**3**

[20] Pirard, C., J. Widart, B. K. Nguyen, C. Deleuze, L. Heudt, E. Haubruge, E. De Pauw and J.-F. Focant. 2007. Development and validation of a multi-residue method for pesticide determination in honey using on-column liquid-liquid extraction and liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1152: 116-123

	Statistical analysis was well described (p 618) and judged to be appropriate to the hypotheses tested.	
Use of GLP and QA/QC.	The study was not GLP and there was no general QA/QC.	**2**
Exposure concentrations	Concentrations of IMI in honey, wax and bees were measured from each of three hives at each of the 16 apiaries. There was geographic characterization of the use of IMI with data from the Agricultural Headquarter of the Walloon Region, the farmers, the vendors of plant protection products, and field observations.	**4**
Transparency of data.	Raw data were not provided. Summary data were provided in Tables and Figures.	**2**
Number of samples and replication.	The colony (hive was the unit measured) and 16 sites were selected (randomly but method not reported). The sites had from 3 to 42 hives but these were not replicated. Score of 2 assigned by SEJ.	**2**
Overall evaluation of methods	Computed mean of above	**2.6**
Score for expert judgment on quality of the study	**1**	**2.6**

Response 1. Relationship between surface area of maize fields treated with IMI and the mortality rate in an apiary.		**Score**
Statistical significance of responses	There was a significant negative correlation ($r_s \leq -0.57$; df = 14; $P \leq 0.02$, Table 2, Fig. 3) between the area of maize treated with IMI and the mortality rates in an apiary. A similar inverse relationship was observed between mortality rate and areal proportion of treated maize/total area of maize ($r_s \leq -0.52$; df = 14; $P < 0.05$).	**4**
Concentration- or dose-response	The area-mortality relationship was characterized.	**4**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	The endpoint measured was death of the hive. This is an apical endpoint.	**4**
Overall evaluation of relevance (computed)		**4.0**
Overall evaluation of relevance (SEJ). A SEJ of zero was assigned because the response to IMI was not adverse.		**0.0**

Response 2. Association between mortality of hives and measurement of IMI in bee-relevant matrices.		**Score**
Statistical significance of responses	No statistical analysis was carried out; none of the colonies where IMI was detected died. Assumed to not be statistically significant.	**0**
Concentration- or dose-response	The area-mortality relationship was not characterized and was not apparent.	**0**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	The endpoint measured was death of the hive. This is an apical endpoint.	**0**
Overall evaluation of relevance (computed)		**0.0**
Overall evaluation of relevance (SEJ).		

Expert Judgement		
QA	Yes	October 2, 2015
SEJ	Yes	October 16, 2015

(Pohorecka et al. 2012b)
Pohorecka K, Skubida P, Miszczak A, Semkiw P, Sikorski P, Zagibajło K, Teper D, Kołtowski Z, Skubida M, Zdańska D, Bober A. 2012. Residues of neonicotinoid insecticides in bee collected plant materials from oilseed rape crops and their effect on bee colonies. Journal of Apicultural Science 56:116-134.

The aim of the study was to measure the concentration of neonicotinoid insecticides (including IMI) in nectar, pollen, honey, bee bread and to assess the potential short- and long-term effects on honeybee colonies. The exposure WoE is addressed separately elsewhere. Other neonicotinoids were used as foliar sprays and are not included in the characterization of exposure. This study was evaluated for field effects and the weight of evidence is provided below for the ecoepidemiology.

Responses 1-6: Quality methods		**Score**
Experimental design and hypotheses	The study was conducted in Poland at an unreported location. In 2010, two winter oilseed rape fields (wOSR) (field A - 41 ha - and field - B 35 ha) were treated with thiamethoxam (CRUISER OSR 322 FS) and IMI CHINOOK PLUS 500 FS) as seed dressing, respectively. In 2012, three separate spring OSR fields (field C - 29 ha, field D - 21 ha and field E - 17 ha) were planted with OSR seed treated with TMX (CRUISER OSR 322 FS), clothianidin (MODESTO 480 FS), and IMI (CHINOOK PLUS 500 FS), respectively. Only the IMI data from Field A for the year 2010 and field E for the year 2012 were assessed in this WoE. Distances between the fields were not reported (*weakness*). All the crops were also treated with herbicides, fungicides, and insecticides from other chemical classes as required by good agricultural practice. The detailed description of the products used was provided (Table 1). The blooming period of wOSR was 31 Apr to 21 May 2010 and, for sOSR, from 14 Jun to 2 Jul 2012. Seed was not analyzed for amount of a.s. and the rate of treatment was not reported (*weakness*). The hives used in this study were assessed for health and disease status prior to use in the study. Ten colonies of honeybees (*Apis mellifera carnica, Apis mellifera caucasica*) with ≈equal colony strengths and similar populations were placed near each oilseed rape field, throughout the flowering period (ca. 3 weeks). In each group, an additional five hives equipped with pollen traps were designated only for collection of pollen loads. Two control groups of hives (one in 2010 and one in 2012) were located in an area where no rape was grown. The hives in 2012 were transported from the field to an apiary on July 30, 2012 for overwintering. Monitoring continued until the end of the season. The population size was measured at set up and every three weeks thereafter until the end of the season and included determinations of colony development and health reflected, in part, by the number of combs covered by bees and the brood area, worker biomass, infections and infestations (diseases and pests), and bee mortality in the hive and at the entrance to the hive (hive bottom boards and white trays on ground in front of hive entrances). Honey yield per colony was determined by weighing the harvested honey. In September, hives were prepared for winter by feeding the honeybees and treating for mites.	**3**

	During flowering of rape samples of nectar were collected from rape flowers protected from insects by 16 m^2 mesh tunnels. A minimum of six samples were collected from each field during the flowering period. Nectar flow from combs (100 g) was taken 7 and 14 d after the colonies were placed on the fields. Honey (100 g) was harvested separately for each colony (from honey chambers only) once, about one week after the blooming period. During the 3-week period of blooming, all of the pollen loads that the bees had collected within a 3 to 4-day period were taken separately from the pollen traps of each of the five colonies. Samples of bee bread (app. 10×10 cm pieces of combs) were taken once, after the blooming period. About 100 of bee workers were taken from brood chamber one time, after the oilseed rape blooming period. Samples of nectar, honey, and pollen were taken for identification of source. Bees were also collected for residue analysis but were not included in this WoE. All collected samples were frozen and stored at a low temperature of about -20°C. The residue analysis was described in detail. The standard of IMI, was from a commercial source and were >93.3% (typically>99%) pure. Internal isotopically-labelled standards were not used (*weakness*). Extractions followed the QuEChERS method. Analysis was by LC-MS/MS, and the analysis followed the guideline of SANCO/825/00 rev 8.1. Mean recovery efficiencies for all collected matrices were from 70-110% and the relative standard deviation was less than 20%. LOQs (Table 2) for IMI in nectar and honey were 1 µg/kg. In pollen and bee-bread LOQs for IMI were 3 µg/kg. No residues of IMI were detected in nectar, honey or pollen from the controls of wOSR (Tables 6 and 7). IMI was detected in nectar and honey from combs of hives located near wOSR (Table 6) and from fresh nectar in combs from hives exposed to IMI-treated sORS in 2012. Statistical analyses were carried out using Statistica 8 software and involved means comparisons (Student's t-test, ANOVA procedures followed by Tukey test for parametric procedures; non-parametric procedures included Mann -Whitney U-test or Kruskal-Wallis test). Means and medians were calculated but other centiles were not (*potential weakness*). Spearman's rank correlation was used to assess relationships between variables. Proportion of pollen from *Brassica napus* in the pollen collected by bees during flowering of rape was large in most samples, up to 98% (Table 3).	
Use of GLP and QA/QC.	Non-GLP only because there is no statement saying GLP was followed. From the description of the materials and methods, the spirit of GLP was followed as purities were stated, source of chemicals identified, and procedures described in detail, blanks were used and calibration described, recovery efficiencies for all matrices were stated, LODs and LOQs.	**2**
Exposure concentrations	One treatment field with IMI and one control field in each year. Concentrations were measured in a variety of matrices to assess exposures.	**4**
Transparency of data.	Summarized and raw data presented in Tables.	**2**
Number of samples and replication.	Multiple measurements taken over time but only one treatment and one control, each with 10 or 5 hives (pseudo-replication).	**1**
Overall evaluation of methods	Computed mean of above	**2.40**
Score for expert judgment on quality of the study	**1**	**2.40**

Response 1. Effects of IMI on adult bee mortality		Score
Statistical significance of responses	Mortality was low while hives were in the field and after transport to the base overwintering apiary (see Section 1.2.1). There was no treatment effect.	**0**
Concentration- or dose-response	There was only one field for each treatment in each year: control and IMI; therefore, a concentration-exposure response was not applicable (NA)	**NA**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 2. Effects of IMI on colony health – infectious diseases and parasites		Score
Statistical significance of responses	The treatments (control and IMI) did not differ significantly (Kruskal-Wallis test, p = 0.793 for 2010 and 0.095 2012) with respect to *Nosema* spp. and clinical symptoms of any diseases were absent in both treatments (Section 1.2.1). No viruses except a few with DWV were found. *Varroa* infestations were low.	**0**
Concentration- or dose-response	There was only one field for each treatment in 2010 and 2012: control and IMI; therefore, a concentration-exposure response was not applicable (NA)	**NA**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 3-4. Effects of IMI on colony strength –population size and brood areas		Score
Statistical significance of responses	There were no significant differences between treatments (P >0.05) in terms of the number of dead bees reported while colonies were developing and waning (Table 9, Group A and Table 10, Group E). All colonies developed normally and there were no significant differences (P >0.05) between treatments for numbers of combs covered by bees and brood areas (Table 9 & 10).	**0**
Concentration- or dose-response	There was only one field for each treatment in 2010 and 2012: control and IMI; therefore, a concentration-exposure response was not applicable (NA)	**NA**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**

Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Response 5-6. Effects of IMI on colony honey production and pollen harvest by bees		**Score**
Statistical significance of responses	In 2010, yield of honey from the IMI exposed hives as small but greater than the control. In 2012, the honey yield for the IMI treated field was 11.8 kg/colony and similar to that for the controls (p = 0.106). Amounts of pollen collected were similar.	**0**
Concentration- or dose-response	here was only one field for each treatment in 2010 and 2012: control and IMI; therefore, a concentration-exposure response was not applicable (NA)	**NA**
Relevance of effect to apical endpoints (survival and/or reproduction, and fitness)	Since no significant response was observed, there was no relevance to the effects.	**0**
Mechanism and or mode of action of effect explained	Since no significant response was observed, no mechanism or mode of action was proposed.	**0**
Overall evaluation of relevance (computed)		**0.00**
Overall evaluation of relevance (SEJ).		

Narrative

There were no negative effects of IMI on honeybee mortality, brood development, colony strength or health, or honey yield. This is not surprising considering that no matrices (bee bread, nectar collected by bees, nectar from plants, hive honey, or pollen) had measured IMI residues above the NOAED for honey bees.

Expert judgement		
QA	Yes	February 14, 2016
SEJ	Yes	February 17, 2016