Appendices to the Final Bee Risk Assessment for Clothianidin (PC code 044309) and Thiamethoxam (PC code 060109)

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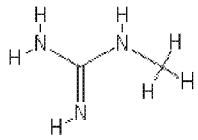
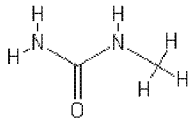
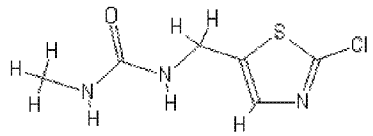
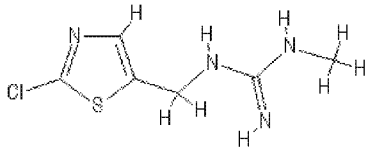
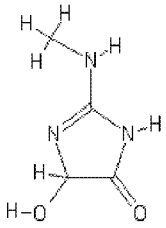
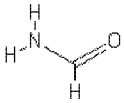
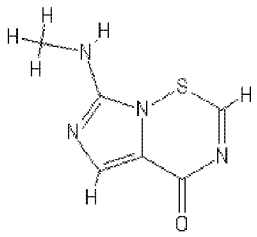
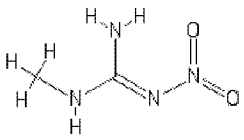
U.S. Environmental Protection Agency
Office of Pesticide Programs
Environmental Fate and Effects Division

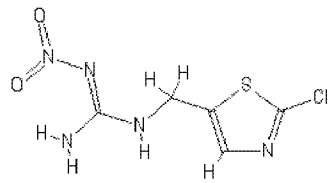
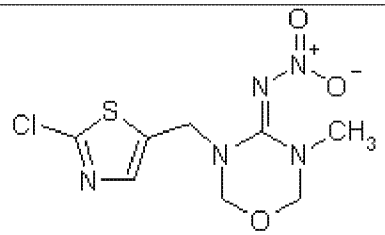
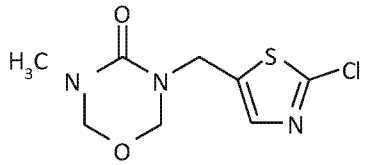
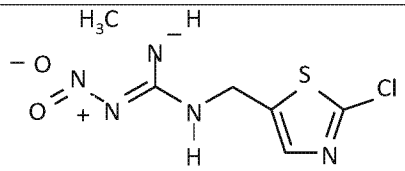
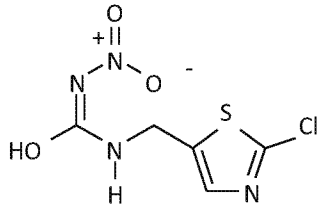
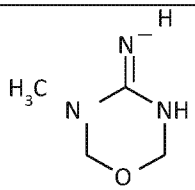
Appendix 1: Chemical Structures of Thiamethoxam and Clothianidin and their degradates

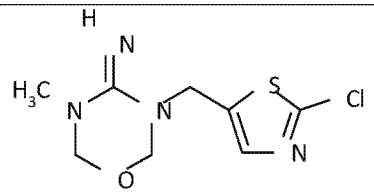
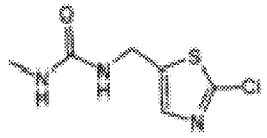
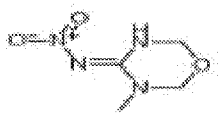
Degradate code / name / CAS Reg. No. / SMILES code	Structure
MG methylguanidine 471-29-4 N=C(NC)N	
MU Methylurea 598-50-5 O=C(NC)N	

TZMU TI-435 urea N-(2-chloro-5-thiazolyl-methyl)-N'-methylurea C1(=CN=C(S1)Cl)CNC(NC)=O	
TMG N-(2-chlorothiazol-5-ylmethyl)-N'-methylguanidine C1(=CN=C(S1)Cl)CNC(NC)=N	

HMIO 4-hydroxy-2-methylamino-2-imidazlin-5-one C1(=NC(C(N1)=O)O)NC	
FA Formamide 75-12-7 O=CN	

MIT 7-methylamino-4H-imidazo[5,1-b][1,2,5]thiadiazin-4-one C12=CN=C([N]1SC=NC2=O)NC	
MNG N-methyl-N'-nitroguanidine C(N)(/NC)=N\[N](=O)=O	

TZNG Desmethyl TI435 N-(2-chloro-5-thizolylmethyl)-N'-nitroguanidine C1(=CN=C(S1)Cl)CN\C(N)=N\[N](= O)=O	
Thiamethoxam (CGA-293343) 3-(2-Chloro-thiazolyl-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-ylidene- N-nitroamine	
CGA-355190 4H-1,3,5-Oxadiazine-4-one, 3-[(2-chloro-5-thiazolyl)methyl]tetrahydro- 5-methyl-.	
Clothianidin (CGA-322704) N-[(2-chloro-5-thiazolyl)methyl]-N'-methyl-N''-nitro-.	
NOA-404617 Urea, N-[(2-chloro-5-thiazolyl)methyl]-N'-nitro-.	
CGA-353042 2H-1,3,5-Oxadiazine-4-amine, 3,6-dihydro-3-methyl-.	

CGA-407475 (NOA-407475) 4H-1,3,5-Oxadiazine-4-imine, 3-[(2-chloro-5-thiazolyl)methyl]tetrahydro- 5-methyl-.	
CGA-309335	
CGA-353968	
CGA-282149	

Appendix 2: Summaries of Clothianidin Pollen and Nectar Residue Studies, Carry-over of Residues in Soil and Monitoring Studies of Hive Matrices

Residues of Concern:

While parent clothianidin is the only stressor of concern in this assessment, many of the residue studies for clothianidin quantified clothianidin metabolites TZNG and TZMU as well as parent clothianidin. As discussed previously in the risk assessment, the toxicity to honey bees of these two metabolites is orders of magnitude less toxic than parent clothianidin (48-hour oral LD_{50} values of 3.95 µg c.e./bee and > 113 µg c.e./bee, respectively, for TZNG and TZMU). Additionally, in the vast majority of the residue studies, these metabolites were less than parent clothianidin (and generally the mean residues of the metabolites were only 10-15% compared to the mean clothianidin residues). Given the comparatively low toxicity and exposures of the metabolites relative to parent clothianidin, the study summaries below do not discuss metabolite residues in detail, with the exception of two studies (MRIDs 49904901 and 49705902) where TZNG residues in some floral matrices were higher than parent clothianidin levels. However, even for these two studies, the emphasis is on clothianidin when comparing those residue concentrations to toxicity data.

Foliar:

There are nine registrant-submitted studies available to characterize clothianidin from foliar applications. Studies are available for clothianidin foliar applications on potatoes, pumpkins, cotton, peaches, apples, grapes, and almonds. In one of the pumpkin studies (MRID 49910601), the potato study (MRID 49705902), and the grape study (MRID 50154305), only one application was performed, although multiple applications are allowed according to label directions. Each foliar study was conducted using differing application regimens. The discussion below and **Table A2-1** summarize the key elements of the available registrant-submitted foliar application residue studies.

In a study of pumpkins in Canada in 2012-2013 (MRID 49602802), clothianidin as the formulated product Clutch™ 50WDG (50.1% w/w; which appears to be similar to the U.S.-registered formulated product Arena® 50 WDG (EPA Reg. #59639-152), was applied twice to two sites for a total application rate of 0.187 lb c.e. (clothianidin equivalents)/A throughout the blooming period. When the pumpkin plants had sufficient numbers of male flowers to generate sufficient quantities of nectar and pollen for residue analysis, flowers and leaf-punches were collected until 28 days after application. In this study, maximum mean clothianidin residues were higher in leaves (21-36 ng c.e./g) than in nectar (3-5 ng c.e./g) but not pollen (46-108 ng c.e./g) across both trials. Additionally, at both application sites clothianidin residues reached mean maximums in leaves and pollen up to 13 days after last application (DALA), and in nectar up to 15 DALA, then declined during subsequent sampling intervals up to 28 DALA. The DT_{50} values of clothianidin were 33 days in pumpkin leaves from Trial 1 and approximately 7 days from Trial 2 (using the maximum formation (highest residue) as time 0 for developing the decline curves), and DT_{50} values of clothianidin in pumpkin pollen and nectar were ca. 2-7 and 4-12 days for both trials, respectively.

In an additional foliar study conducted on pumpkin (MRID 49910601), Belay® 50 WDG was applied at 3 sites using a single application rate of 0.1 lb c.e./A at Biologische **B**undesanstalt, **B**undessortenamt und **CH**emische Industrie (BBCH) growth stage of approximately 14 (3rd true leaf on main stem unfolded). While the foliar application was conducted at the maximum single application rate, two foliar applications (minimum seven-day retreatment interval) are permitted at the maximum single application rate according to the label. Therefore, the residues observed may underestimate the potential residues following multiple foliar

applications as permitted by current labels. Flowers were collected approximately between 21 and 53 days after application. The first sampling was when the first pumpkin flowers were open in the field plot. Thereafter, samples were taken from new flowers and leaves at around 3–5 days, 7-10 days, 12–16 days, and 19–23 days after the first flower collection. Pollen residue levels were greatest in the California sites (maximum measured: 3.03 ng c.e./g; maximum mean: 1.5 ng c.e./g). However, the highest nectar concentration was measured in North Dakota (maximum measured: 1.86 ng c.e./g; maximum mean: 0.69 ng c.e./g), and the highest leaf concentration was also measured in North Dakota (maximum measured: 17,487 ng c.e./g; maximum mean: 16,417 ng c.e./g). Due to the low residues in pollen and nectar (<Limit of Quantification [LOQ] of 1 ng c.e./g), DT50 values were not calculated.

In the case of one foliar cotton study conducted at three sites (*i.e.*, Missouri, Texas and California; MRID 49904901), detectable residues of clothianidin were found in pima cotton nectar, extra floral nectar, pollen and leaves following the single pre-bloom foliar application of 0.083-0.086 lb c.e./A, between BBCH 59 (petals visible, floral buds still closed) and BBCH 61 (beginning of flowering). This rate is slightly less than the highest labelled single application rate and less than the annual application rate of foliar application on cotton (0.1 and 0.2 lb c.e./A, respectively). Maximum measured concentrations were highest in extra floral nectar compared to floral nectar and pollen in both Missouri and Texas. While in California, the maximum concentration was greatest in pollen compared to floral nectar and extra floral nectar. Overall, the maximum measured concentrations for pollen, floral nectar, and extra floral nectar were 1,216, 11.5, and 4,383 ng c.e./g, respectively. Additionally, the maximum mean concentrations for pollen, floral nectar, and extra floral nectar were 911, 8.2, and 3,364 ng c.e./g, respectively. The DT50 values of clothianidin ranged from 1 to 4 days in floral nectar, 2 to 4 days in extra floral nectar, 2 to 6 days in pollen and 2 to 6 days in leaves, suggesting that after a single foliar application, residues may dissipate relatively quickly.

A second foliar cotton study (MRID 49733302) was conducted in California on two varieties of cotton (pima and acala). This study was conducted under conditions more likely to result in upper bound exposures, with two applications conducted at the maximum rate (0.1 lb c.e./A) with the minimum application interval (7 days) and the second application made immediately prior to bloom. As with the Missouri and Texas sites in MRID 49904901 (but in contrast to the California site from that study), clothianidin residues were consistently highest in extra floral nectar (maximum mean clothianidin concentrations of 3,393 and 210 ng c.e./g, respectively, in acala and pima varietals) compared to either floral nectar (maximum averages of 142 and 95.8 ng c.e./g in acala and pima, respectively) or pollen (maximum averages of 419 and 130 ng c.e./g, respectively). Residues in the acala cotton variety were generally higher than for the pima variety. The DT50 values of clothianidin were approximately 3 days in leaves, 3-4 days in floral nectar, 3-9 days in extra floral nectar and 3-4 days in pollen, again suggesting relatively quick dissipation.

In the study conducted on potatoes (MRID 49705902), a single foliar application at the maximum single label rate of 0.05 lbs c.e./A was applied at plant BBCH growth stage 31 to 59 (beginning of crop cover to appearance of petals of first inflorescence visible), and application was avoided between approximately 5 to 7 days prior to potato bloom and petal fall. However, multiple applications are allowed per the label (up to 0.2 lb c.e./A) and therefore, this study may underestimate actual residues. Leaf and flower samples were collected 1-56 and 6-20 days after application, respectively. The first sampling was when potatoes were at around 10-30% of bloom (early bloom). Thereafter, samples were taken at mid-bloom (40-50% plants in bloom) and at late bloom (70-85% of plants in bloom) from new flowers and leaves. The maximum measured and maximum mean concentrations for anthers and pollen were 21.8 and 17.4 and 116 and 76.1 ng c.e./g, respectively. Residues of clothianidin's metabolite, TZNG, were up to 6.6 times higher than parent clothianidin, with maximum measured and maximum mean concentrations for anthers and pollen of 39.4 and 29.1 and 129 and 94.5 ng c.e./g, respectively. The DT50 values were not calculated for anthers or pollen due to the number of samples taken.

In a study conducted on peaches (MRID 50154303), a foliar application of Belay® 2.13 SC at the maximum single label rate of 0.1 lb a.i./A was applied at post-bloom, 35-40 days before harvest (DBH), and a second application at 0.1 lb a.i./A was applied 10 days after the first application but at least 21 DBH for two years. Pollen and nectar were sampled approximately 233-281 days after application. The maximum measured and maximum mean concentrations for pollen were 130 and 49.7 ng/g, respectively. The maximum measured concentration could potentially have been an outlier, as the replicate values were 9.16, 130, and 9.96 ng/g. The maximum measured and maximum mean concentrations for nectar were < 1.0 and < 1.0 ng/g, respectively. The DT50 values were not calculated for nectar or pollen due to the number of samples taken.

In a study conducted on apples (MRID 50154304), a foliar application of Belay® 2.13 SC or Clutch® 50 WDG at the nominal rate of 0.1874 lb/A (slightly below the maximum U.S. rate of 0.2 lb a.i./A) was applied at post-bloom, 7 DBH for two years. Pollen and nectar were sampled approximately 218-248 days after application. The maximum measured and maximum mean concentrations for pollen were 57.4 and 31.2 ng/g, respectively. The maximum measured and maximum mean concentrations for nectar were < 1.0 and < 1.0 ng/g, respectively. The DT50 values were not calculated for nectar or pollen due to the number of samples taken.

In a study conducted on grapes (MRID 50154305), a foliar application of Belay® 2.13 SC or Clutch® 50 WDG at a maximum single label rate of 0.1 lb a.i./A was applied either at post-bloom at BBCH 71 or at pre-bloom at BBCH 14. Pollen was sampled approximately 325-360 (for the post-bloom application scenario) or 17-44 (for the pre-bloom application scenario) days after application. The maximum measured and maximum mean concentrations for pollen for the post-bloom application were 31.9 and 18.1 ng/g, respectively. The maximum measured and maximum mean concentrations for pollen for pre-bloom were 1,564 and 1,306 ng/g, respectively. The DT50 values were not calculated for pollen for either application scheme due to the number of samples taken.

In a study conducted on almonds (MRID 50154302), a foliar application of Belay® 2.13 SC at the maximum single label rate of 0.1 lb a.i./A was applied at post-bloom at BBCH 7.5 and a second application at the maximum single application rate of 0.1 lb a.i./A as applied at 21 days before harvest (DBH) for two years. Pollen, anthers, and nectar were sampled approximately 139-252 days after application. The maximum measured and maximum mean concentrations for pollen were 20.0 and 13.4 ng/g, respectively. The maximum measured and maximum mean concentrations for anthers were 88.1 and 43.4 ng/g, respectively. The maximum measured and maximum mean concentrations for nectar were 2.04 and 1.23 ng/g, respectively. The DT50 values were not calculated for anthers, nectar, or pollen due to the number of samples taken.

Table A2-1. Summary of available registrant submitted foliar application residue studies for clothianidin

Crop Group (Crop Tested)	No. Sites/ Location/ Duration	Formulation, Appl. Rate, Interval, Timing	Matrix[1]	Max. Measured Residue[2] (ng c.e./g)	Max. Mean Residue[3] (ng c.e./g)	DALA[4] (days)	Study Notes and Limitations	Classification (Reference)
Cucurbit Vegetable – 9 (Pumpkin)	2 sites (Canada) 1 year (2012 - 2013)	Clutch® 50 WDG; **2 x 105 g a.i/ha/application – 2-4 days apart (0.187 lb. c.e./A-total rate);** pre-bloom (~5 weeks after planting; at least 9 days prior to sufficient flowers for sampling)	Pollen Nectar Leaves Soil	123 6.51 42.1 42.5	108 4.86 36.1 24.0	9-28	• Only 1 geographical region evaluated (2 sites in 1 region)	Supplemental (Bondarenko, 2015; MRID 49602802)
	3 sites North Dakota, California, Oregon 1 year (2015)	Belay® 50 WDG; **1 x 0.1 lb. c.e./A;** post-emergence (~3-4 weeks after planting; at least 21 days prior to first open flower)	Nectar Pollen Leaves	1.86 3.03 17,487	0.69 1.51 16,417	1-64 (leaves) 21-53 (flowers)	• Only one foliar application was performed, although multiple foliar applications are allowed (up to 0.2 lb c.e./A)	Supplemental (Bondarenko, 2016; MRID 49910601)
Oilseed – 20 (Cotton)	3 sites, Missouri, Texas, California 1 year (2015)	Belay® **1 x 0.083 - 0.086 lb. c.e./A;** pre-bloom (70-76d after planting petals visible, floral buds still closed to beginning of flowering)	Flr Nectar XF Nectar Pollen	11.5 4,383 1,216	8.17 3,364 911	6-35 (flowers) 3-50 (Leaves)	• Foliar rate used was less than maximum labeled foliar rate and only one applications when 2 are allowed	Supplemental (Gould *et al.* 2016; MRID 49904901)
	2 sites, California 1 year (2012-13)	Belay®; **2 x 0.1 lb c.e./A;** pre-bloom, 1st app 7d prior to bloom, 2nd app at bloom	**Acala:** Flr Nectar XF Nectar Pollen **Pima** Flr Nectar XF Nectar Pollen	142 4163 761 182 213 246	142 3393 419 95.8 210 130	5-28	NA	Acceptable (Rose, 2015; MRID 49733302)

Crop Group (Crop)	No. Sites/ Location/ Duration	Formulation, Appl. Rate, Interval, Timing	Matrix[1]	Max. Measured Residue[2] (ng c.e./g)	Max. Average Residue[3] (ng c.e./g)	DALA[4] (days)	Study Notes and Limitations	Classification (Reference)
Root and Tuber Vegetable – 1 (Potato)	4 sites North Dakota, California, Oregon	Belay® 2.13 SC; **1 x 0.05 lb c.e./A;** Post-emergence (~4-8 weeks after planting; at least 6d before early bloom)	Anthers Pollen Leaves Soil	21.8 116 4,723 0.65	17.4 76.1 4,486 0.65	1-56 (leaves) and 6-20 (flower)	• Only one foliar application was applied, although multiple are allowed • Pollen collected from only 2 sites	Supplemental (Bondarenko, 2016, MRID 49705902)
Stone Fruits - 12 (Peach)	3 sites Georgia, South Carolina, California (2015-2016)	Belay® 2.13 SC; 2 x 0.1 lb a.i/A/yr for 2 years Post-bloom (35-40 DBH) and then at least 10 days after the previous application and at least 21 DBH	Pollen Nectar Leaves Soil	130[5] < 1.0 13.3 73.9	49.7[5] < 1.0 9.33 56.0	233-281 (pollen and nectar) 251-314 (leaves) 0-282 (soil)	• None	Acceptable (Bondarenko, 2017, MRID 50154303)
Pome Fruits - 11 (Apple)	3 sites Ontario and Oregon (2015-2016)	Belay® 2.13 SC or Clutch® 50 WDG 1 x 0.2 lb a.i/A/yr for 2 years Post-bloom, 7 DBH	Pollen Nectar Leaves Soil	57.4 < 1.0 < 5.0 76.3	31.2 < 1.0 < 5.0 63.2	218-248 (pollen, nectar, and leaves) 0-247 (soil)	• Although 3 trials were included in the study, 2 were fairly close together in Oregon. EPA recommends that trials are conducted in 3 different geographical locations to account for the effects of potential geographical variability.	Supplemental (Bondarenko, 2017, MRID 50154304)
Berry and Small Fruits – 13-07 (Grapes)	3 sites Ontario, California, Oregon (2015-2016)	Belay® 2.13 SC or Clutch® 50 WDG 1 x 0.1 lb a.i/A Post-bloom, BBCH 71	Pollen Leaves Soil	31.9 15932 6430	18.1 14188 4376	325-360 (pollen) 1-371 (leaves) 273-327 (soil)	• None	Acceptable (Bondarenko, 2017, MRID 50154305)
		Belay® 2.13 SC or Clutch® 50 WDG 1 x 0.1 lb a.i/A Pre-bloom, BBCH 14	Pollen Leaves	1564 12781	1306 10862	17-44 (pollen) 1-63 (leaves)		

Crop Group (Crop)	No. Sites/ Location/ Duration	Formulation, Appl. Rate, Interval, Timing	Matrix[1]	Max. Measured Residue[2] (ng c.e./g)	Max. Average Residue[3] (ng c.e./g)	DALA[4] (days)	Study Notes and Limitations	Classification (Reference)
Tree Nuts - 14 (Almond)	9 sites California (2015-2016)	Belay® 2.13 SC 2 x 0.1 lb a.i/A/yr for two years Post-bloom, BBCH 7.5 and 21 DBH	Pollen Anthers Nectar Leaves Soil	20.0 88.1 2.04 15.4 99.8	13.4 43.4 1.23 10.1 92.4	139-252 (pollen and nectar) 156-283 (leaves) 0-252 (soil)	• None	Acceptable (Bondarenko, 2017, MRID 50154302)

1 Refers to hand-collected pollen and nectar

2 Acute EEC chosen as the maximum reported concentration

3 Chronic EEC chosen as the maximum average concentration

4 DALA = Days after the last application of the pesticide, DBH = days before harvest

5 Concentrations for pollen may have involved a potential outlier, as replicate values were 9.16, 130, and 9.96 ng/g.

Soil:

There are eleven registrant-submitted studies available to characterize the total residues of parent clothianidin in pollen, nectar, leaves and/or anthers following soil applications. Studies on oranges, corn, cucumbers, melons, pumpkins, potatoes, squash, popcorn, and grapes are available. Key elements of these are summarized in the discussion below and in **Table A2-2.**

In one study of oranges (MRID 49317901), the formulated product Belay® Insecticide (% active ingredient not reported) was applied to two or three-yr old orange trees by soil drench and trunk spray with a single application rate of 0.08 fl oz/ tree (equivalent to 0.0013 lb a.i./tree or 0.2 lb a.i./A). This rate is the maximum single application rate for 3-5 year old; however, a second application is allowed after 112 days. In 2012, samples were collected 21 days after application when there were a sufficient number of nectar-containing blooms. In 2013, trees were treated with one application approximately 1- to 6- months prior to bloom. Only residues in nectar were measured in this study. The highest clothianidin concentration in nectar was detected in the 2012 trial (18.7 ng c.e./g, 21 days after application), and the highest average concentration was detected in 2013 (8.50 ng c.e./g, 139 days following treatment). It is noted that even as the interval between application and bloom increased (*i.e.*, 1 to 6 months), the residues in nectar did not change substantially from 0 to 188 DALA. However, residues following treatment approximately one month prior to bloom in the 2012 and 2013 trials were variable (range of <LOD (0.2 ng c.e./g) to 18.7 ng c.e./g in 2012 and 0.5 to 11.4 ng c.e/g).

In a second citrus study (MRID 49944702), various treatment regimens were used, incorporating applications of Belay (clothianidin, 2 soil drench applications at 0.6 g a.i./tree), Platinum® (thiamethoxam, 1 soil drench application at 0.6 g a.i./tree), and Admire® (imidacloprid, 1 soil drench applications at 1.63 g a.i./tree). Applications were made 80-284 days prior to bloom. Only residues in nectar were measured in this study. The maximum measured and maximum mean concentrations for nectar were 15.0 and < 2.5 ng/g, respectively. DT50 values were not calculated for nectar due to the number of samples taken.

In a third citrus study (MRID 50478201), navel orange and lemon trees were treated with Belay at 6, 3, and 1 month prior to bloom. Three of the plots (TRT 2-4) received a soil drench application at a rate of 0.0013 lb a.i./tree/application/year (0.2 lb a.i./A/year), with the fourth plot (TRT 5) receiving two soil drench applications, spread 133-165 days apart, at the maximum label rate of 0.0013 lb a.i./tree/application/year (0.4 lb a.i./A/year). Residues in nectar and pollen were collected. The maximum measured and maximum mean concentrations for nectar were 114 and 64.6 ng/g, respectively. The maximum measured and maximum mean concentrations for pollen were 631 and 412 ng/g, respectively. DT50 values were not calculated for nectar and pollen as there was no clear trend in the concentrations to make an accurate estimation.

In a cereal grain study (MRID 49372102), 0.2 lb c.e./A (Belay® 2.13 SC) was applied to soil in- furrow at corn planting. Pollen samples were collected approximately 60 days after application when tasseling was occurring. At nine of the sites that received only in-furrow application, residues ranged from <LOD (0.25 ng c.e./g) to 5.34 ng c.e./g. However, at one site in Nebraska, the maximum clothianidin concentration detected in pollen following treatment was 27.9 ng c.e./g for which clothianidin was also measured at the corresponding control site with a maximum concentration of 12.4 ng c.e./g. The LOQ

for this study was 1.0 ng c.e./g. According to the study authors, possible reasons for the control contamination at this site include residual levels of clothianidin in soil at the time of planting (*i.e.*, carry over from treatments the previous year), seeds inadvertently contaminated with clothianidin prior or during planting or accidental drift from application of clothianidin to an adjacent field. Excluding the site in Nebraska, the next highest measured clothianidin concentration was 5.34 ng c.e./g (from the Polk, Indiana site). The mean clothianidin concentration across all treatment sites (no control sites included) was 5.5 ng c.e./g. Excluding the highest value from the site in Nebraska, the mean measured residue concentration was 3.1 ng c.e./g.

There are four studies available for cucurbit vegetables. All studies tested the formulated product Belay® Insecticide and applications were made either at planting or post-emergent. In- furrow and chemigation methods were utilized. In MRID 49602801 in California, applications at 0.2 lb c.e./A were made either at planting (9 sites) or post-emergently (3 sites; at BBCH 201- 229; formation of side shoots) for 3 consecutive years. Considering residues across the three years, concentrations in pollen and nectar appeared to be higher following the post-emergent applications (maximum mean concentrations of 37.9 and 17.0 ng/g c.e. in pollen and nectar, respectively), compared to applications at planting (maximum means of 15.5 and 5.8 ng/g c.e. in pollen and nectar, respectively). Following the post-emergent applications, pumpkin grown in coarse soils appeared to have higher (~4-5x) concentrations in nectar and pollen (means of 9.1 and 24.1 ng/g c.e., respectively) than pumpkin grown in fine soils under this scenario (means of 2.4 and 4.4 ng/g c.e. in nectar and pollen, respectively). In contrast, for the pre-emergent application scenario, concentrations did not greatly differ (<2x difference) between coarse (mean concentrations of 2.4 and 5.7 ng/g c.e. in nectar and pollen, respectively) and fine (mean concentrations of 3.9 and 9.2 ng/g c.e. in nectar and pollen, respectively) soils. As multiple sampling events per year were not made in this study, DT_{50} values were not determined.

In MRID 49910601, soil applications of 0.2 lb c.e./A were also made either at planting or post-emergently (~BBCH 14; third leaf on main stem unfolded) to pumpkin plants grown in North Dakota, California and Oregon. The maximum average residue concentrations were observed in the California sites, regardless of whether the applications were pre-emergent (22.2 and 5.0 ng c.e./g in pollen and nectar, respectively) or post-emergent (28.0 and 9.55 ng c.e./g in pollen and nectar, respectively). The DT_{50} values following the pre-emergent application ranged from 9-14 days in pollen and 13-43 days in nectar; DT_{50} values following the post-emergent application ranged from 11-14 days in pollen and 22-43 days in nectar.

MRID 49705901 reported on clothianidin residues in four cucurbit crops (pumpkin, squash, cucumber and melon) grown at a single site in California following a 0.2 lb c.e./A soil application at planting. In this study, pollen samples could not be collected from the cucumber and melon flowers, though anther samples were taken for all four cucurbit species. Maximum mean residues in pumpkin pollen, anthers and nectar were 16.4, 9.2, and 5.4 ng c.e./g, respectively. For squash, the maximum mean residues in pollen, anther and nectar were 12.0, 7.4, and 5.4 ng c.e./g, respectively. Compared to the pumpkin and squash data, cucumber and melon maximum mean residues appeared to be higher and were 32.6 and 10.9 ng c.e./g, in nectar, respectively, and 32.0 and 16.8 ng c.e./g in anthers, respectively, where the LOQ for pollen, nectar and anthers was 1.0 ng c.e./g. Across the tested cucurbits, DT_{50} values ranged from 11 to 20 days for anthers, 13-18 days for nectar (only able to be calculated for pumpkin and squash), and 13-16 days for pollen (pumpkin and squash only).

In a study of melons (MRID 50154306), one application of Belay® 2.13 SC was made at plant using the maximum single label application rate of 0.2 lb a.i./A. Residues in bee-collected pollen, hand-collected pollen, bee-collected nectar, and hand-collected nectar were measured in this study. The maximum measured and maximum mean concentrations for bee-collected pollen were 32.5 and 25.4 ng/g, respectively. The maximum measured and maximum mean concentrations for hand-collected pollen were 39.5 and 39.5 ng/g, respectively. The maximum measured and maximum mean concentrations for bee-collected nectar were 11.5 and 7.19 ng/g, respectively. The maximum measured and maximum mean concentrations for hand-collected nectar were 65.5 and 65.5 ng/g, respectively. An attempt was made to separate pollen particles from the hand-collected nectar samples at the North Carolina trial site; however, the author stated that hand-collected nectar samples contained significant amounts of pollen and that the presence of pollen particles in nectar during shipping and storage may change clothianidin concentration in nectar samples. This may also be a general difficulty in collecting nectar samples that translates across most types of crops, but is not always noted in the study reports. It is also notable that in this study, while multiple replicates were obtained for all the bee-collected samples, only a single replicate was collected at each time point for the hand-collected samples. Therefore, no estimates of variability in residues are available for the hand-collected residue samples. The DT50 values for pollen and nectar ranged from 12.1-21.3 and 8.63-24.9 days, respectively.

In a study of potatoes (MRID 49705902), 0.2 lb c.e./A (Belay® 2.13 SC) was applied in-furrow at planting. Samples were collected 35 to 91 days after application to capture the appropriate bloom stages (~10-30% of bloom (early bloom), 40-50% of plants in bloom (mid-bloom), and 70-85% of plants in bloom (late bloom)). The maximum and maximum mean residue concentrations for anthers were 47 and 27 ng c.e./g, respectively, while maximum and maximum mean residue concentrations for pollen were 188 and 93 ng c.e./g, respectively.

In a study conducted in Germany, clothianidin (Clothianidin™ FS 600; unknown if similar to a U.S. registered formulation) was applied to soil at 0.08 lb c.e./A and incorporated and 22 days later untreated oil seed rape (canola) was planted (MRID 49073624). With the beginning of the flowering period (approximately two months later), a tunnel was set up and honey bees (~3000) were placed within these enclosures on the fields; pollen and nectar were collected from the bees for the course of about two weeks. The overall mean pollen residue concentrations were 3.5 ng c.e./g (average of single residue sample collected at the 7 different time points). Given the limited amount of nectar extracted from the bees, the nectar was pooled, analyzed and was 2.2 ng c.e./g (LOQ = 1.0 ng c.e./g).

In a study on field corn and popcorn (MRID 50009301), one in-furrow application of Poncho® 600 FS was made at 0.1 lb a.i./A at plant. It should be noted that the maximum single soil application rate, based on an Experimental Use Permit, is 0.2 lb a.i./A (no section 3 registrations are currently available for soil applications of clothianidin to corn). Residues in pollen and grain/stover were measured in this study. The maximum measured and maximum mean concentrations for pollen were 129 and 60.0 ng/g, respectively. The maximum measured and maximum mean concentrations for grain/stover were 34 and 32 ng/g, respectively. The DT50 values were not calculated for pollen due to the number of samples taken.

In a study on grapes (MRID 50154305), one application of Belay® 2.13 SC or Clutch® 50 WDG was made pre-bloom at a maximum single label application rate of 0.2 lb a.i./A. Only residues in pollen were measured in this study. The maximum measured and maximum mean concentrations for pollen were

206 and 160 ng/g, respectively; DT50 values were not calculated for pollen due to the number of samples taken.

Table A2-2. Summary of available registrant submitted soil application residue studies for clothianidin

Crop Group (Crop)	No. Sites/ Location/ Duration	Formulation, Appl. Rate, Interval, Timing	Matrix[1]	Max Measured Residue[2] (ng c.e./g)	Max Average Residue[3] (ppb)	DALA[4] (days)	Study Notes and Limitations	Classification (Reference)
Citrus – 10 (Orange)	1 Site Florida 2 years (2012-2013)	Belay® Insecticide; **1 x 0.08 fl oz/tree** soil drench (0.2 lb c.e./A); ~1-6 months prior to bloom	Nectar	**18.7 (2012)** **13.2 (2013)**	**8.18 (2012)** **8.5 (2013)**	21 0 - 180	• No soil climate characteristics provided • Analytical measurements were only made in nectar	Supplemental (Bondarenko, 2014; MRID 49317901)
	1 site Florida (2015)	Belay® Insecticide; **2 x 0.6 g a.i/tree** soil drench (0.4 lb a.i./A); **Platinum®** Insecticide (thiamethoxam) **1 x 0.6 g a.i/tree (0.2 lb a.i/A)** Admire® Insecticide (imidacloprid) 2 x 1.63 g a.i/tree (1.0 lb ae/A) 80-284 days prior to bloom	Nectar	15.0	<2.5	80-203	• Trials were conducted in only one location. • Residues were not measured in pollen, leaves or soil. • Nectar samples were only collected at two sampling intervals for each treatment • No measurements of imidacloprid	Supplemental (Bondarenko, 2017; MRID 49944702)

Crop Group (Crop)	No. Sites/ Location/ Duration	Formulation, Appl. Rate, Interval, Timing	Matrix[1]	Max Measured Residue[2] (ng c.e./g)	Max Average Residue[3] (ppb)	DALA[4] (days)	Study Notes and Limitations	Classification (Reference)
							or thiamethoxam .	
Citrus – 10 (Lemon and navel orange)	2 sites Arizona (lemon) and Florida (orange) (2015-2016)	Belay® 2.13 SC 1 x 0.0013 lb a.i./tree/ application/yr (0.2 lb a.i./A/yr) (TRT 2-4) 2 x 0.0013 lb a.i./tree/ application/yr (0.4 lb a.i./A/yr) (TRT 5) 6, 3, 1 month before bloom for two years	Pollen Nectar Leaves	631 114 1599	412 64.6 988	48-566 (pollen and nectar) 31-624 (leaves)		Supplemental (Bondarenko, 2017; MRID 50478201)
Cereal Grains –15 (Corn/Maize)	10 sites Minnesota, Iowa, Illinois, Indiana, Virginia, Nebraska (2013)	Belay® Insecticide; **0.2 lb c.e./A** in-furrow soil; at-planting	Pollen	**5.34, 27.9[5]**	**5.26, 26.6[5]**	~60	• Only one plot per treatment evaluated at any given site.	Supplemental (Bonderenko, 2014; MRID 49372102)
Cucurbit Vegetable - 9 (Cucumber, Melon, Pumpkin, Squash)	1 site California (2015)	Belay® Insecticide; **0.2 lb c.e./A** In-furrow application by chemigation; at planting	Pollen Nectar Anthers Leaves	**40.2 39.7 34.3 477**	**16.9 32.6 32 308**	25-75 (leaves); 33-65 (flowers)	• Trials conducted in only one location • Residues in pollen from cucumber and melon not	Supplemental (Bondarenko, 2016; MRID 49705901)

Crop Group (Crop)	No. Sites/ Location/ Duration	Formulation, Appl. Rate, Interval, Timing	Matrix[1]	Max Measured Residue[2] (ng c.e./g)	Max Average Residue[3] (ppb)	DALA[4] (days)	Study Notes and Limitations	Classification (Reference)
							measured	
Cucurbit Vegetable - 9 (Pumpkin)	3 sites North Dakota, California, Oregon (2015)	Belay® 50 WDG Insecticide; **0.2 lb c.e./A** chemigation/in - furrow; at-planting	Nectar Pollen Leaves	**5.84** **41.3** **200**	**4.98** **22.2** **129**	22-94 (leaves) 42-79 (flowers)	NA	Acceptable (Bondarenko, 2016; MRID 49910601)
		Belay® 50 WDG Insecticide; **0.2 lbs. a.i/A** chemigation/in - furrow; post-emergence (at BBCH 14)	Nectar Pollen Leaves	**11.3** **34.5** **357**	**9.6** **28.0** **319**	1-64 (leaves) 21-53 (flowers)		
Cucurbit Vegetable - 9 (Pumpkin)	9 sites (pre-emergence)/3 sites (post emergence) California 3 years (2012-2014)	Belay® Insecticide; **0.2 lbs. a.i/A** chemigation/in - furrow; at planting	Pollen Nectar Leaves	**25.8** **9.6** **167**	**15.5** **5.8** **150**	38-68 days (leaves and flowers)	• Trials conducted in only one geographical region (CA), but with a large number of sites in a wide variety of soil types • Only 2 replicates made in floral matrices at a single sampling event per year	Acceptable (Rose, 2015; MRID 49602801)
		Belay® Insecticide; **0.2 lbs. a.i/A** chemigation/in - furrow); post-emergence (side shoots visible on main stem)	Pollen Nectar Leaves	**44.5** **18.0** **450**	**37.9** **17.0** **431**	8-31 days (leaves and flowers)		
Cucurbit Vegetable – 9	3 sites	Belay® 2.13 SC	Pollen (b)	32.5	25.4	33-64 days (pollen and nectar)	None	Acceptable

Crop Group (Crop)	No. Sites/ Location/ Duration	Formulation, Appl. Rate, Interval, Timing	Matrix[1]	Max Measured Residue[2] (ng c.e./g)	Max Average Residue[3] (ppb)	DALA[4] (days)	Study Notes and Limitations	Classification (Reference)
(Melon)	California, Georgia, North Carolina	1 x 0.2 lb a.i/A at plant	Pollen Nectar (b) Nectar Leaves	39.5 11.5 65.5 233	9.50 7.19 65.5 177	19-78 days (leaves)		(Bondarenko, 2017, MRID 50154306)
Root and Tuber Vegetables – 1 (Potato)	4 sites North Dakota, California, Oregon (2015)	Belay® Insecticide; **0.2 lbs. a.i/A** in-furrow; At-planting	Anthers Pollen Leaves Soil	**47.1** **188** **484** **<LOD (1.3)**	**27.0** **92.5** **323** **<LOD**	35 to 91 days	• Pollen collected from only 2 sites	Acceptable (Bondarenko, 2016, MRID 49705902)
Oilseed – 20 (Rape)	1 site Germany (2005)	Clothianidin FS 600; **0.08 lb a.i./A**; (pre-emergence soil application)	Nectar (b) Pollen (b)	**2.2** **4.0**	**2.2** **3.5**	~3 months	• One plot per control and treatment	Not Reviewed (Neumann *et al*. 2005, MRID 49073624)
Cereal Grains –15 (Field corn and popcorn)	21 sites North Dakota, Kansas, Illinois, Pennsylvania, Virginia, Iowa, Indiana, Minnesota, Nebraska, Oklahoma, Wisconsin, Missouri (2014-2015)	Poncho 600 FS 0.1 lb a.i/A (at plant, in furrow)	Pollen Grain, stover	129 34	60.0 32	57-76 days 84-165 days	• Most sites only collected two pollen samples per trial • Unusually high clothianidin residues in 4 sets of pollen samples from 2 control plots,	Acceptable (Lam and Jenkins, 2016, MRID 50009301)
Berry and Small Fruit Crop – 13-07 (Grape)	3 sites Ontario, California, Oregon (2015-2016)	Belay® 2.13 SC or Clutch® 50 WDG 1 x 0.1 lb a.i/A Pre-bloom, BBCH 71	Pollen Leaves	206 417	160 219	31-59 days (pollen) 15-78 days (leaves)		Acceptable (Bondarenko, 2017, MRID 50154305)

NR: Not reported; DALA: Days after last application

[1]Refers to hand collected pollen and nectar unless otherwise specified: "b" (bee collected)

[2] Acute EEC chosen as the maximum reported concentration

[3] Chronic EEC chosen as the maximum average concentration

[4] DAA = Days after the last application of the pesticide

[5] The first value is based on excluding the site where clothianidin was measured in the control site, and the second value includes residue concentrations from this site.

Seed:

Table A2-3 and the discussion below summarize the key elements of the available registrant-submitted seed- treatment residue information. In addition to residue field tirals, there are there are several other registrant-submitted studies that were either a semi-field tunnel or full-field study designs that were conducted primarily in Europe evaluating seed treatments on canola, sunflower, and corn. These studies typically had a residue component in addition to characterizing the effects of clothianidin on honey bee colonies. While these studies will not be individually discussed and the results are aggregated (generally by use site, study type, and clothianidin formulation) in the tables below, they generally reported residues in pollen and nectar (hand-collected from plant, bee-collected, and hive sources) ranging from <LOD/LOQ (LOD: 0.3-1.5 ng c.e./g, LOQ: 1-5 ng c.e./g) to 8.6 ng c.e./g. It is noted that adverse weather conditions (*i.e.*, rain) occurred during some of these studies which may have influenced residue levels. Due to several deficiencies, these studies are classified as supplemental from an exposure (*i.e.*, residue information) standpoint.

In a study with soybeans (MRID 49803701), seeds were treated (PONCHO® (48% clothianidin) and VOTiVO® (21.5% Bacillus firmus)) at the labelled rate of 0.13 mg c.e./seed. Flowers were collected 56 to 71 days after treated seeds were planted. Enclosures were then erected on each plot and a single bee hive placed within each enclosure. One sample of nectar was taken from each hive 0 to 1 day after flower collection from the same plot (nectar collected from empty frame with drawn comb previously placed in the hive). Pollen was also to be sampled using bees; however, there was an insufficient amount of pollen to analyze. There were no residues in flowers or nectar above the LOD (0.08 or 0.63 ng c.e./g for nectar and flowers, respectively).

In a seed-treatment study of canola conducted in Alberta, Canada in 2011 (MRID 49754401), clothianidin (as Prosper® FX, EPA Reg. No. 264-1034) was applied at a rate of 0.016 mg c.e./seed which is less than the EPA labelled rate of 0.0356 mg c.e./seed based on the reported seed weight. Pollen was collected from free foraging bees (pollen traps) while nectar was collected from the inside of the hive from the edge of the brood nest. Though variation was high and residue concentrations were relatively low, there was a general increase in residue concentrations in both nectar and pollen throughout the bloom period. Overall, mean clothianidin residue concentrations across all 30 fields were 1.29 ng c.e./g in pollen and 0.50 ng c.e./g in nectar at the beginning of bloom and 1.82 ng c.e./g in pollen and 0.58 ng c.e./g in nectar at the end of bloom (while planting dates were not provided in study, samples were collected over a one-week interval). The maximum and maximum mean residue concentrations in pollen were 4.14 and 2.79 ng c.e./g, respectively, while maximum and maximum mean residue concentrations in nectar were 1.44 and 1.84 ng c.e./g, respectively. The percentage of canola pollen in the bee-collected pollen samples ranged from 12 to 99% with an average of 72% across all samples.

In MRID 49754402, corn was seed-treated with PONCHO® 500 or PONCHO® 1250 at rates of 0.5 mg c.e./seed and 1.25 mg c.e./seed, respectively. Fifty-three sites were tested across states with notably high corn production (planting dates not provided). In the PONCHO® 500 study (Illinois and Indiana combined), 70% of the bee-collected pollen samples were below the LOD of 0.44 ng c.e./g; however, corn pollen constituted less than 16% of bee-collected pollen on average (range 0-74%). Additionally, in the PONCHO® 1250 study (Nebraska), 55% of pollen samples were below the LOD with corn pollen constituting less than 25% of the bee-collected pollen on average (range 0-89%).

Clothianidin concentrations generally did not vary throughout the tasseling period or among different sites.

In a cotton study (MRID 49904901), residues of clothianidin were detected in extra-floral nectar, pollen and leaves following a seed treatment. Residues in pollen and extra-floral nectar were low (<5 ng c.e./g) and residues of clothianidin in floral nectar samples were all <LOD (0.2 ng c.e./g). In the California site, the maximum clothianidin residues were detected in pollen (4.57 ng c.e./g), while in the Missouri and Texas sites, the maximum clothianidin residues were detected in extra-floral nectar (3.84 and 2.32 ng c.e./g, respectively).

Residues from seed treated melon (0.33 mg c.e./seed) grown in Spain were also examined (MRID 47961202). Honey bees were used to collect nectar and pollen and were placed in tunnels as soon as enough flowers were present to support bee foraging. In the melon study, pollen and nectar residues were less than the LOQ (1 ng c.e./g). It is noted that clothianidin residues were detected in control flowers/plants but were generally below the LOQ (at <LOQ to 3.2 ng c.e./g for flowers, and <LOD (0.3 ng c.e./g) to 2.5 ng c.e./g for plants).

In a study with corn (MRID 50154301), seeds were treated with Poncho® 500 + Votivo®, or Poncho® 500 or Poncho® 250 at a rate of 0.25 to 1.25 mg a.i./seed, for a total application rate of 0.039-0.099 lb a.i/A. Pollen was collected 64 to 76 days after treatment. The maximum measured and maximum mean concentrations for pollen were 7.78 and 4.38 ng/g, respectively.

In a study with soybeans (MRID 50025901), seeds were treated with PONCHO® at a rate of 60 g a.i./100 kg seed (a rate per seed was not available from the study) for a total application rate of 0.048 lb a.i./A. Pollen, bee-collected pollen, nectar, and bee-collected nectar were collected 61 to 70 days after treatment. The maximum measured and maximum mean concentrations for all media were < 0.3 and < 0.3 ng/g, respectively.

In a second study with soybeans (MRID 50025902), seeds were treated with PONCHO® at a rate of 60 g a.i./100 kg seed (a rate per seed was not available from the study) for a total application rate of 0.048 lb a.i./A. Hand-collected pollen, bee-collected pollen, nectar, and bee-collected nectar were collected 63 to 78 days after treatment. The maximum measured and maximum mean concentrations for all media were both < 0.3 ng/g.

Table A2-3. Summary of the registrant-submitted seed treatment application residue studies for clothianidin

Crop Group Crop)	No. Sites/ Location/ Duration	Formulation, Appl. Rate, Interval, Timing	Matrix[1]	Max Measured Residue[2] (ng c.e./g)	Max. Mean Residue[3] (ng c.e./g)	DALA[4] (days)	Study Notes and Limitations	Classification (Reference)
Oilseed – 20 (Oilseed rape/ canola)	30 sites, Canada (2011)	Prosper FX **0.016 mg c.e./seed**	Pollen (t) Nectar (h)	4.14 1.84	2.79 1.44	Early-, mid-, and late-bloom	• Samples were bee-collected (pollen traps) and sites were not tented to ensure foraging only on the treated crop • No soil and weather data or planting dates provided • Application rate less than EPA label max.	Supplemental (Bromenshenk *et al.* 2015; MRID 49754401)
Cereal Grain - 15 (Corn/Maize)	53 sites, Indiana, Illinois, Nebraska (2010, 2011)	PONCHO® 500, **0.5 mg c.e./seed** PONCHO 1250, **1.25 mg c.e./seed**	Pollen (t) Pollen	5.47[5] 23.8	1.12[5] 4.91	During tasseling	• No soil information provided • No planting dates	Supplemental (Bromenshenk *et al.* 2015; MRID 49754402)
Legume Vegetables - 6 (Soybean)	3 sites, North Carolina, Georgia, California (2012)	PONCHO®/VOTiVO®, **0.13 mg c.e./seed,**	Flower Nectar (h)	<LOD	<LOD	56-71	• Pollen was not collected • Plots were tented for bee collection	Supplemental (Murphy *et al.* 2015; MRID 49803701)
Oilseed – 20 (Cotton)	3 sites, Missouri, Texas, California (2015)	PONCHO®/VOTiVO®, **0.353 mg c.e./seed, (0.045 lb c.e./A)**	Nectar Extfl Nectar Pollen	<LOD 3.84 4.57	<LOD 1.97 2.35	78-111 (flowers) 65-125 (leaves)	None	Acceptable (Gould *et al.* 2016; MRID 49904901)

Oilseed – 20 (Oilseed rape/ canola)	Tunnels / Germany, Sweden, Britain, France / 1998-2010	Elado™, Poncho™ FS 500, Clothianidin® FS 600 / **0.025-0.06 lb c.e./A** **9.7-10.6 mg c.e./seed** / Varied	Nectar (b) Nectar Bees Blossoms	8.6 7.2 1.4 4.1	8.6 4.2 NA	Varied (10 days – ca. 8.5 months)	LOD: 0.3-1.5 ppb, LOQ: 1-5 ppb	Supplemental MRIDs: 49073627; 49073626; 47699422-25; 47699418-19; 45422432-33; 45422436-37; **45422431**[6]
Oilseed – 20 (Oilseed rape/ canola)	2 sites, Ontario, Canada, and Minnesota, USA (2000)	Clothianidin® Tech + Vitavax® (carboxin and thiram), **0.04 lb c.e./A** (Ontario), 05/03/2000 Clothianidin® Tech + Vitavax® (carboxin and thiram), **0.04 lb c.e./A** (MN), 05/16/2000	Nectar (b) Pollen (b) Nectar (b) Pollen (b)	3.7 3 1.1 2.8	2.3 2.3 0.6 2.5	61 68 50 57	• Full field study, Ontario Loam soil (Ontario), no soil information for MN component • Vitavax® (carboxin and thiram) + Lindane was used as control • % foraging on crop not quantified LOD: 0.3 ppb, LOQ: 1 ppb	Supplemental (exposure only) (Scott-Dupree *et al.*, 2001 – MRID 45422435)
Oilseed – 20 (Oilseed rape/ canola)	4 sites Ontario (2005)	Prosper® FL & Poncho 600 FS, **4 g c.e./kg seed,** Seeds sown 05/20- 21/2005	Nectar (b) Pollen (b) Honey (b) Beeswax (b)	2.24 2.59 0.93 <0.5	NA (all matrix)	~30 (all matrix)	• Field study • LOQ: 0.5 ppb	Not reviewed (Cutler and Scott-Dupree 2006 - 46907802)
Cereal Grain – 15 (Corn)	Field/field tunnel studies/ France / (2004-2008)	Clothianidin™ FS 600, Clothianidin™ FS 600B G / **0.5 mg c.e./seed** / Varied	Pollen Pollen (h) Pollen (b) Beeswax Plants	6 3 8 <LOD 12	1.5 0.72 3.11 NA 8.78	Varied (ca. 2-4 months	LOQ: 0.5-1 ppb LOD: 0.3	Supplemental MRIDs: 49073613, **49073616**[6]-18

Cereal Grain – 15 (Corn)	5 sites in Upper Rhine Valley, Germany (2008)	Poncho™ Pro, **1.25 mg c.e./seed** Seeds sown April/May, 2008	Pollen Pollen (t) Bee Bread	10.4 11.4 3.3	3.94 1.61 1.33	~2-3 months	• Exact distance between hives and fields and descriptions of additional surrounding vegetation were not provided • No phylogenetic analysis was conducted of pollen in bee traps	Supplemental (Staedtler 2009-48298801)
Oilseed – 20 (Sunflower)	Tunnels / Germany (2000)	Poncho™ / **25.6 g c.e./ha [0.023 lb a.i/A] or 0.29 ng c.e./seed** / Varied	Nectar (h) Pollen (h) Pollen	<LOQ 2.9 2.4	NR 2.9 2.4	~90 (all matrix)	LOD: 0.3 ppb, LOQ: 1 ppb	Supplemental MRIDs: 49073620 & 21
Cucurbit Vegetable – 9 (Melon)	1 site in Valencia, Spain (2009)	Clothianidin & Imidacloprid™ WS 56.25 + 18.75% w/w **1.0 mg c.e./seed**, transplants planted on May 2008	Pollen(h) Nectar(h) Flowers Plants	<LOQ <LOQ 3.0 11,000	<LOQ <LOQ NR	~3-4 months	• Field Tunnel study • Residues detected in control flowers • **LOQ: 1 ppb LOD: 0.3 ppb**	Supplemental (Bocksch 2010—47961202)
Cereal Grain – 15 (Corn)	6 sites Iowa, Illinois, Indiana, Ohio (2015-2016)	Poncho® 500 + Votivo®, or Poncho® 500 or Poncho® 250 (seed, 0.25-1.25 mg/seed) 0.039-0.099 lb a.i/A	Pollen Soil	7.78 59.2	4.38 N/A	64-76 days	• Pollen and soil samples were only collected at a single sampling interval in each trial • Stability samples did not demonstrate stability of the analytes in the corn pollen test samples through to the date of analysis	Supplemental (Bondarenko, S., and A. Newcombe. 2017, MRID 50154301)

							• Test sites had relatively clayey soils	
Legume Vegetables - 6 (Soybean)	1 site Brazil (2013-2015)	Poncho™ (60 g a.i/100 kg seed, 0.048 lb a.i/A)	Nectar (b) Nectar Pollen (b) Pollen Flowers Leaves Soil	< 0.3 < 0.3 < 0.3 < 0.3 < 0.3 < 0.3 < 2.0	< 0.3 < 0.3 < 0.3 < 0.3 < 0.3 < 0.3 < 2.0	61-70 days	• QA samples (*i.e.*, field blanks, transit blanks and spikes, and storage stability spikes) 1 were not provided. • Only a single field site was used in the study.	Supplemental (Bocksch S. 2016 MRID 50025901)
Legume Vegetables - 6 (Soybean)	1 site Brazil (2013-2015)	Poncho™ (60 g a.i/100 kg seed, 0.048 lb a.i/A)	Nectar (b) Nectar Pollen (b) Pollen Flowers Leaves Soil	< 0.3 < 0.3 < 0.3 < 0.3 < 0.3 < 0.3 < 2.0	< 0.3 < 0.3 < 0.3 < 0.3 < 0.3 < 0.3 < 2.0	63-78 days	• QA samples (*i.e.*, field blanks, transit blanks and spikes, and storage stability spikes) 1 were not provided. • Only a single field site was used in the study.	Supplemental (Bocksch S. 2016 MRID 50025902)

NR: Not reported; LOQ: limit of quantitation; LOD: limit of detection; NA = not applicable

[1]Unless delineated as "h" (hive collected), "b" (bee collected), or "t" (trapped pollen), nectar and pollen refer to hand collected pollen and nectar

[2] Acute EEC chosen as the maximum reported concentration

[3] Chronic EEC chosen as the maximum average concentration

[4] DALA = Days after seeds were sown

[5] Given that the 2010 samples were sown at a rate less than the maximum allowable rate (which was done in 2011), if the residues were scaled upwards 2.5X to reflect the maximum rates, the maximum measured and maximum mean residues would be 59.5 and 12.3 ng c.e./g for hand collected pollen and 24.4 and 7.83 ng c.e./g for bee-collected pollen.

Combined:

There are two registrant-submitted studies available to characterize the total residues of parent clothianidin in pollen and nectar following applications made via two different methods (*i.e.*, a combination of two applications via seed treatment, soil, or foliar methods). **Table A2-4** and **Table A2-5** and the discussion below summarize the key elements of the seed treatment + foliar and seed treatment + soil residue studies.

In a study assessing residues from the combined seed and foliar applications to cotton (conducted in Missouri, Texas and California; MRID 49904901; same as previous foliar study), one seed application of 0.353 mg c.e./seed followed by a foliar application of 0.083 - 0.086 lbs a.i/A for a total rate that approximates the highest annual application rate for clothianidin on cotton. Maximum clothianidin residues were detected in extra-floral nectar in Missouri (409 ng c.e./g) and Texas (3442 ng c.e./g), and in pollen in California (1283 ng c.e./g). The DT_{50} values of clothianidin ranged from 1.81 to 5.47 days in nectar, 1.39 to 4.53 days in extra-floral nectar, 1.95 to 3.66 days in pollen and 1.80 to 4.33 days in leaves.

In the seed plus soil treatment study of corn (conducted in Minnesota, Iowa, and Indiana; MRID 49372102), Nipslt INSIDE® 5FS was applied to corn seed (0.25 mg a.i/seed; 0.06 lbs a.i/A) after which a single application Belay® Insecticide was applied in-furrow to the soil (0.18 lbs. a.i/A). The maximum measured clothianidin residue in pollen was 4.37 ng c.e./g. The maximum mean clothianidin concentration in pollen across all sites was 4.09 ng c.e./g.

In a study with field corn and popcorn (MRID 50009301), seeds were treated with PONCHO® 600 FS at the labelled rate of 0.5 mg a.i./seed followed by an in-furrow application at a rate of 0.06 lb a.i./A, for a total application rate of 0.1 lb a.i./A. Pollen was collected 57 to 76 days after treatment. Grain/stover was collected at 124-152 days after application. The maximum measured and maximum mean concentrations for pollen were 14.2 and 7.5 ng/g, respectively. The maximum measured and maximum mean concentrations for grain/stover were 18.0 and 15.0 ng/g, respectively.

In a study with corn (MRID 50154301), seeds were treated with Poncho® 500 + Votivo®, or Poncho® 500 or Poncho® 250 at a rate of 0.25 to 1.25 mg a.i./seed, followed by an in-furrow treatment of Ampex® 1.73 SC at 0.16 lb a.i/A, for a total application rate of 0.18-0.26 lb a.i/A. Pollen was collected 64 to 76 days after treatment. The maximum measured and maximum mean concentrations for pollen were 6.15 and 4.86 ng/g, respectively.

In two other studies which utilized a combined application scenario, clothianidin was applied in 2005 to the soil and incorporated after which treated winter barley seeds were sown (MRID 49073623 and 49073625). After the barley was harvested, untreated winter oilseed rape (canola) seeds were sown on the same plot in 2006 and allowed to flower after which a tunnel was placed in the field and honeybees were allowed to forage on the flowering rape plants. Clothianidin concentrations in the pollen (from bee pollen baskets; corbicular pollen) or nectar (from honey stomach) were below or at the LOQ (1 ng c.e./g) or LOD (0.3 ng c.e./g).

Table A2-4. Summary of the registrant-submitted combined application method residue studies (seed treatment + foliar spray)

Crop Group (Crop)	No. Sites/ Location/ Duration	Formulation, Appl. Rate, Interval, Timing	Matrix	Residue-based Acute EEC[2] (ppb)	Residue-based Chronic EEC[3] (ppb)	DALA[4] (days)	Study Notes and Limitations	Reference
Oilseed – 20 (Cotton)	3 sites, Missouri, Texas, California 1 year (2015)	PONCHO®/VOTIVO® **0.353 mg c.e./seed (0.045 lbs. c.e./A** BELAY® Insecticide **0.083 - 0.086 lbs. c.e./A** (pre-bloom)	Nectar (b) ExNectar Pollen (t)	**10.2** **3,442** **1,283**	**7.11** **2,634** **905**	6-35 (flowers) 3-50 (Leaves)	• Foliar rate used was less than maximum labeled foliar rate	Acceptable (Gould *et al.* 2016; MRID 49904901)

[1]Unless delineated as "b" (bee collected), or "t" (trapped pollen), nectar and pollen refer to hand collected pollen and nectar

[2] Acute EEC chosen as the maximum reported concentration

[3] Chronic EEC chosen as the maximum average concentration

[4] DALA = Days after the last application of the pesticide

Table A2-5. Summary of the registrant-submitted combined application method residue studies (seed treatment + soil application)

Crop Group (Crop)	No. Sites/ Location/ Duration	Formulation, Appl. Rate, Interval, Timing	Matrix	Max Measured Residue[2] (ng c.e./g)	Max Mean Residue[3] (ng c.e./g)	DALA[4] (days)	Study Notes and Limitations	Reference
Cereal Grain –15 (Corn/Maize)	5 sites Minnesota, Iowa, Indiana (2013)	Nipslt INSIDE® 5FS **0.25 mg c.e./seed** Belay® Insecticide, **0.18 lbs. c.e./A** in-furrow soil	Pollen	**4.37**	**2.5**	~ 60	• Pollen samples only collected once from each treatment area	Supplemental (Bonderenko, 2014; MRID 49372102)
Cereal Grain -15 and Oilseed –20	1 site (tunnel)	Clothianidin™ FS 250, **0.08 lbs. c.e./A** in-furrow soil [treated on 09/26/2005]	Pollen Nectar	<LOQ <LOQ	NA	approx. 1.5 years	• Only one plot per treatment	Not Reviewed (Przygoda *et al.* 2007; MRID

Crop Group (Crop)	No. Sites/ Location/ Duration	Formulation, Appl. Rate, Interval, Timing	Matrix	Max Measured Residue[2] (ng c.e./g)	Max Mean Residue[3] (ng c.e./g)	DALA[4] (days)	Study Notes and Limitations	Reference
(Barley/Rape)	Germany (2005-07)	Clothianidin FS 250 **0.5 mg c.e./g seed** [sown on 09/26/2005]					evaluated • LOQ = 1 ppb; LOD = 0.3 ppb	49073623)
Cereal Grain -15 and Oilseed –20 (Barley/Rape)	1 site (tunnel) Germany (2005-07)	Clothianidin™ FS 250, **0.08 lbs. c.e./A** in-furrow soil [treated on 09/27/2005] Clothianidin™ FS 250 **0.5 mg c.e./g seed** [sown on 09/27/2005]	Pollen (b) Nectar (b)	<LOQ <LOQ	NA	approx. 1.5 years	• Semi field tunnel study • Only one plot per treatment evaluated	Not Reviewed (Maus *et al.* 2007; MRID 49073625)
Cereal Grain – 15 (Field corn and popcorn)	21 sites North Dakota, Kansas, Illinois, Pennsylvania, Virginia, Iowa, Indiana, Minnesota, Nebraska, Oklahoma, Wisconsin, Missouri (2014-2015)	Poncho® 600 FS 0.5 mg a.i/seed followed by in-furrow application at 0.06 lb a.i/A (total app rate of 0.1 lb a.i/A), at plant	Pollen Grain, stover	14.2 18.0	7.5 15.0	57-76 days (pollen) 124-152 days (grain, stover)	• Most sites only collected two pollen samples per trial • Unusually high clothianidin residues in 4 sets of pollen samples from 2 control plots,	Acceptable (Lam and Jenkins, 2016, MRID 50009301)
Cereal Grain – 15 (Corn)	6 sites Iowa, Illinois, Indiana, Ohio (2015-2016)	Poncho® 500 + Votivo®, or Poncho® 500 or Poncho® 250 (seed, 0.25-1.25 mg/seed) + Ampex® 1.73 SC (in-furrow, 0.16 lb a.i/A) 0.18-0.26 lb a.i/A total	Pollen Soil	6.15 94.6	4.86 N/A	64-76 days	• Pollen and soil samples were only collected at a single sampling interval in each trial • Stability samples did not demonstrate stability of the analytes in the corn pollen test samples through to the	Supplemental (Bondarenko, S., and A. Newcombe. 2017, MRID 50154301)

Crop Group (Crop)	No. Sites/ Location/ Duration	Formulation, Appl. Rate, Interval, Timing	Matrix	Max Measured Residue[2] (ng c.e./g)	Max Mean Residue[3] (ng c.e./g)	DALA[4] (days)	Study Notes and Limitations	Reference
							date of analysis Test sites had relatively clayey soils	

NR: Not reported; LOQ: limit of quantitation; LOD: limit of detection

[1] Refers to hand collected pollen and nectar unless otherwise stated: "(b)" refers to bee collected.

[2] Acute EEC chosen as the maximum reported concentration

[3] Chronic EEC chosen as the maximum average concentration

[4] DAA = Days after the last application of the pesticide

Carry-over of Residues in Soil:

A confined rotational crop study with radio-labelled clothianidin technical (^{14}C- nitroimino]clothianidin (aka TI-435)), formulated as a soluble concentrate (SC), was conducted within a greenhouse and applied to bare soil within a planting container (total area of 1 m^2) at a rate of 0.293 lb c.e./A (328 g c.e./ha) (MRID 45422618; HED memo D282446). Rotational turnip, Swiss chard, and wheat were planted 29, 153, and 314 days following treatment. Total radioactive residues were in the range of 0.106-362 ng c.e./g (ppb) (turnip tops), 7-16 ppb (turnip roots), 115- 253 ppb (Swiss chard), 296-391 ppb (wheat forage), 360-534 ppb (wheat hay), 1,230-2,430 ppb (wheat straw), and 44-112 ppb (wheat grain).

In another rotational crop study using clothianidin-treated corn seeds (at a rate of 1.6X the maximum seed treatment rate; MRID 45422619) residues of clothianidin were analyzed in the rotational crops of mustard greens, turnips and wheat planted 1 to 12 months following the planting of the treated corn seeds (corn plants were disced and tilled back into the soil prior to planting the appropriate rotational crop. Clothianidin residues were measured in turnip tops, wheat forage and wheat hay at all plant back intervals (PBI) except the 12-month PBI. The maximum clothianidin residues occurred at the 8-month PBI for mustard greens (23 ng c.e./g), turnip tops (21 ng c.e./g) and wheat forage (19 ng c.e./g) and the 1-month PBI for wheat hay (25 ng c.e./g) (LOQ = 10 ng c.e./g).

The study data suggest that clothianidin is available for uptake in rotational crops; however, while residue levels in rotational plants ranged up to 2,430 g c.e./g following a soil treatment, maximum residues in rotational crops following treated corn seed were 25 ng c.e./g and were relatively close to the LOQ.

Monitoring Studies:

In addition to the crop monitoring studies discussed above, studies are available from the open literature that survey residues in in-hive pollen, wax, nectar, and dead bee samples, for multiple chemicals, including clothianidin and thiamethoxam. These studies were not reviewed for their potential utility in terms of quantitative or qualitative use for this assessment for the exposure and effects assessments. Rather, these studies serve to qualitatively characterize the potential extent to which bees are exposed to clothianidin and thiamethoxam in the field. These studies are limited in their utility since the relationship between actual field pollen and nectar concentrations to potential exposures of study hives to clothianidin and thiamethoxam are not known, only that the in-hive residues that have had some degree of processing (*e.g.* mixing pollen with bee secretions to make bee bread). Similarly, individual dead bee samples provide residue loads in bees following some unknown level of metabolic breakdown. - Studies conducted in the US are summarized below.

Mullin *et al*. (2010) collected honey bee matrix samples during 2007 and 2008 from bee colonies belonging to migratory and other beekeepers across 23 states in the U.S. and one Canadian province. Samples were relevant to several agricultural cropping systems. Samples were analyzed using modified the broad spectrum multi-residue QuEChERS (for Quick, Easy, Cheap, Effective, Rugged and Safe) method. The study identified up to 121 different pesticides and metabolites in beebread, trapped (corbicular) pollen, wax, adult bees and brood. In this study, thiamethoxam was detected in 1 of 350

samples of pollen at a concentration of 46.6 (LOD = 5.0) ng c.e./g. Clothianidin was not detected in any of the samples.

Stoner and Eitzer (2013) collected pollen samples from honey bee colonies in Connecticut. Areas where bees were located included urban, rural and agricultural land covers. Samples were collected from 2007-2010 and analyzed using a modified multi-residue QuEChERS method. Thiamethoxam was quantified in 3 (0.96%) of 313 samples at concentrations ranging 1.5-4.1 ng c.e./g (LOD = 1 ng c.e./g; LOQ unknown). Clothianidin was not detected (LOD = 2.0 ng c.e./g; LOQ unknown).

Pettis (2013) collected pollen samples of almond (California), apple (Pennsylvania), blueberry (Maine), cranberry (New Jersey), cucumber (New Jersey), pumpkin (Pennsylvania) and watermelon (Delaware) pollen in pollen traps from returning honey bee foragers. Hives were placed in three fields surrounding each crop and separated from each other by at least 3.2 km. Samples followed the LC/MS-MS and GC/MS methods for pollen analysis. For hives placed in blueberry, cranberry, cucumber, watermelon and pumpkin fields, foraging bees collected relatively little pollen from the crop they were co-located with (<1.2%) while the majority of collected pollen in hives in apple (74%) and almond (99%) fields did come from the field crop. Clothianidin and thiamethoxam were not detected in any sample. Imidacloprid was only detected in apple pollen samples, but not from any of the other crops with mean concentrations of 2.8 ng ai/g and a maximum sample concentration of 36.5 ng ai/g.

USDA APHIS has been collecting pollen samples (stored pollen in brood comb) data since 2011 as part of the National Honey Bee Survey Pesticide Report[1]. Out of 1078 collected samples sampled between 2011 and 2017, Clothianidin was detected in 1.2% of samples with mean concentrations of 28.8 ng c.e./g and a maximum concentration of 62.8 ng c.e./g. Thiamethoxam was also detected in 1.2% of samples with mean concentrations of 13.2 ng a.i./g and maximum concentrations of 39.6 ng a.i./g.

Finally, in Lu *et al.* (2015), monthly pollen and honey samples were collected between April and August 2013 from 62 hives across the state of Massachusetts. Clothianidin and thiamethoxam were detected above the LOQ (0.1 ng/g) in 27 (12%) and 7 (3%), respectively, of the pollen samples. The concentrations were reported to range from <LOQ to 8.09 ng/g for clothianidin and from <LOQ to 2.5 ng/g for thiamethoxam. In honey, clothianidin was not detected above the LOQ and thiamethoxam was above the LOQ in 2 (4%) of 53 samples; concentrations ranged from <LOQ – 0.5 ng/g.

Available survey data suggest that although thiamethoxam and clothianidin are widely used and have been detected in targeted crop monitoring studies, their frequency and magnitude of detections in non-target monitoring studies of honey bee colony matrices are relatively low.

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[1] USDA, 2018. National Honey Bee Survey Pesticide Report. Retrieved from https://bip2.beeinformed.org/state_reports/pesticides/ on September 12, 2018.

Appendix 3: Summaries of Thiamethoxam Pollen and Nectar Residue Studies, Carry-over of Residues in Soil and Monitoring Studies of Hive Matrices

Residue Data

As discussed earlier in the risk assessment, both thiamethoxam and clothianidin are considered as stressors of concern in this assessment (of thiamethoxam applications). Consequently, the residue studies for thiamethoxam quantified clothianidin as well as parent thiamethoxam. Residues of both compounds were variable in the context of percentages with some crops having significant clothianidin residues after thiamethoxam application. Thiamethoxam measured residues have been converted into clothianidin equivalents (c.e.) then summed with clothianidin measured values to yield total residues as clothianidin equivalents.

Foliar:

There are twelve registrant-submitted studies available to characterize the residues of thiamethoxam in plant tissues, pollen and/or nectar following foliar applications. Each study was conducted using differing application regimens. **Table A3-1** and the discussion below summarize the key elements of the available registrant-submitted foliar application residue studies including: tomato, cucumber, cranberry, stone fruit, cotton, strawberry, soybean, apple, pumpkin, blueberry, citrus and ornamentals.

Tomato

A field study with tomatoes (MRID 49804101) was conducted in the United States to provide data on thiamethoxam and clothianidin in pollen, leaves, soil and whole flowers after two foliar applications at the maximum labeled rate of 0.086 lb a.i./A with a 5-day retreatment interval (total rate of 0.172 lb a.i/A) with Actara® 25WG (A9584C; EPA Reg. No. 100-938). Thiamethoxam® WG (25 % w/w) was applied to commercial varieties of tomatoes.

Leaves, whole flowers, and pollen were collected 5 days after the last application (5 ± 1 DALA) as an early bloom sampling event, at 10 ± 2 DALA for mid-bloom, and 15 ± 3 DALA for late bloom. Additional leaf samples were collected before and after bloom to evaluate changes in residues in the plant during the growing season and to establish a residue decline curve. Soil samples were collected before test substance application and post-bloom after the last leaf sampling interval to establish residue levels in soil.

Thiamethoxam and clothianidin residues were present in leaves, pollen, and whole flowers throughout the blooming period across the three sampling locations. Trends in total and individual residue concentrations following foliar application in pollen were relatively comparable between the three regions, with concentrations declining over time. Concentrations in whole flowers also declined following the early bloom sampling; however, a further decline in whole flowers between the mid and late bloom sampling intervals was not evident. The limited sampling events of pollen and whole flowers reduces the ability to accurately quantify potential trends in residue levels in these matrices. The maximum and maximum mean concentrations for pollen and whole flowers were 14,540 and 8,909 and 1,318 and 1,164 ng c.e./g, respectively.

Single First Order (SFO) DT50 values of thiamethoxam for tomato leaves at the Kansas, Alabama and California sites were 0.65, 0.64 and 18.4 days, respectively. The SFO DT50 values of clothianidin for tomato leaves at the Kansas, Alabama and California sites were 6.06, 6.38 and

57.7 days, respectively. The SFO DT50 values for total residues (expressed as clothianidin equivalents) for tomato leaves at the Kansas, Alabama and California sites were 0.97, 0.86 and 28.5 days, respectively. Due to insufficient sampling intervals for pollen and flowers, no other DT50 values could be determined for these two matrices.

In a follow-up (MRID 49973701) to the previous tomato study (MRID 49804101), residue data for thiamethoxam and clothianidin in the leaves, flowers, and pollen of fruiting vegetables and cucurbit vegetables were requested by the California Department of Pesticide Regulation (CDPR). The objective of the study was to obtain residue data in California for thiamethoxam and clothianidin in leaves and flowers from fruiting vegetables (e.g., tomato) crops grown in fields that received thiamethoxam application(s) of Actara® 25WG, Platinum® 2SC, or Platinum® 75SG and that had also received application of the thiamethoxam during the previous growing season.

Eight commercial tomato production locations were identified in California (Fresno and Kings Counties) where thiamethoxam had been soil-applied in 2009 and 2010. These locations represented major commercial tomato production areas in California and were located on soils ranging from coarse (sand) to fine (clay) textures. Leaf and flower samples were collected from each site at 18-74 days after the 2010 thiamethoxam application. Three independently-collected samples of leaves and flowers were included from each trial site. For whole flowers, the maximum (149 ng c.e./g) and maximum mean (76 ng c.e./g) total residues from this study were lower than the previous tomato study (MRID 49804101).

Cucumber

A cucumber field study (MRID 49804105) was conducted in the United States to provide data on thiamethoxam and clothianidin in leaves, flowers, pollen, nectar and soil after two foliar treatment applications at the maximum labeled rate of 0.086 lb a.i/A with a 5-day retreatment interval (total rate of 0.172 lb a.i/A) with Actara® 25WG (A9584C; EPA Reg. No. 100-938). Thiamethoxam® WG (25 % w/w) was applied to commercial varieties of cucumbers.

Leaves, whole flower, pollen and nectar were collected at 5, 10 and 15 DALA to evaluate early-, mid- and late-bloom sampling events. Additional leaf samples were collected before and after bloom to evaluate changes in residues in the plant during the growing season and to establish a residue decline curve. Soil samples were collected before test substance application and post-bloom following the last leaf sampling interval to establish residue levels in soil. The maximum measured and maximum mean concentrations for pollen were 1,228 and 1,049 ng c.e./g, respectively, while the maximum measured and maximum mean concentrations in nectar were and 297 and 168 ng c.e./g, respectively.

The SFO DT50 values of thiamethoxam for cucumber leaves at the Georgia, North Carolina, and California sites were 0.79, 3.88 and 1.75 days, respectively. The SFO DT50 values of clothianidin for cucumber leaves at the Georgia, North Carolina, and California sites were 2.66, 2.84 and 6.61 days, respectively. The SFO DT50 values for total residues (expressed as clothianidin equivalents) for cucumber leaves at the Georgia, North Carolina, and California sites were 0.80, 3.81 and 1.7 days, respectively. Due to an insufficient number of sampling intervals from flowers, pollen and nectar, no other DT50 values could be determined.

Cranberry

A field study with cranberries (MRID 49804102) was conducted in the United States to provide data on thiamethoxam and clothianidin in leaves, flowers, pollen, nectar and soil after three foliar treatment applications of Actara® 25WG (A9584C; EPA Reg. No. 100-938) at the maximum labeled rate of 0.0626 lb a.i/A with a 7-day retreatment interval for New York and Oregon and 5- day retreatment interval for Wisconsin (total rate of 0.188 lb a.i/A) with). Thiamethoxam® WG (25 % w/w) was applied to commercial varieties of cranberry.

Leaves, whole flower, pollen and nectar were collected at 5 ± 1 DALA as an early-bloom sampling event, 10 ± 2 DALA as a mid-bloom sampling event, and 15 ± 3 DALA as a late-bloomsampling event. Additional leaf samples were collected before and after bloom to evaluate changes in residue concentrations in the plant during the growing season and to establish a residue decline curve. Soil samples were also collected before test substance application and post bloom after the last leaf sampling interval to establish residue levels in soil.

The maximum and maximum mean concentrations for pollen were 1,932 and 1,186 ng c.e./g, respectively, the maximum and maximum mean concentrations for nectar were 2,107 and 1,057 ng c.e./g, respectively.

The SFO DT_{50} values of thiamethoxam for cranberry leaves from the New York, Wisconsin, and Oregon sites were 1.95, 3.39 and 3.58 days, respectively. The SFO DT_{50} values of clothianidin for cranberry leaves at the New York and Oregon sites were 56.1 and 16 days, respectively; however, a clothianidin DT_{50} value for Wisconsin could not be calculated. The SFO DT_{50} values for total residues (expressed as clothianidin equivalents) for cranberry leaves at the New York, Wisconsin, and Oregon sites were 2.54, 3.62 and 3.8 days, respectively. No DT_{50} values could be derived for flowers, pollen or nectar due to insufficient sampling intervals.

Stone Fruit

A field study for stone fruit (peach, plum, and sweet cherry; MRID 49819501) was conducted in the United States (California) to provide data on thiamethoxam and clothianidin in leaves, flowers, pollen and nectar. The two-year study was initiated in 2013 to determine the magnitude of thiamethoxam and clothianidin residues in leaves, flowers, anthers, pollen, and nectar following foliar applications with Actara® 25WG (25% a.i; EPA Reg. No. 100-938). The study consisted of 10 trials located in the Pacific Northwest, each trial with an untreated control plot and a treated plot large enough to ensure adequate plants for collection. Over two consecutive growing seasons, thiamethoxam, formulated as Actara® 25WG, was applied to treated plots as a broadcast foliar spray twice (7-day interval) during each growing season at the maximum labeled-use rate of 0.086 lb a.i/A for each application. Applications were targeted 21- and 14-days before normal harvest of mature fruit. Composite samples of leaves, flowers, anthers, pollen and nectar were collected for residue analysis from untreated control and treated plots during bloom the following spring.

Detectable residues of thiamethoxam and clothianidin was present in pollen and nectar in various stone fruit. The maximum and maximum mean concentrations for pollen were 328 and 160, respectively, while the maximum and maximum mean concentrations for nectar were 5.49 and 2.48 ng c.e./g, respectively.

Cotton

A cotton field study with (MRID 49686801) was conducted in the United States (California) to provide data on thiamethoxam and clothianidin in leaves, flowers, pollen, nectar and extra floral nectar. The study included nine trials each consisting of an untreated control plot and three-replicate treated plots conducted on coarse-, medium- and fine-textured soils. Thiamethoxam, formulated as Centric® 40WG (38.9% a.i.) was applied as a foliar broadcast spray two times during the growing season at a target rate of 0.063 lb a.i/A for two consecutive years. The interval between applications was 5 days with the last application targeted 12 days before flowering. This study is also discussed in the subsequent seed treatment section as three of the nine trials included a three-replicate plot planted with seed treated with Cruiser® 5FS (47.5% thiamethoxam) at a targeted rate of 0.375 mg a.i per seed in the first year.

Samples of leaf, whole flower, pollen, nectar and extra floral nectar were collected from all trial sites in Year 1 (2013) and Year 2 (2014) of the study. The target sampling period at all trials (including seed treatment trials) was at early bloom stage (50-75% bloom). In the foliar-application trials, sampling was targeted to occur 12 days after the second (last) thiamethoxam application. Additionally, for Year 2, at six trial sites extra-floral nectar was collected at 3 additional target intervals: 5 days after first application, 5 days after second application, and 24 days after second application. These samples were collected to characterize residues of thiamethoxam and clothianidin of extra-floral nectar during bloom.

Detectable residues of thiamethoxam and clothianidin were present in pollen, nectar, and extra-floral nectar from foliar applications to cotton. The maximum and maximum mean concentrations in pollen were 316 and 54.76 g c.e./g, respectively; maximum and maximum mean concentrations in nectar were 9.83 and 3.06 ng c.e./g; and, maximum and maximum mean concentrations in extra-floral nectar were 675 and 80.84 ng c.e./g, respectively.

Strawberry

A strawberry field study (MRID 50265502) was conducted in California to provide data on thiamethoxam and clothianidin in leaves, flowers, pollen, nectar and soil after three foliar treatment applications at the maximum labeled rate of 0.063 lb a.i/A with a 10-day retreatment interval (total rate of 0.189 lb a.i/A) with Actara® 25WG (A9584C; EPA Reg. No. 100-938). Thiamethoxam, a (25 % w/w) WG formulation was applied to commercial varieties of strawberries.

Leaf and whole flower samples were collected at 5 days after the third application (5 DALA) during bloom. Flowers were also collected for the processing of pollen and nectar. In addition, leaf samples were collected before and after bloom to establish a residue decline curve. Representative soil samples were collected before application of the test substance and post-bloom following the last leaf sampling interval to establish residue levels in soil.

The maximum measured and maximum mean concentrations for pollen were 6,463 and 5,799 c.e./g, respectively, while the maximum measured and maximum mean concentrations in nectar were 567 and 334 ng c.e./g, respectively.

The SFO DT50 values of thiamethoxam for strawberry leaves at the six California sites ranged from 1.28 to 3.63 days. The SFO DT50 values of clothianidin for strawberry leaves could not be calculated except

for a single site (CA-7 DT50 = 19.6 days). The SFO DT50 values for total residues (expressed as clothianidin equivalents) for strawberry leaves at the six California sites ranged from 1.29 to 3.69 days.

Soybean

A soybean field study (MRID 50265503) was conducted in the United States to provide data on thiamethoxam and clothianidin in leaves, flowers, anthers, nectar and soil after two foliar treatment applications at the maximum labeled rate of 0.063 lb a.i/A with a 7-day retreatment interval (total rate of 0.126 lb a.i/A) with the thiamethoxam formulated product Endigo® ZCX (A18481A; EPA Reg. No. 100-1458) was applied to commercial varieties of soybean.

Samples of soybean leaves were collected at pre-flowering (0 DALA), early-bloom (5 to 10 DALA), mid-bloom (8 to 15 DALA), late bloom (13 to 20 DALA), and 30 and 60 DALA and analyzed for residue concentrations; whole flower, anther, and nectar samples were collected at early-bloom (5 to 10 DALA), mid-bloom (8 to 15 DALA) and late-bloom (13 to 20 DALA). Soil samples were collected from each trial just prior to first treatment (-1 to 0 DALA) and 60 DALA to characterize background residues.

The maximum measured and maximum mean concentrations for flowers (representing pollen) were 545 and 486 ng c.e./g, respectively, while maximum measured and maximum mean concentrations in nectar were 44.3 and 42.5 ng c.e./g, respectively. Anthers were sampled but concentrations were less than flower samples.

Single First Order (SFO) DT50 values of thiamethoxam for soybean leaves at the North Carolina, Louisiana and Iowa sites were 1.82, 1.07 and 1.26 days, respectively. The SFO DT50 values for total residues (expressed as clothianidin equivalents) for soybean leaves at the North Carolina, Louisiana and Iowa sites were 1.84, 1.07 and 1.27 days, respectively. DT50 values were not calculated for clothianidin.

Apple

An apple orchard study (MRID 50265504) was conducted in the United States to provide data on thiamethoxam and clothianidin in leaves, flowers, pollen, nectar and soil after a single foliar treatment application at the maximum nominal application rate of 0.086 lb a.i/A with Actara® 25WG (A9584C; EPA Reg. No. 100-938; 25 % w/w) was applied to commercial varieties of apple.

At all field sites leaves, whole flower, pollen and nectar were collected at approximately 5, 10 and 15 days after application to fulfill early-, mid- and late-bloom sampling events. In addition to those collected during bloom, leaf samples were collected before and after bloom to determine residue decline. Representative soil samples were collected before application of the thiamethoxam formulated product and post-bloom following the last leaf sampling interval to establish residue levels in soil.

The maximum measured and maximum mean concentrations in pollen were 2,124 and 1,756 ng c.e./g, respectively, while maximum measured and maximum mean concentrations in nectar 660 and 496 ng c.e./g, respectively.

Single First Order (SFO) DT50 values of thiamethoxam for apple leaves at the New York, Virginia and Washington sites were 8.57, 4.27 and 3.07 days, respectively. Single First Order (SFO) DT50 values of clothianidin for apple leaves at the New York, Virginia and Washington sites were 4.82, 5.25 and 3.29 days, respectively. The SFO DT50 values for total residues (expressed as clothianidin equivalents) for apple leaves at the New York, Virginia and Washington sites were 8.39, 4.32 and 3.1 days, respectively.

Pumpkin

A pumpkin field study (MRID 50265506) was conducted in the United States to provide data on thiamethoxam and clothianidin in leaves, flowers, pollen, nectar and soil after two foliar treatment applications with formulated thiamethoxam product Platinum® 75 SG (A9549C; EPA Reg. No. 100-1291; 75% w/w) at two treatment rates, *i.e.*, 0.086 lb a.i/A with a 5-day retreatment interval (total rate of 0.172 lb a.i/A) and 0.023 lb a.i/A with a 5-day retreatment interval (total rate of 0.046 lb a.i/A).

At all field sites, nectar, pollen, whole flower, and leaf samples were collected at five days after the last application (5 ± 1 DALA) to fulfill an early bloom sampling event, at 10 ± 2 DALA, 15 ± 2 DALA (except in MO with the Bloom 3 sampling at 19 DALA), and 20 ± 3 DALA for the mid-bloom sampling events, and 25 ± 3 DALA to fulfill a late-bloom sampling event.

Additional leaf samples were collected after each application at each field trial; 30 ± 3 DALA; 60 ± 3 DALA at all sites (except in NC with leaves taken at 52 DALA because of accelerated crop disease) to determine the residues in the plant over time. Representative soil samples were collected before application of the test substance (except in NC) and post-bloom following the last leaf-sampling interval to establish residue levels in soil.

The maximum measured and maximum mean concentrations in pollen were 80.4 and 30.7 ng c.e./g, respectively, while maximum measured and maximum mean concentrations in nectar were 26.6 and 23.8 ng c.e./g, respectively.

The SFO DT50 values of thiamethoxam for pumpkin leaves across the three sites and two treatment rates ranged from 1.47 to 4.32 days. The SFO DT50 values of clothianidin for pumpkin leaves across the three sites and two treatment rates ranged from 4.79 to 12.4 days. The SFO DT50 values for total residues (expressed as clothianidin equivalents) for pumpkin leaves across the three sites and two treatment rates ranged from 1.43 to 4.39 days. Some half-life values were also calculated for flowers, pollen and nectar and were within the same order of magnitude as pumpkin leaves.

Blueberry

A field study with blueberries (MRID 50425901) was conducted in the United States (2 sites) and Canada (1 site) to provide data on thiamethoxam and clothianidin in leaves, flowers, pollen, nectar and soil after foliar applications of the thiamethoxam formulated product Actara® 25WG (A9584C; EPA Reg. No. 100-938; 25 % w/w) at two treatment rates (3 applications at 0.063 lb a.i/A with a 7-day retreatment interval (total rate of 0.189 lb a.i/A) and (1 application at 0.063 lb a.i/A with a 7-day retreatment interval (total rate of 0.063 lb a.i/A).

At all field sites leaves, whole flower, pollen, and nectar were collected at early-, mid-, and late-bloom sampling events. In addition, leaf samples were collected before and after bloom to determine the amount of thiamethoxam and clothianidin in the plant over the course of

the growing season. Representative soil samples were collected before application of thiamethoxam and after the last leaf sampling interval to establish residue levels in soil.

The maximum measured and maximum mean concentrations in pollen were 868 and 810 ng c.e./g, respectively, and the maximum measured and maximum mean concentrations in nectar were 647 and 593 ng c.e./g, respectively.

The SFO DT50 values of thiamethoxam for blueberry leaves across the three sites and two treatment rates ranged from 2.19 to 7.55 days. The SFO DT50 values of clothianidin for blueberry leaves across the three sites and two treatment rates ranged from 11.2 to 41.2 days. The SFO DT50 values for total residues (expressed as clothianidin equivalents) for blueberry leaves across the three sites and two treatment rates ranged from 2.32 to 9.17 days.

Citrus

A field study with sweet orange (MRID 50425902) was conducted in the United States to provide data on thiamethoxam and clothianidin in leaves, flowers, pollen, nectar and soil after foliar applications of the thiamethoxam formulated product Actara® 25WG (A9584C; EPA Reg. No. 100-938; 25 % w/w) with targeted application timing at three treatment rates (2 applications at 0.086 lb a.i/A with a 7-day retreatment interval (total rate of 0.147 lb a.i/A), (2 applications at 0.086 lb a.i/A with a 7-day retreatment interval (total rate of 0.147 lb a.i/A) and (1 application at 0.086 lb a.i/A).

At all field sites leaves, whole flower, pollen, and nectar were collected at early-, mid-, and late-bloom sampling events. In addition, leaf samples were collected before and after bloom to determine the amount of thiamethoxam and clothianidin in the plant over the course of the growing season. Representative soil samples were collected before application of thiamethoxam and after the last leaf sampling interval to establish residue levels in soil.

The maximum measured and maximum mean concentrations in pollen were 878 and 703 ng c.e./g while the maximum measured and maximum mean concentrations in nectar were 12.1 and 10.0 ng c.e./g, respectively.

The SFO DT50 values of thiamethoxam for sweet orange leaves across the three sites and three treatment rates ranged from 4.18 to 13.4 days. The SFO DT50 values of clothianidin for sweet orange leaves across the three sites and three treatment rates ranged from 6.21 to 25.7 days. The SFO DT50 values for total residues (expressed as clothianidin equivalents) for sweet orange leaves across the three sites and three treatment rates ranged from 4.99 to 15.5 days.

Ornamentals

A field study (MRID 50425903) was conducted on five ornamental species (crabapple, cotoneaster, lily, mock orange, lilac) in the United States to provide data on thiamethoxam and clothianidin in leaves, flowers, pollen, nectar and soil after foliar or soil treatment applications of the thiamethoxam formulated product Meridian® 25WG (A9584C; EPA Reg. No. 100-943; 25%,w/w) with various application timings of two applications at 0.133 lb a.i/A with a 7-day retreatment interval (total rate of 0.266 lb a.i/A).

For all trials, nectar, pollen, whole flower, and leaf samples were generally collected once during early bloom, mid-bloom, and late-bloom, with additional leaf samples typically collected before application, 0-1 DAFA (days after first application), ca. 3 DAFA, 7 DAFA, and 28-30 and 90 days after bloom to determine the residues in the plant over time.

In lilacs, the maximum measured and maximum mean concentrations in pollen were 3127 and 1238 ng c.e./g, respectively, and the maximum measured and maximum mean concentrations in nectar lilac were 1192 and 796 ng c.e./g, respectively.

For the foliar treatments, the SFO DT50 values of thiamethoxam for ornamental leaves across the five-species ranged from less than 1 day to 5.55 days. The SFO DT50 values of clothianidin for ornamental leaves across the five species ranged from less than 1 day to 126 days. The SFO DT50 values for total residues (expressed as clothianidin equivalents) for ornamental leaves across the five species ranged from less than 1 day to 6 days.

For the soil treatments, the SFO DT50 values of thiamethoxam for ornamental leaves across the five species ranged from 1 day to 152 days. The SFO DT50 values for total residues (expressed as clothianidin equivalents) for ornamental leaves across the five species ranged from less than 1 day to 45 days. DT50 values were not calculated for clothianidin alone.

Table A3-1. Summary of registrant-submitted foliar application residue studies

Crop	No. Sites/ Location/ Duration	Formulation Application Rate Interval Timing	Matrix[1]	Max. Measured Residue[2] (ng c.e./g)	Max. Average Residue[3] (ng c.e./g)	Range of % of clothianidin in total residues	DALA[4] (days)	Study Notes and Limitations	Classification (Reference)
Fruiting Vegetable-8 (Tomato)	3 sites Kansas Alabama California 1 year (2015)	Actara® 25WG 2 x 0.074 lb c.e./A (total: 0.147 lb c.e./A) 5-day interval BBCH 61 (KS-1) BBCH 63 (KS-2) BBCH 51 (AL-1) BBCH 51 (AL-2) BBCH 63 (CA-1) BBCH 64 (CA-2) pre-bloom	Pollen Flowers	14504 1318	8909 1164	5.28-90 38-89	5 4	• Residues detected in control leaf, pollen and flower samples from all trials • 2 different plant varieties were used possibly introducing variability • Residues measured over a single growing season • Whole flowers (instead of nectar) were collected for analysis. LOQ: 0.856 ng c.e./g LOD: 0.428 ng c.e./g,	Acceptable MRID 49804101 (MRID 4997370 Addendum)
Cucurbit Vegetable – 9 (Cucumber)	3 sites Georgia North Carolina California 1 year (2015)	Actara® 25WG 2 x 0.074 lb c.e./A (total: 0.147 lb c.e./A) 5-day interval BBCH 204 (GA-1) BBCH 206 (GA-2) BBCH 51 (NC-1) BBCH 55 (NC-2) BBCH 204 (CA-1) BBCH 206 (CA-2) pre-bloom	Pollen Nectar	1228 297	1049 168	1.73-45.3 1.87-18.1	5 5	• 3 different plant varieties were used possibly introducing variability • Residues measured over a single growing season • Pollen LOQ: 0.856 ng c.e./g; Pollen LOD: 0.428 ng c.e./g • Nectar LOQ: 0.428 ng c.e./g; Nectar LOD: 0.214 ng c.e./g	Acceptable MRID 49804105
	3 sites	Actara® 25WG 3 x 0.0536 lb c.e./A (total: 0.161 lb c.e./A)						• 3 different plant varieties were used possibly introducing variability • Residues measured over a single growing season • Prior to testing, soil samples from NY and	

Berry and Small Fruit-13 (Cranberry)	New York Wisconsin Oregon 1 year (2015)	5-day interval (WI) 7-day interval (NY, OR) BBCH 55 (NY-1) BBCH 59 (NY-2) BBCH 61 (NY-3) BBCH 56 (WI-1) BBCH 60 (WI-2) BBCH 61-62 (WI-3) BBCH 56 (OR-1) BBCH 59 (OR-2) BBCH 59 (OR-3) pre-bloom	Pollen Nectar	1932 2107	1186 1057	1.35-18 2.02-12	9 14	OR had detectable amounts of clothianidin and thiamethoxam, respectively. • Pollen LOQ: 0.856 ng c.e./g; Pollen LOD: 0.428 ng c.e./g • Nectar LOQ: 0.428 ng c.e./g; Nectar LOD: 0.214 ng c.e./g	Acceptable MRID 49804102
Stone Fruit-12 (Peach, Plum, Sweet Cherry)	10 sites California 2 years (2013, 2014)	Actara® 25WG 2 x 0.074 lb c.e./A (0.147 lb c.e./A) 7-day interval post-bloom; last application made 14 days before normal harvest of mature fruit	Pollen (cherry) Nectar (plum)	328 5.49	160 2.48	0.06-63 1.69-94	14 14	• Spatial variability unknown since all 10 sites were from California • Pollen LOQ: 0.856 ng c.e./g; Pollen LOD ≤ 0.428 ng c.e./g • Nectar LOQ: 0.428 ng c.e./g; Nectar LOD ≤ 0.214 ng c.e./g	Acceptable MRID 49819501
Oilseed-20 (Cotton)	6 sites California 2 year (2013 and 2014)	Centric® 40WG 2 x 0.063 lb a.i/A (0.126lb. a.i/A) 5-day interval pre-bloom	Pollen Nectar Extra Floral Nectar	316 9.83 675	54.76 3.06 80.84	4.38-39 1.46-59 1.24-35	12 12 5-24	LOQ: 0.856 ng c.e./g; LOD: 0.428 ng c.e./g	Acceptable MRID49686801
		Actara® 25WG						• For year 1, poor weather conditions resulted in	

Berry and Small Fruit-13 (Strawberry)	9 sites California 1 year (2015)	3 x 0.0539 lb c.e./A (total: 0.162 lb c.e./A) 10-day interval BBCH 56 (CA-1) BBCH 56-59 (CA-2) BBCH 72-74 (CA-3) BBCH 72-74 (CA-4) BBCH 56-68 (CA-5) BBCH 87 (CA-6) BBCH 87 (CA-7) BBCH 87 (CA-8) BBCH 87 (CA-9) pre-bloom	Pollen Nectar	6463 567	5799 334	0.61-7.93 0.14-5.09	6 8	poor flower production and loss of residue analysis. • Residues > LOQ were found in all untreated control matrices. • Residues measured over a single growing season • Pollen LOQ: 0.856 ng c.e./g; Pollen LOD: 0.428 ng c.e./g • Nectar LOQ: 0.428 ng c.e./g; Nectar LOD: 0.214 ng c.e./g	Acceptable MRID 50265502
Legume-6 (Soybean)	3 sites North Carolina Louisiana Iowa 1 year (2015)	Endigo® ZCX 2 x 0.0539 lb c.e./A (total: 0.108 lb c.e./A) 7-day interval BBCH 51, 55 (NC) BBCH 14-15 and 51-55 (LA) BBCH 60, 64 (IA) pre-bloom	Flower Anther Nectar	545 67.8 44.3	486 56.3 42.5	5.3-35 13.2-54 11.9-97	5 5 9	• Residues > LOQ were found in untreated control matrices. • Residues measured over a single growing season • Flower LOQ: 0.856 ng c.e./g; Flower LOD: not reported Nectar LOQ: 0.428 ng c.e./g; Nectar LOD: not reported Anther LOQ: 0.856 ng c.e./g; Anther LOD: not reported	Acceptable MRID 50265503
	3 sites New York Virginia	Actara® 25WG 1 x 0.0736 lb c.e./A (total: 0.0736 lb c.e./A)		2124	1756	1.26-52	6	• Residues > LOQ were found in untreated control samples. • Residues measured over a single growing season	

Pome Fruits-11 (Apple)	Washington 1 year (2016)	BBCH 57 (NY) BBCH 57 (VA) BBCH 57 (WA) pre-bloom	Pollen Nectar	660	496	1.97-8.52		• Pollen LOQ: 0.856 ng c.e./g; Pollen LOD: Not reported Nectar LOQ: 0.428 ng c.e./g; Nectar LOD: not reported	Acceptable MRID 50265504
Cucurbit Vegetables-9 (Pumpkin)	3 sites North Carolina Missouri California 1 year (2016)	Platinum® 75 SG <u>2 Treatments</u> 2 x 0.0736 lb c.e./A (total: 0.147 lb c.e./A; 5-day interval) 2 x 0.02 lb c.e./A (total: 0.039 lb c.e./A; 5-day interval) BBCH 62-63 (NC) BBCH 61&65 (MO) BBCH 61 (CA) pre-bloom	Pollen Nectar	80.4 26.6	30.7 23.8	3.38-93 3.73-99	6 6	• Residues > LOQ were found in untreated control matrices. • Residues measured over a single growing season • Pollen LOQ: 0.856 ng c.e./g; Pollen LOD: 0.428 ng c.e./g Nectar LOQ: 0.428 ng c.e./g; Nectar LOD:0.214 ng c.e./g	Acceptable MRID 50265506
Small Fruit and Berry-13 (Blueberry)	3 sites California Washington Canada (Quebec)	Actara® 25WG <u>2 Treatments</u> 3 x 0.0539 lb c.e./A (total: 0.162 lb c.e./A; 7-day interval) 1 x 0.0539 lb c.e./A (total: 0.0539 lb c.e./A; 7-day interval)	Pollen Nectar	868 647	810 593	4.04-62 33-92	5 8,5	• Residues > LOQ were found in untreated control matrices. • Residues measured over a single growing season • Pollen LOQ: 0.856 ng c.e./g; Pollen LOD: Not reported Nectar LOQ: 0.428 ng c.e./g; Nectar LOD: not reported	Acceptable MRID 50425901

	1 year (2016)	BBCH 57-58 (CA) BBCH 52-56 (WA) BBCH 7-9 and 51 and 55 (Canada) pre-bloom							
Citrus-10 (Sweet Orange)	3 sites Florida (2) California (1) 1 year (2017)	Actara® 25WG <u>3 Treatments</u> 2 x 0.0736 lb c.e./A (total: 0.147 lb c.e./A; 7-day interval) 2 x 0.0736 lb c.e./A (total: 0.147 lb c.e./A; 7-day interval) 1 x 0.0736 lb c.e./A (total: 0.0736 lb c.e./A) BBCH 57-58 (FL1) BBCH 52-56 (FL2) BBCH 52-56 (CA1) pre-bloom	Pollen Nectar	878 12.1	703 10.0	5.77-84 4.63-77	22 21	• Residues > LOQ were found in untreated control matrices. • Residues measured over a single growing season • Pollen LOQ: 0.856 ng c.e./g; Pollen LOD: 0.428 ng c.e./g Nectar LOQ: 0.428 ng c.e./g; Nectar LOD:0.214 ng c.e./g	Acceptable MRID 50425902
	3 sites New York Wisconsin	Meridian® 25WG <u>Foliar **OR** Soil</u> 2 x 0.114 lb c.e./A (total: 0.228 lb c.e./A; 7-day interval)	Pollen Nectar	3127 1192	1238 796	1.44-98 1.19-98	7 7	• Residues > LOQ were found in untreated control matrices. • Residues measured over a single growing season • Pollen LOQ: 0.856 ng c.e./g; Pollen LOD:	Acceptable

Non-Ag; Ornamentals	Oregon 1 year (2016)	BBCH (*see MRID*) Timing varied according to variety						0.428 ng c.e./g Nectar LOQ: 0.428 ng c.e./g; Nectar LOD:0.214 ng c.e./g	MRID 504425903

NR: Not reported; LOQ: limit of quantitation; LOD: limit of detection; BBCH = Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie plant development stage.

[2] Acute EEC chosen as the maximum reported concentration of total residues

[3] Chronic EEC chosen as the maximum average concentration of total residues

[4] DALA = Days after the last application of the pesticide

Soil:

There are seven registrant-submitted studies available to characterize the residues of thiamethoxam in plant tissues, pollen and/or nectar following soil applications. Each study was conducted using differing application regimens. The discussion below and **Table A3-2** summarize the key elements of the available registrant-submitted soil application residue studies including: pepper, citrus, cucumber, tomato, strawberry and cucurbits (pumpkin, squash, muskmelon).

Pepper

A field study of peppers (*Capsicum annuum*) (MRID 49804103) was conducted in the United States to provide data on thiamethoxam and clothianidin in leaves, flowers, pollen, nectar and soil after one soil treatment application at the maximum labeled rate of 0.172 lb a.i/A in Kansas, Alabama and California. Platinum® 75SG (thiamethoxam 75% w/w (75 % a.i.; EPA Reg. No. 100-1291) water soluble granule (SG) formulation was applied to commercial varieties of pepper. Whole flowers and pollen samples were collected at early-, mid-, and late-bloom for each trial. Nectar was collected in Kansas at early-, mid-, and late-bloom periods, and in California at early- and mid-bloom; however, nectar was not available for collection in Alabama. Leaf samples were collected from each trial site approximately two weeks after application, at early- (BBCH 61-69 stage), mid- (10±2 days after early bloom), and late-bloom (20±2 days after early bloom), and at 30±5 and 60±5 days after the late-bloom sampling interval. Soil samples were collected before test substance application and post-bloom following the last leaf sampling interval to establish residue levels in soil.

Thiamethoxam and clothianidin residues were present in leaves, flowers, pollen and nectar throughout the blooming period across the three sampling locations. Trends in total and individual residue concentrations following soil application in pollen were variable between the three regions; however, concentrations of both thiamethoxam and clothianidin declined from early/mid bloom to late-bloom, except for pollen samples in California, where maximum thiamethoxam residues were observed at mid-bloom and only declined slightly by late-bloom. Thiamethoxam, clothianidin, and total residues in nectar exhibited similar declines at the Kansas and California trial sites; however, nectar was not available at the Alabama site.

The maximum and maximum mean concentrations in pollen were 268 and 238 ng c.e./g, respectively, while the maximum and maximum mean concentrations in nectar were 1,384 and 534 ng c.e./g, respectively. The SFO) DT50 values of thiamethoxam for pepper leaves at the Kansas, Alabama, and California sites were 12, 18.1 and 19.3 days, respectively.

Single First Order (SFO) DT50 values of clothianidin for pepper leaves at the Kansas, Alabama, and California sites were 33.6, 31.4 and 38.9 days, respectively. Single First Order (SFO) DT50 values for total residues (expressed as clothianidin equivalents) for pepper leaves at the Kansas, Alabama, and California sites were 13.6, 20.7 and 21.6 days, respectively. Due to insufficient sampling intervals from flowers, pollen and nectar, no other DT50 values could be determined.

Florida Citrus

A citrus study (MRID 49881002) was conducted over three years in Florida to investigate the effect of application timing and rate on potential concentrations of thiamethoxam and clothianidin residues in leaves, flowers, anthers, pollen, and nectar. The potential for residue carry-over and subsequent uptake by orange trees was investigated by repeating the application of the thiamethoxam to the same sites over two consecutive cropping seasons. Thiamethoxam formulated as Platinum® 75SG (75% w/w) was applied as a soil drench in commercial orange groves (under the tree drip line) at five concentrations equivalent to 0.086, 0.129, 0.172, 0.257, and 0.556 lb a.i./A, respectively, at three intervals of approximately 120, 75, and 45 days prior to bloom (DPB). Due to the varying application rates, the pollen and nectar values described below were normalized to the typical citrus application rate of 0.172 lbs a.i./acre. Additionally, the 0.129 lb a.i./A rate was terminated after the Year 1 sample collection and the 0.086 lb a.i./A and 0.172 lb a.i./A rates were then added to the study design.

Leaf samples from each treatment group were collected prior to the application, specific days prior to bloom (DPB), and at bloom. In the 120 DPB application interval, leaf samples were collected prior to application, and at approximately 75, 45, and 0 DPB. In the 75 DPB application interval, leaf samples were collected prior to application and at 45 and 0 DPB. In the 45 DPB application interval, leaf samples were collected prior to application and at 0 DPB. Other matrices (*i.e.*, whole flowers, nectar, pollen, and anthers) were collected at 0 DPB only.

Detectable residues of thiamethoxam and clothianidin was present in leaves, flowers, anther, pollen and nectar throughout the growing period. In the majority of matrices, application times, and treatment levels, thiamethoxam residues exceeded those of clothianidin, and residues of both analytes were highest in pollen. Generally, magnitudes increased with increasing application rate, and carry-over led to greater concentrations in Year 2 compared to Year 1.

The maximum and maximum mean concentrations for pollen were 323 and 69.47 ng c.e./g, respectively, while in nectar the maximum and maximum mean concentrations were 23.71 and 12.80 ng c.e./g, respectively.

This study was not designed to quantify the rate of decline over time within a growing season. Therefore, no DT_{50} analyses were conducted.

California Citrus

A citrus study (MRID 49881001) was conducted in California to investigate the effect of application timing and rate on potential concentration of thiamethoxam and clothianidin residues in leaves, flowers, anthers, pollen and nectar following soil drench applications with Platinum® 75SG (thiamethoxam 75% a.i.; EPA Reg. No. 100-1291). The potential for residue carry-over and subsequent uptake by orange trees was investigated by repeating the study in the second growing season using the same plots as in the first year of the study. The three-year study was initiated in 2012 and consisted of two trials, each with an untreated control plot and nine treated plots. Each treated plot was divided into three replicate plots where thiamethoxam was applied under the tree drip line at target rates of 0.086, 0.172, and 0.558 lb a.i./A at one of three intervals: 150, 90, and 45 days prior to bloom (DPB). Orchards producing navel oranges were selected. However, since navel oranges do not produce pollen,

the navel orange sites were abandoned and the trials were closed following the final sampling for Year 1. Two new trials using sites with pollen-producing Valencia oranges, were started with a full two-year duration.

The maximum and maximum mean concentrations in pollen were 410 and 107 ng c.e./g, respectively, while the maximum and maximum mean concentrations in nectar were 65.22 and 19.78 ng c.e./g, respectively. Pollen and nectar residue values were normalized to the typical citrus application rate of 0.172 lbs a.i./acre.

This study was not designed to quantify the rate of decline over sampling time within a growing season. Therefore, no DT_{50} analyses were conducted.

Cucumber

A two-year study of cucumbers in California (MRID 49550801) was conducted in 2011 and 2012 to determine the magnitude of residues in cucumber leaves, flowers, pollen, and nectar. The study involved three trials each consisting of an untreated control plot and three replicated treated plots. The trials were conducted on coarse-, medium- and fine- textured soils, which were characterized as a sandy loam with 9% clay, a sandy loam with 14% clay, and a clay loam with 38% clay, respectively. Platinum® 75SG (thiamethoxam; 75% a.i.) was applied as an in-furrow treatment at a target rate of 0.172 lb a.i/A in Years 1 and 2. Composite samples of leaves, female flowers, male flowers, pollen, and nectar were collected for residue analysis from the untreated control plot and treated plots at 43 to 57 days after planting in Year 2. Leaf and flower samples were analyzed for thiamethoxam and clothianidin residues.

The maximum and maximum mean concentrations for pollen and nectar were 10.02 and 6.98 ng c.e./g, respectively, and the maximum and maximum mean concentrations in nectar were 11.84 and 9.50 ng c.e./g, respectively. No DT50 analyses were conducted.

Tomato

A tomato field study (MRID 50023201) was conducted in California to provide data on thiamethoxam and clothianidin in leaves and flowers from fruiting vegetables (i.e., tomatoes) treated with Actara® 25WG (thiamethoxam 25% a.i.), Platinum® 2SC (thiamethoxam 21.6% a.i.), or Platinum® 75SG (thiamethoxam 75% a.i.) and that received application(s) of the thiamethoxam during the previous growing season. Eight commercial tomato production locations were identified in Fresno and Kings Counties where thiamethoxam had been soil-applied in 2009 and 2010. These locations represented major commercial tomato production areas in California and were located on soils ranging from coarse (sand) to fine (clay) textures. Leaf and flower samples were collected from each site at 18-74 days after the 2010 thiamethoxam application. Three independently collected samples of leaves and flowers were collected from each trial site. No pollen or nectar samples were collected.

The maximum and maximum mean concentrations for whole flowers were 147 and 141 ng c.e./g, respectively. No DT50 analyses were conducted.

An additional tomato field study (MRID 50265507) was conducted in the United States to provide data on thiamethoxam and clothianidin in leaves, flowers, pollen and soil after a soil application at two treatment rates, 0.125 lb a.i./A and the maximum labeled rate of 0.172 lb a.i/A in Kansas, Illinois, and

California. Platinum® 75SG (thiamethoxam 75% w/w (75 % a.i.); EPA Reg. No. 100-1291), a water-soluble granule (SG) formulation, was applied to commercial varieties of tomato.

At all field sites leaves, whole flowers, and flowers for pollen collection were sampled during early bloom (BBCH 61-69), approximately 10 ± 2 days after early bloom for a mid-bloom collection, and 20 ± 2 days after mid-bloom for a late-bloom sampling.

At all field sites additional leaf samples were collected pre- and post-bloom (S1 targeting 14 ± 2 days after last application, S5 at 30 ± 2 days after S2, and S6 at 60 ± 2 days after S2) to evaluate changes in residues in the plant during the growing season. Representative soil samples were collected before application of the test substance and post-bloom during the last leaf sampling interval to establish residue levels in soil.

The maximum measured and maximum mean concentrations in pollen were 306 and 220 ng c.e./g, respectively, and the maximum measured and maximum mean concentrations and whole flowers were 330 and 261 ng c.e./g, respectively.

The SFO DT50 values of thiamethoxam for tomato leaves across the two treatment rates ranged from 1.64 to 20.4 days. The SFO DT50 values of clothianidin for tomato leaves across the two treatment rates ranged from 8.95 to 50.8 days. The SFO DT50 values for total residues (expressed as clothianidin equivalents) for tomato leaves across the two treatment rates ranged from 1.64 to 25.7 days.

Strawberry

A field study of strawberry (Fragaria L.) (MRID 50266001) was conducted in the United States to provide data on thiamethoxam and clothianidin in leaves, flowers, nectar and pollen after one soil drip application at two rates, i.e., 0.129 lb a.i./A and the maximum labeled rate of 0.188 lb a.i/A in Florida and California. Platinum® 75SG (thiamethoxam 75% w/w (75 % a.i.); EPA Reg. No. 100-1291) water soluble granule (SG) formulation was applied to commercial varieties of strawberry.

At all field sites samples of leaves, whole flower, pollen, and nectar were collected at early-, mid- and late-bloom sampling events. In addition to those collected during bloom, leaf samples were collected before and after bloom to determine residue decline. Representative soil samples were collected before application of thiamethoxam and post-bloom after the last leaf sampling interval to establish residue levels in soil.

The maximum and maximum mean concentrations for pollen were 1,669 and 1,126 ng c.e./g, respectively, while the maximum and maximum mean concentrations in nectar were 186 and 86.9 ng c.e./g, respectively. At the 0.188 lb a.i/A application rate, the (SFO) DT50 values of thiamethoxam for strawberry leaves at the Winter Garden, FL, Oviedo, FL, and California sites were 8.57, 21.1 and 15.8 days, respectively. The (SFO) DT50 values of clothianidin for strawberry leaves at the Winter Garden, FL, Oviedo, FL, and California sites were 12.5, 21.7 and 19.2 days, respectively. The (SFO) DT50 values for total residues (expressed as clothianidin equivalents) for strawberry leaves at the Winter Garden, FL, Oviedo, FL, and California sites were 8.72, 22.2 and 15.9 days, respectively.

At the 0.129 lb a.i/A application rate, the (SFO) DT50 values of thiamethoxam for strawberry leaves at the Winter Garden, FL, Oviedo, FL, and California sites were 8.82, 24.1 and 13.8 days, respectively. The (SFO) DT50 values of clothianidin for strawberry leaves at the Winter Garden, FL, Oviedo, FL, and

California sites were 14.3, 27.3 and 17.6 days, respectively. The (SFO) DT50 values for total residues (expressed as clothianidin equivalents) for strawberry leaves at the Winter Garden, FL, Oviedo, FL, and California sites were 9.02, 25.5 and 13.9 days, respectively.

Cucurbit

A cucurbit field study (MRID 50265501) with three species (pumpkin, squash, muskmelon) was conducted in the United States to provide data on thiamethoxam and clothianidin in leaves, flowers, pollen, nectar and soil after a soil application at two rates, 0.125 lb a.i./A (pumpkin) and the maximum labeled rate of 0.172 lb a.i/A (pumpkin, squash, muskmelon) in North Carolina, Missouri, and California. Platinum® 75SG (thiamethoxam 75% w/w (75 % a.i.; EPA Reg. No. 100-1291) a water-soluble granule (SG) formulation was applied to commercial varieties of cucurbit.

For all trials, nectar, pollen, whole flower, and leaf samples were collected at early bloom (BBCH 61-62), mid-bloom (5±1, 10±3, and 15±3 DAB1; Days After Bloom 1), and late-bloom (20±4 DAB1), with additional leaf samples collected through 85 to 104 days post-treatment to determine the residues in the plant over time, with the exception of the squash trial at the North Carolina site where sampling was terminated early due to crop damage caused by fungus after a large amount of rainfall due to a hurricane. Soil samples were generally collected prior to the test application and after the last bloom sampling event.

The maximum measured and maximum mean concentrations for pollen were 755 and 310 ng c.e./g and maximum measured and maximum mean concentrations in nectar were 57.6 and 28.7 ng c.e./g, respectively.

The SFO DT50 values of thiamethoxam for cucurbit leaves across the three species and two treatment rates ranged from 1.81 to 28.3 days. The SFO DT50 values of clothianidin for cucurbit leaves across the three species and two treatment rates ranged from 5.29 to 52.4 days. The SFO DT50 values for total residues (expressed as clothianidin equivalents) for cucurbit leaves across the three species and two treatment rates ranged from 1.83 to 28.3 days.

Table A3-2. Summary of registrant-submitted soil application residue Studies

Crop	No. Sites/ Location / Duration	Formulation, Appl. Rate, Interval, Timing	Matrix[1]	Max. Measured Residue[2] (ng c.e./g)	Max. Average Residue[3] (ng c.e./g)	Range of % of clothianidin in total residues	DALA[4] (days)	Study Notes and Limitations	Classification (Reference)
Fruiting Vegetable-8 (Pepper)	3 sites Kansas Alabama California 1 year (2015)	Platinum® 75SG 1 x 0.147 lb c.e./A BBCH 26 (KS) BBCH 14 (AL) BBCH 12 (CA) at planting	Pollen Nectar	268 1384	238 534	6.02-95 13-68	62 53	• Residues detected in control leaf, pollen and nectar samples • 3 different plant varieties were used possibly introducing variability • Residues measured over a single growing season • Clothianidin treated seeds were planted in California one year prior to the trial • Pollen LOQ: 0.856 ng c.e./g; Pollen LOD: 0.428 ng c.e./g Nectar LOQ: 0.428 ng c.e./g Nectar LOD: 0.214 ng c.e./g	Acceptable MRID 49804103
Citrus-10 (FL Citrus)	1 site Florid a 3 years (Dec. 2012 – March 2015)	Platinum® 75 SG <u>lb c.e./A</u> 0.074 0.110 0.147 0.220 0.476 <u>Prior to bloom (DPB)</u> 120 75 45	Pollen Nectar	323 23.71	69.47 12.80	3.52-89 7.48-73	120 DPB	• Residues normalized to max application rate allowed on label (*i.e.*, 0.147 lb c.e./A). • Residues were only collected from a single geographical location which limits the study's ability to compare residue magnitude and trends across varying climatic regions with different soil types. • Pollen LOQ: 0.856 ng c.e./g; Pollen LOD: 0.300 ng c.e./g • Nectar LOQ: 0.428 ng c.e./g Nectar LOD: 0.159 ng c.e./g	Supplemental (Quantitative) MRID 49881002
						24-54		• Residues normalized to max	

Crop	No. Sites/ Location / Duration	Formulation, Appl. Rate, Interval, Timing	Matrix[1]	Max. Measured Residue[2] (ng c.e./g)	Max. Average Residue[3] (ng c.e./g)	Range of % of clothianidin in total residues	DALA[4] (days)	Study Notes and Limitations	Classification (Reference)
Citrus-10 (CA Citrus)	1 site California 2 years (Nov. 2012 – Sept 2015)	Platinum® 75 SG lb c.e./A 0.074 0.147 0.219 0.478 Prior to bloom (DPB) 150 90 45	Pollen Nectar	410 65.22	107 19.78	8.55-54	90 DPB	application rate allowed on label (*i.e.*, 0.147 lb c.e./A). • Residues were only collected from a single geographical location which limits the ability to compare residue magnitude and trends across varying climatic regions with different soil types. • Pollen LOQ: 0.856 ng c.e./g; Pollen LOD: 0.428 ng c.e./g • Nectar LOQ: 0.428 ng c.e./g Nectar LOD: 0.214 ng c.e./g	Acceptable MRID 49881001
Cucurbit Vegetables-9 (Cucumber)	3 sites California 2 years (2011-2012)	Platinum® 75 SG 1 x 0.147 lb c.e./A at plant	Pollen Nectar	10.02 11.84	6.98 9.50	11-33 14-20	43-57 (Year 2)	• study not designed for temporal analysis of declining concentrations but to provide a snapshot of residue concentrations during flowering. Only one sample of each matrix was collected and analyzed from each plot; therefore, it is not possible to determine if concentrations were increasing or decreasing. • LOQ: 0.856 ng c.e./g LOD: 0.428 ng c.e./g	Acceptable MRID 49550801
Cucurbit Vegetables	3 sites North Carolina Missouri California	Platinum 75SG lb c.e./A 0.211	Pollen Nectar	755 57.6	310 28.7	1.92-98 3.28-76	48 47, 35	• Residues detected in control leaf, flower, pollen and nectar samples • 3 different plant varieties were used possibly introducing variability	Acceptable

Crop	No. Sites/ Location / Duration	Formulation, Appl. Rate, Interval, Timing	Matrix[1]	Max. Measured Residue[2] (ng c.e./g)	Max. Average Residue[3] (ng c.e./g)	Range of % of clothianidin in total residues	DALA[4] (days)	Study Notes and Limitations	Classification (Reference)
	1 year (2016)	0.145 at planting						• Residues measured over a single growing season • Pollen LOQ: 0.856 ng c.e./g Pollen LOD: 0.428 ng c.e./g Nectar LOQ: 0.428 ng c.e./g Nectar LOD: 0.214 ng c.e./g	MRID 50265501
Fruiting Vegetables-8 (Tomato)	8 sites California 2 years (2009-2010)	Platinum 75SG 2009 1 x 0.067 lb c.e./A (Kings County) 1 x 0.147 lb c.e./A (Fresno County) 2010 2 x 0.070 lb c.e./A 30-day interval (Kings County) 1 x 0.147 lb c.e./A (Fresno County) at plant	Flowers	147	141	66-88	18-74 (Year 2)	• Whole flower data only. • Study not designed for temporal analysis of declining concentrations but to provide a snapshot of residue concentrations during bloom.	Acceptable MRID 50023201
								Only one sample of each matrix was collected and analyzed from each plot; therefore, it is not possible to determine if concentrations were increasing or decreasing. • LOQ: 0.856 ng c.e./g LOD: 0.428 ng c.e./g	
Fruiting Vegetables-8 Tomato	3 sites Kansas Illinois California	Platinum 75SG lb c.e./A 0.107 0.147 at transplant	Pollen Flowers	306 330	220 261	33.3-83 55.1-93	37 41	• Residues detected in control pollen samples • 3 different plant varieties were used possibly introducing variability • Residues measured over a single growing season • Pollen LOQ: 0.856 ng c.e./g	Acceptable MRID 50265507

Crop	No. Sites/ Location / Duration	Formulation, Appl. Rate, Interval, Timing	Matrix[1]	Max. Measured Residue[2] (ng c.e./g)	Max. Average Residue[3] (ng c.e./g)	Range of % of clothianidin in total residues	DALA[4] (days)	Study Notes and Limitations	Classification (Reference)
	1 year (2016)							Pollen LOD: 0.428 ng c.e./g Flower LOQ: 0.856 ng c.e./g Flower LOD: 0.428 ng c.e./g	
Berry and Small Fruit-13 Strawberry	3 sites Florida (2) California 1 year (2016)	Platinum 75SG lb c.e./A 0.211 0.145 at planting	Pollen Nectar Flowers	1699 186 680	1126 86.9 299	1.02-60.4 0.64-30.0 3.36-45.1	70 61 42	• Residues detected in control leaf, flower, pollen and nectar samples • 3 different plant varieties were used possibly introducing variability • Residues measured over a single growing season • Pollen LOQ: 0.856 ng c.e./g Pollen LOD: 0.257 ng c.e./g Nectar LOQ: 0.428 ng c.e./g Nectar LOD: 0.128 ng c.e./g Flower LOQ: 0.856 ng c.e./g Flower LOD: 0.257 ng c.e./g	Acceptable MRID 50266001

NR: Not reported; LOQ: limit of quantitation; LOD: limit of detection; BBCH = Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie plant development stage

[2] Acute EEC chosen as the maximum reported concentration of total residues

[3] Chronic EEC chosen as the maximum average concentration of total residues

[4] DALA = Days after the last application of the pesticide

[5] DPB = Days prior to bloom

Seed:

There are thirteen registrant-submitted studies available to characterize the residues of thiamethoxam in plant tissues, pollen and/or nectar following seed treatment applications. Each study was conducted using differing planting regimens. **Table A3-3** summarizes the key elements of the available registrant-submitted seed treatment residue studies including: soybean, canola, corn, cotton and sunflower.

Soybean

A field study of soybeans (MRID 49804104) was conducted in the United States to provide data on thiamethoxam and clothianidin in nectar, anthers, whole flowers, leaves and soil after a seed treatment application at equivalent to the maximum application rate 0.042 lbs a.i./A using Cruiser® 5FS (thiamethoxam 47.6% a.i) applied as a seed coating to commercial varieties of soybean seed.

Leaves, flowers, anther, and nectar were collected at the R1, R2, and R3 bloom stages to represent early-, mid-, and late-bloom stages, respectively, to quantify residues of thiamethoxam and clothianidin. Leaves were collected prior to bloom at vegetative growth stages of V3 and V5, as well as after bloom at reproductive growth stages of R4 and R5. Soil samples were collected before planting of the treated seed and post-bloom following the last leaf sampling interval to measure residue levels in soil. Samples of treated seed were collected prior to planting but were not analyzed.

Thiamethoxam and clothianidin residues were present in nectar, anthers and whole flowers throughout the blooming period across the three sampling locations. There were no consistent trends for thiamethoxam or clothianidin in any matrix across all three regions. The majority of measured values were less than the limit of quantitation (LOQ). The maximum measured and maximum mean concentrations in anthers were 4.05 and 2.84 ng c.e./g, respectively; maximum measured and maximum mean concentrations in flowers were 6.08 and 4.14 ng c.e./g, respectively; and, the maximum measured and maximum mean concentrations nectar were, and 5.15 and 2.91 ng c.e./g, respectively.

The SFO DT_{50} values of thiamethoxam for soybean leaves at the Louisiana, North Carolina and Iowa sites were 2.63, 6.41 and 10.3 days, respectively. The SFO DT_{50} values for total residues (expressed as clothianidin equivalents) for soybean leaves at Louisiana, North Carolina and Iowa sites were 2.66, 6.45 and 10.9 days, respectively. Due to insufficient sampling intervals or clothianidin formation in other matrices, no other DT_{50} values could be determined.

A second soybean study (MRID 49210901) was designed to measure the magnitude of thiamethoxam residues in whole flowers, upper / lower leaves, and reproductive organ tissue from soybean plants following treatment with Cruiser® 5FS (thiamethoxam 47.6% a.i) treated seeds equivalent to application rates of 0.0375 lb a.i./A and 0.0750 lb a.i./A. Three separate trials were conducted in Oregon MO; Richland IA, and Fisk, MO; each trial consisted of two treated plots and a single untreated control plot. Samples of whole flowers, stamen, pistol, nectary, and leaves were collected 46-54 days following planting. No further samples were collected; thiamethoxam residues in each matrix were not determined over time. Thiamethoxam concentrations were highest in the leaves and lowest in the stamens, pistols, and nectaries. The maximum measured and maximum mean concentrations for reproductive structures (combined stamen, pistol, nectary) were 23.14 and 15.64 ng c.e./g, respectively. No DT50 analyses were conducted.

Canola

The registrant submitted data on two canola studies conducted in Canada. One study (MRID 49775702) consisted of four canola crop field trials conducted over two years in Canada. The test substance, HELIX® XTra Seed Treatment (thiamethoxam (20.7% a.i) co-formulated with the fungicides, difenoconazole (1.25% a.i.), metalaxyl-M and S-isomers (0.39% a.i.) and fludioxonil (0.13% a.i.)), was applied to canola seed at a nominal rate of 438.3 g a.i/100 kg seed. At all field trial locations, samples of canola flowers (2012 only), pollen and nectar were collected at full flowering (50-75% flowering) which corresponded to 43- 59 days after planting in Year 1 (2012) and 44-56 days after planting in Year 2 (2013). The maximum and maximum mean concentrations for pollen were 7.69 and 3.17 ng c.e./g; and, the maximum and maximum mean concentrations in nectar were 2.64 and 1.48 ng c.e./g, respectively. No DT50 analyses were conducted.

In a second study (MRID 49819502), four trials were in Canada where trials were carried out over 2 years. In Year 1 the formulated thiamethoxam product ACTARA™ 240SC (21.6% a.i.), was applied as an in-furrow treatment to potato seed pieces at a rate equivalent to an application of 140 g a.i/ha. In Year 2, the same plots were planted with either untreated canola or canola seed treated with HELIX® XTra at a total rate of 438.3 g a.i/100 kg seed. Samples of canola pollen and nectar were collected in Year 2 at full flowering (50-75% flowering) which corresponded to 41-57 days after planting. The maximum measured and maximum mean concentrations for pollen and nectar were 46.89 and 46.89 ng c.e.,/g (only 1 sampling interval), respectively, while the maximum measured and maximum mean concentrations in nectar were 13.34 and 8.08 ng c.e./g, respectively. No DT50 analyses were conducted.

Corn

Concentrations of thiamethoxam and clothianidin are available for corn pollen from three separate tunnel studies (MRIDs 49158914 – 49158916). Corbicular pollen from corn (treated via seeds) was collected from forager bees that were confined to tunnels. The studies were carried out in different locations in Northern and Southern France (near the cities of Zellwiller, Champagne, and Grisolles) in 2005 and 2006. Treatments were made at rates of 0.87-0.95 mg a.i./seed, which is consistent with the mass allowed in the United States for seed treatments to corn (*i.e.*, 0.59-1.3 mg a.i./seed). Over the tree studies maximum concentration in pollen ranged from 5.19 to 12.47 ng c.e./g; maximum mean concentrations in pollen ranged from 3.33 to 6.45 ng c.e./g.

Cotton

In the cotton field study (MRID 49686801) mentioned in the foliar section, three of the nine trials also included three-replicate plots planted with Cruiser® 5FS (thiamethoxam 47.6% a.i.) treated seed at a targeted rate of 0.375 mg a.i/seed in the first year. Detectable residues of thiamethoxam and clothianidin were present in pollen, nectar, and extra-floral nectar. The maximum and maximum mean concentrations for pollen were both 1.0 ng c.e./g; maximum measured and maximum mean concentrations in nectar were 1.54 and 1.18 c.e./g, respectively, and maximum measured and maximum mean concentrations in extra floral nectar were 1.74 and 1.25 ng c.e./g, respectively.

Sunflower

There are six field studies (MRIDs 46163104 to 46163108; 49158906) conducted in Europe or South America evaluating seed treatments on sunflower. These studies typically had a residue component in addition to characterizing the effects of thiamethoxam on honey bee colonies. While these studies will not be individually discussed, the results are presented in the **Table 3.21**. Measured residues in pollen ranged from 0.86 to 2.7 ng c.e./g while nectar samples were < LOQ of 0.856 ng c.e./g.

Combined:

Brazilian Citrus

A Brazilian citrus study (MRID 49346601; Supplemental-Qualitative) was conducted to determine the magnitude of thiamethoxam and clothianidin residues in citrus flowers, nectar and pollen after various treatment methods (foliar or soil drench) with three thiamethoxam-containing products at their maximum application rates. This study included 5 locations throughout the state of São Paulo, Brazil. At one location, flowers were collected twice due to early flowering and the second flowering was recorded as a separate location. As a result, the study reported collection results from 6 "locations."

Each location had four test plots, three of which were treated with six applications of various chemical products. The products contained thiamethoxam alone, a thiamethoxam/*lambda*-cyhalothrin mixture, or a *lambda*-cyhalothrin and chlorantraniliprole mixture.

The six chemical applications were carried out over a nine-month period that was initiated in November 2011 and completed in August of 2012. The commercial names of the chemicals used in this study are:

- Actara™ 250 WG (thiamethoxam 250 g a.i./L)
- Actara™ 750 SG (thiamethoxam 750 g a.i./L)
- Engeo™ Pleno (thiamethoxam 141 g a.i./L+ *lambda*-cyhalothrin106 g a.i./L)
- Karate™ Zeon 50 CS (*lambda*-cyhalothrin 50 g a.i./L)
- Ampligo™ (*lambda*-cyhalothrin 50 g a.i./L + chlorantraniliprole 100 g a.i./L)

The samples were collected between one and two months after the final chemical application. **Table 3.22** summarizes the range of residues expressed as clothianidin equivalents along withthe study profile for the Brazilian citrus study. In general, residues in nectar and pollen ranged from <1 to 16.3 ng c.e./g and 3.7 to 31.4 ng c.e./g, respectively.

Corn

In a study assessing residues from the combined seed + foliar applications to corn (conducted in Pennsylvania, Iowa and Oklahoma; MRID 50265505), one seed application of 1.25 g a.i./seed followed by two foliar applications of 0.043 - 0.063 lbs a.i/A for a total rate that approximates the highest annual application rate for thiamethoxam on corn.

Each location contained an untreated control plot (CP1), a treated seed plot (P2, Cruiser® 5S), and four plots that contained seeds treated with Cruiser® 5S followed by foliar applications with either Endigo® ZC or Endigo® ZCX. Two plots (P3 [Endigo® ZC] and P5 [Endigo® ZCX]) received foliar applications at the V8 growth stage 7 days after first application, and the other two plots (P4 [Endigo® ZC] and P6 [Endigo® ZCX]) received foliar applications at initial silk emergence 7 days after first application.

At all field sites, leaf and pollen sample collections were determined based on their respective plots (*i.e.*, CP1, P2, P3, P4, P5, and P6). The leaf samples in P2 were collected at 60 ± 3 days after planting, at pollen shed, 30-days after pollen shed and 60-days after pollen shed. The pollen samples in P2 were collected at pollen shed. The leaf samples in P3 and P5 were collected immediately following the first and second foliar applications, at pollen shed, 30-days after pollen shed and 60-days after pollen shed. The pollen samples in P3 and P5 were collected at pollen shed. The leaf samples at P4 and P6 were collected immediately following the first application, at pollen shed, immediately following the second application, 30 days after pollen shed and 60-days after pollen shed. The pollen samples in P4 and P6 were collected at pollen shed and 2- to 5-days after the second foliar application. The leaf and pollen samples in CP1 were collected at the same intervals as the P2 through P6 plots. Leaf samples collected at pollen shed and at 30- and 60-days after pollen shed for all plots were used to establish a decline curve. Representative soil samples were collected prior to planting with the treated seeds and after the last leaf sampling interval to establish residue levels in soil.

The maximum measured and maximum mean concentrations for pollen were 864 and 604 ng c.e./g, respectively. The study design did not include nectar sampling.

The SFO DT50 values of thiamethoxam for corn leaves ranged from 2.52 to 5.25 days. The SFO DT50 values of clothianidin for corn leaves ranged from 1.41 to 199 days. The SFO DT50 values for total residues (expressed as clothianidin equivalents) for corn leaves ranged from 1.94 to 5.55 days.

Table A3-3. Summary of the registrant submitted seed treatment application residue studies

Crop	No. Sites/ Location / Duration	Formulation, Appl. Rate, Interval, Timing	Matrix[1]	Max. Measured Residue[2] (ng c.e./g)	Max. Average Residue[3] (ng c.e./g)	Range of % of clothianidin in total residues	DALA[4] (days)	Study Notes and Limitations	Classification (Reference)
Soybean	3 sites Louisiana North Carolina Iowa 1 year (2015)	Cruiser® 5FS 0.036 lb c.e./A BBCH 00 (LA) BBCH 00 (NC) BBCH 00 (IA) seed treatment	Nectar Anthers Flowers	5.14 4.05 6.08	2.91 2.84 4.14	9.71-61 27-71 32-57	54 43 54	• 3 different plant varieties were used possibly introducing variability. • Residues only measured over a single growing season. • LOQ: 0.856 ng c.e./g LOD: 0.428 ng c.e./g	Acceptable MRID 49804104
Soybean	3 sites 1 Iowa 2 Missouri 1 year (2012)	Cruiser® 5FS 0.129 lb c.e./A seed treatment	Reproductive Structures	23.14	15.64	4.01-54	49	• Although samples included both pollen (stamens) and nectar (nectaries), there is uncertainty on bee attractiveness to reproductive structures. • LOQ: 0.856 ng c.e./g LOD: 0.257 ng c.e./g	Supplemental MRID 49210901
Canola	4 sites Canad a 2 year (2012 and 2013)	HELIX® XTra 375 g c.e./100 kg seed seed treatment	Pollen Nectar	7.69 2.64	3.17 1.48	8.36-54 19-54	46 46	• Low concentrations in all matrices. • LOQ: 0.856 ng c.e./g LOD: 0.188 ng c.e./g	Supplemental MRID 49755702
		Year 1 Actara®	Pollen Nectar	46.89 13.34	46.89 8.08	13-33 3.75-44	53 52	The highest atypical clothianidin value (759 ppb)	

Crop	No. Sites/ Location / Duration	Formulation, Appl. Rate, Interval, Timing	Matrix[1]	Max. Measured Residue[2] (ng c.e./g)	Max. Average Residue[3] (ng c.e./g)	Range of % of clothianidin in total residues	DALA[4] (days)	Study Notes and Limitations	Classification (Reference)
Canola	4 sites Canada 2 year (2013 and 2014)	240SC 120 g c.e./ha Year 2 HELIX® XTra 346 g c.e./100 kg seed seed treatment						excluded; see page 32 of MRID 49819502. Next highest value (46.89 ppb) presented. Max and mean value are identical because there was only a single sampling interval. • LOQ: 0.856 ng c.e./g LOD < 0.103 ng c.e./g	Supplemental MRID 49819502
Corn	2 sites Northern France (Zellwiller) 2 year (2005 and 2006)	A10590C 0.07 lb c.e./A seed treatment	Pollen	7.98	5.02	11-54	78	• Tunnel Study • LOQ: 0.856 ng c.e./g LOD not reported	Supplemental MRID 49158914
Corn	2 sites Southern France (Grisolles) 2 year (2005 and 2006)	A10590C 0.07 lb c.e./A seed treatment	Pollen	5.19	3.33	31-58	85	• Tunnel Study • LOQ: 0.856 ng c.e./g LOD not reported	Supplemental MRID 49158915
Corn	2 sites Northern France (Champagne) 2 year (2005 and 2006)	A10590C 0.07 lb c.e./A seed treatment	Pollen	12.47	6.45	37-70	86	• Tunnel Study • LOQ: 0.856 ng c.e./g LOD not reported	Supplemental MRID 49158916

Crop	No. Sites/ Location / Duration	Formulation, Appl. Rate, Interval, Timing	Matrix[1]	Max. Measured Residue[2] (ng c.e./g)	Max. Average Residue[3] (ng c.e./g)	Range of % of clothianidin in total residues	DALA[4] (days)	Study Notes and Limitations	Classification (Reference)
Cotton	3 sites California 2 year (2013 and 2014)	Cruiser® 5FS (0.321 mg c.e./seed) at plant	Pollen Nectar Extra Floral Nectar	316 9.83 675	54.76 3.06 80.84	4.38-39 1.46-59 1.24-35	12 12 5-24	• LOQ: 0.856 ng c.e./g; • LOD: 0.428 ng c.e./g	Supplemental MRID 49686801
Sunflower	1 site Argentina (2003)	Cruiser® 350 FS (0.006 lb c.e./acre) at plant	Pollen Nectar	< LOQ	<LOQ	--	--	• LOQ: 0.856 ng c.e./g; LOD: not reported •	Supplemental MRID 46163104
Sunflower	1 site Hungary (2001)	Cruiser® 350 FS (0.015 lb c.e./acre) at plant	Pollen Nectar	< LOQ	<LOQ	--	--	• LOQ: 0.856 ng c.e./g; • LOD: not reported	Supplemental MRID 46163105
Sunflower	1 site Hungary (2001)	Cruiser® 350 FS (0.014 lb c.e./acre) at plant	Pollen Nectar	< LOQ	<LOQ	--	--	• LOQ: 0.856 ng c.e./g; • LOD: not reported	Supplemental MRID 46163106
Sunflower	1 site Spain (2003)	Cruiser® 70 WS (0.021 lb c.e./acre) at plant	Pollen Nectar	0.86 – 0.94 (n = 4) <LOQ	<LOQ <LOQ	--	--	• LOQ: 0.856 ng c.e./g; • LOD: not reported	Supplemental MRID 46163107
Sunflower	1 site Italy (2001)	Cruiser® 70 WS (0.02 lb c.e./acre) at plant	Pollen Nectar	1.7 – 2.7 (n = 2) <LOQ	<LOQ <LOQ	--	--	• LOQ: 0.856 ng c.e./g; LOD: not reported	Supplemental MRID 46163108
Sunflower	1 site France (2001-2002)	Cruiser® 70 WS (0.015 – 0.020 lb a.i./acre) at plant	Pollen Nectar	< LOQ	<LOQ	--	--	• LOQ: 0.856 ng c.e./g; • LOD: not reported	Supplemental MRID 49158906

NR: Not reported; LOQ: limit of quantitation; LOD: limit of detection; BBCH = Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie plant development stage.

[2] Acute EEC chosen as the maximum reported concentration of total residues

[3] Chronic EEC chosen as the maximum average concentration of total residues

[4] DALA = Days after the last application of the pesticide

Combined Application Method Residue Studies

There are two registrant-submitted studies available to characterize the residues of thiamethoxam in plant tissues, pollen and/or nectar following applications made via two different methods (*i.e.*, a combination of two applications via seed treatment, soil, or foliar methods). **Tables A3-4** and **A3-5** below summarize the key elements of the combined application methods.

Table A3-4. Brazilian citrus summary with residue profile

TRT	Products	A.I.	Rate	Appl. Time	Appl. Method	Chemical	Nectar (µg c.e. /kg)	Pollen (µg c.e. /kg)
1	Actara 250 WG+ Karate™ Zeon 50 CS	TMX[1]	1.25 g/ tree height	Oct/Nov	Drench	Thiamethoxam (c.e.)	2.1 to 9.7	6.6 to 25.7
		LCY	300 mL/ha					
	Actara™ 250 WG+ Engeo™ Pleno	TMX	1.25 g/tree height	60 DAA1*	Drench			
		TMX + LCY	200 mL/ha		Foliar			
TRT	**Products**	**A.I.**	**Rate**	**Appl. Time**	**Appl. Method**	**Chemical**	**Nectar (µg c.e. /kg)**	**Pollen (µg c.e. /kg)**
	Karate™ Zeon 50 CS	LCY	300 mL/tree	60 DAA2**	Foliar	Clothianidin	< 1 to 5.0	3.7 to 21.0
	Engeo™ Pleno	TMX + LCY	200 mL/ha	April	Foliar			
	Engeo™ Pleno	TMX + LCY	200 mL/ha	June	Foliar			
	Engeo™ Pleno	TMX + LCY	200 mL/ha	August	Foliar			
2	Actara™ 750 SG+ Karate™ Zeon 50 CS	TMX	0.5 g/tree height	Oct/Nov	Furrow	Thiamethoxam (c.e.)	1.3 to 16.3	4.9 to 31.4
		LCY	300 mL/ha		Foliar			
	Actara™ 750 SG + Engeo™ Pleno	TMX	0.5 g/tree height	60 DAA1	Furrow			
		TMX + LCY	200 mL/ha		Foliar			

	Karate™ Zeon 50 CS	LCY	300 mL/ha	60 DAA2	Foliar	Clothianidin	< 1 to 8.3	5.0 to 28.3
	Engeo™ Pleno	TMX + LCY	200 mL/ha	April	Foliar			
	Engeo™ Pleno	TMX + LCY	200 mL/ha	June	Foliar			
	Engeo™ Pleno	TMX + LCY	200 mL/ha	August	Foliar			
3	Actara™ 750 SG	TMX	0.5 g/tree height	Oct/Nov 60 DAA1	Furrow	Thiamethoxam (c.e.)	0.7 to 10.3	4.3 to 23.1
	Ampligo™	LCY+ CTPR	30 mL/100 L	60 DAA2	Foliar			
	Ampligo™	LCY+ CTPR	30 mL/100 L	April	Foliar	Clothianidin	< 1 to 8.0	4.0 to 24.0
	Ampligo™	LCY+ CTPR	30 mL/100 L	June	Foliar			
	Ampligo™	LCY+ CTPR	30 mL/100 L	August	Foliar			

* DAA1 stands for days after first application.

**DAA2 stands for days after second application.

[1] TMX- thiamethoxam, LCY-*lambda*-cyhalothrin, CTPR-chlorantraniliprole

Limit of Quantification (LOQ) of 1.0 µg/kg for flowers and pollen and 0.5 µg/kg for thiamethoxam in nectar.

Table A3-5. Summary of the registrant-submitted combined application method residue studies (seed treatment + 2 foliar sprays)

Crop	No. Sites/ Location/ Duration	Formulation, Appl. Rate, Interval, Timing	Matrix[1]	Max. Measured Residue[2] (ng c.e./g)	Max. Average Residue[3] (ng c.e./g)	Range of % of clothianidin in total residues	DALA[1] (days)	Study Notes and Limitations	Classification (Reference)
Corn	Pennsylvania Iowa Oklahoma 3 sites 1 year (2015)	Cruiser® 5S/600FS Endigo® ZCX Endigo® ZC <u>Seed treatment + 2 foliar sprays</u> 1.25 g a.i./seed 2 x 0.0368 lb c.e./A (total: 0.0736 lb c.e./A; 7-day interval) 2 x 0.0539 lb c.e./A (total: 0.1079 lb c.e./A; 7-day interval) <u>BBCH (all sites)</u> P2-P6: at plant P3, P5: V8-V10.5 P4, P6: R1-R2	Pollen	864	604	1.3-74	2	• Residues > LOQ were found in untreated control matrices. • Residues measured over a single growing season • Pollen LOQ: 0.856 ng c.e./g; Pollen LOD: 0.428 ng c.e./g	Acceptable MRID 50265505

[1] Days after last application

Carry-over of Thiamethoxam Residues in Soil

Two sets of studies on the accumulation of residues from [14C]thiamethoxam in rotational crops are available. For details see the USEPA 2000 (HED Memo D252021) The first set of studies (MRID 44703531) depicts the accumulation of 14C-residues in confined rotational crops following a spray application of radiolabeled thiamethoxam (either [thiazol-2-14C] or [oxadiazin-4-14C]) at 0.077 lb c.e./A to bare soil. Control and treated plots remained fallow until rotational crops were planted. At 30, 120, and 365 days after treatment a subplot was planted with turnips, mustard, and wheat. For the 365-day PBI, spinach was planted instead of mustard. Results are presented below in **Table A3-6**. Generally, the total residues decreased over the PBI with exception of wheat. The metabolite CGA-322704 (clothianidin) was also detected in each commodity (except grain) at various PBIs and accounted for 6.4-47.8% total radioactive residues (TRR; 1-20 ng c.e./g or 0.001 – 0.020 ppm). This study was conducted at ~0.4x the max seasonal application rate.

Table A3-6 Total residues (ppm) based on plant back intervals (PBI) in rotational crop study MRID 44703531

Radiolabel	PBI	Mustard Leaves	Spinach Leaves	Turnip Tops	Turnip Roots	Wheat Forage (25% mature)	Wheat Forage (50% mature)	Wheat Straw	Wheat Grain
[Thiazol-^{14}C]	30	0.019	--	0.051	0.008	0.036	0.036	0.169	0.019
	120	0.015	--	0.014	0.004	0.018	0.008	0.026	0.006
	365	--	0.017	0.026	0.003	0.015	0.021	0.050	0.009
[Oxadiazin-^{14}C]	30	0.016	--	0.010	0.006	0.022	0.031	0.113	0.017
	120	0.023	--	0.029	0.003	0.024	0.010	0.021	0.007
	365	--	0.031	0.027	0.003	0.015	0.029	0.045	0.010

-- At the 365-day PBI, spinach was planted instead of mustard. So residues were not collected for spinach at the 30 or 120 day PBI or mustard at the 365 PBI

In addition to MRID 44703531, data are available on the accumulation of 14C-residues in confined rotational crops following a broadcast spray application of either [thiazol-2-14C] or [oxadiazin-4-14C]thiamethoxam at ~200 g a.i/ha (0.18 lb a.i/A; rate is ~0.8x maximum seasonal rate) to the soil surface (MRIDs 447155116 and 44715117). Thiamethoxam was diluted (with water) and applied at a rate equivalent to 0.15 lb c.e./A. Lettuce, radishes, and spring wheat were planted as representative rotational crops at approximately 1, 4, and 12 months following the soil application. In addition, winter wheat was also planted at 6 months post-treatment. Each subplot was planted with a single rotational crop, and following harvest, was replanted with a different rotational crop for a subsequent PBI. Results are presented below in **Tables A3-7 and A3-8**. Generally, the TTR decreased over the PBI with exception of wheat. Residues generally decreased with the increased PBI with the exception of wheat grain.

Table A3-7. Total residues (ng c.e./g) based on plant back intervals (PBI) in rotational crop studies with lettuce and radish (MRIDs 44715116 and 44716117).

Radiolabel	PBI (d)	Lettuce Leaves	Radish Tops	Radish roots
Thiazol-14C	29	30	99	6.0

	119	11	9.4	1.7
	362	3.4	7.7	2.6
Oxadiazin-14C	29	29	66	4.3
	119	10	9.4	1.7
	362	6.8	6.8	1.7

Table A3-8. Total residues (ng c.e./g) based on plant back intervals (PBI) in rotational crop studies with wheat (MRIDs 44715116 and 44716117).

Radiolabel	PBI (d)	Forage	Straw	Husks	Grain
Thiazol-14C	29	96	645	312	25
	104	26	147	112	126
	180	12	44	45	4.3
	362	7.7	70	50	3.4
Oxadiazin-14C	29	57	445	334	17
	104	48	199	154	73
	180	20	49	59	5.1
	362	8.6	68	62	6.0

Monitoring Studies:

In addition to the crop monitoring studies discussed above, studies are available from the open literature that survey residues in in-hive pollen, wax, nectar, and dead bee samples, for multiple chemicals, including clothianidin and thiamethoxam. These studies were not reviewed for their potential utility in terms of quantitative or qualitative use for this assessment for the exposure and effects assessments. Rather, these studies serve to qualitatively characterize the potential extent to which bees are exposed to clothianidin and thiamethoxam in the field. These studies are limited in their utility since the relationship between actual field pollen and nectar concentrations to potential exposures of study hives to clothianidin and thiamethoxam are not known, only that the in-hive residues that have had some degree of processing (*e.g.* mixing pollen with bee secretions to make bee bread). Similarly, individual dead bee samples provide residue loads in bees following some unknown level of metabolic breakdown. - Studies conducted in the US are summarized below.

Mullin *et al.* (2010) collected honey bee matrix samples during 2007 and 2008 from bee colonies belonging to migratory and other beekeepers across 23 states in the U.S. and one Canadian province. Samples were relevant to several agricultural cropping systems. Samples were analyzed using modified the broad spectrum multi-residue QuEChERS (for Quick, Easy, Cheap, Effective, Rugged and Safe) method. The study identified up to 121 different pesticides and metabolites in beebread, trapped (corbicular) pollen, wax, adult bees and brood. In this study, thiamethoxam was detected in 1 of 350

samples of pollen at a concentration of 46.6 (LOD = 5.0) ng c.e./g. Clothianidin was not detected in any of the samples.

Stoner and Eitzer (2013) collected pollen samples from honey bee colonies in Connecticut. Areas where bees were located included urban, rural and agricultural land covers. Samples were collected from 2007-2010 and analyzed using a modified multi-residue QuEChERS method. Thiamethoxam was quantified in 3 (0.96%) of 313 samples at concentrations ranging 1.5-4.1 ng c.e./g (LOD = 1 ng c.e./g; LOQ unknown). Clothianidin was not detected (LOD = 2.0 ng c.e./g; LOQ unknown).

Pettis et al. (2013) collected pollen samples of almond (California), apple (Pennsylvania), blueberry (Maine), cranberry (New Jersey), cucumber (New Jersey), pumpkin (Pennsylvania) and watermelon (Delaware) pollen in pollen traps from returning honey bee foragers. Hives were placed in three fields surrounding each crop and separated from each other by at least 3.2 km. Samples followed the LC/MS-MS and GC/MS methods for pollen analysis. For hives placed in blueberry, cranberry, cucumber, watermelon and pumpkin fields, foraging bees collected relatively little pollen from the crop they were co-located with (<1.2%) while the majority of collected pollen in hives in apple (74%) and almond (99%) fields did come from the field crop. Clothianidin and thiamethoxam were not detected in any sample. Imidacloprid was only detected in apple pollen samples, but not from any of the other crops with mean concentrations of 2.8 ng ai/g and a maximum sample concentration of 36.5 ng ai/g.

USDA APHIS has been collecting pollen samples (stored pollen in brood comb) data since 2011 as part of the National Honey Bee Survey Pesticide Report[2]. Out of 1078 collected samples sampled between 2011 and 2017, Clothianidin was detected in 1.2% of samples with mean concentrations of 28.8 ng c.e./g and a maximum concentration of 62.8 ng c.e./g. Thiamethoxam was also detected in 1.2% of samples with mean concentrations of 13.2 ng a.i./g and maximum concentrations of 39.6 ng a.i./g.

Finally, in Lu *et al*. (2015), monthly pollen and honey samples were collected between April and August 2013 from 62 hives across the state of Massachusetts. Clothianidin and thiamethoxam were detected above the LOQ (0.1 ng/g) in 27 (12%) and 7 (3%), respectively, of the pollen samples. The concentrations were reported to range from <LOQ to 8.09 ng/g for clothianidin and from <LOQ to 2.5 ng/g for thiamethoxam. In honey, clothianidin was not detected above the LOQ and thiamethoxam was above the LOQ in 2 (4%) of 53 samples; concentrations ranged from <LOQ – 0.5 ng/g.

Available survey data suggest that although thiamethoxam and clothianidin are widely used and have been detected in targeted crop monitoring studies, their frequency and magnitude of detections in non-target monitoring studies of honey bee colony matrices are relatively low.

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Appendix 4: Summary of available bee toxicity data for clothianidin

Tier I

Adult Acute Contact Toxicity

***Apis* – Registrant-Submitted and Open Literature Studies**

There are two studies to characterize the acute contact toxicity of clothianidin technical grade active ingredient (TGAI, purities range from 96 - 99±2%) to adult honeybees. These studies were conducted in accordance with one or more recognized protocols for testing the acute contact toxicity to honey bees. The observation period (*i.e.,* study duration) was 48 hours and the resultant LD50 values ranged from 0.0275 – 0.0439 µg c.e./bee. Clinical signs of toxicity, including varying levels of paralysis or loss of coordination, were observed in one (MRID 45422426) of the studies. **Table 1** summarizes the available registrant-submitted and open literature acute contact toxicity studies to adult honey bees. It is noted here that the most sensitive quantitative adult acute contact toxicity endpoint for clothianidin is 0.0275 µg c.e./bee (MRID 49950102).

There were four studies evaluated from the open literature that investigated the acute contact toxicity to honey bee adults (**Table 1**). These studies generally used novel methods that did not follow any of the protocols available for the acute contact toxicity testing to honey bees (with the exception of Thompson *et al.*, 2014a). The observation period (*i.e.,* study duration) ranged from 24 – 72 hours. The acute contact LD50 values ranged from 0.0218 – 0.256 µg c.e./bee. As noted previously, these studies were classified as qualitative primarily due to their absence of raw data with which to statistically verify the results. In contrast to the suite of registrant-studies, clinical signs of toxicity were generally not reported in the open literature studies.

Table 1. Summary of adult acute contact toxicity studies to *Apis* bees evaluated from the registrant-submitted and open literature

Test Substance (% a.i)	Study Duration	Endpoint (95% CI) (expressed in terms of µg c.e./bee)	Comments	Classification (Reference, MRID)
TGAI (99±2)	48-hr	**LD50: 0.0275 (0.0227 – 0.0340)**	Clinical (behavioral) signs of toxicity were noted to be absent in treated bees. Older bees (22-32 days) were used than recommended by study guidelines.	Acceptable (49950102)
TGAI (96%)	48-hr	LD50: 0.0439 (0.0296 –0.0652)	Clinical signs of toxicity included paralysis and loss of coordination, and were observed in all treatment groups ≥ 0.0019 µg a.i./bee.	Acceptable (45422426)
TGAI (>99)	24-hr	LD50: 0.0218 (0.0102 – 0.0465)		Qualitative (Iwasa 2004, 47523404)
TGAI (99.9)	48-hr	LD50: 0.0350 (0.0155 – 0.0607)		Qualitative (Thompson 2014a, 49750606)
TGAI 95	24-hr	LD50: 0.256 (0.128 – 0.384)	Only carrier control used (no negative control).	Qualitative (Bailey 2005, (47800528)

			Study authors reported the LC50 as 0.0002% solution. The LD50 assumes an average bee weight of 0.128 g.	
TEP 50%	24-hr	LC50: 4,490 µg/kg	-Study authors reported endpoints in ppm only as the absorbed amount of clothianidin could not be determined. Further, it was unclear whether the reported endpoints were in terms of a.i. or formulation -Tests at the different treatment levels were not concurrently tested -Study tested indirect contact (*i.e.* test compound was on leaf material in cages with the bees) -Unclear if reported endpoints are based on the formulation or corrected for %a.i.	Qualitative (Laurino 2011, 48498301)
	48-hr	LC50: 2,970 µg/kg		
	72-hr	LC50: 2,670 µg/kg		

[1] Standard Error
NA: not available; TGAI: technical grade active ingredient; TEP: typical end use product
Bolded value represents value used for risk estimation

There were additional studies evaluated from the open literature that assessed the effects of acute contact exposure to adult honey bees that cursory review determined to be unsuitable for discussion in this assessment due to various uncertainties and limitations.

Non-*Apis* – Registrant-Submitted and Open Literature Studies

There is one registrant-submitted study (MRId 49570701) available to characterize the acute contact toxicity of clothianidin to adult bumble bees (*B. terrestris terrestris*) using TGAI clothianidin (**Table 2**). This study had a 48-hr LD50 of 0.1483 µg c.e./bee, which is close to an order of magnitude less sensitive than the 48-hr acute adult contact LD_{50} of 0.0275 µg c.e./bee for honey bees.

There was 1 study evaluated from the open literature that characterize the acute contact toxicity to non-*Apis* bees including bumble bees (B. *impatiens*), mason bees (*O. lignaria*), and alfalfa leaf cutting bees (*M. rotundata*). This study did not estimate endpoints in terms of dose (*i.e.*, µg a.i/bee) and did not provide sufficient information for estimating dose per bee (**Table 2**).

Table 2. Summary of registrant submitted and open literature adult acute contact toxicity studies for non-Apis bees.

Test Species	Test Substance (% a.i)	Study Duration	Endpoint (95% CI) (expressed in terms of µg c.e./bee unless otherwise noted)	Comments	Classification (Reference, MRID)
Bombus terrestris terrestris	TGAI (99.2)	48-hr	LD_{50}: 0.1483 (0.117-0.200)	none	Supplemental (49570701)
		96-hr	LD50: 0.1451 (0.114-0.196)		
Bumble bee (*Bombus impatiens*- (females only)			LD50: 3.9 µg/kg test solution (No CIs)	- The test groups were presented in terms of percent active ingredient in solution as opposed to actual	Qualitative

Alfalfa leaf cutting bee (Megachile rotundata)	Tech - TGAI (>95)	48-hr	LD50: 0.8 µg/kg test solution (No CIs)	treatment concentrations. These concentrations were converted to µg/kg by assuming the density of the test solution was 1 g/mL	(Scott-Dupree 2009, 48191904)
Blue orchard bee (*Osmia* lignaria)			LD50: 1.0 µg/kg solution (No CIs)		

Summary of Adult Acute Contact Exposure Route to *Apis* and non-*Apis* Bees

In total, there are six studies (from both registrant-submitted and open literature sources) available that tested the acute contact toxicity of clothianidin to adult honey bees. Overall, in these studies, clothianidin's acute contact toxicity to bees was observed to range approximately over an order of magnitude, between 0.0218 to 0.256 µg c.e./bee. However, of these studies, only three (MRIDs 45422426, 49750606, 49950102), tested using TGAI clothianidin, had similar (48-hr) durations and provided sufficient information to allow for dose-based comparisons, making these studies the most comparable. These had very similar endpoints, between 0.0275 and 0.0439 µg c.e./bee.

There was no clear trend in toxicity, based on the available studies, relative to study duration (*e.g.*, 48, 72, 96 hours), however in the studies that extended observations beyond 48-hours (Laurino *et al.* 2011 and MRID 49570701), most of the observed mortalities occurred in the first 48-hours, as evidenced by negligible differences between the 48-hr and 72- or 96-hr endpoints. The only study testing the toxicity of a clothianidin TEP (Laurino *et al.*, 2011) tested the indirect toxicity of the formulation and is not directly comparable to the other acute contact endpoints where clothianidin was applied directly (topically) to honey bees.

The registrant-submitted study investigating clothianidin acute contact toxicity to bumblebees (MRID 49570701) appears to suggest that bumble bees have similar sensitivity (within 5x) to clothianidin than honey bees when adjusting the toxicity values for body weight (**Table 3**).

Table 3. Acute contact LD50 values for honey bees (Apis mellifera) and bumble bees (Bombus terrestris terrestris).

Species	Contact LD_{50} (µg c.e./bee)	Body Weight-Adjusted Contact LD_{50} (µg c.e./g-bw)
Honey bee*	0.0218-0.0439	0.17-0.34
Bumble bee**	0.1451	0.57

*Calculated using BW of 0.128 g/bee.

**Calculated using BW of 0.2556 g/bee (mean control bee weight from study report).

Adult Acute Oral Toxicity

***Apis* – Registrant-Submitted and Open Literature Studies**

There are two studies to characterize the acute oral toxicity of clothianidin to adult honey bees with TGAI (purities range from 96.0 - 99.2%) (**Table 4**). These studies were generally consistent with OECD TG 213 and LD50 values ranged from 0.00368 µg c.e./bee – 0.0157 µg c.e./bee. Clinical signs of toxicity (including varying levels of paralysis or loss of coordination) were noted in all treatments in one study (MRID 45422426), but were not observed in the other study (MRID 49950102). From the suite of registrant-submitted Tier I adult acute oral toxicity studies (for which raw data were available), the most sensitive *Apis* acute oral toxicity endpoint is 0.00368 µg c.e./bee (MRID 45422426).

Discussed below in **Table 4** are also those studies from the open literature that investigated the toxic effects of clothianidin to honey bees following oral exposure. All of these studies evaluated a single oral exposure to 5 or more concentrations followed by a 24-72 hour observations period that is generally consistent with OECD TG 213 with the exception of the study reported by Bailey et al., 2005 (MRID 47800528), which exposed bees to corn pollen following seed treatment and did not measure residues in the corn pollen. As a result, this study did not estimate an endpoint (i.e., an LD50 and does not appear in **Table 4** summarizing the adult acute oral exposure studies from the open literature. In Laurino et al., (2013; MRID 49719620), multiple trials with different honey bee strains were conducted with typical end-use product (TEP), yielding several estimates of acute oral toxicity within the same study. Observations of mortality were recorded every 24 hours for three days, and LD50 values were calculated on each day in this study.

Table 4. Summary of adult acute oral toxicity studies for honey bees (Apis spp.) evaluated from the open literature and registrant-submitted studies

Test Substance (% a.i)	Duration	Endpoint (95% CI) (expressed in terms of µg c.e./bee)	Comments	Classification (Reference/ MRID)
TGAI (96.0)	48-hr	**LD50: 0.00368** (0.0030 – 0.0045)	--Clinical signs of toxicity including paralysis and lower coordination were observed in all treatment groups.	Acceptable (45422426)
TGAI (99.2)	48-hr	LD50: 0.0157 (0.0135—0.0181)	- No clinical signs of toxicity observed in any treatment group. -LD50 based on sucrose consumption rates (differs from reported LD50 based on nominal rates)	Acceptable (49950102)
TEP (50)	24-hr	LD50: 0.0028 (0.0017 –0.0040)	-Treatment concentrations do not appear to have been tested concurrently -Unclear from the study report whether the LD50 values were for the formulation or are corrected by the % a.i.	Qualitative (Laurino, 2010, 48498301)
	48-hr	LD50: 0.0027 (0.0017—0.0037)		
	72-hr	LD50: 0.0026 (0.0019—0.0033)		
TGAI (99.9)	48-hr	LD50: 0.0074 (0.0061—0.0090)	-Control mortality not reported. -Treatment doses not reported.	Qualitative (Thompson 2014a, 49750606)
TEP		LD50 range: 0.0011—0.0054 (confidence intervals NA)	- Study states that 42% of the data presented are from another source making this study both a primary source and review (secondary source) article (no way of discriminating the primary and secondary source data from the available information in the study - The actual number of exposed bees per treatment group is not specified.	Qualitative (Laurino 2013, 49719620)

Test Substance (% a.i)	Duration	Endpoint (95% CI) (expressed in terms of µg c.e./bee)	Comments	Classification (Reference/ MRID)
(Dantop ™ 50 WG – 50%)	24--72-hr	LD50 range: 0.0061—0.0068 (confidence intervals NA)	- There was no mention of whether a dose response was present. -Testing methodology may not have been consistent between trials, making definitive statements regarding subspecies differential toxicity uncertain.	
TGAI 99	48 hr	LD50 = 0.0269 ± 0.0049[1]	-Winter worker bees -Daily repeated dose of treated sucrose solution -LD_{50} appears to be based on daily dose (based on nominal concentration) -No negative control (solvent control only) or positive control -6 test concentrations -20 bees x 3 replicates per level -Unclear if feeding solution measurements were adjusted for potential evaporation Bees from a single colony	Qualitative Alkassab and Kirchner (2016)
	72 hr	LD50 = 0.018 ± 0.0044[1]		
	96 hr	LD50 = 0.0151 ± 0.0036[1]		
TGAI 99	24 hr	LD50 = 0.00168 (0.0012-0.00204)	-Single dose sucrose solution -Unclear level of replication -Unclear if LD_{50} is based on nominal or measured concentration -No positive control -Unclear how bees were experimentally distributed among source colonies or how many colonies were sampled. -Unclear if feeding solution measurements were adjusted for potential evaporation <u>LD_{50} determination</u> -6 test concentrations for *A. mellifera* (0.25 to 8 ng/bee nominal, factor of 2 progression). N = 210	Qualitative Sgolastra et al (2016)

[1] Standard Error
NA: not available; TGAI: technical grade active ingredient; TEP: typical end use product
Bolded value is used for risk estimation

Non-*Apis* – Registrant-submitted and Open Literature Studies

In an acute oral toxicity study (MRID 49570701), bumble bees (*B. terrestris*) were exposed for 96 hours to TGAI clothianidin. The study report indicated that food consumption rates were adjusted for evaporation loss and "unreal values" (values deemed by the study author to be errors). Clinical signs of toxicity included stumbling and knockdown behaviors at all treatment concentrations. As the study report did not make clear how the data were corrected for evaporative loss and censored by excluding

"unreal values", these data were considered of qualitative value for risk assessment purposes. The 96-hour LD50 was determined by EPA/PMRA review to be 0.00199 µg c.e./bee (95% CI: 0.0017—0.0023). The study authors reported similar 48- and 96-hr endpoints (~0.0019 µg/bee), but the review was unable to verify the 48-hr endpoint (**Table 5**).

In a study by Thompson *et al.* 2014b (MRID 49719632), clothianidin was tested on bumble bees (B. *terrestris*) in a sucrose solution for four days.Mortality was <10, <10, <10, and 100% for the control, 1.0, 10, and 100 µg c.e./L groups, respectively. Limitations in this study include the fact that mortality data were excluded if 100% mortality was reached before the end of the experimental period and raw data were not available with which to confirm any of the statistical findings. Also, the large range of concentrations spanning two orders of magnitude is indicative of a range-finding study, rather than a definitive study designed to achieve an LD_{50}. As such, the study's ability to provide quantitatively useful information on the acute toxicity of clothianidin to bumble bees is limited. Since the test duration only lasted 4 days, it is also insufficient to provide meaningful information relating to the potential chronic toxicity of clothianidin to bumble bees.

A third study, by Sgolastra *et al.* (2016) tested TGAI clothianidin on *Bombus terrestris* and *Osmia bicornis* bees (as well as *Apis melifera* as shown in **Table 4**). This study derived 24-hr endpoints for *B. terrestris* (0.00312 µg a.i./bee) and 72-hr endpoints for *O. bicornis* (0.00117 µg a.i./bee), but the level of replication was unclear and it could not be determined whether the estimated endpoints were based on nominal or measured concentrations and if feeding solution measurements were adjusted for potential evaporation.

Table 5. Summary of adult acute oral toxicity studies for non-*Apis* honey bees evaluated from the open literature and registrant-submitted studies

Test Species	Test Substance (% ai)	Exposure Period	Endpoint (95% CI) (expressed in terms of µg c.e./bee)	Comments	Classification (Reference / MRID)
B. terrestris	TGAI (99.2%)	96-hr	LD_{50} =0.00199 µg c.e./bee (0.0017—0.0023	• study report did not make clear how the data were corrected for evaporative loss and censored by excluding "unreal values"	Supplemental 49570701
B. *terrestris*	TGAI (>99%)	96-hr	<10% mortality at 1 and 10 µg/L. 100% mortality at 100 µg/L	• Mortality and some food consumption data were excluded • Age of workers bees unknown • Dose spacing too large to derive reliable LD_{50} endpoints.	Qualitative 49719632 Thompson et al (2014b)
Bombus terrestris	TGAI (99%)	24-hr	LD50 = 0.00312 (0.00232-0.00396)[2] µg ai/bee	• Unclear level of replication • Unclear if LD_{50} is based on nominal or measured concentration • No positive control • Only bees consuming 100% of the test solution (*B. terrestris* and *O. bicornis*) were included in statistical analyses • Unclear how *B. terrestris* were distributed among source colonies or how many colonies were sampled • Unclear if feeding solution measurements were adjusted for potential evaporation	Qualitative Sgolastra et al (2016)
Osmia bicornis	TGAI (99%)	72-hr	LD50 = 0.00117 (0.00093-0.00145)[2] µg ai/bee		

				• 6 test concentrations for *B. terrestris* (0.5 to 16 ng/bee nominal, factor of 2 progression). N = 212 • 5 test concentrations for *O. bicornis* (0.2 to 16 ng/bee nominal, factor of 3 progression). N = 179	

Summary of Adult Acute Oral Exposure Route to *Apis* and non-*Apis* Bees

In total, there were 8 studies (from both registrant-submitted and open literature sources) that tested the acute oral toxicity of clothianidin to adult honey bees. Similar to the acute contact data, the acute oral LD50 values span roughly one order of magnitude, ranging from 0.00168 – 0.0269 µg c.e./bee (inclusive of registrant-submitted and open literature studies testing TGAI and clothianidin formulations, observations periods of 24 – 72 hours). The more reliable and comparable data (MRIDs 45422426, 49750606, 49950102, which were all 48-hr studies that tested TGAI) had relatively similar (<5x difference) endpoints, between 0.00368 and 0.0157 µg c.e./bee. From the suite of available studies, the most sensitive quantitative *Apis* adult acute oral toxicity endpoint was a 48-hour LD50 value of 0.00368 µg c.e./bee (MRID 45422426).

In comparing sensitivities of *Apis* and non-*Apis* (bumblebee and mason bee), the acute oral LD50 values (for the acceptable honey bee studies) were adjusted based on body weight to allow comparisons with the most sensitive non-Apis data (**Table 6**). Based on this comparison, non-Apis bees appear slightly more sensitive, but relatively similar (<5x difference) as honey bees on an acute oral exposure basis. However, there is uncertainty in the consumption rates in the bumble bee and mason bee studies, resulting in less confidence in the results. As such, there is also uncertainty in the reliability of this comparison. Taken at face value and considering confidence intervals that narrowly do not overlap, the data suggest that non-*Apis* adults may be slightly more sensitive to clothianidin on an acute oral basis, however given the uncertainty of consumption rates and the Agency's established policy of using honey bee data quantitatively to assess potential risk to other pollinators, non-Apis acute oral data are not used in risk estimation, but are considered in the risk characterization section of the assessment.

Table 6. Acute oral LD50 values for honey bees (Apis mellifera) and bumble bees (Bombus sp).

Species	Oral LD50 (and 95% CIs;µg c.e./bee)	Oral LD50 (and 95% CIs; µg c.e./g-bw)*
Honey bee (48-hr)	0.00368 (0.0030 – 0.0045) – 0.0157 0.0135—0.0181	0.029 (0.023-0.035) -0.12 (0.11-0.14)
Bumble bee(96-hr)	0.00199 (0.0017—0.0023)	0.0078 (0.0066-0.0090)
Mason bee (72-hr)	0.00117 (0.00093-0.00145	0.0095 (0.0075-0.0117)

*Calculated using body weight of 0.128 g/bee for honey bees (USEPA, 2012) and 0.256 g/bee for bumble bee (mean control bee weight from study report; MRID 49570701), Mason bee weight-adjusted results reported directly from Sgolostra *et al* (2016)

Adult chronic oral toxicity

Apis Registrant-Submitted and Open Literature Studies

There are 4 studies available from combined registrant-submitted and open literature sources that examine the chronic toxicity of clothianidin through dietary exposure for adult honey bees (results combined in **Table 7**). Where available, the no observed adverse effect concentration (NOAEC) and the lowest observed adverse effect concentration (LOAEC) are provided; otherwise, a description of the report's effects is tabulated.

The lowest reported NOAEC comes from the open literature (Boily, 2013—MRID 49750601) following the feeding of adult bees with treated sucrose solution for 10 days. However, that study found no

effects on apical endpoints of survival or bee weight up to the highest concentration tested (5.85 µg c.e./L, intake of 0.00024 µg/bee/day). The most sensitive NOAEC associated with a study with a definitive LOAEC endpoint was from the registrant-submitted study, MRID 48414901, which had a NOAEC of 7.7 µg c.e./L (intake of 0.00036 µg/bee/day) based on significantly ($p<0.05$) higher mortality at the 15 µg c.e./L treatment group (intake of 0.00072 µg/bee/day) after adults were similarly fed treated sucrose solution for 10 days. When adjusted for the density of sugar solution, this is considered equivalent to a NOAEC of 9.10 µg c.e./kg diet. This study is considered suitable for quantitative risk assessment purposes. The NOAEC from this study is similar to the other reported values.

Table 7. Summary of registrant-submitted and evaluated open literature studies assessing the chronic oral toxicity of clothianidin to *Apis mellifera* adults.

Test Substance (% purity)	Exposure Period	Exposure concentrations	Reported Effects	Comments	Classification (Reference / MRID)
TGAI (99.2%)	10 days	<LOD (control), 7.7, 15, 39, and 80 µg c.e./L (mean-measured) 0 (control), 0.00036, 0.00072, 0.00174, and 0.0040 µg c.e./bee/day	**NOAEC/LOAEC (mortality-based): 0.00036/ 0.00072 µg c.e./bee/day (7.7/15 µg c.e./L, equivalent to 9.10/17.73 µg/kg)**	- Statistically significant decreases in food consumption were observed in all concentrations, but did not follow a dose-response relationship (inhibitions of 12-18% relative to controls) -No other sublethal effects evaluated -sugar solution mean density of 1.182 g/cm^3.	Acceptable (48414901)
TGAI (99.9)	10 days	0 (control), 0.00003, 0.00006, 0.00012, and 0.00024 µg c.e./bee/day (nominal)	NOAEC/LOAEC (mortality and body weight): 0.00024/>0.00024 µg c.e./bee (5.85 µg c.e./L—equivalent to 7.20 µg/kg)	- mortality and bee wt. were not significantly different between treatments and controls. -no clinical signs of toxicity observed at any dose -DMSO was used as a solvent.	Quantitative (Boily 2013, 49750601)
TGAI (99)	10 days	0 (control), 0.1, 1.0, 10.0 µg c.e./L (nominal) 0 (control), 0.0000018,	NOAEC/LOAEC: 0.00019/>0.00019 µg c.e./bee/day (10.0 µg c.e./L—	-Solvent control mortality was 15.4%, which is slightly higher than acceptable for the OECD guideline. -DMSO was used as a solvent and no negative control was used -fed treated diet for 10h followed by untreated for 14h.	Qualitative (49950110)

Test Substance (% purity)	Exposure Period	Exposure concentrations	Reported Effects	Comments	Classification (Reference / MRID)
		0.000023, 0.000189 µg c.e./bee/day	equivalent to 11.9 µg/kg)	-Maximum mortality in treatment groups was 5% higher than in controls. (p>0.05) and showed no dose- response. - No effects observed for food consumption -Sugar solution density of 1.19 g/cm^{3}.	
TGAI (99)	10 days	Solvent control, 1, 10, 20, 50, 100, 200 µg ai/kg diet (nominal)	LD50 = 0.0095 ± 0.0029[1] µg ai/bee	-Winter worker bees -Daily repeated dose (sucrose solution) -LD_{50} appears to be based on daily dose (based on nominal concentration) -No negative control (solvent control only) or positive control -20 bees x 3 replicates per level -Unclear if feeding solution measurements were adjusted for potential evaporation -Bees from a single colony -10-d NOAEC and LOAEC endpoints not reported	Qualitative Alkassab and Kirchner (2016)
TGAI (99)	12 days	Solvent control, 1, 5, 10, 15 µg ai/kg diet (nominal)	NOAEC = 10 µg/kg LOAEC = 15 µg/kg based on memory (reduced specificity of early long-term memory tests (e-LTM)). No statistically significant effects on sucrose responsiveness, mid-term memory test, or habituation of the proboscis extension	-Winter worker bees -Daily repeated dose -No negative control (solvent control only) -Sucrose responsiveness test was conducted over three days rather than examining individuals concurrently -Although there was replication during the exposure period (20 bees x 5 replicates per level) it is unclear if the bees used for the subsequent experiments were equally distributed among the replicates from the exposure phase. -It is unclear if any or all	

Test Substance (% purity)	Exposure Period	Exposure concentrations	Reported Effects	Comments	Classification (Reference / MRID)
				the same individual bees were used in more than one of the experiments conducted after the exposure phase. -It is unclear how the subset of bees was selected for the experimental phase and if the selection was random -Unclear if feeding solution measurements were adjusted for potential evaporation -Bees from a single colony -Effects on clear apical endpoints not reported	

Bolded value used for risk estimation
[1] ± standard error
NA: not available; TGAI: technical grade active ingredient; TEP: typical end use product

Non-Apis Registrant-Submitted and Open Literature

No registrant-submitted studies are available looking at the chronic oral toxicity of clothianidin to adult non-*Apis* bees. Two studies looking at the chronic toxicity of clothianidin to adult non-*Apis* bees are available (**Table 8**). Sandrock (2014a) fed adult red mason bees (*O. bicornis*) treated nectar containing 2.87 µg/g- diet thiamethoxam (2.457 µg c.e./g-diet) and 0.450 µg/g-diet clothianidin for 40 days (for a total combined clothianidin equivalent concentration of 2.91 µg c.e./g-diet), observed daily mortality and nest production and after overwintering observed subsequent larval development, weight and sex ratios of the following generation. No significant effects were observed on adult body weight or mortality, but the treatment group had significantly fewer total brood cells in completed nests (44% decrease; $p<0.001$), higher larval mortality/lack of development (50% increase; $p<0.001$) and a male-biased sex ratio (19% increase; $p<0.003$) of emerged bees relative to controls. The study lacked any true replication and is considered qualitative. In the second study, Piiroinen *et al.* (2016) exposed *B. terrestris* to sugar solution containing 0.001 µg/g-diet clothianidin for 34 days. No statistically significant effects were observed on survival, fecundity, sugar water collection or learning, however bees that were exposed and then subsequently subjected to additional stress through other tests (Proboscis Extension Reflex) were reported to have increased mortality. Both of these studies are considered qualitative.

Table 8. Summary of evaluated open literature studies assessing the chronic oral toxicity of clothianidin to non-Apis adults.

Test Species	Test Substance (% ai)	Exposure Period	Exposure concentrations	Reported Effects	Comments	Classification (Reference / MRID)
Osmia bicornis	Analytical standard (exact purity not provided)	40 days	450 ng c.e./g-diet clothianidin + 2,870 ng/g-diet thiamethoxam (2457 ng c.e./g-diet) in nectar (total combined clothianidin equivalent concentration of 2907 ng c.e./g- diet)	NOAEC < 2907 ng c.e./g-diet (total offspring mortality and male-biased offspring sex ratios)	-Adults fed treated nectar for entire life span of adults (~40d) -Study lacked true replication -parameters measured were bee mortality, weight, offspring production and sex ratio, larval mortality, nest number and brood cell numbers - no effects on any parameter except for ~44% reduction in brood cells, 50% reduction in total offspring mortality, male-biased offspring production {56%}	Qualitative Sandrock, 2014a (49579003)
Bobus terrestris audax	Unspecified clothianidin (% ai not reported)	34 days	1 ng/g-diet (nominal)	NOAEC ≥ 1 ng/g No statistically significant effects on survival, fecundity, sugar water collection, or learning; however, pesticide exposure did reduce survival of bees that were harnessed in the learning experiments	-Treatment (sugar water solution) renewed every 3 days + *ad-lib* supply of untreated pollen -Only a single exposure concentration -10 bees x 4 replicates per level (only 8 bees per replicate were treated). Each replicate is a microcolony -Food consumption was not reported -Treatments were clothianidin, clothianidin + parasite inoculum, and parasite inoculum -Only a single control (unclear if acetone or	Qualitative Piiroinen et al (2016)

Test Species	Test Substance (% ai)	Exposure Period	Exposure concentrations	Reported Effects	Comments	Classification (Reference / MRID)
					negative) and no positive control -Bees that were subject to the learning experiments were returned to the colony for the duration of the experiment -Conducted in lab	

Summary of Adult Chronic Oral Exposure Route to *Apis* and non-*Apis* Bees

In total there were four studies evaluating the chronic toxicity of clothianidin to adult honey bees and two studies evaluating its chronic toxicity to non-*Apis* bees. The studies on *Apis* all showed a lack of effect around similar concentrations (5-10 µg/kg), with two of the studies showing definitive effects on mortality and learning ability at 15-18 µg/kg. In contrast the two non-*Apis* studies had very different results, with one (Piiroinen *et al.,* 2016) showing no effects at 0.001 µg/kg while the other (Sandrock *et al*. 2014a) reported significant effects at 0.0029 µg/kg on fecundity and subsequent larval (F1 generation) mortality. Given that non-*Apis* studies tested over a significantly longer exposure duration (>1 month) and each only tested a single concentration, so that no dose-response effects could be observed, no reasonable comparisons can be made between potential differences in sensitivity of *Apis* and non-*Apis* bees on a chronic oral toxicity basis. The 10-d NOAEC of 0.00036 µg c.e./bee/day (MRID 48414901) will be used in the risk assessment to estimate potential chronic risks to adult bees.

Larval Acute Toxicity

***Apis* and Non-*Apis* – Registrant-Submitted and Open Literature Studies**

No open literature is available that examined acute effects to *Apis* or non-*Apis* bee larvae. Additionally, there are no registrant-submitted studies or studies that determined definitive endpoints for acute exposure (single dose) to honey bee larvae. Two submitted studies (MRIDs 48448803 and 48876801), designed to evaluate the chronic toxicity of clothianidin to honey bee larvae following repeated exposure (and discussed further in the following section on chronic larval toxicity) provide limited information to suggest that acute effects to larvae are not observed at doses up to 0.040 µg c.e./g-diet (0.00528 µg c.e./larva) and 4.40 µg c.e./g-diet (dose not determined), respectively. Taken together, these two studies suggest that the likelihood of acute effects to honey bee larvae at concentrations below 0.040 µg c.e./g-diet (0.00528 µg c.e./larva) is very low and are more likely to be observed at concentrations above 4.40 µg c.e./g-diet.

Larval Chronic Toxicity

***Apis* – Registrant-Submitted and Open Literature Studies**

No open literature is available that investigated the chronic oral toxicity of clothianidin to honey bee larvae. There are two registrant-submitted studies designed to investigate the chronic toxicity of clothianidin to larval honey bees following repeated exposure (**Table 9**) of larvae and monitoring through pupation and emergence over the course of 22 days (also discussed briefly above regarding acute exposures to larva). MRID 48448803 was generally conducted in accordance with what was at the time draft OECD guidance[3] concerning repeated dose tests with larvae, except that the treated royal jelly diet was only administered for three days rather than the draft guidance recommendation of four days, and sublethal effects were not reported. Additionally, daily mortality data and raw data by replicate were not provided, preventing statistical analysis by EPA and no analysis of the test chemical concentrations in stock solutions or in diet were conducted. The study author conducted four separate 22-day test runs (conducted under identical conditions but at different times), of which three met the guidance performance criteria of <15% larval mortality and >70% adult emergence for controls. The authors reported that no significant effects were observed in two of these test runs up to the highest nominal dose of 0.040 µg c.e./g-diet (0.00528 µg c.e./larva), although in one of these two runs, mortality in the highest treatment (45.9%) was more than double that of the control group (21.6%), though no obvious dose-response pattern could be determined. In the third valid test run, the study author reported significant effects at the highest treatment dose (0.040 µg a.i./g-diet) with 39.6% mortality, compared to 18.8% mortality in controls with treatments showing some evidence of a dose-response relationship (mortality in treatments ranged from 20.8—39.6%). The NOAEC for this test run was considered 0.020 µg a.i./g-diet (0.00264 µg c.e./larva or 0.0009 µg c.e./larva/day). Based on a meta-analysis of all three valid test runs, included as an appendix to the study report, the study author suggested that the NOAEC should be considered 0.040 µg c.e./g-diet with a LOAEC of > 0.040 µg c.e./g-diet. Several significant limitations were present in this study including: a) dosing only occurred for three days, rather than the guidance recommendation of four days; b) residues in neither the diet nor stock solution were analyzed; c) no sublethal effects were reported; d) the three valid test runs were not conducted concurrently; and, e) replicate data were not provided for each test run. Therefore, this study is considered of qualitative use only. Although the study author provided both dietary and dose-based endpoints, food consumption was not measured in the study, unconsumed food was removed daily and the reported dose assumes that larval bees consumed all the diet provided, which introduces additional uncertainty into the dose-based endpoint. This study provides some evidence that chronic effects to larvae from clothianidin exposure are not observed at doses up to 0.020 µg c.e./g-diet (0.00264 µg c.e./larva) and potentially up to 0.040 µg c.e./g-diet (0.00528 µg c.e./larva).

A second registrant-submitted study, MRID 48876801, also investigated chronic effects to larvae following repeated clothianidin dosing, but did not follow the OECD guidance for such studies. This study generally followed the methodology used in Huang (2009) which differs from the OECD guidance in that the test starts with 2nd instar larvae which are fed treated royal jelly diet for 6 days and greater volumes

[3] http://www.oecd.org/chemicalsafety/testing/Draft_GD_honeybee_larval_tox_repeated_exposure_25_February_2014.pdf (Accessed January 9, 2020)

of diet are used daily compared to the OECD method. Additionally, some aspects of laboratory rearing and bee husbandry differ between the two methods (e.g., in the Huang method, larvae are moved to clean pupal plates following defecation to reduce pupal mortality in controls). This study measured larval mortality/adult emergence and the incidence of deformed wings and tested higher concentrations than were tested in MRID 48448803: 0.33, 0.68, 1.50, 4.40 and 15.0 µg c.e./g-diet (measured in stock solutions at test initiation). Each treatment contained five replicates of 24 larvae that were fed either 100 or 200 µL diet per day (the larger amount for the last 4 days of feeding) for six days, but food consumption was not measured and unconsumed diet was removed each day. Mortality showed a dose-response relationship (**Table 9**); the study NOAEC was 0.330 µg c.e./g-diet, with significant overall mortality at Day 22 of30% at the 0.680 µg c.e./g-diet treatment group, compared to 20% in controls. Deformed wings were also observed to follow a dose-response trend, with clear increases in deformed wings at dietary concentrations above 1.50 µg/g-diet. In comparison to the previously discussed study, this study was considered supplemental for quantitative use in risk assessment, as residues were measured (in stock solution), replicate data were provided and large sample sizes were used. As with MRID 48448803, food consumption was not measured and unconsumed diet was removed daily (and in this case, the study author did not report dose-based endpoints). Overall comparisons of any potential differences in sensitivity between the Huang method and the OECD guidance method across different chemicals are not currently available. For the Tier I assessment, the dietary-based toxicity value from MRID 48876801 (NOAEC=0.330 µg c.e./g-diet) is used to calculate RQs. Due to the absence of suitable endpoints, dose-based chronic RQs for larvae are not calculated, but the concentration-based endpoint of 0.330 µg c.e./g-diet is compared to estimated EECs in the risk assessment.

Table 9. Summary of registrant-submitted and evaluated open literature studies assessing the chronic toxicity of clothianidin to Apis larvae

Test Substance (% purity)	Exposure Period	Exposure concentrations	Reported Effects	Comments	Classification (Reference / MRID)
TGAI (99.5%)	22 days	control, 5, 10, 20, and 40 ng a.i./g-diet (nominal) 0 (control), 0.00066, 0.00132, 0.00264, and 0.00528 µg a.i./larva (based on nominal concentrations)	One trial run concluded that the NOAEC was 20 ng/g-diet (0.00264 µg/larva) with a LOAEC of 0.040 µg/g-diet (0.00528 µg/larva), while two trials concluded LOAECs of > 0.040 µg/g-diet.	-Treated diet were only fed to larvae for 3 days, not the draft guidance recommended 4 days. -Residues were not measured in either stock solutions or treated diet -3 separate trial runs conducted that met performance criteria -Larvae only came from 2 colonies -Raw data by replicate (colony) were not provided -No sublethal effects evaluated	Supplemental-(4844803)

				-Not reported whether any diet was unconsumed.	
TGAI (99.0)	22 days	0 (control), 330, 680, 1500, 4400 and 15000 ng a.i/g-diet (measured in stock solution at test initiation)	**NOAEC/ LOAEC (pupal & overall mortality/ emergence): 330/680ng a.i./g-diet** (larval mortality): 4,400/15,000 ng a.i./g-diet	-Test followed the Huang protocol -Food consumption was not measured and unconsumed food was removed daily, therefore dose-based endpoints are unavailable -Larvae fed up to 7 days -Test larvae were derived from only 1 colony -Underdeveloped wings occurred in 0,2,5, 14, 38 and 53% of control, 0.330, 0.68, 1.50, 4.40 and 15.0 µg a.i./g-diet.	Supplemental- (48876801)

Non-*Apis*—Registrant-Submitted and Open Literature Studies

No registrant-submitted studies were submitted investigating the chronic oral toxicity of clothianidin to non-*Apis* larvae. In a study by Abbott et al. (2008, MRID 47812301), the eggs and 1st instar larvae of alfalfa leafcutter bees (*M. rotundata*) were exposed on one of seven test initiation days to varying concentrations of TGAI (99.75% purity) in pollen provisions throughout the 12-13-day larval stage. Bees were subsequently incubated through pupation and then maintained in a refrigerator to overwinter, prior to incubation the following spring, representing a total study duration of approximately 1 year (July, 2005—June, 2006). Parameters assessed prior to overwintering included the time to complete cocoon and time to darken cocoon; following overwintering measurement endpoints included the time until emergence and emerged bee weight. Confirmation of clothianidin concentrations in the final pollen provisions yielded clothianidin levels for the low, medium, and high treatments of 0.0027, 0.035, and 0.276 µg a.i./g-diet, respectively. None of the treatments was observed to have a consistent effect on any measured parameter. However, the study authors reported a significant ($p<0.05$) effect in the time for females to complete spinning a cocoon, which was significantly shorter for control than for the high treatment bees on two of seven test initiation days, but was not significantly different on the remaining days.

In a second study by Nicholls *et al.* (2017), mason bee (*O. bicornis*) larvae were fed a single pollen provision, that took ~30 days to consume, containing between 0.00076 to 0.0086 µg/g-diet and evaluated through overwintering (>300 days post-exposure). No statistically significant effect on larval development time, overwintering survival, adult weight, or adult metabolic rate occurred at up to the highest concentration.

Table 10. Summary of registrant-submitted and evaluated open literature studies assessing the

chronic toxicity of clothianidin to Non-Apis larvae

Test Species	Test Substance (% purity)	Exposure Period	Exposure concentrations	Reported Effects	Comments	Classification (Reference / MRID)
Megachile rotundata	TGAI (99.75)	12-13 days	Pollen patties containing: 0 (control), 6, 30, 300 ng/g-diet (nominal). 0.0027, 0.035, 0.276 µg/g-diet (measured)	NOAEC/LOAEC 35/276 ng/g- diet (measured) Time to complete spinning cocoon (only on 2 of 7 initiation dates)	-Feeding began at either the egg or 1st instar stage and continued throughout larval period (12-13 days) -Study continued through cocoon stage, overwintering and until emergence (~1 year) -endpoints were time to spin cocoon, time to darken cocoon, time to emergence following overwintering, and weight at emergence.	Qualitative Abbott, 2008 (47812301)
Osmia bicornis	TGAI (un-specified %)	Larval development (egg stage) through adult emergence * Single dose of pollen provision that is reported to take ca. 30 days to consume starting with egg hatch	Pollen patties containing: Solvent control, 0.001, 0.003, and 0.010 µg/g-diet ai (nominal) 0.000757, 0.00240, 0.00886 µg/g-diet ai (mean measured)	NOAEC ≥ 10 ppb (nominal; 8.86 ppb mean measured) No statistically significant effect on larval development time, overwintering survival, adult weight, or adult metabolic rate	-Single dose of treated pollen provision (pollen + nectar) -Exposure during larval development period (21-42 days) -Exposure and observation period in lab -No negative control (solvent control only) or positive control -All pollen provision was consumed -Residues were measured 24 hr and 28 days after application (concentrations were ca. 10%-25% below nominal and stable) -31-38 eggs per level -Unclear level of replication. "Nest blocks" were used but it is not clear how many blocks were used per level or how many eggs per block	Nicholls et al (2017)

					-Source of eggs was from adults that emerged from cocoons within six nests placed in an orchard that had been "organic" for 10 years prior. The pollen used for exposure was collected by adult bees at the same field site. -The pollen was analyzed for select neonicotinoids (confirmed no residues of thiamethoxam, imidacloprid, acetamiprid, thiacloprid, or clothianidin)	

Summary of Larval Chronic Oral Exposure Route to *Apis* and non-*Apis* Bees

In total there were two studies evaluating the chronic toxicity of clothianidin to larval honey bees and two studies evaluating its chronic toxicity to non-*Apis* larval bees. The studies on *Apis* larvae combined showed a lack of effects up to at least 0.040—0.330 µg/g-diet with clear significant dose-responsive effects on mortality at 0.680 µg/g-diet and above. The studies on non-Apis larvae suggested no effects up to at least 0.0086 µg/g-diet, but inconsistent effects at 0.276 µg/g-diet. On the surface, this might suggest that the non-Apis larvae are slightly more sensitive (LOAEC of 0.276 µg/g-diet for non-Apis compared to 0.680 µg/g-diet for *Apis*). However, these LOAECs are still within 5x of each other and the endpoints are very different (inconsistent effects on the non-apical endpoint of time to spin cocoons, compared to clear effects on mortality), making comparisons between the taxa's sensitivity inappropriate, based on the available data. The 22-d NOAEC of 0.330 µg/g-diet for honey bees (MRID 48876801) is used in the risk assessment in comparison to estimated concentrations in nectar and pollen, but valid dose-based endpoints are not available.

Tier I data for clothianidin degradates

The registrant submitted additional toxicity data evaluating the toxicity of the clothianidin metabolites TMG, MNG, and TZMU (**Table 11**). These studies suggest that these metabolites are practically non-toxic to adult honey bees on an acute oral exposure basis. However, the metabolite TZNG was found to be moderately toxic to honey bees in an acute oral toxicity study (LD_{50} = 3.95 µg/bee: MRID 45422430). These studies were conducted using adult worker bees, and, therefore, there is uncertainty regarding the potential effects of clothianidin degradates on honey bee larvae. However, TZNG is less toxic than parent clothianidin on an acute oral basis to adults by two orders of magnitude. Additionally, as noted in the measured residue data discussion in the risk assessment and clothianidin residue appendix, the maximum mean residues of TZNG (and TZMU) in a sampling period were generally much lower than residues of parent clothianidin (*e.g.* 10-15% formation of parent in MRIDs 49910601, 49317901, 49705901; ~45% formations in MRID 49602802); given the percent formation in these studies and the relative toxicity to parent clothianidin, this metabolite is not included as a residue of concern for honey bees. However, for those residue studies where TZNG formation was greater than parent (*e.g.*

MRID 49904901, with maximum mean TZNG concentrations up to 8 times parent clothianidin concentrations in floral nectar of cotton plants), the differential toxicity and exposure profiles of the degradate may be considered in risk characterization to evaluate whether their inclusion impacts the risk picture. Toxicity data for clothianidin degradates on other species of bees were not available.

Table 11. Summary of the Acute Toxicity Endpoints from Submitted Adult Acute Oral Honey Bee Toxicity Studies for Clothianidin Degradates: TZNG, MNG, TMG, and TZMU

Study Type	Species	Toxicity Value	Acute Toxicity Classification	MRID & Status
Acute Oral Toxicity Non-GLN	Honey bee *Apis mellifera*	TMG (96.0% a.i.) LD50 > 152 µg/bee	Practically non-toxic	45422427 Supplemental
Acute Oral Toxicity Non-GLN	Honey bee *Apis mellifera*	MNG (99.2% a.i.) LD50 > 153 µg/bee	Practically non-toxic	45422428 Supplemental
Acute Oral Toxicity Non-GLN	Honey bee *Apis mellifera*	TZMU (98.8% a.i.) LD50 > 113 µg/bee	Practically non-toxic	45422429 Supplemental
Acute Oral Toxicity Non-GLN	Honey bee *Apis mellifera*	TZNG (98.6% a.i.) LD_{50} = 3.95 µg/bee	Moderately toxic	45422430 Supplemental

Tier II

This section summarizes the available Tier II (*i.e.*, tunnel and feeding study design) studies that were conducted on behalf of the registrant for clothianidin. A summary of the results and associated uncertainties is provided within the discussion of each study.

Registrant submitted

Colony Feeding Studies Using Spiked Sucrose Solution

MRID 49836101

This colony feeding study was conducted with honey bees to assess the potential for long-term effects, including possible impacts to overwintering survival, resulting from exposure to clothianidin in spiked sugar diet. The study was conducted in twelve test areas of low agricultural activity in North Carolina from June 17, 2014 (when hives were moved to the study sites) to April 27, 2015 (final colony condition assessment).

In this study, eighty-four hives were divided according to hive strength (number of brood frames). At each apiary, five test hives were continuously fed with 50% sugar solution either untreated or spiked with clothianidin at 10, 20, 40, 80 or 160 µg c.e./L for six weeks in the field, with two hives at each apiary serving as controls. Assuming the density of a 50% sugar solution is 1.2296 g/ml, the reviewer calculated that the test concentrations at 10, 20, 40, 80 or 160 µg/L are equivalent to 8.1, 16.3, 32.5, 65.1, and 130 ng c.e./g, respectively. Residue analysis of the dosing solutions provided mean-measured concentrations

of <LOD (<1 ng/g), 9.5, 19.0, 35.6, 71.8 and 140 ng c.e./g, for the control and 8.1, 16.3, 32.5, 65.1, and 130 ng c.e./g treatment groups, respectively.

Nine Colony Condition Assessments (CCAs) were conducted during the study. Multiple parameters were included in each assessment, such as hive weight, number of individuals at different life stages in the hive, hive honey and pollen stores. Three CCAs (CCA1 - 3; May 12, June 2 and 18, respectively) were conducted prior to feeding to determine hive strength and initial hive conditions. A CCA was conducted during exposure (CCA4; July 15) with another one conducted within one week after termination of the 6-wk exposure period (CCA5; August 5) which characterize hive conditions during exposure. Two more CCAs were conducted at 5 (CCA6; Sept. 8) and 10 (CCA7; Oct. 14) weeks after termination of the exposure phase of the study (or 11 for hives in the 71.8 and 140 ng c.e./g treatment groups, only) to assess effects following the exposure phase and to characterize pre-overwintering hive conditions. Two final CCAs were conducted after overwintering in mid-March 2015 (CCA8; Mar 17-19 for all treatment groups except for the 71.8 ng c.e./g treatment group whose CCA was delayed to April 2) and mid-late April (CCA9; April 22-27) to assess colony-level effects.

Statistically significant ($p<0.05$) dose-related effects were observed in the 35.6, 71.8, and 140 ng c.e./g (40, 80, and 160 µg/L) treatment groups across multiple CCAs for the majority of response variables such as decreased number of adults, brood (eggs, larvae, and pupae), total live (adults + brood), and pollen stores. For the 71.8 and 140 ng c.e./g treatment groups, significant ($p<0.05$) effects (decreases) were determined for every response variable, except for honey and total food stores, and these effects remained statistically different from controls across multiple CCAs. The 35.6 ng c.e./g treatment group also showed statistically significant effects for multiple response variables (such as decreases in the number of adults, pupae, total live (adults + brood), total brood and pollen storage) across multiple CCAs. However, in the 9.5 and 19 ng c.e./g treatment groups there was a general lack of statistically significant effects ($p>0.1$) and in cases where significant effects were detected, they either did not show strong dose-responsiveness and/or did not persist across multiple CCAs. This was the case for the statistically significant effects noted in pollen storage at CCA5 at 9.5 and 19 ng c.e./g (where effects did not persist at subsequent CCAs), in the number of eggs at CCA5 at 19 ng c.e./g (but no statistically significant effects at 35.6 ng c.e./g and the effect did not persist at subsequent CCAs), and in the number of adults at CCA6 in the 9.5 ng c.e./g treatment group (but no statistically significant effect at 19 ng c.e./g and the effect did not persist to CCA7). Therefore, in the colony feeding study, the overall NOAEC was determined to be 19 ng c.e./g and the LOAEC was 35.6 ng c.e./g. However, it is noted that this NOAEC does not take into account the overwintering period due to poor control overwintering survival. As such, this assessment focused on the CCA leading up to overwintering and did not include data subsequent to overwintering since poor control colony overwintering survival precluded further statistical analysis.

MRID 50312501

This sucrose-based colony feeding study was conducted to address the uncertainties associated with the lack of overwintering success in the previous feeding study (MRID 49836101). The same study design (*e.g.* dosing, similar location) was utilized as the original MRID and the details of study design are not discussed here. No elements of the study design were expected to be significantly different[4] than the

[4] Comments were made by EFED via protocol review for minor differences including dosing regimen, increased frequency of hive matrix sampling, marking queens, increased supplemental feeding, and an earlier exposure period.

first study. This study was conducted April of 2016-April 2017 with a 6-week exposure period initiated on July 13, 2016. The nominal doses of clothianidin were 10, 20, 30, 40, and 80 µg a.i/L (ppb) corresponding to measured concentrations of <0.113 (control), 9.5, 19, 28, 37, and 75 ng c.e./g.

Decreases in multiple endpoints (significant reduction [$p<0.05$] relative to controls) and declining trends were observed over multiple CCAs in colonies exposed to 75 ng c.e./g including significant reduction in pollen stores and numbers of adults, uncapped brood (larvae) and capped brood (pupae) with effects observed consistently and across multiple time points. In the 37 and 28 ng c.e. /g treatment groups there were significant reduction in pollen stores at CCA 3 and capped brood at CCA4 (after exposure ended). There were also marginally significant ($0.05<p<0.1$) reductions in numbers of pollen cells in CCAs 4 and 5 at the 37 ng c.e./g treatment level and in pollen cells in CCA4 at the 28 ng c.e./g treatment level. There were other marginally significant reductions ($0.05<p<0.1$) in the number of eggs at CCA5 and honey stores in CCA 1 for the 75 ng c.e./g treatment group with an additional reduction at CCA7 for honey stores in the 9.5 ng c.e./g treatment group.

Overwintering survival in the control colonies averaged (83% 4/24 dead) with 75%, 67%, 92%, 75%, and 25% percent surviving colonies in the 9.5, 19, 28, 37, and 75 ng c.e./g treatments, respectively. Most colony losses occurred between CCA6 in October 2016 and CCA7 in March 2017, *i.e.*, during overwintering. At the 75 ng c.e./g treatment level colonies declined noticeably during the exposure period. Colony strength continued to decline after the exposure period into the fall, at which time it was concluded that most of these colonies were unlikely to successfully overwinter and may become targets for robbing by bees from other colonies (treatment groups) in the apiary. All hives at the 75 ng c.e./g treatment level were therefore moved to a high-rate holding apiary in late fall to prevent any robbing of in-hive food stores in the event of colony winter mortality. The effects in the spring were somewhat confounded by swarming across all treatment rates.

For colony apical endpoints, the NOAEC and LOAEC are 37 and 75 ng c.e./g, respectively, based on significant and consistent impacts on the number of adults, larvae, and pupae as well as overwintering success (hive mortality), in each colony. This endpoint can be used quantitatively for risk assessment, but is less sensitive than that determined in the previous CFS study (MRID 49836101). The analysis also determined a food storage NOAEC and LOAEC of 19 ng c.e./g, based on consistent impacts on pollen storage in the 28 ng c.e./g treatment group. Effects on pollen storage are not considered apical endpoints and this endpoint should only be used qualitatively in the risk assessment.

Colony Feeding Studies Using Spiked Pollen

MRID 50478501

Clothianidin was provided in fortified pollen patties (spiked with treated sucrose) *ad libitum* at nominal rates of <1.0 ppb (negative control), 100 , 400 , and 1600 ppb (mean concentrations 86 g, 372 g and 1460 ng c.e./g) in a field setting to free-foraging honey bees (*Apis mellifera*) in a colony feeding study in rural North Carolina, USA.

The study consisted of four treatment groups: 1 untreated control group (UTC) and 3 clothianidin treatment groups with 8 replicates in the untreated control group and 8 replicates of each clothianidin treatment group for a total of 32 colonies. Colonies were divided into 4 groups and placed at 4 different

locations (apiaries). Pollen patty amounts of 200 g for each feeding; patties were placed inside hives in the middle of the brood nest between two combs containing brood and renewed three times a week over a six-week exposure period (total amount 3600 g per hive). Actual consumption of pollen patties in the control hives averaged 1500 g over the entire six-week exposure period. Colony development and strength (presence of various life stages and frame area covered with adult bees, brood, food stores) were assessed (referred to as colony condition assessments or CCAs) at six-time points during the study: before exposure (2), during exposure (1), and after exposure (3). Samples of uncapped nectar and bee bread were collected from the treatment and control hives to evaluate potential movement of the test material into hive food stores during the study.

Analyses of the CCA data indicate apparent effects on apical colony endpoints at the 1460 ng c.e./g treatment level. The largest treatment-related effects on apical endpoints observed were a significant ($p<0.05$) decrease in the mean number of adults starting at CCA3 (during exposure), but also included decreases in pupae (beginning at CCA4 (shortly after exposure had concluded). These effects were observed consistently at multiple time points during and after the exposure period, and exhibited dose-response relationships. Additional effects were also observed on food matrices including decreased pollen storage and uncapped nectar (both beginning at CCA4). Decreases in uncapped nectar storage were also observed on the 372 ng c.e./gtreatment groups. However, effects on honey-alone storage and combined nectar + honey production as well as pollen storage were only observed in the 1460 ng c.e./gtreatment group.

Overall, statistically significant ($p<0.05$) and consistent effects were observed in the 1460 ng c.e./g treatment group with effects observed in all assessment endpoints and overall colony survival. Although treatment-related effects on uncapped nectar stores and pollen patty consumption were observed at the 372 ppb and 1460 ppb treatment groups; these were not considered apical endpoints. With respect to the concurrently conducted sucrose colony feeding study (MRID 50312501), effects observed on food storage (pollen storage at the biological NOAEC of 19 ppb) did not impact overall colony survival, even following overwintering (see DER for MRID 50312501 for more details). Therefore the biological NOAEC and LOAEC for the spiked pollen study, based on consistent effects to apical endpoints was determined to be 372 and 1460 ng c.e./g, respectively.

Other Registrant-submitted Studies

In addition to the three registrant-submitted colony feeding studies, there are several registrant-submitted Tier II studies that employed a tunnel design. These studies were previously mentioned in the risk assessment and/or clothianidin residue appendix regarding their residue information. These studies generally involved exposure to smaller (nucleus) honey bee colonies foraging on seed- treated canola, maize or sunflower within a netted enclosure (*i.e.* tunnel) over different study durations (2-52 days). These studies generally monitored mortality and foraging activity. However, most of these studies, while serving as a line of evidence in terms of the residue information provided, have deficiencies (such as extended confinement durations, adverse weather which likely reduced foraging activity, and/or only examining a single colony) that limit their utility for evaluating potential effects. The effects parts of these studies are presented in **Appendix 4** (effects data classified as invalid and not used in the risk assessment).

Tier II Open Literature Studies

This section summarizes the available Tier II (*i.e.*, tunnel and feeding study design) studies that were evaluated from the open literature. Many of these were evaluated as part of a joint review between EPA, PMRA, and CDPR prior to the preliminary neonicotinoid bee risk assessments. Where sufficient information is available, their summarized results are presented in **Table 12**. Information from these studies considered here is focused on effects to apical endpoints (effects on growth, reproduction and survival of the colony). Additionally, limitations of each study are provided within each summary.

Tier II Apis

The evaluated Tier II studies from the open literature examined several of the same endpoints (*e.g.*, effects on brood development and number of adults) as were captured in the Tier II registrant-submitted colony feeding study. Additionally, some of these studies also evaluated endpoints not captured in the registrant-submitted feeding studies such as foraging activity. As noted above, these higher-tiered studies were considered qualitative for use in the risk assessment as there were limitations associated with each study (*e.g.*, only one concentration tested, absence of raw data).

Synthesis of Available Tier II Apis Studies

While the studies were varied in their exposure duration, concentrations tested, and endpoints assessed, the following is a discussion of endpoints that aims to put into context the results of these studies with those of the registrant-submitted Tier II colony feeding study described above.

Effects on presence of various life stages:

In the first registrant-submitted colony feeding study (MRID 49836101; sucrose feeding design, 6 wks. exposure, 9.5-140 ng c.e./g) the numbers of adults (↓24-30%), pupae (↓16-47%) and total brood (↓23-38%) were significantly ($p<0.05$) reduced at 35.6 ng c.e./g (NOAEC= 19 ng c.e./g). In this study, overwintering survival was low in all treatments including the control (only 35%), so inferences about overwintering success were not possible. However, based on the repeat registrant-submitted sucrose based study (MRID 50312501), which had successful overwintering (75%) in the control group, no endpoints following overwintering were more sensitive than the effects endpoints (LOAEC of 75 ng c.e./g) noted prior to overwintering. However, in one of the open literature studies that tested both clothianidin and thiamethoxam (Sandrock *et al.* 2014b; pollen feeding design, 46 days exposure), the numbers of adults and brood were reduced at the single concentration tested (6.6 ng a.i./g (clothianidin equivalents) after the exposure phase of the study and after overwintering, but not prior to overwintering. Additionally, at this concentration, 60% of the queens were superseded (replaced by workers) within a year and only 20% of the hives swarmed after overwintering compared to 90% in the control. In another study using spiked pollen (36-day exposure), Williams *et al.* 2015, the number of queens surviving after 4 weeks was not significantly different ($p>0.05$) at 4.5 ng a.i./g (clothianidin equivalents). However, in this study, the number of eggs laid and production of worker offspring was significantly reduced ($p<0.05$).

In the registrant-submitted Tier II tunnel study using spiked pollen, no effects were reported on mortality or colony development over different doses (5.4-19.7 ng a.i./g). However, the confidence in the results of this study is reduced given the limitations in the study design (*i.e.* limited replication within treatment groups).

Summary

Based on the colony feeding studies (and other available studies,) exposure to clothianidin affected adult and brood development. These effects were observed consistently at multiple time points at concentrations of 35.6 and 75 ng c.e./g in the registrant colony feeding studies (MRIDs 4983610 and 50312501 respectively) using sucrose and at concentrations of 1460 ng c.e./g in the spiked pollen CFS. These three registrant submitted studies (2 – sucrose CFS, and 1 pollen CFS), share similarities in the reported effects in that the number of adults and brood were reduced. Particularly effects to adults are seen early in exposure periods (during and immediately following exposure), which is not surprising given the toxicity to individual adults seen in laboratory studies. Decreased brood effects are also seen in these studies; however, these effects were typically delayed for one CCA after the adult effects were observed.

Table 12. Tier II Registrant-Submitted and Open Literature Studies for Apis

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[1] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Clothianidin Tech – (*Apis mellifera*)	Sucrose solution (10-160 µg c.e./L) [mean measured 9.5-140 ng c.e./g]	6 weeks (10.5 months)	84 hives divided according to strength into 6 treatment groups (12 apiaries) and fed treated sucrose soln. for 6 wks. CCA conducted prior to exposure, after exposure and after overwintering. Colony activity and health was monitored.	Multiple parameters, such as hive weight, number of individuals at different life stages in the hive, hive honey and pollen stores, and hive overwintering survival -- (Yes)	-At 71.8 and 140 ng c.e./g significant effects were determined for every response variable, except for honey and total food stores. At 35.6 ng c.e./g significant effects for multiple response variables (adults, pupae, total live, total brood and pollen storage	-high control mortality during overwintering	Supplemental (49836101)
Clothianidin Tech- (*Apis mellifera*)	Sucrose solution (10-80 µg c.e./L (mean measured of 9.5-75 (ng c.e/g)	6 weeks (10.5 months	84 hives divided according to strength into 6 treatment groups (12 apiaries) and fed treated sucrose soln. for 6 wks. CCA conducted prior to exposure, after exposure and after overwintering. Colony activity and health was monitored.	Multiple parameters, such as hive weight, number of individuals at different life stages in the hive, hive honey and pollen stores, and hive overwintering survival -- (Yes)	At 75 ng c.e./g significant effects were determined for every response variable, except for number of eggs and honey/nectar stores. At lower doses (28-37.5 ng c.e./g), consistent significant effects were only observed on pollen storage - (Yes)		Acceptable (50312501)
Clothianidin Tech- (*Apis mellifera*)	Pollen Patties (100, 400 and 1600 ng c.e./g	6 weeks (14 weeks)	32 hives divided according to strength into 4 treatment groups (placed into 4 apiaries) and fed	Multiple parameters, such as hive weight, number of	-at 1460 ng c.e./g consistent significant effects were determined for every response	-Pilot study with relatively lower replication than the sucrose	Supplemental (50478501)

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[1] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
	(mean measured of 86, 372 and 1460 ng c.e./g		treated pollen patties for 6 wks. CCA conducted prior to exposure and after exposure. Colony activity and health monitored	individuals at different life stages in the hive, hive honey and pollen stores, and hive overwintering survival -- (Yes)	variable except for honey storage. -At 372 ng c.e./g, consistent significant effects were only observed for uncapped nectar storage. When nectar storage was combined with honey storage, significant effects were not observed	based CFS studies - study not conducted through overwintering	
Clothi + Thia Analytical grade- 99.9% (*Apis mellifera* carnica and A. *mellifera mellifera*)	Pollen paddies [pollen+yeast+ sucrose] (mean measured: 2.05 µg clothianidin /kg +5.31 µg thiamethoxam/kg; total of 8kg pollen/colony) [6.6 (±1.8 S.D.) ng a.i./g expressed as	46 days (~1 year)	Bee colonies (n=12/group) were fed pollen paddies 3X/week during May/June and maintained in the field until next June. CCAs conducted before exposure (in May), 2 days and 3.5 days after exposure (July, Oct.) and then weekly until following April. Last CCA done in June.	# of brood, adults, presence of queen, storage of pollen and honey, swarming -- (Yes)	-End of exposure period: ↓# of adult bees (28%) & ↓# of brood (eggs+larvae) (13%) -Prior to overwintering: no effects -After overwintering: ↓# of adults and brood (eggs+larvae+pupae) -Pollen storage: ↓50% -Honey storage: ↓29% -Queens: 60% queens superseded within a year -Swarming: after overwintering 90% control group swarmed, only 20% in treatment group -Significant differences observed in responses between two	-Only one concentration was tested, therefore, NOAEC and LOAEC values could not be determined. -The bees from the A.m. carnica were from an area characterized by intense agriculture yet there was no screen of potential pesticide	Qualitative Sandrock, *et al*. 2014 (49719628 50153901)

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[1] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
	clothianidin equivalents				strains (effects to A. m. *mellifera* more pronounced)	exposure before feeding began	
Clothi + thia- Purity not reported (*Apis mellifera* carnaca)	Pollen + honey (3:1) (4.16 µg a.i./kg thiamethoxam and 0.96 µg a.i./kg clothianidin) [combined clothianidin + thiamethoxam = 4.5 ng a.i./g expressed as clothianidin]	36 days (~64 days)	Nucleus colonies (n=3) that contained sister queens were exposed to spiked pollen (pollen traps used) after which queens were produced from each colony and monitored for 4 weeks.	Survival, production of worker offspring, flight behavior, reproductive anatomy (ovaries) and reproductive physiology (stored sperm) -- (Yes)	- eggs laid: ↓38% -worker offspring: ↓34% -ovary size: ↑6.8% -stored sperm ↓20% and viable sperm ↓9%	-Only one concentration tested -Sample size was low (n=3)	Qualitative Williams *et al.*, 2015 (50153902)

[1]Most studies not associated with NOAEC/LOAEC values. Reported are effects statistically derived or otherwise observed difference relative to the control.
[2]Generally, only subset of limitations are listed here.

Tier II Non-Apis (Bombus and Osmia)

It is noted that non-*Apis* studies described below for both clothianidin are generally considered by the agency to be supplemental information for risk assessment purposes. While Tier II (and Tier III) studies are not necessarily used in a quantitative sense (*e.g.*, calculate risk quotients), these studies are used as a weight-of-evidence in characterizing potential effects to non-*Apis* bees.

While workers and the queen bee undergo overwintering in honey bee colonies, andsubsequently build up again the following spring, only the *Bombus* queen overwinters. Therefore, there was no overwintering component included in any of the open literature *Bombus* studies as distinguished from *Apis*.

Additionally, colonies of *Bombus* are much smaller than those of *Apis* and typically range from several dozens to several hundred bees at most. In contrast, *Apis* colonies consist of thousands of bees and can reach sizes up to several tens of thousands. It is therefore expected to some extent that *Apis* colonies are able to compensate for greater losses of their adult population before colony failure as compared to *Bombus*. The forage range of non-Apis bees such as Bombus is considerably smaller (e.g., square mile) whereas, the honey bee forage cam travel up to 8 miles; therefore, the foraging area is 202 square miles.

Bombus

There are several open literature studies examining the effects of clothianidin on *Bombus* species (**Table 13**). In Larson *et al.*, 2013, *B. impatiens* workers and queen were contained either on foliar-treated turf with clover (0.4 lb c.e./A) for six days or on turf that was treated after it was mowed to remove the clover flowers for two weeks. When the bees were exposed to treated turf and clover (clothianidin nectar concentrations in clover blooms was 171 ± 44 ng c.e./g), the worker mortalities significantly increased as well as significant decreases in colony weight, number of adults and honey pots. When exposed after mowing, no effects were detected; however, the residues in clover flowers that grew following mowing were not measured.

In another study with *B. impatiens* (Scholer and Krischik 2014), colonies contained in a greenhouse were fed a treated sucrose solution (7.3-62 ng c.e./g) for 11 weeks and colony development was monitored. Significant decreases were reported in the number of live and total brood (at ≥32 ng c.e./g), colony weight and worker bee activity (at 14 ng c.e./g), and food consumption, number of wax pots and syrup weight (≥7.3 ng c.e./g). Queen mortality was also significantly ($p<0.05$) increased at concentrations of 14 ng c.e./g and greater. However, there is uncertainty in the exposure concentrations due to infrequency of sampling and low residues in wax pots (food source for queen). It is noted that 11 weeks is a fairly long exposure period that may not be reflective of typical exposure durations for colonies at the higher concentrations. Colonies were not observed post-exposure.

In another study, *B. terrestris* were fed pollen and sugar water were treated with both clothianidin and thiamethoxam (4.94 ng c.e./g clothianidin equivalents) or a combination of clothianidin/thiamethoxam-spiked food and a gut parasite (*Crithidia bombi*) for nine weeks. The researchers reported significant ($p<0.05$) decreases in worker production and longevity, food collection, and colony sexual investment (reportedly calculated as the number of male offspring plus two times the number of gynes) and mother queen survival (Fauser-Misslin *et al.* 2014). However, in

this study, it appears that the results of the spiked food only and spiked food plus parasite were combined for statistical analysis which limits an understanding of the potential effects without the parasite.

A fourth open literature study (Arce *et al*., 2017) tested *Bombus terrestris audax* colonies with sugar water containing 5 ng c.e./g clothianidin for five weeks. The study evaluated a number of forage activity endpoints, but also a number of apical endpoints including number of adults, reproductive bees, larvae, and pupae. Treated colonies were reported to have fewer eggs, workers, and reproductive individuals, but had greater number of larvae and pupae. Colonies were only observed during the exposure phase without any continued monitoring to see if colony recovery occurred or if the observed effects continue post-exposure.

Osmia

Osmia bicornis males and females were placed in cages, fed artificial nectar containing both clothianidin and thiamethoxam (2.92 ng c.e./g) for approximately 40 days, and allowed to forage and reproduce freely before being monitored for five months post exposure (Sandrock *et al*. 2014b). The number of nests completed (↓22%), total brood cells (↓44%) and offspring development (↓50%) were significantly ($p<0.05$) decreased in the treated group compared to the control. However, it was reported that none of the larval provisions or bees had detectable residues of clothianidin or thiamethoxam.

Table 13. Summary of Tier II non-Apis studies

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[1] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Clothi + Thia – analytical standard (*Bombus terrestris*)	Pollen+sugar water (4 µg/kg thia+1.5 µg/kg clothi) [4.94 µg/kg (ng/g) clothianidin equivalents]	9 weeks (9 weeks)	Colonies (n=10) with queen & 10 workers were exposed under laboratory conditions to (1) spiked sugar water/pollen only (N), (2) gut parasite *Crithidia bombi* (via contaminated sugar water) only (P), or (3) combination of spiked food + parasite (NP). Control (no spiked food or parasites) was included (C).	Worker production (newly emerged workers); worker & mother queen longevity; colony sexual investment (newly emerged young queens and males); colony pollen and sugar water collection -- (Yes)	-Worker production: ↓ @ wks 4 & 7 (N+NP combined) -Worker longevity: ↓ (N+NP combined) -Sexual investment: ↓43% in males & ↓77% in queens (N+NP combined) -Mother queen survival: ↓ survival time (NP only) -Sugar water collection: ↓ @ weeks 1, 2, 4-9 (N+NP combined) -Pollen collection: ↓ @ weeks 6-9 (N+NP combined)	-Since generally no effects from parasite only exposure according to study authors, results and statistics presented as generally two groups (non neonic exposed-C+P and neonic exposed-N+NP) so effects from clothi-only not explicitly reported.	Qualitative Fauser-Misslin *et al.*, 2014 (49579004)
Arena – 50% (*Bombus impatiens*)	Turfgrass w/30% white clover (*Trifolium ripens*) (0.401 lb c.e./A)	Part A. 6 days (7 weeks) Part B. 2 wks (2 wks) Part C. 7	Part A. 20 workers & fertilized queen in enclosures on treated (foliar) turf/clover then	Part A. mean numbers of: living and dead adults, queens; honey pots; living and dead brood; live adult and queen weights, foraging	Part A. ↓foraging activity (~100%) (day 5 & 6); ↑# dead workers (~92%); ↓hive wt gain (@ day 7, 15, 28, but not day 42); marginally significant effects (p=0.052-0.09) on #	-Current label language for turf restricts applications when blooming plants present or 5 days after, which limits utility of the data	Qualitative Larson, *et al.* 2013

		days (7 days)	moved to horse farm Part B. Colonies enclosed on treated turf/clover or enclosed after mowing turf to remove clover before treatment Part C. # bees monitored on treated plots & untreated borders	activity (number of bees); hive weight gain Part B. numbers of: living and dead adults, honey pots; living and dead brood; live adult and total hive weights Part C. # bees foraging on treated plots and borders -- (Yes)	live adults (↓36%), honey pots (↓53%), colony wt (↓17%); no production of new queens Part B. Non-mowed: ↑worker (3.7x) & brood (3.3x) mortality; ↓honey pots (35%); Mowed: no differences Part C. No differences in # of bumble or honey bee foraging	-residues in clover blooms following mowing were not measured	
Clothi Analytical standard – NR (*O. bicornis*)	Artificial nectar-sucrose, fructose, glucose (2.87 µg/kg thiamethoxam + 0.45 µg/kg clothianidin) [2.92 µg/kg (ng/g) clothianidin equivalents]	~40 days (5 months)	Males and females placed in cages in an environmentally - controlled room and allowed to forage and reproduce freely. Treated nectar placed in artificial flowers. Pollen pellets, nesting blocks and substrate also provided.	Mortality, # nests, hatching success, sex ratio, body wts -- (Yes)	-Nest completion: ↓22% -Total brood cells: ↓44% -Offspring development/mortality: ↓~50% -Offspring sex ratio: male-biased (44.4% control vs. 52.9% treated) -Body wt, female longevity: no effects	-Only one replicate per treatment group -None of the larval food provisions or bees had detectable residues -Unclear if outliers excluded from statistical analysis -Exposure and observation periods not clearly stated	Qualitative Sandrock *et al*. 2014b (49579003)

Clothi Tech – 98.4% (*Bombus impatiens* Cresson)	Sucrose solution (50%) (nominal 10, 20, 50, 100 ppb [µg/L, assumed], measured 9, 17, 39 & 76 ppb) [if using a density of 1.23 g/mL for a 50% solution, the measured conc. in ng c.e./g (ppb) are 7.3, 14, 32, & 62	11 weeks (11 weeks)	Caged bumblebee colonies (n=8, 1 queen, 30-50 workers) exposed under greenhouse conditions. Supplemental pollen was provided. Bees were allowed to forage away from nest.	Queen status (alive, dead, absent), worker movement, number of wax sugar syrup pots, bees on nest, bee weight, colony consumption, individual bee consumption, brood and bee caste production -- (Yes)	-Queen mortality: ↓~38&63% and ~75&100% @32&62 ppb (wks 6&11, respectively); ↓~50%@14ppb (wk 11) Colony weight: ↓69%, 74% and 81% @ 14-62 ppb (wk 11); Bee weight: different between control and 14 ppb # wax pots: ↓64, 94, 105, 110% @ 7.3-62 ppb # live and total brood & bees on nest: ↓ @ 32-62 ppb; # males: ↓95 & 97% @ 32-62 ppb; no sign effect on daughter production (↓trend) or worker production	- Test solutions were measured infrequently and variability in the measured test solutions was observed. Also,	Qualitative Scholer & Krischik, 2014
Unspecified (% unspecified) *Bombus terrestris audax*	5 ppb ai (nominal) Sucrose solution	5 weeks (*foraging*: starting day 3 of exposure to day 33; *colony growth*: test initiation to	20 colonies (10/level) paired by initial colony size (treatment & control) in locations around non-agricultural parkland (110 ha site). Colonies within pairs were 8-10 m apart and	Sucrose solution consumption *Foraging behavior (by time of day and across days):* Foraging activity/hour, proportion of foragers carrying pollen/hour,	-Changed brood composition (treated colonies had fewer number of eggs, workers, drones, and gyne and greater number of larvae and pupae) -Statistically significant treatment effects for other endpoints	-Single treatment level -Potential exposure to other pesticides in surrounding landscape, which is multi-purpose use -No screen of pollen returned by bees for potential exposure to other pesticides -No analytical	Arce et al (2017)

		exposure terminatio n	pairs were at least 25 m apart. Exposed to spiked sucrose solution (replenished 3 days/week) One treatment level and one negative control	pollen brought back/hour (mean and total) measured as (a) weight pollen and (b) surface area of the bee that the pollen covered *Colony growth:* Colony weight, # eggs, # larvae, # pupae, # workers, # drones, # gyne -- (Yes)	depending on how the data were analyzed (by hour or by day of observation): (1) decreased mean foraging time in treatment group (by hour), (2) proportion of foragers carrying pollen was initially higher in the treatment group on day 3 and 5 of exposure (3) no consistent pattern in mean surface area of pollen over observation hour and day (treatment higher or lower than control), and (4) no consistent pattern in mean pollen weight over observation day (treatment higher or lower than control)	measurements to confirm exposure concentration in sucrose solution -Single trial -Unknown if bees returning without pollen was treatment-related or due to forging on crops containing nectar	

Tier III

Apis

Registrant Submitted Full Field Studies

There are five full-field registrant-submitted studies (**Table 14**), in which honey bee colonies were placed in fields that contained either treated canola (in Canada) or maize seeds (in France). In the two field studies conducted in Canada using clothianidin formulation (Poncho® 600 FS or Prosper© FX) treated canola seeds, colonies were placed during the bloom period and maintained in the fields for either 14 or 21 days and then moved to a fall apiary and evaluated for overwintering success (MRID 46907801 and46907802,). Colony health and development were monitored as well as residues in various bee matrices. While no significant differences (p>0.05) between the treated and control sites were detected, there were several limitations associated with these studies, including clothianidin residues detected in control nectar or pollen samples (at levels within an order of magnitude of treated fields) and a loss of queens in the control and treatment group. Similar results are reported in MRID 49248301 (currently in review). Additionally, in a series of field studies conducted in France, colonies were placed in maize fields (seeds were treated with a clothianidin formulation; Clothianidin™ FS 600B G) during bloom for 10-11 days after which they were moved to another area and maintained overwinter (interim reports: MRID 48298802, 48298803, and 48298804). The study authors report that pollen residue concentrations ranged from 1-5 ng c.e./g (LOQ = 1 ng c.e./g). The study authors reported that pollen was also collected from foraging bees but an insufficient amount was collected for analysis. As with the other canola studies, according to the study authors, there were no significant differences in any parameter measured (visual only, statistical analyses not conducted). The study authors did report signs of disease and competition from nearby attractive crops (bees foraging on an alternative [untreated] source of food) or bad weather which may have influenced foraging activity in the maize fields.

In addition to the Tier III studies described above, several other studies are available which evaluate colony-level effects from exposure to dust-off (MRID 47972301-04) and/or guttation water (MRID 49073634, PMRA 2355469) from planting treated seeds. Additionally, there are open literature studies which evaluated potential effects due to simulated seed treatment dust-off (Pistorius *et al*. 2015) and evaluating guttation water (Reetz *et al*. 2015). However, while acknowledged as a potential route of exposure, the Agency lacks information to understand the relative importance of guttation water and/or methods to quantify potential for adverse effects from these routes of exposure.

Open Literature Apis Full Field Studies

There are three honey bee, full-field studies where colonies are placed in or near treated and untreated control fields, that were evaluated in the open literature. These are summarized below (and in **Table 14**). All studies involved limited true replication (*i.e.* bees were placed in/adjacent to a single control or treated field).

Apis

In Pohorecka, 2013, honey bee colonies were placed in maize fields (seeds were treated with clothianidin formulation (Modesto® 480 FS) at 150 mL/50 kg seed) during the flowering period for 21

days and monitored for approximately 3 months. In this study, brood area was significantly ($p>0.05$) increased at two sequential CCAs in the clothianidin-treated field, but there were no reported effects on mortality or the number of combs covered by bees. Clothianidin residues were measured in the pollen loads (10-41 ng c.e./g) with 25% of the pollen collected by bees originating from maize, but clothianidin was not detected in bee bread or adult bees sampled from the colonies. Also, other neonicotinoids (i.e., acetamiprid and thiacloprid) were also detected in the pollen loads in the treated field and control field.

Rolke *et al.* (2016) placed honey bee colonies in one seed-treated (formulation Elado containing clothianidin and cyfluthrin) and one control field in Northern Germany during canola bloom and observed following bloom until autumn. The study found no treatment-related adverse effects on numbers of adult bees, brood, honey production or pathogen infestations. As only a single field was used for each control and treatment site, the study lacked true replication. Measured clothianidin residues were not detected in 19% of treatment colonies; it was not clear whether this was due to colonies foraging in other locations or due to the already generally low residues in bee collected nectar and pollen from the treatment site (mean residues of 1.6 and 2.7 ng/g in nectar and pollen, respectively).

In another study (Rundolf et al., 2014) that examined Apis, Bombus and Osmina species (results described below for non-Apis), honey bee colonies were placed in flowering oilseed rape fields in which the seeds were treated with clothianidin (Elado® - 400 g/L formulation at 0.06 lb c.e./A); bees were monitored through overwintering (location/description not provided) and the number of adults counted. There were no significant differences in the number of adults between the treated and control fields.

Open Literature Non-Apis Full Field Studies

There are two bumble bee and one solitary mason bee species full-field studies (as well as an additional study that examined all both genera in addition to honey bees) where colonies are placed in or near treated and untreated control fields, that were evaluated in the open literature. These are summarized below (and in **Table 15**). All studies involved limited true replication (*i.e.* bees were placed in/adjacent to a single control or treated field).

Bombus

Cutler et al. 2014, examined bumble bee (B. impatiens) colony responses when placed adjacent to clothianidin and/or thiamethoxam seed-treated (conventional fields) or reported organic corn fields. B. impatiens colonies were placed in corn fields for 5-6 days during pollen shed in Canada in which seeds were treated with either a clothianidin formulation or a combination of clothianidin and thiamethoxam (Poncho® (clothianidin) and/or Cruiser® 5FS (thiamethoxam) at 0.25 mg a.i./seed for both chemicals). After the exposure period (5-6 days), the colonies were moved to a site reported to be isolated from neonicotinoid treatments. The number of workers was significantly ($p<0.05$) reduced (↓25%) in the neonicotinoid-treated fields (combined trials) compared to the organic fields, and while not significant ($p>0.05$), worker and drone weights were reduced by more than 25%.

In the study by Rundolf et al., 2014, oilseed rape seeds were treated with a clothianidin formulation (Elado® - 400 g/L) and during flowering the number of wild bees at field sites and field borders was

examined. In addition, *Bombus* colonies (study authors could not separate a number of *Bombus* species including *B. terrestris, B. locorum, B. magnus and B. cryptarum* and treated these wild-collected bees as one group) were placed adjacent to the treated fields and colony development was examined such as number of queens and worker/male cocoons, weight of cocoons, larvae and nest structure, and number of cells used for nectar and pollen storage. The number of wild solitary and bumble bees per flower was reduced in the treated field and field borders. Also, for *Bombus* colonies, there was a significant decrease in the mean number of queen and worker/male cocoons per colony and a decreasing change and rate of growth (weight). For *Bombus* bees foraging in the oilseed rape fields, 75% of the pollen was from oilseed rape from the treated fields, and the clothianidin nectar concentration (from bees) was 5.4 ng c.e./g.

A similar study on bumble bees was conducted by Sterk *et al.* (2016) in Northern Germany in a canola field treated with Elado seed treatment. In this study, *Bombus terrestris* colonies were placed in either the treated field or an untreated control field for 22 days during the canola bloom period and were then removed to a non-agricultural site for continued observation. No adverse effects were found on any parameter including hive weight, number of workers, young queens, and queen brood cells.

Other bee species

In the same Rundolf *et al*. study, mason bees (*O. bicornis*) colonies placed adjacent to treated oilseed rape fields had reduced median number of brood tubes (6/8 females in control and 0/8 females in treated group started to build brood cells). However, while oilseed rape pollen was observed in examined cells and accounted for 35% of the collected pollen, because there was no nesting activity in the treated fields, the authors could not assess whether pollen collection occurred from the treated fields.

A similar study was conducted by Peters *et al* (2016) where mason bees (*O. bicornis*) were placed adjacent to either a seed treated (Elado) or untreated control canola field for approximately one month during the canola bloom period before removal to a non-agricultural area for continued observation for 10.5 months post-exposure. No adverse treatment-related effects were observed on the measured parameters including (during exposure): emergence rate of cocoons, number of nesting females or number of sealed nesting holes, (fall post-exposure): number of undeveloped eggs or brood and parasitization rate, (following overwintering): emergence rate of cocoons, number of emerged males and females, number of undeveloped adults or number of undeveloped pupae.

Table 14. Summary of Tier III (full-field) studies available from the Registrant-submitted and Open Literature for Apis bees

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[1] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Poncho® 600 FS – 48% & Prosper FL – 9.49% (*Apis mellifera*)	Treated canola seed (4 g c.e./kg seed)	21 days (~10 months)	Colonies were placed at 4 sites during canola bloom period in Canada each with two 1-ha fields (1 treated & 1 control (formulation blank). Colonies (4 per field; 16 per treatment) were evaluated during exposure period and moved to a Fall apiary to evaluate overwintering success.	-Bee mortality; Worker longevity; brood development; presence/absence of queen, eggs & larvae; area of sealed brood; # of frames of workers; colony weight gain; residues in honey, beeswax, worker-gathered pollen and nectar; overwintering success. -- (Yes)	-No significant effects for any parameter -Canola pollen was observed in pollen samples (% not reported) -75% of samples had no detectable clothianidin residues.	-Clothianidin was detected in control nectar samples -Extent to which bees actually foraged in the treated site area uncertain -Loss of queens occurred in both treated and control hives	Supplemental Cutler, 2005 & 2006 (46907801 & 46907802)

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed – (Statistical analysis conducted – Yes/No)	Effects[1] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Prosper FX – 22.51 (*Apis mellifera*)	Canola seed treatment (3.77 g c.e./kg seed)	14 days (~10 months)	Colonies (n=4) were placed in the summer of 2012 in either 1 of 5 treated canola fields or in 1 of 5 fields treated with a formulation blank in Ontario, Canada (20 colonies per treatment). A strip of untreated soybeans was planted around the plot perimeters. Colonies were evaluated and moved to a Fall apiary and monitored for overwintering success.	-Mortality; honey yield; colony weight; brood production; adult strength; queen assessments; overwintering colony assessment; pest and disease counts; residues in nectar, honey, pollen, beeswax; pollen source -- (Yes)	-No significant effect for any parameter -Canola pollen accounted for 88% of total pollen in traps and dropped by end of week 2 (46%) -Overwinter loss (dead) was 37% in control and 26% in treatment by April -Pollen residues ranged from 0.5-1.9 ppb from traps in treated fields and from 0.5-1.3 ppb from 2 (out of 5 samples) samples in control	-Clothianidin was detected in control (blank) pollen samples at conc. similar to treated fields and cross-foraging may have occurred -A loss of queens occurred in both the control and treated hives	Currently in review Cutler *et al*. 2014b (49248301)

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[1] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Clothianidin FS 600B G – 600g/L (*Apis mellifera*)	Treated maize seeds (0.5 mg c.e./seed)	10 days (~8 months)	Colonies (n=6) placed in either one treated (1.8ha) or one control (2.8ha) field during maize bloom period (July) in Alsace, France in 2008. Colonies evaluated during exposure period and then moved to a Fall apiary to evaluate overwintering success in following April (2009).	Mortality; flight & foraging activity; behavior; brood development; colony weight; disease-incident; worker-collected pollen identification; residues in pollen, honey, and plants. -- (No)	-According to study author, no distinct differences in any parameter control and treatment -Maize pollen accounted for <19% of pollen collected by bees in treated plots with the majority (<72%) of the pollen types from white clover or dandelion -Low concentrations in pollen from treated fields were detected (≤5 ppb); LOQ = 1 ppb	-Results between control and treatment were not statistically analyzed -Due to bad weather, foraging activity was very low for the first 4 days and day 8 of a 10-day exposure period	Not Reviewed (Interim Report) Hecht-Rost, 2009 (48298802)

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[1] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Clothianidin FS 600B G – 600 g/L (*Apis mellifera*)	Treated maize seeds (0.5 mg c.e./seed)	11 days (~8 months)	Colonies (n=6) were placed in either one treated (2.06 ha) or control (3.2 ha) field during maize bloom period (July-Aug) in Languedoc-Roussillon, France in 2008. Colonies evaluated during exposure period and then moved to a Fall apiary to evaluate overwintering success in following March (2009).	Mortality; flight & foraging activity; behavior; brood development; colony weight; disease-incident; worker-collected pollen identification; residues in pollen, honey, plants -- (No)	-According to study author, no distinct differences in any parameter control and treatment -However, mean colony strength appears to be weaker in treatment (based on only 2 time points), but did overwinter - Maize pollen accounted for 16-99% in treated field and 7-36% in control field (other attractive plants also detected up to 88%) -Low concentrations in pollen from treated fields were detected (≤3 ppb); LOQ = 1 ppb	-Results between control and treatment were not statistically analyzed -A number of colonies had disease symptoms and/or Varroa mite infestations -Attractive crops close to control field may have influenced foraging ratio between control (0.3 bee/ 30 plants/day) and treatment (0.9 bee/ 30 plants/day)	Not Reviewed (Interim Report) Hecht-Rost, 2009 (48298804)

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[1] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Clothianidin FS 600B G – 600 g/L (*Apis mellifera*)	Treated maize seeds (0.5 mg c.e./seed)	10 days (~8 months)	Colonies (n=6) were placed in either one treated (1.97 ha) or control (1.94 ha) field during maize bloom period (Aug) in Champange, France in 2008. Colonies evaluated during exposure period and then moved to a Fall apiary to evaluate overwintering success in following April (2009).	Mortality; flight & foraging activity; behavior; brood development; colony weight; disease-incident; worker-collected pollen identification; residues in pollen, honey, and plants. -- (No)	-According to study author, no distinct differences in any parameter control and treatment -However, mean colony strength appears to be weaker in treatment (based on only 2 time points), but colony did overwinter successfully. -% of maize pollen in samples was low -Low concentrations in pollen from treated fields were detected (≤1ppb); LOQ = 1 ppb	-Results between control and treatment were not statistically analyzed -Attractive crops close to control field may have influenced foraging ratio between control (0.1 bee/ 30 plants/day) and treatment (0.5 bee/ 30 plants/day	Not Reviewed (Interim Report) Hecht-Rost, 2009 (48298803)

Elado oilseed rape (OSR) seeds (10 g clothianidin/kg seed + 2 g β-cyfluthrin / kg of seed) *Apis mellifera*	Clothianidin-treated OSR seed Blooming plants (Northern Germany) *Clothianidin residues in bee collected pollen & nectar and in honey*: < LOD (0.3) – 2.7 (pollen), 1.6 (nectar), 2.1 (honey) µg/kg (treatment sites) < LOD (0.3 µg/kg (pollen, nectar, honey) (reference sites)	During OSR bloom period 28 days (1 season; 157 days; April 22 to September 26)	-8 colonies/location (96 colonies total) Two circular study sites (Reference +Treatment) of ca. 65 km^2 each; 6 locations/site -R site planted with untreated OSR seed. T site planted with Elado treated OSR seed -At each site (R & T), 3 locations adjacent to the edge of an oilseed rape field and 3 locations 400m away from the nearest OSR field. -Colonies moved to 4 post-exposure sites (2 hives from each exposure site were placed in each post-exposure site) -The proportion of OSR sourced pollen was determined in the pollen load on returning bees and in honey during the exposure period Clothianidin residues were	Number of adult bees, areas of capped and open brood, colony weight gain, honey yield, *Varroa* and *Nosema* infestation, adult mortality -- (Yes)	No clearly adverse treatment-related effects	-Single "treatment" level OSR fields also treated with β-cyfluthrin -Potential exposure to other pesticides in surrounding landscape, including overwintering sites Significantly (statistically) more OSR pollen collected at the T site -Measurable clothianidin residues were not detected in 9 of 48 colonies (19%) at the T site (< LOQ or LOD in all pollen, nectar, and honey samples). Measurable residues were detected in only a single source (pollen, nectar, or honey) in 38% of the colonies at the T site. -Heavy infestation of Varroa may have stressed colonies	Qualitative Rolke et al (2016)

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed – (Statistical analysis conducted – Yes/No)	Effects[1] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
			determined in pollen, nectar, and honey sampled during the exposure period			(ca. 20% of R + T colonies collapsed) No over-wintering period	
Modesto® 480 FS-NR (*Apis mellifera*)	Corn (156 mL/50k seeds)	21 days (approx. 3 months)	-One control plot (unknown size), one treatment plot (15 acres) -Colonies assessed every 3-4 weeks during observation period)	-Mortality, number of combs covered by bees, brood area -- (Yes)	Brood area: ↑134% (mid-September CCA), ↑92% (early October CCA); no effects on mortality, number of combs covered by bees	-While clothianidin was detected in pollen load (10-41 ppb), not detected in bee bread or adult bees (Pollen analysis indicated ~25% of pollen collected originating from treated crop; 12% corn pollen grains in bee bread); -Other neonicotinoids (acetamiprid and thiacloprid) were also detected in pollen loads -Two fungicides (metalaxyl and fludioxonil) were seed treated along with clothianidin.	Qualitative Pohorecka, 2013 (49719625)

Elado oilseed rape (OSR) seeds (10 g clothianidin/kg seed + 2 g β-cyfluthrin / kg of seed) *Apis mellifera*	Clothianidin-treated OSR seed Blooming plants (Northern Germany) *Clothianidin residues in bee collected pollen & nectar and in honey*: < LOD (0.3) – 2.7 (pollen), 1.6 (nectar), 2.1 (honey) µg/kg (treatment sites) < LOD (0.3 µg/kg (pollen, nectar, honey) (reference sites)	During OSR bloom period 28 days (1 season; 157 days; April 22 to September 26)	-8 colonies/location (96 colonies total) -Two circular study sites (Reference +Treatment) of ca. 65 km^2 each; 6 locations/site -R site planted with untreated OSR seed. T site planted with Elado treated OSR seed -At each site (R & T), 3 locations adjacent to the edge of an oilseed rape field and 3 locations 400m away from the nearest OSR field. -Colonies moved to 4 post-exposure sites (2 hives from each exposure site were placed in each post-exposure site) -The proportion of OSR sourced pollen was determined in the pollen load on returning bees and in honey during the exposure period Clothianidin residues were determined in pollen, nectar, and honey sampled during the exposure period	Number of adult bees, areas of capped and open brood, colony weight gain, honey yield, *Varroa* and *Nosema* infestation, adult mortality -- (Yes)	No clearly adverse treatment-related effects	-Single "treatment" level -OSR fields also treated with β-cyfluthrin -Potential exposure to other pesticides in surrounding landscape (predominantly agricultural land), including overwintering sites (no screen of pollen, nectar, or honey for other pesticides) -Pollen and nectar were only sampled twice and honey once for clothianidin residues; pollen sampled only twice and honey once for source -Significantly (statistically) more OSR pollen collected at the T site -Measurable clothianidin residues were not detected in 9 of 48 colonies (19%) at the T site (< LOQ or LOD in all pollen, nectar, and honey samples). - Measurable residues were detected in only a single source	Qualitative Rundolf *et al.*, 2014

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed – (Statistical analysis conducted – Yes/No)	Effects[1] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
						(pollen, nectar, or honey) in 38% of the colonies at the T site. -Heavy infestation of Varroa may have stressed colonies (ca. 20% of R + T colonies collapsed) -No over-wintering period	

[1]Most studies not associated with NOAEC/LOAEC values. Reported are effects statistically derived or otherwise observed difference relative to the control.
[2]Generally, only subset of limitations is listed here.

Table 15. Summary of Tier III (full field) studies available for non-Apis bees.

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed – (Statistical analysis conducted – Yes/No)	Effects[1] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Poncho® (clothianidin) and/or Cruiser 5FS (thiamethoxam) - NR (*Bombus impatiens*)	Corn seed treatment (0.25 mg a.i./seed for both clothianidin & thiamethoxam)	5-6 days (36-41 days)	Colonies were placed in corn fields in Ontario, Canada treated with either conventional pesticides (either Poncho only or a combination of either Poncho or Cruiser) or treated organically (n=24 total colonies). Each unit was provided Biogluc as a carbohydrate source. After 5-6 days in fields, colonies moved to another site reported to be isolated from crops treated w neonic-treated seeds.	colony weight; # of: honey pots, pollen pots, brood cells, drones, queens and workers; and worker, drone, and queen weight -- (Yes)	-# of workers: ↓25% @ conventional fields compared to organic fields -Worker and drone weight ↓ >25% but not statistically significant	-Seeds not treated at the maximum rate (1.25 mg a.i./seed) -No residue analysis was conducted on the bee-collected pollen -Some conventional sites were not tested for residues of both clothianidin and thiamethoxam which was a problem in field planted with both a.i.s -Convention fields were treated with various fungicides and seed was modified for Bt endotoxin	Qualitative Cutler *et al.* 2014a

Elado® - 400 g/L (*Bombus terrestris* L complex (multiple B. species)), *Apis mellifera*, and *Osmia* bicornis L)	Oilseed Rape Seed (OSR) Treatment (0.06 lb c.e./A)	Varied depending on study design, but generally ~1-2 months (A. *mellifera* overwintered)	Eight pairs of OSR field were used with one planted w/Elado® + fungicide and the other one was the control treated w/fungicide. Four components to study. 1. Monitor wild bee density & flower cover 3X at field sites and field borders. 2. Monitor O. bicornis colonies (n=3) on-field 3. Monitor B. *terrestris* colonies (n=6) on-field Monitor A. *mellifera* colonies (n=6) on-field.	Varied based on study design 1. Wild bees (# of bees) /flower cover 2. # tubes w/brood cells, % emerging 3. # of queens and worker/male cocoons, weight of cocoons, larvae and nest structure, # of cells used for nectar and pollen storage 4. # of adults -- (Yes)	Wild bees: ↓# of solitary and bumble bees. O. bicornis: ↓median number of tubes (6/8 females in control and 0/8 females in treated group started to build brood cells B. *terrestris*: ↓mean # of queen and worker/male cocoons per colony; ↓ change and rate of growth (weight) A. *mellifera*: no effects	-A description of the site where the honey bee hives were relocated after the oilseed rape flowering period. concluded was not provided. -Exposure through pollen to O. bicornis cannot be confirmed since none were found nesting in the treated fields (therefore no pollen to collect from provisions). -Treated fields contained clothianidin, pyrethroid, and a fungicide, whereas control fields just contained fungicide.	Qualitative Rundolf *et al.*, 2014

Elado oilseed rape seeds (OSR) (10 g clothianidin/kg seed + 2 g β-cyfluthrin / kg of seed) *Osmia bicornis*	Clothianidin-treated OSR seed Blooming plants (Northern Germany) *Clothianidin residues in bee collected pollen:* < LOQ (1.0) – 1.7 µg/kg (treatment sites) < LOD (0.3 µg/kg) (reference sites)	During OSR bloom period 32 days (ca. 11.5 months total, ca. 10.5 months post-exposure)	-1500 cocoons/location (18,000 cocoons total) -Two circular study sites (Reference +Treatment) of ca. 65 km^2 each; 6 locations/site -R site planted with untreated OSR seed. T site planted with Elado treated OSR seed -At each site (R & T), 3 locations adjacent to the edge of an OSR field and 3 locations 100m away from the nearest OSR field -Nesting blocks were moved to a sheltered warehouse for the post exposure period. Cocoons were overwintered (ca. 5 months) in a refrigerator -Pollen in brood cells was sampled at each location to determine (1) proportion of OSR sourced pollen (2 dates during exposure period) and (2) clothianidin concentrations (1 date during exposure period)	Emergence rate of cocoons released at the field sites, # nesting females, # sealed nesting holes *Reproduction (Autumn after exposure)* # undeveloped eggs, # undeveloped larvae, parasitization rate *Reproduction (Spring after exposure and over-wintering)* Emergence rate of cocoons, # emerged males & females, # undeveloped males & females, # undeveloped pupae -- (Yes)	-No clearly adverse treatment-related effects -Increased # completed nesting holes, decreased # undeveloped eggs and larva, increased # emerged males after over-wintering, and decreased # undeveloped females and pupae after over-wintering	-Single "treatment" level -OSR fields also treated with β-cyfluthrin -Potential exposure to other pesticides in surrounding landscape (predominantly agricultural land) (no screen of pollen for other pesticides) -Pollen was sampled only twice for source and once for clothianidin residues -Significantly (statistically) less OSR pollen collected at the T site -Measurable clothianidin residues were detected in the pollen samples at only 2 of the 6 locations at the T site (residues were < LOQ at 3 sites) Single trial	Rundolf *et al.*, 2014 (con't)

Elado oilseed rape seeds (OSR) (10 g clothianidin/kg seed + 2 g β-cyfluthrin / kg of seed) *Bombus terrestris dalmatinus*	Clothianidin-treated OSR seed Blooming plants (Northern Germany) *Clothianidin residues in bee collected pollen*: < LOQ (1.0) – 1.3 µg/kg (treatment sites) < LOD (not reported) µg/kg (reference sites)	During OSR bloom period 22 days (43 days total, 21 days post-exposure)	-10 hives/location (120 hives total) -Two circular study sites (Reference +Treatment) of ca. 65 km^2 each; 6 locations/site -R site planted with untreated OSR seed. T site planted with Elado treated OSR seed -At each site (R & T), 3 locations adjacent to the edge of an OSR field and 3 locations 400m away from the nearest OSR field -Hive moved to nature park (forest. Lake, heath) for post-exposure period -Pollen load on retuning bees was sampled at each location from a single hive to determine (1) proportion of OSR sourced pollen (2 dates during exposure period) and (2) clothianidin concentrations (1 date during exposure period)	Hive weight, # workers, # young queens # queen brood cells, observations for abnormal flight activity, guarding and cooling behavior -- (Yes)	-No clearly adverse treatment-related effects -Greater number of queen brood cells at T site; however, no difference between T and R sites based on the sum of young queens and queen brood cells	-Single "treatment" level -OSR fields also treated with β-cyfluthrin -Potential exposure to other pesticides in surrounding landscape (predominantly agricultural land) (no screen of pollen for other pesticides) -Pollen was sampled only twice for source and once for clothianidin residues -Significantly (statistically) more OSR pollen collected at the T site -Measurable clothianidin residues were detected in the pollen samples at only 3 of the 6 locations at the T site (residues were < LOQ at 3 sites) -Single trial	Qualitative Sterk et al (2016)

Elado oilseed rape seeds (OSR) (10 g clothianidin/kg seed + 2 g β-cyfluthrin / kg of seed) *Osmia bicornis*	Clothianidin-treated OSR seed Blooming plants (Northern Germany) *Clothianidin residues in bee collected pollen:* < LOQ (1.0) – 1.7 µg/kg (treatment sites) < LOD (0.3 µg/kg) (reference sites)	During OSR bloom period 32 days (ca. 11.5 months total, ca. 10.5 months post-exposure)	-1500 cocoons/location (18,000 cocoons total) -Two circular study sites (Reference +Treatment) of ca. 65 km^2 each; 6 locations/site -R site planted with untreated OSR seed. T site planted with Elado treated OSR seed -At each site (R & T), 3 locations adjacent to the edge of an OSR field and 3 locations 100m away from the nearest OSR field -Nesting blocks were moved to a sheltered warehouse for the post exposure period. Cocoons were overwintered (ca. 5 months) in a refrigerator -Pollen in brood cells was sampled at each location to determine (1) proportion of OSR sourced pollen (2 dates during exposure period) and (2) clothianidin concentrations (1 date during exposure period)	Emergence rate of cocoons released at the field sites, # nesting females, # sealed nesting holes *Reproduction (Autumn after exposure)* # undeveloped eggs, # undeveloped larvae, parasitization rate *Reproduction (Spring after exposure and over-wintering)* Emergence rate of cocoons, # emerged males & females, # undeveloped males & females, # undeveloped pupae -- (Yes)	-No clearly adverse treatment-related effects -Increased # completed nesting holes, decreased # undeveloped eggs and larva, increased # emerged males after over-wintering, and decreased # undeveloped females and pupae after over-wintering	-Single "treatment" level -OSR fields also treated with β-cyfluthrin -Potential exposure to other pesticides in surrounding landscape (predominantly agricultural land) (no screen of pollen for other pesticides) -Pollen was sampled only twice for source and once for clothianidin residues -Significantly (statistically) less OSR pollen collected at the T site -Measurable clothianidin residues were detected in the pollen samples at only 2 of the 6 locations at the T site (residues were < LOQ at 3 sites) -Single trial	Peters et al (2016)

[1]Most studies not associated with NOAEC/LOAEC values. Reported are effects statistically derived or otherwise observed difference relative to the control.
[2]Generally, only subset of limitations is listed here

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Appendix 5: Summary of available bee toxicity data for thiamethoxam

This appendix includes a summary of the bee toxicity data available for thiamethoxam from registrant submitted studies and the scientific literature. The appendix describes Tier I (individual level laboratory toxicity studies), Tier II (semi field tunnel or feeding studies) and Tier III (full field) studies, focusing on endpoints that are relevant to survival, growth or reproduction of individuals or colonies.

Tier I

Adult Acute Contact Toxicity

***Apis* – Registrant-Submitted Studies**

Several studies are available to characterize the acute contact toxicity of thiamethoxam to honey bee adults (**Table 1**). Studies include TGAI as well as several formulated products. The LD50 values for contact exposure range 0.02-0.39 µg c.e./bee. Comparison of LD50 values for TGAI and formulated products indicate that there is no substantial difference in toxicity for four formulated products, with the TGAI LD50 of 0.021 µg c.e./bee, being within an order of magnitude of four different formulated products. The TGAI LD50 is an order of magnitude less sensitive than the LD50 for Actara® and an order of magnitude more sensitive than the LD50 for Actara® 75 WG.

Table 1. Thiamethoxam Tier I acute contact toxicity data for adult honey bees (*Apis mellifera*) (48-h study duration) reported in terms of thiamethoxam active ingredient and clothianidin equivalents (c.e.)

Test material (% a.i.)	LD_{50} Value (95% CI; units: µg a.i./bee)		MRID/source	Classification
	Thiamethoxam	Clothianidin equivalents		
Thiamethoxam® WG (25)	0.019 (0.014-0.024)	0.016	49950111	Acceptable
Thiamethoxam® 240SC (21.6)	0.0198 (0.0163-0.0237)	0.0169	49950105	Acceptable
TGAI (98.6)	0.024 (0.021-0.027)	0.021	44714927	Acceptable
Cruiser® 600 FS (NA)	0.066 (0.012-1093)	0.056	49950114	Supplemental (qualitative)
Actara® 75 WG (74.8)	0.46 (0.34-0.68)	0.39	49950106	Acceptable
Thiamethoxam® WG (25)	23.5 (22.2-28.7) 48-hr LC_{50}	20.1	49950119	Supplemental (qualitative)
Thiamethoxam Cruiser 350 FS	Not Calculated**	Not Calculated**	49950122	Supplemental (qualitative)
Thiamethoxam Formulation*	0.5 formulation (0.37-0.69)	0.428	49950116	Unacceptable

*This formulation contained 81.9 g a.i./L. The results were reported in terms of mg wm/mL. "WM" meant "whole material" which were presumed to be formulation. It was not clear if the liquid formulation was weighed or if the weight of thiamethoxam was calculated when making the dosing solutions. The authors did note they were not adjusted for purity.

**There were only 2 doses tested

Apis – Open Literature Studies

The open literature studies considered include two acute, contact-based studies with adult honey bees (**Table 2**), both involving TGAI. The LD50 value generated by Iwasa *et al.* (2004), *i.e.*, 0.0256 µg c.e./bee, is similar to the registrant-submitted study with TGAI (LD50 0.021 µg c.e./bee; MRID 44714927). The LD50 value reported by Thompson *et al.* is 5-fold greater than the other two TGAI values.

Table 2. Thiamethoxam Tier I acute contact toxicity data for adult honey bees (*Apis mellifera*) (48-h study duration)

Test material (% a.i.)	LD_{50} Value (95% CI; units: µg a.i./bee)		MRID/source	Classification
	Thiamethoxam	Clothianidin equivalents		
TGAI (>99)	0.0299 (NA)	0.0256	Iwasa *et al.* 2004	Qualitative
TGAI (99.7)	0.124 (0.0768-0.328)	0.106	Thompson *et al.* 2014	Qualitative

NA = not applicable

Non-*Apis* – Registrant-Submitted studies

One registrant-submitted study is available for adult bumble bees (B. *terrestris* (L.)) exposed to thiamethoxam via contact (**Table 3**). The contact LD50 value is 0.094 µg c.e./bee (MRID 49950109). This LD50 is an order of magnitude higher (*i.e.*, less sensitive) than the honey bee value for the same formulated product (*i.e.*, LD50 = 0.00475 µg c.e./bee; MRID 49950125).

Table 3. Summary of registrant submitted adult acute contact toxicity studies for non-Apis bees (*Bombus terrestris terrestris*) exposed to thiamethoxam

Test material (% a.i.)	Study Duration (Type)	LD_{50} Thiamethoxam (95% CI; units: µg a.i./bee)	Clothianidin equivalents	Comments	Classification (Reference, MRID)
Actara 25 WG (25.2)	72-hr	0.11 (0.10-0.13)	0.094	none	Acceptable (49950109)

Non-*Apis* – Open Literature Studies

Sechser *et al.* 2002 exposed bumble bees (B. *terrestris* L.) to thiamethoxam (Actara™ WG 25) via contact with glass plates that were sprayed at levels representative of an application rate of 8.6 g c.e./ha. All exposed bees died within 7 d. The doses received by bees were not quantified (**Table 4**).

Valdovinos-Núñez *et al.* (2009) exposed stingless bees (*Nannotrigona perilampoides*) to thiamethoxam (TGAI) via contact exposure at levels of 0.009, 0.09, 0.4 and 0.9 µg c.e./bee. After 24 hours, the LD50 was 0.003 µg (95% CI: 0.002-0.005) c.e./bee; however, there is considerable uncertainty associated with this endpoint as it is below the lowest level tested.

Table 4. Summary of open literature adult acute contact toxicity studies for non-Apis bees exposed to thiamethoxam.

Test material (% a.i.)	Study Duration (Type)	LD_{50} Thiamethoxam (95% CI; units: µg a.i./bee)	Clothianidin equivalents s	Test Organism	Classification (Reference, MRID)
Actara™ WG 25 (25)	7 d	10 g c.e./ha	8.6 g c.e./ha	Bumble bee *Bombus terrestris*	Qualitative (Sechser *et al*. 2002)
TGAI	24 h	0.004 (0.003-0.006)	0.003	Stingless bees (*Nannotrigona perilampoides*)	Qualitative (Valdovinos-Núñez *et al*. (2009)

Adult Acute Oral Toxicity

***Apis* – Registrant-Submitted Studies**

Several studies are available to characterize the acute oral toxicity of thiamethoxam to honey bee adults (**Table 5**). Studies include TGAI as well as several formulated products. Comparison of LD50 values for TGAI and formulated products indicate that there is no substantial difference in toxicity (all within the same order of magnitude). The LD50 values for oral exposure range from 0.0031 to 0.0067 µg a.i./bee (or 0.0026-0.0057 µg c.e./bee as clothianidin equivalents). These data indicate that thiamethoxam is more toxic to bees exposed through diet compared to through direct contact exposure.

Table 5. Thiamethoxam Tier I acute oral toxicity data for adult honey bees (*Apis mellifera*) (48-h study duration) expressed in terms of active ingredient (a.i.) and clothianidin equivalents (c.e.)

Test material (% a.i.)	LD_{50} Value (95% CI; units: µg a.i./bee)		MRID/source	Classification
	Thiamethoxam	Clothianidin equivalents		
Thiamethoxam® 240SC (21.6)	0.00309 (0.00256-0.00366)	0.00265	49950105	Acceptable
TGAI	0.0044 (NA)	0.0038	49005702	Acceptable
TGAI (98.6)	0.005 (0.004-0.006)	0.004	44714927	Acceptable
Formulated product (20.6% thiamethoxam, 20.6% cyantraniliprole)	0.0064* [0.031 µg test material/bee]	0.0055	48432530	Acceptable
Thiamethoxam® SG (72.8)	0.00668 (0.00571-0.00773)	0.00572	49950115	Acceptable
Thiamethoxam Cruiser 350 FS	Not Calculated**	Not Calculated**	49950122	Supplemental (qualitative)
Thiamethoxam Formulation*	0.085 (0.065-0.11)	0.073	49950116	Unacceptable

NA = Not Applicable

*This formulation contained 81.9 g a.i./L. The results were reported in terms of mg wm/mL. "WM" meant "whole material" which were presumed to be formulation. It was not clear if the liquid formulation was weighed or if the weight of thiamethoxam was calculated when making the dosing solutions. The authors did not they were not adjusted for purity.

**There were only 2 doses tested.

Apis - Open Literature Studies

Three qualitative studies are considered from the literature (**Table 6**). The LD_{50} values are within the range of the registrant-submitted LD_{50} values reported above.

Table 6. Thiamethoxam Tier I acute oral toxicity data for adult honey bees (*Apis mellifera*) (48-h study duration) expressed in terms of active ingredient (a.i.) and clothianidin equivalents (c.e.)

Test material (% a.i.)	LD_{50} Value (95% CI; units: µg a.i./bee)		MRID/source	Classification
	Thiamethoxam	Clothianidin equivalents		
Actara® 25 WG	0.0026-0.0044 (NA)	0.0022-0.0038	Laurino *et al.* 2010	Qualitative
TGAI (92.6)	0.00428 (NA)	0.00366	Oliveria *et al.* 2013	Qualitative
TGAI (99.7)	0.0112 (0.00915-0.0135)	0.00959	Thompson *et al.* 2014	Qualitative

NA = Not Applicable

Non-*Apis* – Registrant Submitted Studies

One registrant submitted study is available for adult bumble bees (*Bombus terrestris* (L.)) exposed to thiamethoxam (**Table 7**). This study determined an acute oral LD_{50} value of 0.017 µg c.e./bee (MRIDs 49950107).

Table 7. Summary of registrant submitted adult acute oral toxicity studies for non-Apis bees (Bombus terrestris terrestris) exposed to thiamethoxam expressed in terms of active ingredient (a.i.) and clothianidin equivalents (c.e.)

Test material (% a.i.)	72-hr LD_{50} Value (95% CI; units: µg a.i./bee)		MRID/source	Classification
	Thiamethoxam	Clothianidin equivalents		
Actara 25 WG (25.2)	0.02	0.017	49550107	Acceptable

Non-Apis – Open Literature Studies

Sechser et al. 2002 exposed bumble bees (B. terrestris (L.)) to thiamethoxam (Actara® WG 25) via dietary exposure at levels representative of an application rate of 8.6 g c.e. /ha. All exposed bees died within 7-d (Table 8).

Table 8. Summary of open literature adult acute oral toxicity studies for non-Apis bees exposed to thiamethoxam

Test material (% a.i.)	Study Duration (Type)	Thiamethoxam (95% CI) (expressed in terms of µg c.e./bee)	Clothianidin equivalents	Test Species	Classification (Reference, MRID)
Actara WG 25 (25)	7 d	10 g c.e./ha	8.6 g c.e./ha	*Bombus terrestris*	Qualitative (Sechser et al. 2002)

Adult chronic oral toxicity

Apis

Seven chronic toxicity studies are available for honey bees exposed to thiamethoxam (**Table 9**). One study is available for deriving risk quotients while the remaining six have limitations such that they are useful for characterizing potential effects of thiamethoxam on bees. All studies were conducted in a laboratory with either *A. mellifera* or the Indian honey bee *A. cerana indica*. The majority of the studies involved exposures via diet (oral exposure to spiked sucrose solution). Several of these studies describe effects related to sublethal endpoints with unknown links to apical endpoints (*i.e.*, survival growth or reproduction of individuals or hives). Of all the studies, effects to apical endpoints were observed in three studies: at 212 mg a.i/kg solution (181 mg c.e./kg solution), 70.3% mortality was observed (MRID 50084901), 428 µg a.i./L (366 µg a.i./L clothianidin- equivalents), bee lifespan was reduced by 41% (Oliveria *et al.* 2013) and at 500 µg a.i./L (428 µg a.i./L clothianidin-equivalents), 25% mortality was observed (Chandramani *et al.* 2008).

Chronic oral toxicity data for adult honey bees (A. *mellifera* L.) are available from three registrant-submitted studies (**Table 9**). In these studies, bees were dosed for 10 days through sucrose solution. In MRID 50084901 significant effects (relative to the control), on mortality was observed at 4.87 ng a.i/bee/day (LOAEC), while food consumption was affected at 1.84 ng a.i./bee/day (4.2 and 1.6 ng c.e./bee/day respectively). No effects were observed in the remaining studies, with the highest tested doses being 0.002 and 0.008 µg c.e./bee (MRIDs 49950110 and 49346603), which correspond to dietary concentrations of 8.6 and 27 µg c.e./L.

In a chronic study with Africanized honey bees (A. *mellifera*, Oliveira *et al* 2013), honey bees (newly emerged worker) exposed for 18 days to 366 µg c.e./L diet thiamethoxam through sucrose had a reduced lifespan (in days). In this exposure, 50% of bees lived 8d in the control; whereas, 50% of bees exposed to thiamethoxam only lived 5.2 days, resulting in a 41% decrease in the lifespan of adult worker bees. Bees exposed for 8 to 36.6 µg c.e./L diet had morphological changes (histological changes in neural mushroom bodies and optical lobes) of the brain and chemical changes (cytotoxicity) to the midgut. Similar to Oliveria *et al.* 2013, the study by Catae *et al.* 2014 exposed Africanized honey bees for 8 d to 36.6 µg c.e./L. Damage (cytotoxicity) to the midgut and Malpighian tubules were reported.

Aliouane *et al.* 2009 exposed adult bees to thiamethoxam via oral or contact exposure at levels of 0.00009 and 0.0009 µg c.e./bee. At the lower level, bees exposed via contact showed a decrease in olfactory memory (via testing proboscis extension reflex), relative to the control (p=0.02). At the higher level, bees exposed via contact had impaired learning (two trials p=0.025,0.033). Also at the higher level, bees exposed via diet had a decrease in proboscis extension reflex (PER) when stimulated with sucrose. This study focused on sublethal effects; however, without information of how these effects related to survival, growth or reproduction of individuals or the colony, the relevance of these effects to the individual bee or colony is unknown.

Table 9. Laboratory chronic toxicity data for adult honey bees (Apis sp.)

Test dose (µg a.i./bee)		Test concentration (ng /g)[a]		Exposure route	Test material (% a.i.)	Duration (d)	Observed effects	Source	Classification
Thia-methoxam	Clothianidin-equivalent	Thia-methoxam	Clothianidin-equivalent						
0.0025/0.0049	0.0021/0.0042	120/212	103/181	Oral*	TGAI (99.5)	10	Mortality	50084901	Acceptable
0.0001	0.000086	NA	NA	Contact	TGAI (97)	11	Decrease in olfactory memory	Aliouane *et al* 2009 (MRID 47800507)	Qualitative
0.001	0.00086	NA	NA	Contact	TGAI (97)	11	Learning impairment	Aliouane *et al* 2009 (MRID 47800507)	Qualitative
0.001	0.00086	NA	NA	Oral*	TGAI (97)	11	Decrease in proboscis extension response to sucrose stimulation	Aliouane *et al* 2009 (MRID 47800507)	Qualitative
0.002	0.0017	10 µg/L	8.6 µg/L	Oral*	TGAI (99)	10	No effects to mortality or food consumption observed. No LOAEC was established.	MRID 49950110	Supplemental (qualitative)
0.00898	0.00768	27	23	Oral*	TGAI (99)	10	No effects to mortality or food consumption observed. No LOAEC was established.	MRID 49346603	Supplemental (qualitative)
NA	NA	42.8 µg/L	36.6 µg/L	Oral*	TGAI (92.5)	8	Cytotoxicity observed in midgut and Malpighian tubules	Catae *et al*. 2014	Qualitative
NA	NA	42.8 µg/L	36.6 µg/L	Oral*	TGAI (92.5)	8	Morphological changes to brain and chemical changes to midgut	Oliveira *et al*. 2013	Qualitative
NA	NA	428 µg/L	366 µg/L	Oral*	TGAI (92.5)	18	Reduced lifespan (41 % reduction)	Oliveira *et al*. 2013	Qualitative

*Bees were fed sucrose solution. NA = not available

[a] Unless specified data are in ng/g.

Larval Toxicity

Apis

Several studies are available to characterize the toxicity of thiamethoxam (TGAI) to honey bee larvae (**Table 11**). MRID 50096607 is an acceptable larval chronic toxicity study for which the acute endpoint was extrapolated. Effects were seen on adult emergence at day 22 and pupal mortality at day 15, with no effects (>50%) to larval mortality seen at day 8. The remaining studies are considered scientifically valid, but have notable limitations that prevent quantitative use of these data (*i.e.*, to derive RQs). Two studies evaluated impacts on larval survival following repeated dietary doses, generating a 48-hour LC_{50} value of 11.7 and a 7-day LC_{50} 23 µg a.i./g-diet and a 7-day LD_{50} of 0.78 µg a.i./larva/day (Tavares *et al*. 2015 and MRID 49950118). Data from a third acute toxicity study (MRID 49346602) failed to generate a definitive LC_{50} with only 29% mortality observed at the highest test level of 113 µg a.i./g diet, which was an order of magnitude above the LC_{50} values estimated for the other two studies. In a chronic repeat-dose study (22-D), significant mortality (12 and 16%) was observed at 0.025 and 0.050 µg a.i./g-diet (respectively), resulting in a 22-day NOAEC of 0.0125 µg a.i./g- diet (MRID 49513601). It is notable that the dose-response observed in this study was very shallow, as mortality only increased 4% relative to controls, despite a two-fold increase in exposure.

Table 11. Tier 1 Acute and Chronic toxicity data for honey bee (Apis mellifera) larvae exposed to thiamethoxam. All studies involved TGAI (≥99% a.i.).

Duration	Endpoints (units)	Thiamethoxam	Clothianidin equivalents	MRID/source	Classification	Comments
Acute – repeat dose	LD_{50} (µg a.i./larva/day)	0.78 (0.05 – 1.88)	0.67	49950118	Supplemental (qualitative)	Study carried out for 7 days
		>0.03	>0.03	50096607	Acceptable	Day 8 mortality endpoint based on Repeat dose on day 4 exposure/4 (>0.120/4)
	LC_{50} (µg a.i./g- diet)	11.7 (2.24-21.1) *	10.0	Tavares et al. 2015	Qualitative	Bees were Africanized.
		23	20	49950118	Supplemental (qualitative)	Value estimated based on concentrations reported by study author
		>113	>96.7	49346602	Supplemental (qualitative)	NOAEC = 35; LOAEC = 51.5 (21% mortality)
Chronic (22 d; repeat dose)	NOAEC (LOAEC) (ng a.i./g-diet)	12.5	10.7	49513601	Supplemental (qualitative)	LOAEC =25 Replicates were run at different times, composition of diet and verification of chemical concentration were not reported.
	NOAEL (LOAEL)	0.028 (0.059)	0.024 (0.05)	50096607	Acceptable	None

	(µg a.i./larvae/ day) day 22 emergence					

* The study author reported a value of 0.01434 µg a.i./µL-diet (95% CI: 0.00275- 0.02594). This value was converted with an assumed density of sucrose diet (50% sugar) of 1.23 g/mL. ng a.i./µL-diet is equivalent to µg a.i./mL-diet.

Non-Apis – Open Literature Studies

In another study with bumble bees (B. *terrestris audax*), Thompson *et al*. 2014 exposed bees via sucrose to 1, 10 and 100 µg a.i./L (TGAI; 0.86, 8.6 and 86 µg c.e./L). After 4 days of continuous exposure, no significant mortality was observed in the 0.86 and 8.6 µg c.e./L test groups while 100% mortality was observed at 86 µg c.e./L (**Table 12**). Feeding was not affected at the lower test levels (*i.e.*, 0.86 and 8.6 µg c.e./L).

Table 12. Summary of open literature adult acute oral toxicity studies for non-Apis bees exposed to thiamethoxam

Test material (% a.i.)	Study Duration (Type)	Thiamethoxam (95% CI) (expressed in terms of µg c.e./bee)	Clothianidin equivalents	Test Species	Classification (Reference, MRID)
TGAI	4 d	10 µg a.i./L	8.6 µg c.e./L	*B. terrestris audax*	Qualitative (Thompson et al. 2014)

Toxicity data are also available to characterize (qualitative) effects of chronic exposures to the stingless bee larvae (*Scaptotrigona aff. depilis*) (Rosa *et al*. 2015). Effects to survival, development and morphology were observed in bees dosed with 0.000044 and 0.0044 µg a.i./larva (0.000038 and 0.0038 µg a.i./larva as clothianidin equivalents).

Tier II

This section summarizes the registrant-submitted Tier II (*i.e.*, tunnel and feeding study design) for thiamethoxam. A summary of the results and associated uncertainties is provided within the discussion of each study. The studies below, along with those outlined in the clothianidin section above indicate that exposure to thiamethoxam affected adults and brood. This conclusion is largely supported by effects seen in the colony feeding studies both sucrose and pollen based exposure test designs.

Registrant submissions - Apis

Colony Feeding Study - MRID 49757201

This registrant-submitted honey bee colony feeding study for thiamethoxam was conducted under similar parameters described for clothianidin (conducted in North Carolina, 12 test apiaries *etc.*) to assess the potential for long-term effects, including colony overwintering survival, resulting from exposure to thiamethoxam. The study was conducted June 27, 2014 to April 28, 2015. Ninety-six hives were divided according to hive strength (number of brood frames) with the strongest 8 hives assigned to Apiary A and the weakest 8 hives assigned to Apiary L (*i.e.*, the study design was stratified to account for differences in colony strength). Within each apiary, 7 hives were randomly assigned to treatment groups where five of

the colonies were provided 50% sugar solution spiked with thiamethoxam at 10.7, 21.4, 32.1, 42.8, or 86.6 µg c.e./L and two of the colonies served as controls and were provided untreated sugar solution for six weeks continuously while bees were allowed to forage freely. The 8th colony at each apiary served as a monitoring hive to characterize the alternative sources of forage (pollen/nectar) for the test colonies as well as to monitor for the potential contamination with other pesticides.

Ten Colony Condition Assessments (CCAs) were conducted during the study. Two CCAs (CCA1 2) were conducted prior to feeding (*i.e.*, pre-exposure phase) to determine hive strength (number of adult and developing bees) and initial hive conditions, CCAs 3-5 were conducted during the exposure phase, CCAs 6-8 were conducted post-exposure and CCA9-10 were conducted after overwintering. Multiple parameters, such as hive weight, number of individuals at different life stages in the hive, hive honey and pollen stores, and hive overwintering survival, were measured during the course of the study.

There are three main limitations associated with the colony feeding study which reduce the utility in this risk assessment:

Late timing of exposure that coincided with normal reductions in bee activity in preparation for overwintering;
Lower than expected performance of controls; and,
Lack of overwintering success.
Almost every parameter for evaluating life stages decreased after exposure ended and there is uncertainty whether these reductions were the result of the late time of the year when the study was initiated or whether the effects were the result of treatments. The natural process of colonies reducing their size/activity in preparation for winter contributed to high variability at the later CCAs. Many of the treatment hives performed similarly to the control, especially after exposure ended. While this could be indicative of a lack of treatment effects, variability limited the extent to which treatment effects could be detected.

Control colony loss after overwintering (79%) also adds uncertainty when considering the results of individual measurements. Because so few control hives survived overwintering (potentially due to poor food stores) and performed similar to the treatment hives during exposure the results have limited utility in evaluating colony-level effects after overwintering. The study is useful for characterizing pre-overwintering effects.

There were significant reductions ($p<0.05$) relative to controls in multiple endpoints over several CCAs in colonies exposed to 86.6 µg c.e./L. In addition, numbers of larvae, pupae, pollen stores and adults declined in the 42.8 µg c.e./L group shortly after exposure. There were statistically significant ($p<0.05$) decreases in pollen stores at CCA5, CCA7 in colonies exposed to 21.4 µg c.e/L, and there were statistically significant decreases in the number of pupae at CCA5 in colonies exposed to 32.1 µg c.e/L. At the lowest test level, i.e., 10.7 µg c.e/L, no significant reductions were noted ($p<0.05$) in any of the parameters tested. There were marginally significant ($0.05<p<0.1$) reductions in numbers of eggs at CCA6 and numbers of cells containing honey at CCA5. Based on the limitations of this study, a NOAEC derived from this study is considered uncertain. There is uncertainty in whether this value is conservative based on the study limitations discussed above.

Colony Feeding Study - MRID 50432101

Similar to clothianidin, this colony feeding study was conducted to address the uncertainties associated with the lack of overwintering success in the previous CFS (MRID **49757201**). The same study design (*e.g.* dosing, similar location) was utilized as the original MRID and the details of study design are not discussed

here. No elements of the study design were expected to be significantly different[5] than the first study. This study was conducted April of 2016-April 2107 with a 6-week exposure period initiated on July 5, 2016. The nominal doses of thiamethoxam were 12.5, 25, 37.5, 50, and 100 µg a.i/kg corresponded to measured concentrations (in c.e) of 10.1, 20.1, 29.0, 43.6, 81.7 µg a.i c.e./kg.

Decreases in multiple endpoints (significant reduction [$p<0.05$] relative to controls) and declining trends were observed over multiple CCAs in colonies exposed to 81.7 µg c.e./kg including significant reduction in larvae, pupae, and pollen in CCAs 3-6 for brood matrices, and CCAs 3-8 for pollen. Pollen reduction relative to controls was also statistically significant ($p<0.05$) at CCA 4 at the 43.6 µg c.e/kg treatment level. There were marginally significant ($0.05<p<0.1$) reductions in numbers of eggs at CCAs 6-8 and the number of cells containing honey at CCA6 in the 81.6 µg a.i c.e./kg treatment level. Numbers of bees and food stores were similar in numbers and trends compared to controls (*i.e.*, no significant differences noted) at the other treatment levels. There were no significant effects detected in the number of adults; however, there was high variability in the number of adult bees, particularly in the overwintering colonies in the highest treatment groups.

Overwintering survival in the control colonies was good (87.5%; 3/24 dead) with 91.7, 83.3, 100, 91.7, and 75 percent surviving colonies in the 10.1, 20.1, 29.0, 43.6, 81.7 µg a.i c.e./kg treatments, respectively. All colonies (3/12) that were dead in the highest treatment level died before overwintering. The study authors also reported the 81.7 µg c.e/kg treatment hives exhibited a significant decreased weight difference compared to the control hives.

In the 100 ppb (T5) treatment level, multiple endpoints were significantly affected at consecutive assessment times prior to overwintering. Therefore, the lowest observed adverse effect level (LOAEL) was determined to be 100 ppb (81.7 µg a.i c.e./kg) and the no observed adverse effect level (NOAEL) was determined to be 43.6 µg a.i c.e./kg, based on significant reductions in brood matrices.

Other Registrant Submissions

In addition to the colony feeding study, there are also registrant-submitted Tier II (tunnel) studies. As with clothianidin, these studies are generally considered qualitatively in the weight-of-evidence approach while noting design flaws and the limitations. In some cases, studies were conducted using protocols which had not been reviewed in advance by EPA to better ensure that the study would address specific uncertainties identified in lower-tier testing. There is a seven Tier II studies considered supplemental by the Agency (**Table 13**)

The Tier II registrant-submitted study examined two separate single foliar applications of Actara® 25 WG (active ingredient: thiamethoxam) at 0.089 lb a.i/A to honeydew melons. For Treatment 1, application was made 10 days before flowering and for Treatment 2, application was made 5 days before flowering. Each tunnel (representing one replicate) contained one hive, covered an area of 150 m^2 and was located in a single melon field. Colonies were confined to foraging on the enclosed melon plants for 8 days (exposure phase) after which time the colonies were relocated to a separate site and allowed to forage freely for 29 days (post-exposure monitoring phase). In Treatment 1, the only effect was increased mortality observed 3 days after exposure started. In Treatment 2, increased mortality (workers and pupae) occurred relative to

[5] Comments were made by EFED via protocol review for minor differences including dosing regimen (not incorporated), increased frequency of hive matrix sampling, marking queens, increased supplemental feeding, and an earlier exposure period.

the control in the subsequent days after exposure began. No biologically relevant effects were observed in behavior or brood indices, and differences were attributed to stress of bees in the tunnels.

MRIDs 50781601 and 05781602 were semi-field tests conducted on fields sown with thiamethoxam seed treated sunflowers. In MRID 50781601 three tunnels were placed over flowering sunflower (*Helianthuus annus)* plants and observed for 7 days. The study authors noted higher dead bees compared to the control on a single day in the treatment tents, but average mortality was higher in the control tents (11.8 bees/day) than the treatment tunnels (9.9) although these numbers are comparable. The overall mean number of eggs, larval, and capped stages were similar in the control and treatment groups. The authors noted a decline in eggs and larvae in both the treatment and controls which was attributed to being in the tunnel. In MRID 50781602 tunnels were placed over seeds applied at increasing rates in two different tunnels. Mortality was higher in the higher application rate tunnel (~4230 bees) compared to the lower rate (~240 bees); however, the control tunnel mortality was the highest in the untreated control (~4700 bees). There was some qualitative observation in a reduction of food stores (honey/pollen); however, no quantitative analysis was done by the study author.

MRIDs 50781603, 50781604, and 50781605 were semi-field tests conducted on oilseed rape fields. MRIDs 50781603 and -05 were conducted under foliar spray conditions, while 50781604 was conducted using treated seed. Similar to the other seed treatment studies, the study authors concluded similar levels in observations of mortality, and decline in brood in both the treatment and the control for thiamethoxam treated seeds. For the foliar application studies there were increased mortality effects noted if sprayed during bee flight, but was generally similar in control and treatment tunnels at the end of the observation periods. Additional declines in brood were attributed to stress from the tunnels and were comparable between control and treatment tunnels. No clear treatment effects (except for mortality when sprayed during bee light) were noted by the study authors.

Additionally, although several Tier II studies described earlier in **Section 4** and which are considered qualitative for their residue information were not considered valid for assessing potential effects and are listed in **Appendix 2**.

Table 13. Tier II Tunnel Thiamethoxam Studies Submitted by the Registrant

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[2] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Thiamethoxam 25% (*Apis mellifera*)	Application to melon	Foliar Application (0.089 lb a.i./A; 0.076 lb c.e./A) (8 days in tunnel 29 days at monitoring site)	3 replicates/trt 1 replicate/ref chemical Hives placed in tunnels prior to full flowering either 10 or 5 days before full flowering Hives: six frame, queen right, 205 brood combs, 2-5 honey/pollen combs, 3-5 brood comb colonies, 8000-11000 adults.	Mortality, Colony condition, brood development (Yes)	Increased mortality in Treatment 2 (days before flowering)	Number of replicates was low (effects data) pollen and nectar residue data used from whole flowers and honeybee guts	Supplemental Bocksch 2011 (49158904)
Thiamethoxam (Actara 25 WG) (*Apis mellifera*)	1 g ai/ha (0.86 g c.e./ha) and 5 g ai/ha (4.3 g c.e./ha) applied via foliar spray to *Phacelia tanacetifolia*	27 d	Two replicate tunnels	Mortality, colony condition, foraging activity	Increased mortality in 5 g a.i./ha treatment	No residue data were collected	Supplemental 50781603
Thiamethoxam (Actara 25 WG) (*Apis mellifera*)	80 g ai/ha or 20 g ai/ha foliar spray to *Phacelia tanacetifolia*	7-10 d	One tunnel	Mortality, flight intensity, behavior	Increased mortality,	One replicate. No residues	Supplemental 50781605
Thiamethoxam (A-9567 B) (*Apis mellifera*)	Seed treatment of sunflower at 350 or 700 g a.i./100 kg seed)	14 d	1 replicate tunnel per treatment	mortality, foraging activity, flight activity and behavior (no)	No reliable differences between treatment and control groups were observed.	Residues were not measured; number of bees per hive were not reported; no replication	Supplemental 50781602

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed – (Statistical analysis conducted – Yes/No)	Effects[2] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Thiamethoxam (*Apis mellifera*)	Seed treatment of sunflower at 339 g a.i./100 kg seed	7 d	Three tunnels	Mortality, foraging activity, flying intensity, behavior, colony condition (number of bees, brood, presence of queen)	None	Residue data not provided. Limited observation period.	Supplemental 50781601
Thiamethoxam (Cruiser 350 FS) (*Apis mellifera*)	Seed treatment of oilseed rape	28 d	Three tunnels	Mortality, flight activity, colony condition, bee brood development	None	No residues were collected	Supplemental 50781604

Open literature

This section summarizes the Tier II (i.e., tunnel and feeding studies) studies that were evaluated from the open literature in addition to those listed for clothianidin (Section 4.2.1) as part of the aforementioned joint review between EPA, PMRA, and CDPR. Many studies consider both thiamethoxam and clothianidin, so overall open literature studies in both sections are considered in the weight-of-evidence for both chemicals. As noted previously, all studies are determined to be of qualitative utility for characterization purposes in this assessment. The limitations discussed below generally add enough uncertainty to warrant a qualitative use of these studies in characterizing the potential for adverse effects from exposure to thiamethoxam and/or clothianidin. These limitations are considered when deciding the weight to give each study in the overall risk conclusions

Henry et al. 2012 monitored individual freely foraging honeybee homing behavior using radio frequency identification (RFID) tagging technology in four separate treatments versus control. There were varying degrees of bee familiarity with the release site, the distance from the release site to colonies, and the type of landscape. Foragers received a single sublethal oral dose of thiamethoxam (1.34 ng/20 µL sucrose) and were released (at different distances from the hives) and assessed for mortality, homing ability for 5 to 7 days post-treatment. The study provides evidence that bees treated with thiamethoxam had fewer returning to their colonies. There were significantly lower proportions of bees returning to colonies compared to the controls control when released 1 km away from either a familiar or random location; however, the variability in the study results fails to convincingly demonstrate/equate return frequency to mortality.

Kessler et al. 2015 examined forager honeybees collected at colony entrances; newly emerged adult workers were also collected from brood comb. Cohorts of 25 bees were placed in rearing boxes and five feeding tubes were provided: (1) one with deionized water; (2) two with 1M sucrose; (3) two with 1M sucrose containing a specific concentration of a neonicotinoid (either thiamethoxam or clothianidin). The number of bees alive in each cohort was counted and food consumption determined 24 h later. The total food consumption of forager honey bees was significantly reduced only when bees fed from solutions containing 100 nM (0.00107 µg/bee/day) or 1000 nM (0.0103 µg/bee/day) thiamethoxam or clothianidin (0.00108 µg/bee/day; 0.0085 µg/bee/day respectively).

Thomazoni et al. 2009 performed a greenhouse study (conducted 2006-2007) with cotton (cultivar FiberMax 933) plants in containers were sprayed with thiamethoxam at a rate of 400 L/ha (200 g a.i./ha) at the flowering stage (≈ 50 – 55 days after germination). Spraying was done at 9:00 AM at 29ºC with 68% relative humidity. The experiment consisted of randomized blocks, with six treatments (different chemicals) and four replicates per treatment. Each plot consisted of a pot containing four plants/pot and 30 adult worker honey bees (about 5-6 days old) and confined in gauze cages 98.5 × 41cm. Spraying with thiamethoxam resulted in 100% mortality at 5.5 hrs.

Table 14. Tier II Open Literature Studies for Apis involving thiamethoxam (THX).

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed – (Statistical analysis conducted – Yes/No)	Effects[2] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Thiamethoxam (NR)	Treated sucrose (1.34 ng/20 µl; 1.34 ng a.i/bee; 1.15 ng c.e./bee (estimated))	Single Dose (RFID tracked)	Varied degree to which the bees were familiar with the release site (Experiments 1 vs 2) the distance from the release site to colonies (Experiments 1 vs 3), or the type of landscape (Experiments 2 vs 4). Experiment 1; bees released 1 km from their colonies Experiment 2: six groups of bees released 1 km from colony at equally spaced random sites at 1- km boundary [circumference].	Lower- and upper-bound estimates of mortality based on homing frequency (Yes)	Experiments 1 and 2, 10.2% and 31.6% of treated bees failed to return to their colonies, respectively; Homing frequency was significantly (p<0.001) lower in treated bees that were unfamiliar with their release site compared to bees that were familiar with their release site Experiment 3 homing failure was reduced compared to Experiment 1 but was still significant (P<0.003) Experiment 4: homing failure was significant (p<0.029)	Purity of test chemical not reported, lack of information about potential exposure to other chemicals in the area. Uncertain of return results to specific colonies.	Supplemental (qualitative) Henry *et al.* 2012 (E159247)

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed – (Statistical analysis conducted – Yes/No)	Effects[2] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Thiamethoxam (NR). And other insecticides	Spray 200 g a.i./ha (thiamethoxam) (171 g c.e./ha)	Greenhouse study conducted on cotton, November 2006 - January 2007 in Brazil	Randomized block with six treatments and four replicates per treatment Number of cotton plants maintained in a pot per plot: 4. Number of A. *mellifera* adult workers per pot per gauze cage (98.5 x 42 cm): 30.	Mortality	Spraying with thiamethoxam lethal for A. *mellifera* causing 100% mortality 330 minutes	Effects on the behavior of the honey bees after treatment application were not documented. No access to the raw data to confirm statistical analyses	Supplemental (qualitative) Thomazoni *et al*. 2009

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed – (Statistical analysis conducted – Yes/No)	Effects[2] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Clothianidin (NR), Thiamethoxam (NR), Imidacloprid (NR)	Treated sucrose: COD (0.07, 0.647, 5.28 and 10.3 ng/bee /24 h) corresponds to 1, 10, 100, and 1000 nM; IMI (0.064, 0.418, 3.98 and 13.9 ng/bee) corresponds to 1, 10, 100 and 1000 nM ; THX (0.105, 1.05, 10.3, 33.6 ng/bee) corresponds to 1, 10, 100, and 1000 nM).	Behavioral two-choice assays: 24 h Honey bee antennal and mouthpart assays: not stated 3. Electrophysiology experiment: 2 s	Three experiments: Behavioral two-choice assays: Honey bee antennal and mouthpart assays: 3. Electrophysiology experiment	Food consumption, survival; The feeding reflex (proboscis extension reflex, or PER); Electrophysiological recordings from taste neurons (Yes)	The authors report honeybees did not avoid concentrations occurring in the range of 1 – 100 nM and the highest concentrations of thiamethoxam and clothianidin tested (1 µM) significantly reduced their survival; Proboscis extension or retraction was not affected; Stimulation with imidacloprid thiamethoxam or clothianidin did not elicit spikes from any of the neurons in the galeal sensilla honeybees or spiking activity of sucrose- sensitive gustatory	Feeding was conducted under stressful conditions.; Choice oral tests were conducted not according to any official protocol; PER and electrophysiology studies are very artificial, conclusions should be made cautiously on this type of lab based experiments where only part of a bee are examined and not the whole (l*et a*lone at the colony level)	Supplemental (qualitative) Kessler *et al.* 2015

Tier II studies - Non-Apis

There were 3 Tier II registrant-submitted studies and 5 open literature studies considered to characterize the colony-level effects on bumble bees (*Bombus* spp.) to thiamethoxam (**Table 4.29**). As with the higher-tier *Apis* open literature studies, exposure duration, concentrations tested, and endpoints assessed varied across these studies, and many of the same limitations are noted.

Some *Bombus* studies are conducted with microcolonies. Microcolonies are queen-less units of a few worker bumble bees where one individual eventually becomes dominant and starts laying unfertilized eggs (*i.e.*, males).

Registrant Submitted Studies

Two studies submitted by Reber (PMRA#s 2364898 and 2364900) looked at effects on bumble bee colonies (B. *terrestris*) in tents following drip or foliar application of thiamethoxam. Colonies were placed in tents immediately after application (Actara™ 25 WG foliar rate 100 g a.i./A; drip irrigation rate – 150 g a.i./A). While overall there were no differences between treated and control groups for foraging activity or behavior following drip irrigation, there were significant ($p<0.05$) reductions in bees foraging activity in the treated group following foliar applications. The foliar application noted affected bumble bees exhibited irritation, erratic motions, were paralyzed and in a dorsal position before dying or that affected bumble bees were hanging on the tomato leaves and died afterwards.

A study submitted by Balluf (2001) looked at foliar applications of 0.1 kg a.i./ha with split applications and different time intervals (21/14 and 9/2 days before exposure). The study did not find any effects of either treatment on mortality, foraging activity, food consumption, or growth of bumble bee colonies.

Open Literature

Mommaerts et al. (2010) examined the effects of Actara™ 25 (25% TGAI) to bumblebees (Bombus terrestris) from oral exposure in sugar water for 11 weeks, under two different conditions in the laboratory, i.e., considering and not considering foraging behavior. Worker bumblebees (four artificial nests each with 5 bumblebee workers per treatment) were exposed to thiamethoxam at concentrations ranging from ranging from 0.01 to 100 ppm via ingestion of spiked sugar water; bees were evaluated for survival, nest development and reproduction (drones produced), and foraging behavior. Increased (compared to control) worker mortality was noted in the thiamethoxam treated groups, and the nests exposed to 100, 10, 1.0 and 0.5 ppm thiamethoxam showed a total loss of reproduction, while at 0.1 ppm the numbers of drones were significantly ($p < 0.05$) lower than the controls with no difference observed at 10 ppm. Some of the limitations of the study included a lack of quantification of test material, potential stress from the study design, and a lack of information on the control group.

Alacrcon et al. 2005 examined effects to bumble bee (Bombus terrestris L.) colonies (30 recently born or just born workers, a queen, and pupae) from thiamethoxam (Actara®) applied through drip irrigation at a rate of 200 g a.i./ha and as a split application (100 g a.i/ha) of the same total rate, and compared to imidacloprid (toxic standard) applied as foliar at 15 g a.i./ha. Treatment took place 2 days after the colonies were introduced into greenhouse containing the treated tomato plants (hives were closed and opened after application). Two consecutive trials (3/9/14-4/26/14 and 4/27/14-6/7/14) were made on the same tomato crop. The duration of each trial was 6 weeks. There were no effects for mortality, foraging activity, or pollination rates in both trials between the treated and control plots. Visual evaluation of the data suggests effects (lower adult, larvae and pupae counts) in the treated hives compared to the control

with more pronounced effects seen from one drip application of 200 g a.i./ha compared to the two drip applications of 100 g a.i./ha each. However, there was poor control performance (especially in the second trials) relative to the reference toxicant adding considerable uncertainty to the results.

Sechser and Freuler 2003 (MRID 49579001) examined the effects of a thiamethoxam drip irrigation application scenario to adult bumble bees (B. terrestris) and brood. Tomato plants within greenhouses (1 replicate) or tunnels (1 replicate) were treated with thiamethoxam at rates from 150 to 161 g a.i/ha (0.13 to 0.14 lb a.i/A). A single bumble bee colony was placed inside a greenhouse (1) or two colonies in a tunnel (1) and bees freely foraged on tomato plants as well as supplemental bumble bee food (nectar) and pollen that were provided within the greenhouses. After 13 to 35 days of exposure, there were no differences between the hives exposed to thiamethoxam and the negative controls. However, limitations included no replication within treatment groups, high variability in the results, and exposure uncertainties (rate of uptake into pollen and nectar and/or lack of measurement to confirm exposure).

Elston et al. 2013 (MRID 49579002) examined the effects on nest building or brood production from dietary exposure of thiamethoxam (or propiconazole) in B. terrestris microcolonies. Bees were exposed for 28 days to thiamethoxam concentrations of 1 or 10 µg/kg in honey water and pollen paste. For thiamethoxam, both dietary exposures reduced consumption of honey-water and the number of wax cells ("honey pots"). At the 10 µg/kg treatment, nest building initiation was delayed, fewer eggs were laid, and no larvae were produced.

Laycock et al. 2014 used microcolonies of B. terrestris L. Workers were exposed to a wide range of dietary concentrations up to 98 µg/kg in syrup for 17 days while also feeding clean pollen. Bumblebee workers survived fewer days relative to controls when presented with syrup at 98 µg/kg, while production of brood (eggs and larvae) and consumption of syrup and pollen in microcolonies were significantly ($p<0.05$) reduced by thiamethoxam only at the two highest concentrations (i.e., 39 and 98 µg kg-1).

Stanley et al. 2015 investigated how exposure to thiamethoxam could affect the ability of bumblebees to pollinate apple trees. Colonies were pre-exposed to thiamethoxam at 0, 2.4 or 10 ppb in artificial sugar water for a period of 13 days (8 colonies per treatment). Afterward, treated colonies were brought to the field, allowed access untreated apple trees, and observations were collected at both the individual- and colony-level behavior. The study authors reported that in the 10 ppb treatment there were lower visitation rates to flowers and lower numbers of bees carrying pollen compared to controls ($p = 0.05$, and 0.008 respectively), in addition to suggesting that thiamethoxam exposure altered how bees behave on flowers.

Stanley and Raine 2017 investigated colony growth of bumblebees by exposing *Bombus terrestris* colonies (via treated sucrose for 27 days) to 2 levels (2.4 and 10 ppb) over 4 weeks and observed them in the lab. The study author's reported no impact of insecticide exposure on colony weight gain, or the number or mass of sexuals produced, although colonies exposed to 2.4 ppb thiamethoxam produced fewer males (this difference was not statistically significant) that were larger than those in the control or 10 ppb exposure group.

Stanley et al. 2016, investigated the impact of chronic exposure (5–43 days) to field-realistic levels of a neonicotinoid insecticide (24 ppb thiamethoxam) on foraging ability, homing success and colony size using radio frequency identification (RFID) technology in free-flying bumblebee colonies. Pesticide treatment colonies received a feeder of 40% sucrose solution in the external chamber that contained approximately 2.4 ppb thiamethoxam. The author's reported individual foragers from pesticide-exposed colonies carried out longer foraging bouts ($P<0.05$) than untreated controls (68 vs. 55 min). Pesticide-exposed bees also

brought back pollen less frequently ($P<0.05$) than controls indicating reduced foraging performance, while no overall impacts to colony size were found relative to the control.

Baron et al. 2017 took wild caught bumblebee queens from 4 species to examine effects of field realistic exposure to thiamethoxam (1.9-5.3 ppb). Queens were fed for 14 days, and observed for 14 days after for signs of mortality, waxing behavior and egg laying as well as ovary development. The authors reported exposure to 5.3 ppb of thiamethoxam resulted in feeding reduction in 2 species ($p<0.05$). No impacts were reported to egg laying; however, it was noted a low number of queens laid eggs during the experiment.

Table 15. Tier II studies characterizing the toxicity of thiamethoxam to non-Apis colonies.

Open Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[2] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Registrant Studies							
Thiamethoxam Actara 25 WG (25%) (*Bombus terrestris* L)	Foliar application (1 hand sprayer 100 g a.i./ha; 85.6 g c.e./ha) Fed 50% sugar solution inside the hive ad libitum	Single Foliar Application (28 days 6/18/98-7/16/98)	Tomatoes (10-12 week old plants, first flower stage), 2 plants per pot (35 cm diameter, 20 L volume) and 16 pots per tent. bumble bee hives placed in tent immediately following application. three rep/trt	Pollination activity, behavior, mortality, and vitality (1, 2, 4, 7 days post trt then 2-3 days until endo of exposure.)	Reduced pollination activity (2 weeks after exposure) Effects on behavior (irritation, uncontrollable motions, paralysis). High mortality. Eggs and larvae could not be monitored since there were no larvae or eggs present at study termination in any treatment group.	Initial number of bees in the hive and/or an estimate of the total number of bees in a hive throughout the experimental duration is not reported The hives were within the same treatment area, which represent repeated measures and not true replicates. It is uncertain what the residues were in the pollen	Supplemental Reber 1999a PMRA 2364900
Thiamethoxam Actara 25 WG (25%)	Foliar application (hand sprayer 150 g a.i./ha; 128 g c.e./ha)	Single Foliar Application (28 days 6/18/98-7/16/98)	Tomatoes (10-12 week old plants, first flower stage),	Pollination activity, behavior, mortality, and vitality (1, 2, 4, 7	No effects on pollination activity of bumble bees.	The hives were within the same treatment area, which represent repeated	Supplemental Reber 1999b

Open Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed – (Statistical analysis conducted – Yes/No)	Effects[2] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
(*Bombus terrestris* L)	Fed 50% sugar solution inside the hive ad libitum		2 plants per pot (35 cm diameter, 20 L volume) and 16 pots per tent. bumble bee hives placed in tent 1 day prior to application. three rep/trt	days post trt then 2-3 days until endo of exposure.)	No effects on behavior and repellency. Overall, the mortality was high in all test groups Eggs and larvae could not be monitored since there were no larvae or eggs present at study termination in any	measures and not true replicates. It is uncertain what the residues were in the pollen	PMRA 2364898
Thiamethoxam Actara 25 WG (25%) (*Bombus terrestris* L)	Foliar application (2 X hand sprayer 100 g a.i./ha; 85.6 g c.e./ha) Fed 50% sugar solution inside the hive ad libitum	TRT 1: Tomatoes, BBCH growth stage was 21 (first primary apical shoot visible) to 29 (nine or more apical shoot visible) TRT 2: 51 (first inflorescence visible) to 62 (second inflorescence first flowers to open) for treatment 2 scenario (27 days)	4 reps/trt Initiated 10/31/00 ad 11/1/00 TRT 1: Application 21 and 14 days before hives TRT 2: 9 and 2 days before hives	Food consumption, Colony weight, Mortality, Foraging activity, Brood	No effect of either treatment scenario on mortality, pollination, consumption of sugar, growth of colonies or brood.	It is uncertain what the residues were in the pollen Uncertain if control group mortality is high	Supplemental Balluf 2001 PMRA 2364997

Open Literature							
Thiamethoxam Actara 25 WG (25%) *Bombus terrestris* L	Treated sugar water (100 µg a.i./L; 85.6 µg c.e./L)	4 colonies with 5 workers each housed in cages for 11-week exposure	Bees were exposed orally to pesticides via treated sugar water in box plain sugar water and no worker mortality was observed after 11 weeks	mortality, drone production -- (Yes)	85% of worker toxicity were observed, significant sublethal effects ($p<0.05$) as the drone production was very low	There was no analytical confirmation of thiamethoxam in the treatment solutions Control performance was not reported	Qualitative Mommaerts, 2010 48151502

Thiamethoxam Actara 25 WG (25%) *Bombus terrestris* L	Greenhouse 1 app Drip irrigation (200 g a.i./ha; 171 g c.e./ha) Split app drip irrigation (100 g a.i./ha; 85.6 g c.e./ha) Fed 50% sugar solution inside the hive ad libitum	Split application (100 g a.i/ha 7 days apart 3/11, 3/18/04 - 4/26/04). Single application (200 g a.i./ha - 3/11/04 - 4/26/04).	1280 m^2 with four plots each measuring 320 m^2 (40 x 8 m) were used	Pollination, Mortality, Food consumption, Brood production, Colony strength	Single app: Significant (p<0.1) mortality Split app: No sig differences observed	Control hives performed worse than reference toxicant Statistical analysis was conducted for the pollination rate but there was no mention on what method of statistical analysis was used. Raw data was not included in the study.	Qualitative Alarcon *et al.* 2005
Thiamethoxam (TGAI) *Bombus terrestris* L	Artificial nectar solution and pollen paste (1, 10 µg a.i./kg; 0.86, 8.6 µg c.e./kg;)	28-day exposure	4 trt (including neg and solvent control) 10 reps replicates in each:	Worker mortality, nest- building activity, egg laying, and bee behavior	reduction in nectar consumption and storage Delayed colony development Fewer eggs/larvae/was cells (10 µg/kg) and reduced nest building	No verification of test substance in pollen or nectar No Raw data to confirm statistical conclusions	Qualitative Elston *et al.* 2013 49579002
Thiamethoxam NR	Treated sugar solution with untreated pollen	17-day exposure	Queenless microcolonies of *Bombus terrestris* L.	Worker mortality, wax covered egg cells, brood	Reduced survival (98) and reduced brood production and food	Bumblebees may forage on mass-flowering crops	Qualitative Laycock *et al.* 2014
Unspecified (% unspecified) *Bombus terrestris*	2.4 ppb ai (nominal) (2.1 ppb c.e.) 1.74-2.34 ppb ai (1.49-2.00 ppb c.e.)	43 days (*foraging*: starting day 5 of exposure to ca. exposure termination;	8 colonies (4/level) located in lab with unrestricted access to forage on flowers outside	*Foraging activity:* # drifters/colony, # days foraged/bee, # foraging bouts/day/bee, #	Increased foraging trip duration per bee per day and proportion of bees that returned when	Single treatment level No negative or positive controls	Stanley et al (2016)

	(range measured in 3 samples) Sucrose solution and acetone	*homing*: starting after 2 weeks of exposure to ca. exposure termination; *colony growth*: test initiation to exposure termination	Exposed in lab to spiked sucrose solution (replenished 3 days/week) One treatment level and one solvent control	visits/day/bee, foraging trip duration/day/bee, # foragers/colony, # foragers retuning to colony, proportion of bees carrying pollen/colony *Homing ability:* Proportion of bees returned from 1 or 2 km away/colony, time taken to return 1 or 2 km/bee, proportion of bees that returned overall/colony, time taken to return overall/bee *Colony growth:* # callows emerged/colony, # dead bees/colony, # dead bees that did not return/colony, colony size, body length/bee -- (Yes)	released 1 km from their nest per colony Decreased proportion of bees that returned carrying pollen per colony No statistically significant differences on colony growth or additional measured variables related to foraging activity and homing ability	Unclear how much sucrose was consumed and therefore unknown actual exposure (per day and cumulative) Analytical measurement of only 3 samples to confirm exposure concentration Potential exposure to other pesticides in surrounding landscape, which is multi-purpose use. No screen of pollen returned by bees for potential exposure to other pesticides Single trial Foraging activity and homing ability analyses represented a range in duration of exposure	

Bombus terrestris L	(dosages s (µg/kg thiamethoxam: 98.43, 39.37, 15.75, 6.30, 2.52, 1.01, 0.40, 0.16, 0.06)		two replicate trials between October and December 2012. Each trial comprised 50 microcolonies	production (eggs/larvae)	consumption (39, 98)	throughout their bloom (> 17 d exposure) Multiple sources of bees used No Raw data to confirm statistical conclusions	

Thaimethoxam (B. terrestris)	Tomato plants within greenhouses (1 replicate) or tunnels (1 replicate) were treated with thiamethoxam at rates from 150 to 161 g a.i/ha (0.13 to 0.14 lb a.i/A; 0.11 lb c.e./A to 0.12 lb c.e./A).	13-35 d	Bees kept in tunnels or greenhouses	Number of larvae, adults, amount of pollen and nectar	None	no replication within treatment groups, high variability in the results, and exposure uncertainties (rate of uptake into pollen and nectar and/or lack of measurement to confirm exposure)	Supplemental Sechser and Freuler 2003 (MRID 49579001)
Analytical standard (% unspecified) *Bombus terrestris*	2.4 and 10 ppb ai (nominal, v/v) (2.1 and 8.6 ppb c.e.) Sucrose solution and acetone	26-27 days (39-41 days; bees monitored an additional 13-14 days after exposure period; colony weight measured weekly and other measures at test termination)	24 mature colonies (8/level) located in lab Each day, three colonies (1/level) began treatment for 8 consecutive days Exposed in lab to spiked sucrose solution (replenished daily) and an equal amount of untreated pollen Two treatment levels and one solvent control	Colony weight # bees (worker, male, queen) Dry weight/bee (worker, male, queen) Total biomass/colony (workers, males, queens) -- (Yes)	None	Two treatment levels No negative or positive controls "*All sucrose solutions were actively consumed*". It is unclear if this indicates that each colony consumed the entire sucrose solution each day. No analytical measurements to confirm exposure concentration Did not screen pollen for pesticides Single trial	Stanley and Raine (2017)

Thiamethoxam (NR) *Bombus terrestris* audax	Treated Sucrose 2.3 and 10 ppb a.i. (2.0 and 8.6 ppb c.e.)	12-15 days (60 mins for 8 days and at test termination)	24 colonies (avg 99 workers) 8/trt exposed to thiamethoxam Brought to apple orchard and observed for pollination services	Entry/exit from colony boxes, bees carrying pollen, flower visitation rate	In 10 ppb lower visitation rates to flowers and lower number of bees carrying pollen	Conservative exposure scenario (not representative of field-level) Orchard details left out No analytical verification text concentrations	Qualitative Stanley *et al*. 2014
Thiamethoxam analytical standard (% unspecified) *B. terrestris, B. lucorum, B. pratorum, B. pascuorum*	Solvent control, 1.87, 5.32 ppb ai (measured) (1.60 and 4.55 ppb c.e.) *Slight contamination in control (0.063 ppb ai)	2 weeks (4 weeks total: 2 exposure followed by 2 post- exposure)	Spring-caught wild queens from a site with known pesticide use 39-50 bees per level; however, fewer were used for analysis: some bees escaped and bees were excluded if found to be infected with parasites. B. lucorum (5-12 bees used in analysis); B. pascuorum (15-17 bees); B. pratorum (15-22 bees); B. terrestris (32-35 bees) Spiked sucrose syrup solution + pesticide-free commercial pollen pellets)	Oocyte length, feeding, survival, waxing behavior	B. terrestris: NOAEC = 1.87 ppb ai LOAEC = 5.32 ppb ai based on reduced length of terminal oocytes No effects on feeding, survival, or waxing behavior B. lucorum: NOAEC = 1.87 ppb ai LOAEC = 5.32 ppb ai based on reduced length of terminal oocytes No effects on feeding, survival, or waxing behavior B. pratorum: NOAEC = 1.87 ppb ai LOAEC = 5.32 ppb ai based on	No negative control (solvent control only) or positive control	Baron et al (2017)

					reduced feeding and length of terminal oocytes No effects on survival, or waxing behavior B. pascuorum: NOAEC = 1.87 ppb ai LOAEC = 5.32 ppb ai based on reduced feeding and length of terminal oocytes No effects on survival, or waxing behavior		

Tier III

The following section describes the Tier III studies either submitted by the registrant or identified in the open literature. Studies considered below and with those listed for clothianidin are considered when evaluating the potential for adverse effects from exposure to thiamethoxam.

Registrant submissions

There are thirteen registrant-submitted Tier III field studies on various crops submitted (**Table 16**). These studies are generally considered to be of limited utility in risk assessment based on the strength of their design and resulting effects. Five honey bee field studies, which are classified as supplemental, including Szentes 2001a (46163102), Szentes 2001b (46163103c), Frana 2003 (46241601) Balluf 2003 (46163103a), Schur 2001 (46163103b) were conducted in Hungary, Argentina, Spain, and Italy and examined the effects of thiamethoxam-treated sunflower seeds on honey bee colonies: mortality, foraging behavior, overall behavior, and colony strength. Two studies were conducted with CRUISER® 70 WS (70%) and three studies were conducted with CRUISER® 350 FS (30-35%). Overall, transient effects were seen on mortality mostly after application with no treatment-related effects detected on brood or adult foraging. Two submitted studies were conducted in France using oilseed rape over 4 years (Hecht-Rost 2009 48053301 and Hecht-Rost 2009 48053302) with no statistically significant effects detected on brood development, and only statistically significant ($p<0.05$) effects noted on honeybee mortality dependent on specific years in the multi-year studies. A study (Mayer 1998 44714929) was conducted on apple orchards in 1998 and again noted no effects relative to control plots.

Additional studies were also submitted on pome fruits, oil-seed rape, and melon. These studies exposed honey bees to treated orchard crops: 44714929, 5076602, and 50766604 (apples), and 48584701 (pears). No effects were detected on bee mortality, flight activity, behavior and brood from pre-bloom or post bloom (via available residues) applications to apple trees treated with 100 to 200 g a.i/ha. No effects were detected on bee mortality from pre-bloom application to peach at 62.5 g a.i/ha (with declining residues over time) when applied 15 days before bloom but higher mortality and reduced foraging activity from pre-bloom application were identified when applied 7 days before bloom. No effects were detected on bee mortality or foraging activity from pre-bloom application to pear at 95 g a.i/ha 5, 8 or 11 days before bloom. However, statistically significant higher mortality (relative to controls) was observed from pre-bloom applications at 1 and 3 days before bloom.

Common limitations noted in these studies include uncertainty of exposure and the origin of the pollen and nectar brought back to the hives, high variability in the data collected (including in control hives), and lack of suitable replication or pseudo-replication. Additionally, close proximity between control, treatment, and both control/treatment plots may have resulted in cross foraging, and intrinsic to field studies, availability of alternate forage which are also uncertainties.

Table 16. Thiamethoxam Tier III Registrant-submitted Studies for Apis.

Test Substance Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects2 (all comparisons made relative to the study's control)	Limitations	Classification Citation (MRID Number)
Thiamethoxam 25.2% (*Apis mellifera*)	Application to apples	Application 4-7 days prior to bloom at 0.04 lb c.e./A (16 days Mortality – daily all days Foraging – 8/16-8/23) Hive strength 8/4, 8/24	Apple (Malus sp.) orchard located in the Yakima Valley of South Central Washington State. Each plot (replicate) measured between 0.24 and 0.40 ha Treatment and control plots were separated by at least 305 m (1000 ft.). 3 colonies per treatment, distributed in the orchards on day 0 after the application.	Mortality, foraging, brood development (Yes)	No abnormal effects on mortality/foraging or hive strength found	# Bees/Colony not provided Uncertainties in the pooled control origins used for comparisons by the study author	Supplemental Mayer 1998 (44714929)
Thiamethoxam 35% (*Apis mellifera* carnaca)	Thiamethoxam Treated Sunflower (*Helianthus annuus*), seed	Application 0.016 lbs a.i/A Observations: Mortality/Foragin/behavior – daily all days Hive strength 6/22/00, 7/4/00 (12 days)	Fields located in Tolna, Hungary 15 hives/trt Plots were 63.2 h (C); 155.0 Ha (trt) with 8000 m between	Mortality, foraging, brood development, residues honey/nectar/pollen and flower heads (No)	No treatment effects mortality, foraging, behavior, or brood development observed	No replication (1 control/ treated field) Lack of pollen analysis to confirm foraging on treated field	Supplemental Szentes 2001a (46163102)

Thiamethoxam 30% (*Apis mellifera* L.)	Thiamethoxam Treated Sunflower (*Helianthus annuus*), seed	Application 0.02 lbs c.e/A Observations: Mortality/Foraging/behavior – day 2-12 Hive strength day 0 and day 13 (12 days)	Fields located in Hungary Control fields were 4.5/15 ha; treated field was 4.5 ha. 15 hives/trt	Mortality, foraging, brood development, residues honey/nectar/pollen and flower heads (No)	Mortality higher up to 7 days post treatment. No apparent treatment effects on behavior, brood development, colony strength	No replication (2 control/ 1 treated field) Lack of pollen analysis to confirm foraging on treated field Attractive melon fields were close 500-1500 m to treatments/control Data were not reported for each hive.	Supplemental Szentes 2001b (46163103c)
Thiamethoxam 34.8% (*Apis mellifera* L.)	Thiamethoxam Treated Sunflower (*Helianthus annuus*), seed	Application 0.006 lbs c.e/A Observations 2/21/01-4/11/01: Mortality/Foraging/behavior – daily Hive strength day 3, 13, 49 days after treatment (9 days mortality/behavior 49 days brood)	Fields (>2km apart) located in Santa Fe, Argentina, 20,448-22050 m2 6 hives/trt	Mortality, foraging, brood development, residues honey/nectar/pollen and flower heads (No)	No treatment effects on honey bee mortality, foraging, behavior, and brood development	Thiamethoxam were detected in the control pollen No replication (1 control/treated [and 1 reference] field)	Supplemental Frana 2003 (46241601)

Thiamethoxam-282 g/L Fludioxonil-8.00 g/L Mefenoxam-33.4 g/L (*Apis mellifera* L)	Seed Treated (A9807C) Oilseed Rape (0.03 lb c.e./A maximum)	4-year study. Observations during first 9 days of exposure and thereafter 2-3 days through the end of exposure (21 days) Brood development assessed at start of exposure and approx. 7 days thereafter	Alsace France at different locations in each year. 2-3 ha, fields separated by 1.8 to 7.5 km. with 6 colonies per control/treatment fields. colonies were set-up and maintained at the exposure location until the end of the flowering period. Colonies were then relocated to their monitoring and over-wintering location (forest near Hegency, France).	Mortality, foraging activity, behavior of the bees daily during Brood development	In 2006, control mortality > treatment mortality (t = 3.66, p = 0.005). No other significant treatment related effects	Lower application rate (and variable over the 4 years) than the highest labeled rate. Different control seed treatments in different years and in different places Limited residue/pollen analysis (LOD not reported)	Supplemental Hecht-Rost 2009 48053302
Thiamethoxam-Cruiser WS 70 (70%) *Apis mellifera* L)	Thiamethoxam Treated Sunflower (*Helianthus annuus*), seed	Application 0.02 lbs c.e/A Mortality/Foraging/behavior – daily (16 days) Brood assessments day 1, 10, 19, and 48 days after treatment (16 days mortality/behavior 48 days brood)	Fields located in SW Spain Fields were ~40000m2 and 3.7 miles apart 6 hives/trt Reference chemical trt imidacloprid	Mortality, foraging activity, behavior of the bees, brood development as well as residues in sunflower blossoms, honey, pollen, bee honey, stomach	Increased mortality and flight intensity 5, 6, and 7 days after treatment No treatment effects on behavior, colony strength, the queen, or brood development	No replication (1 control/treated [and 1 reference] field); Short observation period	Supplemental Balluf 2003 (46163103a)

Thiamethoxam-Cruiser WS 70 (70%) *Apis mellifera* L)	Thiamethoxam Treated Sunflower (*Helianthus annuus*), seed	Application 0.02 lbs c.e./A Mortality/Foraging/behavior – daily (10 days) Brood assessments at 2, 9, and 40 days after treatment (10 days mortality/behavior 49 days brood)	Fields located in Central Italy, Fields were ~20000m2 and > 1.2 miles apart 6 hives/trt Reference chemical trt imidacloprid	Mortality, foraging activity, behavior of the bees, brood development as well as residues in sunflower blossoms, honey, pollen, bee honey, stomach	Increased mortality days 7 and 8 and flight intensity day 8 significant reduction in the number of capped cells No treatment effects on behavior, colony strength, the queen, or most of brood development.	No replication (1 control/treated [and 1 reference] field); residues of Thiamethoxam were detected in one control pollen sample Short observation period	Supplemental Schur 2001 (46163103b)
Actara 25 WG (25.0%) *Apis Mellifera*	Pre-bloom application to pears	1 pre-bloom application at different times (6 TRTS) at 0.07 lb c.e./A observed for 14 days	1 control and 6 treatments. Hives placed in plots 10 acres with 40 trees and 10 /replicate Application 11, 8, 5, 3, and 1 day before bloom	Mortality, foraging, colony strength	Statistical differences in mortality when sprayed 3 and 1 days prior to bloom; author notes there was high variability regardless of treatment group	One plot per treatment No residue analysis was conducted Treatment plots were next to each other (potential cross contamination)	Supplemental 48584701
Actara 25 WG (25.0%) *Apis Mellifera*	Drip irrigation to honeydew melon plants	1 application at 0.15 l.b. c.e./A observed for 14 days	Two treatment (T1/T2) fields, one control, one reference 2 km apart 4 hives/field T1 – treated 1 wk after planting T2 – treated during bloom	Mortality, foraging activity, colony condition, hive weight, behavior (Yes)	No significant effects on colonies noted. Transient mortality effects in treatments on Day 2 after application	T1 and T2 were different application scenarios as well as reference (foliar). No residues were taken to confirm exposure/no melon pollen was found in pollen traps	Supplemental 50766601

Actara 25 WG (25.0%) *Apis Mellifera*	Post-bloom application to apples (*Malus domesticus*)	2 post bloom apps 7 days apart @ 0.08 lb c.e./A 21 days in field observed for 8 weeks post application	Application was made during bee flight after fruit fall and when frit was 10-20mm 1 trt and 1 control field 5 km apart 4 hives/field	Mortality, foraging activity, colony condition, hive weight, behavior (Yes)	No significant effects on colonies noted.	Although used in accordance with the label directions, post bloom applications mostly limits exposure to contact only in during the 21 days in the field	Supplemental 50766602
Actara 25 WG (25.0%) *Apis Mellifera*	Post-bloom application to apples (Gala, Elstar)	1 post bloom application at 0.08 lb c.e./A 14 days (mortality behavior) in the field and observed for 29 days (colony condition)	One treated field (no control) with 4 hives Sprayed during bee flight	Mortality, foraging activity, colony condition (Yes)	. No significant effects on colonies noted. No effects on mortality during exposure.	Although used in accordance with the label directions, post bloom applications mostly limit exposure to contact only. Adjacent fields had also flowered, so forage distance as likely high (and would also limit drift exposure). No control for comparison limits interpretation of results	Supplemental 50766604

Thiamethoxam WS (70.1) *Apis Mellifera*	Seed treatment to oilseed rape (*Brassica napus*)	Oilseed was sown at 0.02 lb c.e./A 11 days for mortality, behavior) and observed for 46 days (colony condition)	One control and one treated field w/6 hives/field during full flowering	Mortality, foraging activity, colony condition, hive weight, behavior (Yes)	No significant effects on colonies noted. Increased morality during exposure period Decreased hive weight	Mortality was higher in control than treatment for one observation period (attributed to robbing by study author). No residue analysis to confirm magnitude of exposure (pollen analysis confirmed foraging on treated field)	Supplemental 50766603

Open literature

Two additional field-level studies were evaluated from the open literature (Table 4.33). Both were determined to be qualitative in nature, as it was uncertain if the test designs were robust enough to evaluate treatment effects.

Thompson et al. 2016 used RFID tags on free-foraging honeybees to evaluate survival and foraging/homing activity at varying distances from either untreated winter oilseed rape or winter oilseed rape grown from seed treated with thiamethoxam (as Cruiser™ OSR). There were no obvious trends (the data were not amenable to statistical analysis) between the control and treated groups across the three tested distances from the fields; however, visual observation indicates colonies located within 1 km from treated fields may be more likely to be impacted (decreased mean foragers life span, total flying days, mean trip durations and mean total flying time per bee for foragers). Pollen was identified to family level, and there was uncertainty as to the actual proportion of oilseed rape pollen utilized by either the control or the treatment colonies, which may have influenced the ability of the study to detect treatment effects.

Tremolada et al. 2010 examined the effects on hives from exposure to residues from sowing operations with Cruiser®- and Celest® xl- treated corn seeds. The study indicated effects on honeybee mortality, during planting while control hives located 200 m away from the test site and protected by a vegetation barrier showed no apparent effect on mortality. The mortality observed in the control hives and the treatment hives were similar before sowing. The control hive mortality did not differ during the day of sowing; however, mortality in the treatment hives increated to >40 dead bees/day. Shortly after the sowing period, bee mortality in the exposure hives decreased back to about 10 bees/day. However, except for the day of sowing, the control hives had higher mortality on all other days compared to the treatment hives. There was also some indication of decreased foraging after planting after a (visually observed) decrease in number of foragers (9.3) compared to controls (23) was observed; however, the number of foragers recovered to pre-planting numbers. This study was comparatively short and measured more individual effects (from exposure during sowing operations) rather than brood development/colony effects (from foraging on treated corn pollen).

Table 17. Thiamethoxam Tier III Open Literature Studies for Apis

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[2] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Thiamethoxam (Cruiser®; 350 g L^{-1}) 47.6% Apis mellifera L	Seed treated winter oilseed rape (0.020 mg thiamethoxam/seed)	5-week exposure during flowering (16 May–20 June 2013). Foraging observations collected from 16 May - 20 June 2013; one disease	Frequency identification transponders (RFID tags) on free-flying honeybees (Apis mellifera L). 36 colonies used with 12 colonies per study field (2 control fields and 1 treated field), 3 apiary sites Study hives were located at the field edge (on-field site), approximately 500m (0.5 km site) or 1000m (1.0 km site) from the fields of oilseed rape.	lifespan and foraging/homing activity	No obvious trends reported between the control and treated groups across the three tested distances from the fields Results do suggest foragers farther away from treated field were affected (homing behavior, lifespan, and reduced foraging).	Lack of replication (1 treated field and 2 controls) Colonies placed at 20% bloom (treatment). Additional forage was possible diluting exposure Residue analysis unclear if robust enough to capture variation in a treated field	Qualitative Thompson et al. 2016

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[2] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Thiamethoxam (Cruiser®; 350 g L^{-1}) Apis mellifera L	Treated corn seed (7.35 g a.i./ha)	Sowing on 6/22/2008 (6 days of observation)	2 hives/treatments 4 hives/control agricultural farm in the south-east of Milan, Italy control hives placed inside the farm garden (approximately 200 m away from the treated fields). The exposure hives were located at the field hedge boundary of the test field	Direct mortality Foraging activity (Yes)	Greater mortality in the exposure hives the day of sowing – decreasing Shortly after significant effect of treatment (p=0.024) and time (p=0.020) on mortality. Foraging bees/minute reduced in both control/treated hive groups but more markedly in treated hives. significant effect of treatment and time (p<0.001 for both) on foraging	monitoring of honey bee mortality and foraging activity was limited due to weather conditions. Study duration short – Pollen/nectar carried by the bees back to the hives after foraging were not identified. Non-inclusion of raw data. Brood parameters no	Qualitative Tremolada et al. 2010

Tier III Effects to Bombus spp.

There were several Tier III studies to characterize the colony-level effects on bumble bees (i.e. various species of Bombus). There was one study available from a registrant submission and two from the open literature (Table 18). As with the higher-tier Apis open literature studies, exposure duration, concentrations tested, and endpoints assessed varied across these studies, and many of the same limitations are noted.

The registrant-submitted study by Wilkins 2014 (49589501) examined effects on bumble bees exposed to flowering rape grown from seeds which were treated with thiamethoxam and seeded at a rate equivalent to 0.02 lb a.i./A. This study included one treated field and two control fields, each with 25 bumble bee colonies (5-week exposure 3-week post exposure monitoring). The author reported no treatment-related colony failures (i.e., a total loss of adult bees or brood), and the mean number of queens produced per colony was similar between the three treatment groups: Control 1 (C1; n=23) contained 18.6 (range 1 to 60); Control 2 (C2; n=21) contained 17.9 (range 1 to 67); and Treatment 1 (Tl; n=22) contained 21.3 (range 1 to 88). The mean numbers of workers and drones produced by all colonies across the treatments were also similar: 54, 47 and 58 workers for C1, C2, and T1, respectively; and, a mean of 33, 34 and 32 drones per colony in C1, C2, and T1, respectively. The author reported that some colonies on site T1 appeared to be increasing in weight until Day 54 and had not started to produce queens, while the other colonies (C1/C2) decreased in weight beginning on Day 47.

For the open literature, Thompson et al. 2015 examined development of bumblebee (B. terrestris audax) colonies where bees had foraged for 5 weeks on flowering winter oilseed rape grown from seed treated with thiamethoxam (as Cruiser™ OSR) using two controls, one treated field. Colony development was evaluated by monitoring the colony mass, forager activity both at the hive and within the crop, and the extent of oilseed rape pollen stored within the colony was analyzed. This study reported an increase[6] in colony mass (13%) relative to controls. No statistically significant effects (see footnote) in foraging activity were observed. Numerically, higher mean numbers of queens/gynes, workers, eggs (2-3x), pupae, and larvae were noted in the thiamethoxam-treated fields. In 2014, Balfour et al. (2017) placed bumblebee colonies (36 per species) adjacent to three large oilseed rape fields (12 colonies per field) planted in 2013 with thiamethoxam treated seeds. Another 36 were in three nearby locations in the same agro-ecosystem, but several kilometers distant from any oilseed rape fields. The study authors report *Bombus* colony growth and reproduction were unaffected by location (distant versus adjacent) following the two month flowering period.

[6] The study author noted the pseudoreplication in the study design and uncertainties in statistical analysis.

Table 18. Thiamethoxam Tier III Registrant Submitted and Open Literature Studies for Bombus sp.

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[2] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Thiamethoxam – 0.03 mg a.i./seed *Bombus terrestris* audax	Treated Oilseed Rape Seed (0.02 lb a.i./A)	38-day exposure (Daily assessments for activity within crop)	Field test – 2 controls 1 treated field with 25 colonies/trt group ~2 ha fields drilled 10/6/12 0.02 lb a.i/A	Foraging activity, hive weights, post study hive dissection, pollen analysis	Similar weight gains in colonies. No treatment effects noted for eggs/pupae although mean number in the treated colonies was higher	Two control fields to 1 treated field The treatment hives performed better in number of eggs/pupae than controls LOD not reported for residue analysis. Potential mixing of bees from different treatments/outside sources	Supplemental Wilkins 2014 (49589501)

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[2] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Thiamethoxam, (Cruiser) 47.6% Clothianidin (Modesto) Imidacloprid (Chinook) *Bombus terrestris*	Treated oilseed rape seed (4.25 kg/ha; 0.029 mg a.i./seed)	38-day exposure 68-day observation	2 control 1 treated field ~ 2 ha and 5 km apart. 20% flower on the treated field colonies placed and moved to monitoring 75 colonies w/10-20 workers	Colony mass (every 5-8 days), foraging activity (daily during exposure), pollen composition (23-27 days post flowering)	Increase in colony mass (treated); Increased foraging (treated) Higher number of queens/gynes, workers, eggs, larvae Lower number of drones	1 treatment replicate Colonies lost to farm/animal damage Uncertain dates of bloom, hive placement Uncertain if study design adequate to observe effects.	Qualitative Thompson *et al.* 2015:

Cruiser oilseed rape (OSR) seed (% unspecified) *Bombus terrestris audax* *Apis mellifera*	Thiamethoxam-treated oilseed rape seed Blooming plants *Thiamethoxam + clothianidin residues in bee collected pollen and in honey*: *B. terrestris* < 0.1 – 0.49 µg/kg (adjacent sites) <0.1 (distant sites) *A. mellifera* < 0.1 – 1.51 µg/kg (adjacent sites) <0.1 – 0.70 µg/kg (distant sites)	During OSR bloom period *B. terrestris:* 42-58 days (42-44 days for half of the colonies and 56-58 days for the other half starting at exposure initiation) *A. mellifera*: 46-51 days (ca. 1 year starting at exposure initiation)	72 colonies each of honeybees and bumblebees (12 honeybee and 12 bumblebee/site) 3 sites "adjacent" to (< 5 m) thiamethoxam-treated OSR fields and 3 sites "distant" (1.25-4.55 km away) from the nearest OSR field boundary All sites within predominantly agricultural land Honeybee colonies moved to a common site after the exposure period One treatment (adjacent sites) level and one control (distant sites) Pollen sampled during exposure period to determine proportion of OSR sourced pollen Pollen and honey sampled to determine thiamethoxam + clothianidin concentrations	*B. terrestris:* Adult bee populations, # of cocoons, nest weight change, final nest volume *A. mellifera*: Hive weight change, frames of brood, colony survival, queen survival / replacement -- (Yes)	*B. terrestris:* Higher # adult males and workers in adjacent (treatment) colonies *A. mellifera*: Differences in colony weight change between adjacent (treatment) and distant (control) during first three months of the 12 months of the study (treatment weight change more or less than control depending on the month) Negative relationship between mean concentration of thiamethoxam + clothianidin in honey and pollen with cumulative colony weight gain Fewer frames of brood in 3 adjacent (treatment) colonies in 3 of	Single "treatment" level OSR fields also treated with 3 fungicides (picoxystrobin, tebuconazole, and thiophanate-methyl) Potential exposure to other pesticides in surrounding landscape (predominantly agricultural land), including overwintering sites for honeybees (no screen of pollen or honey for other pesticides) No true negative control (thiamethoxam + clothianidin residues were detected in pollen and honey of *Apis* colonies at distant (control) sites; pollen analysis showed OSR foraging by both species at the control sites, potential pesticide use and exposure at control sites may have differed from	Balfour et al (2017)

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[2] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
					the 4 final months of the experiment (Dec, Feb, Mar)	adjacent (treatment) sites) Single trial	

Tier III Effects to *Osmia spp.*

The three field studies with the mason bee (*Osmia bicornis L.*) were similar in design. All three studies involved exposures of mason bees to thiamethoxam following seed treatments (20.1 µg thiamethoxam/seed) to oil seed rape. Each study took place in a different location in Germany in 2015-2016. The exposure involved parent bees and their offspring. The major limitation of all three studies is that they lacked true replication. Each study included one treated field and one control field, which multiple nesting mason bee sites placed on each. Each nesting site represents a pseudoreplicate. Since there is only one treated field and one control, there is no replication. These studies are considered scientifically valid and useful for characterization purposes. Bees were assessed for hatching rate, nest occupation, cell production, flight and foraging activity, cocoon production, failure and parasitation rate, hatching success and offspring vigor. Significant differences in control and thiamethoxam treated sites were observed; however, results differed by location (**Tables 19 and 20**). When all three studies are taken together, it is unclear whether seed treatments of thiamethoxam to oil seed rape impact mason bees.

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Table 19. Thiamethoxam Tier III Registrant Submitted and Open Literature Studies for *Osmia bicornis*.

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed – (Statistical analysis conducted – Yes/No)	Effects (all comparisons made relative to the study's control)	Limitations	Classification Citation (MRID Number)
Thiamethoxam Formulation A9807F	Treated oilseed Rape seeds 20.1 µg thiamethoxam/seed (17.2 ug c.e./seed) Thiamethoxam was measured in several pollen samples collected from the treated site at 3-4 ng a.i./g.	Approximately 1 month	Field test, 1 treated field, 1 control, 8 nesting sites per field.	hatching rate, nest occupation, cell production, flight and foraging activity, cocoon production, failure and parasitation rate, hatching success and offspring vigor (Yes)	A significant reduction was observed in female foraging 7 days after exposure (DAE); no other significant differences were detected in this endpoint at different times. None of the other endpoints had significant decreases in the treatment compared to the control. Total nest occupation and cell production were higher in the treatment group compared to the control.	No true replication, only pseudoreplication included in study design	Supplemental 50096602

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects (all comparisons made relative to the study's control)	Limitations	Classification Citation (MRID Number)
Thiamethoxam Formulation A9807F	Treated oilseed Rape seeds 20.1 µg thiamethoxam/seed (17.2 ug c.e./seed) Thiamethoxam was detected once in pollen samples collected from the test item treatment field at 1 ng a.i./g and once in nectar samples at 4.1 ng a.i./g.	Approximately 1 month	Field test, 1 treated field, 1 control, 8 nesting sites per field.	hatching rate, nest occupation, cell production, flight and foraging activity, cocoon production, failure and parasitation rate, hatching success and offspring vigor (Yes)	The following endpoints were significantly lower in the treatment group compared to the control group: - nest occupation at 6, 9, and 12 days after exposure (DAE), - total cell production, - cell production increases at 6, 9, 12, 15, and 18 DAE, - flight activity at all observations except 18 DAE.	No true replication, only pseudoreplication included in study design	Supplemental 50096604

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects (all comparisons made relative to the study's control)	Limitations	Classification Citation (MRID Number)
Thiamethoxam Formulation A9807F	Treated oilseed Rape seeds 20.1 µg thiamethoxam/seed (17.2 ug c.e./seed) Thiamethoxam was detected in one pollen sample at 1 ng a.i./g and clothianidin was detected in one pollen sample at 4 ng a.i./g	Approximately 1 month		hatching rate, nest occupation, cell production, flight and foraging activity, cocoon production, failure and parasitation rate, hatching success and offspring vigor (Yes)	The following endpoints were significantly lower in the treatment group relative to the negative control: - nesting females per unit 15 DAE, females entering the test unit at 3, 9, 13, 15, and 25 DAE, - cocoons per nesting unit and cocoons per hatched female, - male cocoon weight, and - male and female offspring weight.	No true replication, only pseudoreplication included in study design	Supplemental 50096605

Table 20. Significant decrease in endpoint observed in mason bee study (relative to control).

Endpoint	MRID 50096602	MRID 50096604	MRID 50096605
Hatching Rate	No	No	No
Nest Occupation	No*	Yes (3 time points)	Yes (1 time point)
Cell Production	No*	Yes (total)	No
Flight and foraging activity	Yes (increased flight activity at one observation period)	Yes (all but one observation period)	No

Cocoon production	No*	No	No
Cocoon failure and parasitation rate	No	No**	
Hatching success	No	No	No
Offspring vigor	No	No	Yes (decreased weight)

*Nest occupation, cell production and cocoon production were greater in treatment group compared to control.
**Failure rate was higher in control compared to treatment.

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Stanley DA, Russell AL, Morrison SJ, Rogers C, Raine NE. Investigating the impacts of field-realistic exposure to a neonicotinoid pesticide on bumblebee foraging, homing ability and colony growth. Journal of Applied Ecology. 2016. Volume 53, Issue 5.

Stanley, DA, Raine, NE. Bumblebee colony development following chronic exposure to field-realistic levels of the neonicotinoid pesticide thiamethoxam under laboratory conditions. Sci Rep 7, 8005 (2017)

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Thompson HM, Fryday SL, Harkin S, Milner S. 2014. Potential impacts of synergism in honeybees (Apis mellifera) of exposure to neonicotinoids and sprayed fungicides in crops. Apidologie 45(5):545-553.

Thompson, H. 2016. Extrapolation of Acute Toxicity Across Bee Species. Integrated Environmental Assessment and Management 12(4): 622 -626

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Tremolada P, Mazzoleni M, Saliu F, Colombo M and Vighi M. 2010. Field trial for evaluating the effects on honeybees of corn sown using Cruiser ® and Celest XL ® treated seeds. Bull Environ Contam Toxicol 85(3):229-234.

Valdovinos-Nunez GR, Quezada-Euan JJ, Ancona-Xiu P, Moo-Valle H, Carmona A and Ruiz Sanchez E. 2009. Comparative toxicity of pesticides to stingless bees (Hymenoptera: Apidae: Meliponini). J Econ Entomol 102(5):1737-1742.

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Appendix 6. Evaluated Registrant-Submitted and Open Literature Toxicity Studies Invalid for Risk Assessment Use

Table 1. Evaluated registrant submitted and open literature toxicity studies for clothianidin that were determined to be invalid.

MRID	Citation	Major Uncertainties
49073620	Maus, C. ; Schoning, R. (2001) Residue Levels of TI 435 FS 600 and Its Relevant Metabolites in Nectar, Blossoms and Pollen of Sunflowers from Dressed Seeds and Effects of these Residues on Foraging Honeybees - Test Location: "Laacher Hof". Project Number: M/031709/01/2, MAUS/AM/005, E/319/1838/3. Unpublished study prepared by Bayer AG. 34p	Only one replicate per treatment; # of bees per colony was low [invalid for effects data, residue data considered supplemental]
49073621	Maus, C.; Schoening, R. (2001) Residue Levels of TI 435 FS 600 and its Relevant Metabolites in Nectar, Blossoms and Pollen of Sunflowers from Dressed Seeds and Effects of these Residues on Foraging Honeybees - Test Location: Farmland "Hoefchen". Project Number: M/031715/01/2, MAUS/AM/008. Unpublished study prepared by Bayer AG. 33p	Only one replicate per treatment; # of bees per colony was low [invalid for effects data, residue data considered supplemental]
45422431	Schmuck, R.; Schoning, R. (2000) Residues of TI 435 in Nectar. Blossoms, Pollen and Honey Bees Sampled from a Summer Rape Field in Sweden and Effects of These Residues on Foraging Honeybees: Lab Project Number: E370 1361-1: 110282. Unpublished study prepared by Bayer AG. 30 p	Only one colony in the treatment group; Rain prohibited/limited foraging/flight activity for 3 of the 5 exposure days [invalid for effects data, residue data considered supplemental]
45422432	Schmuck, R.; Schoning, R. (2000) Residues of TI 435 in Nectar. Blossoms, Pollen and Honey Bees Sampled from a British Summer Rape Field and Effects of These Residues on Foraging Honeybees: Lab Project Number: E 370 1657-6: 110024. Unpublished study prepared by Bayer AG. 31 p	Only one colony in the treatment group; Rain occurred on Day 1 (of a 2 day exposure period) which may have limited foraging/flight activity [invalid for effects data, residue data considered supplemental]
45422433	Schmuck, R.; Schoning, R. (2000) Residues of TI 435 in Nectar. Blossoms, Pollen and Honey Bees Sampled from a French Summer Rape Field and Effects of These Residues on Foraging Honeybees: Lab Project Number: E 370 1359-8: 110046. Unpublished study prepared by Bayer AG. 29 p	The hive for the control plot could not be installed and so no control group with bees; Only one colony (replicate) in the treatment group; Rain occurred on Day 1 (of 2 day exposure period) which may have limited foraging/flight activity [invalid for effects data, residue data considered supplemental]
45422434	Maus, C.; Schoning, R. (2001) Effect of Diet (Sugar Solution) spiked with TI 435 Technical on Behaviour and Mortality of Honey Bees (Apis mellifera) and on the Weight Development of Bee Colonies Under Field Conditions: Lab Project Number: E370 1911-2: 110294. Unpublished study prepared by Bayer AG. 52 p.	Measures were not taken to ensure that bees remained in intended plots and mortality data were unreliable because wasps were observed removing dead bees from the study plots
45422435	Scott-Dupree, C. D.; Spivak, M. S.; Bruns, G.; Blenskinsop, C.; Nelson, S (2001). The Impact of GAUCHO and TI-435 Seed Treated Canola on Honey Bees, Apis mellifera L	Environmental data of beta-cyfluthrin uncertain; it is not known to systemically translocate; Flowering data / schedule lacking through study; Multiple hives used but only one treatment season; Only one plot per treatment; Treatment hives evidently stronger than the control hives; possible masking of treatment

MRID	Citation	Major Uncertainties
		effects; Additional reps would help elucidate uncertainties about hive health; Prior pesticide exposure of hives unknown
45422436	Maus, C.; Schoning, R. et al. (2001) Residue Levels of TI-435 FS 600 and its Relevent Metabolites in Nectar, Blossoms, and Pollen of Summer Rape from Dressed Seeds and Effects of These Residues on Foraging Honeybees (Test Location: Farmland Laacher Hof): Lab Project Number: 110295: E 319 1839-4. Unpublished study prepared by Bayer AG. 34 p	Only one colony in the treatment group; Weather reportedly affected rape density and supplemental food was needed [invalid for effects data, residue data considered supplemental]
45422437	Maus, C.; Schoning, R. et al. (2001) Residue Levels of TI-435 FS 600 and its Relevant Metabolites in Nectar, Blossoms, and Pollen of Summer Rape from Dressed Seeds and Effects of These Residues on Foraging Honeybees (Test Location: Farmland Hofchen): Lab Project Number: E319 1836-1: 110177. Unpublished study prepared by Bayer AG. 36 p	Only one colony in the treatment group; Rain occurred during most of the sampling period (of a 22-day exposure period) which may have limited foraging/flight activity [invalid for effects data, residue data considered supplemental]
45422438	Maus, C. and R. Schoning (2001) Residue Levels of TI-435 FS 600 and its Relevant Metabolites in Pollen of Maize Plants from Dressed Seeds (Test Location: Farmland "Laacher Hof") Laboratory: Bayer AG Crop Protection-Development. Sponsor: Bayer AG Crop Protection-Development.	Residues of TI-435 were detected in the control pollen samples (1.7 and 1.1 ug/kg) and the source of the contamination could not be traced;
45422439	Maus, C. and R. Schoning (2001) Residue Levels of TI-435 FS 600 and its Relevant Metabolites in Pollen of Maize Plants from Dressed Seeds (Test Location: Farmland "Hofchen") Laboratory: Bayer AG Crop Protection-Development. Sponsor: Bayer AG Crop Protection-Development	Residues of TI-435 were detected in the control pollen sample (3.8 ug/kg) and the source of the contamination could not be traced
45422440	Maus, C.; Schoning, R. et al. (2001) Effects of TI-435 Technical Residues in Pollen on the Development of Small Bee Colonies and on Behavior and Mortality of Honey Bees: Lab Project Number: E319 1833-8: 110059. Unpublished study prepared by Bayer AG. 60 p	Only one replicate per treatment; # of bees per colony was low
PMRA 2355468	Maus, C.; Schoning, R. et al. (2011) Evaluation of the effects of residues of TI 435 in maize pollen from dressed seeds on honeybees (Apis mellifera) in the semifield	Differences in the granulation of the pollen between the control and treatment due to differences in weather during harvesting; Residues in pollen from treated seeds were <LOQ; Bees maintained in tunnel for 43 days which may have caused stress to colonies due toconfinement
49073616	Claben, C. (2009) Clothianidin FS 600B G: A Residue Study with Clothianidin FS 600B G Treated Maize Seed, Investigating Residues in Crop, Soil and Honeybee Products in Languedoc-Roussillon (France). Project Number: M/347742/01/2, S08/01377, S08/01377/01/BZEU. Unpublished study prepared by Eurofins - GAB GmbH. 250p	Only one hive in the control group; Author stated that colonies had difficulties adapting to tunnel conditions [invalid for effects data, residue data considered supplemental]
49073617	Claben, C. (2009) Clothianidin FS 600B G: A Residue Study with Clothianidin FS 600B G Treated Maize Seed, Investigating Residues in Crop, Soil and Honeybee Products in Alsace (France). Project Number: M/347727/01/2, S08/02437, S08/02437/01/BZEU. Unpublished study prepared by Eurofins - GAB GmbH. 236p	Only one hive in the control group; Bad weather affected foraging and collection of forager bees; Author stated that colonies had difficulties adapting to tunnel conditions

MRID	Citation	Major Uncertainties
49073618	Claben, C. (2009) Clothianidin FS 600B G: A Residue Study with Clothianidin FS 600B G Treated Maize Seed, Investigating Residues in Crop, Soil and Honeybee Products in Champagne (France). Project Number: M/347748/01/2, S08/02438, S08/02438/01/BZEU. Unpublished study prepared by Eurofins - GAB GmbH. 242p	Only one hive in the control group; Author stated that colonies had difficulties adapting to tunnel conditions [invalid for effects data, residue data considered supplemental]
49750603	Jeyalakshmi T., Shanmugasundaram R., Saravanan M., Geetha S., Mohan S.S., Goparaju A. and Balakrishna Murthy R. 2011. Comparative toxicity of certa.in insecticides against Apis cerana indica under semi field and laboratory conditions. Pestology 35(12):23-26.	- There was no evidence of a control was evident for the laboratory component - There was no husbandry information on the bees used provided There was no analytical verification of clothianidin in the test solutions
49719623	Lu, C., K. M. Warchol, R. A. -Callaha. 2014. Sub-lethal exposure to neonicotinoids impaired honey bees winterization before proceeding to colony collapse disorder. Bulletin of Insectology 67 (1): 125-130.	- Purity and source of clothianidin was not known - Colonies were repeatedly monitored during the late fall and winter months with average temperature at or below freezing for much of the sampling period, likely causing an added level of stress that may have amplified effects observed in the study - It is unclear of the accuracy of the sampling method by which the sizes of clusters were measured by only counting the numbers at the top of the hive only. Presumably this was done to minimize exposure to the outdoor temperatures at the time of sampling - Prior to treatment, *Varroa* mite counts were at a level that has been indicated to be sufficient for colony loss. Although treatment knocked these numbers, *Varroa* mite numbers were not provided during the latter part of the study. - Potential exposure from pesticides other than neonicotinoids was not provided - Actual test dose is not known - No information on adult honey bee mortality during the course of the study No information on the stability of the test compound
49719627	Sanchez-Bayo F, Goka K. 2014. Pesticide residues and bees--a risk assessment. PLoS ONE 9(4):e94482	Review article – not primary source of information

MRID	Citation	Major Uncertainties
49719630	Stanley J, Sah K, Jain SK, Bhatt JC, Sushil SN. 2015. Evaluation of pesticide toxicity at their field recommended doses to honeybees, apis cerana and A. mellifera through laboratory, semi-field and field studies. Chemosphere 119:668-674	No indication of performance of the control group.

Table 2 Evaluated registrant submitted and open literature toxicity studies for thiamethoxam that were determined to be invalid.

MRID	Citation	Major Uncertainties
49158920	Knabe, S. (2010) Thiamethoxam: Thiamethoxam (CGA293343) - A Semi-Field Study with A9700B + A9638A Treated Maize Seed, Followed By Untreated Flowering Crop(s), Investigating Residues in Crop(s), Soil and Honeybee Products in Alsace (France), in 2009: Final Report. Project Number: S08/01279, TK0005524. Unpublished study prepared by Eurofins - GAB GmbH. 184p.	Effects invalid - a lack of replication in the control group
49158901	Sagan, K. (2013) Thiamethoxam: Two Field Trials to Determine the Effects of HELIX Seed Treatment on Honeybees Foraging on Canola Flowers: Final Report Amendment 2. Project Number: CER03214/99, TK0180599. Unpublished study prepared by Syngenta Crop Protection Canada, Inc. 345p.	Effects invalid – limited exposure and potentially confounded via exposure to other pesticides
49158902	Hecht-Rost, S. (2010) Thiamethoxam: Thiamethoxam (CGA293343) - A Field Study with A9700B + A9638A Treated Maize Seed, Investigating Effects on Honeybees (Apis mellifera L.) over Four Years in Southern France: France Report. Project Number: 20061138/F3/BFEU, 2032756, TK0005515. Unpublished study prepared by Eurofins - GAB GmbH. 548p.	Effects invalid – due to potential confounding exposures to other pesticides
49158903	Hecht-Rosts, S. (2010) Thiamethoxam: Thiamethoxam (CGA293343) - A Field Study with A9700B + A9639A Treated Maize Seed, Investigating Effects on Honeybees (Apis mellifera L.) over Four Years in Lorraine (France). Project Number: 20061138/F2/BFEU, 2032755, TK0005516. Unpublished study prepared by Eurofins - GAB GmbH. 527p.	Effects invalid - a lack of replication, confounding exposures to other pesticides, and very limited exposure levels (based on the pollen identification data)
49158905	Bocksch, S. (2011) Thiamethoxam: Thiamethoxam WG (A9584C) - A Field Study to Evaluate Effects on the Honeybee (Apis mellifera; Hymenoptera, Apidae) in Peach in Italy: Final Report. Project Number: S10/00375, TK0025676. Unpublished study prepared by Eurofins - GAB GmbH. 134p.	Effects invalid – No replication in study design

MRID	Citation	Major Uncertainties
49158913	Hecht-Rost, S. (2009) Thiamethoxam: Thiamethoxam - Thiamethoxam (CGA293343) - A Field Study with A9700B Treated Maize Seed, Investigating Effects on Honeybees (Apis mellifera L.) Over Four Years in Alsace (France): 3rd Interim Report. Project Number: 20061138/F1/BFEU, 2032754, TK0180412. Unpublished study prepared by Eurofins - GAB GmbH. 253p.	Effects invalid – exposure uncertainty due to potential confounding exposures to other pesticides, supplemental feeding in treatment hives but not control
49435001	Schuld, M. (2001) Thiamethoxam: Field Test: Effects of Oil-Seed Spring-Rape Grown from Seeds Dressed with CGA293343 WS 70 (A95667B) on the Honey Bee (Apis mellifera L.).: Final Report Project Number: 99125/01/BFEU, 991559, TK02413090. Unpublished study prepared by Arbeitsgemeinschaft GAB Biotechnologie. 59p.	Effects invalid - Control not run concurrently with treatment and no replication.
49435002	Schur, A. (2001) Thiamethoxam: Field Test - Effects of Oil-Seed Winter-Rape Grown from Seeds Dressed with Cruiser OSR (A9807C) on the Honey Bee (Apis mellifera L.):Final Report. Project Number: 99393/01/BFEU, 991568, TK0241389. Unpublished study prepared by Arbeitsgemeinschaft GAB Biotechnologie. 80p.	Effects invalid – No replication in study design
49435004	Barth, M. (2001) Thiamethoxam: Assessment of Side Effects of CGA293343 + CGA329351 + CGA173506 FS 321.3 (A9807C) Applied as Seed Dressing of Brassica napus on the Honeybee Apis mellifera L.: Final Report. Project Number: 00/10/48/016, 2003626, TK0241388. Unpublished study prepared by Biochem Agrar, Labor fuer Biologische und Chemische. 59p.	Effects invalid – No replication in study design
49158914	Hecht-Rost, S. 2007. Thiamethoxam: Thiamethoxam (CGA 293343) and its Metabolite (CGA322704) - A Residue Study with A10590C Treated Maize Seed, Investigating Residues in Crop, Soil and Honeybee Products in Southern France: Final Report. Project Number: 20061138/F1/BFEU. Performed by Eurofins- GAB GmbH, NiefernÖschelbronn, Germany. Sponsored by Syngenta Crop Protection, LLC, Greensboro, NC.	Rationale: brood development between treatment and control groups was compared only for 9 days with huge data variation. One of the major study limitations is that the samples were not analyzed immediately but were stored at ≤ -18°C for ≥ 4-5 months. The residue stability is uncertain. No independent laboratory method validation was reported. The limit of detection (LOD) was not reported.
49158915	Hargreaves, N. 2007. Thiamethoxam: Thiamethoxam (CGA293343) and its Metabolite (CGA322704) - A Residue Study with A10590C Treated Maize Seed, Investigating Residues in Crop, Soil and Honeybee Products in Northern France. Project Number: T003256/05/REG, TK0180410. Unpublished study prepared by Syngenta Jealotts Hill International Research Centre.	Rationale: a compound mixture (A10590C) of three active ingredients was used for the test. Limited brood exposure period and observation time (1 day preexposure and 7-8 days postexposure. No matrix spikes associated with the samples that were stored for over 1 – 4 months at various temperatures from ambient temperature to ≤ -18 °C. The residue stability is uncertain. No independent laboratory method

MRID	Citation	Major Uncertainties
		validation was reported. The limit of detection (LOD) was not reported.
49158916	Hecht-Rost, S. 2007. Thiamethoxam: Thiamethoxam (CGA293343) and its Metabolite (CGA322704) - A Residue Study with A10590C Treated Maize Seed, Investigating Residues in Crop, Soil and Honeybee Products in Alsace, France: Final Report. Project Number: 20051149/F1/BZEU, 2032748, TK0180389. Unpublished study prepared by Eurofins - GAB GmbH. Sponsored by Syngenta Crop Protection, LLC, Greensboro, NC.	a compound mixture (A10590C) of three active ingredients was used for the test. Limited brood exposure period and observation time (1 day preexposure and 7-8 days postexposure. No matrix spikes associated with the samples that were stored for over 4 months at temperatures ≤ -18 °C. The residue stability is uncertain. No independent laboratory method validation was reported. The limit of detection (LOD) was not reported
49158917	Hecht-Rost, S. 2007. Thiamethoxam: Thiamethoxam (CGA293343) and its Metabolite (CGA322704) - A Residue Study with A9807C Treated Winter Oil-Seed Rape Seed, Investigating Residues in Crop and Honeybee Products in Southern France: Final Report. Project Number: 20051041/F3/BZEU, 2032747, TK0180385. Unpublished study prepared by Eurofins - GAB GmbH. Sponsored by Syngenta Crop Protection, LLC, Greensboro, NC.	Rationale: a compound mixture (A9807C) of three active ingredients was used for the test. Limited brood exposure period (7-8 days). No matrix spikes associated with the samples that were stored for over 4-8 months at temperatures ≤ -18 °C. The residue stability is uncertain. No independent laboratory method validation was reported. The limit of detection (LOD) was not reported.
49158918	Hecht-Rost, S. 2007. Thiamethoxam: Thiamethoxam (CGA293343) and its Metabolite (CGA322704) - A Residue with A9807C Treated Winter Oil-Seed, Investigating Residues in Crop and Honeybee Products in Northern France: Final Reports. Project Number: 20051041/F2/BZEU, 2032746, TK0180382. Unpublished study prepared by Eurofins - GAB GmbH. Sponsored by Syngenta Crop Protection, LLC, Greensboro, NC.	a compound mixture (A9807C) of three active ingredients was used for the test. Limited brood exposure period (10 days). No matrix spikes associated with the samples that were stored for over 8 months at temperatures ≤ -18 °C. The residue stability is uncertain. No independent laboratory method validation was reported. The limit of detection (LOD) was not reported.
49158919	Hecht-Rost, S. 2007. Thiamethoxam: Thiamethoxam (CGA293343) and its Metabolite (CGA322704) - A Residue Study with A9807C Treated Winter Oil-Seed Rape Seed, Investigating Residues in Crop and Honeybee Products in Alsace (France): Final Report. Project Number: 20051041/F1/BZEU, 2032745, TK0180378. Unpublished study prepared by Eurofins - GAB GmbH. Sponsored by Syngenta Crop Protection, LLC, Greensboro, NC.	the data for the visual assessments on brood development are of limited value because the thiamethoxam formulation included two other active ingredients. In addition, the exposure time (13 days) was short.

MRID	Citation	Major Uncertainties
49158920	Knäbe, S. 2010. Thiamethoxam (CGA293343) – a semifield study with A9700B + A9638A treated maize seed, followed by untreated flowering crop(s), investigating residues in crop(s), soil and honeybee products in Alsace (France), in 2009. Report number S08-01279. Performed by Eurofins GAB GmbH, Niefern-Öschelbronn, Germany. Sponsored by Syngenta Crop Protection, LLC, Greensboro, NC.	There was no replication in the control, thus the effects in the control could not be compared to the treatment groups.

Appendix 7 – Refined Tier I RQs based on empirical residue data

What follows is a summarization of RQs for each use pattern where there are residue data available in pollen and/or nectar. The information is presented based on application method and presents RQs for caste of bees as well as where appropriate (multiple data points over time) exceedances over time which allows to characterize exceedances reflected in short-term, infrequent 'spikes' of chemical in pollen and nectar residues from those that reflect long-term, frequent occurrences. For oral RQs, the acute and chronic EECs (**Table 7.1** and **7.2**) which are based on the maximum reported concentration among all individual replicates following application and the maximum mean reported concentration respectively are compared against the most toxicity endpoints and where appropriate (e.g. soil applications) fate properties[7] (**Table 7.3**). As discussed previously, the refined Tier I assessment focuses only on the oral route of exposure and not contact. Finally, although Bee-REX includes consumption rates for royal jelly, residue information for this matrix is not available from any residue study for imidacloprid. As royal jelly constitutes the exclusive diet of the larval and the adult queen, refined Tier I oral RQs are not provided for the queen (larval and adult). Additionally, due to a non-definitive endpoint (>) larval acute RQs are not presented. Refined RQs based on empirical residue values and (where available) over time are presented in **Tables 7.4 – 7.X** and **Figures 7.1-7.X**.

Table 7.1. Summary of the maximum single value and maximum mean residue concentration in pollen and/or nectar from the residue studies for clothianidin (ng/g).

Application Method	Crop	Max concentration in pollen	Max concentration in nectar	Max mean concentration in pollen	Max mean concentration in nectar
Foliar	Potato (49705902)	119	--	76.1	--
	Pumpkin (49602802)	123	6.51	108	4.86
	Cotton (49904901)	1216	4883	911	3393
	Peach (50154303)g	130	< 1.0	49.7	< 1.0
	Apple (50154304)	57.4	< 1.0	31.2	< 1.0
	Grapes, post-bloom (50154305)	31.9	--	18.1	--
	Grapes, pre-bloom (50154305)	1564	--	1306	--
	Almond (50154302)	20.0	2.04	13.4	1.23
Soil	Potato (49705902)	188	--	92.5	--
	Pumpkin [pre-emergence] (49910601)	41.3	5.84	22.2	4.98
	Pumpkin [post-emergence] (49910601)	34.5	11.3	28	9.55
	Pumpkin [from 4 cucurbit study] (49705901)	40.2	7.28	16.9	5.39

[7] See exposure section. Fate properties of clothianidin were used for soil application for both thiamethoxam and clothianidin.

	Cucumber (49705901)	--	39.7	---	32.6
	Melon (49705901)	--	14.7	--	10.8
	Squash (49705901)	14.8	4.51	12	4.46
	Orange (49317901)	--	18.7	--	8.2
	Corn (49372102)	27.9	--	26.6	--
	Citrus (49944702)	--	15.0	--	< 2.5
	Popcorn (50009301)	129	--	60	--
	Grapes (50154305)	206	--	160	--
	Melon, bee-collected (50154306)	32.5	11.5	25.4	7.19
	Melon, hand-collected (50154306)	39.5	65.5	39.5h	65.5h
	Citrus (50478201)	631	114	412	64.6
Seed	Corn (scaled)e (49754402)	59.5	--	12.3	--
	Corn (unscaled)e (49754402)	23.8	--	4.91	--
	Canola (49754401)	4.14	1.84	2.79	1.44
	Cotton (49904901)	4.57	3.84	2.35	1.97
	Popcorn (50009301)f	14.2	--	7.5	--
	Corn (50154301)f	6.15	--	4.86	--
	Corn (50154301)	7.78	--	4.38	--
	Soybean (50025901)	< 0.3	< 0.3	< 0.3	< 0.3
	Soybean (50025902)	< 0.3	< 0.3	< 0.3	< 0.3

Table 7.2. Summary of the maximum single value and maximum mean residue concentration in pollen and/or nectar from the residue studies for thiamethoxam (ng c.e./g).

Application Method	Crop (MRID)	Max TR conc. in pollen	Max TR conc. in nectar (EFN conc.)	Max mean TR conc. in pollen	Max mean TR conc. in nectar (EFN conc.)
Foliar	Tomato (49804101)	14504	--	8909	--
	Cucumber (49804105)	1228	297	1049	168

	Cranberry (49804102)	1932	2107	1186	1057
	Stone Fruit (49819501)	328	5.49	160	2.48
	Cotton (49686801)	316	9.83 (675)	54.76	3.06 (80.84)
	Strawberry (50265502)	6463	567	5799	334
	Soybean (50265503)	545[b]	44.3	486[b]	42.5
	Apple (50265504)	2124	660	1756	496
	Pumpkin (50265506)	80.4	26.6	30.7	23.8
	Blueberry (50425901)	868	647	810	593
	Citrus (50425902)	878	12.1	703	10.0
	Ornamentals (50425903)	3127	1192	1238	796
Soil	Cucumber (49550801)	10.02	11.84	6.98	9.50
	Pepper (49804103)	268	1384	238	534
	FL Citrus (49881002)	323[a]	23.71[a]	69.47[a]	12.80[a]
	CA Citrus (49881001)	410[a]	65.22[a]	107[a]	19.78[a]
	Strawberry (50266001)	1669	186	1126	86.9
	Cucurbit (50265501)	755	57.6	310	28.7
	Tomato (50265507)	306	330[i]	220	261[i]
Seed	Soybean (49804104)	6.08[b]	5.15	4.14[b]	2.91
	Soybean (49210901)	23.14[c]	--	15.64[c]	--
	Canola (49819502)	46.89[d]	13.34	46.89[d]	8.08
	Canola (49755702)	7.69	2.64	3.17	1.48
	Cotton (49686801)	1.0	1.54 (1.74)	1.0	1.18 (1.25)
	Corn (49158916)	12.47	--	6.45	--
	Corn (49158914)	7.98	--	5.02	--
	Corn (49158915)	5.19	--	3.33	--
Seed + Foliar	Corn (50265505)	864	--	604	--

TR = Total Residue

EFN = extra floral nectar concentrations, where available (cotton).

a = concentrations normalized to typical citrus application rate of 0.172 lb a.i./acre.
b = no pollen data. Whole flower and anther data available. Highest values presented from whole flower data.
c = no pollen or nectar data. Values represent reproductive organ structure (stamen, pistol, nectary) data.
d = highest clothianidin value (759 ppb) excluded. Next highest value (47 ppb) presented. Max and mean value are identical because there was only a single sampling interval.
e=for this use, the "scaled" residue values are empirically measured residue concentrations which were adjusted upwards 2.5X to account for the maximum allowable rate for corn seed treatment. The "unscaled" values are the empirically measured residue concentrations before adjusting.
f = this application consisted of treated seed plus an in-furrow application
g = values for pollen could include a potential outlier. Replicate residues registered 9.16, 130, and 9.96 ng/g.
h = mean and max concentrations are the same, as there was only one sample.
i = no nectar collected. Whole flower data

Table 7.3. Toxicity value and Fate Properties for Refined Tier I Risk estimation.

Inputs	Clothianidin	Thiamethoxam
Adult contact LD50	0.0275	**0.021**
Adult oral LD50	**0.0037**	0.0038
Adult oral NOAEL	**0.00036**	0.00251
Larval LD50	N/A	**>0.03**
Larval NOAEL	680 (conc)	**0.0037**
Kow	**0.64**	-0.13
Koc	**160**	70.4

Bold values were used in calculations.

Thiamethoxam - Refined Tier I Foliar Applications

Table 7.4. Refined RQs for foliar applications of thiamethoxam to apples.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.03	**8.9**
		5	120	3.6			0.07	**17.8**
	Drone	6+	130	3.6			0.07	**19.1**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.05	**14.68**	0.04	**115.10**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.11	**30.71**	0.09	**239.72**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.04	**11.72**	0.03	**90.96**
	Worker (foraging for pollen)	>18	43.5	0.041	0.03	**7.78**	0.02	**60.13**
	Worker (foraging for nectar)	>18	292	0.041	0.19	**52.11**	0.14	**402.51**
	Worker (maintenance of hive in winter)	0-90	29	2	0.02	**6.37**	0.02	**49.71**
	Drone	>10	235	0.0002	0.16	**41.92**	0.12	**323.78**

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

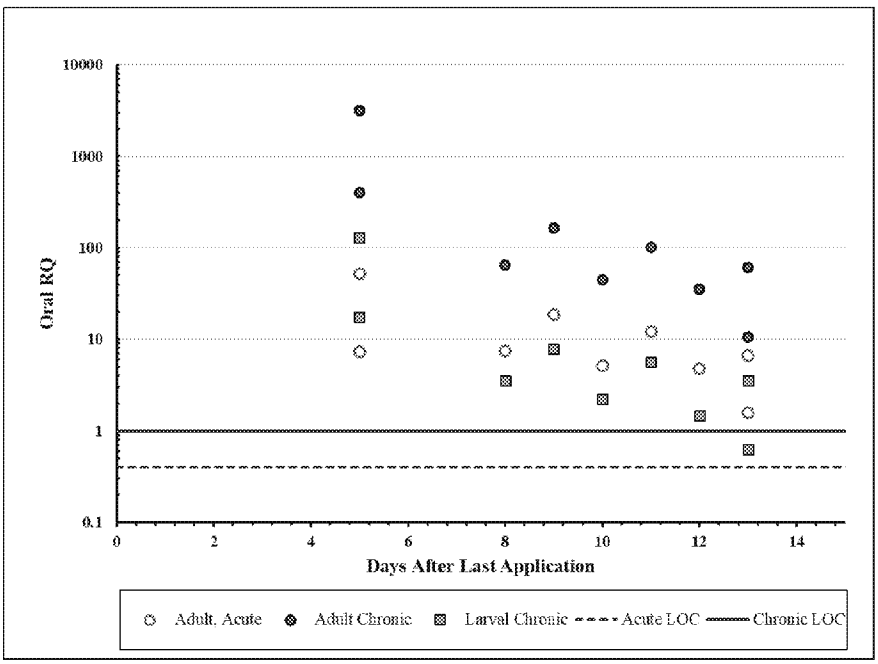

Figure 7.1. RQs over time for foliar applications of thiamethoxam to apples.

Table 7.5. Refined RQs for foliar applications of thiamethoxam to blueberry.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.037038	**10.0**
		5	120	3.6			0.074076	**20.0**
	Drone	6+	130	3.6			0.080006	**21.6**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.044592	**12.05**	0.040967	**113.8**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.098913	**26.73**	0.090796	**252.2**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.040296	**10.89**	0.036957	**102.7**
	Worker (foraging for pollen)	>18	43.5	0.041	0.02818	**7.62**	0.025829	**71.7**
	Worker (foraging for nectar)	>18	292	0.041	0.18896	**51.07**	0.173189	**481.1**
	Worker (maintenance of hive in winter)	0-90	29	2	0.020499	**5.54**	0.018817	**52.3**
	Drone	>10	235	0.0002	0.152045	**41.09**	0.139355	**387.1**

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

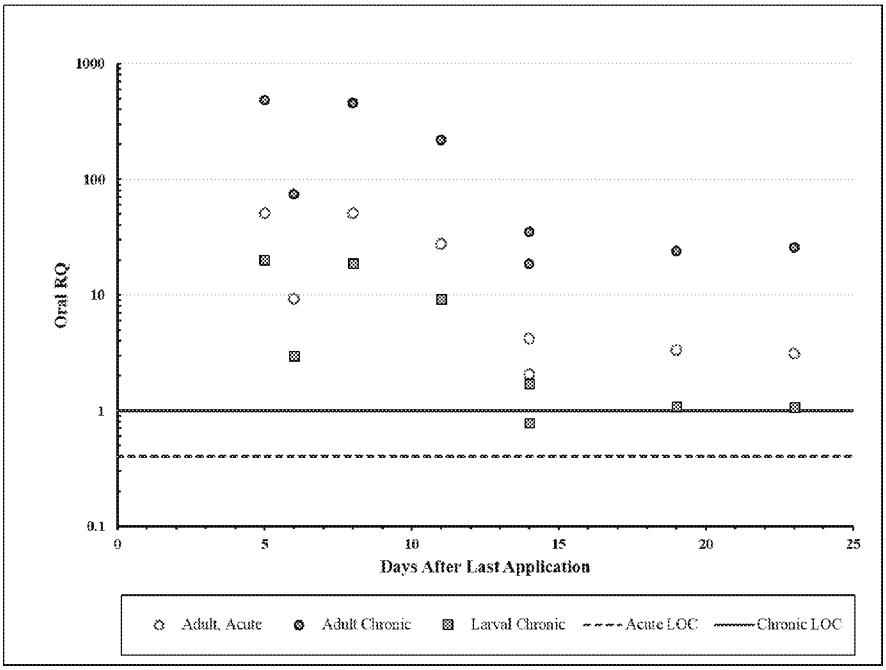

Figure 7.2. RQs over time for foliar applications of thiamethoxam to blueberry.

Table 7.6. Refined RQs for foliar applications of thiamethoxam to orange.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.5
		5	120	3.6			0.00	**1.0**
	Drone	6+	130	3.6			0.00	**1.0**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.01	**1.77**	0.01	**14.65**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.01	**2.74**	0.01	**22.64**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	**0.60**	0.00	**4.99**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.15	0.00	**1.29**
	Worker (foraging for nectar)	>18	292	0.041	0.00	**0.96**	0.00	**8.19**
	Worker (maintenance	0-90	29	2	0.00	**0.57**	0.00	**4.71**

	of hive in winter)							
	Drone	>10	235	0.0002	0.00	**0.77**	0.00	**6.53**

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

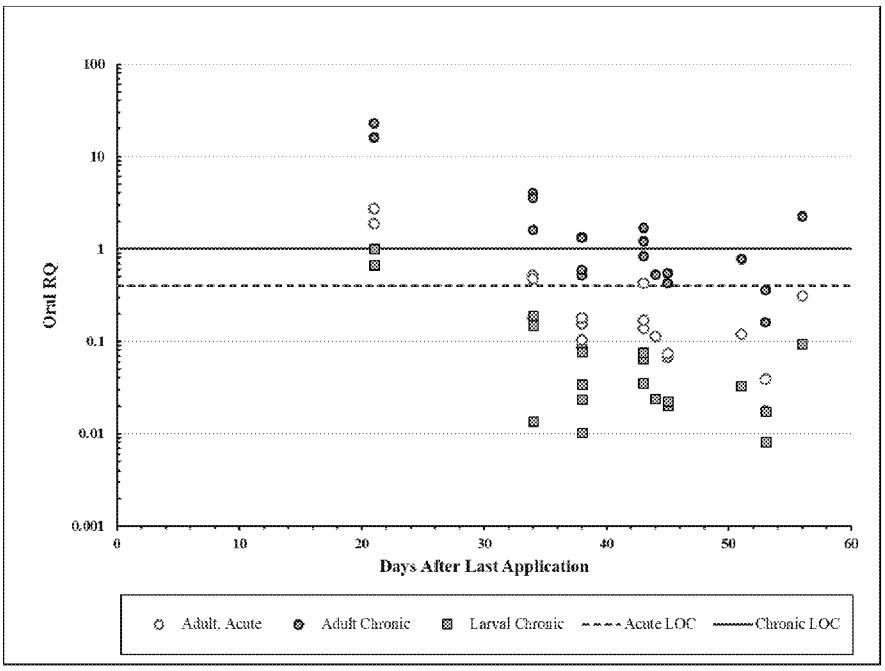

Figure 7.3. RQs over time for foliar applications of thiamethoxam to citrus (orange).

Table 7.7. Refined RQs for foliar applications of thiamethoxam to cotton*.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	**1.3**
		5	120	3.6			0.01	**2.7**
	Drone	6+	130	3.6			0.01	**2.9**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.01	**1.77**	0.01	**14.48**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.01	**2.74**	0.01	**32.90**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	**0.60**	0.00	**13.73**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.15	0.00	**9.77**
	Worker (foraging for nectar)	>18	292	0.041	0.00	**0.96**	0.02	**65.58**
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	**0.57**	0.00	**6.82**
	Drone	>10	235	0.0002	0.00	**0.77**	0.02	**52.77**

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

*Although cotton pollen is not attractive to honeybees, analysis includes pollen residues to be protective of other oilseed crops.

Table 7.8. Refined RQs for foliar applications of thiamethoxam to cranberry.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.07	**17.7**
		5	120	3.6			0.13	**35.4**
	Drone	6+	130	3.6			0.14	**38.3**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.14	**37.64**	0.07	**198.07**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.31	**84.74**	0.16	**442.68**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.13	**35.06**	0.07	**181.77**
	Worker (foraging for pollen)	>18	43.5	0.041	0.09	**24.79**	0.05	**127.86**
	Worker (foraging for nectar)	>18	292	0.041	0.62	**166.30**	0.31	**857.48**
	Worker (maintenance of hive in winter)	0-90	29	2	0.06	**17.56**	0.03	**91.74**
	Drone	>10	235	0.0002	0.50	**133.82**	0.25	**689.99**

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

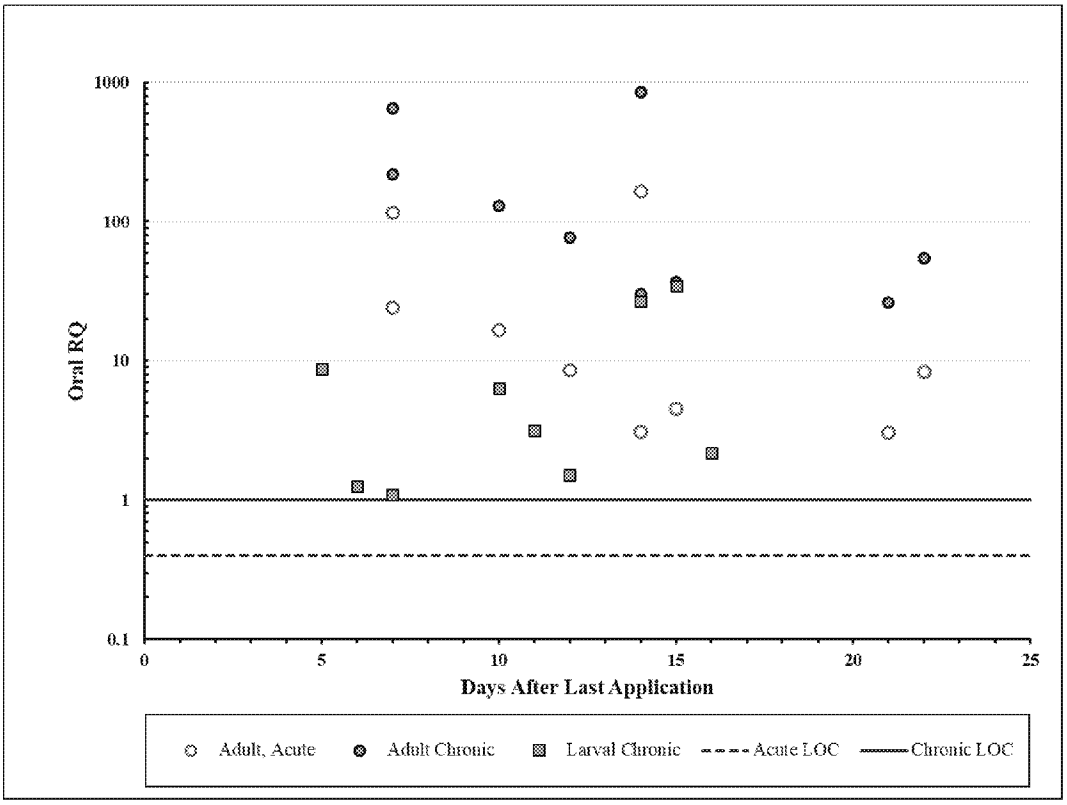

Figure 7.4. RQs over time for foliar applications of thiamethoxam to cranberry.

Table 7.9. Refined RQs for foliar applications of thiamethoxam to cucumber.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.10	**27.8**
		5	120	3.6			0.21	**55.5**
	Drone	6+	130	3.6			0.22	**60.0**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.03	**7.02**	0.11	**299.38**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.05	**14.42**	0.25	**681.31**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.02	**5.38**	0.10	**284.95**
	Worker (foraging for pollen)	>18	43.5	0.041	0.01	**3.51**	0.07	**203.12**

	Worker (foraging for nectar)	>18	292	0.041	0.09	**23.45**	0.49	**1362.79**
	Worker (maintenance of hive in winter)	0-90	29	2	0.01	**2.99**	0.05	**141.16**
	Drone	>10	235	0.0002	0.07	**18.86**	0.39	**1096.67**

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

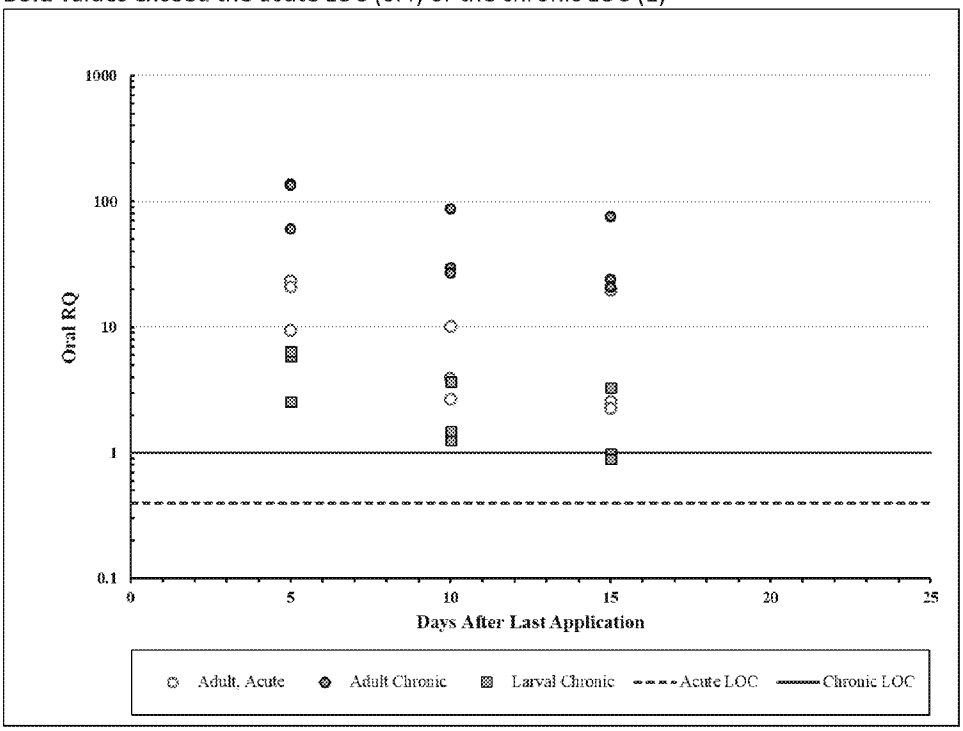

Figure 7.5. RQs over time for foliar applications of thiamethoxam to cucumber.

Table 7.10. Refined RQs for foliar applications of thiamethoxam to ornamentals.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.05	**13.5**
		5	120	3.6			0.10	**27.0**
	Drone	6+	130	3.6			0.11	**29.2**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.09	**24.95**	0.06	**155.54**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.20	**53.22**	0.12	**342.57**
	Worker (comb building, cleaning and	11 to 18	60	1.7	0.08	**20.77**	0.05	**138.51**

	food handling)							
	Worker (foraging for pollen)	>18	43.5	0.041	0.05	**14.05**	0.03	**96.32**
	Worker (foraging for nectar)	>18	292	0.041	0.35	**94.11**	0.23	**645.79**
	Worker (maintenance of hive in winter)	0-90	29	2	0.04	**11.03**	0.03	**71.00**
	Drone	>10	235	0.0002	0.28	**75.71**	0.19	**519.61**

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

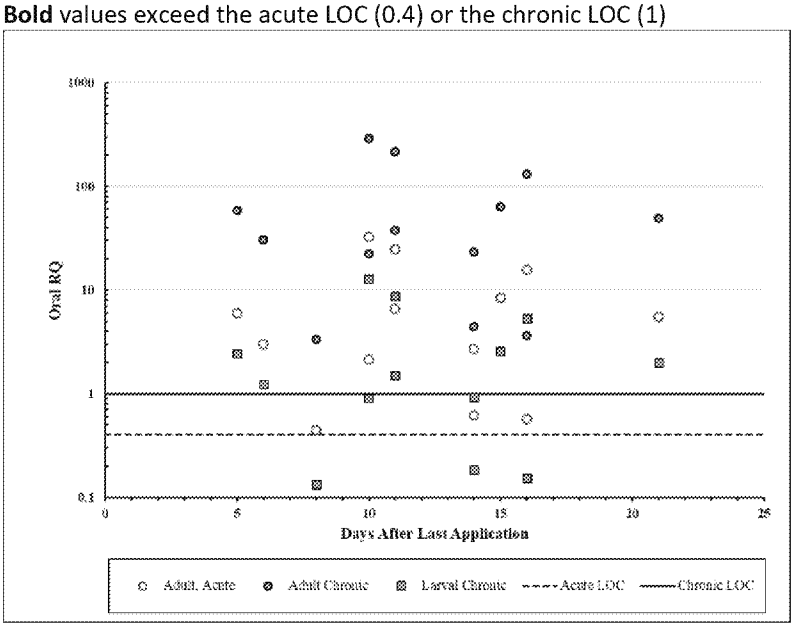

Figure 7.7. RQs over time for foliar applications of thiamethoxam to ornamentals[8].

[8] Data include stargazer lilly, common lilac, and mock orange. RQs were calculated only when residues values were available at the same corresponding DALA (+/- 1 day) for both pollen and nectar at the same site.

Table 7.11. Refined RQs for foliar applications of thiamethoxam to pumpkin.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.4
		5	120	3.6			0.00	0.8
	Drone	6+	130	3.6			0.00	0.9
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	**0.58**	0.00	**4.53**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	**1.22**	0.00	**10.07**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	**0.47**	0.00	**4.11**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.31	0.00	**2.88**
	Worker (foraging for nectar)	>18	292	0.041	0.01	**2.10**	0.01	**19.31**
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	**0.25**	0.00	**2.09**
	Drone	>10	235	0.0002	0.01	**1.69**	0.01	**15.54**

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

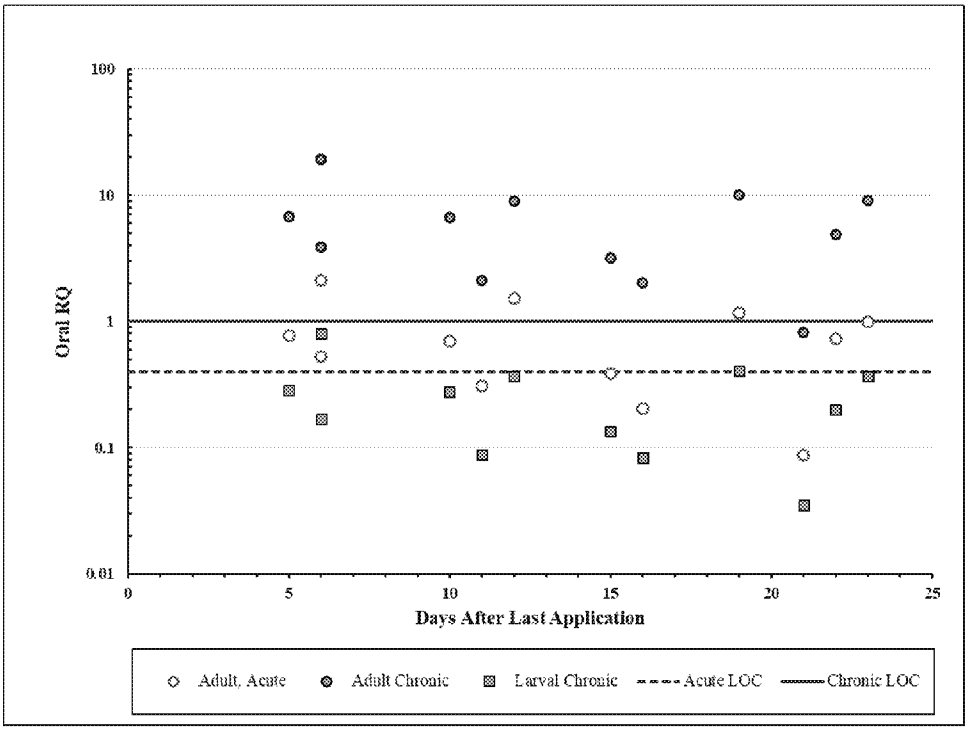

Figure 7.8. RQs over time for foliar applications of thiamethoxam to pumpkin.

Table 7.12. Refined RQs for foliar applications of thiamethoxam to soybean.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.9
		5	120	3.6			0.01	**1.9**
	Drone	6+	130	3.6			0.01	**2.0**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.01	**1.70**	0.01	**16.06**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.01	**3.09**	0.01	**29.49**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	**0.97**	0.00	**9.38**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	**0.53**	0.00	**5.19**
	Worker (foraging for nectar)	>18	292	0.041	0.01	**3.50**	0.01	**34.53**
	Worker (maintenance	0-90	29	2	0.00	**0.64**	0.00	**6.12**

	of hive in winter)							
	Drone	>10	235	0.0002	0.01	**2.81**	0.01	**27.74**

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

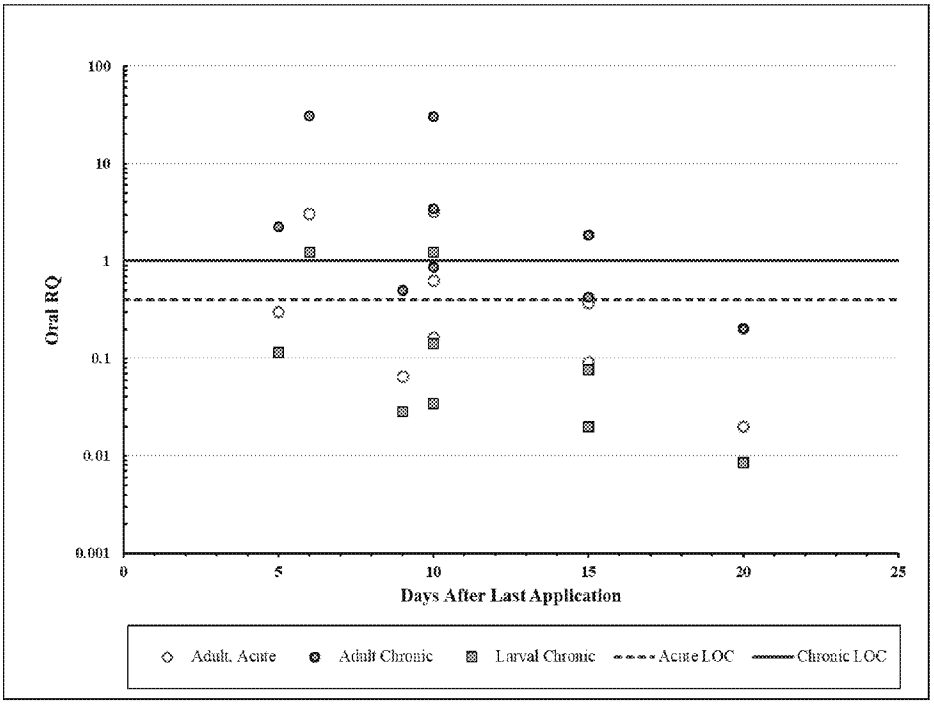

Figure 7.9. RQs over time for foliar applications of thiamethoxam to soybean

Table 7.13. Refined RQs for foliar applications of thiamethoxam stone fruit

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.1
		5	120	3.6			0.00	0.2
	Drone	6+	130	3.6			0.00	0.2
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	**0.68**	0.00	**3.37**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	**1.06**	0.00	**5.23**

	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	0.24	0.00	**1.17**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.07	0.00	**0.32**
	Worker (foraging for nectar)	>18	292	0.041	0.00	**0.44**	0.00	**2.03**
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.22	0.00	**1.09**
	Drone	>10	235	0.0002	0.00	0.35	0.00	**1.62**

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

Table 7.14. Refined RQs for foliar applications of thiamethoxam to strawberry.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.03	**8.2**
		5	120	3.6			0.06	**16.5**
	Drone	6+	130	3.6			0.06	**17.4**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.08	**20.81**	0.06	**162.79**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.14	**38.22**	0.10	**284.53**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.05	**12.16**	0.03	**83.05**
	Worker (foraging for pollen)	>18	43.5	0.041	0.02	**6.74**	0.01	**41.02**
	Worker (foraging for nectar)	>18	292	0.041	0.17	**44.82**	0.10	**271.57**
	Worker (maintenance of hive in winter)	0-90	29	2	0.03	**7.94**	0.02	**59.12**
	Drone	>10	235	0.0002	0.13	**36.01**	0.08	**218.03**

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

Table 7.15. Refined RQs for foliar applications of thiamethoxam to tomato*.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.02	**4.3**
		5	120	3.6			0.03	**8.7**
	Drone	6+	130	3.6			0.03	**8.7**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.10	**26.07**	0.06	**164.57**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.14	**37.63**	0.09	**237.57**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.02	**6.66**	0.02	**42.07**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.16	0.00	**1.01**
	Worker (foraging for nectar)	>18	292	0.041	0.00	0.16	0.00	**1.01**
	Worker (maintenance of hive in winter)	0-90	29	2	0.03	**7.84**	0.02	**49.49**
	Drone	>10	235	0.0002	0.00	0.00	0.00	0.00

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

*Based on pollen concentrations only

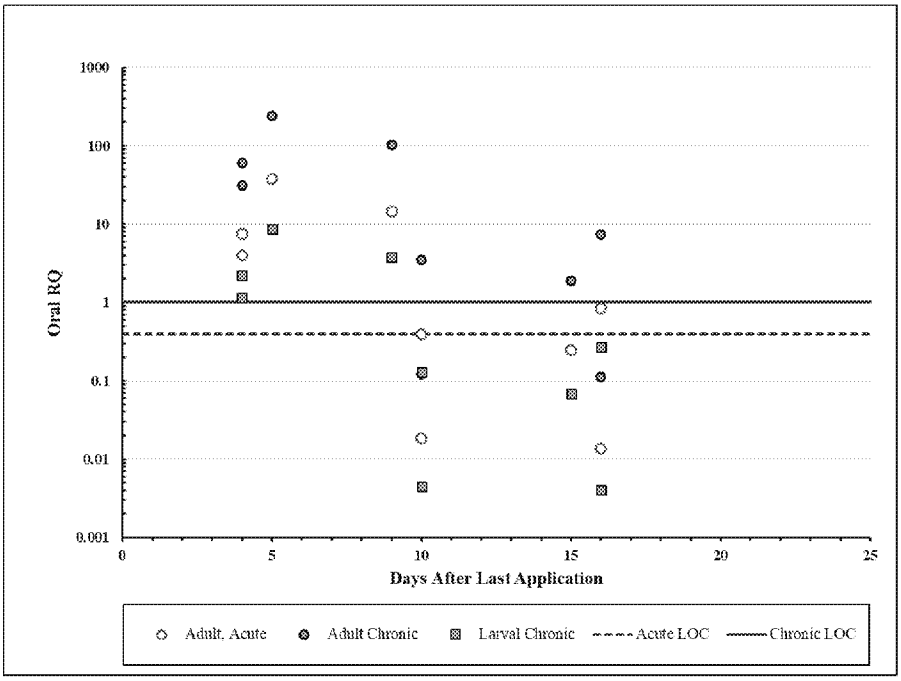

Figure 7.10. RQs over time for foliar applications of thiamethoxam to tomato.

Thiamethoxam - Refined Tier I Soil Applications

Table 7.16. Refined RQs for soil applications of thiamethoxam to cucumber.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.2
		5	120	3.6			0.00	0.3
	Drone	6+	130	3.6			0.00	0.3
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	**0.21**	0.00	**1.71**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	**0.47**	0.00	**3.88**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	0.20	0.00	**1.62**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.14	0.00	**1.15**
	Worker (foraging for nectar)	>18	292	0.041	0.00	**0.93**	0.00	**7.71**

	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.10	0.00	0.80
	Drone	>10	235	0.0002	0.00	**0.75**	0.00	**6.20**

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

Table 7.17. Refined RQs for soil applications of thiamethoxam to Citrus (FL*).

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.2
		5	120	3.6			0.00	0.5
	Drone	6+	130	3.6			0.00	0.5
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	**0.96**	0.00	**3.42**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.01	**1.73**	0.00	**6.83**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	**0.53**	0.00	**2.46**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.28	0.00	**1.55**
	Worker (foraging for nectar)	>18	292	0.041	0.01	**1.87**	0.00	**10.39**
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.36	0.00	**1.42**
	Drone	>10	235	0.0002	0.01	**1.51**	0.00	**8.36**

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

*A study with California citrus (MRID 49881001) is also available, but residues are similar (within 3X) and would not significantly alter the information above or below. RQs may be slightly higher based on residue difference.

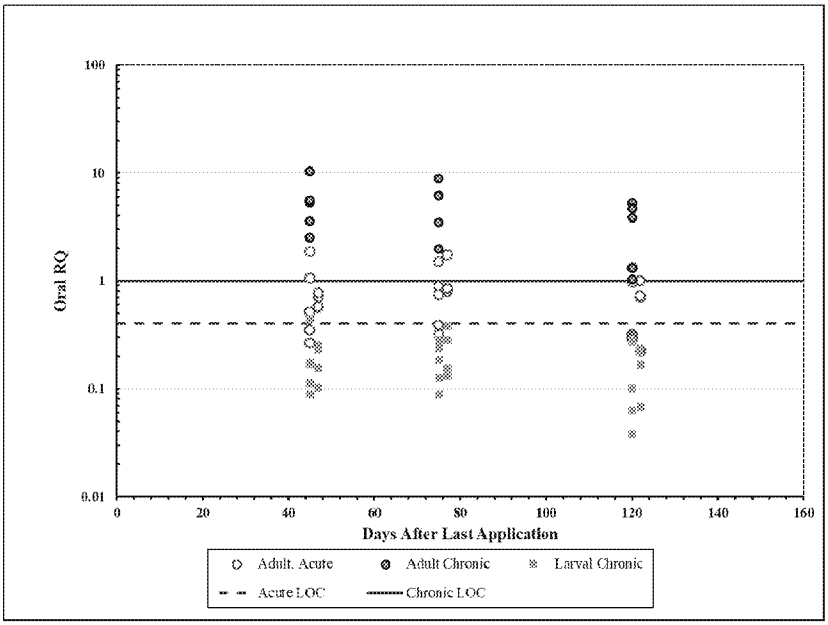

Figure 7.11. RQs over time (in days before bloom) for soil applications of thiamethoxam to citrus (FL).

Table 7.18. Refined RQs for soil applications of thiamethoxam to chili peppers.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.03	**8.8**
		5	120	3.6			0.06	**17.6**
	Drone	6+	130	3.6			0.07	**19.0**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.08	**22.92**	0.03	**93.40**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.20	**53.06**	0.08	**214.01**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.08	**22.57**	0.03	**90.12**
	Worker (foraging for pollen)	>18	43.5	0.041	0.06	**16.27**	0.02	**64.55**
	Worker (foraging for nectar)	>18	292	0.041	0.40	**109.23**	0.16	**433.16**
	Worker (maintenance	0-90	29	2	0.04	**10.99**	0.02	**44.34**

	of hive in winter)							
	Drone	>10	235	0.0002	0.33	**87.90**	0.13	**348.58**

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

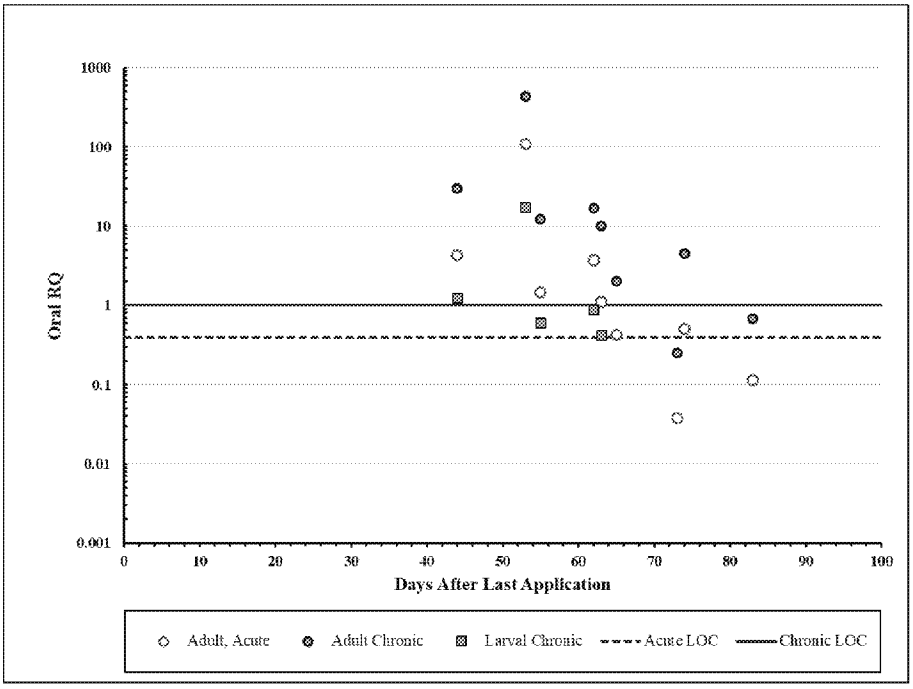

Figure 7.12. RQs over time for soil applications of thiamethoxam to chili peppers.

Table 7.19. Refined RQs for soil applications of thiamethoxam to tomato*.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.1
		5	120	3.6			0.00	0.2
	Drone	6+	130	3.6			0.00	0.2
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	**0.55**	0.00	**4.06**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	**0.79**	0.00	**5.87**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	0.14	0.00	**1.04**

	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.00	0.00	0.03
	Worker (foraging for nectar)	>18	292	0.041	0.00	0.00	0.00	0.03
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.17	0.00	**1.22**
	Drone	>10	235	0.0002	0.00	0.00	0.00	0.00

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

*RQs are based on residues (pollen only) using 2 different app rates (0.125 and 0.172 lb a.i./A).

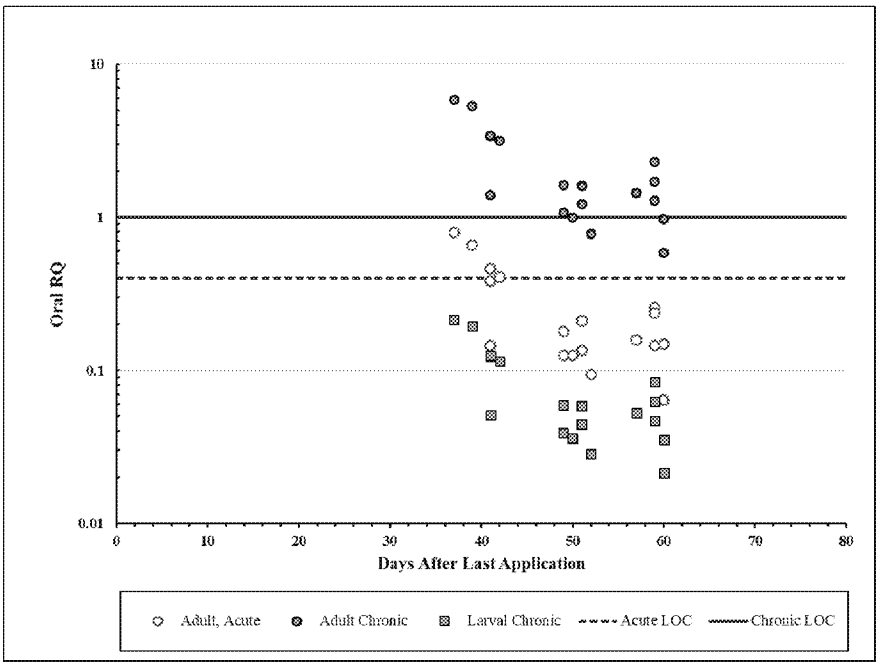

Figure 7.13. RQs over time for soil applications of thiamethoxam to tomato*.

Table 7.20. Refined RQs for soil applications of thiamethoxam to strawberry.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.01	**2.0**
		5	120	3.6			0.01	**3.9**
	Drone	6+	130	3.6			0.02	**4.1**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.02	**6.02**	0.01	**35.28**
	Worker (brood and queen	6 to 17	140	9.6	0.04	**11.37**	0.02	**63.82**

	tending, nurse bees)							
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.01	**3.78**	0.01	**19.80**
	Worker (foraging for pollen)	>18	43.5	0.041	0.01	**2.21**	0.00	**10.63**
	Worker (foraging for nectar)	>18	292	0.041	0.05	**14.70**	0.03	**70.61**
	Worker (maintenance of hive in winter)	0-90	29	2	0.01	**2.36**	0.00	**13.26**
	Drone	>10	235	0.0002	0.04	**11.81**	0.02	**56.73**

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

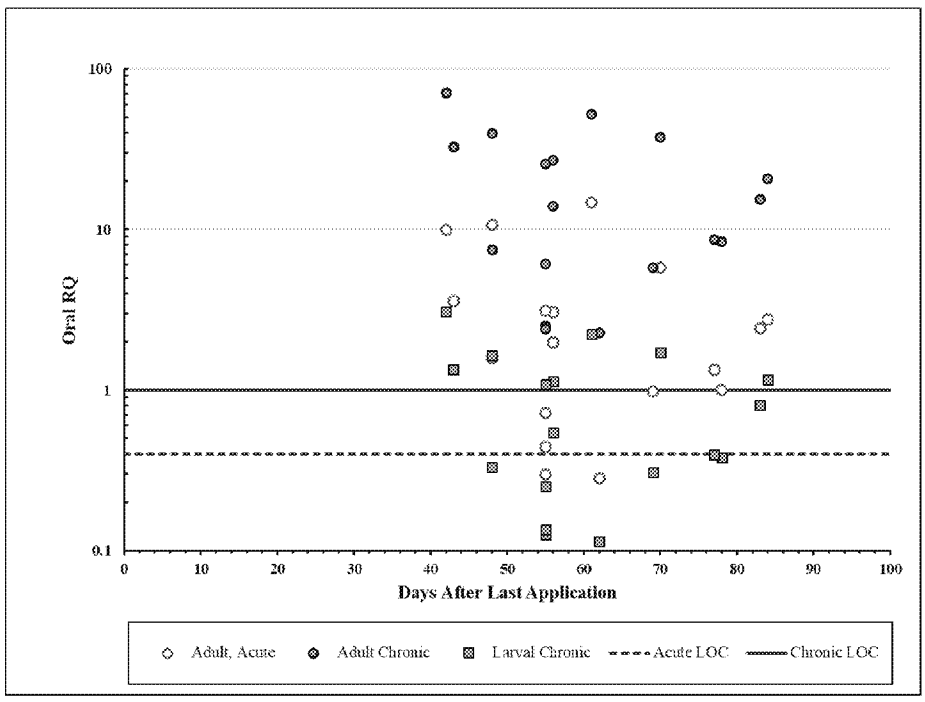

Figure 7.14. RQs over time for soil applications of thiamethoxam to strawberry*.

Table 7.21. Refined RQs for soil applications of thiamethoxam to muskmelon*.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.6
		5	120	3.6			0.00	**1.2**
	Drone	6+	130	3.6			0.00	**1.3**

Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.01	**2.29**	0.00	**10.51**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.02	**4.14**	0.01	**19.43**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	**1.28**	0.00	**6.25**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	**0.69**	0.00	**3.50**
	Worker (foraging for nectar)	>18	292	0.041	0.02	**4.55**	0.01	**23.31**
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	**0.86**	0.00	**4.03**
	Drone	>10	235	0.0002	0.01	**3.66**	0.01	**18.73**

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)
*Study was conducted with pumpkin, muskmelon, and summer squash. Melon residues were used for this analysis for the maximum residue value.

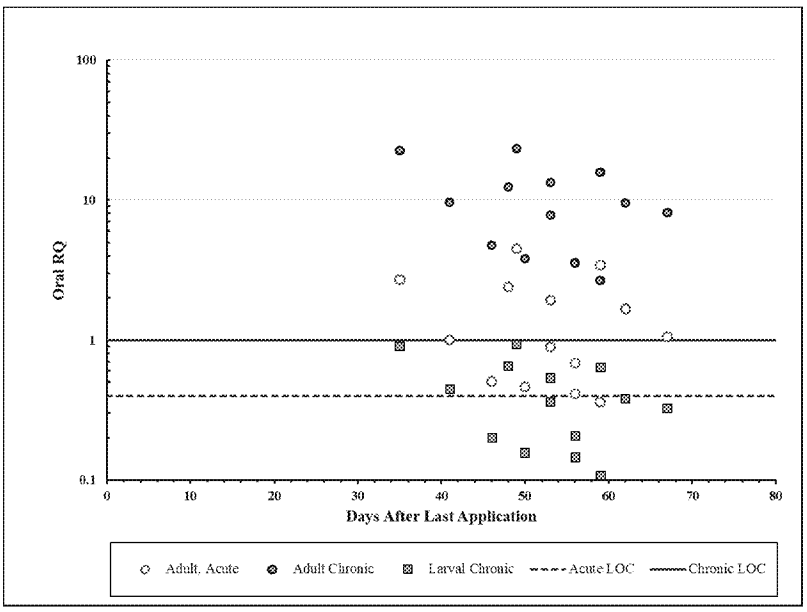

Figure 7.14 RQs over time for soil applications of thiamethoxam to muskmelon*.

Table 7.23. Refined RQs for soil applications of thiamethoxam to ornamentals*.

			Consumption Rates[5]				

Life stage	Caste or task in hive	Average age (in days)	Nectar (mg/day)	Pollen (mg/day)	Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.6
		5	120	3.6			0.00	**1.1**
	Drone	6+	130	3.6			0.00	**1.2**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.01	**2.29**	0.00	**10.51**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.02	**4.14**	0.01	**19.43**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	**1.28**	0.00	**6.25**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	**0.69**	0.00	**3.50**
	Worker (foraging for nectar)	>18	292	0.041	0.02	**4.55**	0.01	**23.31**
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	**0.86**	0.00	**4.03**
	Drone	>10	235	0.0002	0.01	**3.66**	0.01	**18.73**

Bold values exceed the acute LOC (0.4) or the chronic LOC (1)

*Study was conducted with sargent crabapple, hedge cotoneaster, common lilac, mock orange, and stargazer lily. Lilac residues were used to calculate RQs based on having available pollen and nectar data. Note the nectar values used were from a single sample (not a composite).

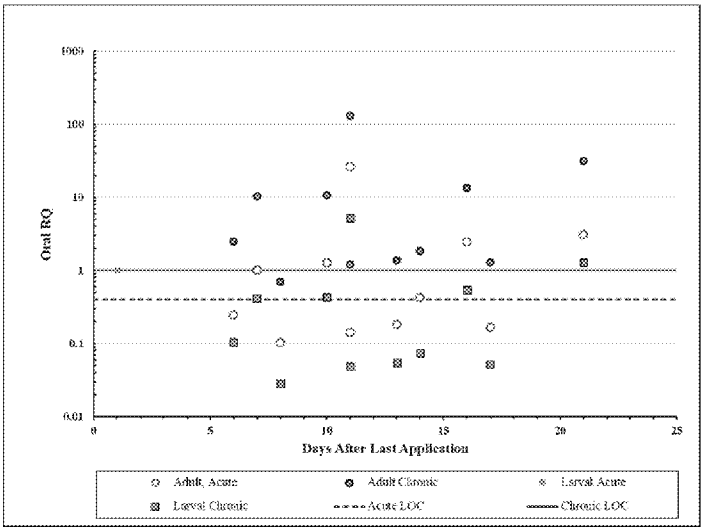

Figure 7.15. RQs over time for soil applications of thiamethoxam to ornamentals*.

Clothianidin - Refined Tier I Foliar Applications

Table 24. Refined RQs for post-bloom foliar applications of clothianidin to almonds

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.03
		5	120	3.6			0.00	0.05
	Drone	6+	130	3.6			0.00	0.06
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	0.07	0.00	0.45
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	0.13	0.00	0.84
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	0.04	0.00	0.27

	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.02	0.00	0.15
	Worker (foraging for nectar)	>18	292	0.041	0.00	0.16	0.00	**1.00**
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.03	0.00	0.17
	Drone	>10	235	0.0002	0.00	0.13	0.00	0.80

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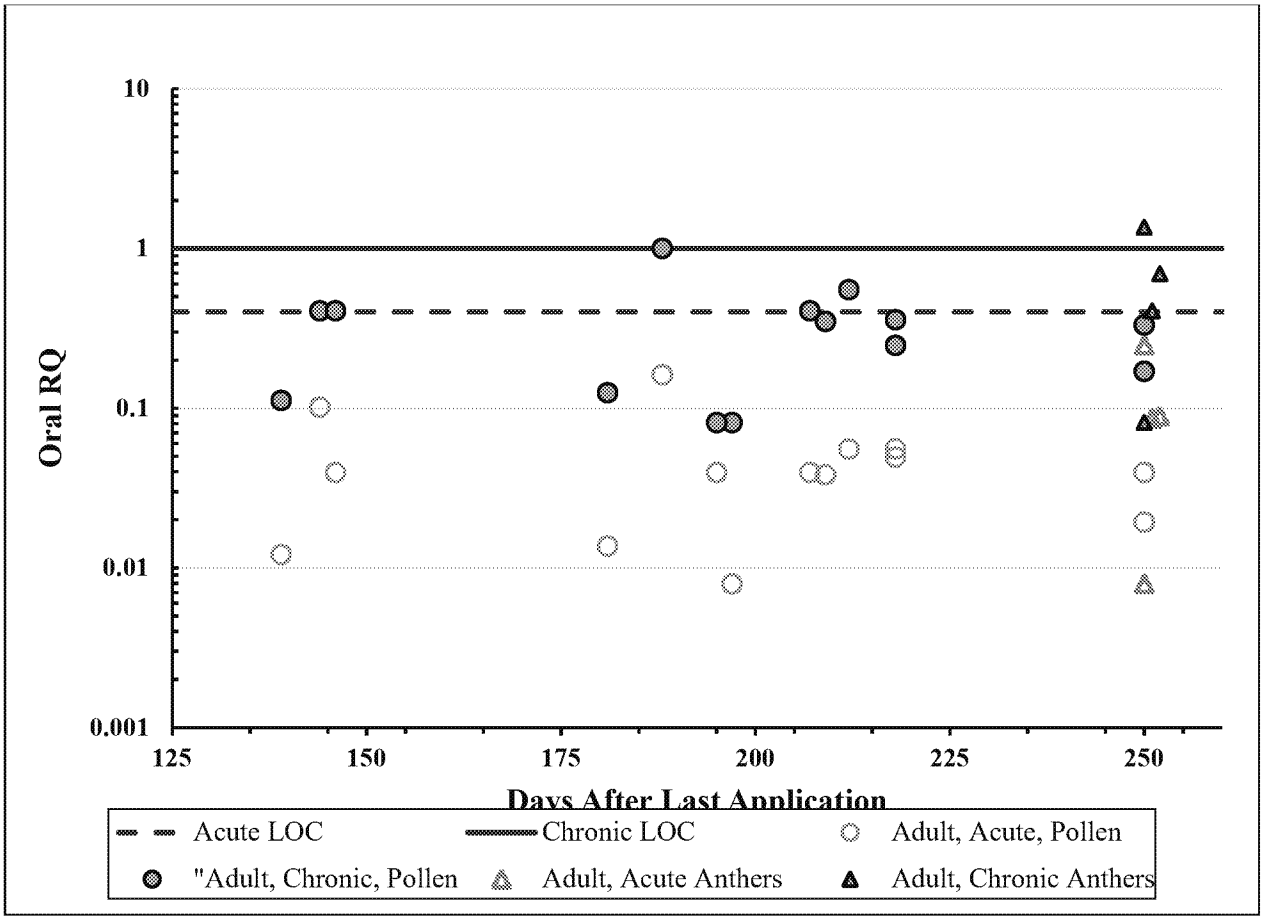

Figure 7.16. RQs over time for foliar applications of clothianidin to almonds

Table 7.25. Refined RQs for Post-Bloom Foliar Applications of clothianidin to apples

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.02
		5	120	3.6			0.00	0.05
	Drone	6+	130	3.6			0.00	0.05
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	0.11	0.00	0.66

	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	0.17	0.00	**1.03**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	0.03	0.00	0.23
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.01	0.00	0.06
	Worker (foraging for nectar)	>18	292	0.041	0.00	0.04	0.00	0.41
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.03	0.00	0.21
	Drone	>10	235	0.0002	0.00	0.03	0.00	0.33

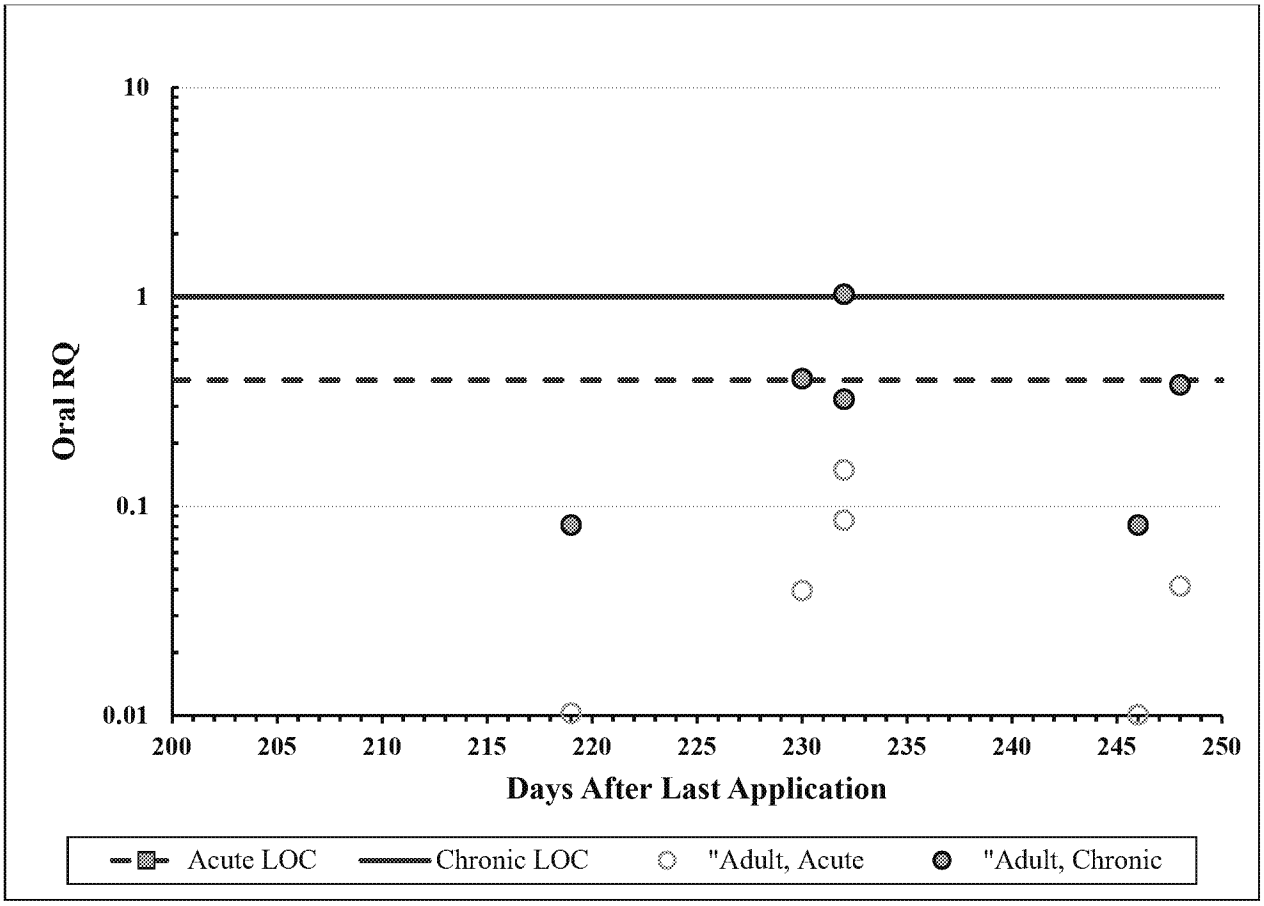

Figure 7.17. RQs over time for foliar applications of clothianidin to apples

Table 7.26. Refined RQs for foliar applications of clothianidin to cotton

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.20	**55.02**
		5	120	3.6			0.41	**110.04**
	Drone	6+	130	3.6			0.44	**119.21**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.26	**71.08**	0.20	**565.50**

	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.61	**165.84**	0.48	**1319.50**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.26	**71.08**	0.20	**565.50**
	Worker (foraging for pollen)	>18	43.5	0.041	0.19	**51.53**	0.15	**409.99**
	Worker (foraging for nectar)	>18	292	0.041	1.28	**345.90**	0.99	**2752.10**
	Worker (maintenance of hive in winter)	0-90	29	2	0.13	**34.35**	0.10	**273.33**
	Drone	>10	235	0.0002	1.03	**278.38**	0.80	**2214.88**

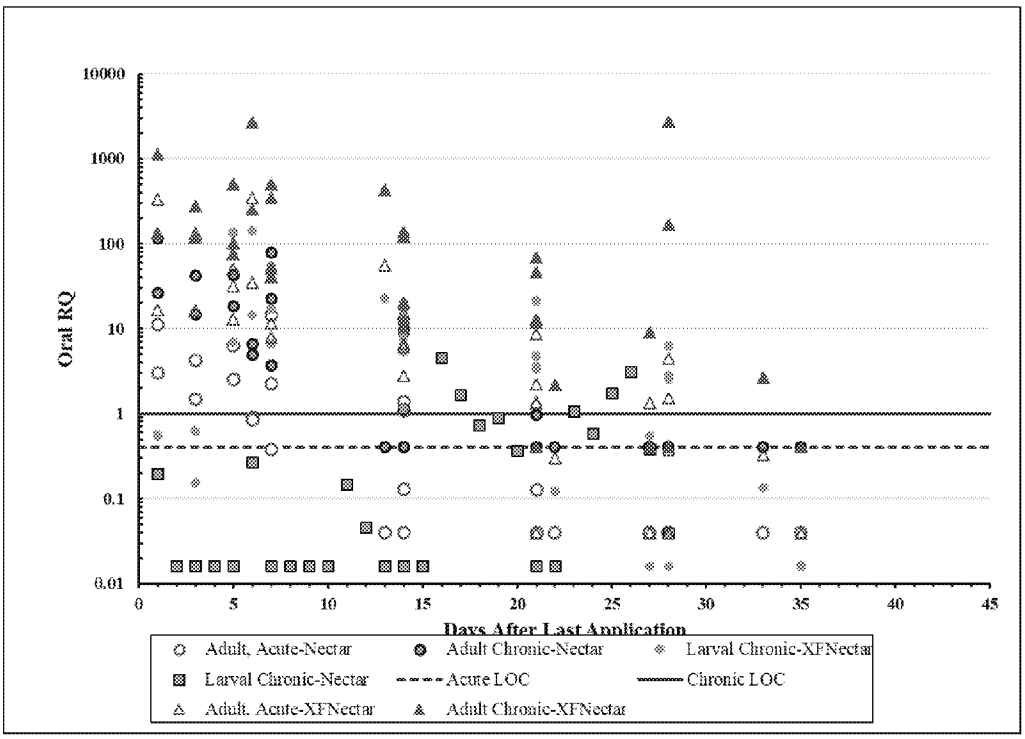

Figure 7.18. RQs over time following foliar applications of clothianidin to cotton.

Table 7.27. Refined RQs for foliar applications of clothianidin to grapes.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.64
		5	120	3.6			0.00	**1.27**
	Drone	6+	130	3.6			0.00	**1.27**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.01	**2.81**	0.01	**24.12**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.02	**4.06**	0.01	**34.83**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	**0.72**	0.00	**6.17**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.02	0.00	0.15
	Worker (foraging for nectar)	>18	292	0.041	0.00	0.02	0.00	0.15
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	**0.85**	0.00	**7.26**
	Drone	>10	235	0.0002	0.00	0.00	0.00	0.00

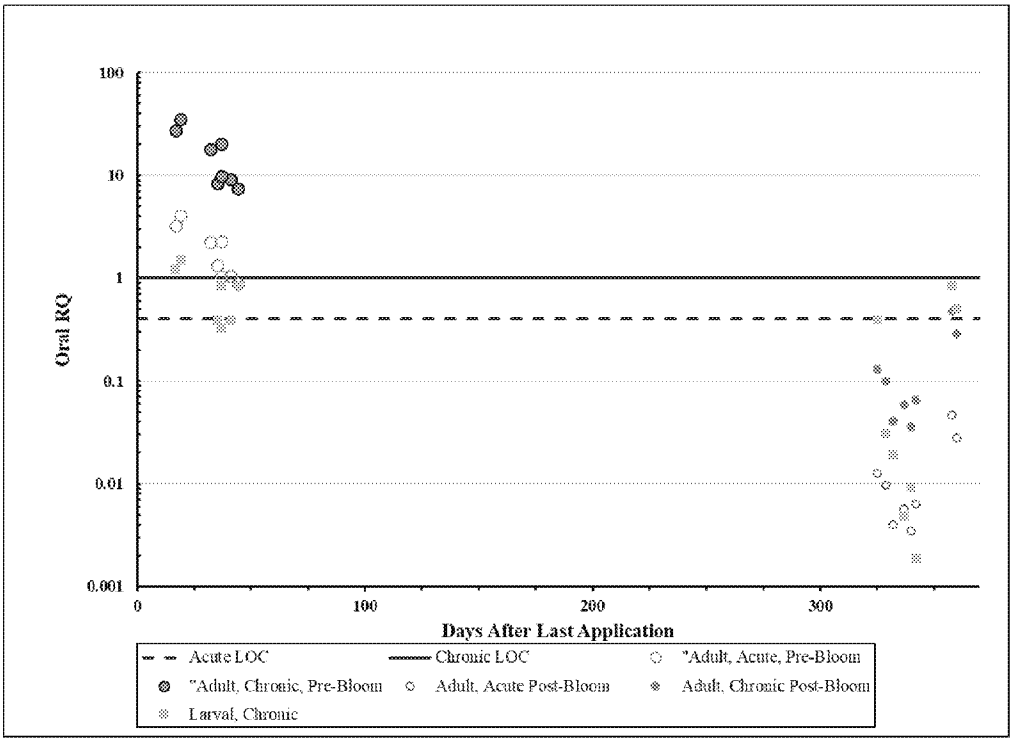

Figure 7.19. Refined RQs over time following foliar applications (pre-bloom or post-bloom) of clothianidin to grapes.

Table 7.28. Refined RQs following post-bloom foliar applications of clothianidin to peach.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.03
		5	120	3.6			0.00	0.06
	Drone	6+	130	3.6			0.00	0.07
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	0.24	0.00	**1.00**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	0.36	0.00	**1.52**

	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	0.07	0.00	0.32
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.01	0.00	0.07
	Worker (foraging for nectar)	>18	292	0.041	0.00	0.04	0.00	0.41
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.07	0.00	0.32
	Drone	>10	235	0.0002	0.00	0.03	0.00	0.33

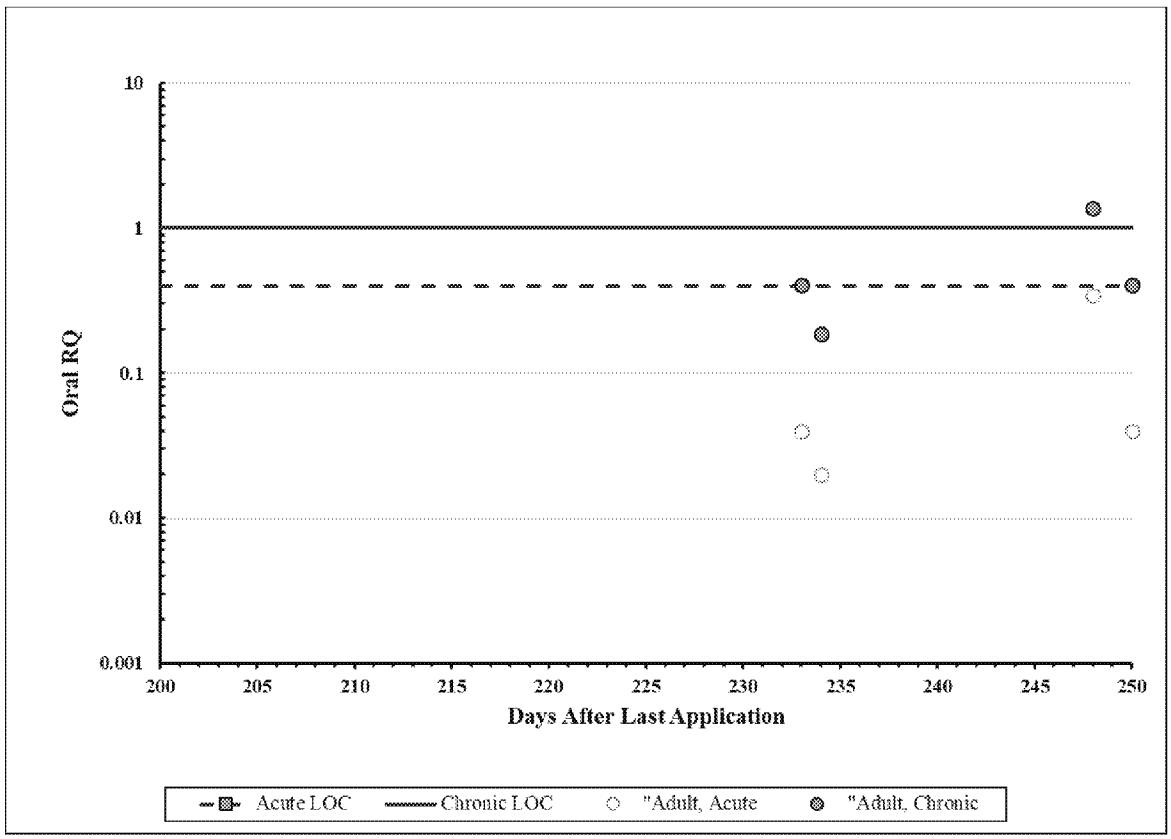

Figure 7.20. RQs over time following foliar post-bloom applications of clothianidin to peach

Table 7.29 Refined RQs following Foliar Applications to Potatoes

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.04
		5	120	3.6			0.00	0.07
	Drone	6+	130	3.6			0.00	0.07
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	0.21	0.00	**1.41**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	0.31	0.00	**2.03**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	0.05	0.00	0.36
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.00	0.00	0.01
	Worker (foraging for nectar)	>18	292	0.041	0.00	0.00	0.00	0.01
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.06	0.00	0.42
	Drone	>10	235	0.0002	0.00	0.00	0.00	0.00

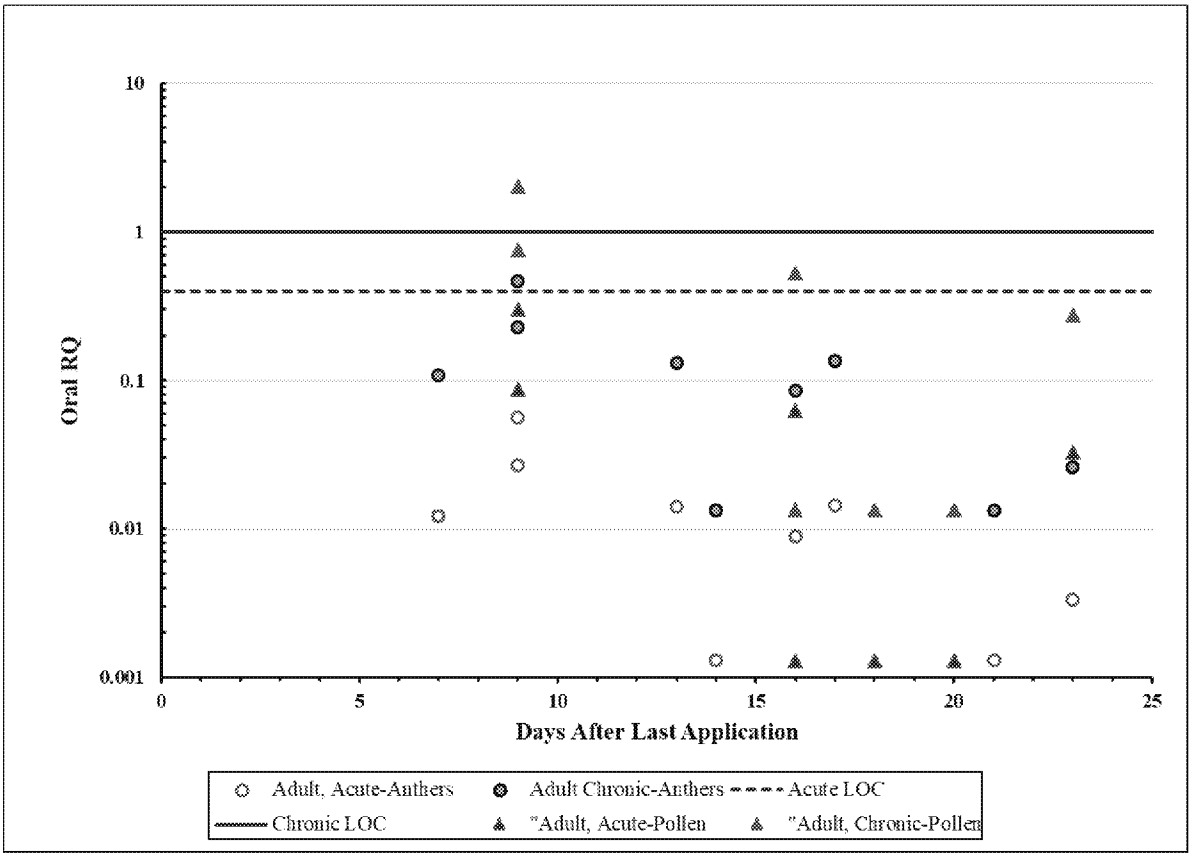

Figure 7.21 RQs over time following foliar applications of clothianidin to potatoes

Table 7.30 Refined RQs following foliar application of clothianidin to pumpkin

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.13
		5	120	3.6			0.00	0.26
	Drone	6+	130	3.6			0.00	0.27
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	0.33	0.00	**2.81**

	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	**0.57**	0.00	**4.77**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	0.16	0.00	**1.32**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.08	0.00	0.60
	Worker (foraging for nectar)	>18	292	0.041	0.00	**0.52**	0.00	**3.95**
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.12	0.00	0.99
	Drone	>10	235	0.0002	0.00	**0.41**	0.00	**3.17**

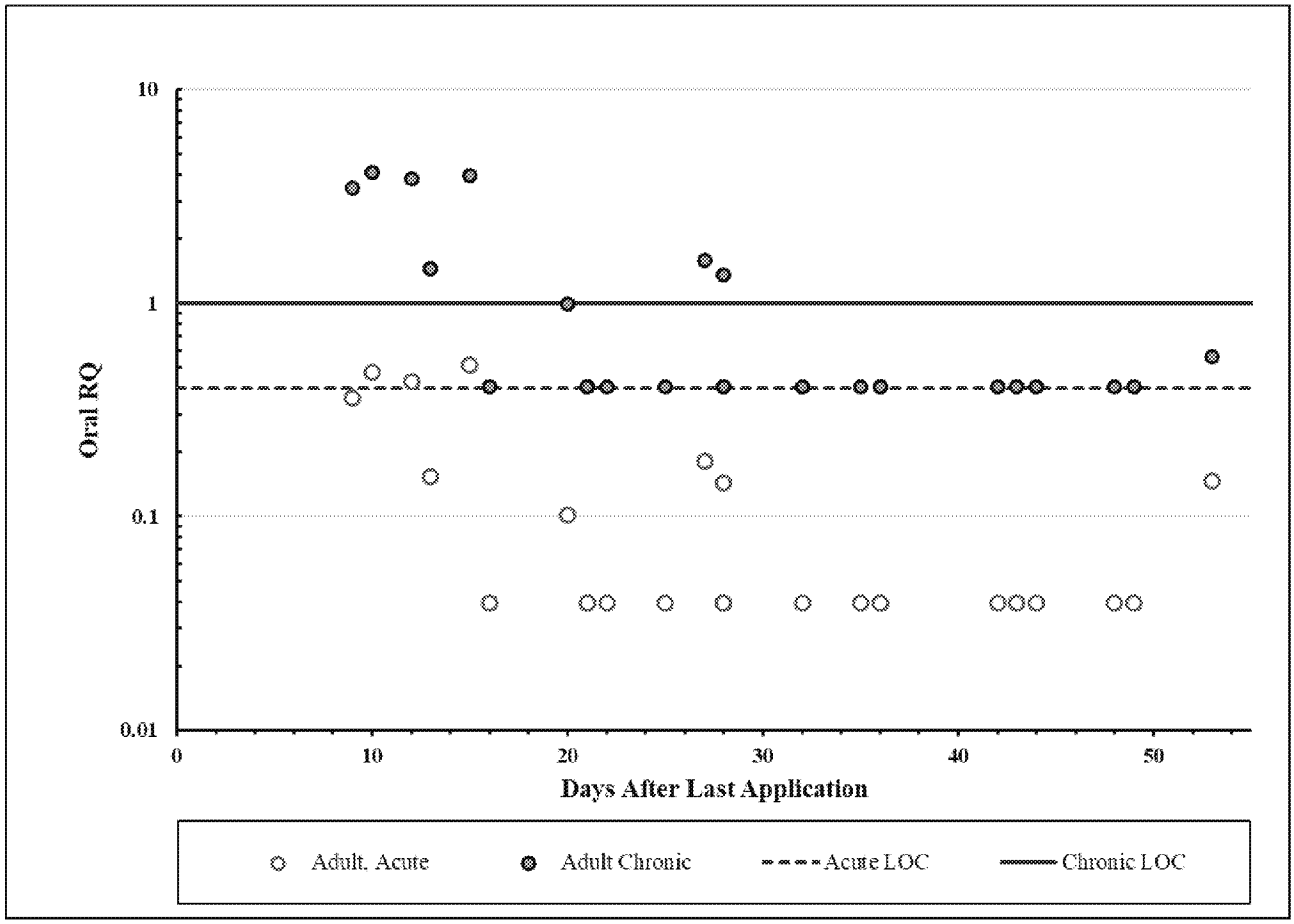

Figure 7.22. RQs over time following foliar applications of clothianidin to pumpkin

Clothianidin - Refined Tier I Soil Applications

Table 7.31 Refined RQs following soil applications of clothianidin to pumpkin (4 cucurbit study, MRID 49705901)

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.10
		5	120	3.6			0.00	0.19
	Drone	6+	130	3.6			0.00	0.21
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	**0.19**	0.00	**1.21**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	**0.38**	0.00	**2.55**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	**0.14**	0.00	**0.98**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.09	0.00	**0.65**
	Worker (foraging for nectar)	>18	292	0.041	0.00	**0.57**	0.00	**4.37**
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	**0.08**	0.00	**0.53**

	Drone	>10	235	0.0002	0.00	**0.46**	0.00	**3.52**

Table 7.32 Refined RQs following soil applications of clothianidin to cucumber (4 cucurbit study, MRID 49705901). Anther residues (maximum and maximum-mean of 34.3 and 32 ng c.e./g, respectively) used in lieu of pollen residue values.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.54
		5	120	3.6			0.00	**1.09**
	Drone	6+	130	3.6			0.00	**1.18**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	**0.71**	0.00	**6.02**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.01	**1.59**	0.00	**13.53**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	**0.66**	0.00	**5.58**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	**0.47**	0.00	**3.94**
	Worker (foraging for nectar)	>18	292	0.041	0.01	**3.13**	0.01	**26.45**
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.33	0.00	**2.80**
	Drone	>10	235	0.0002	0.01	**2.52**	0.01	**21.28**

Table 7.33. Refined RQs following soil applications of clothianidin to cucumber (4 cucurbit study, MRID 49705901). Anther residues (maximum and maximum-mean of 20.8 and 16.8 ng c.e./g, respectively) used in lieu of pollen residue values.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.18
		5	120	3.6			0.00	0.37
	Drone	6+	130	3.6			0.00	0.40
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.28	**2.83**	0.00	**2.11**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.61	**6.27**	0.00	**4.65**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.25	**2.55**	0.00	**1.88**
	Worker (foraging for pollen)	>18	43.5	0.041	0.17	**1.78**	0.00	**1.31**
	Worker (foraging for nectar)	>18	292	0.041	1.16	**11.93**	0.00	**8.76**
	Worker (maintenance of hive in winter)	0-90	29	2	0.13	**1.30**	0.00	0.96
	Drone	>10	235	0.0002	0.93	**9.60**	0.00	**7.05**

Table 7.34. Refined RQs following soil applications of clothianidin to squash (4 cucurbit study, MRID 49705901)

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.08
		5	120	3.6			0.00	0.16
	Drone	6+	130	3.6			0.00	0.17
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	0.10	0.00	0.97
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	0.21	0.00	**2.05**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	0.08	0.00	0.80
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.05	0.00	0.54
	Worker (foraging for nectar)	>18	292	0.041	0.00	0.36	0.00	**3.62**
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.04	0.00	0.43
	Drone	>10	235	0.0002	0.00	0.29	0.00	**2.91**

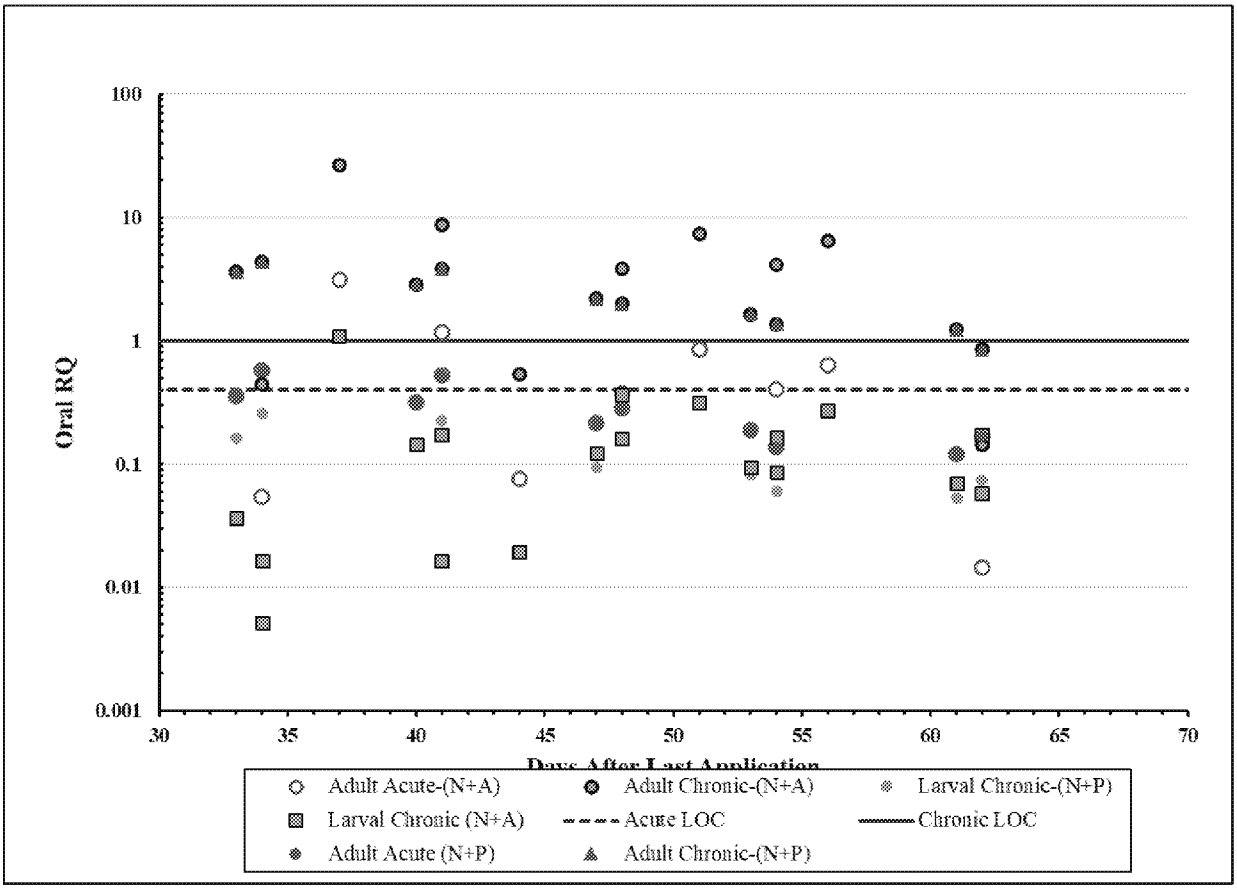

Figure 7.23. RQs over time for soil applications cucurbit crops (MRID 49705901). N+A reflects RQs calculated using nectar and anther data (as a direct surrogate for pollen). N+P reflects RQs calculated using nectar and pollen data (and was only available for pumpkin and squash).

Table 7.35. Refined RQs for soil applications to pumpkin (MRID 49910601).

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.17
		5	120	3.6			0.00	0.34
	Drone	6+	130	3.6			0.00	0.36
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	0.26	0.00	**2.11**

	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	**0.53**	0.00	**4.46**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	0.20	0.00	**1.72**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.13	0.00	**1.16**
	Worker (foraging for nectar)	>18	292	0.041	0.00	**0.89**	0.00	**7.75**
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.11	0.00	0.92
	Drone	>10	235	0.0002	0.00	**0.72**	0.00	**6.23**

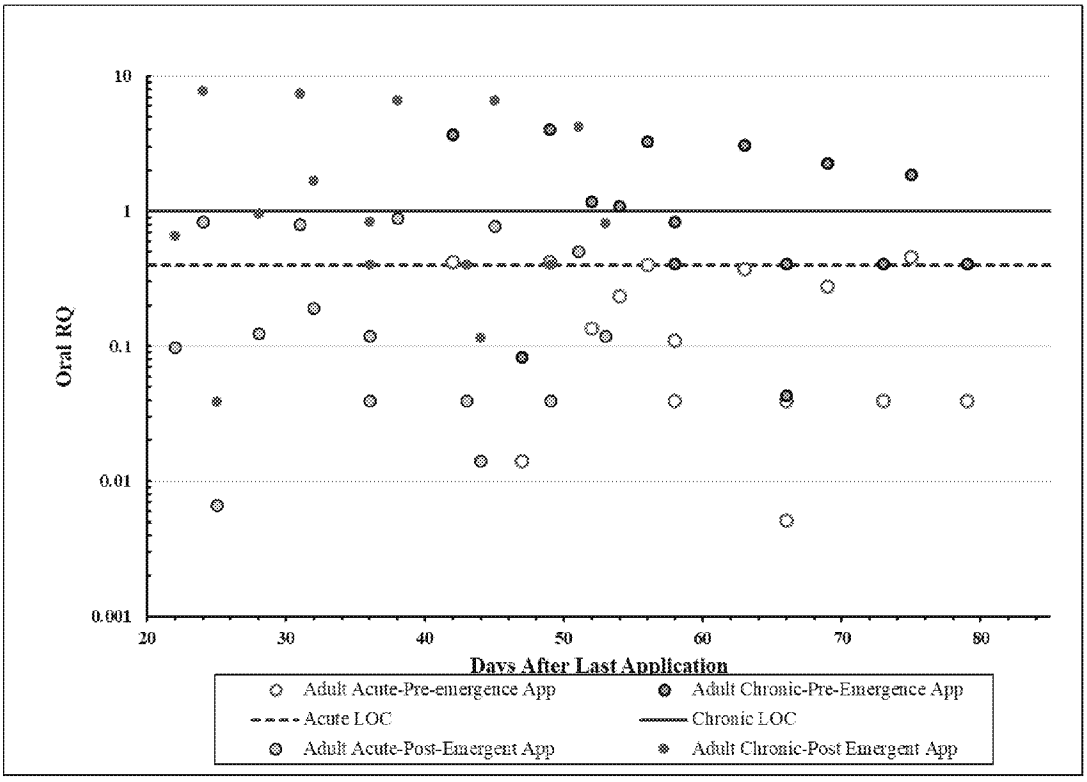

Figure 7.24. RQs over time following soil applications to pumpkins (MRID 49910601).

Table 7.36. Refined RQs for soil applications to melons (MRID 50154306) using hand-collected samples (only 1 sample/time period so acute and chronic doses do not differ).

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	**1.08**
		5	120	3.6			0.01	**2.16**
	Drone	6+	130	3.6			0.01	**2.34**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	**1.13**	0.00	**11.65**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.01	**2.58**	0.01	**26.53**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	**1.08**	0.00	**11.10**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	**0.77**	0.00	**7.92**
	Worker (foraging for nectar)	>18	292	0.041	0.02	**5.17**	0.02	**53.13**
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	**0.53**	0.00	**5.50**
	Drone	>10	235	0.0002	0.02	**4.16**	0.02	**42.76**

Table 7.37. Refined RQs for soil applications to melons (MRID 50154306) using bee-collected samples

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]	Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]

			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.13
		5	120	3.6			0.00	0.26
	Drone	6+	130	3.6			0.00	0.28
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	0.24	0.00	**1.67**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	**0.52**	0.00	**3.47**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	0.20	0.00	**1.32**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.14	0.00	0.87
	Worker (foraging for nectar)	>18	292	0.041	0.00	**0.91**	0.00	**5.83**
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.11	0.00	0.72
	Drone	>10	235	0.0002	0.00	**0.73**	0.00	**4.69**

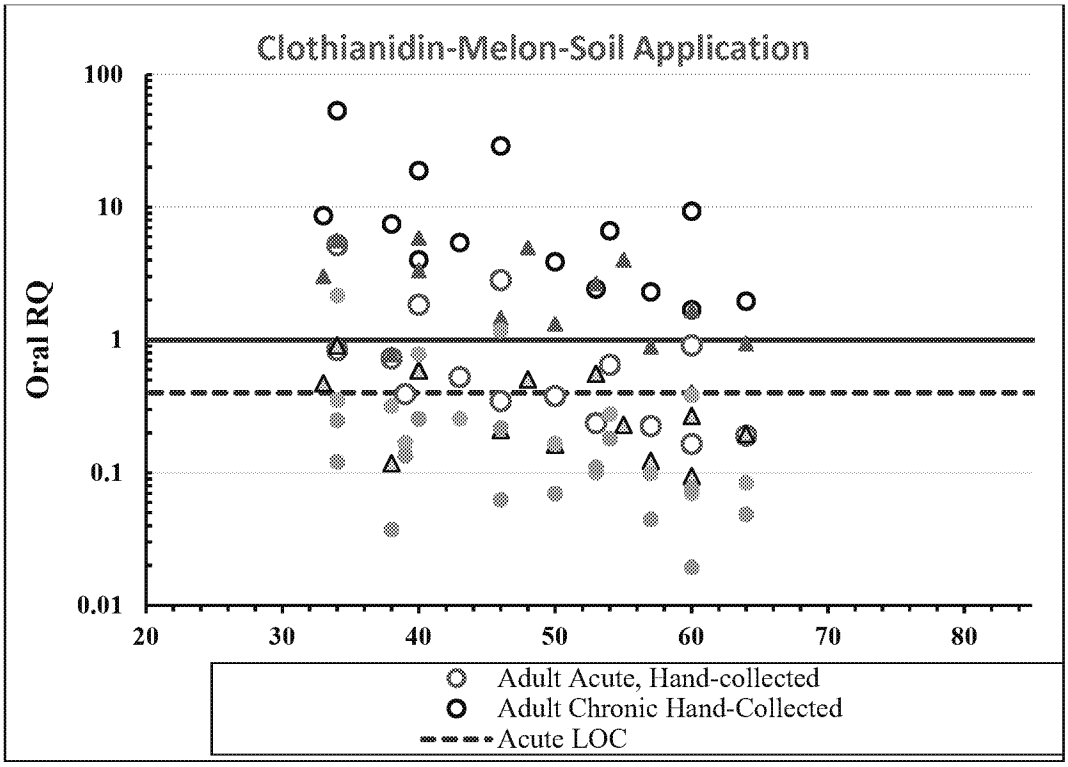

Figure 7.25. RQs over time following soil applications to melons (MRID 50154306) using hand-collected (n=1) or bee-collected samples (n=5).

Table 7.38. Refined RQs for citrus (lemon and orange) crops following soil applications

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	**1.22**
		5	120	3.6			0.01	**2.43**
	Drone	6+	130	3.6			0.01	**2.60**
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.01	**2.98**	0.01	**18.38**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.02	**5.95**	0.01	**36.11**

	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.01	**2.14**	0.00	**12.71**
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	**1.35**	0.00	**7.85**
	Worker (foraging for nectar)	>18	292	0.041	0.03	**9.00**	0.02	**52.44**
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	**1.23**	0.00	**7.49**
	Drone	>10	235	0.0002	0.03	**7.24**	0.02	**42.17**

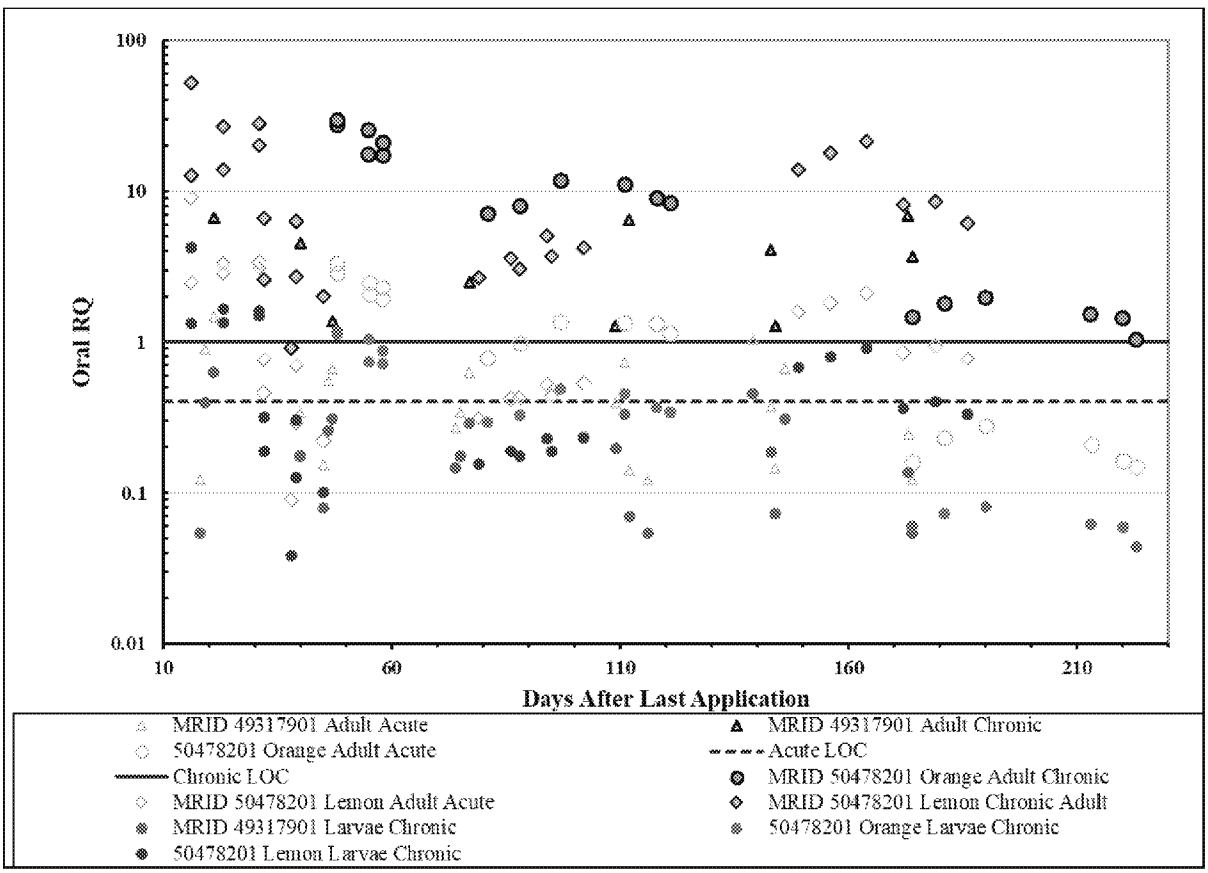

Figure 7.26. RQs over time following soil applications to citrus crops. RQs for MRID 49317901 are based on empirical residues in orange nectar and Bee-REX generated modeled residues in pollen (40.2 ng c.e./g). MRID 50478201 values are for empirical residues in either orange (located in Florida) or lemon (located in Arizona)

Table 7.39. Refined RQs following pre-bloom soil applications to grapes (based solely on residues in pollen).

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.08
		5	120	3.6			0.00	0.16
	Drone	6+	130	3.6			0.00	0.16
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	0.37	0.00	**2.96**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	**0.53**	0.00	**4.27**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	0.09	0.00	0.76
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.00	0.00	0.02
	Worker (foraging for nectar)	>18	292	0.041	0.00	0.00	0.00	0.02
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.11	0.00	0.89
	Drone	>10	235	0.0002	0.00	0.00	0.00	0.00

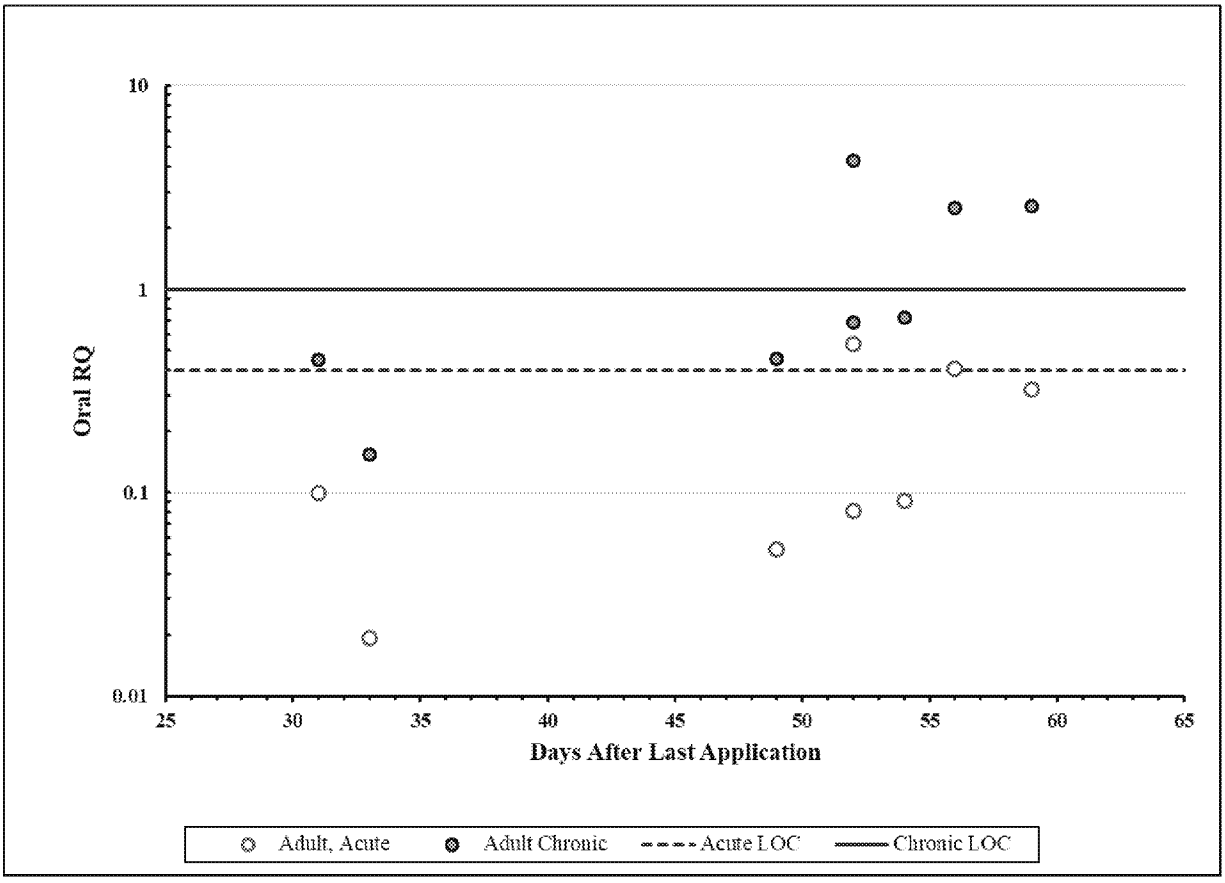

Figure 7.27. RQs over time following pre-bloom soil applications to grapes (pollen residues only).

Table 7.40. Refined RQs following soil applications to potatoes (pollen residues only)

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.05
		5	120	3.6			0.00	0.09
	Drone	6+	130	3.6			0.00	0.09
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	0.34	0.00	**1.71**
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	**0.49**	0.00	**2.47**
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	0.09	0.00	0.44
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.00	0.00	0.01
	Worker (foraging for nectar)	>18	292	0.041	0.00	0.00	0.00	0.01
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.10	0.00	0.51
	Drone	>10	235	0.0002	0.00	0.00	0.00	0.00

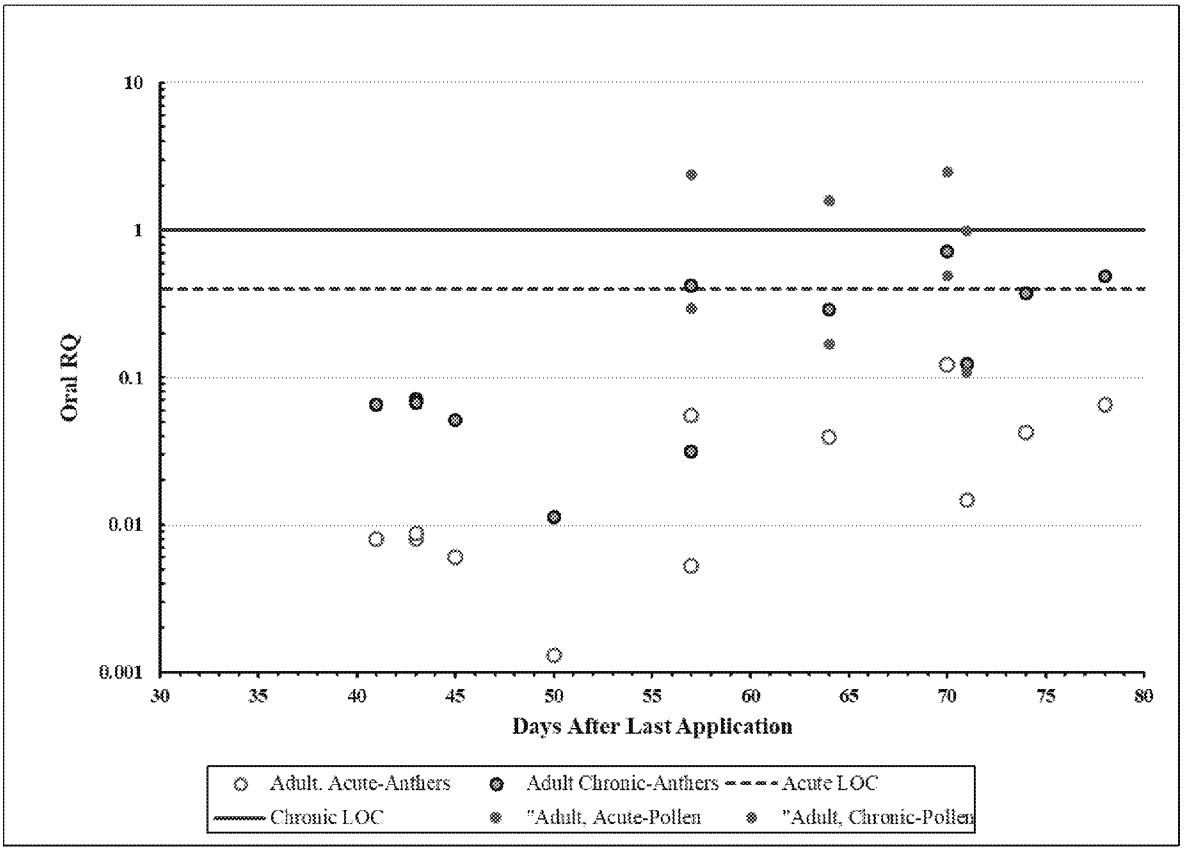

Figure 7.28. RQs over time following soil applications to potatoes. RQs are based on either residues in pollen- or anthers-alone (anthers serving as a direct analog in lieu of pollen data)

Table 7.41. Refined RQs for corn-in furrow clothianidin soil applications on top of seed treatments

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.00
		5	120	3.6			0.00	0.10
	Drone	6+	130	3.6			0.00	0.10
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	0.05	0.00	0.49
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	0.07	0.00	0.71

	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	0.01	0.00	0.13
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.00	0.00	0.00
	Worker (foraging for nectar)	>18	292	0.041	0.00	0.00	0.00	0.00
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.02	0.00	0.15
	Drone	>10	235	0.0002	0.00	0.00	0.00	0.00

Clothianidin - Refined Tier I Seed Treatments

Table 7.42. Refined RQs for seed treatment applications to canola seeds.

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.00
		5	120	3.6			0.00	0.10
	Drone	6+	130	3.6			0.00	0.10
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	0.04	0.00	0.29
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	0.08	0.00	0.63
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	0.03	0.00	0.25
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.02	0.00	0.17
	Worker (foraging for nectar)	>18	292	0.041	0.00	0.15	0.00	**1.17**
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.02	0.00	0.13
	Drone	>10	235	0.0002	0.00	0.12	0.00	0.94

Table 7.43. Refined RQs following seed treatment applications to cotton-seed

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.00
		5	120	3.6			0.00	0.10
	Drone	6+	130	3.6			0.00	0.10
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	0.07	0.00	0.37
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	0.16	0.00	0.83
	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	0.06	0.00	0.34
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.05	0.00	0.24
	Worker (foraging for nectar)	>18	292	0.041	0.00	0.30	0.00	**1.60**
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.03	0.00	0.17
	Drone	>10	235	0.0002	0.00	0.24	0.00	**1.29**

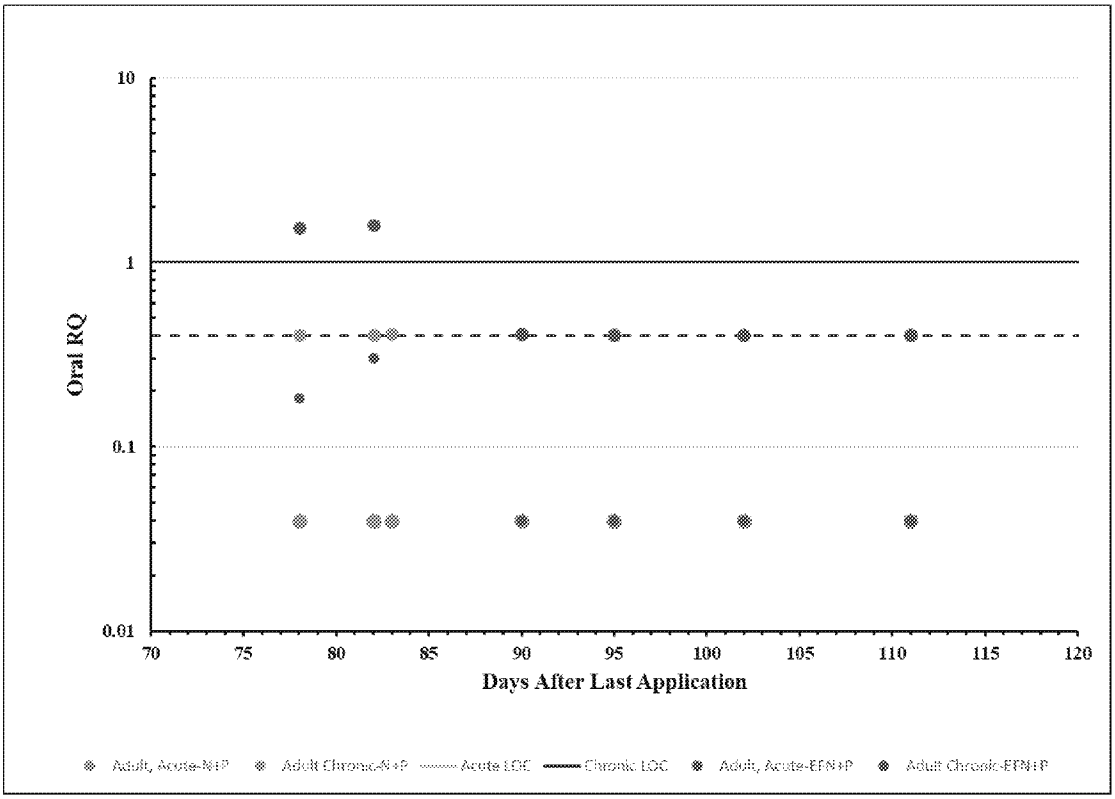

Figure 7.29. Refined RQs over time following seed treatment applications to cotton seed.

Table 7.44. Refined RQs following seed treatment in corn (residues in pollen only), scaled to reflect max seed treatment rate

Life stage	Caste or task in hive	Average age (in days)	Consumption Rates[5]		Acute dose (µg a.i./bee)	Acute RQ[2]	Chronic dose (µg a.i./bee)[6]	Chronic RQ[3]
			Nectar (mg/day)	Pollen (mg/day)				
Larval	Worker	4	60	1.8	Not-calculated		0.00	0.00
		5	120	3.6			0.00	0.10
	Drone	6+	130	3.6			0.00	0.10
Adult	Worker (cell cleaning and capping)	0-10	60	6.65	0.00	0.11	0.00	0.23
	Worker (brood and queen tending, nurse bees)	6 to 17	140	9.6	0.00	0.15	0.00	0.33

	Worker (comb building, cleaning and food handling)	11 to 18	60	1.7	0.00	0.03	0.00	0.06
	Worker (foraging for pollen)	>18	43.5	0.041	0.00	0.00	0.00	0.00
	Worker (foraging for nectar)	>18	292	0.041	0.00	0.00	0.00	0.00
	Worker (maintenance of hive in winter)	0-90	29	2	0.00	0.03	0.00	0.07
	Drone	>10	235	0.0002	0.00	0.00	0.00	0.00