Imidacloprid Preliminary Pollinator Assessment Table of Appendices

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

Table A-1. Evaluated registrant submitted and open literature studies determined to be invalid for quantitative or qualitative use

Citation	MRID	Major Uncertainties
Abrol, D. P., Sharma, D. 2007. Morphogenic and toxic effects of pesticide on honey bee brood. *Journal of Research*. Vol. 6, No. 2, pp 133-148.	49719601	- Article is not a primary source
Ambolet B, JF Crevat and HW Schmidt. 1997. Research on secondary effects of seed treatment with imidacloprid on the behaviour of honey bees on flowers of sunflower. Proceedings of the fourth international conference on pests in agriculture; 6-8 January 1997; Montpellier, France; Association Nationale pour la Protection des Plantes (ANPP).	49719602	- Purity and source of imidacloprid not provided - Plots in the semi field and field tests were treated with a carbamate insecticide (pirimicarb) one week before introduction of the hives into the field - No residue samples from pollen, or within the hive to confirm the presence or absence of imidacloprid. Nectar that was collected was used for an acute toxicity test to aphids. - Very few details provided throughout the study as this information was drawn for conference proceeding and therefore there is no evidence that the study has undergone a scientific peer review.
Ambolet B, Crevat JF, Cure G, Schmuck R and Vincinaux C. 1999. Influence under field condition of imidacloprid on honey bees. Proceedings of the fifth international conference on pests in agriculture, Part 3; 7-9 December 1999; Montpellier, France. Association Nationale pour la Protection des Plantes (ANPP).	49719603	- Article is not a primary source but rather a review of the results from other citations - No information on the methodology used for the residue trails is provided (including application rates)
Belien, T., J. Kellers, K. Heylen, W. Keulemans, J. Billen, L. Arckens, R. Huybrechts, B. Gobin. 2009. Effects of sublethal doses of crop protection agents on honey bee (*Apis mellifera*) global colony vitality and its potential link with aberrant foraging activity. *Communications in Agricultural and Applied Biological Sciences*. 74/1. Pp 245-253.	49719604	- Lack of replication as one colony per treatment was utilized and so the variability of the responses could not be measured - One time treatment of imidacloprid where it was not quantified the amount of sucrose consumed - No in hive measurements and so, coupled with no quantification of sucrose consumed, exposure not adequately confirmed
Bortolotti, L., E. Grazioso, C. Porrini and G. Sbrenna. 1999. Effect of pesticides on the bumble bee Bombus terrestris L. in the laboratory. Hazards of pesticides to bees, Avignon (France), September 07-09, 1999, Ed. INRA, Paris.	47875304	- Source of pesticide not available - Not stated what the doses were for acute oral and contact portions of the study - Not stated how many bees per treatment level were used - Unclear of the duration of exposure for the oral portion - No mention of a control used for the study - No mention of source or husbandry of bees
Carrillo, M.P, de Souza Bovi, T., Negrão, A.F., de Oliverira Orsi, R. 2013. Influence of agrochemicals fipronil and imidacloprid on the learning behavior of Apis mellifera L. honey bees. Acta Scientiarum: Animal Science, Vol. 35 Issue 4, p 431.	49719608	- Purity of imidacloprid not provided - No duration associated with the LD50 values - No analytical conformation of imidacloprid in the test solutions - No information regarding honey bee source and husbandry - No indication of control performance
Chandramani, P., Rani, B.U., Muthiah, C., Kumar, S. 2008. Evaluation of toxicity of certa.in insecticides to	49750602	- There was no endpoint calculated - There was no information regarding the source of the bees and husbandry conditions

Citation	MRID	Major Uncertainties
India honey bee, *Apis cerana indica* F. Pestology 32(8):42-43.		- The purity of imidacloprid was not reported
Choudhary, A., Sharma, D.C., 2008. Dynamics of pesticide residues in nectar and pollen of mustard (*Brassica juncea* (L.) Czern.) grown in Himachal Pradesh (India). Environmental Monitoring and Assessment, 144: 143-150.	49719609	- Source and purity of imidacloprid was not available - No information provided on the amount of active ingredient used on treated seeds, nor could one be back calculated from the information provided - No information on how much time bee were allowed to forage on treated crop - Unclear to what extent sampled pollen and nectar was from treated crop vs neighboring areas - The LOD of the analytical method used was 10 ppb which is noted as being relatively high compared to other studies (generally 1 ppb or less)
Colin, Y Le Conte, J.P. Vermendere. 1999. Managing nuclei in insect-proof tunnel as an observation tool for foraging bee: sublethal effects of deltamethrin and imidacloprid. *Hazard of pesticides to bees.* Ed. INRA, Paris, 2001.	47800505	- No information on the purity or source of imidacloprid - No information on health or source of colonies - No information on total amount of treated sucrose consumed - No statistical analysis of the data - No analytical confirmation of the exposure concentrations
Cure, H.W. Schmidt, R. Schmuck. 1999. Results of a comprehensive field research programme with the systemic insecticide imidacloprid (Gaucho). Hazards to of pesticides to bee. Ed. INRA. Paris 2001.	47875302	- Article is a review of other citations and not itself a primary source
Devillers, J., A. Decourtye, H. Budzinski, M.H. Pham-Delegue, S. Cluzeau, G. Maurin. 2003. Comparative Toxicity and Hazards of Pesticide to Apis and non-Apis Bees. A Chemometrical Study. SAR and QSAR in Environmental Research, Vol. 14 (5-6), October - December 2003, pp. 389-403.	47812305	- Primary source of data is outdated - Toxicity results were based on a scale and not a quantified measure of toxicity (*e.g.* an LD_{50}) - The duration of exposure was not identified - The actual concentrations of imidacloprid were not identified - The route of exposure was not identified - It was not stated how many bees were exposed per treatment - There was no mention of an experimental control
Dively, G.P., M. Embrey, T. Patton, and A. Miller. 2007. Determination of Imidacloprid Residue Concentrations in Seedless Watermelon Flowers: Final Report submitted to the Foundation for the Preservation of Honey Bees	49719613	- The interval between exposure and analysis of samples was not clear - This study appears to be pilot work for determining the parameters to be used in subsequent work - The limit of quantitation and limit of detection were not provided - Results from this study were not published in a scientific journal and thus were not peer reviewed
Harris J. L.1999. Evaluation of Gaucho seed dressing, applied to canola on the honye bee (*Apis mellifera*) at Indian Head, Saskatchewan (Indian Head Research Station Site, RM# 156, SW-19-18-12-W2) in 1999.	49766213	- Residues in canola nectar and pollen were not measured - No replication of tunnels within treatment groups - Food storage in the hives was not reported - Colony strength was estimated as a function of mark/recapture but no details on how this was done
Husain D, Qasim M, Saleem M, Akhter M, Khan KA. 2014. Bioassay of insecticides against three honey bee species in laboratory conditions. Cercetari Agronomice in Moldova 47(2):69,79.	49719619	- Level of control mortality is unknown - Source of imidacloprid not provided - No husbandry information of the bees - Results provided in time to LD5- rather than concentration to LD50 - Indirect contact toxicity rather than direct contact toxicity assessed

Citation	MRID	Major Uncertainties
Incerti, F., L. Bortolotti, C. Porrini, A. M. Sbrenna, G. Sbrenna. 2003. An extended laboratory test to evaluate the effects of pesticide on bumble bees. *Bulletin of Insectology*. 56, pp. 159-164.	47800519	- Test was trying to validate a new methodology which was had several flaws that included cages not being large enough to provide enough forage for the bumble bees - No measurements made on the plant tissue and so no data are available to provide a measure of dose
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.	49750603	- 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 imidacloprid in the test solutions
Khan RB and Dethe MD. 2004. Median lethal time of new pesticides to foragers of honey bees. Pestology 28(1):28-29.	49750604	- It was not reported what concentrations the bees were actually exposed to - There was no analytical verification of imidacloprid in the test solutions - Although a control was included, it was not reported whether there was any control mortality - Route of exposure was contact toxicity of residues on film rather than on treated foliage
Kirchner, W. (2000) The Effects of Sublethal Doses of Imidacloprid, Hydroxy-Imidacloprid and Olefine-Imidacloprid on the Behaviour of Honey bees. Project Number: 110634, M/031852/02/2. Unpublished study prepared by Bayer CropScience LP. 13 p.	47699406	- No replication of the hives among treatment groups, precluding a statistical analysis - The means of the responses were no presented, only graphical representations which were difficult to discern - Chemical degradates were not measured - Source, age, and husbandry of the bees was not provided - The number of exposed bees per treatment group was not provided - No mention of whether the colonies had prior exposure to insecticides
Laurino D, Manino A, Patetta A, Ansaldi M and Porporato M. 2010. Acute oral toxicity of neonicotinoids on different honey bee strains. Redia - Giornale Di Zoologia; 2010.93:99-102.	N/A	- Although a control was included, it was not reported whether there was any control mortality - Vomiting was observed in the higher concentration that likely reduced exposure although it is unknown to what degree - The amount of ingested material did not appear to be calculated
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.	49719623	- Purity and source of imidacloprid 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

Citation	MRID	Major Uncertainties
Maus, C.; Schoning, R. (2001) Effects of Residues of Imidacloprid in Maize Pollen from Dressed Seeds on Honey Bees (Apis mellifera). Project Number: E/319/1912/6, M/052637/01/2, MAUS/AM/012. Unpublished study prepared by Bayer Ag. 53 p.	47699416	- Two replicate colonies for each of the control and treatment groups resulted in in high variability in the responses - Hives may have been stressed after confinement of 35 days within the flight cage
Maus, CH. (2002): Evaluation of the effects of residues of Imidacloprid FS 600 in maize pollen from dressed seeds on honey bees (Apis mellifera) in the semifield Bayer AG, unpublished report No: MAUS/Am018; 2002-03-07. Bayer study No. E 319 2046 - 5 : Evaluation of the effects of residues of Imidacloprid FS 600 in maize pollen from dressed seeds on Honey bees (Apis mellifera) in the semifield Bayer AG, unpublished report No: MR-547/01; 2001-11-28	47699414	- Hives confined in tunnels for 45 days which may have caused undue stress to the colonies - Replication of colonies among treatment groups but low number (3 per group) led to high variability in the responses - Long duration (6 months) between pollen collection in the field and analysis - Treatment groups consumed more pollen collectively which was speculated by the study authors as having to do with a different consistency for the pollen in the treatment groups as compared to the control
Moncharmont, Francois-Xavier, Axel Decourtye "Statistical Analysis of Honey bee survival after chronic exposure to insecticide" Environmental Toxicology and Chemistry,(2002): Vol.22 3088-3094	47800501	- No explicit duration of exposure - No presentation of LD50 endpoints - No statement of number of treated bees per test level
Morandin, Lora A. and Winston, Mark L. "Effects of Novel Pesticides on Bumble Bee (Hymenoptera: Apidae) Colony Health and Foraging Ability." *Environmental Ecology*; 32 (3), 555-563	47699432	- Explicit duration of exposure not stated - No analytical confirmations of imidacloprid in the pollen - Statistical results only presented instead of the means for all endpoints - Foraging observations took place in late October and November, presumably when the bees are preparing for overwintering
H. W. Schmidt. 1988. Tolerability of seed treatments to bees (bee tunnel I). Bayer Report No. : VAZ 4/88	49766211	- Minimal details on methodology of the study - Minimal information for results presented, with no means, percent differences, or statistical results present.
H. W. Schmidt. 1990. Bee IV:Soil application of NTN 33893 at sowing time. Report No : VAZ 10/90	49766216	- Unknown duration in the tunnel for the summer rapeseed trial - No summer rape flowers in the control plots - All plots, including controls, were sprayed with Rubitox (Phosalone) and pirimicarb during the trial
H. W. Schmidt. 1995. Bees VI: flower visits after seed treatment. Bayer Programme No. VAZ-P-H3-003/95. Report No : VAZ 34a/95	49602719	- Unknown duration of exposure - No information on initial hive condition and the size of the test hives used - No statistical analysis conducted on the data - Timing of measurements on foraging activity not provided - Pollen samples from the pollen traps indicated that only 3% of pollen originated from the treated crop, suggesting limited exposure - Distance between control and treated fields not provided
H. W. Schmidt. 1997. Bees I: systemic nature of insecticidal seed treatment. Report No: DVG 10/97	N/A	- No replications of flight tunnel among treatment groups - Hives were confined in the flight tunnel for 24 days, which may have caused undue stress to the colonies reduced in AKD treatment; reasons for declines uncertain due to lack of replication

Citation	MRID	Major Uncertainties
H. W. Schmidt. 1998. Flower visits to sunflowers seed-treated with Gaucho. Bayer Report No. : Bees 1/98	49766212	- No replications of test fields among treatment groups - Demonstration of exposure was not adequately characterized as treatment fields were 1.5 hectares big and bees would be expected to forage outside this area. There was no pollen analysis conducted to characterize the other sources of forage. - The control field was noted to have lower germination in the crop as compared to the control group and therefore less available forage. - Flower conditions and timing varied among treatment plots - Small test plots suggested bees were foraging outside of treatment areas
H. W. Schmidt. 1999. Beobachtungen im Zeltversuch mit Bienen nach Beizung von Sommerraps. Report No: DVG7/98	49766215	- No replication across the treatment groups, precluding a statistical analysis of the results - No information provided on the species of bee used, source, or other husbandry methods.
H.W. Schmidt, R. Schmuck, R. Scheming. 1998. The impact of Gaucho 70 WS seed treated sunflower seeds on honey bees. REPORT NUMBER BF 1/98	49766206	- No replication of the test fields, precluding a statistical analysis of the results - Demonstration of exposure was not adequately characterized as treatment fields were 1.5 hectares big and bees would be expected to forage outside this area. There was no pollen analysis conducted to characterize the other sources of forage.
Schmuck R. "No causal relationship between Gaucho seed dressing in sunflowers and the French bee syndrome". Pflanzenshtuz Nachrichten (1999) 52/99.	47800508	- Article is not a primary source but rather a review of other citations
Schmuck, R., Schöning, R. and O. Schramel, 1999, Residue Levels of Imidacloprid and Imidacloprid Metabolites in Nectar, Blossoms and Pollen of Summer Rape, sunflowers, and maize Cultivated on Soils with Different Imidacloprid Residue Levels and Effects of These Residues on Foraging Honey bees, Bayer AG, Crop Protection-Development Institute for Environmental Biology, D-51368, Leverkusen-Bayerwerk, Laboratory No. E 370 1548-8, Bayer Report No. 109566, Bayer Corporation, September 28, 1999.	47699417	- One tunnel per treatment group, precluding a statistical analysis of the results - Hives confined to tunnels for 39 days; which may have caused undue stress to the hives - Background residue of imidacloprid in the treatment fields in the soil up to 17.3 ppb prior to test initiation.
Schmuck, R.; Schoning, R. (1999) Residue Levels of Imidacloprid and Imidacloprid Metabolites in Nectar, Blossoms, Pollen and Honey Bees Sampled from a Summer Rape Field in Sweden and Effects of these Residues on Foraging Honey bees. Project Number: E/370/1360/0, 109492, M0/03/011933. Unpublished study prepared by Bayer Ag. 32 p.	47699418	- One tunnel per treatment group, precluding a statistical analysis - Long-term confinement in the tunnels (39d) may have stressed out the bees
Schmuck, R.; Schoning, R. (1999) Residues of Imidacloprid and Imidacloprid Metabolites in Nectar, Blossoms, Pollen and Honey Bees Sampled from a French Summer Rape Field and Effects of these Residues on Foraging Honey bees. Project Number:	47699419	- One tunnel per treatment group, precluding a statistical analysis - Long-term confinement in the tunnels (39d) may have stressed out the bees

Citation	MRID	Major Uncertainties
E/370/1358/7, 109496, M/006815/01/2. Unpublished study prepared by Bayer Ag. 31 p.		
Schmuck, R.; Schoning, R.; Schramel, O. (1999) Residue Levels of Imidacloprid and Imidacloprid Metabolites in Nectar, Blossoms and Pollen of Sunflowers Cultivated on Soils with Different Imidacloprid Residue Levels and Effects of these Residues on Foraging Honey bees. Project Number: E/370/1549/9,109563, M0/03/006522. Unpublished study prepared by Bayer Ag. 48 p. Schmuck, R.; Schramel, O.; Schoning, R. (1999) Residue Levels of Imidacloprid and Imidacloprid Metabolites in Nectar, Blossoms and Pollen of Summer Rape Cultivated on Soils with Different Imidacloprid Residue Levels and Effects of these Residues on Foraging Honey bees. Project Number: 109566, E/370/1548/8, SXR/AM/008. Unpublished study prepared by Bayer Ag. 46 p. Schmuck, R.; Schoning, R.; Schramel, O. (1999) Residue Levels of Imidacloprid and Imidacloprid Metabolites in Pollen of Maize Plants Cultivated on Soils with Different Imidacloprid Residue Levels. Project Number: E/370/1550/1, 109567, M0/03/006517. Unpublished study prepared by Bayer Ag. 41 p.	47699422/ 47699425/ 47699423	- One tunnel per treatment group precluded any statistical analysis - Background residue of imidacloprid in the treatment fields soils up to 24.5 ppb prior to test initiation.
Schmuck, R.; Schoning, R.; Schramel, O. (1999) Residue Levels of Imidacloprid and Imidacloprid Metabolites in Nectar, Blossoms and Pollen of Sunflowers Cultivated on Soils with Different Imidacloprid Residue Levels and Effects of these Residues on Foraging Honey bees. Project Number: E/370/1552/3, 109495, M0/03/011939. Unpublished study prepared by Bayer Ag. 45 p. Schmuck, R. ; Schramel, O. ; Schoning, R. . (1999) Residue levels of imidacloprid and imidacloprid metabolites in nectar, blossoms and pollen of summer rape Cultivated on Soils with Different	47699421 /49073643 /47699424	- One tunnel per treatment group, precluding any statistical analysis - Short exposure period (3 days in the tunnel)

Citation	MRID	Major Uncertainties
Imidacloprid Residue Levels and Effects of these Residue on Foraging Honey bees. ''Hoefchen'' 1999. Project Number:M/016842/02/2, 109568, E/370/1553/4. Unpublished study prepared by Bayer AG. 45p. Schmuck, R.; Schoning, R.; Schramel, O. (1999) Residue Levels of Imidacloprid and Imidacloprid Metabolites in Pollen of Maize Plants Cultivated on Soils with Different Imidacloprid Residue Levels. Project Number: E/370/1551/2, 109569, M0/03/006519. Unpublished study prepared by Bayer Ag. 39 p.		
Schuld, M. (2002) Field Test: Side Effects of Oil-Seed Rape Grown from Seeds Dressed with Imidacloprid and Beta-Cyfluthrin FS 500 on the Honey Bee (Apis mellifera L.). Project Number: M/066846/01/2, 99398/01/BFEU. Unpublished study prepared by Arbeitsgemeinschaft GAB Biotechnologie. 77p.	49073605	- No true experiment control as the control polots were seed treated with thiram - No pollen analysis conducted to ascertain the extent to which bees were foragin on the treated crop
Schnier, H.F., Wenig, G., Laubert, F., Simon, V., Schmuck, R. 2003. Honey bee safety of imidacloprid corn seed treatment. *Bulletin of Insectology*. 56 (1): 73-75.	47800523	- No treatment replicates; no statistical inferences possible - No bees tested on Maize - Uncertainty regarding photolysis of imidacloprid through time - Hives lost weight over 10d period of study - Hives were small and subjected to heavy sampling; uncertainty on impact of study methods on the colonies
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.	45422435	- 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 effects - Additional reps would help elucidate uncertainties about hive health - Prior pesticide exposure of hives unknown
Singh, N. and Karnatak AK. 2005. Relative toxicity of some insecticides to the workers of *Apis mellifera* L. Shashpa 12(1):23-25.	49750605	- Although mortality in the treatment group was adjusted using Abbott's correction, it was not stated the level of control mortality. - There was no analytical verification of imidacloprid in the test solutions - Route of exposure was contact toxicity of residues on film rather than on treated foliage

Citation	MRID	Major Uncertainties
Stanley J, Sah K, Ja.in SK, Bhatt JC, Sushil SN. 2015. Evaluation of pesticide toxicity at their field recommended doses to honey bees, A*pis cerana* and A. mellifera through laboratory, semi-field and field studies. Chemosphere 119:668-674	49719630	- No LD50 established - Acute contact test conducted formulated product - No level of control mortality reported - No residue analysis available to confirm exposure in the semi field study - lack of any colony health parameters in the semi field study - Very little information on methodology - Age of foragers not uniform - Minimal cage size, colonies not located in the "tent" in this trial - The design of this trial would cause stress on individual bees since they were unable to return to colony for 48 h. This level of stress could have contributed to unreliable mortality results. - No residue analysis was conducted to confirm exposure level.
Szentes, C. 1999. Field test of Gaucho 350 FS seed dressed sunflowers on honey bee colonies. Test code: 3103/99. Archives No: 13/T-4/1999	49766210	- One field per treatment group, precluding statistical analysis - High variability was noted, making it difficult to discrn treatment related effects - Hive conditions, including weight, were not uniform at the beginning of the study in the control and treatment groups
Valdovinos-Nunez G.R., Quezada-Euan J.J., 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.	49750607	- LD_{50} value was extrapolated outside (lower) than the range of treatment concentrations - There was no analytical verification of imidacloprid in the treatment concentration.
Wallner, K. 2001. Tests regarding effects of imidacloprid on honey bees. Hazards of pesticides to bees. Avignon (France), September 07-09, 1999.	48077901	- There were no replicates in the study (one tunnel per test group and one hive per tunnel). - It is not known how much imidacloprid was applied. Gaucho was applied at 0.005 g a.i/m2 as a seed treatment but it was not stated how much a.i was on each seed, nor was a seeding rate provided to back calculate to get this information. Consequently, the amount of imidacloprid applied is unclear. - 6. The LOD and LOQ (3 and 10 ppb) are markedly higher than observed in other studies (LOQ as low as 1 ppb)
Wang, D., W. Yu, Y. Yuan and J. Zhao 2003. Sensitivities of bumble bees to some pesticides commonly applied to tomato Acta Agriculturae Shanghai, 19(4): 67-69.	49750608	- The age of foragers (which were used in imidacloprid and thiamethoxam) was unknown. - The level of exposure of imidacloprid to the test bees could not be estimated due to the lack of information on neither the deposition of imidacloprid on the test bees and filter paper, nor the spray conditions, such as spray apparatus, spray duration and other relevant application information

Appendix B. Observational Residue Monitoring Study Summaries

Soil Application Studies

In a study by Dively and Kamel (2012), parent imidacloprid and its metabolites (IMI-olefin, IMI-5-OH, desnitro-imidacloprid, urea metabolite, and 6-chloronicotinic acid) were measured in the pollen and nectar of pumpkins (*Cucurbita pepo*) treated with varying application regimens. The study was conducted across 2 years (2009 and 2010) to yield two sets of residue data in both pollen and nectar. For both trial years, pumpkins were grown with untreated seeds in a greenhouse. In early June of both trial years, plants were transplanted from the greenhouse to plastic mulch beds. Foliar applications of the fungicide chlorothalonil was used several times throughout pumpkin crop cycle to prevent leaf disease (although it was not stated by the study how many times this occurred).

The experimental design for treatment plots was randomized, but consisted of four replicates per treatment regimen. The treatment regimens were selected based on a telephone survey of crop consultants and entomologists, which indicated that usage and application methods can vary depending on the pest. Specifically, it was stated that in areas where only early-season pests were an issue, bedding-tray drenches and transplant water treatments were typically employed. In areas with season-long pest pressure, foliar treatments and chemigation through drip irrigation were more often used.

For the 2009 trial period, 8 different treatment regimens with various neonicotinoid insecticides (as well as a negative control plot, with 4 plots per treatment for a total of 36 plots) were used. For the purposes of this assessment, only the treatments concerning imidacloprid will be discussed. The treatment regimens were:

1) bedding tray drench (Admire Pro, 55% active ingredient) applied at a rate of 0.005 g per plant (0.027 lbs a.i/A);
2) transplant water treatment (Admire Pro) applied during planting (0.25 lbs a.i/A);
3) transplant water treatment (Admire Pro) applied during planting (0.38 lbs a.i/A);
4) split treatments (Admire Pro) applied as half rate in transplant water (0.19 lbs a.i/A) and the remaining half rate applied 3 weeks later by drip irrigation; and
5) untreated control.

For the 2010 trial, the same regimens were assessed, but treatment 3 above replaced with a non-imidacloprid regimen. For both trial years, flowering began about 5 weeks after transplanting. During this time wax-coated paper bags were placed over staminate flower buds that had not yet bloomed to prevent pollinator visits. Nectar and pollen samples were collected 2 – 3 times for a period of 7 – 10 days for both trial years to ensure enough for analysis. In 2010, leaf samples were also collected, which was done after the second foliar treatment application. All samples were stored in 15 mL centrifuge tubes at -80°C until ready for residue analysis. The reference materials gathered from the U.S. EPA National Pesticide Standard Repository of parent imidacloprid and its degradates were of 97.5% purity or greater.

Samples were analyzed for residues using LC-MS/MS. The LOD and LOQ of imidacloprid was 0.2 ppb and 0.66 ppb, respectively in contrast to the LOD for all degradates (identified previously) ranging from 0.2 to

3 ppb and LOQ values of 0.66 and 6 ppb, respectively. Residue data for the parent and metabolite compounds were averaged by year to calculate means and ranges for each treatment regimen. Non-detected (ND) samples were scored a value of half the LOD, whereas samples with detected traces between LOD and LOQ were scored 0.2 ppb (*i.e.* the LOD) for the parent neonicotinoids and the LOD value of each metabolite (3 ppb). Control data was not included in the analyses because the primary aim according to the study authors was to test for differences among treatment regimens

Table B-1 below summarizes the residue values of parent imidacloprid and its metabolites in pollen, nectar, and leaf samples (2010 only) across both trial years for the various treatment regimens. Overall, residue levels in all sampled matrices were higher in 2009 than in 2010. It was noted by the study authors that these lower residues in 2010 were likely due to extreme environmental conditions that were observed, specifically with the pumpkin plants being heat and moisture stressed during most of the interval between transferring to plastic mulch beds to flowering. Drip irrigation had to be applied several times to maintain plant growth and flower production. Temperatures were noted to be higher overall in 2010 with 43 days over 32°C (89.6 F) compared with 11 days in 2009. Rainfall was noted as being suboptimal for both trial years.

As shown below the residues in pollen and nectar varied significantly from one another depending on the treatment regimen. As noted previously, the mean residues of each treatment regimen were not compared to a negative control but rather between one another. For parent imidacloprid pollen residues in 2009, the means from the bedding drench regimen increased approximately 7-fold for low transplant rate (0.25 lbs a.i/A), increased 12-fold for the high transplant rate (0.38 lbs a.i/A, and increased 16-fold for the transplant/drip regimen. For nectar, factor increases from the bedding drench regimen were approximately 14 for the low transplant rate, 19 for the high transplant rate, and 28 for transplant/drip regimen. While the factor increases for nectar residues are higher than those for pollen, it is noted that the maximum residues found in pollen are 8-fold higher than for nectar (transplant/drip regimen). There were 5 metabolites of imidacloprid detected in pollen and nectar samples, which represent 26.3% of total residue concentrations. The majority of the total residues of metabolites were identified as IMI-olefin (35.6%) and IMI-5-OH (52.3%).

Table B-1. Summary of residues in pollen a nectar from 2009 trial of various treatment regimens of imidacloprid on pumpkins (Dively and Kamel, 2012)

Treatment Regimen	Parent imidacloprid (ppb)			Metabolites (ppb)[1]		
	Mean[2,3]	Min	Max	Mean[2]	Min	Max
Pollen (2009)						
Bedding Drench (0.005 g/plant, 0.027 lbs a.i/A)	4.9 c	3.3	6.7	0.7 b	0.1	2.7
Transplant (0.25 lbs a.i/A)	36.7 b	30.1	40.1	11.4 a	8.3	16.6
Transplant (0.38 lbs a.i/A)	60.9 ab	40.5	86.6	17.5 a	10.6	21.9
Transplant-Drip (0.19 lbs a.i/A 2X	80.2 a	52.3	101	19.1 a	13.2	27.5
Nectar (2009)						
Bedding Drench (0.005 g/plant, 0.027 lbs a.i/A)	0.4 c	0.3	0.5	0.1 c	0.1	0.2
Transplant (0.25 lbs a.i/A)	5.7 b	3.8	7.3	1.8 bc	0.1	4.0

Transplant (0.38 lbs a.i/A)	7.4 ab	4.7	11.9	3.4 ab	0.2	5.9
Transplant-Drip (0.19 lbs a.i/A 2X	11.2 a	9.0	13.7	6.4 a	5.0	9.4
Pollen (2010)						
Bedding Drench (0.005 g/plant, 0.027 lbs a.i/A)	0.1 c	0.1	0.1	NA	NA	NA
Transplant (0.25 lbs a.i/A)	18.2 ab	13.2	23.9	NA	NA	NA
Transplant-Drip (0.19 lbs a.i/A 2X	31.8 a	23.9	44.0	NA	NA	NA
Nectar (2010)						
Bedding Drench (0.005 g/plant, 0.027 lbs a.i/A)	0.1 c	0.1	0.1	0.1	0.1	0.1
Transplant (0.25 lbs a.i/A)	6.1 ab	4.8	6.7	0.1	0.1	0.1
Transplant-Drip (0.19 lbs a.i/A 2X	9.1 a	6.7	16.0	0.1	0.1	0.1
Leaf Tissue (2010)						
Bedding Drench (0.005 g/plant, 0.027 lbs a.i/A)	5.0 cd	4.0	6.0	8.0 bc	5.0	10.5
Transplant (0.25 lbs a.i/A)	22.7 cd	18.3	31.0	39.1 ab	18.0	77.5
Transplant-Drip (0.19 lbs a.i/A 2X	39.5 bc	22.0	53.0	34.4 ab	27.5	40.5

NA = Not analyzed

[1]Imidacloprid metabolites included IMI-olefin, IMI-5-OH, urea metabolite, desnitro-olefin imidacloprid, desnitro-HCl-imidacloprid, and 6-chloronicotinic acid.

[2]Means within a column followed by the same letter are not statistically significant (p>0.05).

[3]Non-detetced samples were scored half of the LOD for statistical analysis (*i.e.* 0.1)

For the 2010 data in pollen and nectar, respectively, the high transplant regimen was not assessed. For the bedding drench method, parent imidacloprid was not detected in both pollen and nectar. The low transplant rate and transplant/drip residue values were roughly half of what they were in pollen and approximately the same in nectar when compared to 2009. No metabolites of imidacloprid were detected in pollen and nectar for any treatment regimen in both pollen and nectar. Leaf residue values of parent imidacloprid were approximately half of the residue levels in the pollen for the same trial year. Imidacloprid residues reached a maximum of 53 ppb in leaves and closely corresponded with residue levels in pollen. However, metabolites of imidacloprid constituted a higher proportion (54.8%) of the total residue amounts in leaf tissue and showed no correlation with metabolite levels in pollen.

The results of this study suggest that several factors can affect the available residues of parent imidacloprid and its metabolites in pollen and nectar. As mentioned previously, weather conditions in 2010 were stated to have potentially affected the residue values. These conditions necessitated more frequent drip irrigation due to the higher temperatures, where applications were made beneath the plastic mulch beds and could have potentially enhanced leaching of imidacloprid, resulting in lower overall residue values. This study also determined that the timing of application significantly impacted the residue levels of parent imidacloprid and its metabolites in pollen and nectar. Specifically, treatment regimens made closer to the time of planting, such as the bedding tray method, resulted in significantly lower residue levels in pollen and nectar as compared to drip irrigation methods applied closer to the time of flowering. Indeed, for the bedding tray treatment regimen, parent imidacloprid residues did not exceed 5 ppb in either the pollen or nectar for the 2009 trial and was not detected in pollen and nectar for the 2010 trial. There are several limitations to this study that include: 1) statistical comparisons were

not made to the untreated control plot, but rather between the different treatment regimens, 2) it is unclear if other factors besides weather conditions (which led to increased frequency of drip irrigation and increased leaching and dissipation of the transplant, as the study authors suggest) contributed to the dramatically lower residue concentrations in 2010 as compared to 2009, and 3) the fungicide chlorothalonil was used "several times," through the pumpkin crop cycle (presumably in all treatment regimens) to prevent leaf disease. It is unclear what effect these applications had to overall residue concentrations.

In a study by Stoner and Eitzer (2012, MRID 49719616), pollen and nectar samples were analyzed for imidacloprid presence in squash following various methods of application. The study was conducted across one trial each from 2009 and 2010. In 2009, yellow summer squash (*Cucurbita pepo*) was planted on black plastic mulch in rows on 1.5 m centers with seed holes spaced at 0.9 m. For direct-seeded application, three seeds were planted per hole. For transplanted application, a cell per hole (each cell contained three seeds which were grown in a greenhouse). Randomized block design consisted of three blocks and five treatments (3 of which concerned either control or imidacloprid treatments which will be discussed): 1) Untreated control, 2) imidacloprid (at 0.32 lbs a.i/A, Admire Pro®) applied by surface spray to soil, and 3) imidacloprid applied at the same rate per acre as with the previous scenario but using an injector through drip irrigation 5 days after transplanting.

For the 2010 trial, the study authors planted the same species of squash (although a different variety was planted) as well as a winter squash in a different field where the neonicotinoid insecticides had not been previously used; 3 blocks planted with summer squash and 1 block planted with winter squash. The same treatments for 2009 were used in 2010 but at an increased rate of imidacloprid (0.37 lbs a.i/A). It was noted by the study authors that both trial years used rates of imidacloprid within the range of labeled rates (0.26 – 0.38 lbs a.i/A, Admire Pro). Because rainfall was significantly different during the two trial years, one additional irrigation of the entire field was applied through drip lines for the 2010 trial.

In the 2009 trial, the total weight of all plant material from a seed hole was determined by randomly choosing an individual seed hole from center row of each plot and gathering all squash plants growing from that hole. Nectar from summer squash was gathered from all three blocks, whereas nectar from all 3 blocks planted with summer squash was obtained in 2010. In 2010, winter squash was collected and examined separately. Hand-collecting techniques were used to collect pollen.

Samples were analyzed using LC-MS/MS. By analyzing the extractions, control sample compounds averaged between 95 ±18% recovery with detection limits ranging from 0.5 to 2 ppb depending on matrix and the amount of sample available. The LOQ was not reported. The study authors reported that the two metabolites of imidacloprid (5-OH imidacloprid and imidacloprid urea) were detected in whole plant samples, but the concentrations of these metabolites were not provided. Whole body plant tissues were not monitored in the 2010 trial.

The interval between treatment and collection of plants parts for sampling varied on the treatment regimen and the sampled matrix. For the soil treatments, collection of plant parts for residue analysis occurred approximately 6 weeks after seeds were planted in the previously sprayed soil. Pollen and nectar

collection came roughly 10 days later. For the drip irrigation treatment, plant parts were sampled approximately 3 weeks after exposure to imidacloprid with pollen and nectar sampling following later by a week.

Table B-2 below summarizes the mean residue values for both treatment regimens in the 2009 and 2010 trials. For the 2009 trial, drip irrigation treatment resulted in residues levels roughly 3 fold higher than the same sampled tissue for soil treatment applications. As samples for the drip irrigation treatment followed exposure to imidacloprid at a 3 week interval as compared to a 6 week interval for soil applications, it is possible this sampling interval explains the observed difference in residues.

Table B-2. Summary of imidacloprid residues in various plant parts of squash following exposure to 2 treatment regimens across 2009 and 2010

Sampled Tissue	Residues (ppb ± SD)	
	Soil Treatment	Drip Irrigation Treatment
2009 Trial (both treatment regimens were 0.32 lbs a.i/A)		
Whole plant	47 ± 37	218 ± 52
Female flower bases	10 ± 5	31 ± 17
Synandria (fused anthers)	15 ± 5	46 ± 4
2010 Trial (both treatment regimens were 0.37 lbs a.i/A)		
Female flower bases	28 ± 10	15 ± 2
Synandria (fused anthers)	9 ± 1	11 ± 2

LOD: 0.5 – 2 ppb (depending on matrix, no further information provided); LOQ: not reported

For residues in pollen and nectar, the data from 2009 and 2010 were pooled and did not differentiate across application method or squash variety. It was reported that that there were no significant differences in pesticide concentration in pollen with treatment in either year. In pollen, the overall mean residues were reported to be 14 ± 8 ppb while in nectar, the overall residues were reported to be 10 ± 3 ppb.

Table B-3. Summary of imidacloprid residues in nectar and pollen (pooled from 2009 and 2010 trials) in squash plants following exposure to imidacloprid.

Sampled Matrix →	Pollen	Nectar
Mean residue (ppb ± SD)	14 ± 8	10 ± 3
Number of samples	12	6
Minimum residue value	6	5
Maximum residue value	28	14

LOD: 0.5 – 2 ppb (depending on matrix, no further information provided), LOQ: not reported

Limitations to this study include: 1) by pooling the data in 2009 and 2010 for pollen and nectar residues, it is not possible to examine whether higher residues result from the soil application or drip irrigation application as was possible for the plant part component of this study, 2) it was stated that various degradates of imidacloprid were screened for but no separate data or statement was made confirming the presence or absence of these residues. It was assumed that the residues provided in this study refer to the total residues of parent imidacloprid and its metabolites, and 3) the number of samples used for each matrix was not reported.

In a study by Rogers and Kemp (2003, MRID 49719626), soil treatments of imidacloprid were made to different crops over the course of a three year period to determine residue carryover in soil. Imidacloprid was applied as an in-furrrow treatment to potatoes in 2001 (designated as Year 1 in the study), to grain in 2000 (Year 2), and clover in 1999 (Year 3). Residues of imidacloprid were determined in the soil with the degradates IMI-olefin and 5-OH imidacloprid in the clover leaves and flowers.

All fields used in the study were stated to have been, at one time, planted with potato and received an in-furrow application of imidacloprid (As the product Admire 240F). Some of these fields received the in-furrow imidacloprid treatment the same year samples were collected (*i.e.* the Year 1 fields). Other fields received the soil treatment (on potatoes) 1 or 2 years prior to sample collection (identified as Year 2 and Year 3 fields, respectively). At the time the study was conducted, Year 2 fields (identified as "grain" above) were planted with either oats or barley and underseeded with a mixture of red clover *(Trifolium pratense),* alsike clover *(Trifolium hybridum),* and timothy (*Phleum pretense)*. Year 3 clover fields were cut mid-season with residues of the aforementioned degradates assessed at both cuttings. This is to say that Year 2 fields were planted for one growing season with potato, followed by one growing season for grain, while the Year 3 field had a potato, grain, clover field history. For both Year 1 and Year 2 fields, there was also edge of field samples taken to investigate the potential residues in runoff.

An in-furrow soil application treatment of Admire 240F was made to all fields at the rate of 0.18 lbs a.i/A. Exceptions to this were two fields that received no imidacloprid treatment that served as control, one field where half received 0.18 lbs a.i/A and the other half received 0.28 lbs a.i/A, and one field that received a foliar treatment of imidacloprid (no further details provided in the study). A total of 23 fields were employed for this study (including the exceptions to the standard 0.18 lbs a.i/A treatment discussed above) and it was unclear from the study the distribution of the number of fields used for Year 1, Year 2, and Year 3. Additionally, 8 honey colonies were placed in Year 3 fields (clover) to supply foraging bees with pollen and nectar that would be collected for residue analysis. These colonies were placed in the fields for two months (mid July – mid September). Samples of soil, clover leaves, clover flowers, honey and pollen were analyzed for imidacloprid and for all matrices except soil, its degradates IMI-olefin imidacloprid and IMI-5-OH. All samples were analyzed using HPLC-MS/MS with a LOQ of 2.0 ppb for imidacloprid, and its two degradates.

Table B-4 below shows that the Year 1 (potato) residues were either not sampled or below LOQ in soil and wild flower samples. Year 2 (cereal feeds) had in field soil residues averaging 32 ppb but these residues were slightly above the LOQ at the edge of the field. In field residues of clover leaves reached as high as 4.4 ppb. Finally, Year 3 (clover) in field residues (inclusive of both flowerings) ranged from 14 – 38 ppb in the soil. For all other matrices (inclusive of samples from 1st and 2nd flowerings), all samples were below LOQ with the exception of one sample from clover leaves at the second flowering which was slightly higher than the LOQ (2.5 ppb). The results of this study suggest that imidacloprid residues can persist in soil and that imidacloprid residues can carry over in soil from the previous growing season to the next for up to 3 years (duration of this study).

Table B-4 below summarizes the residue levels of all fields and matrices assessed (average residue level, with range if applicable, in ppb).

Matrix	Year 1 (Potato fields)		Year 2 (Underseeded Cereal fields)		Year 3 (Clover fields)	
	PEI In-field	**PEI Edge of field (n=3)**	**PEI In-field (n=3)**	**PEI Edge of field (treatment n=3, control n=1)**	**PEI 1st Flowering, In-field (n=3)**	**PEI/NB 2nd Flowering, In-Field (treatment for all matrices but clover flower, n=4, clover flower: n= 8, control n=1)**
Soil	NS	<LOQ	32 (27-38)	<LOQ-3.7	24.6 (16-38)	Treatment: 20 (14-25) Control: <LOQ
Clover Flower	NS	NS	NS	NS	<LOQ	Treatment (PEI and NB): <LOQ[1] Control: <LOQ
Clover leaves	NS	NS	<LOQ-4.4	NS	NS	PEI Treatment: <LOQ-2.5 Control: <LOQ NB Treatment: <LOQ[1] Control: <LOQ
Wild Flower	NS	<LOQ	NS	Treatment: <LOQ Control: <LOQ	NS	Not sampled
Pollen	NS	NS	NS	NS	NS	Treatment: <LOQ[2] Control: <LOQ
Nectar	NS	NS	NS	NS	NS	Treatment: <LOQ[2] Control: <LOQ
Uncapped Honey	NS	NS	NS	NS	NS	Treatment: <LOQ[3] Control: <LOQ

PEI: Prince Edward Island; NB: New Brunswick; NS: not sampled

[1] One of the non-detections was from a field with foliar application (no application information provided) and this field was included in the n value.

[2] In two fields, the foraging bees were collected at the hive entrance.

[3] Samples collected on two occasions (August 22 and September 14)

While providing residue information on the hive products pollen and nectar, the honey bee component of this study yields limited information to the overall health of the hives exposed to these application regimens. This is primarily due to the fact that the hives were assessed for overall colony health only once during the 2 months exposure period, no adequate demonstration of exposure (no foraging measurements, pollen composition analysis) and qualitative measures of various life stages and food stores on combs (*e.g.* "some" vs "lots").

Limitations for this study include: 1) while it appears that the crop rotation in Year 3 sites was potato-grain-clover, this was not completely clear from the study methodology, 2) the application rate used in the study was 0.18 lbs a.i/A (except for 1 field site where it was 0.28 lbs a.i/A), which is below the current maximum single application rate for soil treatments to potatoes, 3) the application timing of imidacloprid and the interval between the imidacloprid treatment and sampling were not provided, 4) the sampling design would not have captured the highest residue levels as no in-field samples were taken from Year 1 potato fields (only runoff areas on the edge of Year 1 fields were sampled) which limits the ability to put the in-field results from Year 2 and 3 fields into context. Observed levels in Year 3 soil were lower than those in Year 2 and also expected to be lower than levels in Year 1 (for which no in-field soil was sampled).

Pollen and nectar residue levels therefore do not reflect the highest potential exposure due to the uptake of imidacloprid from the soil, 6) there was a high density of hives in the test field (8 hives in a 5 acre field) and thus bees may have been forced to find alternate food sources, suggesting that there may have been a dilution effect / reduced exposure to imidacloprid.

Seed Treatment Residue Studies

In a study by Donnarumma et al. (2011, MRID 49719614), seed treated corn (Gaucho 350 FS at 1.0 mg/seed) were planted and samples were collected at 30, 45, 60, 80, and 130 days after initial sowing. Samples included soil, roots, leaves, stems, kernals, panicles, and pollen. The study was conducted at the Agricultural Research Council in Rome, Italy. The composition of the soil of the test fields was 54.3% clay, 43.4% silt, and 2.3% sand. At the rate described above, imidacloprid dressed seeds (as well as undressed seeds serving as a control) were planted at a sowing destiny of 123,000 seeds/ha (timing of sowing not reported). There were three replicate plots (0.40 acres each) for each treatment group.

Pollen samples were obtained at the tasseling stage and kernels during the harvest period. Analytical verification of imidacloprid residues in samples was conducted using HPLC-MS/MS, with an LOQ of 1 ppb.

Table B-5 below shows the mean residue value of each sampled matrix at a given time point after seeds were sown. The level of imidacloprid in the leaves and stems decreased as the corn plants grew. The amount of imidacloprid residue present in the different plant parts were highest during the first sampling time (30 days after sowing for leaves, 45 days after sowing for stems) and declined markedly in subsequent sampling times. Residue values in leaves were as high as 253 µg/kg 30 days after sowing but were reduced to 13 µg/kg roughly two weeks after. Similar patterns were present for other plant matrices where the first sampling period (depending on the plant matrix) was always the highest value with a decline in residues below 5 µg/kg for leaves, stems, and panicles by 80 days after sowing. Pollen was only sampled once (130 days after sowing) and showed a value below the LOQ.

Table B-5. Summary of residue values in various corn matrices following imidacloprid seed treatment (Donnarumma 2011)

Sampled Matrix	Control[2]	Mean Residues by time point (µg/kg±SE)[1]				
		30 days after sowing	45 days after sowing	60 days after sowing	80 days after sowing	130 days after sowing
Soil	5 ± 4	652 ± 210	330 ± 54	241 ± 57	52 ± 25	11 ± 3
Root	4 ± 1	--	290 ± 155 a	118 ± 79 a	53 ± 36 a	--
Leaf	<1	253 ± 50	13 ± 3 b	5 ± 1 b	2 ± 1 b	--
Stem	<1	--	8 ± 2 b	6 ± 2 b	3 ± 2 b	--
Panicle	<1	--	--	--	2 ± 1 b	--
Kernals	<1	--	--	--	--	<1
Pollen	<1	--	--	--	--	<1

LOQ = 1 ppb

[1]Means within columns for each sampling time with different letters differ significantly ($p<0.05$)

[2]Samples measured at time of harvest (130 days after sowing)

At 130 days after sowing, an average soil concentration of 11 µg/kg was determined. The study authors suggest that the persistence of imidacloprid in plant tissue, at different sampling times, is due to a

prolonged accessibility of root uptake. It is noted that for control plots (using undressed corn seeds) that the imidacloprid concentration in the soil was 5 ppb and 4 ppb in the roots. These samples were taken at the time of harvest and although it was not stated in the study whether the plots used for the control had been previously treated with imidacloprid, the residue data suggest that this may have been the case. Despite this, the mean residue value of pollen (which represent the plant matrix sampled in this study most pertinent to foraging bees) was below the LOQ at the time of harvest (130 days after sowing). Limitations for this study include 1) lack of sampling in the control group at similar time points as those sampled in the treatment group, 2) although the soil composition was provided, it was not reported what weather conditions were like during the time of the study that could have been informative in explaining the variability that was seen for some of the sampled plant material, and 3) there was no tracking of the various degradation products of imidacloprid within the plant.

In a study by Laurent and Rathahao (2003, MRID 48077902), the uptake and distribution of seed treated-imidacloprid in sunflowers was examined under controlled conditions in the laboratory and uncontrolled conditions using an outdoor lysimeter.

Sunflower seeds, dressed with Gaucho 70 WS (1 mg a.i/seed). The dressed seeds were also radiolabeled with ^{14}C (radiochemical purity of >97%). For the laboratory experiment seeds were planted in1 L pots and grew for 1 month in a climate-controlled cabinet at 25°C day and 20°C night with a 16 h photoperiod. After one month, leaves, pairs of cotyledons, stems (cut into sections above leaf insertions) and unmerged tops of plants (apexes) were gathered. For the outdoor lysimeter experiment, seed treated sunflower seeds were grown for 4-5 days in the lab and following emergence were transplanted into an outdoor lysimeter.

Samples were collected at three stages: 1) B4 stage (four whole expended leaves) 1 month of growth; 2) E4 stage (star buds), 2 months of growth; and 3) F stage, 2/3 of the florets blossoming. Stems and leaves were gathered as a single sample at the B4 stage or as two samples (top and bottom fractions) at the E4 and F stages. For E4, flower heads were cut in two segments: involucre (bracts and flower head back) and floral dishes with florets. During blossoming, pollen was obtained by using a brush to sweep up the pollen sample, this was conducted every other day. At the F stage, flower heads were gathered and cut into three factions: involucre, flower head back, and floret dishes. Analytical verification of imidacloprid in the sampled plant parts was conducted using radio-HPLC-MS with an LOQ of 0.5 ppb. It was not reported the total number of plants or samples upon which mean concentrations were determined.

At the B4 stage in the lab component, approximately 5% of the radioactivity was taken up from the seed dressing. At the B4 stage for plants grown in the outdoor lysimeter, imidacloprid uptake varied from 3 to 10% of the total applied radioactivity, that is, similar to uptake under controlled conditions.

Table B-6 below shows the mean residues from various plant parts at different stages of sunflowers grown in the outdoor lysimeter. As indicated by the residues, the concentration of imidacloprid decreases with the growth of the plant where residues in leaves at the F stage (2/3 of florets blossoming) are roughly 1/5 of their value at the B4 stage (1 month of growth). The decrease in residues in even more dramatic in stems through the various stages of growth (130 ppb at B4 compared to 1.0 ppb at F). Residue values in the various parts of the sunflower infloresence range from 4 – 33 ppb. It is noted that for this data as well

as the leave and stem data that variability was very high with in some cases the mean residue value and standard deviation equaling each other.

Table B-6. Summary of residue values in seed treated sunflower (Laurent and Rathahao, 2013)

Residues in leaves and stems (ppb ± SD)			
Stage → Plant part ↓	**B4**	**E4**	**F**
Leaves	2880 ± 2440	2760 ± 890 (top leaves) 140 ± 110 (bottom leaves)	520 ± 210
Stem	130 ± 81	14 ± 6 (top leaves) 76 ± 29 (bottom leaves)	1.0 0.3
Residues in sunflower inflorescences			
Plant part	**Stage**	**Mean residue (ppb ± SD)**	
Involucre	E4	33 ± 19	
Floret dish	E4	10 ± 6	
Involucre	F	22 ± 12	
Floret dish	F	14 ± 7	
Flowerhead back	F	4 ± 12	
Seeds	F	28 ± 28	
Pollen	F	13 ± 13	

SD: Standard deviation

In roots, only parent imidacloprid was detected. In shoot samples, imidacloprid was always the major compound detected, however, 3 metabolites (guanidine, IMI-5-OH and IMI-olefin) were also detected. In cotyledons, 1/3 of the radioactivity was present as metabolites. At the E4 and F stages, metabolites accounted for 30-50% of radioactivity in leaf samples although the concentrations of each were not provided.

This study indicated that the uptake of imidacloprid on sunflower seeds is approximately 5% in the cabinet (climate controlled) conditions and varied from 3-10% in outdoor conditions in a lysimeter. Imidacloprid concentrations were shown to decrease in plant parts with the growth of the plant. Importantly, the authors report that variability in imidacloprid residues in plants increased substantially for plants grown outdoors, as indicated by the residue values presented above. This study also suggests that the distribution radiolabeled imidacloprid residues was a function of time and was therefore correlated with leaf age. Specifically, the results indicate that imidacloprid residues predominantly moved upward from the roots to all other plants parts (consistent with xylem transport). The level of imidacloprid that remained deposited on the coated seed was estimated to be around 90% of the applied radioactivity. Pollen was determined to have an average residue value of 13 ppb. It is worth noting that the concentration in nectar was not determined in this study.

Limitations for this study include: 1) the sample size supporting the mean residue values was not stated, 2) it was noted that variability was substantially for several sampled concentrations at all stages with the mean value equaling the standard deviation in some cases, 3) results may not be completely representative of field conditions as even seed in the lysimeter experiment was grown for 4-5 days in controlled laboratory conditions and then transplanted, 4) the concentrations of imidacloprid and its

metabolites were not assessed in the nectar, 5) it is not known from this study if the F stage is reached at variable times across sunflowers. The B4 and E4 stages are stated to be one month and 2 months old, respectively whereas the F stage is described as 2/3 of the florets blossoming. This information would be helpful in getting an idea of the residues in pollen in a given number of days after the sowing of the seeds, and 6) it was not stated what weather conditions were present for the outdoor lysimeter part of the experiment.

In Stadler 2003 (MRID 47796301), while the primary focus was to evaluate the effects of imidacloprid on honey bee colonies exposed to seed treated sunflower, the residues in bee-collected honey and pollen as well as hive wax were quantified using HPLC/MS analysis with an LOD of 1.5 ppb in all matrices. The sampling occurred in the spring following an overwintering period of honey bee colonies that were exposed to imidacloprid seed-treated sunflower for 10 days. Parent imidacloprid and its metabolites IMI-olefin and 5-OH imidacloprid were below the LOD in all matrices assessed. The interval between samples being collected and analyzed was approximately 216 days.

Schmuck et al. (2001), which will be discussed later in the context of toxic effects of imidacloprid and its metabolites to adult honey bees, conducted an uptake and metabolism study of seed treated imidacloprid with sunflowers in a greenhouse as well a residue component of imidacloprid treated sunflower seeds in a honey bee field study.

For the greenhouse component, sunflower seeds were dressed with [methylene-^{14}C]imidacloprid formulated as the commercial 700g/ kg WS (Gaucho WS 70) at a rate of 0.7 mg a.i/seed. Flowering started 62 - 66 days after sowing and lasted for two weeks during which time nectar and pollen samples were collected. Pollen and nectar samples of two plant rows were sampled, stored and analyzed separately (two replicates). Radioactivity was measured using liquid scintillation counting followed by identification of the radioactive residues by thin layer chromatography. The ^{14}C recovery of the purification procedure used was 97.3 (±2.5)% (pollen) and 98.1 (±14.8)% (nectar). It was reported that a radiolabeled compound was used for the analytical methods that had an LOD of <1 ppb.

The field residue component were performed on the farms Höfchen (Burscheid, Germany and Laacher Hof, Germany). Twenty soil samples were taken from each study site to determine the soil characteristics of the study plots. At each farm, study plots with different histories of imidacloprid treatment were selected. The control plots and all plots which had received an imidacloprid treatment during 1996-1998 were drilled with imidacloprid-free sunflower seed (treated only with a combined fungicide of carbendazim, metalaxyl and copper oxyquinolate). The application rate was 1 mg a.i/seed.

Pollen and nectar samples were collected as well as male and female sunflower florets. Soil and biological samples were analyzed by HPLC confirmed additionally by LC-MS/MS. The limit of quantification (LOQ) and the limit of detection (LOD) for soil analyses were 6 and 2 ppb, respectively. The LOQ in the analyses of the biological samples were 5 ppb for imidacloprid and the hydroxy-metabolites (IMI-5-OH and 4,IMI-5-OH) and 10 ppb for the IMI-olefin-metabolite. The LOD for the biological samples were 1.5 ppb for imidacloprid and the hydroxy-metabolites and 3 ppb for the IMI-olefin-metabolite.

For the greenhouse metabolism component, it was reported that that a total of 3.5g nectar and 9.6g pollen were harvested from the sunflower plants (total of 22 plants). The total radioactive residues (TRR) in the samples were similar for pollen (3.9 ppb) and nectar (1.9 ppb). The residues consisted entirely of imidacloprid and no known or unknown imidacloprid metabolites were detected. For the residue component of the full field study with honey bees, the recovery of imidacloprid at fortification levels of 6 and 60 ppb in the soil matrix was 93.5 (±1.6)% with a mean coefficient of variation from duplicate samples was 6.2%. There were no residues of imidacloprid or its metabolites that could be detected in the nectar and pollen of plants raised from imidacloprid-dressed seed (actual mean residue of each sampled matrix not provided in the study). It is noted that the plots used for this component of the study had soil samples analyzed for prior imidacloprid use and were found to contain residues of up to 17.8 ppb before control or imidacloprid dressed sunflower seeds were planted.

Limitations for this study include: 1) the greenhouse results appear to be from a single pooled sample and 2) seeds in the field study with honey bee component were also treated with systemic fungicides yet their residue levels were not reported.

Appendix C. Observational Residue Monitoring Study Summaries

Agricultural Monitoring Studies

In a study by Bonmatin et al. (2005, MRID 47523411), various parts of corn plants (stems, leaves, panicles, and pollen) were collected from fields at the time of tasseling when honey bees would be expected to be foraging on pollen. Samples were collected from corn fields grown with 1) treated imidacloprid seed, 2) organically grown corn (untreated for at least 3 years), and 3) fields that used insecticides, but not imidacloprid. The samples were then analyzed with using high performance liquid chromatography (HPLC) with tandem mass spectroscopy (MS/MS). The limit of detection (LOD) was 0.1 ppb for stems, leaves and panicles (branched clusters of flowers) and an LOD of 0.3 ppb for pollen. The limit of quantification (LOQ) was 1 ppb for all sampled plant parts.

Samples from organically farmed areas and samples from areas untreated with imidacloprid for the 3 years prior to the sampling conducted for the study did not have any detections of imidacloprid above the LOD. It was reported that for trapped pollen samples, corn pollen comprised approximately one third of the samples. **Table C-1** below shows the distribution of the number of samples in the various sampled plants parts according to their analytically confirmed level of imidacloprid. For stems and leaves, there were 13/17 (76%) samples determined to have an imidacloprid level above the LOQ with concentrations ranging from 1 – 10 ppb. In panicles, 39/48 (81%) of the samples were determined to have an imidacloprid concentration greater than tan the LOC, with no samples being below the level of the LOC. Acceding to the study authors, 23% of these samples had a concentration of >10 ppb. It was noted by the study authors that the variance of this sample set was "quite high" which was attributed to variation in soil composition, corn variety, and weather conditions. The maximum concentration was 33.6 ppb. In plant collected pollen, 23/47 (49%) of the samples had imidacloprid residues above the LOQ, while only 2/47 samples had residues above 10 ppb. Finally, for pollen collected from traps, 4/11 (36%) of samples contained residues greater than the LOQ, with no samples containing more than 10 ppb.

Table C-1. Distribution of samples from corn fields according to their concentration of imidacloprid (Bonmatin, 2005)

Sampled Matrix	Number of samples	Number of samples below LOD[1]	Number of samples between LOD and LOQ[1,2]	Number of samples above LOQ[2]	Mean concentration (ppb ±SD)	Variance
Corn pollen	47	6	18	23	2.1 ± 2.7	7.1
Trapped pollen	11	5	2	4	0.6 ± 1.0	1.0

[1] LOD = 0.1 ppb for stems, leaves, and panicles; 0.3 ppb for pollen
[2] LOQ = 1 ppb for all plant parts

While this study is informative in providing the relative frequency of samples from various parts of the corn plant above a 1 ppb threshold, it does not tie an application rate or timing of application to residue values but rather only serves as a survey of residues resulting from seed treatment applications. Additionally, while the maximum residue levels measured in pollen collected directly from the plant may

correspond with levels suspected to cause sub-lethal effects on individual honey bees (max concentration of 33.6 ppb), the mean residue levels in both plant collected pollen and trapped pollen were relatively close to the LOQ of 1 ppb (mean residues of 2.1 and 0.6 in corn pollen and trapped pollen, respectively). Finally, this study determined that even at the time of corn flowering, (tasseling) that the majority (approximately 2/3) of pollen brought back to the hive is not from corn but rather from other sources. This last point has an uncertainty as this study was conducted in France, and it is unknown how the makeup of pollen brought back to the hive at the time of corn flowering would compare for honey bee colonies in the United States.

Other limitations include: 1) the authors measured the sources of the trapped pollen, but did not discuss any results of this analysis other than stating that corn pollen represented 30% of the pollen sampled by bees, 2) the mean residue of trapped hive pollen was 0.6, which is below the study LOQ of 1, and 3) the study determined that the coefficient of variation was 129 and 167% for the panicles and bee-collected pollen, respectively suggested that the reported means were not particularly reliable measures of central tendency.

In further work by Bonmatin (2007), imidacloprid was measured in the pollen and flowers of corn and sunflowers plants gathered across a wide area of agricultural locations in France from 1998 to 2005. The methodology for analytical verification was similar to that described in Bonmatin, 2005, with LOD and LOQ values of 0.3 and 1 ppb, respectively.

Flowers and pollen from organically grown farming crops were used as controls, which did show no detects for imidacloprid presence and were the basis for calibration and comparisons. For sunflower pollen, a total of 24 samples were analyzed with the LOD (0.3 ppb) being exceeded in 83% of the samples. For 25% of the samples, the residues did not exceed the LOQ of 1 ppb. In 58% of the samples, imidacloprid residues were able to be quantified with a mean value of 3 ppb (range of 1 – 11 ppb). For corn pollen, a total of 47 samples were available, with 50% of the samples having quantifiable residues with a mean value of 2 ppb.

Table C-2. Distribution of samples from corn and sunflower fields according to their concentration of imidacloprid (Bonmatin, 2007)

Sampled Matrix	Number of samples	Percentage of samples exceeding LOQ[1]	Mean concentration (ppb)
Corn pollen	47	2	2.0
Sunflower pollen	24	58%	3.0

[1]LOQ: 1 ppb

While this study in informative in providing a range of residue values in corn and sunflower pollen following seed treatment application, it has the same limitations as described above in Bonmatin 2005, particularly relating to the inability to tie an application rate or interval between application and sampling time directly to a residue level. Other limitations include: 1) while it was stated that Gaucho is registered in France for seed treated corn and Regent TS for seed treated corn and sunflower, it was unclear which product was associated with a given residue value, 2) although it was stated that the metabolites of imidacloprid were screened for and not detected, the LOD and LOQ for the metabolites, as well as which

metabolites of imidacloprid were screened for was not provided, 3) variability in the soil characteristics and environmental conditions were not reported, and 4) residues of imidacloprid found in the trapped pollen at the hive entrance was not reported, which was stated in the methods section as having been analyzed.

Hive Monitoring Studies

In a study by Chauzat et al. (2006), a field survey was conducted in 2002 in apiaries throughout France to monitor the health of honey bee colonies. Colonies were selected from apiaries in 5 regions of France (a total of 125 colonies was used for the study). The observation periods were conducted before winter (October/November, 2002), after winter (March/April, 2003), before summer (May/June, 2003), during summer (July/August, 2003), and before winter (October, 2003). Pollen loads were gathered at each observation which were subsequently analyzed for pesticides residues (41 total screened, including various metabolites of certain chemicals).

The analysis of residues in hive pollen was conducted using LC-MS/MS. Imidacloprid and its degradate 6-chloronicotinic acid (6-CNA) were screened with an LOD and LOQ for imidacloprid at 0.2 and 1 ppb, respectively and 0.2 and 0.6 ppb for 6-CNA, respectively. Imidacloprid was the most frequently detected chemical, with positive findings in 40 of 81 samples (49.4%) with residues ranging from >LOD – 5.7 ppb. Similarly, 6-CNA was detected in 36 of 81 samples (44.4%) with residues ranging from >LOD – 9.3 ppb. Notably, despite the high frequency of imidacloprid in the analyzed samples, the mean concentration was 1.2 ppb, which is approximately the level of the LOQ. This was also the mean concentration for detections of 6-CNA.

In a study conducted by the Bee Research Institutes across Germany (2008, MRID 47699438), the regional institutes, as well as 125 beekeepers provided data on over 7,000 colonies across 4 years (2004-2007) on overwintering survival, honey production, disease incidence, and hive residues of pesticides. A screen of over 250 pesticides was conducted on bee bread samples at varying limits of quantitation (imidacloprid = 1 ppb). Samples were stated to always have been taken in the spring.

In the first year of the survey (2004), there were no residues of imidacloprid found in nectar and 2 of 48 samples (4.2%) in pollen contained imidacloprid residues approximately at the LOQ. In 2005 and 2006, 105 bee bread samples were analyzed from colonies that were stated to have been heavily exposed to seed treated canola residues. There were 42 different chemicals detected in the samples analyzed with the three most frequently detected were the hive miticide coumaphos (46%) the fungicide boscalid (35%), and the herbicide terbuthylazine (32%). There were no samples that had detectable imidacloprid residues. A further 110 bee bread samples were analyzed from 2007. Similar to the previous years, a total of 42 different pesticides were detected with boscalid (67%), the neonicotinoid thiacloprid (62%), and terbuthylazine (48%) being the 3 most frequently detected chemicals. Imidacloprid was found in one sample at 3 ppb.

In similar work as discussed above, Chauzat et al., (2009), saw a more extensive survey conducted across France for a 3 year duration (2002-2005). This study is distinct from the previous work discussed above

in that residues of pesticides were examined in a greater number of matrices including honey and beeswax. The same 5 sites that were used for the 2006 study discussed above (representing the main zones of French honey production) were used for this study. A total of 41 chemicals were screened for, of which 30 were insecticides and 11 were fungicides. Imidacloprid and its degradate 6-CNA were analyzed using LC-MS/MS with LOD and LOQ values of 0.3 and 1 ppb, respectively for imidacloprid and 0.3 and 0.6 ppb, respectively for 6-CNA.

For trapped pollen samples, imidacloprid and 6-CNA were positive (defined by the study as being above the LOD) in 57.3% of samples. The mean concentration of imidacloprid was 0.9 ppb, which is noted as being between the LOD and LOQ. The mean residue concentration of 6-CNA was 1.2 ppb, 2X the value of the LOQ. Total residues of imidacloprid and 6-CNA were positive in 29.7% of 239 honey samples, with average residues of 0.7 ppb for imidacloprid (between LOD and LOQ) and 1.2 ppb for 6-CNA (2X the LOQ). Similarly, combined residues were positive in 26.2% of 187 samples of honey bee samples with average residues of imidacloprid of 1.2 ppb (slightly above LOQ) and 1.0 ppb for 6-CNA. There were no residues of imidacloprid or 6-CNA detected in beeswax samples.

In Mullin et al. (2010), a total of 749 samples from beebread (honey bee processed mixture of pollen and honey), trapped pollen, wax, brood, and adult bee samples from 23 states and one province in Canada (states and province not provided) were analyzed with either gas chromatography (GS/MS) or liquid chromatography (LC-MS/MS) methods. The samples were collected across 2007 and 2008 and were part of an effort to investigate threats to colony health from chemical and non-chemical sources. The colonies from which the samples originated were diverse both in overall health and proximity to known agricultural areas with some samples being designated as being from colonies diagnosed with colony collapse disorder. Over 200 chemicals and various degradation products of these chemicals were screened.

In over 92% of the 749 total samples, there were 2 or more chemicals present across all sampled matrices. The most frequent combination (78%) of chemicals were the miticides fluvalinate and coumaphos, which are widely used to control the *Varroa* mite. In the 208 wax samples, imidacloprid was detected twice (1%). The two samples were 2.4 and 13.6 ppb (LOD = 2.0 ppb). Fluvalinate and coumaphos were detected in 98% of the samples. Similarly, in 350 pollen samples, imidacloprid was detected 10 times (2.9%). The level of imidacloprid in these samples ranged from 6.2 – 912 ppb (LOD = 2) (mean was 39 ppb, median was 20.5 ppb). Again, the most frequently detected chemicals in pollen were fluvalinate (88%) and coumaphos (75%). Additionally, two degradation products, 5-OH imidacloprid and IMI-olefin, had one detect each in pollen (152 and 554 ppb, respectively; LOD = 25 ppb). There were no dead bee samples that yielded any detections of imidacloprid. It was not further characterized in the study whether the imidacloprid detections in beeswax and pollen originated from colonies characterized to be healthy or diseased or from areas in close proximity to agricultural fields.

Wiest et al. (2011) employed similar methodology as discussed above with Mullin 2010, but on a smaller scale with honey, pollen, and bee samples (142, 145, 140 samples, respectively) originating from 16 apiaries across Western France in 2008 and 2009. Residue analysis was performed using GC/MS or LC-MS/MS, depending on the chemical (LC-MS/MS for imidacloprid in all matrices). The LOD and LOQ differed depending on the matrix. The LOD and LOQ for honey, pollen, and bees was 0.2, 3.9; 2.6, 12; and

0.4, 9.6 ppb, respectively. A total of 36 chemicals were detected, with 10 chemicals being detected in all matrices including the hive miticides fluvalinate and coumaphos.

Imidacloprid was detected in 2% of honey samples (maximum value was below LOQ), 1% of pollen samples (maximum value was below LOQ), and was not detected in bee samples. Notably, this study did not test for the presence any degradates of imidacloprid.

In a study by Stoner and Eitzer (2013), pollen was collected from 5 apiary locations in Connecticut from a variety of land pattern uses (*e.g*, urban, rural, agricultural fields) for a 2 - 5 year period. These samples were then screened for 60 pesticides and various degradation products for the purposes of comparing the residue levels to the acute contact and oral LD_{50} values to honey bees.

Pollen was collected using pollen traps at the 5 apiary locations, which were stated to not have a history of problems with honey bee health. For *Varroa* mite management, all hives at one apiary site were treated annually in September with Apiguard (thymol) according to label instructions. Other apiaries from which the samples were collected were managed by local beekeepers, who used formic acid for mite control. Fluvalinate and coumaphos were not used on any hive for at least 2 years prior to the start of sampling. Terramycin was used for control of American foulbrood and fumagillin for *Nosema* as needed.

A total of 313 pollen samples were analyzed using LC-MS/MS. Imidacloprid, as well as IMI-5-OH, IMI-olefin, and urea imidacloprid, were screened for with LOD values of 1, 5, 10, and 3 ppb, respectively. Parent imidacloprid was detected in 38 samples (12%) of the samples with values ranging from 1 – 70 ppb (median value of 2.8 ppb, mean value of 5.2 ppb). There was a single detection of 5-OH imidacloprid of 5.6 ppb, which is approximately at the LOD. The degradates IMI-olefin and urea imidacloprid were not detected.

In a study by Pettis et al. (2013), although the primary focus of the study was to examine the interaction between pesticides and the fungal pathogen *Nosema ceranae*, this study also screened for multiple pesticides in pollen originating from various crops. Pollen was collected from bees returning to 9 hives representing 7 crops (almond, apple, blueberry, cranberry, cucumber, watermelon, and pumpkin). The crops sites were mostly confined to states in New England and the mid-Atlantic with the exception of almonds, which were grown in California. The hives in these fields were employed for pollination services. Pollen samples were analyzed for the genus or species from which they originated. The samples were then screened for a battery of pesticides (35 total chemical detected) that included several classes of insecticides (*e.g.* neonicotinoids, pyrethroids) as well as herbicides and fungicides using either LC-MS/MS or GC/MS methodology depending on the chemical. The LOD and LOQ for each chemical was not reported in the study.

Out of 19 total pollen samples analyzed, imidacloprid was detected in 3 samples (15.7%) with a mean concentration of 2.8 and maximum value of 36.5. These detections were associated with apple pollen only. Apple was also associated with the highest pesticide diversity of all assessed crop samples. The fungicide chlorothalonil had the most detections (89%) and was associated with 6 of the 7 identified crops

form which the samples originated. Other frequently detected residues was the hive miticide fluvalinate (84%) and the fungicides azoxystrobin and fenbuconazole (53% each).

A study by Kasiotis et al. (2014) surveyed residues from different sites across Greece known to have reported incidents for honey bee losses. A total of 71 samples of pollen, honey, and bees were collected over the course of 3 years (2011-2013). The samples were analyzed using LC-MS/MS with an LOD and LOQ of 0.4 and 1.5 ppb, respectively in all matrices. A total of 115 pesticides were screen for in this study which included parent imidacloprid but not any of its major degradates. Imidacloprid was detected in 2 of 34 (5.9%) honey bee samples (range of 0.3 – 5.24 ppb), and in 1 of 14 (7.1%) of pollen samples (73.9 ppb). There were no detects in the available honey samples. There was no further information of additional information of the surrounding area or pesticide use history of where the positive samples originated.

In a report by Johnston et. al. (2014, Greenpeace Research Laboratory Report – *The Bees' Burden*), an extensive survey of pesticide residues in beebread and trapped pollen was conducted across two years (2012-2013) and 12 European countries. A total of 25 bee bread samples and 107 trapped pollen samples were analyzed with either LC-MS/MS or GC-MS/MS with an LOD and LOQ of 0.3 and 1 ppb, respectively. The results found that 68 and 67% of all bee bread and trapped pollen samples, respectively, had at least one pesticide (53 chemicals screened). Fungicides, notably the sterol biosynthesis inhibiting chemicals were the most prevalent. As with other reports discussed thus far, fluvalinate and coumaphos were prevalent in the hive bee bread as well as the miticide amitraz. Imidacloprid was detected 6 of 107 (5.6%) trapped pollen samples. Four of these 6 samples were from Spain where concentrations ranged from 7.6 – 149 ppb and 2 samples were from Italy (1.7 and 11 ppb). There were no imidacloprid residues detected in any bee bread samples.

Finally, in recent work by Lu et al. (2015), monthly pollen and honey samples were collected between April and August 2013 from 62 hives across the state of Massachusetts. Individual beekeepers were informed of the study through beekeeping associations and voluntarily supplied samples to support the study. A total of 219 pollen samples and 53 honey samples were screened for 8 neonicotinoid pesticides (imidacloprid, thiamethoxam, clothianidin, dinotefuran, nitenpyram, acetamiprid, flonicamid, and thiacloprid). Samples were analyzed using LC/MS/MS with an LOQ for all chemicals of 0.1 ppb, except for flonicamid which was 0.5 ppb.

Imidacloprid was detected above the LOQ in 124 pollen samples (57%). The range of concentrations was reported to be <LOQ – 43.1 ppb. The mean concentration in pollen was 0.1 ppb (equivalent to the LOQ) ppb and the median concentration was 0.7 ppb. In honey, imidacloprid was above the LOQ in 28 of 53 samples (53%). Concentrations ranged from <LOQ – 14.7 ppb. The mean concentration was 0.58 ppb, and the median was 0.08 ppb (noted to be below the LOQ).

Appendix D. Effects Study Summaries

Tier I

Adult Acute Contact Exposure

Apis - Registrant Submitted Studies

In an adult acute contact study conducted with TGAI (99.8%) conducted in accordance with OCSPP 850.3020 (MRID 42273003), two replicates of 10 honey bees (*Apis mellifera*) each were exposed to nominal contact treatments 0.025, 0.050, 0.10, 0.20, and 0.40 µg a.i/bee (including negative control for both tests). It is noted that clinical signs of toxicity (if any) were not reported. The 48-hour contact LD_{50} was reported to be 0.078 (0.068 – 0.090) µg a.i/bee. This study is classified as acceptable.

An adult acute contact study with TGAI imidacloprid (MRID 49766209) conducted in accordance with EPPO Guideline 170 (98.6%) exposed honey bees to nominal test concentrations of 0.045, 0.085, 0.125, 0.166, and 0.207 µg a.i/bee (including a negative control). Three replicates of 10 adult bee each were exposed per treatment concentration. Clinical signs of toxicity included behavioral abnormality observations of paralysis, spasms, or frozen behavior and were observed at all treatment concentrations at 4 hours post exposure and up to 48 hours after exposure. The 72-hour LD_{50} was determined to be 0.104 (0.080 – 0.131) µg a.i/bee. This study is classified as acceptable.

In an acute contact toxicity study conducted in accordance with OCSPP 850.3020 (MRID 49602715), 3 replicates of ten honey bees each were exposed to nominal doses of 0.04, 0.056, 0.078, 0.110, and 0.140 µg a.i/bee (including a negative control). Observations of mortality indicated the frequency of the effect increased over time. Clinical signs of toxicity included bees observed to have been knocked down and stumbling or otherwise moving in a poorly coordinated manner. These effects were noted at all treatment concentrations. The 72-hour LD_{50} was determined to be 0.048 (0.041 – 0.057) µg a.i/bee. This study is classified as acceptable.

An acute contact toxicity test with TGAI (98.6%) that was conducted according to EPPO 170, exposed 3 replicates of 10 bees each per concentration (MRID 49602717). Test bees received nominal doses of 0.040, 0.056, 0.078, 0.110, and 0.0154 µg a.i/bee (including a negative control). During the 48 observation period, clinical signs of toxicity included observations of lying on back and problems standing up as well as slow motions and coordination issues. These observations were frequent in all treatment concentrations although their prevalence did not appear to be dose-dependent. The 96-hour contact LD_{50} was reported to be 0.043 (0.026 – 0.055) µg a.i/bee. This study is classified as acceptable.

In an acute contact toxicity study (MRID 49602714), TGAI imidacloprid (98.6% purity) was exposed as a single contact exposure with a 96 hour observation period. The test was conducted following the guideline recommendations of EPPO 170 and the OECD Guideline 214. The test exposed 3 replicates of bees per treatment concentration with 10 bees per replicate. Bees were exposed to nominal doses of 0.040, 0.056, 0.084, 0.110, and 0.0154 µg a.i/bee including a negative control). In the acute contact

toxicity test, the 96-hour LD_{50} was determined to be 0.069 (0.056 – 0.085) µg a.i/bee. Clinical signs of toxicity included apathy discoordinated movements, and immobility but the study report does not indicate at what treatment levels these observations occurred. This study is classified as acceptable.

In a test conducted with formulated imidacloprid (Imidacloprid SL 200, Confidor 200, MRID 49602707), bees were exposed via direct contact exposure and observed for 96 hours. Assuming the density is 1 g/L, the percent purity of imidacloprid is 20%. There were three replicates with ten honey bees per test concentration. Bees were exposed to doses of 0.0029, 0.0057, 0.011, 0.023, 0.046, and 0.091 µg a.i/bee (including a negative control). Clinical signs of toxicity included bees showing uncoordinated movement while walking or were inactive at the 4 highest treatment concentrations, which was observed primarily between 48 and 72 hours. The 96-hour LD_{50} for acute contact exposure is 0.045 (0.034 – 0.060) µg a.i/bee (246 ng product/bee). This study is classified as acceptable.

Apis - Open Literature Studies

Iwasa et al. (2004, MRID 47523403), exposed technical imidacloprid (>99% purity) via topical application to the honey bee thorax. Bees were exposed to 5-7 doses of imidacloprid (exact doses not provided in the study) and were observed for 24 hours following application for mortality. The 24-hour LD_{50} was determined to be 0.018 µg a.i/bee (0.009 – 0.032). Additionally, this study exposed bees via contact exposure to imidacloprid along with potential synergistic compounds including piperonyl butoxide (PBO), triflumizole, and propiconazole (doses of each chemical not available from the study). There were no significant differences ($p>0.05$) determined for the LD_{50} values for of imidacloprid alone and imidacloprid plus each of the potential synergistic agents. It is noted that this study included only a 24 hour observation period that is distinguished from Tier I registrant submitted studies (*i.e*. OCSPP and OECD guidelines) that employ longer (*i.e* 48 – 96 hour) observation periods.

In a study conducted by Schmuck et al. (2001, MRID 47812303), *Apis mellifera carnica*, a subspecies of the European honey bee (*Apis mellifera*) was tested. The study was conducted according to guidance provided in EPPO 170. Three test facilities (identified as United Kingdom, Netherlands, and Germany) were employed for the tests. Technical imidacloprid (>98% purity) was tested along with two different formulations (WG 70, 70% a.i and SC 200, purity not reported but assumed to be 20% assuming density of 1 g/L). The 48-hour contact LD_{50} values for technical imidacloprid ranged from 0.081 (0.055 – 0.12) to 0.23 (95% CI not available) µg a.i/bee, depending on the testing facility. The 48-hour contact LD_{50} values for the WG 70 and SC 200 formulations were 0.24 (0.17 – 0.35) and 0.060 (0.039 – 0.093) µg a.i/bee, respectively. Limitations of the study include no information on the dose-response relationship and no indication of control performance although the tests were conducted according to EPPO 170 which stipulates control mortality to be below 15%.

In further work by Schmuck et al. (2003, MRID 47796304), similar methodology was carried out as for the Schmuck 2001 in that multiple testing facilities were employed, the tests was conducted according to EPPO 170 guidance, and the subspecies *Apis mellifera carnica* was tested. For one testing facility, the 72-hour LD_{50} was determined to be 0.14 µg a.i/bee (0.83 - 0.13). All other testing facilities yielded 48-hour

contact LD_{50} values ranging from 0.042 (0.020 – 0.59) to 0.075 (0.062 – 0.091) µg a.i/bee. Limitations were similar to those noted in Schmuck et al 2001.

In a study by Suchail et al. (2000, MRID 47800513), technical imidacloprid (98% purity) was exposed to honey bees (both *Apis mellifera and Apis mellifera caucasia*) closely following methods described in OCSPP 850.3020 and OECD TG 214 with the trail replicated 3 times. During the course of the 48-hour observation period, several neurotoxic symptoms were noted that included trembling, tumbling, and lack of coordination within 24 hours after exposure of both species tested. The 48-hour LD_{50} values were determined to be 0.024 (0.022 – 0.027) for *Apis mellifera mellifera* and 0.013 (0.010 – 0.016) µg a.i/bee for *Apis mellifera caucasica*. The study authors reported an unusual dose response profile for the *Apis mellifera mellifera* subspecies results. Specifically, the rate of mortality increased at low doses of imidacloprid, then decreased at intermediate doses, and increased again at higher contact doses. The cause of this biphasic dose response (as purported by the study authors) which occurred across all three replications of the study, is unknown. It is also noted that this study did not report the doses to which the bees were exposed.

Thompson et al. (2014) investigated the potential of neonicotinoids (including imidacloprid) and fungicides to exhibit synergistic effects on the acute contact and oral exposure to honey bees. Imidacloprid (99.9% purity) was administered topically to worker honey bees that were observed 48 hours post administration, following guidance recommended in OECD TG 214. Stumbling and knockdown effects were observed at 4 hours post administration (numbers of bees affected at specific doses not available from the study). The 48-hour LD_{50} for imidacloprid alone was determined to be 0.067 (0.043 – 0.010) µg a.i/bee. As mentioned previously this study also assessed the contact toxicity of imidacloprid and several other fungicides, specifically myclobutanil (0.16 µg a.i/bee), propiconazole (0.22 µg a.i/bee), flusilazole (0.36 µg a.i/bee), and tebuconazole (0.45 µg a.i/bee). The 48-hour LD_{50} values for imidacloprid with the other 4 individual fungicides were generally at the level of those with imidacloprid alone, with no more than a 2-fold increase in toxicity. This corroborates similar work discussed above in Iwasa 2004 in which triflumizole and propiconazole were not determined to have significantly different toxicity when administered with imidacloprid as compared to imidacloprid alone (48 hour observation period in Thompson as opposed to 24 hour observation period in Iwasa).

In a study by Biddinger et al. (2013, MRID 49719605), the contact toxicity of formulated imidacloprid (Provado, 17.4%) was investigated with a varying doses (not specified in the study) of the test substance applied to the thorax, followed by a 48 hour observation period. Based on the experimental design information and the number of bees used for the study, a total of 10 bees per replicate and 5 replicates per treatment group were exposed. Control mortality was stated to be 2.7%. The 48-hour contact toxicity LD_{50} was determined to be 0.03 µg a.i/bee (95% CI – 0.017 – 0.05 µg a.i/bee). Limitations to this study include no explicit statement of the range of doses tested, no mention of the presence of a dose response.

Non-*Apis*– Registrant Submitted studies

In an acute contact toxicity study conducted with TGAI (98.6% purity) imidacloprid on the bumble bee (*Bombus terrestris*), 30 bumble bees per treatment concentration were exposed alongside a control (MRID

49766208). The nominal doses were 0.1, 4.0, 8.0, 31, and 65 µg a.i/bee. In all but the lowest treatment concentration, mortality was 90% or greater by 24 hours post exposure. In the lowest treatment concentration, mortality was continuously increasing (3, 40, and 60% at 24, 48, and 72 hours, respectively) at test termination at 72 hours. Therefore, a definitive LD_{50} could not be determined. Clinical signs of toxicity that were described in the study report as "frozen behavior" were observed in all test concentrations It is noted that the concentration used for this study are markedly higher than the range tested in the available laboratory contact toxicity studies on adult honey bees (*i.e. Apis)*. As a definitive LD_{50} could not be determined, this study is classified as supplemental.

An acute toxicity study conducted with formulated imidacloprid (FS 350, 30.4% purity) tested bumble bees (*Bombus terrestris*) under laboratory conditions (MRID 495332101). Although no formal guideline is currently available for the testing of bumble bees, this study based its test design on EPPO 170, OECD TG 214, and on the methods summarized in the open literature review by Van der Steen (2001).[1] Bumble bees, in groups of 10 bees per replicate and 3 replicates per treatment group, were exposed to single topical doses on the thorax of 1.23, 3.70, 11.1, 33.3, and 100 µg a.i/bee (including a negative control). Observations of mortality were made for 96 following dose administration. Control mortality at 96 hours was 0%, while the reference control (dimethoate at 12 µg a.i/bee) yielded 77% mortality. Clinical signs of toxicity included moribund (defined by study report as bees not being not be able to walk and show only feeble movements of legs and antennae and only weak response to stimulation), affected (defined as bees still upright and attempting to walk but showing signs of reduced coordination), and apathy (defined as bees showing only low or delayed reaction to stimulation). These effects occurred at all treatment groups although no clear dose response is clear in their prevalence across the treatment groups. The percent mortality was 20, 33, 27, 53, and 47% in the 1.23, 3.70, 11.1, 33.3, and 100 µg a.i/bee treatment groups, respectively. The study author 96-hour LD_{50} was determined to be 85.3 µg a.i/bee. Due to there not being a clear indication of a dose-response, reliable 95% confidence intervals were not available.

Non-*Apis* – Open Literature Studies

In a study conducted by Gradish et al. (2009, MRID 48194902), the acute contact toxicity of imidacloprid (>95% purity) was investigated on individual bumble bees (*Bombus impatiens*). Rather than topical administration of the test substance as described in OCSPP and OECD guidelines, exposure was achieved via a Potter Spray Tower. Concentrations that were the equivalent of 0.05, 0.5, and 5 lbs. a.i/A were administered to test bees which were then observed for 72-hours for mortality. As there were only 3 doses administered, a LD_{50} was not estimated. After 72 hours, there was 72, 96, and 100% mortality in the 0.05, 0.5, and 5 lbs a.i/A, respectively. It is noted that the doses levels administered for this test were relatively high, particularly the highest treatment dose.

Marletto et al. (2003, MRID 47796306) exposed bumble bees (*Bombus terrestris*) to imidacloprid (purity not reported) via topical administration to individuals, which were observed for 72 hours for mortality. A droplet of 10 µL of the test solution, or water as the control, was placed between the coaxer of anesthetized bees using a pipette. After the test solution evaporated, the bumbles were then put into

[1] Van Der Steen, J. (2001): Review of the methods to determine the hazard and toxicity of pesticides to bumble bees. Apidologie 32, pp 399-406.

cages and observed for mortality. The 72-hour contact LD_{50} value was determined to be 0.02 µg a.i/bee (95% CI not available). Uncertainties from this study include the purity of imidacloprid not being specified, the doses that were administered to the bees not being specified, no indication of whether a dose response was present, and no information on the performance of the control. The study does state however, that repetitions of the test in which over one individual in the control had died were not considered, suggesting control mortality was low.

In Biddinger et al. (2013, 49719605), which was discussed previously as it related to the contact toxicity to honey bees (*Apis mellifera)*, formulated imidacloprid (17.4% purity) was administered topically to Japanese orchard bees (*Osmia cornifrons*) and observed for 48 hours for mortality. Although 6 doses were stated to have been administered to the bees, it was not reported in the article what these doses were. Immobilized bees were placed in a cage (10 bees per cage) and 1 µL/bee was applied on their thorax. Mean control mortality was reported to be 2.7%. The 48-hour contact LD_{50} was determined to be 0.66 (0.30 – 2.19) µg a.i/bee. This study also tested formulated imidacloprid with formulated (22.9%) fenbuconazole to investigate potential synergistic effects. The 48-hour LD_{50} of bees exposed to contact residues of imidacloprid + fenbuconazole was 1.12 (0.24 – 1.63) µg a.i/bee. Limitations for this include no explicit statement of what the dose levels actually were administered to the bees and no indication of a dose response from the data provided.

In a study by Scott-Dupree et al. (2009, MRID 48191904), the direct contact toxicity of imidacloprid was investigated on bumble bees (*Bombus impatiens*, females only), alfalfa leaf-cutting bees (*Megachile rotundata*, males and females), and blue orchard bees (*Osmia lignaria*, males and females). Technical imidacloprid (>95% purity) was administered at 4-6 dose concentrations (not available from the study) to four to replicates per treatment group (9-11 bees per replicate). A Potter Spray Tower was used to administer the treatment concentrations, after which bees were transferred to waxed cups covered with petri dishes with 50% sucrose solution and observed for mortality for 48 hours. While the exact level of control mortality was not provided, it was stated to have not exceeded 10%. The results presented in this study are presented in terms of concentrations expressed as a percentage of solution (weight:volume). The amount of pesticide deposited on bees was therefore not determined and without actual test doses, LD_{50} values in terms of µg a.i/bee cannot be calculated. It is possible to convert the values reported by the study author's results from % solution to µg a.i/kg, however no information on the density of the test solution is available from this conversion. Assuming that the density of the test solution is 1 g/mL, the 48-hour LD_{50} may be estimated to be 32.2, 1.7, and 0.7 µg a.i/kg for bumble bees, alfalfa leaf-cutting bees, and blue orchard bees, respectively.

In a study conducted by Tomé et al. (2015, MRID 49719633), the lethal and sublethal effects to the stingless bee *Melipona quadrifasciata* were investigated following exposure to imidacloprid and spinosad, an allosteric modulator of the nicotinic acetylcholine receptor. Although the primary focus of this study was to determine effects resulting from spinosad exposure, imidacloprid (reported as 700 g a.i/L, 70% purity assuming a density of 1 g/L) was used as a positive control. Six concentrations, ranging from 0.005 – 0.090 ng a.i/bee, were spiked into a 50% sucrose solution and provided to 30 adult bees per treatment concentration. Once the bees had completely depleted the sucrose solution, observations of mortality, flight behavior, and respiratory rate (by placing bees in completely closed chamber systems where CO_2

production was measured) were made for 24 hours. After 3 hours of exposure, bees treated with imidacloprid showed a decreased activity level as compared to the negative control but this was reversed after 24 hours where treated bees showed an activity level 4 times higher than the negative control (statistical results not provided). Imidacloprid also significantly affected flight activity of bees, as there were no bees observed to fly above 35 cm, whereas the majority of bees in the control group were able to reach the light source at 125 cm. The respiratory rate of treated bees was also significantly reduced relative to the control (statistical results not provided). The 24-hour LD_{50} was determined to be 0.024 µg a.i/bee.

Uncertainties in this study include: 1) no analytical verification of imidacloprid in the treatment concentrations, 2) the purity of imidacloprid not being provided, 3) the level of control mortality is not explicitly stated. While it was stated that the morality levels determined in the treatment groups were adjusted for control mortality, it is uncertainty what the level of mortality in the control actually was, 4) the species of stingless bee used for this study does not have a range that extends into North America and its appropriateness to serve as a surrogate for other stingless bees is unknown, and 5) a purity of 70% is assuming a product density of 1 g/L which is recognized as an uncertainty.

Adult Acute Oral Exposure

Apis – Registrant Submitted Studies

There are nine available acute studies to characterize the oral toxicity of imidacloprid to adult honey bees with technical grade active ingredient (TGAI, purities range from 98.6 - 99.8%) and one study conducted with a formulated typical end use product (TEP, 200 g/L, 20% a.i., assuming density of 1 g/L).

In an adult acute oral study conducted with TGAI (99.8%) (MRID 42273003), two replicates of 10 honey bees (*Apis mellifera*) each were exposed to nominal oral treatments of 0.0015, 0.0031, 0.0063, 0.013, and 0.025 µg a.i/bee (including negative control). It is noted that clinical signs of toxicity (if any) were not reported. The 48-hour oral LD_{50} was determined to be 0.0039 (0.0027 – 0.0054) µg a.i/bee. This study is classified as acceptable.

In an acute oral toxicity study (MRID 49766202), TGAI (98%) imidacloprid was exposed to adult honey bees ranging in age from 4-6 weeks old. This study was conducted according to EPPO 170. Three replicates of 10 bees each were exposed per treatment group. The treatment groups, as reported as the mean dose levels consumed by bees were, 0.0001, 0.006, 0.0012, 0.0024, 0.0056, 0.010, 0.018, and 0.036 µg a.i/bee (including a negative control). Clinical signs of toxicity that were observed included coordination problems, apathy, and nervousness that were observed in the four highest treatment groups. It was reported that there was continuously increasing mortality up to 48 hours after treatment and so the test was prolonged a further 48 hours (for a 96-hour total observation period). There was no treatment group that elicited a 50% or greater mortality response (47% mortality in the highest treatment group). The 96-hour LD_{50} is therefore determined to be >0.036 µg a.i/bee. This study is classified as acceptable.

In an acute oral toxicity study (MRID 49766205), three replicate groups of ten bees each were exposed to nominal concentrations of TGAI (98.6%) imidacloprid at 0.0049, 0.0086, 0.013, 0.016, and 0.021 µg a.i/bee (including a negative control). This corresponded to actual mean dose levels consumed by the bees of 0.0042, 0.0077, 0.012, 0.014, and 0.020 µg a.i/bee. Clinical signs of toxicity included paralysis, spasms, and frozen behavior that was observed in bees at the for highest treatment groups. The 48-hour LD_{50} was determined to be >0.020 µg a.i/bee. This study is classified as acceptable.

In an acute oral toxicity study conducted with TGAI (98.6%), three replicates of ten honey bees per test concentration were observed for mortality for 48 hours following exposure (MRID 49602716). This study was conducted following guidelines recommended by EPPO 170 and OECD TG 213. The nominal treatment concentrations were 0.0009, 0.0028, 0.0082, 0.025, and 0.074 µg a.i/bee (including a negative control). Clinical signs of toxicity included stumbling and bees being observed to be "knocked down" in the three highest treatment concentrations with increasing prevalence as the dose increased. The mean maximum dose determined to be consumed was 45 ng, which is 39% less than the actual highest test concentration offered. The 48-hour LD_{50} was determined to be >0.045 µg a.i/bee as there was no concentration tested that elicited a 50% or greater lethal response. This study is classified as acceptable.

An acute oral toxicity test with TGAI (98.6%) that was conducted according to EPPO 170, exposed 3 replicates of 10 bees each per concentration (MRID 49602717). The nominal treatment concentrations were 1, 3, 9, 27, and 81 ng a.i/bee (including a negative control). This corresponded to measured (based on actual ingestion of the test item) concentrations of 0.0009, 0.0028, 0.007, 0.018, and 0.035 µg a.i/bee. During the course of the 96-hour observation period, there were 3 instances of bees observed to be lying on their backs with problems standing up in the highest concentration (34.7 ng a.i/bee). Additionally, food uptake of the test substance decreased in a dose dependent manner with increasing concentration with percent food uptake of 87.4, 93.7, 96.6, 77.9, 65.9, and 42.8% for the control, 0.0009, 0.0028, 0.007, 0.018, and 0.035 µg a.i/bee, respectively. The 96-hour oral LD_{50} was determined to be >0.035 µg a.i/bee. This study is classified as acceptable.

In an acute oral test (MRID 49766204),TGAI (99.4%) imidacloprid was orally exposed to adult (4-6 week old) honey bees at nominal test concentrations of 0.001, 0.005, 0.010, 0.020, 0.040, 0.080, and 0.160 µg a.i/bee (including a negative control). This study was conducted in accordance with EPPO 170. These concentrations corresponded to single oral doses of 0.0012, 0.0046, 0.0104, 0.019, 0.039, 0.082, and 0.160 µg a.i/bee, respectively. The 96-hour LD_{50} was determined to be 0.15 µg a.i/bee. The very wide 95% confidence interval (0.078 – 1.86) indicates this endpoint is subject to uncertainty with the cause likely arising from the high variability observed between the replicates. There were also other deviations (from EPPO Guideline 170) noted that included older age of test bees than recommended, and unknown sugar concentration of the diet. This study is classified as supplemental as the reliability of the endpoint is determined to be low due to the wide 95% confidence intervals.

In an acute oral toxicity study with TGAI imidacloprid (99.4% - MRID 49766204) that was conducted according to EPPO 170 guidance, 4-6 week old adult honey bees were exposed to nominal oral doses of 0.0001, 0.0006, 0.0013, 0.0025, 0.005, 0.010, 0.020, and 0.040 µg a.i/bee (including a negative control). This corresponded to single oral doses of 0.0001, 0.0008, 0.00015, 0.0031, 0.006, 0.012, 0.023, and 0.041

µg a.i/bee. Due to the increasing mortality between 24 and 48 hours, the test was extended for a further 48 hours up to 96 hours. Three replicates of ten bees were exposed per treatment concentration. Clinical signs of toxicity included apathy, coordination problems, laziness, nervousness, and sitting in one corner of the chamber were observed in the five highest treatment concentrations. While the study authors determined the 96-hour LD_{50} to be 0.041 µg a.i/bee, an independent review of the data yielded an 96-hour LD_{50} of 0.048 µg a.i/L (95% CI: 0.0135 – 3980). It is noted that the estimated endpoint is above the range of concentrations tested in this study. Additionally, the very wide confidence intervals are likely to be the result of variable responses among the replicates within the treatment groups and the lack of a clear dose response (mortality was 10, 0, 0, 6.9, 33.3, 33.3, 30, 16.7, and 53% in the control and 0.0001, 0.0006, 0.0013, 0.0025, 0.005, 0.010, 0.020, and 0.040 µg a.i/bee treatment groups, respectively). As the study did not test high enough to allow for a proper convergence of the regression model (*i.e.* as indicated by the wide confidence intervals), this study is classified as supplemental.

In an acute oral toxicity study (MRID 49602714), TGAI imidacloprid (98.6% purity) was exposed to adult honey bees following the guideline recommendations of EPPO Guideline 170 and OECD TG 213. There were 3 replicates of bees per treatment concentration with 10 bees per replicate. Mean measured concentrations were 0.001, 0.003, 0.009, 0.027, and 0.070 µg a.i/bee (including a negative control). Clinical signs of toxicity included apathy, discoordinated movements, and immobility observed 4 and 24 hours after oral dosing in the two highest treatment concentrations (prevalence of the findings not available from the study report). There was no concentration that elicited a 50% or greater effect on mortality and therefore the 48-hour LD_{50} was determined to be >0.70 µg a.i/bee. This study is classified as acceptable.

In a test conducted with formulated imidacloprid (Imidacloprid SL 200, Confidor 200, 20% purity assuming a product density of 1 g/L, MRID 49602707), bees were exposed orally and observed for 96 hours. There were three replicates with ten honey bees per test concentration for each component of the study. Clinical signs of toxicity included bees showing uncoordinated movement while walking or were inactive at the 2 highest treatment concentrations, which was observed primarily between 48 and 72 hours. The 96-hour LD_{50} for acute oral exposure is 53 ng a.i/bee (290 ng product/bee), respectively. This study is classified as acceptable.

***Apis* - Open Literature Studies**

In a study conducted by Ramirez-Romero (2005, MRID 47796305), individual honey bees were exposed to a single concentration of technical imidacloprid (98% purity) for 4 days that was preceded and followed by 4 day periods of untreated syrup. In this way, there was no true experimental control as the experiment group alternated between bees being exposed (48 ppb in sucrose solution) and unexposed to imidacloprid. This study assessed effects on mortality, food consumption, foraging ability, and olfactory performance following an alternating exposure to untreated syrup and imidacloprid. There were significant ($p<0.05$) reductions in food consumption, foraging activity (reported as the number of visits to an artificial floral array utilized in the study), and olfactory learning performance (reported as percent of visits to scented sites vs unscented sites) determined during the treatment period and compared to before and after the treatment periods. Mortality was not significantly ($p>0.05$) impacted. For all endpoints,

there appeared to be recovery although the level was still significantly reduced from before the treatment period. It is noted that the interpretation of statistical results from this study is uncerta.in given that one colony was employed, and therefore there was no replication to capture colony to colony variation. Other uncertainties include no analytical confirmation of imidacloprid in the treatment solution and no information on the pesticides used as a weekly sanitary control for the *Varroa* mite.

In a study by Schmuck et al (2001, MRID 47812303), which was discussed above with the acute contact toxicity results, honey bees were orally exposed to technical (>98% purity) as well as the WG 70 and SC 200 formulations. The methods were similar to that of the contact toxicity component of the study in that multiple research facilities were used and the exact concentrations that bees were exposed to were not available from the study. Additionally, the tests adhered to guidance recommended in EPPO 170 as well as exposing *Apis mellifera carnica* honey bees. The 48-hour oral LD_{50} values for bees exposed to technical imidacloprid ranged from 0.0037 (0.0026 – 0.0053) to 0.041 (95% confidence limits not available) µg a.i/bee. For the WG 70 and 200 SC formulations, the 48-hour oral LD_{50} value was determined to be 0.012 (0.0073 – 0.018) µg a.i/bee and 0.021 (0.0o15 – 0.030) µg a.i/bee, respectively. Limitations to this study include the concentrations of imidacloprid were not analytically verified in the sucrose, no information on the dose response relationship was present, and no raw data were provided to allow for an independent verification of the statistical results.

Similarly, in a study by Schmuck et al (2003, MRID 47796304), which is also discussed previously as it relates to acute contact toxicity, the acute oral toxicity was investigated using methods similar to that of Schmuck 2001 for the oral exposure component. The testing occurred at 7 research facilities and the level of control mortality was not stated, although the test was in accordance with EPPO 170, that stipulates control mortality be below 15%. The 48-hour oral LD_{50} values ranged from >0.020 to >0.081 µg a.i/bee. The limitations of this study are previously discussed above.

Suchail et al (2000, MRID 47800513) was previously discussed in the context of acute contact results but this study also orally exposed two subspecies of honey bees to varying concentrations of imidacloprid (98% purity). The methods employed for this study closely followed those of OECD TG 213. Although both 24 hour and 48-hour oral LD_{50} values were determined, the results from 48 hours of observation yielded more sensitive endpoints. The 48-hour LD_{50} values for *Apis mellifera mellifera* and *Apis mellifera caucasica* were 0.0048 (0.0045 – 0.0051) and 0.0065 (0.0047 – 0.0083) µg a.i/bee, respectively. As with the contact portion of the study, there were observations of clinical signs of toxicity within 24 hours of exposure that include coordination problems, trembling, and tumbling. Limitations for this study are previously discussed above.

In another study by Suchail et al (2001, MRID 47523402), technical imidacloprid (97% purity) was oral dosed in sucrose to honey bees ranging from 1 to 1000 ng a.i/bee. The acute oral tests closely followed guidance in OECD TG 213. Following oral exposure to imidacloprid, clinical signs of toxicity included hyper-responsiveness, hyperactivity, and trembling, but it was not detailed in the study the frequency of these observations and which treatment groups these signs occurred in. The 48, 72, and 96 oral LD_{50} values were determined to be 0.057, 0.037, and 0.037 µg a.i/bee, respectively. Limitations of this study include

no analytical confirmation of imidacloprid in the test concentrations, and no raw data being provided to allow for an independent verification of the statistical results.

In a study by Thompson et al (2014a, MRID 49750606) which was previously discussed in the context of acute contact results, honey bees were also orally exposed to varying concentrations of imidacloprid (99.9% purity) and observed for mortality for 48 hours post treatment. The 48-hour oral LD_{50} was determined to be 0.536 (0.339 – 1.18) µg a.i/bee. The study also investigated the potential synergism or oral exposure to imidacloprid along with several ergosterol biosynthesis inhibitor fungicides including myclobutanil, propiconazole, flusilazole, and tebuconazole. Similar to that of the results of the contact toxicity portion of this study, imidacloprid + any of the tested fungicides did not yield an endpoint that was significantly lower than that of imidacloprid alone (LD_{50} values of 1.08, 1.50, 1.18, and 0.893 µg a.i/bee for imidacloprid + myclobutanil, propiconazole, flusilazole, and tebuconazole, respectively. Although the study appeared to generally adhere to guidance recommended in OECD TG 213, there was no mention of any control mortality in the study.

Laurino et al (2013, MRID 49719620) exposed two subspecies of *Apis mellifera* (*Apis mellifera mellifera* and *Apis mellifera ligustica*) to varying concentrations of formulated imidacloprid (Confidor 200 SL, 17.4% purity) and observed test bees for mortality at 24, 48, and 72 hours. Tests were carried out on honey bees taken from nine hives. Ten foraging honey bees were placed in each cage not later than 15 minutes from capture in order to minimize stress. Two or three cages were used for each replication and the tests were replicated two or three times. Replications with control mortality above 10% were discarded, although it was not clear from the study how often this occurred. Honey bees were provided a 25% sucrose solution, for untreated controls and the treatment groups were provided known amounts of the compounds. Solutions were made available to the honey bees for one hour; then honey bees could feed on sugar candy throughout the remaining part of the trial. Mortality responses were notably irregular, particularly at 24 hours. Despite this, the *Apis mellifera ligustica* colonies that were used for these tests yielded comparable LD_{50} values when compared to that of *Apis mellifera mellifera*. The results of certain colonies were so erratic that LD_{50} values and confidence intervals could not be calculated after 24 hours. The 72-hour LD_{50} values for the sole colony of *Apis mellifera mellifera* honey bees used was 193 ng a.i/bee (95% confidence intervals not provided). The 72-hour LD_{50} values for the 4 colonies of *Apis mellifera ligustica* used ranged from 25 – 65 ng a.i/bee. It is worth noting that this study also tested two other neonicotinoid chemicals, thiamethoxam and clothianidin, were also tested in this study on 2 (clothianidin) and 3 (thiamethoxam) subspecies of *Apis mellifera.* The 72-hour LD_{50} values indicated that there were was not the same discrepancy of toxicity values across multiple subspecies for these two chemicals as there was for imidacloprid (same subspecies tested, with the addition of *Apis mellifera carnica* for thiamethoxam). This comparison also would have been more informative if the study had tested technical grade imidacloprid and not formulated product. Other limitations include no information on control performance (although it was stated that control replicates showing 10% or more mortality were discarded and not used for the test) and no analytical verification of imidacloprid in the treatment concentrations.

Chronic Adult Oral Toxicity

Apis– Registrant Submitted Studies

In a chronic 10-day study (MRID 49511703), honey bees were continuously exposed to sucrose spiked with varying concentrations of technical grade imidacloprid (99.4% purity). The nominal treatment concentrations of imidacloprid in the 50% sucrose solution were control, 10, 20, 50, and 100 µg a.i/L. Daily observations of mortality, clinical signs of toxicity, and behavior were made throughout the course of the 10-day study. Additionally, the overall mean daily consumption of the sucrose syrup was assessed. While part of the Tier I suite of toxicity studies, there has at present, been no formal guideline released for this study type.

After 10-day of continuous exposure to imidacloprid spiked sucrose solutions, the level of mortality at all treatment concentrations was not significantly different ($p>0.05$) as compared to the control group (cumulative control mortality was 2.67% at test termination). Mortality in the 10, 20, 50, and 100 µg a.i/L treatment groups was 4.00, 0, 1.00, and 4.00% at test termination. It was noted that starting on Day 5 and persisting until test termination (Day 10), that bees in the 100 µg a.i/L treatment group were very calm and inactive compared to the bees in the lower treatment groups and control. The overall mean daily consumption rate (average value over 10 days) was 47.1, 37.7, 39.8, and 33.3 mg/bee in the 10, 20, 50, and 100 µg a.i/L groups, respectively, which were all significantly lower as compared to the control group (54.2 mg/bee). Therefore, based on a significant decrease on overall mean daily food consumption at the lowest treatment level (13% decrease from the negative control), a definitive NOAEC could not be established. The 10 µg a.i/L treatment group was equivalent to an average intake of 3.9 ng a.i/bee/day, which is noted to be approximately the value of the most sensitive acute oral toxicity value (48-hour LD_{50}) of 3.9 ng a.i/bee. This study is classified as supplemental due to failure to achieve a definitive NOAEC.

Apis – Open Literature Studies

In a study by Boily et al, 2013 (need MRID), the chronic oral toxicity to adult honey bees (*Apis mellifera*) was investigated over a 10-day exposure period. Although the study used formulated imidacloprid (Admire 240F), the doses were provided in terms of active ingredient per bee. Bees were provided nominal concentrations of imidacloprid that were equivalent to 0, 0.08, 0.16, 0.24, and 0.30 ng a.i//bee spiked into sucrose solution. There were 30 individual bees per cage with three replicate cages per treatment group. Sugar solutions were changed daily during the 10-day exposure period.

After 10 days, survival and body weight was significantly ($p<0.05$) reduce at the two highest treatment groups (0.24 and 0.30 ng a.i/bee). It was reported that from Day 3 to Day 6, survival decreased steadily and remained around 30% for the 0.24 ng a.i/bee treatment group, while survival at the highest dose (0.30 ng a.i/bee), the survival dropped from 40% at Day 6 to 2/5% by Day 10. Clinical signs of toxicity that were observed include tumbling and trembling that appeared on Day 1 in the 0.30 ng a.i/bee group and at Day 3 for the lower doses. From Day 4 to Day 6, the percentage of bees exhibiting these symptoms was significantly higher in the 0.24 and 0.30 ng a.i/bee treatment groups as compared to the control. Raw data was made available by the study author which provided for verification of the test results. The

NOAEC was for this study based on mortality and body weight was determined to be 0.16 ng a.i/bee. As discussed previously, the chronic 10-day registrant submitted study (MRID 495117093) did not achieve a definitive NOAEC due to reduced food consumption determined at all treatment concentrations including the lowest concentration of 3.9 ng a.i/bee. Therefore, the results of this study will be used to estimate chronic risk to adult honey bee resulting from the oral route of exposure at the Tier I level.

In a study by Alaux et al (2010, MRID 48077922), although the primary focus of the study was to investigate the additive or synergistic effect of imidacloprid and exposure to the fungal pathogen *Nosema ceranae*, there was an imidacloprid exposure only component of the study that is relevant to the chronic oral exposure discussion. Imidacloprid (purity not reported) was spiked into a 50% sucrose solution at nominal concentrations of 0.7, 7, and 70 ppb. Fresh imidacloprid solutions were prepared each day and bees (9 replicates per treatment group with 30 bees per replicate) were offered the solution for 10 hours daily while the reminder of the day they were offered candy (30% honey, 70% powdered sugar) and water *ad libitum*. Mortality at test termination (day 10) was 10.4, 16.3, and 17.4% in the 0.7, 7.0, and 70 ppb groups, respectively, as compared to the negative control group (5.60%). It is noted that despite all treatment groups having a significantly ($p<0.05$) higher mortality rate than the control group, mortality remained relatively stable despite imidacloprid concentrations increasing 100 fold across treatment groups. Additionally, there was no significant differences as compared to the control for food sucrose consumption rates in all treatment groups ($p>0.05$).

As an additional component of this study, individual and social immune endpoints were assessed. Individual immune endpoints that were assessed included phenoloxidase activity and total hemocyte count. Phenoloxidase is purported to play a role a central role in invertebrates' immune response, implicated in the encapsulation of foreign substances through melanization (Decker and Jaenicke, 2004). Additionally, total hemocyte count has been stated to give an indirect measurement of immune cell function and is involved in processes such as phagocytosis and the encapsulation of a parasite (Tanada and Kaya, 1993). Social immunity endpoints that were examined included glucose oxidase enzymatic activity which catalyzes the oxidation of β-D-gluconic acid and hydrogen peroxide (White et al, 1963). Taormina et al. (2001) and Brudzynski et al (2006) determined the level of hydrogen peroxide in honey is positively correlated with the inhibition of pathogens. The size of the hypopharyngeal glands, where this enzyme is primarily expressed (Ohashi et al, 1999), was also assessed as an endpoint. After the 10-day exposure to imidacloprid, there were no individual or social immunity endpoints assessed that were significantly different ($p>0.05$) in any imidacloprid treatment group as compared to the negative control. Limitations of this study include no analytical confirmation of imidacloprid in the sucrose solutions and no raw data being provided to allow for an independent verification of the statistical results. .

In a study Cresswell (2012, MRID 49719610), the toxicity of honey bees and bumble bees was compared following a 6-day oral exposure of imidacloprid spiked in sucrose solution. Although the purity was not reported in the study article, email correspondence with the author (03/16/15) indicated the test substance was technical grade imidacloprid. Bees were exposed to test concentrations of control, 0.08, 0.2, 0.51, 1.3, 3.2, 8.0, 20, 50, and 125 µg a.i/L. Daily observations were made for food consumption and mortality. Locomotory activity was quantified by video monitoring of each test age. Individual honey bees were reported to be capable of ingesting the sucrose at the highest treatment concentration, 125

µg a.i/L (4.9 ng a.i of imidacloprid per day) without an evident effect on any endpoint. It is suggested by the study author that if this dose was taken in a single meal, that this level would be enough to produce a 50% lethal effect (Cresswell, 2012) and that the honey bees in this study survived because they ingested imidacloprid gradually, allowing the chemical to be continuously metabolized. This suggestion cannot be confirmed with the results of this study alone. Further work by this author (Cresswell, 2013), described below, that examines the clearance of imidacloprid from honey bees sought to further characterize this assertion.

In another study by Cresswell (2013, MRID 49719611), honey bees were provided a nominal dose of 125 µg a.i/L (the highest treatment concentration in Cresswell, 2012) in sucrose solution either continuously for 8 days, or as part of a 'pulsed' exposure. Honey bees (10 bees per replicate, 3 replicates per group) in the pulsed exposure group were provided with imidacloprid for 3 days, followed by 5 days of untreated sucrose. Honey bees exposed to dosed syrup for 8 days consumed an average of 2.2 ng a.i/bee per day for a cumulative total ingestion of 17.4 ng a.i/bee over the course of 8 days. The daily clearance rate (estimated using whole body residues) was determined to approximately 100%. Mean individual daily feeding rates and locomotory activity did not differ between treated bees and the control group (both in the continuous and pulsed exposure scenarios). The study authors contend that that this supports the notion that honey bees can recover from short-term exposures, yet also note that the uptake, depuration, and behavioral recovery of worker beers observed in this study should be assumed to have an equivalent effect on the colony as a whole. Limitations of this study include no analytical verification of imidacloprid in the single treatment concentration and information on control mortality was not available. Additionally, the endpoints assessed in this study were not directly linked to the apical endpoints including bee survival, development, and reproduction.

Non-*Apis* – Open Literature Studies

In Cresswell, 2012 (MRID 49719610), which was discussed above as it relates to chronic oral exposure to adult honey bees exposed honey bees to varying concentrations of imidacloprid in sucrose, bumble bees (*Bombus terrestris*) were orally exposed to nominal test concentrations of control, 0.08, 0.2, 0.51, 1.3, 3.2, 8.0, 20, 50, and 125 µg a.i/L of technical imidacloprid for 6 days. Daily observations were made for food consumption and mortality. Locomotory activity was quantified by video monitoring of each test age. Individual bumble bees consumed more syrup per day than honey bees, and the rate of feeding responded to the dosage of imidacloprid only in bumble bees. Each additional nanogram of imidacloprid in an individual bumble bee's daily diet reduced syrup consumption by approximately 6% relative to that of undosed bees. There were significant effects to daily feeding rate but no impacts on longevity or locomotive activity. Individual honey bees were capable of ingesting up to 4.9 ng of imidacloprid per day without an evident effect where an equivalent intake was shown to cause a 38% reduction in feeding by bumble bees. The consequence of this reduction in feeding by bumble bees at the colony level or the impact on pollination services is uncerta.in. A limitation to this study is that it did not determine an endpoint (*i.e.* NOAEC) but rather the observations for a given response variable for one species are compared against the response of the other.

In Cresswell, 2013 (MRID 49719610 - also previously discussed with regards to chronic oral exposure to adult honey bees) bumble bees (*Bombus terrestris*) were provided a nominal dose of 125 µg a.i/L (the highest treatment concentration in Cresswell, 2012) in sucrose either continuously for 8 days, or as part of a 'pulsed' exposure in similar methodology as that used for honey bees. Individual bumble bees (33 per treatment concentration) consumed a mean of 6.7 ng imidacloprid/day for a cumulative total ingestion of 53.8 ng imidacloprid/bee over the 8 days of exposure. Mean whole body residues of 2.4 ng/bee (12.9 ng/g) were reported between days 4 to 8 of exposure. The daily clearance rate in bumble bees was estimated as 88% on the first day of ingesting imidacloprid and 68% thereafter. After removal of imidacloprid following 3 days of exposure, bumble bees depurated 100% of the whole body residues by 48 hr. Bumble bees exposed to 125 µg/L (nominal) imidacloprid experienced statistically significant reductions in daily food consumption and locomotion ($p<0.05$). Limitations to this study are previously discussed for this study in the section discussing *Apis* open literature studies.

Larval Chronic Oral Toxicity

Non-*Apis* – Open Literature Studies

In a study by Abbott et al (2008, MRID 47812301), the eggs of blue orchard bees (*Osmia lignaria*) were exposed to varying concentrations of technical imidacloprid (97.5% purity) into microwell plates and continuously exposed through all life stages until adult (egg, larvae, pupae, adult), a duration of up to approximately 30-40 days. The bees were obtained as over-wintering adults in cocoons and kept in storage until the start of the experiment.

Cocoons were then placed in microwell plates and allowed to emerge as adults, after which eggs to be used for the study were collected along with their pollen provisions. Two different laboratory methodologies were used to determine the most effective method. The first method involved injecting the pollen provisions with 10 µL of the four varying pesticide concentrations (control, 3, 30, and 300 ppb spiked in pollen). The second method involved replacing the pollen provision with a preblended pollen mixture containing the aforementioned concentrations of imidacloprid. Pollen traps on honey bee hives were used for the collection of pollen for these preblended mixtures. These pollen samples were ground up with an electric food processor and mixed with a 30% sucrose solution that was added to the ground pollen in a 2:1 pollen to sucrose solution ratio. The newly laid eggs were then transferred onto these new pollen provisions in the laboratory.

Parameters that were assessed included the timing and completion of larval development, the number of days between the egg stage and the beginning of each larval stage, and the start of cocoon formation and its completion, including darkening. Time was also recorded from the date of first observation (egg stage) to the date each larva finished spinning a thin white cocoon around itself, and to the date the darkened cocoon was completed. Confirmation of the concentrations of the final pollen provisions were performed by Bayer CropScience and yielded levels for the low, medium, and high treatments of 2.7, 35, and 276 ppb, respectively for imidacloprid.

For the first method (using the bees' own pollen provisions), there was no significant effect on all endpoints assessed as compared to the control ($p>0.05$) with the exception of decreased time for females to finish darkening a cocoon at the 300 ppb concentration (effect not observed in females) and increased weight of male bees at all concentrations (effect not observed in females) after emergence from the cocoon. Similarly, there were no significant effects on any parameters assessed in the second method (using new pollen provisions) with the exception of decreased time in days for females to emerge from the cocoon in the 300 ppb treatment group (effect not observed in males).

Table D-1. Summary of results from Abbott et al, 2008.[1]

Response variable	3 ppb	30 ppb	300 ppb
Lab Component – Own Pollen			
Time to reach last larval stage (days)	NS	NS	NS
Time to spin a cocoon (days)	NS	NS	NS
Time to finish darkening a cocoon (days)	NS	NS	Males: NS Females: ↓
Time to emerge from cocoons (days)	NS	NS	NS
Weight of bees after emergence from cocoon (grams)	Males: ↑ Females: NS	Males: ↑ Females: NS	Males: ↑ Females: NS
Lab Component – New Pollen			
Time to reach last larval stage (days)	NS	NS	NS
Time to spin a cocoon (days)	NS	NS	NS
Time to finish darkening a cocoon (days)	NS	NS	NS
Time to emerge from cocoons (days)	NS	NS	Males: NS Females: ↓
Weight of bees after emergence from cocoon (grams)	NS	NS	NS
Field Component			
Time to reach last larval stage (days)	NS	Males: NS Females: ↑	Males: ↑ Females: NS
Time to spin a cocoon (days)	NS	Males: ↑ Females: NS	NS
Time to finish darkening a cocoon (days)	NS	Males: ↑ Females: ↑	Males: ↑ Females: NS
Time to emerge from cocoons (days)	NS	NS	NS
Weight of bees after emergence from cocoon (grams)	NS	NS	NS

[1] Means not presented. Arrow up or down denotes significant ($p<0.05$) increase or decrease from control, respectively; NS = not significant ($p>0.05$)

This study also had a field component (discussed here as it was conducted on individuals rather than on the colony), in which the same concentrations of imidacloprid used for the laboratory component were spiked into the bees own pollen provisions in the field and allowed to develop to adults in natural environmental conditions. There was a significant effect the time elapsed between the egg and last larval stage for both females and males. The time to reach the last larval stage for female bees in the 30 ppb treatment was longer than in the control and 3 ppb treatments, and those treated with 300 ppb took longer than the 3 ppb group. Males in the 300 ppb group took longer to reach the last larval stage than bees in the control and 3 ppb group. It is evident that a dose response is not clearly established with this endpoint, however. Similarly, males took significantly longer than control in the time to darken a cocoon at the 30 and 300 ppb level while females took longer at only the 30 ppb treatment. Despite the statistical significance of these findings, the dose response curve was relatively flat across treatments, particularly

for the males in time to finish darkening a cocoon. There was no significant effect ($p>0.05$) for either males or females in terms of the time until emergence or weight.

Generally, the study authors report that bees that were initiated earlier in the season had longer developmental times, and larger weights. There were no significant differences in survival until cocoon completion and until adulthood across treatments. It was stated that the study authors expected initiation date effects because fresh pollen was treated over a period of a few weeks, and the numbers of bees treated on those days were not equal. As the magnitude of effects for the statistically significant findings appeared to be small (although there were no percent effect changes reported in the study), there is uncertainty as to what the ecological significance of the observed effects in this study would have. It is noteworthy that the study authors considered the sub-lethal effects observed in this study as "negligible," but encouraged further testing of imidacloprid and clothianidin to examine mating success, reproductive output, foraging, and nesting ability before stating imidacloprid as safe to *Osmia lignaria.* Limitations for this study include small sample sizes for the laboratory component of this experiment as a result of difficulty in successfully rearing larvae for successful cocoon spinning, uncertainty in imidacloprid being evenly distributed throughout the spiked pollen provisions, and no chemical analysis conducted on the pollen used for this study collected from field pollen traps for other chemicals

Toxicity of Residues on Foliage

In a study by Mayer *et al.* (1991), the residual foliar toxicity to honey bees, alfalfa leaf cutting bees and alkali bees (*Nomia melanderi*) was investigated over 24 hours (MRID 42480503). The procedures used in the Mayer *et al*. study do not follow the protocol recommended by EPA (OCSPP 850.3030). The tests were conducted with imidacloprid TEP (FS 240, purity not specified) applied to first and second growth alfalfa at levels of 0.025, and 0.05, and 0.1 lbs. a.i/A. Residual test exposures were replicated 4 times by caging 30 - 40 worker honey bees, 20 - 25 alfalfa leaf cutting bees, or 20 – 25 alkali bees with each of four foliage samples per treatment and time interval. While there was mortality in all treatment groups for all species (ranging from 2-28% for honey bees, 9 – 18% for alfalfa leaf cutting bees, and 12 – 20% for alkali bees), there was no clear dose response apparent for any species. Imidacloprid residues aged for less than 8 hrs. resulted in less than 25% mortality, the study concluded that late evening applications, when bees have ceased foraging, are "probably safe." As this study was not conducted according to EPA guidelines, as well as the non-dose responsiveness of the data, this study is classified as supplemental.

Acute and Chronic Toxicity of the Degradation Products of Imidacloprid

Acute Toxicity

In a study by Schmuck et al (2003, MRID 47796304), which was previously discussed in the acute oral and contact results for *Apis*, honey bees were exposed to varying concentrations of IMI-olefin, IMI-5-OH, 4,5-OH imidacloprid, urea metabolite, and 6-chloronicotinic acid (6-CNA) (all with purities stated to be at least 98%). Although the study duration was not specified, it was assumed to be 72-hours as with the parent imidacloprid acute oral tests described in the study. As with the parent imidacloprid tests, the range of concentrations tested and the number of bees treated per concentration were not specified. The oral

LD_{50} values were determined to be >36, 159, >49, >99,500, and >121,500 ng a.i/bee for IMI-olefin, IMI-5-OH, 4,5-OH imidacloprid, urea metabolite and 6-CNA, respectively. Both IMI-olefin and 4,5-OH imidacloprid were within an order of magnitude of the toxicity of the most sensitive oral LD_{50} value for parent imidacloprid for this study (41 ng a.i/bee). It is worth noting that relative toxicities cannot be compared precisely without uncertainty as the LD_{50} values for olefin imidacloprid and 4,5-OH imidacloprid were non-definitive. The oral LD_{50} values for urea metabolite and 6-CNA were 3 and 4 orders of magnitude less toxic than parent imidacloprid, respectively (same uncertainty regarding comparing the relative toxicity values of non-definitive endpoints). Limitations for this component of the study include no mention of the level of control mortality, although it was stated that these tests followed guidance recommended in EPPO 170 (<15%).

In further work by Schmuck et al (2004, MRID 47800520), the chronic oral toxicity of two degradates of imidacloprid (urea metabolite and 6-CNA) were tested at similar levels as in Suchail et al, 2001 (discussed below). Similar to other studies by Schmuck, testing was conducted across several testing facilities in Europe.

In the previously discussed Suchail et al (2001), the acute (96-hour) and chronic (10-day) oral toxicity to honey bees (*Apis mellifera*) of the various degradation products was investigated alongside that of parent imidacloprid. For the acute tests, 3 replicates of 20 bees each were exposed to varying concentrations of IMI-olefin, IMI-5-OH, 4,5-OH imidacloprid, desnitro-imidacloprid, urea metabolite, and 6-CNA (all degradates at least 97% purity, only range of concentrations provided and not actual concentrations for each degradate). The 96-hour LD_{50} values for olefin imidacloprid, 5-OH imidacloprid, 4,5-OH imidacloprid, desnitro-imidacloprid, urea metabolite, and 6-CNA were 23, 222, >1000, >1000, >1000, and >1000, respectively. After exposure began, it was noted that early symptoms of neurotoxicity including hyper-responsiveness, hyperactivity, and trembling, were observed but it was not stated at which levels or for which degradates these observations occurred.

For chronic toxicity component, honey bees were continuously fed 50% (w/v) sucrose solution containing 0, 0.1,1, and 10 mg/L of each of the degradates tested in the acute component for 10 days. Three replicates of 30 bees each per treatment were dosed. Cumulative doses were calculated directly from sucrose solution consumption, the concentration of the products tested, and the density of the sucrose solution. While an endpoint was not calculated for this component of the study, it was stated that 50% mortality was reached at approximately 8 days. The study authors state that when considering the sucrose was consumed at the mean rate of 12 µL/day/bee, after an 8-day period the accumulated doses were approximately 0.01, 0.1, and 1 ng/bee. This would make all tested compounds toxic at doses 30 to 100,000 times lower than those required to produce the same effect in the acute toxicity studies. Limitations of this study include no measure of control mortality for the acute component, no analytical confirmation of the concentrations of the degradates of imidacloprid in the treatment concentrations, and as mentioned earlier, no statistically derived endpoint from the chronic component of this study.

Tier II

Apis

Single Oral Dose Studies (Nectar/Sucrose)

In a study by Bortolotti et al (2003; MRID 47800505), formulated imidacloprid (Confidor, percent active ingredient not reported) was spiked into sucrose at nominal concentrations of 100, 500, and 1000 ppb. One colony of honey bees (*Apis mellifera*) was exposed per treatment group. Additionally, there were two control groups (one colony each group) with one control serving for the 100 and 500 groups, and one control for the 1000 ppb group. Bees were trained to forage on an artificial feeder containing uncontaminated 50% sucrose solution. Once trained, the bees were allowed to forage on the feeders containing untreated (control) or imidacloprid-treated sucrose solution for one week during which observations were made of bee behavior and number of bees returning to the feeder and the hive entrance. Observation intervals were set at 0-2 hours after initial release, 4-5 hours after release, and 24-25 hours after release. It is noted that it was not stated the amount of time the bees were foraging on the sucrose solution.

The authors noted that the sucrose solutions containing 500 and 1000 ppb appeared to have a repellent effect on the honey bees, suggested by the bees in to these treatment groups not visiting the feeder. As a result, the authors could not collect 30 bees (the target number for each group with which to make foraging observations) from these groups. In the first trial, 30 control bees and 20 bees in the 1000 ppb group were collected. In the second trial, 29, 30, and 10 bees were collected from the control, 100 and 500 ppb groups, respectively.

During the first observation period (0 to 2 hours after release), 80 and 72% of the Control 1 and Control 2 bees, respectively, returned to the hive and 33 and 31% returned to the feeder, respectively. By contrast, 57% of the bees in the 100 ppb treatment group returned to the hive and only 3% returned to the feeder (insufficient numbers for the 500 and 1000 ppb groups). During the second observation period (4 to 5 hours after release), 87 and 79% of the Control 1 and 2 bees, respectively, returned to the hive and 77 and 76% returned to the feeder, respectively. Similar to the first observation period, 57% of 100 ppb treatment group bees returned to the hive and 7% returned to the feeder. In the last observation period (24 to 25 hours after release), 90 and 87% of the Control 1 and 2 bees, respectively, returned to the hive, while 84 % of 100 ppb treatment group bees returned to the hive and 73% had restarted foraging.

As noted previously, the 10 and 20 marked bees in the 500 and 1000 ppb groups, respectively, were not seen returning to the hive or the feeder in any of the observation time periods and therefore the target 30 bee sample for foraging observation was not achieved in these treatment groups. The study authors reported that marked bees in these treatment groups appeared to show symptoms of intoxication and often fell into the grass and their flight direction was not towards the hive.

Robust conclusions from this study cannot be reliably made because of substantial limitations in the study design. Results from this study are therefore only considered suggestive that 30 marked bees exposed to

100 ppb (nominal) imidacloprid as formulated product (Confidor®) dissolved in a 50% sucrose solution for an unspecified time experienced temporary delays in homing ability as well as temporary inhibition of foraging activity that is restored after 24 hours. Ten and 20 bees exposed to 500 and 1000 ppb groups, respectively were not observed returning to the hive or feeding at the sucrose station through 24 hours after the test. Other limitations to this study include: 1) lack of replication of treatment hives which precludes any statistical analysis of the results; 2) use of formulated product to which bees would have limited oral exposure; 3) unspecified duration of exposure of bees to the spiked sucrose solution; 4) lack of analytical confirmation of imidacloprid concentrations, 5) use of exceptionally high concentrations tested relative to the 48-h LD_{50} for imidacloprid TGAI (as low as 3.9 ng a.i/bee); 6) no information on the condition of the bees or hive prior to or after treatment; 8) small sample size (n = 10 to 30 bees per treatment); 9) no evaluation of the accuracy of the marking/observation methodology used; and 10) bees were only marked by color for the treatment groups instead of being individually tagged to track individual flight patterns.

In a study by Tan et al (2014), the authors tested the effects of field-realistic doses of imidacloprid on *Apis cerana* foraging behavior and decision-making with respect to food source danger, the hornet *V. velutina*, a native Asian predator and an emerging threat to *A. mellifera* in Europe. The trials were conducted from October to November 2013 which corresponds to when *V. velutina* actively hunts honey bees at the field site of Yunnan Agricultural University in Kunming, China.

Imidacloprid (purity not stated in the study) was spiked into sucrose at concentrations of 10, 20, and 40 ppb. The feeder was located 130 m from the colony and consisted of a 70 mL vial with an aerated lid, which the bees were trained to. After bees made 10 trips to the training feeder, it was replaced with an identical feeder containing one of four different treatment concentrations of imidacloprid. The bees were allowed to sample the treatment feeder and then it was recorded which bees subsequently returned to this feeder. A bee that made a single return within 1 hour was counted as a returning bee. The proportion of bees that continued to forage at the different imidacloprid concentrations was then calculated. In total, 360 bees were trained with 90 bees per treatment group (including the control group).

A second component of the study quantified the nectar brought back during foraging trips. After 10 training trips, the feeders were changed to those containing one of the four imidacloprid treatments. After the 10th visit, the bee was captured at the nest upon its return. Bees were anaesthetized, weighed, then gently squeezed by its abdomen and the nectar contents were collected onto tissue paper and immediately weighed again. The volume collected per bee was then calculated. In total, 84 bees were trained with 21 bees per treatment group (including the control group).

A third component of the study investigated predator avoidance to a hornet species known to prey upon the bees at the field site. Bees were trained as previously described and were exposed to imidacloprid once the training feeder was placed inside the foraging cage. Each forager was randomly assigned to one of treatment concentrations. One feeder was labelled ‘‘dangerous’’ and had a captured *V. velutina* hornet tethered 10 cm above the feeder with a stiff wire wrapped between the thorax and abdomen and the other was labelled ‘‘safe’’ with a similar wire, but with no hornet. Five successive choices of our trained bees were monitored. Thus, each bee was exposed over approximately 1.5 hours for 15 total visits to a

given treatment. After each visit, the feeders were replenished to eliminate potential odor marks and randomly swapped dangerous and safe feeder positions to avoid potential site bias. In total, 80 bees were trained for the predator avoidance experiment with 20 bees per treatment (including the control).

The results are presented below in **Table D-2**. The study authors reported that none of the foragers died from exposure to imidacloprid treatments during the experiments. The proportion of bees that returned to feeder was only significantly reduced from the control in the highest treatment group (40 ppb). The average volume of nectar collected was reduced in the middle and high treatment groups. Based upon the average nectar volume collected per trip, each bee collected (but did not necessarily absorb into its hemolymph) 0.27, 0.39, and 0.52 ng of imidacloprid per trip from solutions with imidacloprid concentrations of 10, 20, and 40 ppb, respectively. However, as noted below, these values likely do not represent the amount of pesticide that bees individually absorbed into their bodies per trip since the majority of this sugar solution was regurgitated to other bees and stored inside the nest. Bees avoiding the feeder with the hornet in the highest treatment group was reduced from the control and two lower treatment groups (not indicated by the study if the results were significant). It is noted that there did not appear to be a dose response for this variable.

While this study demonstrates that field relevant concentrations of imidacloprid in sucrose (nectar) can lead to a decreased proportion of bees returning to the feeder, it is unclear how these observations made on individual bees would translate to the colony level. Additionally, while the average volume of nectar brought back from the feeder was quantified, it is unclear of the true dose that the bees received as the majority of the sugar solution was stated to have been regurgitated. Finally, there was no clear dose response pattern for the predator avoidance component. It is also unknown at whether the time that the trials were conducted (October – November) had an impact on the results as western honey bees (*Apis mellifera*) are typically preparing for overwintering during this time. Despite the study being conducted with *Apis cerana* (not present in North America), it is unclear if these bees follow a similar life history.

Table D-2. Summary of results from Tan et al 2014.

Response Variable	Effect (treatment group)[1]	Comments
Proportion of bees that returned to feeder	**↓ 23% (40 ppb)**	
Average volume of nectar collected	**↓ 46% (20 ppb); ↓63% (40 ppb)**	Bees were anaesthetized, weighed, then gently squeezed by its abdomen and the nectar contents were collected onto tissue paper and immediately weighed again.
Predator avoidance	- 85, 80, and 85% of the bees in the control, 10, 20 ppb groups, respectively chose the feeder without the hornet. - 65% in the 40 ppb chose the feeder without the hornet	

[1]Means were not provided in the study, only the percent increase or decrease and whether or not this effect as significant at $\alpha = 0.05$ (shown in **bold**).

Limitations to this study include 1) no information on the purity of imidacloprid, and 2) and the study authors state that the overall exposure to imidacloprid at the hive was “minimal,” due to the observance of the majority of the sugar solution being regurgitated.

Schneider et al (2012) investigated the foraging activity of honey bees (*Apis mellifera carnica)* by employed radiofrequency identification (RFID) technology to monitor the homing ability of bees as well as the duration of foraging trips and interval between trips within the hives. The experiments were conducted during the summer of 2009 and 2010 at a research facility in Oberursel, Germany. Each trial included several training steps, individual pesticide treatment, and a subsequent observation period of up to 48 hours.

Bees were labeled with RFID tags by gluing the transponder to the bees dorsal part of their thorax. Each RFID contains a unique ID number and were used to retrieve the exact date and time to register when bees entered and left the hive or feeder. For tracking bee movements, specifically designed scanners were positioned in front of the hive entrance. The same design system was used with the feeder. To train the bees, the study authors first let the bees forage from the feeder freely, then they placed the feeder inside the feeder compartment for approx. 30 minutes. A lid was next placed over the compartment which required the bees to enter through the opening.

To create dosing solutions, powered imidacloprid (analytical standard, personal communication 01/29/15), was spiked into sucrose at nominal doses (per 10 µL) of 0.15, 1.5, 3 and 6 ng a.i/bee. Controls were fed with unspiked sucrose solution containing acetone; acetone percentage did not surpass 0.01% (v/v). The study consisted of 2 individual trials conducted consecutively. There were 12 individual bees per treatment group per trial.

Results are summarized in **Table D-3** by foraging behavior endpoint and time at which that endpoint was assessed for imidacloprid. Bees that returned to the hives after post-treatment release at the feeder was 100% for the control, 0.15 ng/bee, and 1.5 ng/bee treatments and 95% with 3 ng/bee treatment. However, only 25% of the bees returned to the hives after post-treatment release at the feeder for the 6 ng/bee treatment. Bees treated with 3 ng and 6 ng that were not directly flying to the hive exhibited reduced mobility, followed by a phase of motionless with occasional trembling and cleaning movements. There were generally no significant effects observed at the lowest dose, that is, 0.15 ng imidacloprid. Significant ($p<0.05$) increases in the time duration of foraging trip, time to feeder, time at feeder, time to hive, and the interval inside the hive between trips were observed at the intermediate treatments of imidacloprid (1.5 and 3 ng). These effects generally occurred within 3 hours. For the median number of feeder visits for bees treated with 1.5 ng and 3 ng a.i/bee reduced to 47% ng and 98% ($p<0.001$ for both treatment groups) compared to the control groups. Bees treated with 6 ng, which had re-entered the hive, did not stop by the feeder until 24-hr after treatment.

Table D-3. Summary of results from Schneider 2012.

Response Variable	Time point	Effect (treatment group – ng a.i/bee)[1,2]
Number of feeder visits	Immediately after treatment	**↓47% (1.5), ↓98% (3)**
	24 hours after treatment	NS
	48 hours after treatment	NS
Foraging trip (sec)	Immediately after treatment	**↑50% (1.5), ↑130% (3)**
	24 hours after treatment	NS
	48 hours after treatment	NA
Time to feeder (sec)	Immediately after treatment	**↑65 (1.5), ↑241 (3)**

Response Variable	Time point	Effect (treatment group – ng a.i/bee)[1,2]
	24 hours after treatment	**↑ (% effect not provided) (1.5)**
	48 hours after treatment	NS
Time at feeder (sec)	Immediately after treatment	**↑28% (1.5), ↑46% (3)**
	24 hours after treatment	NS
	48 hours after treatment	NA
Time to hive (sec)	Immediately after treatment	**↑20% (1.5), ↑210% (3)**
	24 hours after treatment	NS
	48 hours after treatment	NA
Interval between foraging trips (sec)	Immediately after treatment	**↑33% (1.5), ↑993% (3)**
	24 hours after treatment	**↑ (6) (% effect not provided)**
Time inside the hive (sec)	Immediately after treatment	**1st stay: ↑972% (3)** **2nd stay: ↑33% (1.5), ↑1077% (3)**

NS = not significantly different from control (p>0.05); NA = Not assessed

[1]Means were not provided in the study, only the percent increase or decrease and whether or not this effect as significant at α = 0.05 (shown in **bold**).

[2]No bees treated with 6 ng imidacloprid returned in the first three hours of observation after treatment

The results of this study indicate that at doses as low as 1.5 ng a.i/bee, that honey bees exhibit a reduced number of visits to a training feeder, increase length of foraging trip, time to feeder, time at feeder, time back to hive, and interval between trips. Bees in the highest treatment (6 ng a.i/bee) were not observed returning to the hive or feeder within the first 3 hours of observation after dosing. It is noted that each trial conducted exposed only 12 bees per treatment group. Additionally, it is unknown the impact that these findings would have on other colony health parameters such as numbers of different life stages and food stores. Finally, it is noted that the effects observed in this study are not observed 24 and 48 hours of treatment suggesting that the bees are able to recover from these effects under the conditions of this study. Additional limitations to this study include: 1) high variability for certain endpoints observed, likely originating from the low number of trials (replicates) and bee per treatment group for each trial and 2) there was no analytical confirmation of the pesticides residues in the sugar solutions.

In a study by Eiri et al (2012), the short term effect on honey bee sucrose response (defined by the study as the lowest sucrose concentration that will elicit a proboscis extension response) and longer term effects on imidacloprid metabolites on honey bee foraging preferences and waggle dancing were investigated. Bees were determined to be nectar or pollen foragers depending on whether they brought back only a nectar or pollen load to the colony before initiation of exposure. The sucrose response component of the study was measured 1 hour after consuming a dose of 7 µL of control, low (0.21 ng/bee or 24 ppb) or high (2.16 ng/bee or 241 ppb) treatment concentrations of imidacloprid (analytical standard) suspended in pure sucrose solution. The test was conducted by presenting the antennae of 7-15 bees per trial for 3 seconds with a series of increasing sucrose concentrations of 0%, 0.1%, 0.3%, 1%, 3%, 10%, 30% and 50% (w/w) prepared with analytical grade sucrose. Each bee was tested with each sucrose concentration presented 2 minutes apart in an ascending concentration series. A trial consisted of a 2-day process capturing bees and feeding it 7 µL of control (pure sucrose) or treatment (24 ppb a.i imidacloprid in pure sucrose) solution on day 1. After a 1-hour incubation, bees were released back to the hive, and on day 2 each bee was observed for the number of visits made to a decreasing series of sucrose concentrations (50%, 30%, 10% and 3% w/w) where each concentration was available for 25 minutes. Each returning

forager was also measured for unloading wait time (which the authors identified as influencing dancing) and number of dance circuits.

Table D-4 below summarizes the results of both components of the study. The total PER/bee was reduced ($p<0.05$) at the high concentration for both pollen and nectar foragers relative to control while only the nectar foragers were reduced from control at the low treatment group. For the sucrose response threshold (defined previously) there was a significant increase in the sucrose concentration relative to control to elicit a PER in both pollen and nectar foragers in the high treatment concentration, as well as for nectar foragers in the low treatment group. When all foragers were examined for the frequency of waggle dance circuits within the hive there was a significant reduction in the frequency of waggle dances observed receiving the 50% sucrose solutions. It is noted that the control group also had a decreased frequency of waggle dances observed when receiving the 30% solution as compared to the 50%.

Table D-4. Summary of results of Eiri and Nieh 2012.[1]

Response variable	Forager type	Treatment groups (means presented with difference as compared to the control)		
		Control	24 ppb (0.21 ng a.i/bee)	241 ppb (2.16 ng a.i/bee)
Single oral dose with observations 1 hour later				
Total PER/bee	Nectar	4.1	**3.0 (↓27%)**	**2.6 (↓37%)**
	Pollen	4.8	4.7 (↓2%)	**3.0 (↓38%)**
Sucrose response threshold (lowest sucrose percentage that will elicit a PER)	Nectar	10.6	**18.9 (↑78%)**	**19.2 (↑81%)**
	Pollen	5.9	5.7 (↓3.4%)	**18.1 (↑206%)**
Single oral dose with observations 24 hours later				
Waggle dancing behavior	All foragers	**↓ waggle dances after receiving 50% sucrose solution**		

[1]All effects listed significant at $\alpha = 0.05$ (shown in bold).

While there are uncertainties (described below) regarding the extent to which changes in sucrose responsiveness over a range of sucrose concentration thresholds or a decrease in the frequency of honey bees' dancing have on the colony as a whole, the results of this study suggest the potential that imidacloprid exposure (under the conditions tested) may have on individual bee foraging ability which could t honey bee colonies under natural foraging conditions where bees may not feed exclusively on pollen/nectar containing similar residue levels. The effects on sucrose response seen in the high imidacloprid treatment (240 ppb a.i) may not be environmentally relevant to all use patterns, particular seed treatment and soil treatment applications. There is potential, however, that some use patterns may result in pollen and nectar loads similar to those tested in the foraging experiment and the low treatment concentration in the sucrose response component (24 ppb a.i).

Limitations in this study include: 1) unclear origin of the pollen in this study and whether it was screened for other pesticides, 2) analytical verification of the treatment solutions to confirm imidacloprid presence was not conducted, 3) it was unclear as to whether the entire dose was consumed, 4) there was no report of any tests for statistical significance nor any statistical measures of central tendency for the effect of treatment and sucrose concentration on PER response, 5) the mean % sucrose response was reported to be highly variable with coefficients of variation of 143% and 224% based on measures of central tendency for control nectar and pollen foragers, respectively 6) the result of the effects described (a 10-15%

difference in sucrose concentrations in sucrose responsiveness threshold for pollen and nectar foragers and a 10.5x and 4.5x reduction in dance circuits for treated bees feeding on 50% and 30% sucrose solution, respectively) on overall colony condition is uncerta.in, 7) despite a 10x difference in dosage (24 ppb and 240 ppb) there was no dose-response relationship in mean sucrose response/bee as for nectar foragers the two treatment concentrations elicited a similar response while for pollen foragers the low treatment was similar to the control while the high treatment resulted in a 15% sucrose increase in mean sucrose response/bee. Similarly, no dose-response relationship was observed for the mean total PER/bee endpoint for both nectar or pollen foragers, and 8) although the authors state that the objective of the study was to examine the longer-term effects of imidacloprid metabolites, residues are not identified and/or measured.

Feeding Study Design (Spiked Sucrose)

In a study by Faucon et al (2005), the effects of imidacloprid on honey bee (*Apis mellifera mellifera*) colony growth, survival, and reproduction were investigated. Two groups of eight colonies were fed with two either 0.5 or 5 ppb imidacloprid (purity not reported) in sucrose syrup located in the hive for a 4 week period during mid-July to Mid-August. There were two control groups (also with each colonies each) consisting of both an unspiked syrup group and an un-supplemented control group that had to rely exclusively on outside sources of forage. The effects on all control and treated hives were monitored until the end of the following winter. The study evaluated adult bee population, capped brood area, frequency of parasitic and other diseases (acarapisosis, nosemosis, varroosis, American foulbrood, European foulbrood, chalkbrood and chronic bee paralysis (CBPV), mortality, number of frames with brood after overwintering, and a global score of health of colonies after overwintering.

The exposure phase started on 12 July, 2000 with each colony initially provided 1 litre of syrup. Feeding was repeated three times per week until 14 August (3 days of exposure), making for a total of 13 distributions (total volume = 13 litres) of syrup per colony. Colonies were visited on 11 and 24 July, 7 and 21 August, 4 September, 25 October 2000 and 21 March 2001 for assessment of overall health. Brood quality, presence of eggs, queen cells and any specific symptoms of diseases were noted during these periods. From 13 July, 2000 to 1 September, 2000, mortality were assessed daily except for weekends. Colonies were weighed early in the morning, before the departure of foragers, using an electronic balance (accuracy = ±0.05 kg). Weighing was done every week from 12 July, 2000 to 2 October, 2000. From October, as the bee activity decreased in preparing for overwintering, the frequency of weighing was decreased. The last weighing was performed on the 16 February, 2001.

Additionally, *Varroa destructor* was preventively managed using amitraz (Apivar®) from 21 March to 29 May, 2000, roughly 6 weeks prior to the exposure phase. Following the observation of bees with deformed wings in front of several hives, the same treatment was repeated from 22 August to 6 November, 2000 (initiated one week after the exposure phase ended). *Varroa* mites were collected in all groups, every 1-3 days during the three-week period that followed the amitraz treatment of 22 August. Coumaphos (Perizin®) was applied on the 7 November, 2000 as the last treatment before winter. Fumagillin (Fumidil B®), an antibiotic recommended for controlling *Nosema apis*, was applied on the 3, 9 and 22 January 2001.

The weather was reported to be typical of a Mediterranean climate (study conducted in southern France) with a dry hot summer and a cool and wet winter. From May to August 2000 monthly mean temperatures ranged from 19.00 to 24.15 °C (66 – 75.5 °F) and rainfall was reported to be scarce. The temperature began to decrease in September 2000: the lowest mean temperature during the period was 9° C (48.2°F) in January 2001. The apiary was closely surrounded by typical Mediterranean forest (*Quercus suber)* and meadows. In addition to Mediterranean oaks, sources of pollen, nectar and honeydew were from a large variety of wild plants such as *Dorycnium pentaphyllum*, sages (*Salvia*), thyme (*Thymus*), rosemary (*Rosmarinus officinalis)*, heather (*Erica arborea*), and arbutus (*Arbutus unedo*).

Table D-5 summarizes the results of this study. Mortality, as well as adult bee population, egg laying, and activity index (defined as bees per minute entering the hive) were not significantly different ($p>0.05$) from either control (fed and unfed) group during any time interval examined during the course of the study that included pre-feeding, during feeding, post feeding, and post overwintering. In contrast, the mean capped brood area was significantly increased over the level of the fed control group and significantly decreased from the level of the fed control group ($p<0.05$ for both findings) at the post overwintering interval only. Mean hive weight for both treatment groups was also significantly increased over the level of unfed control group but not the fed control group during the feeding period but was reduced from the unfed control group after the overwintering period. While there was a significant reduction in the mean capped brood area in the high treatment group after the overwintering period, it is uncertain the extent of the impact on the colony as a whole given the lack of effects of other response variables including mortality, egg laying, adult bee population, and activity indexat this treatment group during and after the feeding period as well as after overwintering.

Table D-5. Summary of results of Faucon et al 2005

Response variable	Control (unfed)	Control (sucrose)	0.5 µg a.i/L	5 µg a.i/L	Comments
Activity index (bees per min entering the hive)	NS				Inclusive of all time intervals, means not presented in the study
Mean capped brood area (number of frames)	6.6	7.0	**8.0 (↑ compared to sucrose control)**	**4.6 (↓ compared to sucrose control)**	- Results refer to after the overwintering period (March 2001) - Not clear from study whether comparisons are significant compared to the unfed control
Mean hive weight (kg)	37.3	37.1	41.6	40.2	- Refers to period when colonies were established (July 2000) - Means not presented for any other time point although the syrup groups (treatment and control) were always significantly ($p<0.05$) heavier than the unfed group but not different from each other ($p>0.05$)
Adult bee population	NS				Inclusive of all time intervals, means not presented in the study
Mortality	- Daily means of dead bees were 3.1, 4.4, 4.3 and 3.3 bees found dead per colony during the feeding period and then 1.1, 1.6, 1.8 and 0.2				Mortality rates determined to be low by the study authors, results not subjected to statistical analysis

Response variable	Control (unfed)	Control (sucrose)	0.5 µg a.i/L	5 µg a.i/L	Comments
	after the feeding period for the unfed control, fed control, 0.5, and 5 ppb groups, respectively.				
Egg laying	During and after the feeding period, the number of colonies that were reported as "surely or probably swarmed" was 2, 3, 1 and 0 in the unfed control, fed control, 0.5, and 5 ppb groups, respectively. The difference was not significant between groups (p>0.05)				Mean totals of actual eggs laid not available from the study

[1]All effects listed significant at α = 0.05 (shown in bold), NS = not significantly different from control (p>0.05)

After the exposure period, four colonies of the unfed control group were infested with *Nosema* (spore levels not provided), and among the syrup-fed groups, colonies of 0.5 ppb group were the less frequently and the less severely infested compared to other groups. Despite the application of amitraz in spring, typical symptoms of *Varroa* infestation (bees with atrophied wings) were seen in front of colonies of all groups (control and treatment) during the course of the study. The total number of *Varroa* mites collected after the feeding period ranged between 3 and 1244 per colony with a geometric mean of 51.4. No significant difference was determined between groups.

The primary limitation to this study concerns the analytical results of the test solutions. Chemical analysis at two different facilities using two analytical methods assessed the presence of imidacloprid and its metabolite 6-CNA in the sugar solution, dead bees, and honey stores. Imidacloprid was quantified in the sugar solution just after preparation and later in the honey stores in the 5 ppb group but there were either trace or no detects for all samples from 0.5 ppb group. The limit of detection and quantification for imidacloprid was 3 and 8 ppb, respectively which raises uncertainty as to what extent the bees were exposed despite being observed to feed on the sucrose solution. Analysis of the 5 ppb sucrose solution just after preparation vs 24 hours later at ambient temperature yielded 4.65 ppb and "not detected," results, respectively. As noted previously, the honey bees were observed feeding on the sucrose solution and this was also confirmed by hive weight measurements throughout the course of the study.

Feeding Study Design (Spiked Pollen)

Feeding Design Studies (Pollen)

In a study by Dively et al (2009) that was submitted by Bayer Crop Science (MRID 47775502), honey bee colonies were exposed to imidacloprid at concentrations of 5 and 20 ppb spiked into pollen cakes provided in the hive, in addition to a control group. Multiple measures of colony health were assessed over the course of the nearly 12-week exposure period (15 May, 2008 – 06 August 2008).

Packages of 900g of bees were obtained from a commercial supplier on April 4, 2008. Sister queens were also obtained from the same breeding line. The authors established 30 colonies in nucleus hive boxes containing five foundation frames with all hive equipment being new. The experimental design consisted of ten replicate colonies assigned to each of three treatment groups: negative control, low treatment group (5 ppb), and a high treatment group (20 ppb) of imidacloprid.

Colonies were located at the University of Maryland research farm at Beltsville, MD. At the start of the experiment, all hives were managed in one apiary and fed sugar syrup and a pollen substitute to allow colonies to build up before they were assigned to treatment groups. On May 15, the study authors inspected the colonies and equalized the strength of the colonies if necessary and colonies were randomly assigned to treatment groups and relocated to five isolated apiaries on the research farm. Each apiary consisted of six colonies representing two replicates of each treatment. Individual hives were placed on wood platforms spaced 3m apart in each apiary (total of 10 replicate colonies per treatment group).

The colonies were allowed to freely forage with which each colony was provided MegaBee diet either untreated or spiked with imidacloprid. Stock solutions of imidacloprid (Admire Pro) were prepared and diluted in distilled water. The final concentration for each treatment dose was achieved by adding an appropriate concentrated solution of imidacloprid to heavy sucrose syrup, which was then added to the MegaBee powder in a 1.7:1 diet to sucrose solution ratio. The pollen patties were formed into 80g, moist dough patties. Three or four patties were placed weekly on the top bars of frames inside each colony to allow the bees *ad libitum* access to the pollen substitute. At each diet placement, the authors removed and weighed remaining portions of the old cakes in order to keep track of the cumulative weight of cakes consumed by each colony. Pollen traps were placed at the entrance of each hive in order to attempt to force the bees to consume the maximum amount of artificial pollen diet.

During the exposure period, the study authors inspected the colonies on a weekly basis to visually estimate the percentage of each frame covered with capped brood, food stores, and bees. The status of the queen was assessed during each inspection, and whether eggs and successive stages of larvae were present. To prevent overcrowding and swarming, frames with stored food and brood were removed and new foundation frames were added to make room for brood expansion. Queen cells were also removed from combs to prevent swarming. Foraging success was recorded by weighing the pollen collected each week in the entrance traps, and on the number of foraging bees returning with and without pollen pellets. Foraging counts at the hive entrance were recorded over a 5-minute period in the morning between the hours of 9 a.m. and 11a.m.

Exposure to imidacloprid ended on August 6, and the pollen traps were removed. Although the study authors refer to a 9-week exposure period, 83 days elapsed between 15 May and 6 August 2008, representing nearly 12 weeks. Additional data on foraging behavior was recorded by marking bees in selected colonies and recording their first appearance and relative numbers at nectar stations at varying distances from the apiary. During early evening while bees were returning to the hives, an extended entrance chamber was used to collect 300 foragers from each colony. Only one colony from each treatment group was marked in each apiary. The bees from each marked colony were given a unique color painted in the dorsal thorax.

Nectar stations (2-5 per apiary) with large sponges saturated with diluted honey water were established at various distances (200 – 500m) from the apiary. The study authors then recorded the number of visiting bees marked with each color at each nectar station every 15 minutes. Counts of marked and unmarked foragers at each hive entrance were also recorded over a 5-minute period every 30 minutes during the day. The study authors used the number and relative frequency of marked bees visiting nectar stations

over time and the number of marked bees drifting to other colonies as measures of orientation behavior of foragers and their ability to communicate to other bees.

Samples of bees and bee bread were collected from each colony and sent to the USDA National Science Laboratory at Gastonia, NC, for a multi-residue analysis to detect imidacloprid and its metabolites. Also collected were 1-week old samples of the patties of each treatment. Finally, during late August, the authors transferred the colony and combs of each nucleus hive to a full hive box with an additional five foundation frames added. On October 15, an in-depth assessment of colony health was performed by examining each frame to estimate brood development, honey and pollen stores, and queen status. Colonies were then overwintered in the apiaries and observations of survival were recorded the following spring.

As this study was extensive in scope in the numbers of response variables examined, the following narrative summarizes the results (as well as in **Table D-6**)

Residue Analysis: Residue analysis in the provided pollen patties indicated that the mean level of imidacloprid (±SE) was 0.0, 8.73 ± 1.73, and 15.7 ± 1.45 ppb in patties from the control, 5, and 20 ppb groups, respectively. Although the authors specify that "two major metabolites" were analyzed, no other information is presented on the identity or concentration of these metabolites. At one week after the final exposure, residue analysis of worker bees determined mean (±SE) imidacloprid levels of 0.6 ± 0.31, 1.58 ± 0.63, and 3.67 ± 1.48 ppb in the control, 5, and 20 ppb groups, respectively, which was significantly different among the groups (ANOVA, $p = 0.03$). Similar levels were found in bee bread averaging 0.2 ± 0.22, 1.62 ± 0.68, and 3.49 ± 1.55 ppb collected from control, 5, and 20 ppb respectively. These results demonstrate that exposure of bees to imidacloprid occurred and followed a dose-dependent pattern. Contamination of control bees and bee bread was suggested by the authors to be to bees drifting among hives given their similar proximity at each apiary.

Disease Incidence: All colonies appeared to be relatively free of diseases and pests during the exposure period, except for one colony in the control group on July 9, and one colony in the high treatment group on July 31, both of which experienced chalkbrood. Later in the summer and early fall, however, chalkbrood and infected mummies were found on the bottom board in three control, two in the 5 ppb group, and four in the 20 ppb treatment group colonies. Small hive beetle and larvae were also detected in six hives (4 controls, one colony in each of the 5 and 20 ppb groups).

Overwintering Success: The bees were not provided with supplemental sucrose solution after the spring honey flow. As a result, all colonies depleted their honey supply by the end of August, and fall feeding of colonies after they were transferred to full hive boxes did not adequately build up bee populations and honey stores to ensure overwintering success. During February, live bees in the clusters of 14 colonies were found but out of 30 colonies, only 1 survived overwintering. The study authors indicated however, that this study was not designed to evaluate overwintering success.

General Observations of Colony Health and Queen Failure: Due to limited space in the colonies, the nucleus box hive populations became congested, particularly during early June. Heavy brood combs were

removed and replaced with foundation frames in 26 of the 30 colonies. An average of 1.4, 1.6, and 1.4 combs per colony were removed from the control, low, and high treatment groups, respectively. Queen cells were removed from 18 colonies. No clear treatment differences were found in the number of brood combs or queen cells removed. Queen failure was relatively high due to the aforementioned congested hive conditions following the spring honey flow. Nine of the 30 colonies lost queens during early June but were replaced within a week to minimize the effect on brood rearing. Another six colonies lost queens during late July and early August but quickly superseded new queens and experienced only a moderate break in brood rearing. Altogether, the control group had queen failure in 5 colonies (50%), 7 in the low treatment colonies (70%), and 3 in the high treatment colonies (30%) suggesting no relationship between queen failure and imidacloprid exposure.

Diet Consumption: Mean daily consumption of the MegaBee patties was not different among the treatment groups ($p>0.05$).

Brood Development: There were no consistent treatment effects on egg-laying activity, or larval development (as indicated by the percentage of comb area occupied with capped brood cells). The overall mean percentage of comb area occupied with capped brood cells was not significant different among the groups ($p>0.05$). It is not clear the extent to which removal of brood and food frames during the exposure period impacted the interpretation of the results regarding brood development. Additionally, it is not known what fraction of brood frames were removed from each colony; just the average number of brood frames. On the final assessment date of October 15 (6 weeks after colonies were transferred to full hive boxes), there were no significant differences in the amount of brood rearing, food stores, and bee strength between imidacloprid colonies and control colonies ($p>0.05$).

Foraging measurements: There was no significant effect on the weight of pollen collected at the entrance traps for the treatment groups as compared to the control ($p>0.05$). Foraging activity was measured by counting the total number of foragers returning to each hive and the percentage of bees loaded with pollen pellets. Overall, there were no significant differences across the control and treatment groups in the mean counts of foragers returning to the hives ($p>0.05$) as well as the percentages of bees loaded with pollen pellets returning to the hive. Additionally, there was a statistically significant reduction ($p<0.05$) in visits of marked bees to the apiary nectar stations in the 5 ppb group but not in the 20 ppb group. Since the number of marked bees represented a very small percentage of the total number of foragers (ranged from 1.8 – 4.7%), the study authors suggest that there was likely bias toward stronger colonies in terms of locating nectar stations quicker and then sending out more foragers (including marked ones) to the source.

Table D-6. Summary of results from Dively 2009.[1]

Response Variable	Control (mean ± SE)	5 ppb (mean ± SE)	20 ppb (mean ± SE)
Queen failure (numbers of affected colonies, percentage of total)	5 (50%)	7 (70%)	3 (30%)
Mean daily consumption during exposure period of 15 May, 2008 – 06 August 6, 2008 (grams)	29.0 ± 0.84	29.3 ± 0.78	31.1 ± 0.85 (↑7.24%)

Response Variable	Control (mean ± SE)	5 ppb (mean ± SE)	20 ppb (mean ± SE)
Mean percentage of comb area with capped brood cells	25.9 ± 1.22	24.7 ± 1.50	29.5 ± 1.36 (↑13.9%)
Mean percentage of frames covered with bees	30.5 ± 4.07	28.0 ± 4.96 (↓8.20%)	30.6 ± 4.95
Mean percentage of frames covered with capped brood	7.2 ± 1.36	6.5 ± 1.04 (↓9.72%)	8.0 ± 1.30 (↑11.0%)
Mean percentage of frames covered with honey cells	18.4 ± 4.61	18.2 ± 3.37	17.9 ± 3.61
Mean percentage of cells with pollen cells	4.2 ± 0.60	4.9 ± 1.16	4.2 ± 0.80
Pollen collection (grams per day)	19.0 ± 1.33	21.3 ± 1.70 (↑12.1%)	23.6 ± 1.94 (↑24.2%)
Overall mean counts of foragers per 5 minutes returning to hive (11 June, 2008 – 14 October, 2008)	121.6 ± 5.80	124.6 ± 7.42	135.4 ± 7.04 (↑11.3%)
Percentage of bees loaded with pollen pellets returning to hive	25.9 ± 1.53	23.2 ± 1.36	25.0 ± 1.50
Relative frequency of marked bees visiting nectar stations in each apiary	36.0 ± 4.24	**23.3 ± 4.72 (↓35.7%)**	40.7 ± 4.36 (↑13.1%)

[1]All effects listed significant at $\alpha = 0.05$ (shown in bold).

This study examined the effects of exposure of honey bee colonies fed imidacloprid-spiked pollen on endpoints related to colony health and foraging success. This study was robust in several elements of its experimental design including g usage of replicate colonies within treatment groups and randomization, use of sister queens for establishing colonies, sampling of in hive residues to confirm imidacloprid exposure, and monitoring of the hives for pathogens and parasites. Overall, the results indicate no clear or consistent treatment-related effects on overall colony health, brood development, food consumption, or foraging measurements from exposure to pollen cake patties spiked with 5 and 20 ppb during the 12 week exposure and subsequent 10 week observation period. Assessing potential impacts of imidacloprid exposure on overwintering success of the colonies is not possible with this study as it was indicated by the authors that no supplemental feeding of nectar or sucrose solution occurred after the spring honey flow as well as well as the crowding conditions which led to over half of the colonies in this study to produce queen cells. There are some uncertainties that may affect interpretation of the results including the high rate of queen loss (and replacement), frequent disturbance of hives for weekly brood examination, and late summer incidences of chalkbrood and small hive beetle.

Other limitations to this study include: 1) this study examined only the pollen route of exposure which is not the primary food source of foraging bees, 2) there was no information on the mention of other crops grown in the area of the study or the quantification of other pesticide residues in the hive residue samples,

3) there may have been confounding effects of queen replacement, food/brood frame removal to address the overcrowding issue and weekly examination of the combs for brood assessment, 4) foraging measurements were taken by way of visual counts which may have been less accurate than RFID technology, and 5) this study used formulated imidacloprid to spike into pollen patties which would also contain other ingredients like surfactants and solvent, for which the extent these translocate to nectar and pollen is unknown.

In a study by Dively et al, 2015, three trials were conducted, one each in 2009, 2010, and 2011. These trials were conducted as a follow on to the Dively 2009 work, with an additional treatment group added (100 ppb) as well as supplemental feeding in the fall to allow for buildup to assess overwintering survival.

2009 Trial

Colonies were established in early April 2009 with starter packages of 900 g (approximately 6,900) of bees obtained from a commercial supplier (Wilbanks Apiaries; Claxton, GA, USA) from laying sister queens. The mean number of colonies in May appeared to be approximately 15,000 bees per colony based on visual inspection. The colonies were also equalized at this time to ensure similar colony strength prior to exposure. The colonies were subsequently randomly assigned to treatment groups and relocated to isolated apiaries on the research farm with hives spaced 10 m apart in each apiary. Colonies were located on the University of Maryland research farm at Beltsville, MD. Crops within the foraging range of about 3 km of the apiaries were exclusively field corn, soybean, and small grains. None of these crops were treated with imidacloprid. A portion of the corn acreage was seed-treated with other neonicotinoids.[2] There were 5 apiary locations, where each location had 2 replicate colonies of each treatment group (control, 5, 20 and 100 µg/kg). There were 10 replicate colonies for each treatment tested for a total of 40 colonies.

The exposure phase began in mid-May 2009 and continued for 12 weeks ending in August. Bees were allowed to freely forage but were also provisioned with a pollen diet substitute either untreated or spiked with imidacloprid (at 5, 20, or 100 µg/kg). Four 80 g diet patties were placed weekly on the top bars of frames inside each colony and at each diet placement; with remaining portions of the old patties removed and weighed to assess the cumulative weight of diet consumed per colony. Pollen traps were installed at the entrance of to ensure the bees fed on the provided spiked pollen within the hive. After 7 days, samples of fresh patties of each treatment group were analyzed for imidacloprid residues. To confirm exposure within colonies, samples of hive bees and bee bread were also analyzed for residues.

The colonies were sampled biweekly by visual estimate for the following parameters: percentage of the frame area covered with drawn cells (defined by study as an empty cells that had been cleaned out by worker bees), adult bees, capped brood (noted by the study author to be the sum of capped brood and older, uncapped larvae), cells packed with beebread, and capped honey. Additionally, the presence and egg laying status of the queen was established during each inspection by either directly observing her, or

[2] Communication with study author (phone – 04/20/15) indicated that either Poncho 250 (clothianidin - 0.25 mg a.i/kernel) or Cruiser 5FS (thiamethoxam - 0.25 mg a.i/kernel) was applied to a very small proportion of the predicted foraging area.

freshly laid eggs. Other observations included any unusual presence of drone cells, dead larvae, abnormal behavior of workers, abnormal brood pattern, and signs of disease or pest presence. To measure foraging activity, data was recorded twice weekly on the weight of pollen collected in the entrance traps, and biweekly on the number of foraging bees returning with and without pollen loads.

In early October, a detailed assessment of colony health was conducted that included an estimation of bee strength, brood development, food stores, and queen status. Additional inspections were conducted in January, February and March, 2010 to assess food stores and overwintering survival. Additionally, another assessment of overwintering survival occurred in May 2010 that included checking for the presence of disease (described in the next paragraph). Eleven queen events (defined as either manual replacement or natural supersedure) occurred at different times during the course of the study across the control and all treatment groups.

From each surviving colony, 100 bees were collected and placed in 70% alcohol, then submitted to the USDA-ARS Bee Research Laboratory at Beltsville, MD, where they were examined for *Varroa* mites, tracheal mites, and *Nosema* presence. Study bees were collected in early November and then again after overwintering in May 2010. Samples of bees and other hive matrices from all experiments were analyzed for residue levels of imidacloprid and its major metabolites (IMI-olefin, IMI-5-OH, 6-CNA, urea metabolite, desnitro IMI-olefin, desnitro imidacloprid HCl). All samples were processed by the Analytical Chemistry Branch, Biological and Economic Analysis Division, Office of Pesticide Programs, U.S. EPA at Fort George G. Meade. For all matrices, the limit of detection was 0.2 for imidacloprid and 0.2–15 µg/kg for the metabolites. The limit of quantification was 0.6 µg/kg.

Results

Residue Analysis: There was no evidence of widespread control contamination with less than 5% of the control samples having trace amounts of imidacloprid. Mean residues in the patties removed after seven days were 0.0, 5.5, 19.8 and 97.5 µg/kg of imidacloprid from the control, 5, 20 and 100 µg/kg colonies, respectively. Consumption of patties varied significantly over the exposure period but were not different among treatment groups, and ranged from 265.3 to 277.2 g per colony. Each colony of the 5, 20 and 100 µg/kg treatment groups was exposed to an average cumulative dose of 16.6, 63.7 and 322.6 µg of imidacloprid, respectively. Where imidacloprid and its metabolites were detected in bee samples and bee bread following the exposure period (in early August), residues ranged from 0.3 to 2.8 µg/kg in bees and 0.4 to 1.6 µg/kg in beebread. Trace residues (<0.4 µg/kg) were detected in 24% of the bee samples collected in October, but residues in 85% of the beebread samples were consistently higher than levels in August but not correlated with exposure dose.

Disease Incidence: All colonies sampled at the October assessment were infested with *Varroa* mites at average (±SE) densities of 7.1 ±1.4, 8.8 ±2.6, 6.6 ±1.2 and 13.3 ±3.0 mites per 100 bees from the control, 5, 20 and 100 µg/kg treatment groups, respectively. The 100 µg/kg treatment group colonies had a significantly higher count based on a contrast test of difference ($p = 0.047$) as compared to the control. An overall positive trend of infestation level with imidacloprid dose was observed ($p = 0.03$). Additionally, three colonies, two in the 5 µg/kg group; one in the 20 µg/kg group, tested positive for *Nosema* (<1.5 million spores). There was no mention in this study of any hive treatment to address either of these

pests.[3] It is noted that United States Department of Agriculture (USDA) guidelines for *Varroa* treatment thresholds are not clearly defined and in a in a recent conference paper, contributors listed thresholds of 1-2 mites/100 bees in spring and 4-20 mites/100 bees in August (USDA Varroa Mite Summit 2014). In the colonies that survived winter, an assessment completed in May 2010 showed that all colonies were again infested with *Varroa* to a level of ≥2 mites/100 bees with no significant differences between treatments. Out of the colonies that survived, 41% were infected with *Nosema* at <2.2 million spores/bee; there was no correlation between spore counts and imidacloprid concentration. Additionally, samples that were taken of dead bees and queens from the 7 colonies that died during the winter were assessed for the presence *Nosema* spores and only one colony in each 5 and 20 µg/kg treatment group tested positive.

Colony health: There were no colonies that showed evidence of overcrowding, unusual queen cell formation, or swarming behavior during or after the exposure period, as distinguished from the Dively 2009 study where overcrowding was an issue early on in the study. Eleven queen events occurred at variable times across the 40 colonies, with the frequency of queen events positively associated with the exposure concentration ($p = 0.009$), that is, higher numbers of queen events with increasing concentration of imidacloprid. Two colonies in each of the 20 and 100 µg/kg treatments that had previously undergone natural queen supersedure, did not build up to be strong enough to sustain themselves through the overwintering stage and were subsequently terminated by the study authors (September) to avoid robbing.

Colony health parameters of bees, capped brood cells, food stores and drawn out cells (as a percentage of coverage of frame) were not significantly different from the control group both after the 12-week exposure period (August 6, 2009) and two months after exposure prior to overwintering (data recorded October 6, 2009, $p>0.05$). The colony size (±SE) averaged over all dates was 17,440 ± 546, 18,541 ± 565, 17,813 ± 540 and 18,850 ± 448 bees in the control, 5, 20 and 100 µg/kg treatment groups, respectively, with no significant differences for the treatment groups compared to control ($p>0.05$).

Foraging measurements: The amount of pollen collected varied over the course of the experiment for the control and all treatment groups but was not significantly different when treatment groups were compared to the control ($p>0.05$). Pollen collected by the control, 5, 20 and 100 µg/kg exposed colonies averaged 56.6 ± 3.0, 60.8 ± 2.9, 57.6 ± 2.6 and 59.8 ± 2.3 g (±SE) per day, respectively. There was also no significant effects ($p>0.05$) determined on the number of foragers returning to each hive as well as the percentage of bees loaded with pollen pellets. The foraging activity of the treated colonies at all dose levels was significantly lower (12% as stated by author, $p = 0.026$) than the control at the last two assessment points on August 26th and September 2nd, which both occurred after the exposure period.

Overwintering Results: Of the 36 remaining colonies surviving on October 6, no significant differences were found in the cells drawn, capped honey, bee bread, capped brood, or bees (Fig. 3). The authors state that honey stores in the 20 and 100 µg/kg exposed colonies were consistently higher at the August and October inspections however; the supporting data is not presented in this article.

[3] Communication with study author (phone – 04/20/15) confirmed no pest control treatments were applied.

The authors considered a colony to have successfully overwintered if it survived to the March inspection with an active queen with brood and was able to buildup in the spring. Out of ten replicate colonies in each treatment group, 10, 8, 7 and 6 colonies survived the winter from the control, 5, 20 and 100 µg/kg groups, respectively. The article did not state in which apiaries the dead hives were found but based on a conversation with the main author, it was confirmed that no block effects were found[4]. Author observations from die-off colony inspections showed smaller clusters that could not reach the stored honey; there were no food shortages or symptoms of colony collapse disorder related to winter mortality. Using Fisher's one-tailed Exact Test, the difference in winter survival between the control and 100 µg/kg exposed colonies was statistically significant.

[4] Communication with study author (phone – 04/20/15) indicated there were no trends of block effects seen in the dead hive location data.

Table D-7. Summary of results from the 2009 trial of Dively, 2015

Measured Parameter	Treatment groups (with response variable mean ± SE if available, percent effect if applicable)				Comments
	Control (n=10)	**5 µg/kg** (n=10)	**20 µg/kg** (n=10, early Sept n=8)	**100 µg/kg** (n=10, early Sept n=8)	
Residue Analysis					
Exposure Dose Verification (µg/kg)	0	5.5	19.8	97.5	Measured in partially consumed patties removed after 7 days
Mean Cumulative Dose (mg imidacloprid)	N/A	16.6	63.7	322.6	- Based on total consumption over 12 weeks. - Consumption was estimated to be within the range of 37.9 – 39.6 g/day by reviewer.
Residues in Bees from August (µg/kg)	0.3 – 2.8				Of the positive detections, residues were generally higher with increasing exposure concentration.
Beebread residues from August (µg/kg)	0.4 – 1.6				
Residues in Bees from October (µg/kg)	Trace (<0.4)				Trace residues were detected in 24% of the bee samples
Mean beebread residues from October (µg/kg)	0.7	1.2	2.8	2.2	Values are from positive detections only.
Disease Monitoring					
Mean *Varroa* Count (fall; per 100 bees)	7.1 ± 1.4	8.8 ± 2.6	6.6 ± 1.2	**13.3 ± 3.0 (↑87%)**	- USDA autumn range of 4-10 mites/100 bees not exceeded - An overall positive trend of infestation level with imidacloprid dose occurred (p = 0.03).
Nosema Positive Detection (fall; spores per bee)	0	2	1	0	- Positive detection by the author was classified as <1.5M spores per bee
General Hive Condition					
Queen Event[1]	1/10 (replaced manually)	2/10 (replaced through superedure)	4/10 (replaced through supersedure)	4/10 (replaced through supersedure)	- The number of queen cells of all types summed over inspections was not significantly affected by the treatments (p>0.05. - The frequency of queen events was positively associated with the exposure dose statistically (p<0.05).
Mean Colony Size Across all Inspection Dates from May to October 2009[2]	17,440 ± 546	18,541 ± 565 (↑6.31%)	17,813 ± 540 (↑2.24%)	18,850 ± 448 (↑8.08%)	

Measured Parameter	Treatment groups (with response variable mean ± SE if available, percent effect if applicable)				Comments
	Control (n=10)	**5 µg/kg** (n=10)	**20 µg/kg** (n=10, early Sept n=8)	**100 µg/kg** (n=10, early Sept n=8)	
					For the 20 and 100 µg/kg treatment groups, means of the last two inspection dates for these groups are based on the eight surviving colonies.
Foraging Success					
Mean amount of pollen collected[3] (grams/day)	56.6 ± 3.0	60.8 ± 2.9 (↑7.42%)	57.6 ± 2.6 (↑1.77%)	59.8 ± 2.3 (↑5.65%)	- The amount of pollen collected twice weekly showed a significant time effect ($p < 0.001$) but no dose or interaction effects on foraging success ($p>0.05$). - No significant imidacloprid related effects on the number of foragers returning to each hive.
Colony Performance Endpoints Collected After 12 Week Exposure Period (Data collected on August 6 after a 12 week exposure period)					
Bees[4] (percentage of total frame covered)	27.6 ± 3.13	31.5 ± 2.75 (↑14.1%)	26.9 ± 3.32 (↓2.53%)	28.5 ± 2.88 (↑3.26%)	--
Capped Brood Cells[5] (percentage of total frame covered)	9.2 ± 1.13	8.9 ± 1.21 (↓3.26%)	8.12 ± 1.6 (↓11.7%)	8.12 ± 1.43 (↓11.7%)	--
Capped honey (percentage of total frame covered)	11.7 ± 2.39	13.1 ± 2.71 (↑12%)	13.1 ± 2.33 (↑12%)	19.3 ± 2.47 (↑65%)	There were consistently higher levels of honey stores in exposed colonies compared to the control but this effect was only significant ($p<0.05$) via a contrast test with adjusted p-values; effect was not significant ($p>0.05$) using Tukey's multiple comparisons test
Beebread (percentage of total frame covered)	5.7 ± 1.16	7.2 ± 1.64 (↑26.3%)	7.6 ± 1.67 (↑33.3%)	8.0 ± 1.28 (↑40.4%)	--
Drawn Out Cells (percentage of total frame covered)	59.5 ± 5.38	66.8 ± 5.17 (↑12.3%)	61.1 ± 4.82 (↑2.69%)	70.3 ± 3.53 (↑18.2%)	--
Colony Performance Endpoints Collected Two Months After Exposure Period and Prior to Overwinter (Data recorded October 6)					
Bees (percentage of total frame covered)	24.5 ± 1.48	21.4 ± 2.10 (↓12.7%)	20.9 ± 2.70 (↓14.7%)	23.1 ± 1.85 (↓5.71%)	--
Capped Brood Cells (percentage of total frame covered)	4.01 ± 0.51	3.7 ± 0.76 (↓7.73%)	3.9 ± 0.77 (↓2.74%)	4.4 ± 0.66 (↑9.73%)	--

Measured Parameter	Treatment groups (with response variable mean ± SE if available, percent effect if applicable)				Comments
	Control (n=10)	**5 µg/kg** (n=10)	**20 µg/kg** (n=10, early Sept n=8)	**100 µg/kg** (n=10, early Sept n=8)	
Capped honey (percentage of total frame covered)	22.8 ± 3.28	21.9 ± 2.85 (↓3.95%)	24.8 ± 2.81 (↑8.77%)	27.6 ± 2.47 (↑21%)	--
Beebread (percentage of total frame covered)	2.4 ± 0.64	4.0 ± 0.72 (↑66.7%)	3.7 ± 0.94 (↑54.2%)	3.3 ± 0.57 (↑37.5%)	--
Drawn Out Cells (percentage of total frame covered)	65.3 ± 4.53	71.6 ± 3.53 (↑9.65%)	68.2 ± 3.71 (↑4.40%)	75.2 ± 2.18 (↑15.2%)	--
Overwinter Survival					
Overwinter Survival 2009 (percentages are reviewer calculated, the denominator is the number of colonies that were alive in October heading into winter	10/10 colonies survived 100%	8/10 colonies survived 80%	7/8 colonies survived 88%	**6/8 colonies survived 75%**	- Two colonies in each of the 20 and 100 µg/kg groups underwent natural supersedure and did not build up enough to survive overwintering and were terminated in September to avoid robbing - Colonies were considered to have successfully overwintered if they survived to March with an active queen/brood

Effects determined to be statistically significant ($p<0.05$) are shown in **bold**.

[1]Queen replacement either by manual replacement or natural supersedure.

[2] Mean colony size was assessed using the Area Under the Curve (AUC) methodology as described in the Statistical Analysis section 11.14. The mean colony size was assessed over the total cumulative bee days for each colony for the whole season.

[3]The mean amount of pollen collected twice weekly in the entrance traps was used as a measure of foraging success.

[4] For bee strength, a linear regression function was used to estimate the number of hive bees per colony from the percentage frame area covered with bees.

[5] The author refers to capped brood in this document which based on wording, the reviewer has assumed to include both capped brood and older (uncapped) larvae in the assessment.

2010 Trial

The methodology in 2010 generally followed that used in 2009 with the following exceptions:

- There were 7 apiary locations where each location had 1 replicate colony of each treatment as opposed to 5 apiaries where each location had 2 replicate colonies of each treatment in 2009.
- There were 7 replicate colonies of each treatment tested for a total of 28 colonies as opposed to 2009 in which there were 10 replicate colonies for each treatment tested for a total of 40 colonies.
- Bees were collected for *Varroa* and *Nosema* presence analysis at the end of the exposure period in August 2010 as opposed to 2009 in which bees were collected in early November 2009 (after exposure), and then aga.in after overwintering in May 2010.

The dataset for the biweekly assessments during exposure started on June 10 and ended on August 19, 2010.

Results

Residue Analysis: Imidacloprid residues were not detected in the control colonies, while residues in bees and beebread collected from treatment group colonies were less frequently detected and lower than levels found in 2009. Individual colonies in treatment groups consumed similar amounts of diet, ranging from an average of 58.8 to 61.7 g per day, which is higher than the reviewer calculated daily amount in 2009 (37.9 - 39.6 g per day). Exposure doses were confirmed by residues in both old and fresh treated patties, all of which were within ± 5% of the targeted concentrations of 5, 20 and 100 µg/kg. Residues of 0.3–2.2 µg/kg in bees were detected on June 30 after four weeks of exposure in two colonies exposed to 100 µg/kg, while only trace amounts of imidacloprid (< 0.5 µg/kg) in bees were detected in three of the 28 colonies sampled on August 19 when the exposure period concluded. Beebread samples collected from all colonies on June 30 had four positive detections out of 28 with residues in the range of 0.2-4.1 µg/kg, on August 16 in 5/28 a range of 0.2-0.5 µg/kg, and on October 7 after the exposure period there were 3/28 detections in a range of 0.3-3.6 µg/kg with five other detections that could not be confirmed because of issues with the ion ratio outside of range. These residues were not related to exposure dose.

Disease Incidence: All colonies sampled on at the end of the exposure period (August 19, 2010) were infested with *Varroa* mites at average densities (±SE) of 2.0 ± 0.39, 1.8 ± 0.56, 2.9 ± 0.99 and 3.9 ± 0.82 mites per 100 bees from the control, 5, 20 and 100 µg/kg treatment groups, respectively. Although mite counts were not significantly different among treatment groups ($p>0.05$), regression results showed a significant increasing trend with exposure concentration ($p = 0.043$). Only one control and two treated colonies tested positive for low levels (not quantified in the article) of *Nosema* spores. There was no mention in this article of any pest control treatment being applied to address either of these pests communication with the study author revealed that the USDA-ARS Bee Research Laboratory determined the level of *Varroa* and *Nosema* infestations to be low and that no treatment was warranted. It is also noted here, as described earlier, that only one sampling event (just after exposure ended) occurred in the 2010 trial where two sampling events (November 2009 and May 2010) occurred for the 2009 trial.

Colony Health: Unlike the 2009 results, the frequency of queen events was not associated with exposure concentration (p = >0.05), nor was there a significant regression relationship between the number of queen cells and concentration (p = >0.05). The control and 5 µg/kg exposed colonies experience nine queen events as compared to five events in colonies exposed to the higher doses (*i.e.* 20 and 100 µg/kg). It is not clear why the queen events were combined for the control and 5 µg/kg groups as well as the 20 and 100 µg/kg groups instead of being split out by treatment group as they were in 2009. Although the reviewer received raw data files from the author, the number of queen events that occurred was not included in the files so the actual number of events per individual treatment cannot be determined.

The 2010 bee populations were generally lower than those in 2009. Colony population estimates (±SE) across all inspection dates averaged 13,822±600, 14,200±790, 13,813±690 and 14,140±613 bees in the control, 5, 20 and 100 µg/kg treatment groups, respectively. The number of bees per colony and percentage of frame area covered with brood (capped cells and older larvae) varied over inspection dates but were not significantly affected by the exposure dose (Fig. 5). Brood production significantly changed over the season, with expected higher levels in all colonies during June, lowest during July, and then a gradual increase through to September. Note that the brood was lowest in control colonies during late July through early September, and the study author attributed this to a higher frequency of queen events and subsequent breaks in reproduction. Although the dose and interaction effects were not significant, a contrast test showed a significant difference in brood production between the control group and exposed colonies grouped together. An analysis of hive inspection data on August 19 after exposure ended revealed no significant dose effects on any of the colony performance endpoints (Fig. 6). Although the 100 µg/kg exposed colonies stored higher amounts of honey on August 19, which is consistent with the 2009 results, the relationship between the honey stores over all dates and exposure dose was not significant. Dose relationships using AUC values were also not significant for bee numbers, capped brood cells and older larvae, pollen, queen cells, or drawn cells.

Foraging measurements: The amount of pollen collected at the entrance of colonies was not significantly different among treatment groups (p = 0.75). Regression results also showed no significant relationship between the cumulative AUC values for collected pollen and imidacloprid dose (p = 0.88). The total amount of pollen collected in the pollen traps was 41% less by weight in 2010 than in 2009 and the authors also noticed that there were 31% more forager bees on average in 2009. This is in agreement with the fact that mean colony size across all inspection dates was larger in 2009 (17,440 – 18,850 bees) than in 2010 (13,813 – 14,200 bees) as well.

Control colonies collected significantly less pollen during July which the authors attributed to breaks in brood rearing and reduced bee strength. Figure 5 confirms that by the July 21 assessment the percentage of frame area covered with capped brood is lower in the control (~5%) compared to the other treatments and remains lower than the other treatments until the last assessment on October 7. However according to the graph in the top of Figure 5, there may be a slight reduction in bee strength at the July 21 and August 5 assessments but visually, it does not appear to be very different than the other treatments and recovers by the August 19 assessment point. Since the authors previously mentioned that there were nine queen events in the control and 5 µg/kg colonies, with no further description of the events or how many

of the nine occurred in the control colonies, the reviewers suggest that the low brood numbers may have been due to issues with the queens and/or natural variability in the control honey bee colonies.

Foraging activity showed no evidence of any dose or interaction effects on the total number of foragers returning or the percentage of bees loaded with pollen pellets (Fig. 8). The authors stated that overall, an average of 151 to 159 foragers returned per 5 min and 38 to 40% were loaded with pollen.

Overwintering Results: All colonies survived to the last inspection (October 7), except for one replicate in each exposed group. These colonies died out during September due to a lack of brood and virtually no stored food, despite the fact that each hive was provisioned with sucrose syrup since mid-August.

Of the remaining 25 colonies going into the winter, results showed no dose response or significant differences across the gradient in colony size, brood or food stores among treatment groups at the last inspection. Overwintering survival was much lower than the levels observed in 2009, particularly illustrated by the control group (100% vs. 57% in 2009 and 2010, respectively). The authors suggested this was a result of over-consumption of food stores due to a mild winter. They also noted that in 2010 the spring season started out cooler than in 2009 and resulted in colonies that were slow to build up their populations. On February 7, hive inspections revealed 2, 2, 3 and 3 dead colonies in the control, 5, 20 and 100 µg/kg treatment groups, respectively, and most of these colonies were low in food stores and had to be provisioned with bee candy. A final inspection on March 17 confirmed another dead colony in each of the control and 5 µg/kg treatment groups. Twelve colonies died out during the winter (10 in February and an additional 2 in March), but only two hives lacked stored food. Out of seven replicate colonies, 4, 3, 3 and 3 in the control, 5, 20 and 100 µg/kg groups, respectively, were able to successfully overwinter; however, Fisher's one-tailed Exact Test showed no statistical significance in the proportions of overwintered colonies. After the March inspection, all remaining colonies were fed sucrose syrup in top feeders and were able to buildup normally in the spring.

Table D-8. Summary of results from the 2010 trial of Dively, 2015

Measured Parameter	Treatment groups (with response variable mean ± SE if available, percent effect if applicable)				Comments
	Control (n=7)	**5 µg/kg** (n=7, Sept n=6)	**20 µg/kg** (n=7, Sept n=6)	**100 µg/kg** (n=7, Sept n=6)	
Residue Analysis					
Exposure Dose Verification	Exposure doses were confirmed by residues in both old and fresh treated patties, all of which were within ± 5% of the targeted concentrations of 5, 20 and 100 µg/kg. Imidacloprid presence in the control colonies was non-detectable.				--
Residues in Bees (µg/kg)	0	0	0	0.3–2.2 in 2/28 colonies; Trace (< 0.5) in 3/28 colonies	Of the positive detections, residue values after 4 weeks of exposure, sampled on Aug 19, 2010.
Bee bread residues	Beebread samples collected from all colonies on June 30 had four positive detections out of 28 colonies (14%) with residues in the range of 0.2-4.1 µg/kg, on August 16, 2010 in 5/28 (18%) a range of 0.2-0.5 µg/kg, and on October 7, 2010 after the exposure period there were 3/28 (11%) detections in a range of 0.3-3.6 µg/kg with 5 other detections that could not be confirmed because of issues with the ion ratio outside of range. These residues were not related to exposure concentration.				
Disease Monitoring (August 19, 2010 data recording)					
Mean *Varroa* Count (per 100 bees)	2.0 ± 0.39	1.8 ± 0.56	2.9 ± 0.99	3.9 ± 0.82	The USDA thresholds were not exceeded according to author but USDA lists a large fall range of 4-10 mites/100 bees.
Nosema Presence	1	2			Only one control and two treated colonies tested positive for low levels of *Nosema* spores.
General Hive Condition					
Queen Event[1]	9/14 replacements		5/14 replacements		The frequency of queen events was not associated with exposure concentration ($p>0.05$)
Mean Colony Size Across all Inspection Dates from May to October 2009[2]	13,822 ± 600	14,200 ± 790 (↑2.73%)	13,813 ± 690	14,140 ± 613 (↑2.30%)	The process of hive packaging of bees started later in April and cooler temperatures slowed the build-up of colonies; thus, bee populations were lower overall as compared to those in 2009.

Measured Parameter	Treatment groups (with response variable mean ± SE if available, percent effect if applicable)				Comments
	Control (n=7)	**5 µg/kg** (n=7, Sept n=6)	**20 µg/kg** (n=7, Sept n=6)	**100 µg/kg** (n=7, Sept n=6)	
Foraging Success					
Mean amount of pollen collected (grams/day)	29.8 ± 2.26	35.1 ± 2.93 (↑17.8%)	39.4 ± 3.74 (↑32.2%)	34.1 ± 2.97 (↑14.4%)	--
Colony Performance Endpoints Collected After 12 Week Exposure Period (Data collected August 19, 2010)					
Bees[3] (percentage of total frame covered)	23.6 ± 2.41	19.9 ± 2.98 (↓15.7%)	19.5 ± 2.37 (↓17.4%)	21.1 ± 1.81 (↓10.6%)	--
Capped Brood Cells[4] (percentage of total frame covered)	6.7 ± 1.51	8.5 ± 2.35 (↑26.9%)	9.05 ± 2.18 (↑35.1%)	10.1 ± 1.60 (↑50.7%)	--
Capped honey (percentage of total frame covered)	3.9 ± 1.54	2.3 ± 0.70 (↓41.0%)	2.2 ± 0.119 (↓43.6%)	8.8 ± 2.82 (↑125%)	--
Beebread (percentage of total frame covered)	9.5 ± 2.68	6.7 ± 2.46 (↓29.5)	5.9 ± 1.73 (↓37.9%)	6.2 ± 0.79 (↓34.7%)	--
Drawn Out Cells[5] (percentage of total frame covered)	73.3 ± 7.03	64.5 ± 5.24 (↓12.0%)	66.4 ± 6.36 (↓9.41%)	70.1 ± 4.51 (↓4.37%)	--
Colony Performance Endpoints Collected Seven Weeks After Exposure Period and Prior to Overwinter (Data Collected October 7, 2010)					
Bees (percentage of total frame covered)	19.1 ± 1.72 (↑1.51%)	19.4 ± 3.82	19.1 ± 2.31	18.4 ± 3.22 (↓3.66%)	--
Capped Brood Cells (percentage of total frame covered)	10.0 ± 1.41	10.8 ± 2.32 (↑8.0%)	8.7 ± 0.94 (↓13.0%)	10.0 ± 1.97	--
Capped honey (percentage of total frame covered)	11.7 ± 2.36	5.85 ± 1.29 (↓50%)	9.25 ± 2.00 (↓20.9%)	10.76 ± 3.16 (↓8.03%)	--
Beebread (percentage of total frame covered)	4.4 ± 1.75	2.1 ± 0.73 (↓52.3%)	2.9 ± 0.94 (↓34.1%)	1.7 ± 0.41 (↓61.4%)	--
Drawn Out Cells (percentage of total frame covered)	77.7 ± 6.49	68.9 ± 5.65 (↓11.3%)	70.3 ± 6.19 (↓9.52%)	74.3 ± 4.07 (↓4.38%)	--
Overwinter Survival					
Overwinter Survival (percentages are reviewer calculated, the denominator is the number of colonies that	February 7, 2011: 5/7 (71%)	February 7, 2011: 4/6 (67%)	February 7, 2011 and March 17, 2011: 3/6 (50%)	February 7, 2011 and March 17, 2011: 3/6 (50%)	- All colonies survived to the last inspection (October 7), except for one replicate in each treatment group.

Measured Parameter	Treatment groups (with response variable mean ± SE if available, percent effect if applicable)				Comments
	Control (n=7)	**5 µg/kg** (n=7, Sept n=6)	**20 µg/kg** (n=7, Sept n=6)	**100 µg/kg** (n=7, Sept n=6)	
were alive in October heading into winter)	March 17, 2011: 4/7 (57%)	March 17, 2011: 3/6 (50%)			- Colonies were considered to have successfully overwintered if they survived to the February 7 or March 17 inspection with an active queen with brood and were able to buildup in the spring. - No statistical significance ($p>0.05$) in the proportions of overwintering survival

[1]Queen replacement either by manual replacement or natural supersedure.

[2] Mean colony size was assessed using the Area Under the Curve (AUC) methodology as described in the Statistical Analysis section 11.14. The mean colony size was assessed over the total cumulative bee days for each colony for the whole season.

[3]The mean amount of pollen collected twice weekly in the entrance traps was used as a measure of foraging success.

[4] For bee strength, a linear regression function was used to estimate the number of hive bees per colony from the percentage frame area covered with bees.

[5] The author refers to capped brood in this document which based on wording, the reviewer has assumed to include both capped brood and older (uncapped) larvae in the assessment.

Overwintering Results (2009/2010): As an additional analysis, the study author pooled the overwintering survival data across 2009 and 2010. The results of this analysis indicated a mean overwintering survival (for years 2009 and 2010) of 82.4, 58.8, 47.1, and 52.9% for the control, 5, 20, and 100 µg/kg treatment groups, respectively. The subsequent statistical analysis of the combined data showed an overall significant treatment concentration effect (p = 0.024), and all contrast tests comparing survival between control and each treatment group were also significant, except for colonies exposed to 5 µg/kg, that is, a significant reduction in colony survival for the 20 and 100 µg/kg treatment groups.

Despite the rationale provided in offering potential reasons for the differences observed of the overwintering survival results in 2009 and 2010, the overwintering results from this study will be considered separate and not from the pooling analysis conducted by the study author. As discussed above, the overwintering survival for the 2009 trial was 100, 80, 88, and 75% in the control, 5, 20, and 100 µg/kg groups, respectively, with a significant ($p<0.05$) reduction determined at the 100 µg/kg group. By contrast, overwintering survival was 57%, 50% 50%, and 50% for the control, 5, 20, and 100 µg/kg treatment groups, respectively in the 2010 trial, with no treatment groups significantly different from the control group ($p>0.05$). The discussion above (Overwintering results for 2010 trial) provides insight from the study author in explaining the differences observed in overwintering survival. The 2010 overwintering data is confounded by the fact that nearly half of the colonies did not survive until the following spring. As a result, it is not considered appropriate to pool two sets of data with such stark differences in control performance and therefore as mentioned previously, the overwintering results of the 2009 and 2010 trials are considered separately.

2011 Trial – Fate of imidacloprid within the colony

An additional trial was conducted in 2011 to track the fate (movement and degradation) of imidacloprid within colonies. Colonies were established in April with 900 g packages of bees and sister queens obtained from the same commercial supplier as with the 2009 and 2010 trials. Prior to the trial, bees were fed sucrose syrup and pollen patties for five weeks and by mid-May; hives were inspected and then assigned to treatment groups. All colonies were fed 2 kg of sucrose syrup and 400 g of pollen patties provisioned each week for six weeks. The different treatments (control, 20 µg/kg spiked sucrose, 100 µg/kg spiked pollen) were incorporated into the sucrose solution or pollen patties depending on the field level residue exposure to simulate. The same total amount of active ingredient (40 µg imidacloprid) was provided in both the sucrose solution and pollen patties per week. There were four apiary locations, where each location had two replicate colonies of each treatment group. Eight replicate colonies for each group was tested for a total of 24 colonies. At two, four, and six weeks during the exposure period (6 weeks) as well as six weeks after exposure, samples of bees (30-40), bee bread (9 cm^2 comb), capped honey, larvae (30-40), and royal jelly (queens removed after 5 weeks of exposure to trigger queen cell production and formation) were collected.

Biweekly checks on queen health and colony performance were conducted during the course of the study. Estimates of bee strength, brood development and food stores were made at 4 and 6 weeks of exposure. The number of inner spaces between frames with bee clusters, number of frame sides with stored honey and beebread, and the number of frame sides covered with at least 25% capped brood and larval cells

were checked at the 2, 4 and 6 weeks of exposure assessments. A third hive box was added after exposure to allow space for further colony growth for which colonies were fed sucrose syrup for several weeks starting in late August to facilitate. A final assessment was conducted on 24 September, 2011 to assess the cumulative impact of the exposure scenarios and to determine strength according to scale provided below (according to the study authors):

- 'weak' (missing queen, very little brood and stored food)
- 'medium strength' (queen and brood but low in stored food)
- 'strong' (queen, brood and sufficient stores of food present)

It is noted here that the study makes no mention of a quantity of brood or food stores (*e.g.* percentage of frame coverage) that is associated with 'weak' 'medium' and 'strong.'

A total of 87 samples were analyzed for the presence of imidacloprid and its metabolites (LOD = 0.2 ppb, LOQ = 0.6 ppb). Eight control colony samples showed trace levels that the authors explained was apparently due to drifting or possible cross-contamination during sampling. One bee sample from an untreated colony contained 2.9 µg/kg and was analyzed twice for confirmation and subsequently considered an outlier by the authors because the value lay more than 1.5 times outside the interquartile range of the residue data. The authors stated it was possibly due to a mislabelled sample.

The frequency of positive detections and mean residue levels were significantly higher in colonies fed 100 µg/kg patties compared to residues in colonies fed 20 µg/kg sucrose syrup. Totaled over all hive matrices, 77.1 and 19.4% of the samples collected during exposure from colonies fed treated diet patties and sucrose syrup, respectively, contained residues of imidacloprid. The highest levels were found in honey (average 2.3–13.4 µg/kg), followed by lower but consistent levels in bees (average 0.2–1.9 µg/kg) and then beebread (average 0.5–1.7 µg/kg). Residues in larvae were below LOD, except for one sample collected after 2 weeks of exposure in each treatment group. At 6 weeks after exposure, residue levels were slightly lower but the frequency of positive samples remained approximately the same in bees, beebread and honey from colonies fed diet patties with 100 µg/kg of imidacloprid. In contrast, residues in colonies fed 20 µg/kg in sucrose syrup declined more quickly after exposure. All samples of royal jelly from colonies fed 100 µg/kg diet patties had detectable levels of imidacloprid residues, ranging from 0.3 to 1.0 µg/kg (average 0.6 µg/kg), whereas no royal jelly samples from colonies fed treated sucrose syrup contained detectable levels. Residues of the imidacloprid metabolites were not detected in any of the samples. However, the LODs were higher (ranging from 0.4 to 4.4 µg/kg) for these degradates.

Overall colony performance of the three treatment groups provided additional data on the sublethal effects of imidacloprid. Colony size was not significantly affected by the two exposure routes during the 6-week period. However, bee strength measured during August inspections showed significant differences in the number frames with bees. Colonies fed 100 µg/kg diet patties had 14–26% fewer frames of bees than the control colonies or colonies fed 20 µg/kg sucrose syrup. All other endpoints including brood rearing and food stores were not significantly ($p>0.05$) affected by the exposure routes. All eight colonies in each treatment group were active on September 24 but 3, 4 and 4 colonies in the control, 20 µg/kg sucrose syrup, and 100 µg/kg diet patty groups, respectively, were rated weak with very little brood and stored food. Nearly an equal number of colonies in each group were rated either medium strength or strong. After the last inspection, weak colonies were combined with stronger ones for overwintering and no further data were recorded.

Table D-9. Summary of results from the 2011 trial of Dively, 2015

Measured Parameter	Treatment groups			Comments
	Control (n=8 colonies)	**40 µg imidacloprid/week in pollen patty (n=8 colonies)**	**40 µg imidacloprid/week in sucrose syrup (n=8 colonies)**	
Mean Colony Size	14,001 ± 476	13,080 ± 338	13,890 ± 408	--
Bee strength (August 2011)	Values not available from article	14-26% fewer frames compared to control or sucrose solution treatment	Values not available from journal article	- A linear regression was used to estimate the number of bees per colony from the percentage frame area - Significant differences in the number frames with bees ($p<0.05$).
Brood rearing	Values not available from article			No significant effects ($p>0.05$)
Food stores	N/A			No significant effects ($p>0.05$)
Number of colonies with positive detections/total samples by life stage (range of imidacloprid provided parenthetically for positive samples in µg/kg)				
Bees	2 weeks exposure: 4/8 (0.2–2.9)	2 weeks exposure: 6/8 (0.2–1.4)	2 weeks exposure: 3/8 (0.2–0.5)	--
	6 weeks exposure: 0/8	6 weeks exposure: 5/8 (0.5–1.9)	6 weeks exposure: 0/8	
	6 weeks after exposure: 0/8	6 weeks after exposure: 8/8 (0.3–0.5)	6 weeks post exposure: 0/8	
Larvae	2 weeks exposure: 0/6	2 weeks exposure: 1/8 (0.4)	2 weeks exposure: 1/8 (0.5)	--
	6 weeks exposure: 0/6	6 weeks exposure: 0/8	6 weeks exposure: 0/8	
	6 weeks after exposure: 0/4	6 weeks after exposure: 0/7	6 weeks after exposure: 0/5	
Beebread	2 weeks exposure: 1/8 (1.1)	2 weeks exposure: 8/8 (0.5–1.7)	2 weeks exposure: 2/8 (0.7–1.0)	--
	6 weeks exposure: 0/8	6 weeks exposure: 8/8 (0.6–1.2)	6 weeks exposure: 3/8 (0.2–0.9)	
	6 weeks after exposure: 0/8	6 weeks after exposure: 8/8 (0.8–1.4	6 weeks after exposure: 2/8 (0.2–0.3)	
Honey	2 weeks exposure: 0/8	2 weeks exposure: 7/8 (4.7-13.4)	2 weeks exposure: 4/8 (0.2–3.7)	--

Measured Parameter	Treatment groups			Comments
	Control (n=8 colonies)	**40 µg imidacloprid/week in pollen patty (n=8 colonies)**	**40 µg imidacloprid/week in sucrose syrup (n=8 colonies)**	
	6 weeks exposure: 0/8	6 weeks exposure: 7/8 (2.8–10.8)	6 weeks exposure: 1/8 (0.9)	
	6 weeks after exposure: 3/7 (0.2-0.3)	6 weeks after exposure: 8/8 (2.3–11.7)	6 weeks after exposure: 2/8 (0.2–0.5)	
Royal jelly - number of colonies with positive detections/total samples and residue range	2 weeks exposure: NA	2 weeks exposure: NA	2 weeks exposure: NA	--
	6 weeks exposure: 0/6	6 weeks exposure: 8/8 (0.3–1.0)	6 weeks exposure: 0/8	
	6 weeks after exposure: NA	6 weeks after exposure: NA	6 weeks after exposure: NA	

LOD = 0.2 µg/kg; LOQ = 0.6 µg/kg), NA = not available

Bombus

Tunnel Design Studies

In a study by Tasei et al (2001), the individual and colony level effects to bumble bees (*Bombus terrestris*) were explored in two components (semi-field, tunnel design, and full field design discussed in the Tier III data summaries). Sunflowers were cultivated in 12 liter pots. Three rows of 16 pots each containing vegetable mold were placed in a greenhouse compartment (3 by 6 m). Three seeds per pot were sown and after germination, only one seedling per pot was grown as a test plant. Treated (0.7 mg/seed Gaucho, imidacloprid percent a.i not specified) and control plants alternated within each row. When sunflower heads started to flower, a colony of *B. terrestris* (~100 workers) was inserted and monitored the following over a 4 day period (June 16th to 19th): 1) the total number of foragers visiting the heads at each flowering stage through 4-8 counts covering flight hours (8.15-16.30) and 2) average visit duration/head was predicted for 75 foragers on 52 control heads and 52 treated heads. Frequencies of short visits were noted as <50 s.

Table D-10 below summarizes the results of this study. The number of foragers visiting the blooming heads did not differ between the treated and control plants significantly ($p>0.05$). The duration of individual visits on heads were between a few seconds to 4 minutes. Short visits, (defined for this study as <50s, and long visits, >50s), did not differ significantly ($p>0.05$) between treated and untreated plants.

Table D-10. Summary of results from Tasei et al 2001.

Response Variable	Control (mean ± SE, if reported)	0.7 mg/seed (mean ± SE, if reported)
Greenhouse Component (4 day exposure)		
Number of workers visiting blooming heads	59	80 (↑36%)
Number of bees with 'short' trips (*i.e.* 1 – 50 seconds)	30	20 (↓33%)
Number of bees with 'long' trips (*i.e.* >50 seconds)	10	16

While this study investigated individual and colony level effects to bumble bees resulting from exposure to seed treated sunflower, it is noted here that there are uncertainties as to what extent the bumble bees were exposed. Despite the finding that nectar foragers and pollen foragers had 98 and 25% of their load originating from sunflowers, there were no confirmatory measurements in both the semi-field and field components to indicate that imidacloprid was present in the nectar or pollen collected from the bumble bees. Instead, the authors cited prior work that was conducted in the same fields used for this study in which residues of imidacloprid were detected and therefore conclude that exposure did actually occur. Specifically, for the semi-field portion the authors cite unpublished data from five nectar samples collected by bumble bees. For the field portion of the experiment, soil samples were analyzed but not pollen or nectar. For the soil samples, it was stated that imidacloprid was detected, however the limit of detection was 5 ppb, notably higher than levels reported from other studies. This level of detail is determined to be insufficient for characterizing the exposure for this study.

Other limitations to this study include 1) no information on the purity of imidacloprid, 2) little information on the husbandry of the bumble bees both during the in-field exposure and when the bees were brought back into the laboratory, 3) the study make reference to nectar gatherers and pollen gatherers but it is unclear how this classification system was employed as bumble bees are able to collect both pollen and nectar on foraging trips. Additionally, while it was stated that 108 nectar gatherers and 133 pollen gatherers were sampled, it is unknown as to how many were from control fields vs treated fields.

In a study by Gels et al (2002), bumble bee (*Bombus impatiens*) colonies were exposed in a flight cage to imidacloprid treated turf grass/white clover. This study had two application scenarios, with one exposing colonies to granularly applied imidacloprid (as Merit 0.5 G, 0.4 lbs a.i/A) followed by 1.5 cm of irrigation from lawn sprinklers and the other with turf sprayed with imidacloprid (as Merit 75 WP, 0.3 lbs a.i/A) which was then either followed up by 1.5 cm of irrigation or did not receive irrigation. Both scenarios exposed bumble bee colonies to the plant residues of imidacloprid within a flight cage over a 28 day period where multiple colony health parameters were observed. The bees were allowed to freely forage in the cages.

For the first exposure scenario, imidacloprid (Merit 0.5 G, Bayer) was applied (0.4lb a.i/A) on a mixed stand of tall fescue, with approximately 25-50% flowering white clover cover (characterized by study as weedy turf). On June 18, 10 plots (3m x 5m) were paired based on percentage of surface area covered by clover. One of each pair was randomly selected for treatment the following day. The other plot of each pair was untreated. Shortly after treatment, all plots received 1.5 cm of irrigation from lawn sprinklers. Plots were then covered with pollination cages (4m x 2m x 1.3m). The first rain was 5 days after treatment. Total rainfall during the 30 day interval following treatment was 7.0 cm.

Bumble bee colonies of similar age were paired according to their initial weights and housed in a cardboard hive containing a fertilized queen, 40-50 workers, and brood. Seven days after treatment, one randomly assigned hive was placed on a concrete block within each cage, facing west. Dry honey bee pollen was provided to each colony at 7g, twice per week, and placed directly into the hive. Foraging activity was monitored in each plot three times per week where the total number of bees foraging on each plot was counted for a 2-min interval between 11a.m. and 1p.m. In addition to foraging activity, the study authors obtained a measure of defensive behavior by tapping the hive with a 30cm wooden ruler three times. The time it took for the first three bees to leave the hive was recorded as the initial response time. The duration of the response was the time elapsed from the initial response until a 25s lapse during which no more bees left the hive. In addition, the total number of bees that left the hive was also counted.

For the second exposure scenario, fifteen plots were established in a stand of tall fescue with 25-50% white clover coverage. Plots of 3 m x 5 m were marked and ranked as described above and placed in five groups of three according to similar clover density. On June 22, two plots of each group were randomly selected for treatment with imidacloprid (Merit 75 WP, Bayer) at a rate of 0.3lb a.i/A. One sprayed plot within each group was randomly selected to receive 1.5 cm of irrigation immediately after treatment with the other plot not receiving irrigation. The third plot in each group served as control so that there were 5 plots for group (control, treatment with irrigation, treatment with no irrigation). Plots were enclosed in pollination cages as described above. One day after treatment, one hive was placed into each flight cage

on a cement block, facing west. Each hive initially contained 20-25 workers, a fertile queen, and brood. The bees were fed 7g of dry honey bee pollen once every 7 days. Foraging and defensive behavior were assessed as described above.

Table D-11 below summarizes the results of both exposure scenarios of this study. In exposure scenario 1 (granular application followed by irrigation), there were no response variables that were significantly different from the control group ($p>0.05$).

In exposure scenario 2, colonies that were exposed to spray applications of imidacloprid that were immediately followed by irrigation did not significantly impact colony health or worker defensive behavior ($p>0.05$). However, in the absence of irrigation, bumble bee colonies had significantly ($p<0.05$) fewer adults, brood chambers, honey pots, weighed less, and had less of defense response in terms of its duration and number of bees responding. Queen weights were not significantly impacted ($p>0.05$.). There were a number of dead bees clinging to the sides of the cages on the non-irrigated, imidacloprid treated plots that was significantly increased over the level of control ($p<0.05$)

Table D-11. Summary of results from Gels et al 2002.

Granular treatment followed by irrigation (Exposure Scenario 1)			
Response Variable	**Control (mean ±SE)**	**0.4 lbs a.i/A (mean ±SE)**	
Colony weight (grams)	2540 ± 52	2690 ±53 (↑5.90%)	
Worker weight (grams)	27.4 ± 5.6	30.3 ± 4.9 (↑10.6%)	
Queen weight (grams)	0.79 ± 0.02	0.73 ± 0.05	
Number of workers	157.2 ± 37.1	116.6 ± 33.8 (↓26%)	
Number of brood chambers	43.6 ± 11.5	76.2 ± 27.4 (↑75%)	
Number of honey pots	132 ± 20	149 ± 11 (↑13%)	
Time to initial defense response (seconds)	7.5 ± 2.6	1.8 ± 0.6 (↓76%)	
Duration of defense response (seconds)	46.6 ± 14.4	44.0 ± 12.7	
Number of bees responding	15.0 ± 2.2	6.2 ± 3.6 (↓59%)	
Spray treatment followed by irrigation/no irrigation (Exposure Scenario 2)			
Response Variable	**Control (mean ±SE)**	**0.3 lbs a.i/A with irrigation (mean ±SE)**	**0.3 lbs a.i/A) with no irrigation (mean ±SE)**
Colony weight (grams)	86.4 ± 6.8	80.6 ± 2.6 (↓6.7%)	**39.6 ± 12.4 (↓54%)**
Worker weight (grams)	7.2 ± 1.0	7.9 ± 0.4 (↑9.7%)	**3.2 ± 0.6 (↓56%)**
Queen weight (grams)	0.7 ± 0.0	0.7 ± 0.1	0.7 ± 0.0
Number of workers	55.4 ± 7.0	48.6 ± 4.4 (↓12%)	**21.8 ± 2.3 (↓60%)**
Number of brood chambers	28.6 ± 4.2	25.0 ± 3.6 (↓13%)	**3.6 ± 0.7 (↓87%)**
Number of honey pots	24.0 ± 2.9	24.2 ± 4.7	**6.8 ± 4.7 (↓72%)**
Dead bees	0.0	1.0 ± 0.7	**13.2 ± 2.3**
Time to initial defense response (seconds)	9.8 ± 0.9	9.2 ± 1.7 (↓6.1%)	**3.2 ± 1.9 (↓67%)**
Duration of defense response (seconds)	46.8 ± 1.0	38.2 ± 7.0 (↓17%)	**12.6 ± 7.2 (↓73%)**
Number of bees responding	6.2 ± 1.9	7.0 ± 0.7 (↑13%)	**1.4 ± 0.8 (↓77%)**

Statistically significant effects as compared to the control ($p<0.05$) shown in **bold**

The results of this study suggest that, under the conditions tested, irrigation following granular or spray application of formulated imidacloprid on turf reduces colony level effects to bumble bees following contact exposure to residues. The results from exposure scenario 1 are associated with high variability for nearly every response variable that precluded the statistical analysis used by the study author to identify significant reductions from the negative control up to and including 76%. Exposure scenario 2, by contrast identified significant effects for all response variables but one (queen weight). Although there was an equal number of plots with flight cages used per treatment group in each scenario, the colonies used for scenario 1 were roughly double the size (40-50 workers and 1 queen) compared to scenario 2 (20-25 workers and 1 queen). This alone would not explain the variability, as greater number of workers in the colony would be expected to decrease variability because of larger sample sizes.

Other limitations to this study include the following: 1) this study did not quantify the in hive residues or pollen and nectar residues of imidacloprid (if any) to confirm the extent and magnitude of exposure, 2) the methods of evaluation of defensive behavior have not been formally tested (or standardized) to determine if they are sensitive and precise measures of effects, and 3) the method of application in the granular treatment scenario (scenario 1) was stated to be via gloved hand. The degree of homogeneity in application rate was not quantified.

Feeding Design Studies (Spiked Sucrose)

In a study by Mommaerts et al (2010), there were three components to the study, examining both microcolonies (described earlier) and queenright colonies.

In the first component, newly emerged individual bumble bee (*Bombus terrestris)* workers were exposed to imidacloprid (Confidor, 20% SC). Adult workers (in the absence of a queen) were exposed to formulated imidacloprid orally to varying concentrations (0.01, 0.02, 0.2, 2.0, 20, and 200 ppm) in sugar water for a period of 11 weeks. Observations for mortality were made daily for the first 3 days and then once weekly for each week subsequent to that. Similar to work in Laycock (2012), one worker bee in each microcolony became the dominant individual within a week, developed her ovaries, and began laying eggs (males). In each group, there were four artificial nests, each containing 5 worker bees each.

In the second component of the study, test conditions in terms of concentrations tested and replicate structure were the same as those tested in the first component, but an additional experimental chamber was introduced which contained the untreated or treated dosing solution. Before exposure to imidacloprid, the bees were trained for a period of two days to 'forage' on the sugar solutions at a location different from that of the nest in a separate, connected chamber. In addition to observations on mortality, food consumption was measured throughout the course of the 11-week exposure.

In the third component, 1 queenright colony (1 queen, 25 workers) in each of 4 treatment groups (control, 2, 10, and 20 ppb) were set up in a greenhouse to monitor foraging behavior. Feeders with untreated or treated sucrose solutions were placed 3 m from the hives as well as an unspiked pollen source. The exposure phase lasted 2 weeks during which time observations were made for several colony health parameters.

Table D-12 below summarizes the results for the three components of the study. In the first part, LC_{50} and NOAEC values for mortality and reproduction were generated showing a lower no effect concentration based on mortality as compared to reproduction. Mortality was 0 and 15% in the 0.01 and 0.02 ppm groups, respectively. It was not stated in the study what the level of control mortality was. Chronic (11-week) exposure to imidacloprid resulted in 100% in the 4 highest (200, 2000, 20000, and 200000 ppb) treatment concentrations. The time to reach 100% mortality <1, 14, 28, and 49 days for these treatment concentrations, respectively. While there were no effects to reproduction (measured as production of drones as these colonies were without a queen) in the two lowest treatment concentrations, reproduction was 100% lower than controls in the 200 ppb treatment group, and no reproduction occurred at the three highest treatment groups.

Similar to the first component, in the second part of the study, there was 100% mortality in the 4 highest concentrations. There was 0% mortality and 50% mortality in the 10 and 20 ppb groups, respectively. As with the first experiment, it was not stated wheat the level of control mortality was. The time to reach 100% mortality was <1, 7, 14, and 49 days for the 200, 2000, 20000, and 200000 ppb treatment groups, respectively. There was a dose dependent reduction in reproduction (as numbers of drones produced) at the three lowest treatment concentrations (62 – 83% reduction) while there was no production of drones at the three highest treatment concentrations.

In the third component (queenright colonies, two week exposure, greenhouse exposure), there were significant effects ($p<0.05$) in the 10 and 20 ppb groups including an increase in mortality and decrease in reproduction as compared to the negative control. Control mortality was reported to be 6%. The mortality level in the 10 and 20 ppb groups precluded any foraging behavior measurements (number of bees observed going in and out of the hive during a 30 minute observation interval) from being recorded. The study authors noted that in the 20 ppb, nearly all of the dead workers were found at or around the feeding station, whereas with the 10 ppb group all of the dead workers were found inside the hives. In addition, all workers in the 20 and 10 ppb treatments appeared to be apathetic in their movements. Finally, total sucrose solution consumption was not significantly different ($p>0.05$) in the 2 ppb group, as compared to the control group. Sucrose consumption results were not available for the 10 and 20 ppb group.

The results of this study suggest that queenright colonies are potentially more susceptible to colony level effects on mortality and reproduction than microcolonies. Interestingly, although a definitive statement of where the NOAEC actually falls cannot be made for component 2, the results for reproduction in component 2 and 3 of this study are generally in line despite the difference in types of colonies exposed, test design, and exposure duration.

Table D-12. Summary of results of Mommaerts et al, 2010.

Response Variable	LC_{50}/EC_{50} (95% CI) (ppb)	NOAEC (ppb)	Comments
Component 1 – Microcolonies, no foraging behavior (11-week exposure)			
Mortality	59 (52 – 68)	10	100% mortality in the 200, 2000, 20000, and 200000 ppb concentrations

Response Variable	LC_{50}/EC_{50} (95% CI) (ppb)	NOAEC (ppb)	Comments
Reproduction	37 (26 – 51)	20	No effect at 10 and 20 ppb. No reproduction at other concentration due to 100% mortality
Component 2 – Microcolonies, foraging behavior observations (11-week exposure)			
Mortality	20 (19 – 21)	10	0% mortality at 10 ppb, 50% mortality at 20 ppb, 100% mortality at all other groups
Reproduction	3.7 (2.5 – 5.5)	<2.5	↓62%, and ↓75% and ↓83% in the 10 and 20 ppb and 200 ppb treatment groups, respectively
Component 3 – Queen-right colonies, foraging behavior observations in a greenhouse (2-week exposure)			
Mortality	NR	2	62 and 92% mortality in the 10 and 20 ppb groups, respectively. 5% mortality in the 2 ppb group
Reproduction	NR	2	No reproduction in 10 and 20 ppb groups, no impact at the 2 ppb group
Foraging behavior (workers in and out of the hive per 30 min interval)	NR	2	No foraging behavior measurements made at 10 and 20 ppb due to effects on mortality, no significant impact at the 2 ppb group

NR = not reported

Limitations for this study include: 1) there was no analytical confirmation of imidacloprid in the treatment solutions, and 2) control performance was not reported in component 1 and component 2 of the study.

In a study conducted by Gill et al (2012), researchers exposed a total of 40 colonies of bumble bees (*Bombus terrestris)* to imidacloprid (10 ppb in sucrose solution), λ-cyhalothrin (sprayed on filter paper near feeders), a mix of both, or an untreated control group for a period of 28 days. Colony development, worker mortality, and various measures of foraging activity were made using radiofrequency identification (RFID) tagging techniques.

Each bumble bee colony used in the study contained a queen and an average of 4 workers (range: 0-10) at the start of the experiment. The study employed a split block design due to 20 colonies being used in July and 20 colonies used in September. The authors indicated that nearby flowering crops were minimized during these times, and that environmental conditions during each experimental replicate were moderate for the time of year and would not have hindered foraging. For each replicate, colonies were ranked according to the number of workers and pupae, with the 4 highest-ranked (largest) colonies assigned to block 1, the next 4 highest assigned to block 2, etc. Each replicate consisted of 5 blocks (n = 20 colonies). Treatments included a control, 10 ppb imidacloprid in sucrose solution, λ-cyhalothrin sprayed on filter paper at equivalent rate of 0.036 lbs a.i/A), and a mix of imidacloprid and λ-cyhalothrin. Within each block, the 4 treatments (control, imidacloprid, L-cyhalothrin, and mixed) were randomly assigned among the 4 colonies. There was no significant difference among treatments in either the number of workers or pupae present at the start of the experiment.

Imidacloprid treatments were provided in the food chamber. Imidacloprid (analytical standard) was used to make a primary stock solution of 1 mg imidacloprid/ml acetone, from which weekly stocks of 10 ppb sucrose solution (40% sucrose, 60% water) were made. During the 28-d exposure period, the sucrose treatment was provided every 2 days (3 days over weekends). Before refilling feeders the volume was

measured of any remaining solution in order to calculate what the bees had collected (n = 12 feeder replenishments per colony during the 28-day exposure period). The study began with 10ml of sucrose treatment per application in week 1, with a 2-ml incremental increase in the volume of sucrose at the start of each subsequent week. The study authors calculated that the amount of sugar provided was approximately 50% less than each colony would typically collect by nectar foraging, based on open literature reports.

Spray treatments of λ-cyhalothrin (analytical standard) were applied once at the start of each experimental week using a new piece of filter paper for each application for the LC and M treatments. Control solution was made by repeating this process but using an acetone stock solution (*i.e.* without λ-cyhalothrin). The spray treatment (new solutions made weekly) was applied using a hand sprayer following OCSPP 850.3030 guidelines for application. However, only around one third of the solution sprayed (mean ± s.e.m. = 0.69 ± 0.046 g; n = 12 sprays) landed on the filter paper, giving a calculated application rate of 0.036 lbs a.i/A.

Colonies were inspected every day to assess the number of newly eclosed workers, the number of dead workers, and the queen condition. At three days before the start of the experiment, fecal samples from each queen were checked for three parasites including the trypanosome *Crithidia bombi*, the microsporidian *Nosema bombi* and the neogregarine *Apicystis bombi*. The parasite assessment was also repeated on the final day of exposure using fecal samples from the queen (if present) and a subset of workers from each next box. In order to monitor foraging, all workers present at the start of the experiment (precise age unknown) were individually RFID tagged, and during the experiment all newly produced workers were tagged within 3 days of eclosion. The authors classified a "foraging bout" as a period of at least 5 minutes between a worker leaving and entering a colony. A worker was classified as a "forager" if it made 4 or more foraging bouts. Pollen foraging was observed in each colony for 1 hour per day (5 days a week) to record activity. Observation periods were always 2 hours and 21 hours after treatment application or renewal. The time that each tagged worker entered a colony was recorded using a stopwatch synchronized with the RFID data logger. The study authors then scored the amount of pollen in the forager's corbiculae (pollen baskets) as small (score of 1), medium (score of 2), or large (score of 3) relative to the size of the worker.

Table D-13 summarizes the results for the foraging activity, individual bee health, and colony development and health endpoints from this study. The mean number of foraging bouts per colony was not significantly different among imidacloprid and controls but was significantly reduced in the mixed treatment relative to controls ($p<0.05$). Imidacloprid exposed foragers returned with significantly smaller pollen loads per foraging bout (mean score = 1.3) compared to control colonies (mean score =1.9), although results were not statistically significant ($\alpha=0.05$) when pollen scores were normalized per the number of pollen bouts. This difference is related to the observation that imidacloprid-exposed foragers collected pollen successfully in a significantly lower percentage of their foraging bouts (59%) compared to control foragers (82%; $p < 0.01$). Furthermore, the average duration of successful foraging bouts was significantly longer ($p<0.05$) for imidacloprid-exposed foragers (~2,600 seconds) than for control foragers (~2,200).

The percentage of workers that were found dead inside the nest box was not significantly different between the imidacloprid from control treatments (mean = 10.3% and 9.4%, respectively). However, bees in the L-cyhalothrin and mixed treatments experienced significantly higher worker mortality (36% and 38.5%, respectively) compared to controls, suggesting that L-cyhalothrin exposure was responsible for the observed worker bee mortality and not imidacloprid. The average size and consumption of sucrose solution was not significantly different from controls across all treatments, suggesting that there was no repellency effect or impact on individual pollen forager growth. On the contrary, the average percentage of workers getting lost (not returning to the colony by the end of the experiment) significantly increased in the imidacloprid treatment (30%) vs. the control (20%; $p < 0.01$). The average percentage of workers found dead or lost was elevated in the imidacloprid treatment (41%) relative to controls (30%), but was not statistically significant at α of 0.05, but was significant at an α of 0.1.

The average number of workers per colony at the end of the experiment (worker production) was significantly lower in the imidacloprid treated colonies (reduced by 27%) and mixed treatment colonies (reduced by 9%) compared to control colonies. Worker production in imidacloprid treated colonies did become significantly lower than control colonies until the end of week 2, and for mixed treatment colonies until the end of week 4. The authors attribute this delayed effect on worker productivity in these colonies to the time taken by workers to develop from egg to adult (approximately 22 days). The average number of larvae and pupae (combined) that were found in colonies at the end of the experiment was also reported to be significantly lower in imidacloprid treated (36/colony) and mixed treatment colonies (43/colony) compared to control colonies (46/colony)

Notably, the average number of bees classified as "foragers" (*i.e.,* having $\geq$ 4 foraging bouts) nearly doubled in the imidacloprid treatment (~10) and mixture treatment (~9) vs. ~4 forager/colony for controls. The authors suggest this represents a greater recruitment of workers into foraging activities presumably due to lower foraging efficiency exhibited by pollen foragers. The weight of the nest structure (wax, pupal silk, remnants of sucrose/nectar and pollen stores) did not differ significantly across controls and treated colonies. Queen loss occurred in 14 colonies, though the loss rate did not differ significantly among treatments. Two of the forty colonies, both in the mixed treatment group, did not survive the experiment.

No parasites were detected in the fecal samples of queens at the start of the experiment. At the end of the experiment, fecal samples were obtained from 25 of the 26 live queens and an average of 3.3 workers per colony. No individuals were infected with *Apicystis bombi,* however, *Nosema bombi*, was present in five bees, each from different colonies, but with no obvious correlation with treatment. *Crithidia bombi* was found in 22 (55%) of the colonies but there was no correlation between infection status and pesticide treatment.

Table D-13. Summary of results from Gill et al 2012

Endpoint	Control	Imidacloprid 10 ppb (% change)[1]	L-Cyhalothrin 0.036 lbs/A (% change)	Mix (% change)
Foraging Activity				
# Foraging bouts / colony	~ 40	~35	~25	**~20 ***
Pollen load score / foraging worker / colony	~ 1.9	**~ 1.3 (↓31%)**	~ 2.2	**1.0 (↓47%)**
Pollen load score / successful pollen bout	~2.4	~2.2	~2.2	~2.1

Endpoint	Control	Imidacloprid 10 ppb (% change)[1]	L-Cyhalothrin 0.036 lbs/A (% change)	Mix (% change)
% Foraging bouts per forager that returned with pollen / colony	82 ± 5.8%	**59 ± 7.3 (↓28%)**	~ 85%	**55 ± 8.6 (↓33%)**
Duration of pollen bouts / successful pollen bout (s)	~2200	**~2600 (↑18%)**	~2500	**~3000 (↑36%)**
Individual Bee Health				
% Worker Mortality (d 28) / colony	9.4 ± 3.6	10.3 ± 3.1	**36.0 ± 7.7 (↑380%)**	**38.5 ± 8.0 (↑410%)**
% Workers getting lost / colony	20 ± 2.9	**30 ± 3.1 (↑50%)**	NR	**31 ± 5.3 (↑55%)**
% Worker loss + mortality / colony	30 ± 5.0	41 ± 4.2 (↑37%)	**51 ± 6.8 (↑70%)**	**69 ± 7.1 (↑130%)**
% Sucrose Consumption	~ 80%	~ 80%	~ 80%	~ 80%
Pollen forager size (thorax width in mm)	4.70 ± 0.080	4.72 ± 0.071	4.64 ± 0.092	4.71 ± 0.070
Colony Development and Health				
# Workers / colony (d 28)	27.0 ± 4.0	**19.7 ± 3.0 (↓27%)**	24.6 ± 2.5 (↓9%)	**24.4 ± 3.2 (↓9%)**
# Foragers/ colony	~ 4	**~10 (↑150%)**	~5	**~9 (↑125%)**
Brood Production (# Larvae + Pupae) / colony	46 ± 9.7	**36 ± 8.0 (↓22%)**	NR	**43 ± 11.7 (↓7%)**
Queen Loss	4	5	2	3
Nest Structure Mass (g)	9.4 ± 1.2	8.3 ± 0.8 (↓12%)	8.6 ± 0.8 (↓8.5%)	8.6 ± 0.9 (↓8.5%)
Colony Failure	0/10	0/10	0/10	2/10

Statistically significant results ($p<0.05$) shown in **bold**; ~ = estimated from author's graphs; NR = not reported

[1]Some percent change calculations were based off of estimates from the study author graphs.

The results showing that the imidacloprid exposed colonies recruited more foragers combined with the lower efficiency of these foragers suggests that the colonies may have been trying to compensate for the reduced efficiency through more frequent foraging bouts. Furthermore, the combination of two pesticides led to further adverse impacts, even to the point of a significantly greater colony failure rate. Finally, this study determined effects at the individual level (pollen load size, pollen foraging time, percentage of workers getting lost) and colony level were associated with significant effects at the colony level (reduced worker and brood production). Interestingly, effects at the individual level were generally associated with foraging activity and not individual health, yet the number of workers of workers per colony after 28 days was still significantly reduced. This suggests that field realistic doses of imidacloprid (10 ppb) in nectar (as simulated by the sucrose solution in this study) can result in bumble bee colony level effects although it is important to note here that no colonies exposed to imidacloprid alone in this study were determined to fail during the course of the study, despite a 40% queen loss incidence in this group.

Limitations to this study include: 1) colony sizes were very small (1 queen, 4 workers) which introduces some uncertainty when results are extrapolated to larger colonies that conceivably may be more resilient due to greater numbers of workers, 2) pesticide residues in food (both naturally foraged plants, and test substance), bees, and hive produces were not measured analytically, and 3) *Crithidia bombi* was found in

55% of the colonies. Although infection did not appear treatment related and was accounted for with controls, the potential interaction of this stressor with pesticide exposure is uncertain.

In a follow up study (Gill and Raine, 2014), the study authors re-examined the foraging data from the 2012 study (summarized above) and described the results more temporally across the 28-day period.

The methods employed for this study are largely the same as those described above for Gill et al 2012 as the same colonies were used. The study authors examined the relationship between specific foraging performance measures (number of foragers, number of foraging bouts and bout duration per day) and the time since the start of the experiment for all colonies within a treatment. Trends across treatment groups were then explored by comparing their respective regression slopes from a linear regression. While the foraging response variables in the Gill et al 2012 study (**Table D-13** above) are collapsed to be inclusive of the entire study duration, the results below in **Table D-14** break out the responses across the 4 weeks of the study to investigate the temporal nature of the effects.

The number of foragers response variable (not included in Gill 2012) was significantly increased ($p<0.05$) over the control group starting after 1 week of exposure and persisting until the end of the study (4 weeks). The number of foraging bouts and foraging bout duration were not significantly different ($p>0.05$) from control (foraging bout duration not included as response variable in Gill 2012). Pollen load size from all foraging bouts (described as "Pollen load score / foraging worker / colony" in Gill 2012 above) was significantly reduced from control in the final 2 weeks of the study only. The same variable from only successful bouts was not significantly different from control at any time interval. Finally, the successful pollen foraging bout duration was significantly increased from control in this study but only at the final time interval (week 4 of the study). When all time points were collapsed in Gill 2012, this response variable was also increased over the level of the control.

The results of Gill 2012 and Gill and Raine 2014 are largely the same with the exceptions being the addition of the number of foragers endpoint (statistically increased over the level of control) and the determination that the results of decreased pollen load size from all foraging bouts and increased duration of successful pollen bout were observed only at the end of the 4 week exposure. This suggests (under the conditions and concentration of imidacloprid used in this study) that bumble bee individuals can compensate certa.in aspects of foraging trips but prolonged exposure can lead to longer foraging trips and smaller loads. Limitations for this study are the same as those listed for Gill 2012 as the new analysis was based on the dataset and methods discussed in Gill 2012.

Table D-14. Summary of results of Gill and Raine 2014.

Variable	Treatment Group	Week 1	Week 2	Week 3	Week 4
Number of foragers	Imidacloprid	↑	↑	↑	↑
	λ-cyhalothrin	↑	NS	NS	↑
	IMI/LambaCy	↑	↑	↑	↑
Foraging bouts	Imidacloprid	NS	↓	NS	NS
	λ-cyhalothrin	NS	↓	NS	↓
	IMI/LambaCy	NS	↓	↓	↓
Foraging bout duration	Imidacloprid	NS	NS	NS	NS
	λ-cyhalothrin	↑	NS	NS	NS

Variable	Treatment Group	Week 1	Week 2	Week 3	Week 4
	IMI/LambaCy	↑	NS	NS	↓
Pollen load size from all foraging bouts	Imidacloprid		NS	↓	↓
	λ-cyhalothrin		NS	NS	NS
	IMI/LambaCy		↓	↓	↓
Pollen load size from successful foraging bouts	Imidacloprid		NS	NS	NS
	λ-cyhalothrin		NS	NS	NS
	IMI/LambaCy		↓	NS	NS
Successful pollen foraging bout duration	Imidacloprid		NS	NS	↑
	λ-cyhalothrin		NS	NS	NS
	IMI/LambaCy		NS	NS	↑

NS = not significantly different from control (p>0.05)
Means not presented in this study but only the direction of effect provided by arrows in relation to the negative control.

In a study by Laycock et al (2012), it was investigated whether field relevant concentrations of imidacloprid in pollen and nectar could affect reproduction in queenless microcolonies of worker bumble bees (*Bombus terrestris*). The study authors also evaluated the effects of imidacloprid treatment on worker bumble bee ovary development using the size of oocytes as a surrogate measure for ovary development. Microcolonies are created by separating a small number of bumble bee workers from the queen. Out of this small number of individuals, one will eventually assume control as her ovaries begin to develop and start to lay her own eggs (all of which will be male as this new 'queen' has not been previously fertilized). Once this process starts, the ovary development of other workers in the microcolony will be suppressed to ensure one dominant individual.

The study authors placed 328 worker bees (originating from 3 queenright bumble bee colonies) into groups of 5 (first trial) or 4 (second and third trials) workers bees each which the authors termed, microcolonies (described above). Allocation of workers into the microcolonies was random, but only contained worker bees from same original queenright colony. Microcolonies were placed into boxes (120 x 120 x 45 mm) and were partitioned into two equal compartments that workers could access through a hole in the center. Microcentrifuge tubes (2 mL) were placed into additional holes in the box to function as syrup feeders that were removed and weighed daily to measure syrup consumption.

Microcolonies were maintained for 14 days in controlled laboratory conditions (24-27^{0}C, 23—43% relative humidity, 10:14 h light: dark photoperiod) and were acclimated 24 hours prior to imidacloprid exposure with control sugar syrup provided *ad libitum*. After the acclimation period, microcolonies were provided an untreated pollen ball (mean 5.4 g, prepared from honey bee hive pollen) and either control or treatment solution for 13 days. Treatment solutions used included the following concentrations: 0.08, 0.20, 0.51, 1.28, 3.20, 8.0, 20.0, 50.0, and 125.0 µg a.i/L. The purity of the test substance was not reported but was indicated by the methods section to be analytical standard. The study used an unbalanced statistical design with different numbers of replicates per treatment level (ranging from 3-17 replicates); however, the authors reported that workers from each parent colony were distributed approximately evenly with dosage treatments and across the three trial periods.

Mortality, brood production, and syrup consumption were measured daily. Pollen ball weight was measured before and after the experiment to assess pollen consumption. The study authors reported that they corrected for water evaporation from syrup and pollen based on the mass change of several

syrup feeders and pollen balls kept in empty microcolony boxes under identical conditions. The study authors reported that all dosages contained appropriate levels of imidacloprid but the actual mean measured concentration for each nominal dose was not reported. The limits of detection and quantification (LOD and LOQ, respectively) of this analytical method were not reported.

The fecundity of the microcolony was considered by the authors to be the total number of brood (*i.e.* eggs and larvae together) produced. After one day of acclimation followed by 13 days of control or imidacloprid-treated syrup exposure, bees were sacrificed and necropsied to remove their ovaries; furthermore, la.id eggs and larvae were removed from the boxes.

The study authors used the mean size of all intact terminal oocytes/ovary and the size of the largest terminal oocyte as measures of ovary development. The length of the radial cell from one forewing per worker was also measured and used as a surrogate for body size. Comparisons were made between control, treatment and from 10 workers (dissected prior to the start of the microcolony experiment) taken directly from each original queenright colony to determine the change in ovary development for workers placed in microcolonies. These measurements were performed by technicians who were unaware of the imidacloprid concentration to which each specimen had been subjected. The mean length of eggs laid in the control syrup fed microcolonies was compared to the longest terminal oocyte inside each bee to determine the maturity of the oocytes in workers in each microcolony. A worker was considered to have mature oocytes if its longest terminal oocyte was at least as large as the egg mean length from the control microcolonies.

Microcolonies began to lay eggs after 7 days. After 14 days, the maximum number of eggs la.id in a microcolony was 39 eggs and some eggs had hatched and produced larvae. The study authors reported that the dosage effect on fecundity was similar across all three trials. Only one bee died during the exposure period (in one of the 125 µg/L microcolonies). Worker fecundity was reported to decline significantly with increasing imidacloprid dietary concentration. According to the study authors, the relationship between dosage and fecundity results in a 42% (95% CI: 33%, 51%) reduction in worker fecundity at an imidacloprid concentration of 1 µg/L (which the study authors stated was equivalent to 1.27 ppb a.i). It was not further reported from the study the level of fecundity as compared to the control at each treatment concentration. Dosage did not affect the time to first oviposition in the microcolony. Increasing imidacloprid dosage was inversely correlated with daily feeding rate of both pollen and syrup; however, bees exposed to higher imidacloprid doses still consumed more imidacloprid despite less syrup consumption. The authors did not provide the exact food consumption values for each concentration tested, but did report that at 1.28 µg a.i/L (1.63 ppb), bees consumed a mean 0.587 ng imidacloprid per day.

Bees collected from the queenright colony did not exhibit the increased oocyte size that the bees in the queenless microcolonies showed. All measures of oocyte size in microcolonies (mean length, width and area) as well as the number of workers with mature oocytes per microcolony were significantly affected by imidacloprid dosage ($p < 0.001$ for each variable). It is noted however, that there was not a dose-response relationship for mean area, with larger-sized oocytes depicted in several imidacloprid treatment groups compared to controls, especially at the lower dosages (*i.e.*, 0.08—0.51 µg a.i/L). It was reported

that microcolonies at the highest treatment level (*i.e*., 125 µg a.i/L, reported to be equivalent to 159 ppb a.i) had smaller mean oocyte size compared to all other dosages ($p<0.001$). There was no significant difference between the number of workers with mature oocytes at any concentration except for bumble bees exposed to the highest concentration of imidacloprid (125 µg a.i/L, $p<0.001$). Colonies with higher mean daily feeding rates also had significantly increased fecundity ($p< 0.01$ both for syrup and pollen feeding rates). Feeding rates for syrup and pollen were not correlated with terminal oocyte size. No correlation was observed between mean terminal oocyte size and individual bee body size (taken as a measure of forewing radial cell size).

This study provides information on the effects of imidacloprid on food consumption and potential reproductive effects of worker bumble bees in artificial microcolonies in the absence of a queen bumble bee. The study raises concerns for the effects of likely exposures of imidacloprid on bumble bee reproductive capacity. It is noted here that the relevance of reproductive effects of imidacloprid on worker bumble bees is highly uncertain for effects to bumble bee colony health given that worker production of males is generally much less of the colony male output and worker bees cannot produce either additional workers or new queens but rather only male drones. Additionally, despite a range of several concentrations and subjecting the data to regression statistical methods, there was no clear endpoint that was calculated from this study. Rather, percent reductions were defined for a given response variable at certain treatment concentrations (*e.g.* 42% reduction in fecundity at 1 µg a.i/L) with not all percent reductions reported for all treatment concentrations. This make it difficult to ascertain the levels at which the several response variables examined were impacted.

Other limitations to this study include: 1) pollen balls were not dosed; however the study authors did not report if they were analyzed for potential imidacloprid residues, 2) analytical measurements were not made of the syrup solutions actually used, but were made in water instead using the same range of nominal experimental dosages reported for syrup. The study authors also did not report the actual measured concentrations from these water-substituted test solutions, but instead provided a linear regression equation for establishing the measured concentration from nominal dosage, 3) where the study author's provided test concentrations converted to concentrations in ppb (*e.g.* 1.0 µg a.i/L reportedly equivalent to 1.27 ppb), the reviewer was unable to replicate the study author's calculations of the actual tested dose from the provided regression equation, 4) the study authors did not report whether other flowering crops were close to the field study site, 5) it is unclear why the number of replicates varies so much across treatments and whether this may be an indication that study conditions were not ideal to ensure adequate survival, 6) the relevance of reproductive effects of imidacloprid on worker bumble bees is highly uncertain for effects to bumble bee colony health given that worker production of males is generally much less of the colony male output and worker bees cannot produce either additional workers or new queens, 7) there is not a clear dose-response pattern in the mean oocyte areas reported in the study. Presumably, the expectation was that the workers were expected to attempt to replace the queen but would only be able to produce males, 8) the study author's do not discuss the relevance and exact correlation of these surrogate measures (*i.e* size of ovaries for ovary development) with more standard apical endpoints of reproduction and growth. Bumble bee weights were not recorded, which may have been a more accurate measure of body size than forewing radial cell size.

In additional work by Laycock and Cresswell (2013), small colonies of bumble bees (*Bombus terrestris)*, were exposed to a 'pulse' of varying concentrations of imidacloprid (reported to be technical grade). The 'pulse' was described by the study authors as 14 days of oral exposure to spiked sucrose syrup followed by a 14-day observation of untreated syrup. This study is distinguished from previous work by Laycock (*i.e*. the 2012 study above) in that the present study exposed small queenright colonies (1 queen, 4 workers) whereas the 2012 study exposed queenless microcolonies.

One colony each was placed in a softwood box (120 x 120x 45 mm). The box contained two 2 mL microcentrifuge tubes that served as syrup (artificial nectar) feeders. Imidacloprid (reported as technical grade) was spiked into sucrose syrup at concentrations of 0, 0.06, 0.16, 0.40, 1.01, 2.52, 6.30, 15.75, 39.37, and 98.43 ppb. Concentrations of imidacloprid in the sucrose solutions were analytically verified by LC/MS. To create a pulsed exposure, the 28-day experimental period was split into two successive periods of 14-days. During the 'on dose' period (day 1 to 14), 60 experimental colonies were supplied *ad libitum* with either undosed control syrup (6 control colonies) or dosed syrup (6 colonies per dosage treatment from those listed above). Fresh syrup with corresponding dosage was supplied to colonies daily. For the 'off dose' period (day 15 to 28), the bees were transferred to new softwood boxes and fed *ad libitum* with undosed control syrup.

At the beginning of the 14 day testing period, each experimental colony was supplied with a fresh ball of undosed pollen to which bees had *ad libitum* access. Pollen balls (avg. mass: 6.1 g) were prepared from ground pollen pellets mixed with water to form dough and were weighed prior to and after placement in colonies to calculate pollen uptake. Study colonies remained in the dark, except when monitored daily for the appearance of wax covered egg cells (indicating that oviposition had occurred), syrup consumption, and individual mortality. In addition, to reduce disturbance to bees, brood production was assessed by collecting all laid eggs and larvae from experimental colony boxes at the end of each 14-day period. The experiment was conducted in two replicate trials, one between October-November 2011 and the other between January-February 2012. Each trial comprised 30 experimental colonies and treatment groups were equally represented in both (3 colonies per treatment).

To establish that the observed recuperation from imidacloprid induced effects under pulsed exposure was caused by the removal of dietary imidacloprid rather than from acclimation to exposure over elapsed time, a separate continuous exposure experiment was conducted. Twelve randomly assigned experimental colonies were randomly assigned to either 28 days feeding on control syrup (7 colonies) or 28 days feeding on the highest concentration of imidacloprid in the diet (98.43 ppb) (5 colonies). This continuous exposure trial was conducted between March and April 2012. The statistical methodology for analysis of the results was noted to be robust in detail.

For both pulsed and continuous exposure studies, *B. terrestris* queens in testing colonies began to produce eggs after approximately two days. There were no queens died during the study and there was minimal worker mortality (one dead worker at the 98 ppb level, two dead at the 39 ppb level, and two dead at the 16 ppb level). During the 14^{th} day of 'on-dose' period of pulsed exposure, there was a dose-dependent repression on brood production. Based on this relationship, the 14-d on-dose EC_{50} values for brood production was estimated to be 1.44 ppb. On the 14^{th} day of off-dose period of pulsed exposure, brood

production exhibited dose-dependent recuperation. Dosage did not significantly impact brood production during the 'off dose' period and taken over the entire 28-day pulsed exposure, the EC_{50} value for brood production was beyond the tested dosage range (>98 ppb). The study authors estimated that a 14-day exposure to dietary imidacloprid at concentrations between 0.3 ppb and 10 ppb may diminish brood production in *B. terrestris* colonies by between 18-84%. When taken off dose, the effects on brood production begin to recuperate, and the impact determined during the on dose period is reduced to 2-19%. Recuperation was not attributable to acclimation over time because brood production remained repressed under continuous exposure at 98 ppb (the highest treatment concentration) over 28 days.
For the colonies subjected to continuous exposure to 98 ppb imidacloprid, brood production was significantly reduced from the negative control but did not vary between the first 14 days and subsequent 14 days of exposure.

In the groups where brood was produced, exposure did not affect the timing of first ovipositon during the on-dose period, but it delayed oviposition in the subsequent off dose period. It is noted however that the data did not show a dose response although the top two concentrations (39 and 98 ppb) appear to show a marked delay in time to oviposition as compared to control. In the colonies in the pulsed exposure component, dose-dependent reductions in the daily consumption of syrup and pollen were determined during the on dose phase. The 14-d on dose EC_{50} and EC_{10} values for reduced pollen consumption were 4.4 ppb and 0.2 ppb, respectively while the equivalent values for reduced syrup consumption were >98 and 23.6 ppb, respectively.

During the off-dose period for pulsed exposure, colonies showed dose-dependent recovery of both syrup and pollen consumption. Dosage did not significantly impact syrup consumption during the off-dose period but pollen consumption significantly increased among colonies previously exposed to higher doses. For the entire 28-day pulsed exposure period, the quantity of syrup and pollen consumed in colonies decreased as imidacloprid dosage increased, demonstrating the recuperation of food consumption was incomplete. From these results, the 28-d pulsed dose EC_{50} values were 43.7 ppb for reduced pollen consumption and >98 ppb for reduced consumption of syrup, while EC_{10} values were 16.2 ppb for pollen and 32.4 ppb for syrup. After analysis to control for the effects of dosage, brood production in experimental colonies increased with greater daily uptake of syrup and pollen.

Table D-15. Summary of results from Laycock and Cresswell (2013)

Effect	14-d "On-Dose" (ppb)	14-d "Off Dose" (ppb)	28-d "Pulsed Dose" (ppb)
Brood production (EC_{50}, EC_{10})	1.44, 0.15	Dose-dependent recuperation	> 98, 2.5
1st Oviposition	No treatment-related effect (p>0.05)	Delayed oviposition (p<0.05)	N/A
Pollen consumption (EC_{50}, EC_{10})	4.4, 0.2	Dose-dependent recuperation (p<0.05)	43.7, 16.2
Syrup Consumption (EC_{50}, EC_{10})	>98, 23.6	Dose-dependent recuperation (p<0.05)	>98, 32.4

N/A = not applicable

The results of this study suggest that bumble bee colonies undergo significant dose-dependent reductions in brood production and food (pollen and nectar) consumption when exposed to oral concentrations of

imidacloprid that they are to recover from when subsequently provided untreated food. This recuperation did not occur during the continuous exposure of imidacloprid that suggests that recuperation is achieved by the reversibility of the effects of imidacloprid rather than acclimation to imidacloprid exposure over time. However, it is noted that the continuous exposure group was only treated with the highest concentration (98 ppb). Pollen and nectar consumption showed similar patterns with dose-dependent recuperation that was incomplete recuperation at the two highest treatment concentrations (39 and 98 ppb). Since the pollen in this study was not dosed, the study authors suggest that imidacloprid reduces the bee overall ability or desire to eat.

A key limitation to this study is it does link these effects to other important measures of colony health such as queen production and worker mortality. This study is informative however in showing that potential transient exposure to imidacloprid may be overcome by colonies that are able to recuperate when imidacloprid exposure ceases.

In Bryden et al (2013), bumble bee (*Bombus terrestris*) colonies were exposed to sublethal levels of imidacloprid (described as analytical standard in the study) for a period of 6 weeks. Response variables that were examined included colony size, mortality, and birth rate. The results of the trials were then populated into models developed by the study authors to explain and predict the dynamics of colonies treated with imidacloprid. As the model used in this study (SubLethal Stress Model, SLS) has not been formally validated by the Agency, all discussion of results from this study will focus on the colony level experiments and not the inferences made from the model.

Prior to the beginning of the test, the colonies were sorted according to size by determining the total number of workers and pupae. The largest two colonies were randomly assigned as control and imidacloprid treated colony. This process was repeated for the second largest pa.ir of colonies, and so on until the eighth pair of colonies was completed. As a result of this process, there was no significant difference in the number of workers or pupae per colony between the control and imidacloprid assigned colonies. A total of 16 queen right bumble bee colonies were used for this study, 8 of these served as controls and the other 8 served as imidacloprid-treated colonies (at 10 ppb in a sucrose solution). All colonies were provided with fresh 40/60% sucrose/water (v/v) solution every two days (or three days over the weekend), with 10 mL provided in the first week and an incremental increase of 2ml for each subsequent week (with 20 mL on the sixth week). The exposure period lasted 42 days.

All workers present in the colonies (their precise age unknown) were individually tagged with a uniquely numbered tag. Each colony was housed in wooden nest boxes (28 × 16 × 11 cm) with a rear chamber and a front chamber. The rear chamber contained the brood and the front chamber housed a gravity feeder containing sucrose solution and a pollen dish. Colonies were kept at room temperature in a naturally lit laboratory throughout the experiment (brood chamber of each colony was covered with cardboard when not being observed to mimic the darkness of a subterranean nest). Additionally, defrosted honey bee-collected pollen was also provided in a plastic dish three times per week, at the same time sucrose feeders were refilled. Each colony was provided with 1g of pollen per refill during weeks 1–3 (*i.e.,* 3g per week), which was increased to 2g of pollen per refill during weeks 4–6 (*i.e*., 6g per week) to account for the

greater demand of larger colonies. Before refilling the pollen dish, old pollen was disposed and the pollen dish was thoroughly rinsed with water.

The results of this study were presented after 21 days and 42 days. It was not indicated from the study on whether the differences were significantly different from control, rather only the means were reported. After 21 days, the mean colony size was 27.4 in control and 18.3 bees in the treated colonies (↓33%). At the same interval, the mean mortality rate in the control colonies and treatment colonies was 3.5 and 3.3 bees, respectively. Finally, mean birth rate in the control and treated colonies was 17.6 and 13.3 bees (↓24.4%), respectively. At 42 days, only the response variable means of the control group were reported. From the raw data provided, worker production at the end of the 42 day exposure averaged 49.8 in the control group and 14.4 in the treatment group, a reduction of 71.3%.

This study indicates that at a field relevant dose of imidacloprid (10 µg a.i/L in sucrose), while bumble bee worker mortality did not appear to be impacted throughout the duration of the study (although not confirmed statistically), worker production was reduced over 70% from the control by the end of the 42-day study. It is noted that control mortality differed dramatically among the 8 colonies in the group, ranging from 9 – 38%, and above 25% for 5 of the 8 colonies. This high level of control mortality in a controlled setting of the laboratory suggests there may have been husbandry issues present. Additionally, while the primary purpose of the study was to inform the population model, the study authors did not report whether the differences observed in the treatment group were statistically significant from that of the control group. Other limitations from this study include: 1) it is not stated why one of the control colonies was started with only 2 bees. This colony subsequently failed to produce any workers and both bees died and 2) no analytical verification of imidacloprid in the sole treatment group.

Feeding Design Studies (Spiked pollen)

In a study by Gradish, 2009, that was previously discussed as it relates to the acute contact toxicity to bumble bees, the chronic oral toxicity to microcolonies of *Bombus impatiens*, was also investigated.

Bumble bee microcolonies (each 3 worker bumble bees where one would eventually become dominant and lay eggs) were provided with pollen spiked with formulated imidacloprid (Intercept® 60 WP) for 30 days, followed by another 30 days of observation where the treatment group microcolonies received uncontaminated pollen. The microcolonies were also provided a 60% sucrose solution *ad libitum*. At test initiation, there were 10 microcolonies in the sole treatment group (19.2 mg a.i/kg pollen) and 20 microcolonies for the control group. During the course of the 60-day test, observations of mortality, food consumption, and time to ovipoistion were made.

Table D-16 summarizes the results of this study below. Worker bees supplied with imidacloprid-spiked pollen at 19.2 mg a.i/kg showed a 3X reduction in life-span (~20d vs. ~60d in controls, $p<0.05$) and consumed less pollen (~0.4 g vs. ~3.8 g in controls, $p<0.05$). Microcolonies that received imidacloprid-spiked pollen did not begin oviposition, and therefore no production of larvae took place.

Table D-16. Summary of results of Gradish et al 2009[1].

Response Variable	Control	19.2 mg/kg in pollen
Longevity (days)	~ 60	~ 20 **(↓66.7%)**
Food consumption (grams)	~ 3.8	~ 0.4 **(↓89.5%)**
Time to oviposition (days)	**~ 6**	No colonies began oviposition

~: Estimated from study author graphs

[1]Means for control and treatment groups not provided in the study, only the indication of significance (p<0.05 shown in **bold**)

While this study indicates significant reductions in longevity, food consumption, and time to oviposition of queen-less bumble bee microcolonies exposed to imidacloprid-spiked pollen, the sole treatment group used is noted to be a remarkably high concentration in pollen. Therefore, the environmental relevance of this concentration is uncertain. An additional limitation is that data was only presented graphically making it difficult to evaluate the responses if each endpoint with precision.

Feeding Design Studies (Spiked sucrose and spiked pollen)

In a study by Whitehorn et al (2012), the colony level effects to bumble bees (*Bombus terrestris)* receiving both imidacloprid (technical grade) treated nectar and pollen were examined over a 14 day exposure period. Two treatment groups (in addition to a control) were initiated with the bees in the low treatment received diets of pollen and nectar containing 6 µg/kg and 0.7 µg/kg (ppb), respectively, while bees in the high treatment received 12 and 1.4 µg/kg, respectively (25 colonies/treatment group). According to the authors, there was no difference in initial colony weight before treatments and the number of worker bees within the colony at the start of the study was recorded to control for variations in initial size (mean: 15.4; range: 5 – 34). After the 14-day exposure, all colonies were placed in the field where they were left to forage independently for 6 weeks (beginning on July 11) while their performance was monitored.

Colonies were housed within a plastic box which was in turn placed within a cardboard box. For weight measurements, the interior plastic box was removed from the cardboard box and weighed. The release site was reported to be close to ornamental gardens, deciduous woodland and mixed farmland so scattered patches of wild and ornamental flowers were available; however, there were no flowering crops within 2 km. Doors of the nest boxes were designed to prevent queens from exiting the colony.

All colonies exhibited an intial weight gain followed by a decline as they switched from their growth phase to producing new queens. Colonies in the low and high group gained less weight over the course of the study compared to controls ($p<0.05$), with an 8% and 12% smaller, reduction in size of 8 and 12% for the low and high groups, respectively, by the end of the study. The rate of colony growth was dependent on the number of workers present; however, there were no significant differences between treatments in terms of the number of males, workers, pupae or empty pupal cells at the end of the study although the number of empty pupal cells was 18% and 30% lower, respectively, in the low and high treatment compared to the controls. The mean number of queens was significantly lower ($p<0.05$) than in the treatment groups with 85 and 90% reductions from the level of the control in new queens produced in the low and high groups, respectively.

The results in this study indicate that chronic (14-day) exposure to environmentally relevant (up to 12 and 1.4 ppb in pollen and sucrose solution, respectively) lead to a reduction on multiple parameters of colony health, most notably a sharp drop in the mean numbers of queens produced. It is also noted that the reductions in colony weight were not significantly different from each other at the low and high treatment groups and did not appear to reflect the two-fold difference in concentration.

Limitations to this study include 1) no analytical verification of imidacloprid in nectar and pollen, 2) it would have been informative to have a measure of food consumption or whether the pollen and nectar stores had to be replenished at any time during the 14-day exposure, and 3) although the authors suggest that imidacloprid may have reduced foraging efficiency in the treated colonies, this study did not include any response variables to evaluate foraging efficiency.

Table D-17. Summary of results for Whitehorn et al 2012.[1]

Response Variable	Control	6 µg/kg, 0.7 µg/kg in pollen and sucrose, respective	12 µg/kg, 1.4 µg/kg in pollen and sucrose, respective
Colony weight	Means not provided	**↓ 8%**	**↓12%**
Numbers of life stages (workers, males, pupae, or empty pupal cells)		↓ 18% (empty pupal cells)	↓30% (empty pupal cells)
Mean number of queens produced		**↓ 85%**	**↓90%**

[1]Mean for control and treatment groups not provided in the study, only percent difference from the control and indications of significance ($p<0.05$ shown in **bold**)

Feltham et al (2014), investigated foraging endpoints from individual bumble bees (*Bombus terrestris*) exposed to similar concentrations as those from those of Whitehorn et al (2012) discussed above. Bumble bees received uncontaminated pollen and nectar (control group) or the treated food items (*i.e.* 6 and 0.7 µg/kg in pollen and sucrose solution, respectively) for a 14-day period. Foraging efficiency endpoints were examined using RFID tags.

A total of six bumble bee colonies were used that were of similar aged and each consisting of the queen and up to 65 workers. Colonies of approximately equal weights were randomly allocated to either a treatment or control group. There were 3 colonies in each of the control and treatment groups. The colonies were supplied *ad libitum* with either treated or untreated (control) sucrose solution and pollen for a total of 14 days in the laboratory with no alternative forage provided during this time.

Following the laboratory feeding phase, Following the laboratory feeding phase, the colonies were placed outside in domestic gardens in an urban area of Stirling in the Central-belt region of Scotland; the nearest farmed area was over 1 km away. Bees were allowed to acclimatize to their surroundings for 24 h and were then fitted with a RFID tag. Foraging observations were made for 4 weeks after the RFID tags were fitted which recorded the exact time individual bees entered and exited the colony.

Between August 7 and September 4 (post-exposure observation period), there were 256 observation sessions. During these sessions 21 foragers from control colonies were recorded making 113 foraging trips (5.4 ± 1.4 trips per bee) and 24 foragers from treated colonies made a total of 142 trips (5.96 ± 1.9 trips per bee). Two bees from the treated group and one bee from the control group failed to return to the

nest during the course of the study. There was no difference in the lifespan of bees from the treated and control groups ($p>0.05$) as all tagged bees survived until the end of the study, with the exception of the three bees that failed to return to the nest.

There was no significant difference in weight of nectar collected and nectar foraging efficiency between the treated and control groups ($p>0.05$). Control bees spent on average 25.44 ± 6.1 minutes foraging for nectar, with a mean weight of 42.6 ± 9.86 mg of nectar collected per bout, resulting in a nectar foraging efficiency rate of 101 ± 10.68 mg/hour. Treated bees spent on average 27.26 ± 8.4 minutes foraging for nectar, with a mean weight of 44.7 ± 12.49 mg of nectar per bout, resulting in a nectar foraging efficiency rate of 99.24 ± 9.67 mg/hour. Neither treatment nor any of the other explanatory variables (time of day, batch and number of days since the bee was tagged) were significant in explaining trip duration, weight of nectar collected or nectar foraging efficiency.

Control bees spent on average 73.8 ± 14.38 minutes foraging for pollen (trips in which the returning bee had visible pollen loads), with a mean weight of 57.32 ± 11.22 mg of pollen collected per bout, resulting in a pollen foraging efficiency rate of 47.71 ± 7.62 mg/hour. Treated bees spent on average 77.85 ± 24.96 minutes foraging for pollen with a mean pollen weight of 41.07 ± 12.72 mg collected per bout, resulting in a mean pollen foraging efficiency rate of 32.97 ± 9.43 mg/hour. The mean pollen weight collected by treatment bees is significantly lower (↓28%) than that collected by control bees. The foraging efficiency rate of treatment bees represents a 31 % reduction compared to control bees. In the control group, the duration of trips remained approximately constant throughout the experiment, however in the treated group, the duration of trips increased with time from tagging.

This study suggests that environmentally relevant concentrations of imidacloprid (6 and 0.7 µg/kg in pollen and sucrose, respectively) can impact individual bumble bee foraging efficiency as indicated by significant reduction in the weight of pollen collected and the foraging efficiency of pollen. It is noted that the concentrations used in this study were that of the 'low' treatment concentration in Whitehorn et al 2012, in which colony level effects were determined (decreased colony weight, decreased number of queens produced). In Whitehorn et al 2012, the authors suggested that imidacloprid exposure may have led to reduced foraging efficiency but did not examine those types of response variables in that study. The results of this study, along with Whitehorn et al 2012, indicate a potential mechanism for decreased queen production as well as a reduction (although not statistically significant) of 18% in the number of empty pupal cells.

Table D-18. Summary of results from Feltham et al 2014.

Response Variable	Control (mean ±SE, percent effect)	Treatment (6 µg/kg and 0.7 µg/kg in pollen and sucrose, respectively)[1] (mean ±SE, percent effect)	Comments
Colony survival (at study end)	20/21 (95%)	22/24 (92%)	--
Weight of nectar collected/bout (mg)	42.6 ± 9.86	44.7 ± 12.49	--

Response Variable	Control (mean ±SE, percent effect)	Treatment (6 µg/kg and 0.7 µg/kg in pollen and sucrose, respectively)[1] (mean ±SE, percent effect)	Comments
Nectar foraging efficiency (mg/hour)	101 ± 10.68	99.2 ± 9.67	--
Weight of pollen collected (mg)	57.3 ± 11.22	**41.1 ± 12.72 (↓28%)**	There were significantly positive correlation between time spent foraging for pollen and the amount of pollen collection in both treated bees ($p<0.001$) and control bees ($p<0.001$).
Pollen foraging efficiency (mg/hour)	47.7 ± 7.62	**33.0 ± 9.43 (↓31%)**	

[1]All effects listed significant at $\alpha = 0.05$ (shown in **bold**) as compared to the control group

Limitations to this study include: 1) the purity of imidacloprid was not provided, 2) no hive residue measurements were available. Additionally, the presence of imidacloprid in the spiked pollen and sucrose solution was not confirmed and, 3) exposure to pollen and sucrose solution containing imidacloprid occurred during a 14-day period when no other food source was provided. In the field, alternative sources of forage may be present that would not be exposed to imidacloprid

Tier III

Apis

In a study conducted by Pohorecka et al (2013) the primary focus of this study was to evaluate the effects to *Apis mellifera* colonies during and after exposure to flowering maize (Zea *mays* L.) grown from seeds coated with imidacloprid (Gaucho 600 FS ; 83.3 mL/50,000 seeds and Courase 350 FS; 150 mL/50,000 seeds) in field realistic conditions. The experimental maize crops were adjacent to other flowering agriculture crops. The study authors situated bee colonies in three different maize fields, during blooming period, and afterwards were relocated to a stationary apiary. Authors gathered samples of pollen loads, bee bread, and adult bees for the assessment of any presence of insecticide residues.

Two field trials were conducted. For the field study conducted in 2011, imidacloprid (Gaucho 600 FS) was applied at a rate of 83.3 mL per 50,000 seeds). For the field study conducted in 2012, maize was planted on a field treated with imidacloprid (Courase 350 FS) at a rate of 150 mL per 50,000 seeds. Maize planted in all field areas were also treated with the fungicide Maxim XL 035 FS (a fungicide containing both fludioxonil and metalaxyl), and all crops were sprayed with herbicides (not specified) one or two months prior to blooming period. The treated crops bloomed from July 12 to August 2, 2011 and from July 9 to July 30, 2012. Treated fields were adjacent to fields with other flowering agriculture crops. There was no mention about the area of the control fields in both years. It was not stated the size of each control field.

Apis mellifera carnica and *A. m caucasia* colonies were established for the study. In the control and sole treatment group from each trial, five hives were installed with pollen traps. The control colonies were placed in a vicinity where no maize plants grew. Prior to relocation to the treated (or control) maize fields, colonies were assessed for biological and health status. Samples of worker bees were gathered from the periphery combs of the brood nest. Laboratory analyses were performed for the presence of pathogens

(*Nosema* spp.), parasites (*Varroa destructor*), and other viruses (chronic bee paralysis virus, acute bee paralysis virus, deformed wing virus, and Israel acute paralysis virus).

Mortality was observed throughout the experiment by calculating the quantity of dead adults on hive bottom boards and in 1x1 m white trays set up on the ground in front of the hive entrances. During September 2011 and 2012, colonies from experimental and control groups were prepared for overwintering. Each colony obtained approximately 20 L of sugar solution. *Varroa destructor* treatment was performed with amitraz (formulations: Biowar 500 and Apiwarol, Biowet, Pulawy, Poland) and 3.5% oxalic acid sugar solution. The status of overwintered bee colonies (adult bees and brood population) was recorded in April 2012.

Pollen samples were acquired at various times during the maize blooming, although it was not explicitly stated how frequently this was in the study. Pollen loads gathered by bees within 3 to 4 days were taken separately from pollen traps of each of the five colonies and weighed. Bee bread (approx. 10 x 10 cm pieces of combs) and adult bees (workers from brood chambers) were taken at the same time, after maize flowering. Pollen and bee bread samples were divided into two parts (subsamples), one for pollen analysis and one for the residue analysis. The LOD for pollen samples were 0.8 ppb for imidacloprid and the LOQ for pollen quantified as 3.0 ppb. The LOD and LOQ for honey bee samples were 0.5, and 2.0 ppb, respectively.

Table D-19 below summarizes the results from this study. For both trials (conducted with 2 formulations of imidacloprid), there were no significant differences on all parameters of colony health evaluated with the exception of an increase in brood area in the 2011 trial (Gaucho 600 FS) over the level of control at the 4th and 5th observation periods only. It is uncerta.in the biological significance of this effect as these time periods (mid-September and early October) represent a time where the colonies are beginning to overwinter as well as the fact that mortality and the number of combs covered by bees was not significantly impacted at any time point. A decline in bee populations (expressed as the numbers of combs covered and brood area), that occurred in August and September (late summer in Poland, where the study was conducted) were stated by the study authors to be due to natural processes of the colonies. All colonies were stated by the study authors to have overwintered successfully. The number of bees that had died during winter was low and similar in all groups (exact numbers not reported from the study). There were no significant differences between the control and treatment hives in the 2012 experiment but overwintering observation were not reported for this trial

Table D-19. Summary of results from Pohorecka et al (2013)

Response variable	Measurement Date	Control (mean ± SD)	Gaucho 600 FS (83 mL/50,000 seeds) (mean ± SD)
2011			
Mortality (dead bees per colony)	12 July – 18 October	132	141
Number of combs covered by bees	12 Jul	20.0 ± 0.0	20.0 ± 0.0
	02 August	17.9 ± 0.9	17.3 ± 1.9 (↓3.3%)
	08 August	16.1 ± 1.2	16.0 ± 2.1
	09 September	9.0 ± 0.0	8.8 ± 0.9 (↓2.3%)
	03 October	8.8 ± 0.4	8.8 ± 1.1
	25 October	7.9 ± 0.7	7.8 ± 0.9 (↓1.0%)

Response variable	Measurement Date	Control (mean ± SD)	Gaucho 600 FS (83 mL/50,000 seeds) (mean ± SD)
2011			
Brood area (dm^2)	12 July	56.7 ± 9.8	53.9 ± 7.3 (↓5.0%)
	02 August	55.3 ± 8.7	53.5 ± 8.4 (↓3.2%)
	08 August	49.0 ±7.2	47.2 ±10.0 (↓3.7%)
	09 September	2.9 ±3.6	**4.2 ± 3.3 (↑44%)**
	03 October	3.9 ± 2.2	**7.3 ±4.3 (↑87%)**
	25 October	0.0	0.0
2012			
Response variable	**Measurement Date**	**Control (mean ± SD)**	**Courase 350 FS (150 mL/50,000 seeds) (mean ± SD)**
Mortality (dead bees per colony)	09 July – 31 August	30	22
Number of combs covered by bees	09 July	20.0 ± 0	20.0 ± 0
	31 July	20.0 ± 0.9	20.0 ± 1.9
	23 August	8.2 ± 1.2	8.2 ± 0.8
Brood area (dm^2)	09 July	57.3 ± 5.3	61.6 ± 11.5 (↑7.5%)
	31 July	55.8 ± 17.3	55.2 ± 10.2 (↓1.1%)
	23 August	67.0 ± 18.4	56.1 ± 5.3 (↑16%)

Bolded values indicate effects was statistically significant ($p<0.05$)

It was evident from the study that the imidacloprid treated maize did not serve as a significant forage source for the bees as indicated by the percentage of total pollen collected (both from traps and combs in both trials) which did not exceed 3%. It was suggested by the study authors that this was likely the reason why there was no imidacloprid detected in the pollen, bee bread, or adult bee samples. Therefore, results from this study ↑have little utility for evaluating the effects of imidacloprid seed treatment on bees, other than suggesting that in this study, bees tended not to forage on corn pollen and preferred other flowering agricultural crops. Potential side-effects of exposure of colonies to blooming maize crops applied with the insecticides as seed dressing might be greater in places intensively sown with maize monocultures without any presence of an alternative flowering crop in adjacent fields.

In addition to the lack of clear demonstration of exposure as demonstrated by the low level of corn pollen collected by the bees in this study, other limitations include: 1) the product Maxim XL 350 FS (a fungicide containing fludioxonil and metalaxyl) was also used on the treated maize. While fludioxonil is not characterized as a systemic fungicide, metalaxyl would be expected to be translocated within the plant and be available in the pollen and nectar with imidacloprid. These fungicides were not assayed in the residue analysis so it is not clear if they were present or interacted with imidacloprid or clothianidin during the course of the study. It is not known to what extent these pesticides affected other relevant endpoints (foraging efficiency, mating success, reproductive output), 2) Purity of both formulations used were not provided, 3) variability in soil characteristics and/or environmental conditions was not reported across the different plots. This information would have potentially been helpful in understanding potential sources of variance in imidacloprid residues in plants and soil, 4) the frequency of pollen collection was not stated, 5) there was no tracking of the various degradation products of imidacloprid within the plant parts. Parent imidacloprid residues are expected to be broken down with the plant with time and degradate to products that have been shown to be of similar toxicity to bees and 6) sample size of pollen loads, bee bread, and bees were relatively small (n ranged from 10 to 30).

Stadler et al (2003) evaluated the effect of imidacloprid on the population development and honey production of beehives subjected to sunflower treated with imidacloprid (Gaucho®) as a seed treatment. Colonies were exposed to the treated sunflower for 10 days after which they were observed for 216 days that included the overwintering period.

The study was conducted for 226 days in Estancia "La Catalina" (Buenos Aires, Argentina). The authors indicated that this study was conducted by University Comahue-Argentina research team in accordance to BBA (1980) and OEPP/EPPO(1992) test guidelines with GLP standards, although few details were provided that documented this statement in the study. The field consisted of two plots, each 24 ha, and planted with 60,000 seeds/ha of sunflower, commercial hybrid (DEKASOL). One plot had seeds treated with Gaucho® FS (imidacloprid 60%; 600ml/100 kg of seed) at a dose of 0.24 mg imidacloprid per seed, and the second plot consisted of untreated seeds, thus being the control plot. Wild flora and other crops adjacent to the study plots were monitored and spots of flowering were removed. Additional information characterizing the nearby flora was not provided.

The study began with hives of approximately 20,000 single *Apis mellifera* bees along with sister queens sharing equal age and attributes. The bees were housed in 16 hives and split into two hive bodies by an excluder to prevent ovi-position on the upper hive body. It was reported that the prevention of bee parasites and infections were handled according to beekeeping practices in Argentina, but no further details such as treatment regimens of the hives, if any, were provided. The hives were established approximately 30 to 35 days prior to initiation of the exposure phase of the study.

Colony assessments were conducted before exposure (after initial acquisition), just after the 10-day exposure (and before relocation away from test field), 28 days post-relocation, and 216 days post relocation to assess overall colony health, behavioral observations, and the production of honey. When the treated sunflower reached 10% bloom, the hives were relocated to the middle of each of the two plots and remained there throughout the flowering period. After a period of 10 days when 80% flowers were without pollen, all beehives were taken to El Gabi Farm, a natural planation in Buenos Aires. The study authors monitored emergence for the following 216 days as well as other response variables that included hive weight, food stores (honey, nectar, and pollen).

During the 10 day exposure phase where hives remained on the seed-treated sunflower fields, mortality was evaluated every 24 h, foraging activity monitored twice daily, and pollen loads were observed and noted in a 3 min interval. For residue analysis, authors used HPLC-MS to detect imidacloprid and its main metabolites in soil, sunflower heads, seeds, wax, honey, and pollen. There were no detected residues of imidacloprid or of its main secondary metabolites (IMI-olefin and IMI-5-OH) in any of the components of the beehives 10 days after their exposure to treated sunflower (LOD < 1.5 ppb).

The populations from treated and control hives presented no significant differences in their development regarding pollen entrance and pollen in the hives, nectar and mortality. However, treated hives were more productive in terms of average weight, honey production, foraging activity, and worker brood and comb foundation probably due to the better physiological state of the treated crop. It was reported that >20%

of the pollen analyzed at 10 days after exposure began and prior to removal of the hives from the treatment fields were form the sunflowers treated for this study, indicating some level of exposure occurred.

No side effects were observed, in the short (10 and 28 days) or in the long-term (216 days) analysis, on the hives exposed to the sunflower plot treated with imidacloprid. The development of the hives or the individual bees was not affected by their exposure during bloom to sunflower plants originated from seeds treated with Gaucho®, under the conditions of the trial.

Table D-20 below summarizes the results of this study. It is noted here, as in the table, that summary data was not provided in the study, only statistical results. Additionally, the results of the last assessment interval (216 days post relocation of the hives) did not appear to be available from the study report. While it was determined that foraging activity was significantly greater in the treated plot compared to the control plot, while mortality and pollen collection (observed at hive entrance) were not significantly different between control and treated hives, the nomenclature used in the summary table of the study regarding statistical significance is not clear, as the use of the asterisk indicating p > 95% is not consistent with the authors' statements in the text.

By the end of the 10-d exposure period, study authors reported a significant increase in mean weight, honey/nectar, pollen, and brood from hives in the treated plots than the control plots. By 28 days after removal from the plots, the authors also reported that the frames surface covered with pollen, nectar and honey was significantly greater in the hives of the treated plots than the control plots. By 216 days after removal from the plots (after overwintering), the authors reported that both groups of hives (treated and control) presented a similar population development, although differences in mean weight, worker brood, comb foundation and honey production in the upper hive body was also observed in T4 before winter. However, it is not clear to upon review if these differences after the overwintering period were statistically significant. All residues of imidacloprid, IMI-olefin, and IMI-5-OH in soil, plant tissue and hive matrices were below the limits of detection used in this study.

Table D-20. Summary of results from Stadler 2013

Response variable	Time point[1]	Effect[2,3]
Hive weight	Before exposure	↑ *
	10 d post exposure, prior to removal from test area	↑ *
	28 d post removal from test area	↑ *
Percent cells occupied with honey and nectar	Before exposure	NS
	10 d post exposure, prior to removal from test area	
	28 d post removal from test area	
Percent cells occupied with pollen	Before exposure	↑ *
	10 d post exposure, prior to removal from test area	↑ *
	28 d post removal from test area	↑ *
	Before exposure	↑ *

Response variable	Time point[1]	Effect[2,3]
Percent cells occupied with worker brood	10 d post exposure, prior to removal from test area	NS
	28 d post removal from test area	↑ *
Percent empty cell	Before exposure	NS
	10 d post exposure, prior to removal from test area	NS
	28 d post removal from test area	↑ *
Foraging activity	Before exposure	Not clear from the article as the summary table differs from the text in the reporting of effects
	10 d post exposure, prior to removal from test area	
	28 d post removal from test area	
Mortality	Before exposure	Not clear from the article as the summary table differs from the text in the reporting of effects
	10 d post exposure, prior to removal from test area	
	28 d post removal from test area	

NS = not significantly different from control (p>0.05); NA = Not assessed

[1] Before exposure time point refers to 35-days post acquisition of the hives

[2] * indicates statistical significance at $\alpha = 0.05$

[3] The means for each response variable not available from the study, only directional effect of statistical significance

This study provides information on the quantity of imidacloprid residues in sunflower and hive products (albeit, below analytical detection) and associated effects on measures of hive health and bee foraging activity. However, the results of this study are of limited value primarily because residues were not detected in sunflowers or soils indicating that exposure was minimal, despite the pollen analysis revealing >20% of the collected pollen originated from sunflowers. It is unclear if the lack of detectable and quantifiable residues of imidacloprid is a function of the soil or other characteristics such as meteorological conditions affecting translocation of imidacloprid in sunflowers. Other limitations include: 1) exposure period of hives was relatively short (10-days); and (3) details of the methods and results were not provided as well as reasons for why no detectable amounts of imidacloprid were found in soil or sunflowers were not provided, 4) Raw or summary data are not reported (only statistical results), 5) descriptive statements about the statistical findings is not clear and ambiguous compared to text, 6) insufficient information on how to determine the performance of control hives, 7) it was stated that the management of bee parasites and diseases was done according to the usual bee keeping practice in Argentina. It is unclear whether these practices include chemical treatment to control for parasites and 8) the methods section states that crops and wild flora in adjacent fields was monitored during the whole study and flowering spots of plants were removed. Presumably, this was to ensure that the test bees foraged only on the sunflower fields used for this study yet it would have been informative to have a greater amount of deta.il regarding the frequency at which these steps were taken or any indications of whether the bees were actually observed to forage on other sources.

Bombus

In a study by Tasei 2001 (previously discussed for the semi-field, tunnel design component) a second component that was a full field design involved two sunflower fields (located in France) where seed-

coating with imidacloprid was not permitted, except in one experimental area. In the experimental area, 90% of sunflower acreage was treated. The treated field (3-km radius) consisted of 1100 acres of sunflowers, including the 40 acre portion in the middle that served as the experimental area. The control field (20 km away) was composed of 1038 acres of sunflower (untreated), that included an 44 acre area in the middle of the field. Seeds from the previous crop, an unspecified cereal in the control field had not been coated with imidacloprid, but had been previously treated in the field serving as treated experimental field. Imidacloprid (as Gaucho, percent a.i not provided) was applied at 0.7 mg/seed. In the beginning of May, the two experimental fields were planted with 70,000 seeds/ha, and the sunflowers started to bloom on July 4 and July 8 for the treated and control fields, respectively. Full bloom was 78 and 73 days after planting for the treated and control fields, respectively.

In June, 20 colonies of bumble bees were acquired for the study with approximately 50 workers each. All foragers were moved to the study fields on July 8th, when all bumble bees were marked with a color spot on the thorax. Ten colonies were placed at even distances in treated and control fields in a single row 10 m long, near an aisle between two lines of plants next to the border of the field. Observations began on July 9th and finished on July 17th when the colonies were relocated to the laboratory, for a total of a 9 day exposure in the field to imidacloprid-treated sunflower. When bees were allowed to forage in the fields, 108 nectar and 133 pollen foragers were sampled in order to distinguish what plant species the bees had foraged. Overall values of nectar and pollen foragers sampled per colony were between 6 to 14 for control and 10 to 15 for treated colonies. Collection of bees was conducted in the morning and afternoon for 6 days.

When colonies were brought to the laboratory, the bees were fed with standard syrup and pollen paste. After 26 days, the study authors recorded marked and unmarked bees to estimate their homing rate during the field period and the growth rate of each colony under standardized settings. At the conclusion of the colony life cycle, emerged queens were captured, recorded, and housed in cages along with male bees for mating purposes.

Table D-21 below summarizes the results of the full field component of this study. Nectar gatherers visited sunflowers almost exclusively in both fields as evidenced by 98% of trapped individuals carried sunflower nectar as the unique or major species. Only 24 and 25% of pollen gatherers in the control and treated field, respectively, carried sunflower pollen. In the control and treated fields, nectar gatherers accounted on average for 40 and 50% of the foragers, respectively. When considering the exposure phase duration (Day 0 to Day 9), the mean loss of marked workers per colony was 33.5% in the treated group as compared to 23.1% in the control group, a difference that was not significant ($p>0.05$). The mean population increase, which was assessed 26 days after the introduction of the hives into the fields, was 86.5 and 78.1 workers/colony in control and treated fields, respectively. This difference was not significant ($p>0.05$). New queens were produced by 8 colonies out of 10 in each field. There were 17 and 24 queens/colony in hives of the control and treated fields, respectively, a difference that was not significant ($p>0.05$).

Table D-21. Summary of results from Tasei 2001.

Response Variable	Control (mean ± SE, if reported)	0.7 mg/seed (mean ± SE, if reported)
Full field Component (9 day exposure)		
Mean loss of marked workers (Day 0-Day 9)	23.1%	33.5%
Population increase (Day 0 – 26)	86.5 ± 17.4	78.1 ± 11.5 (↓8.65%)
Mean number of new queens	17	24 (↑41.2%)

While this study investigated individual and colony level effects to bumble bees resulting from exposure to seed treated sunflower, it is noted here that there are uncertainties as to what extent the bumble bees were exposed. Despite the finding that nectar foragers and pollen foragers had 98 and 25% of their load originating from sunflowers, there were no confirmatory measurements in both the semi-field and field components to indicate that imidacloprid was present in the nectar or pollen collected from the bumble bees. Instead, the authors cited prior work that was conducted in the same fields used for this study in which residues of imidacloprid were detected and therefore conclude that exposure did actually occur. Specifically, for the semi-field portion the authors cite unpublished data from five nectar samples collected by bumble bees. For the field portion of the experiment, soil samples were analyzed but not pollen or nectar. For the soil samples, it was stated that imidacloprid was detected, however the limit of detection was 5 ppb, notably higher than levels reported from other studies. This level of detail is determined to be insufficient for characterizing the exposure for this study.

Other limitations to this study include 1) no information on the purity of imidacloprid, 2) little information on the husbandry of the bumble bees both during the in-field exposure and when the bees were brought back into the laboratory, 3) the study make reference to nectar gatherers and pollen gatherers but it is unclear how this classification system was employed as bumble bees are able to collect both pollen and nectar on foraging trips. Additionally, while it was stated that 108 nectar gatherers and 133 pollen gatherers were sampled, it is unknown as to how many were from control fields vs treated fields.

Appendix E. Registrant Submitted Residue Study Summaries

Foliar Application Studies

Citrus
(MRID 49521301; Murphy et al 2014)

Study Objectives and Design. This study was designed to measure the magnitude of imidacloprid residues in bee-relevant pollen and nectar samples and in/on flowers, and leaves from citrus trees following two pre-bloom foliar applications of 0.25 lbs. a.i/A per year (current U.S. label rate) over two successive years (2012 and 2013). Three separate trials were conducted in Florida citrus groves (NT004, NT005 and NT006), each with a treated (TRTD) and untreated control (UTC) plot. Treated plot sizes ranged from 0.4 to 0.7 acres. It is evident that Trials NT005 and NT006 were located in neighbouring fields near Lake Peal in Umatilla, FL. Trial NT004 was approximately 60 miles to the southeast near Bithio, FL. In general, soil type (sand), plot size, row/plant spacing were similar among the three study sites/trials. The variety of orange differed between NT004/NT005 and NT006 (Valencia vs. Hamlin, respectively). Also, orange trees were considerably younger in the NT004 trial (8 years old) vs. NT005/006 (23-28 years). Total rainfall was similar across sites and each site was irrigated.

Nature of Pesticide Application. Two pre-bloom foliar applications of Admire Pro Systemic Protectant (550 g a.i./L) approximately 0.25 lb a.i./A were applied at least 10 days prior to bloom in successive years at each of the three treatment sites (total annual application rate = 0.5 lb a.i./A). All applications were made with adjuvant (Dyne-Amic, 0.25% to 0.50%) using a ground-based Skid Mount Radiarc Sprayer. The volume of application was similar across trials and years. The interval between the 1st and 2nd applications was 8-10 days. All applications were made between BBCH growth stages 51 and 59 (BBCH 51: inflorescence buds swelling: buds closed, light green scales visible; BBCH 59: most flowers with petals forming a hollow ball), except for year 2 in trial NT004. In that year/trial, applications were made between BBCH growth stages 31 and 61 (BBCH 31: beginning of shoot growth, axes of shoots visible; BBCH 61: beginning of flowering, about 10% of flowers open) in year 2.

Due to an oversight by the Principle Investigator, the plots in trial NT004 were sprayed with PROVADO, an insecticide containing imidacloprid (1.6 lb a.i/gal, corresponding to 0.1 lb a.i/acre application), in both 2010 and 2011, which was not known at the time of the experiment. Additionally, PREY, another insecticide containing imidacloprid, was used as a maintenance pesticide on both the UTRD and UTC plots of this trial in September 2012 and September 2013 (single application of 0.15 lbs./ac each year). **Because of the additional imidacloprid added to the plots prior to the study, the residue values are presumably higher in the NT004 than what would be expected from the study applications alone.**

Residue Sampling. In all trials, five composite samples (separate runs through the plot) of citrus flowers for direct analysis, flowers to be processed for nectar and pollen, and leaves were collected by hand at each sampling period from plot TRTD in years 1 and 2. Each composite sample of flowers contained a minimum of 125 g collected from at least 5 individual trees. Each composite sample of leaves contained a minimum of 100 g collected from at least at least 5 individual trees. Citrus flowers were collected at four sampling periods, when the citrus trees were at growth stages of BBCH 61, 64, 65, and 67 (BBCH 61:

beginning of flowering, about 10% of flowers open; BBCH 67: flower fading, majority of petals fallen), corresponding to 4 to 38 days after the last application (DAA). Exceptions are trial NT006 year 2, when only BBCH 64 and 65 flowers were collected, and trial NT004 year 2, when samples were collected at BBCH 60 and not at BBCH 64. Citrus leaves were also collected but are not discussed further in this summary. Nine soil samples were collected prior to treatment and at the end of the growing season per plot per year, except in trial NT006, when only seven samples were collected prior to the year 2 applications. It is evident that the citrus developed at different rates across different years and trials, such that the DAA varied somewhat for the same BBCH stage.

Analytical Methods and QA/QC. The residues of imidacloprid, IMI-5-OH, and IMI-olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labelled internal standards. For total imidacloprid, the LOQ and LOD were 1 ppb and 0.7 ppb in nectar and 1 ppb and 0.5 ppb in pollen, respectively. All samples were stored within the limits of storage stability trials and spike recoveries were within 70%-120% with a standard deviation of less than 20%.

Magnitude of Residues. Summary statistics of the overall magnitude of total IMI in citrus nectar and pollen are shown in **Table E-1.** These statistics reflect analysis of individual composite samples (usually 5/sampling date) among all of the sampling times within each trial. Within each trial year, mean residues of total IMI in citrus nectar range from **70 - 178 ppb** while maxima range from **79 - 450 ppb**. As noted earlier, multiple inadvertent applications of pesticide containing IMI occurred prior to and during trial NT004. Therefore, these residues may be higher than expected based on the four foliar applications which occurred during the trial.

Within a trial and year, mean residues of total IMI in citrus pollen range from **257 - 4,160 ppb** and maxima range from **480 - 7,400 ppb** (**Table E-1)**. Overall, mean values of total IMI in pollen are about 7X to 45X those measured in nectar. The trial year with the greatest overall concentrations of total IMI in both pollen and nectar is NT004 (2013), although residues levels from trial NT006 (2012) are comparable. This suggests that the contribution of inadvertent IMI applications in trial NT004 did not result in residues which were elevated beyond the realm of environmental variability.

Table E-1. Magnitude of total imidacloprid residues in citrus pollen and nectar

Statistic	Trial NT004		Trial NT005		Trial NT006	
	TRTD1		TRTD1		TRTD1	
	2012	2013	2012	2013	2012	2013
Total IMI in Citrus Nectar (ppb)						
Mean	70	178	111	42	155	39
Min.	36	54	46	17	52	10
Med.	68	150	69	37	120	29
90th	87	361	270	69	298	70
Max.	120	450	280	79	**430**	110
Total IMI in Citrus Pollen (ppb)						
Mean	1107	4160	2664	1877	2780	257

Statistic	Trial NT004		Trial NT005		Trial NT006	
	TRTD1		TRTD1		TRTD1	
	2012	2013	2012	2013	2012	2013
Min.	520	1700	1800	980	1400	87
Med.	1200	3700	2900	1850	2600	330
90th	1520	6770	3300	2460	4060	416
Max.	1600	7400	3400	2700	**4100**	480

Source: MRID 49521301.Total IMI = sum of imidaclopridIMI-5-OH, and IMI-olefin.
Bold values indicate overall maximum values from the study (NT004 data were not used, see text for details).

Across the three trials, the mean proportion of total IMI as olefin-IMI and 5-OH-IMI in nectar ranged from 4-10% and that for pollen ranged from 5-8%.

Temporal Variability in Residues. Plots of the mean concentrations of total IMI vs. sampling time (relative to the last application) are shown in **Figure E-1**. With the exception of trial NT004 (2012), concentrations of total IMI in nectar declined substantially with time from 4 DAA through up to 45 DAA. The dissipation half-life (DT_{50}) values could be reliably calculated for 3 trial years with nectar. These DT_{50} values are **8.9 days** (NT005, 2012), **14.1 days** (NT005, 2013) and **10.1 days** (NT006, 2012). Unlike nectar, concentrations of total IMI in citrus pollen did not decline as rapidly as seen for nectar in most trials. For three trial years, total IMI in pollen remained relatively constant with time (both years in NT005, year 1 in NT006). This may reflect differences in the temporal dynamics associated with imidacloprid translocation into pollen vs. nectar. Only one DT_{50} value could be calculated (15.8 days for NT006, 2012). An important limitation to these DT_{50} values is that they reflect a targeted pre-bloom application interval of 10 days. While this is consistent with existing label restrictions with citrus, the DT_{50} values may not be appropriate when applied to other situations (*e.g.,* for estimating residues associated with shorter pre-bloom intervals).

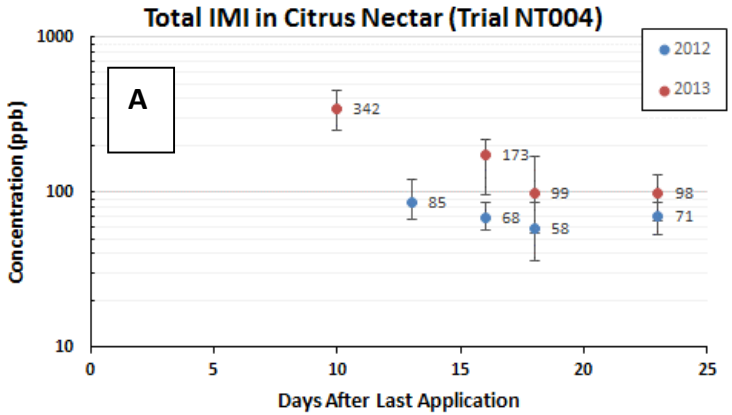
Spatial Variability in Residues. All three sites for the citrus study were in relatively close proximity. In fact, two sites (NT005 and NT006) were adjacent. Soil types reflect sandy compositions (96-98%) and low organic carbon content (0.35-1.9%). As expected, weather conditions (temperature and precipitation) were similar across the three trials. As a result of the close proximity of trial sites, this study provides very limited information on how differences in environmental conditions across different areas of the US may affect accumulation of total IMI in pollen and nectar.

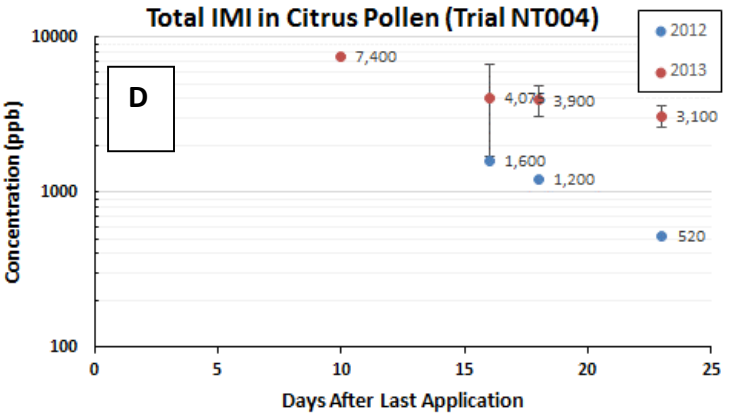
Pesticide Carryover. The extent to which prior year applications of imidacloprid contributed to year-to-year carryover in nectar and pollen concentrations could not be reliably assessed due to several study limitations. These include the inadvertent pesticide applications in trial NT004 and more importantly, the different sampling times (and BBCH growth stages) employed during the two trial years (NT005 and NT006). Based on the results from soil sampling for NT005 and NT006, total IMI concentrations at the end of the second growing season (57 and 44 ppb, respectively in 2013) declined or were comparable to those from the end of the first growing season (120 and 54 ppb, respectively in 2012). However, since the application method was foliar spray, concentrations in soil may not be reliable indicators of pesticide year-to-year carryover.

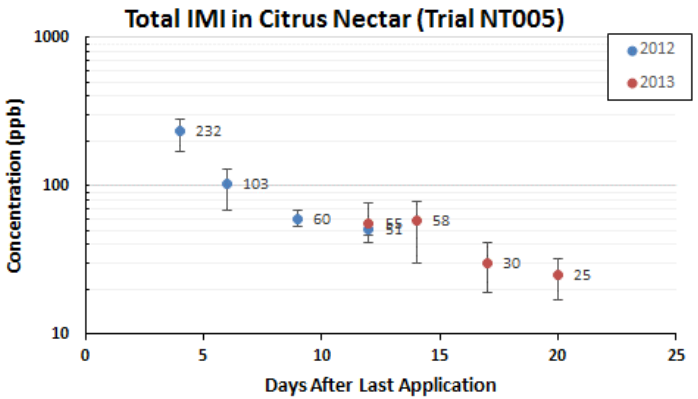
Classification/Utility for Bee Risk Assessment. This study is classified as supplemental primarily due to inadvertent pesticide applications which may have confounded results from trial NT004. Results from trials NT005 and NT006 are considered acceptable for quantitative use in risk assessment, although are essentially considered replicate trials due to their close proximity to each other.

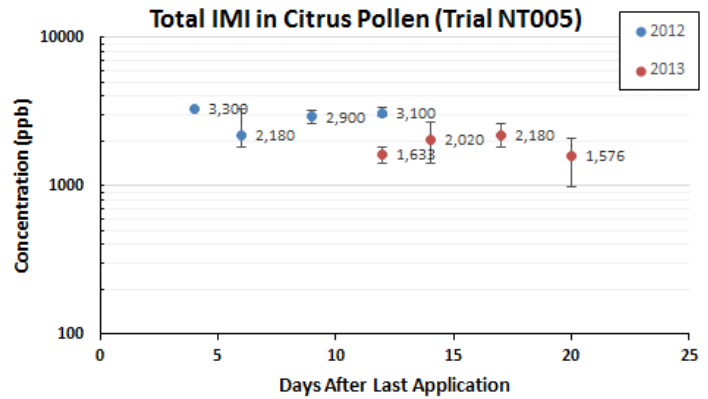
Reference

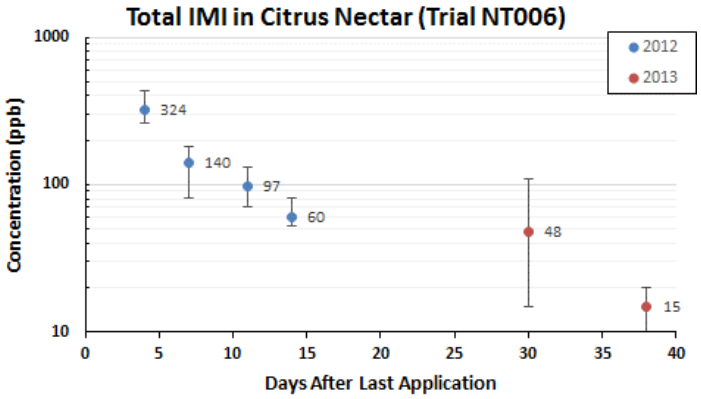
Murphy, I., Bowers, L., Dyer, D., and Jerkins, E. (2014). ADMIRE PRO - Magnitude of the Residues of Imidacloprid and its Metabolites IMI-5-OH and IMI-olefin in Bee Relevant Matrices Collected from Citrus Trees following Foliar Applications of Imidacloprid Over Two Successive Years. Bayer Crop Science, Research Triangle Park, NC. MRID 49521301.











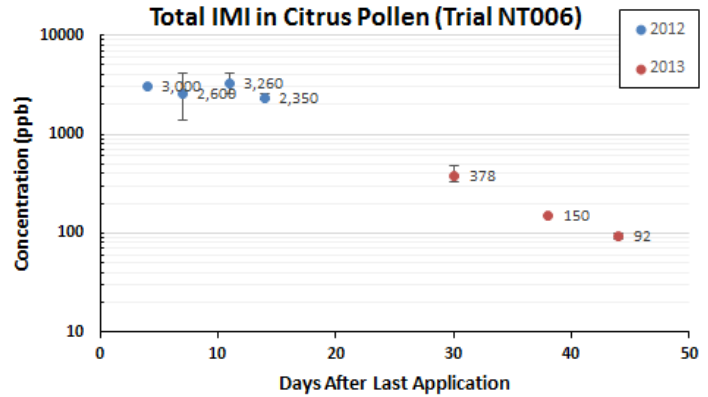


Figure E-1. Mean (and range) of total IMI concentrations reported in citrus nectar and pollen

Cherry
(MRID 49535601; Miller et al., 2014)

Study Objectives and Design. This study was designed to measure the magnitude of imidacloprid residues in bee-relevant pollen and nectar samples and in/on flowers, leaves from cherry trees following five post-bloom foliar applications of 0.10 lbs. ai/A per year (current maximum U.S. label rate) over two successive years (2012 and 2013). Four separate trials were conducted in New York and Oregon cherry orchards (NT007, NT008, NT016 and NT017), each with a treated (TRTD) and untreated control (UTC) plot. Treated plot sizes ranged from 0.2 to 0.5 acres. It is evident that the two NY trial sites (NT007 and NT008) were in close proximity as were the two OR sites (NT016 and NT017). As a result, the NY sites shared the same weather data as did the OR sites (Figure 1). Therefore, to the extent that variation in weather affects residue levels in cherry trees, the NY sites and OR sites can be considered replicates from a climate perspective. In general, soil type (sandy loam), tree age, and row/plant spacing were similar among the four study sites/trials. The variety of cherries tested included Montmorency (NT007/NT008), Skema (NT0016) and Bing (NT017). The NY trial sites received much greater rainfall during the summer of 2013 compared to the OR sites. As a result, the NY trial sites were not irrigated during the study period while the OR sites were irrigated (2-6 in/month from May-Sept of each year).

Nature of Pesticide Application. Five foliar applications of approximately 0.1 lbs. a.i./A were applied at intervals from 8-11 days apart at each of the four treatment sites (total annual application rate = 0.5 lbs. a.i./A). All applications were made with adjuvant (Dyne-Amic, 0.3%) using a ground-based air blast equipment. The volume of application was similar across trials and years. All first year applications were made after cherry harvest at BBCH growth stages 91 (shoot growth completed, foliage still fully green). All second year applications were initiated before cherry harvest between BBCH growth stages 73 and 75 (second fruit fall – fruit about half size).

Residue Sampling. Samples that were treated in 2012 were harvested in 2013 (this period inclusively considered year 1 of the study), and samples treated in 2013 were harvested in 2014 (year 2 of the study**.** Each treated plot (TRTD) was divided into 5 subplots containing 3 trees each. A single composite sample cherry flowers (for direct analysis), flowers (for nectar and pollen), and leaves was collected by hand at each sampling period from each subplot (5 composites/sampling event). In trial NT017, no samples after the fall 2013 applications were collected because the trees were removed from the orchard by the grower. Samples were taken from the same trees at each sampling interval and in successive years. Cherry flowers were collected when the trees were at growth stages of BBCH 61 (beginning of flowering, about 10% of flowers open) and BBCH65 (full flowering, at least 50% of flowers open and first petals falling). These sampling times corresponding to 205 to 218 days after the last application (DAA) in year 1 and 274 to 303 DAA in year 2, except for trial NT007 from which BBCH 61 flowers were not collected in year 2 due to weather. Cherry leaves were collected but are not discussed further in this summary. Nine soil samples were collected twice during the each year of the study and generally corresponded to pre-application and post residue sampling time periods.

Analytical Methods and QA/QC. The residues of imidacloprid, IMI-5-OH, and IMI-olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and

LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labelled internal standards. For total imidacloprid, the LOQ and LOD were 1 ppb and 0.7 ppb in nectar and 1 ppb and 0.5 ppb in pollen, respectively. All samples were stored within the limits of storage stability trials and spike recoveries were within 70%-120% with a standard deviation of less than 20%.

Magnitude of Residues. Summary statistics of the overall magnitude of total IMI in cherry nectar and pollen are shown in **Table E-2.** These statistics reflect analysis of individual composite samples (usually 5/sampling date) among all of the sampling times within each trial. Within each trial year, mean residues of total IMI in cherry nectar range from **1.1 -4.8 ppb** while maxima range from **2.0-10 ppb**. Values near 1 ppb reflect non-detect samples where concentrations are assumed to be ½ the detection limit.

Within a trial and year, mean residues of total IMI in citrus pollen range from **1.7 - 422 ppb** and maxima range from **2.6 – 1,000 ppb** (**Table E-1)**. Overall, mean values of total IMI in pollen are up to 100X those reported in nectar. The 2013 trial years of NT007, NT008 and NT016 showed the greatest mean and maximum of nectar, while just the 2013 year of NT007 and NT008 showed the greatest concentrations for pollen. The reason for the generally large variation between the NY and OR trial sites is not clear, but may be partially a function of the heavy precipitation received at the NY trial sites during summer 2013.

Table E-2. Magnitude of total imidacloprid residues in cherry nectar and pollen

	Trial NT007 (NY)		Trial NT008 (NY)		Trial NT016 (OR)		NT017 (OR)	
Statistic	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**
Total IMI in Cherry Nectar (ppb)								
Mean	4.8	1.4	3.9	1.1	3.4	1.7	1.6	*n.d.*
Min.	2.4	1.1	1.1	0.8	1.9	1.1	0.8	*n.d.*
Med.	4.7	1.1	3.1	1.0	3.1	1.2	1.1	*n.d.*
90th	7.7	n.d.	7.1	1.4	4.7	2.2	2.2	*n.d.*
Max.	8.3	2.0	**10.0**	2.1	6.0	4.9	3.4	*n.d.*
n	*10*	*5*	*10*	*10*	*10*	*10*	*10*	*n.d.*
Total IMI in Cherry Pollen (ppb)								
Mean	153	21	422	18	13	1.7	21	*n.d.*
Min.	87	5.8	140	6.9	4.8	1.0	1.8	*n.d.*
Med.	130	15	400	12	7.6	1.7	12	*n.d.*
90th	197	n.d.	658	36	28	2.5	46	*n.d.*
Max.	350	58	**1000**	52	38	2.6	67	*n.d.*
n	10	5	10	10	10	10	10	*n.d.*

Source: MRID 49535601.Total IMI = sum of imidacloprid, 5-OH imidacloprid, and IMI-olefin.
Bold values indicate overall maximum values from the study. The level of quantification of total IMI in nectar and pollen was 0.7 and 1.0 ppb, respectively. N.D. = not determined.

The proportion of total IMI occurring as parent and metabolites (IMI-olefin, IMI-5-OH) could not be reliably determined for nectar samples since a large fraction of residue data were below the LOQ or LOD. With pollen in trials NT007 and NT008, the mean % of total IMI as IMI-olefin in pollen was 6% (range 2-17%) and the mean % of total IMI as IMI-5-OH was 8% (range 2-29%). Parent IMI was present at 86% of total IMI in pollen on average (range 61-97%).

Temporal Variability in Residues. Plots of the mean concentrations of total IMI vs. sampling time (relative to the last foliar application) are shown in **Figure E-2** for nectar and **Figure E-3** for pollen. Within each trial, it can be seen that applications during **late summer/early fall** resulted in greater residues measured in nectar during year 1 (2013) compared to applications made in **late spring / early summer** as subsequent measurement in year 2 (2014). The lower residue values measured during year 2 may reflect the greater amount of time available for dissipation of IMI residues between application and residue measurement (275-300 days) compared to that from year 1 (about 205-215 days). Lower residues in year 2 may also reflect temporal differences in imidacloprid translocation to nectar when applied in late summer/early fall vs. spring/early summer. It should be noted that except for 1 value from trial NT016, residues of total IMI in nectar from year 2 (2014) largely reflect levels below the limit of analytical detection or quantification where ½ the LOD or LOQ was assumed.

Spatial Variability in Residues. The two trials within NY and OR were each within close proximity, such that they shared the same weather data. Soil types reflect sandy loam compositions (53-67% sand) and moderate to low organic carbon content (0.9-3.4%). Although within NY and OR the sites were in close proximity, this study at least encompasses regional differences in cherry cultivation which may affect accumulation of total IMI in pollen and nectar.

Pesticide Carryover. A qualitative evaluation of the potential for 'year-to-year' accumulation of total IMI residues in nectar and pollen could be conducted for 3 of the 4 trials, since data from year 2 were missing from trial NT017. Examination of Figures 2 and 3 (and Table 8 and 9) indicates that total IMI residues in nectar and pollen decline from year 1 to year 2. Similarly, residues of total IMI in flowers and leaves also decline from year 1 to year 2. Although this suggests that year-to-year accumulation of total IMI is not evident, the impact of differences in pesticide application timing between year 1 to year 2 on the resulting residues in pollen and nectar is not clear. In other words, the lower residues measured in year 2 may reflect longer time

Classification/Utility for Bee Risk Assessment. This study is classified as acceptable and appropriate for quantitative use in risk assessment.

Reference

Miller, A., Bowers, L., Dyer, D., and Jerkins, E. (2014). Determination of the Residues of Imidacloprid and its Metabolites IMI-5-OH and IMI-olefin in Bee Relevant Matrices Collected from Cherry Trees following Foliar Application of Imidacloprid over Two Successive Years. Study ID EBNTY008. Bayer Crop Science, Research Triangle Park, NC. MRID 49535601.

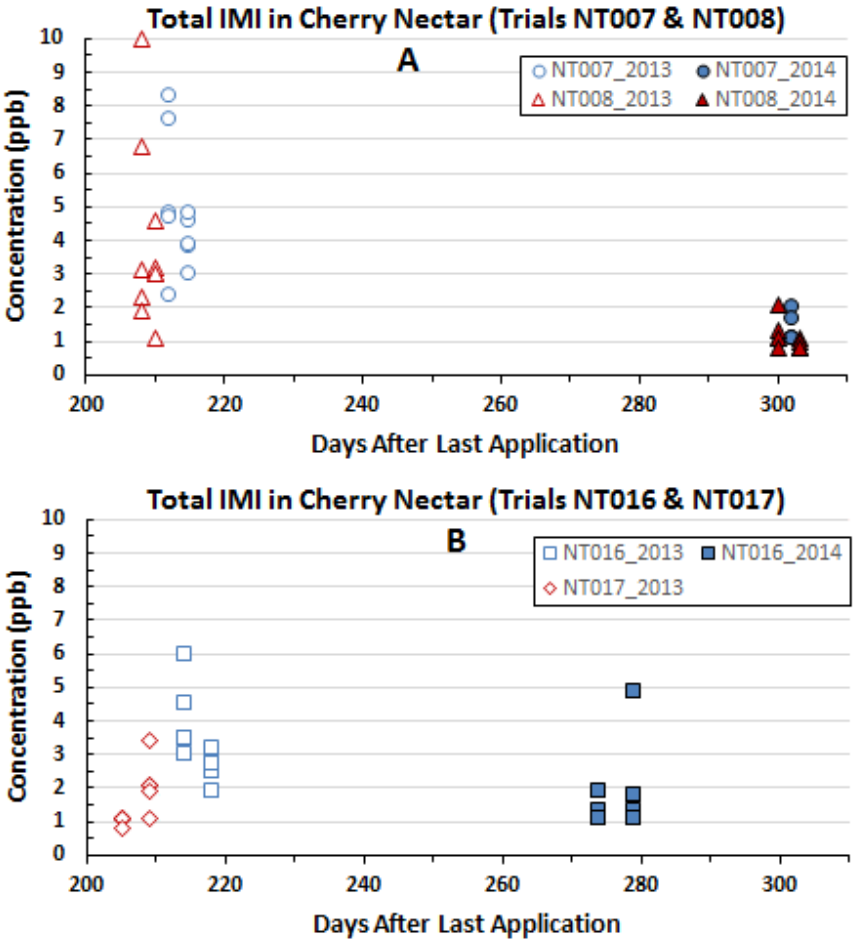


Figure E-2. Occurrence of total IMI in cherry nectar following post-bloom foliar applications (5 x 0.1 lbs. ai/A)/yr. of imidacloprid

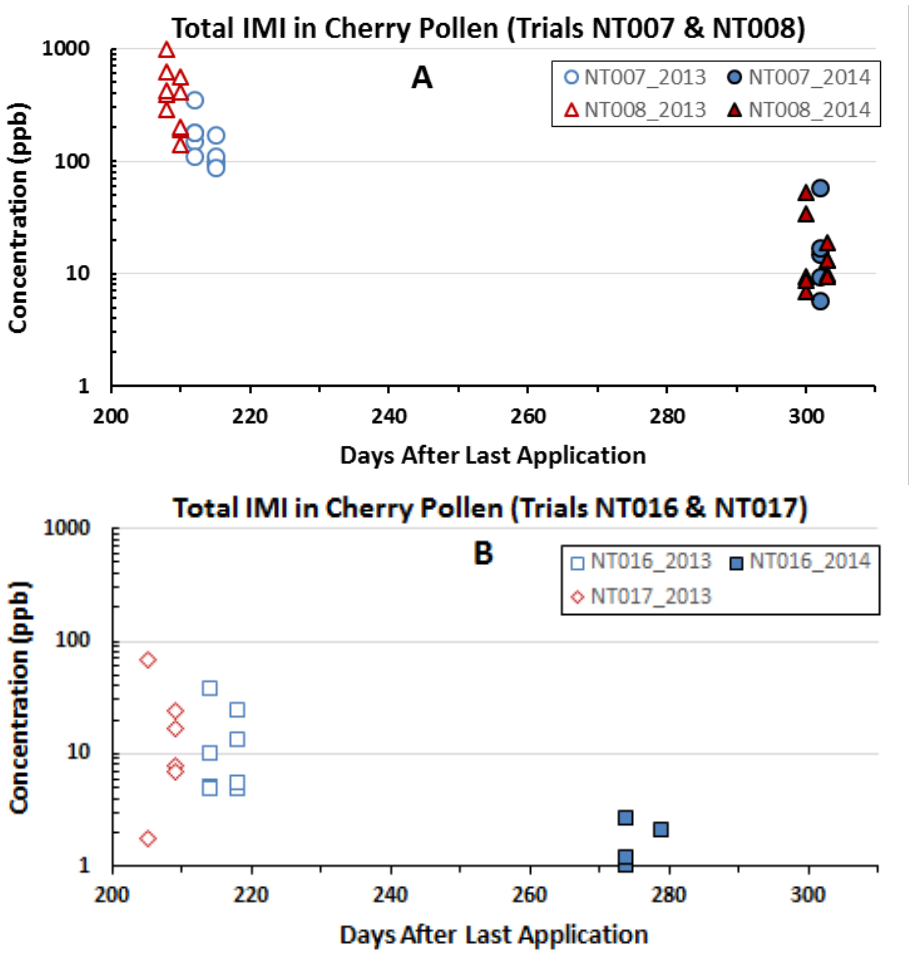


Figure E-3 Occurrence of total IMI in cherry pollen following post-bloom foliar applications (5 x 0.1 lbs. ai/A)/yr. of imidacloprid

Cotton

(MRID 49103301; Beedle and Harbin 2011)

Study Objectives and Design. This study, conducted in 2010 in Kern County, California, measured the concentrations of imidacloprid and its metabolites (IMI-5-OH and IMI-olefin) in leaves and nectar samples. The cotton was grown in locations treated with one aerial application of imidacloprid in 2010 and at least one chemigation application in preceding years. The 2010 application rates were 0.063 lbs. ai/A (well below the maximum label rate of 0.5 lbs. ai/A). Chemigation applications in previous years (2009 and/or 2008) ranged from 0.22 to 0.38 lbs. ai/A/year. Bees were not used to collect nectar in this study. Samples of cotton nectar and leaves were collected by hand during flowering. All five field sites had fine textured soil.

Nature of Pesticide Application. All five sites had aerial (foliar) applications of 0.063 lbs. ai/A in 2010. Four of the five sites had chemigation applications in 2008 and 2009 (NT001, NT002, NT003, NT005). One of the five sites had two chemigation applications in 2009 but no application at all in 2008 (NT004). The end products used were Provado 1.6F (for aerial applications) and Admire Pro (for chemigation).

Residue Sampling. Quadruplicate samples of cotton nectar and leaves were collected during flowering. Pre-application samples were collected two to seven days before the 2010 aerial application. Post-application samples were collected six days after the 2010 aerial application. Nectar and leaf samples were collected from the same plants. Leaf samples were 500 grams and were composed of leaves collected from high, low, inside, and outside areas of each plant. Nectar samples were one gram and were collected from a minimum of 20 plants. This study did not distinguish between floral and extra-floral nectar.

Analytical Methods and QA/QC. Residues of imidacloprid, IMI-5-OH, and IMI-olefin were analyzed using high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) using stable, isotopically labelled internal standards. The Limit of Quantification (LOQ) for of imidacloprid, IMI-5-OH, IMI-olefin, and total imidacloprid was 1 ppb for nectar, and 2 ppb for leaves. The Limit of Detection (LOD) values varied according to the matrix and the analyte. Samples were frozen before analysis for a maximum of 149 days. The overall mean values of the recoveries for each matrix ranged from 70% to 120%.

Magnitude of Residues. Total imidacloprid was calculated as the sum of imidacloprid, IMI-5-OH, and IMI-olefin in parent equivalents. Summary statistics of the overall magnitude of total imidacloprid in cotton nectar and leaves are shown below in **Table E-3.** In cases where individual analyte residues were less than the LOD, a value of half the LOD was used to calculate total imidacloprid. The average total imidacloprid residue in cotton leaves in the five sites ranged from 4.3 ppb to 23 ppb before the 2010 aerial (foliar) application. Then, six days after application, the average total imidacloprid residue in leaves ranged from 230 ppb to 1700 ppb. The average total imidacloprid residue in nectar ranged from 1.4 ppb to 4.2 ppb prior to the 2010 aerial application. Then, six days after application, the average total imidacloprid residue in nectar ranged from 16 ppb to 56 ppb.

Table E-3. Magnitude of total imidacloprid residues in cotton nectar and leaves.

Commodity	Soil Texture Category[a]	Days After Treatment (DAT)[b]	Total Imidacloprid Residue Levels (ppb)[c]						
			N	Min	Max	Highest Avg. Site Residue	Median	Mean	Standard Deviation
Cotton Nectar	Fine	Pre-App (-7 to -2 DAT)	10	1.2	433	4.2	2.7	2.8	1.0
Cotton Nectar	Fine	Post-App (6 DAT)	10	13	66	56	21	29	18
Cotton Leaves	Fine	Pre-App (-7 to -2 DAT)	10	3.3	25	23	14	14	7.8
Cotton Leaves	Fine	Post-App (6 DAT)	10	150	1900	1700	360	770	670

[a] Classification of the soils was obtained from the Soil Survey Geographic (SSURGO) Database provided by the Natural Resources Conservation Service and converted to soil texture categories.

[b] Duplicate samples of nectar and leaves from the five trials were analyzed at pre- and post- application sample intervals.

[c] Abbreviations used are as follows: Min is the lowest treated residue value; Max is the highest treated residue value; Median is the geometric median of the treated residue values; Mean is the mathematical average of the treated residue values; Standard Deviation is the standard deviation for a small population of "N" samples.

Temporal Variability in Residues. Samples were collected 2-7 days before and 6 days after the aerial application in 2010 but a decline in residues was not recorded because only one time point was sampled after application. This study was not designed for temporal analysis of declining concentrations, but rather, to provide a snapshot of residue concentrations during flowering.

Spatial Variability in Residues. All five sites were in Kern County in the Southern Central Valley (i.e., San Joaquin Valley) of California. All of the sites had similar climatic conditions. All five sites had soil with fine texture. Medium and coarse soils were not included in this study.

Pesticide Carryover. The extent to which prior year applications of imidacloprid contributed to year-to-year carryover was not analyzed by the study authors.

Classification/Utility for Bee Risk Assessment. This study is classified as supplemental because the application rate is below the maximum annual application rate and therefore does not represent a worst case scenario). This study was repeated and residues have been shown to be much higher when soil and foliar applications are made in the same season and the maximum annual application rate is used.

Reference

Beedle, E.C., and A.M. Harbin (2011). Determination of the Residues of lmidacloprid and its Metabolites 5-Hydroxy lmidacloprid and lmidacloprid Olefin in Bee Relevant Matrices Collected from Cotton, Grown at Locations Treated with lmidacloprid at Least Once Per Year During Two Successive Years. Project Number: EBNTL056-01. Bayer CropScience, Stilwell, KS. 147p. MRID 49103301.

Soil Application Studies

Tomato

(MRID 49090503; Freeseman and Harbin, 2011)

Study Objectives and Design. Studies were conducted to determine the residues of imidacloprid and its metabolites (IMI-5-OH and IMI-olefin) in anthers (pollen) and leaves collected from tomato plants grown at locations treated with imidacloprid at least once per year for two years. Nine trials were conducted in fine-textured clayey or medium-textured loamy soils, classified as "heavy" or "medium" respectively, in the study report. All sites were located in California, in either Kings County (four fine-textured soils and two medium-textured soils) or Kern County (three medium-textured soils). The sites in Kern County typically had a higher average maximum temperature than those located in Kings County, however total rainfall was similar between all sites. Each site was irrigated as needed.

Nature of Pesticide Application. Each trial received application(s) of Admire Pro in 2010 at the same rates as in 2009. The six sites located in Kings County received two applications of Admire Pro at 3.5 fl oz. product/A/application (0.13 lbs. ai/A/application) for a total seasonal rate of 7.0 fl oz. product/A (0.25 lbs. ai/A). The first applications were made at or closely following transplanting with the second applications 52 to 57 days following the first applications. The three sites located in Kern County received a single application of Admire Pro at 0.18 lbs. ai/A (5.0 fl oz. product/A) 2 to 25 days following transplanting.

The growth stages of the plants at the times of applications were not documented but likely occurred at growth stages of BBCH 21 to 51 (first primary shoot to first inflorescence visible) for the first applications and BBCH 61 to BBCH 69 (flowering but prior to fruiting) for the second applications. All applications were made through drip chemigation (buried lines).

Imidacloprid use in 2008 and prior years was unknown.

Residue Sampling. Quadruplicate samples of tomato anthers and leaves were collected from tomato plants at indeterminate flowering and fruiting growth stages as typical for solanaceous fruits (BBCH 61 to BBCH 79). The intervals from last imidacloprid application to sampling ranged from 72 to 102 days for the nine trials. Anthers and leaves were collected from the same plants. A sample weight of approximately 0.5 anthers was collected from a minimum of 20 plants. For every anther collected, a leaf sample was also collected. Leaf samples were a composite from high, low, inside, and outside areas of the plant.

Analytical Methods and QA/QC. The residue(s) of imidacloprid, IMI-5-OH, and IMI-olefin were quantified by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) using stable isotopically labeled internal standards. The total imidacloprid residue is the sum of the individual measured residue values of imidacloprid, IMI-5-OH, and IMI-olefin in parent equivalents. For the purpose of calculating total imidacloprid residues where individual analyte residue values were less

than the limit of detection (LOD), the residues were assigned a finite value of half the value of the respective LOD.

All samples extracts were analyzed within two days of extraction. Acceptable recoveries measured concurrently with each set of samples ensured the integrity of the sample extract during the period of time between extraction and analysis.

Transit stability samples (control anthers and leaves fortified with imidacloprid, IMI-5-OH, and IMI-olefin) monitored the stability of the analytes during sampling, transit, and storage. The average recovery of all analytes in these samples ranged from 79% to 99%, demonstrating that residues were stable under the practices used in this study. The maximum storage period of frozen samples in this study was 158 days for tomato anthers and 164 days for tomato leaves.

The residue data were not corrected for any in-storage decomposition.

Magnitude of Residues. Summary results for concentration of total imidacloprid in anthers are summarized in **Table E-4** The average total imidacloprid residue found in tomato anthers grown in fine-textured soil was 21 ppb (n=8). The average total imidacloprid residue found in tomato anthers grown in medium-textured soil was 34 ppb (n=10). The average total imidacloprid residue found in tomato leaves grown in fine-textured soil was 93 ppb (n=8). The average total imidacloprid residue found in tomato leaves grown in medium-textured soil was 110 ppb (n=10).

The maximum total residues in tomato anthers were measured to be 30 ppb (mean ± SD, 21 ± 5) and 54 ppb (mean ± SD, 34 ± 12) in tomato anthers grown in fine and medium-textured soils, respectively. Concentrations were similar between the two soil types.

Table E-4. Magnitude of total imidacloprid residues in tomato anthers and leaves.

Commodity	Soil Texture Category[a]	Days After Last Treatment (DALT)[b]	Total Imidacloprid Residue Levels (ppb)[c]						
			N	Min	Max	Highest Avg. Site Residue	Median	Mean	Standard Deviation
Tomato Anthers	Fine	72-79	8	14	30	27	22	21	5
Tomato Anthers	Medium	79-102	10	16	54	46	36	34	12
Tomato Leaves	Fine	72-79	8	57	140	120	89	93	26
Tomato Leaves	Medium	79-102	10	38	230	200	100	110	61

[a] Classification of the soils was obtained from the Soil Survey Geographic (SSURGO) Database provided by the Natural Resources Conservation Service and converted to soil texture categories.

[b] All trials received one or two applications of Admire Pro. DALT are the days following the last application when two applications were made.

[c] Abbreviations used are as follows: Min is the lowest treated residue value; Max is the highest treated residue value; Median is the geometric median of the treated residue values; Mean is the mathematical average of the treated residue values; Standard Deviation is the standard deviation for a small population of "N" samples.

Temporal Variability in Residues. The intervals from last imidacloprid application to sampling ranged from 72 to 102 days for the nine trials. This study was not designed to measure temporal variability in residues.

Spatial Variability in Residues. All sites were located in Kern or Kings County. The sites in Kern County typically had a higher average maximum temperature than those located in Kings County; however, total

rainfall was similar between all sites. There were no analyses of effects of soil type or location on imidacloprid residues in plant matrices in this study. However, it seems that imidacloprid residues in both anthers and leaves are slightly higher in tomatoes grown in medium soil compared to heavy soil.

Pesticide Carryover. This study was not designed to measure pesticide carryover. All samples were taken in 2010, 72 to 102 days after the last application.

Classification/Utility for Bee Risk Assessment. The study is considered to be informative but does not represent the worst-case application scenario due to the low test rates. The tested rate of 0.18 lbs. ai/A (equivalent to 202 g ai/ha) is lower than the labeled maximum soil application rate for tomato in the U.S. (0.5 lbs. ai/A) and Canada (559.9 g ai/ha). This study will be considered as an additional line of evidence for the estimation of exposure to bees.

Reference

Freeseman, P.L.; Harbin, A. (2011) Determination of the Residues of Imidacloprid and its Metabolites IMI-5-OH and IMI-olefin in Bee Relevant Matrices Collected from Tomato, a Fruiting Vegetable, Grown at Locations Treated with Imidacloprid at Least Once Per Year During Two Successive Years: AMENDED. Project Number: EBNTL056/05/1/AMENDED. Unpublished study prepared by Bayer Cropscience and California Agricultural Research, Inc. 144p. MRID 49090503.

Melon

(MRID 49090501; Beedle 2012)

Study Objectives and Design. This study, conducted in 2011 in the Imperial Valley in Southern California, measured the concentrations of imidacloprid and its metabolites (IMI-5-OH and IMI-olefin) in melon leaves and bee-collected pollen and nectar samples. The melons were grown in locations treated with imidacloprid at least once annually from 2008 to 2011. The 2011 application rates ranged from 0.00 to 0.38 lbs. ai/A/application. Two mesh tents were placed in ten field sites, with each tent (24' x 100' x 10' tall) covering three rows of melons, shortly after the beginning of flowering in 2011. Each tent contained one honey bee colony and each site contained a treated area of at least one acre. Five of the field sites had fine textured soil and the other five field sites had medium textured soil.

Nature of Pesticide Application. All applications were made between 2008 and 2011 through chemigation either by injection to a drip irrigation system or with a drench application over the seed-line. ADMIRE PRO® Insecticide was applied at rates ranging from 0.23 to 0.38 lbs. ai/A/application (0.26 to 0.43 kg ai/ha/application). The study author stated that melons were "grown at locations treated with imidacloprid at least once per year during two successive years" but two of the sites (NT204 and NT205) only had a single application in 2011, with no applications in 2009 and 2010, and with no data for 2008. Four of the sites (NT202, NT203, NT207, and NT208) had applications in 2009 and 2011, but not in 2010 (i.e., not successive). One of the sites (NT206) had no application at all in 2011, when samples were collected, only in 2009 and 2010. That site (NT206) had two applications of imidacloprid in 2009, but this is only reflected in one of the two application history tables presented in the report. Another two sites (NT209 and NT210) had applications in 2008, 2009, and 2011, but not in 2010. Only NT 201 had two successive applications in 2010 and 2011.

Residue Sampling. There are issues with the methods employed to collect samples of nectar and pollen in this study. When the colonies were moved into the tent new brood frames were introduced into the hives but the bees did not deposit any nectar into these frames. Therefore, composite nectar samples were collected from brood frames starting one to two days after the hives were placed into the tents. Nectar sample collection continued for two weeks but the majority of nectar samples were collected from 9 to 11 days after the hives were placed in the tents. The study author stated that nectar stores were low because this variety of melon is a "minimal nectar resource to bees." Pollen was collected using plastic pollen traps mounted to the hive entrances. Composite pollen samples consisted of pollen that was allowed to accumulate in the pollen traps over a period of two to three weeks. Leaf samples were composited from at least twelve plants in each tent. Basal, terminal, and middle leafs were collected from each plant sampled.

Analytical Methods and QA/QC. Residues of imidacloprid, IMI-5-OH, and IMI-olefin were analyzed using high performance liquid chromatography/tandem mass spectrometry (LC/MS/MS) using isotopically labelled internal standards. The Limit of Quantification (LOQ) for imidacloprid, IMI-5-OH, IMI-olefin, and total imidacloprid was 1 ppb for nectar, and 10 ppb for pollen and leaves. The Limit of Detection (LOD) values varied according to the matrix and the analyte. Samples were frozen before analysis for a maximum

of 20 months but freezer storage stability studies have indicated that degradation is less than 30% during 24 months of freezer storage using other crops. Freezer temperatures varied from -18.8°C to -20.0°C during the study but it is unclear at what temperature the freezer storage stability studies were conducted.

Magnitude of Residues. Summary statistics for concentration of total imidacloprid in melon nectar, pollen and leaves are contained in **Table E-1**. Total imidacloprid was calculated as the sum of imidacloprid, IMI-5-OH, and IMI-olefin in parent equivalents. In cases where individual analyte residues were less than the LOD, a value of half the LOD was used to calculate total imidacloprid. Values that were less than the LOQ but greater than the LOD were used to calculate the sum, even though they were below the LOQ, but if the sum was less than the LOQ for total imidacloprid then the total imidacloprid concentration was reported as <LOQ.

In all matrices (nectar, pollen, and leaves) concentrations of total imidacloprid were higher in melons grown in medium soil than in fine soil. Melons grown in coarse soil were not included in this study. The maximum total residues in melon pollen were measured to be 12 ppb (mean ± SD, <10 ± 2.8) and 32 ppb (mean ± SD, 13 ± 8.6) grown in fine and medium-textured soils, respectively. The maximum total residues in melon nectar were measured to be 5.3 ppb (mean ± SD, 3.0 ± 1.5) and 8.0 ppb (mean ± SD, 3.9 ± 2.5) grown in fine and medium-textured soils, respectively. The maximum total residues in melon leaves were measured to be 28 ppb (mean ± SD, 16 ± 6.7) and 71 ppb (mean ± SD, 27 ± 25) grown in fine and medium-textured soils, respectively.

Table E-5. Magnitude of total imidacloprid residues in melon pollen, nectar and leaves.

Commodity	Soil Texture Category[a]	Total Imidacloprid Residue Levels (ppb)[b]						
		N	Min	Max	Highest Avg. Site Residue	Median	Mean	Standard Deviation
Bee collected (hive deposited) melon nectar	Fine	10	1.2	5.3	3.9	2.4	3.0	1.5
Bee collected (hive deposited) melon nectar	Medium	10	1.6	8.0	4.9	3.0	3.9	2.5
Bee collected (pollen traps) melon pollen	Fine	10	<10	12	11	<10	<10	2.8
Bee collected (pollen traps) melon pollen	Medium	10	<10	32	19	<10	13	8.6
Melon leaves	Fine	10	<10	28	27	13	16	6.7
Melon leaves	Medium	10	<10	71	55	10	27	25

[a] Classification of the soils was obtained from the Soil Survey Geographic (SSURGO) Database provided by the Natural Resources Conservation Service and converted to soil texture categories.

[b] Abbreviations used are as follows: Min is the lowest treated residue value; Max is the highest treated residue value; Median is the geometric median of the treated residue values; Mean is the mathematical average of the treated residue values; Standard Deviation is the standard deviation for a small population of "N" samples.

Temporal Variability in Residues. Applications were made from January 3 to January 20, 2011 (except the one site that did not receive any application in 2011), and sampling was conducted from April 23 to May 8, 2011. This study was not designed for temporal analysis of declining concentrations, but rather, to provide a snapshot of residue concentrations during flowering.

Spatial Variability in Residues. All ten sites in this study were in Imperial County in Southern California. All of the sites had similar climatic conditions. Five of the sites had soil with fine texture and five sites had soil that was described as medium texture. Coarse soil was not included in this study. Concentrations of total imidacloprid were higher in melons grown in medium soil than in fine soil.

Pesticide Carryover. The extent to which prior year applications of imidacloprid contributed to year-to-year carryover was not analyzed by the study authors. There was substantial variability in the application history but the study authors did not consider application history in their data analysis. Therefore, the effects of pesticide carryover in melons are unknown.

Classification/Utility for Bee Risk Assessment. This study is classified as supplemental primarily due to the many uncertainties which may have confounded the results and could have caused the reported residues to be not representative of actual field conditions. The study design outlined by the Department of Pesticide Regulation (DPR) requested that sites be selected with a preference to sites receiving soil applications of imidacloprid during two successive years. However, half of the field sites used in this study did not have sequential applications. In two sites there was only one application in 2011, with no data indicating any previous applications had been made.

Reference

Beedle, E.C. (2012). Determination of the Residues of Imidacloprid and its Metabolites 5-Hydroxy Imidacloprid and Imidacloprid Olefin in Bee Relevant Matrices Collected from Melons Grown at Location Treated with Imidacloprid at Least Once Per Year During Two Successive Years. Bayer CropScience, Research Triangle Park, NC. Bayer Report No. EBNTL056-02. 244p. MRID 49090501.

Citrus

(MRID 49090504; Byrne et al 2011; MRID 49090505 Fischer and Bowers 2012)

Study Objectives and Design. This study was designed to measure the magnitude of imidacloprid residues in bee-relevant nectar samples from citrus trees following a soil application of imidacloprid in five different field investigations.

Tunnel Cage Study (Section 2):
In this first trial, oranges (Washington Parent Navels on C35 rootstock) in a 3.9 acre block were treated in the fall of 2009 with 0.50 lbs. ai/A (the maximum current U.S. label rate). The trial was located at the Lindcove Research and Extension Center (LREC) in Exeter, CA. The soil type at LREC is a loam soil consisting of 15-25% clay and 0.5-1% organic matter.

Nectar was sampled directly from the flower by hand using a micro-capillary tube. The target volume for each sample was 150 µl, with nine composite nectar samples collected, three from each tree. Samples were also collected using honey bees that were contained over the three trees by a tunnel constructed from transparent netting (Combined Clear Net 13%) with 1" PVC tube frames. The bees were intercepted at the hive entrance and chilled with dry ice for 30 seconds. A composite sample from at least 5 bees was collected. In addition, stored nectar was collected from new combs within each hive. A pooled sample was collected from cells near each other with a target of ten samples per hive. Nectar sugar analysis was measured by refractometry.

Open Field Study (Section 3):

The second trial, also at the LREC, consisted of an open field area with multiple citrus varieties. A very large contiguous area of citrus had been treated in 2009 with various imidacloprid commercial formulations at 0.25 lbs. ai/A (half the maximum label rate).

Five large 10-frame colonies were situated in the citrus orchard area. Pollen was successfully collected from only two of five hives via pollen traps that were operated for 24 hours. Nectar was either hand collected from randomly selected citrus trees, extracted from honey stomachs of honey bees allowed to forage in the treated orchard and intercepted at the hive, and from the hive as uncapped honey. Nectar sugar analysis was measured by refractometry.

One Year Citrus Nectar Collections (Section 4):

The third trial was conducted at the LREC and a commercial citrus farm in Bakersfield, CA. Navel oranges (LREC) and Valencia oranges (Bakersfield) were treated with either 1X (the maximum label rate) or 2X the label rate in Fall 2009. Hand collected nectar was quantified in Spring 2010.

Citrus Nectar Collections From Field Sites Treated in Successive Years (Section 5):

The fourth trial was conducted to determine if imidacloprid residues in nectar could accumulate from year to year following successive year applications at three different locations (Hemet, Temecula, and LREC). Hand collected nectar samples were obtained with either 1X or 2X soil applications were made in two successive years (2008, 2009) prior to sampling during bloom 2010.

Hemet site (sandy loam) consisted of a 10-acre block of Ruby Red grapefruit trees having received imidacloprid applications in Fall 2008 and second treatments applied in Fall 2009. Temecula site consisted of six different treatment plots, primarily Star Ruby Grapefruit (five plots) and Valencia Orange. LREC site consisted of five different treatment plots with a mix of citrus varieties, primarily navel (Atwood, Carcara, and Parent) and Red Valencia.

Citrus Nectar Collections from Field Sites Treated in Successive Years (Supplemental):

In 2011, the researchers followed up with a third year of treatments at the Temecula, LREC, and Hemet sampling sites. Nectar was again collected from six groves previously treated in Temecula where the soil type is sandy loam and five citrus blocks at the LREC with a loam soil (20% clay).

In addition, sites were added at University of California, Riverside (UCR), Ventura County and Tulare County to address different soil types not previously represented. Nectar was collected from six citrus groves in Tulare County grown in Porterville clay (clay content 40%). Of these six sites, five had been treated with 1X imidacloprid for the past three years and the remaining site was treated similarly the past two years. A new site in Ventura County with a soil consisting of 23% clay/35% sand was sampled following applications at the full label rate of imidacloprid at different times during the season. The treatment timings for this site were May, July and September 2010 and untreated in 2009. Also, a citrus block from the farm at UCR, where the soil type is loam, was treated in October 2010 and sampled in 2011.

In Temecula and Tulare County, the 2010 treatments at the 1X label rate were made in mid-May to mid-June. The LREC and UCR 2010 treatments at 1X label rate were made in September and October. Treatments at the four Hemet sites were 1X-1X-1X, 1X-2X-0X, 2X-1X-0X, or 2X-2X-2X representing years 2008, 2009 and 2010, where 1X-1X-1X represents a single application at the maximum label rate per year for three consecutive years.

Analytical Methods and QA/QC. The residues of imidacloprid, IMI-5-OH, and IMI-olefin were quantified by high performance liquid chromatography (HPLC) mobile phase and pollen was extracted sing QuEChERS methodology. The final extracts were analyzed by LC/MS/MS using stable isotopically internal standards. The Limit of Detection (LOD) of imidacloprid, IMI-5-OH, and IMI-olefin was 0.018, 0.36 and 0.30 ppb. The Limit of Quantitation (LOQ) was and 0.6, 1.2 and 1 ppb respectively.

Magnitude of Residues. Summary statistics of the overall magnitude of total imidacloprid in citrus nectar and pollen are summarized below**.** These statistics reflect analysis of individual composite samples among all of the sampling times within each trial.

Tunnel Cage Study (Section 2):

The mean residues of total imidacloprid in hand or bee collected citrus nectar ranged from 13.97 – 21.19 ppb, while mean residues in hive stored nectar ranged from 44.65 – 72.81 ppb. The highest residues were found in hive stored nectar, corresponding with the highest sugar concentration.

Open Field Study (Section 3):

In this trial, the mean residues of total imidacloprid in hand collected citrus nectar ranged from 3.50 – 9.42 ppb while the mean residues in nectar extracted from foraging honey bees ranged from 1.11 – 7.59 ppb. Also, the mean residues of total imidacloprid in hive stored nectar ranged from 6.95 - 11.63 ppb. Moreover, the maximum mean residues for hive pollen were found to be 10.2 ppb while the average residue measurement was 9.39 ppb.

Table E-6. Summary of imidacloprid residues in nectar collected from orange trees within four tunnel enclosures that had been treated the previous two years as a soil drench at the maximum labelled rate. Maximum application rate applied to individual trees on 9/3/2009.

Lindcove Research and Extension Center (LREC) Spring 2010 Loam Soil [15-25% clay, 0.5 – 1% organic matter] Mean Residue Concentration (ppb) (minimum – maximum)						
Tunnel	Hand Collected Nectar N[1] = 9 [3 samples from each tree, 3 trees per tunnel] Sampling occurred 4/22/2010		Bee Collected Nectar N[1] = 20 [one hive per tunnel, mean of 20 samples] Sampling occurred 4/22/2010		Uncapped Stored Nectar N[1] = varies [one hive per tunnel, mean of up to 10 samples] Sampling occurred 4/24/2010	
	IMI	Total IMI[2]	IMI	Total IMI[2]	IMI	Total IMI[2]
Control	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1	8.3 (2.86 – 13.64)	13.96 (5.30 – 22.73)	10.61 (5.6 – 21.22)	17.55 (9.17 – 37.12)	25.49 (19.71 – 36.06)	44.65 (35.40 – 62.75)
2	7.64 (4.38 – 12.38)	14.01 (8.06 – 22.84)	8.97 (3.02 – 16.17)	15.08 (4.92 – 29.75)	27.33 (24.81 – 30.83)	49.65 (42.52 – 57.58)
3	12.81 (8.72 – 21.91)	21.19 (9.18 – 34.64)	8.00 (1.66 – 18.99)	13.38 (2.81 – 34.15)	40.12 (27.14 – 54.14)	72.81 (48.75 – 95.18)
Mean for Tunnels 1-3	**9.58 (7.64 – 12.81)**	**16.39 (13.96 – 21.19)**	**9.19 (8.00 – 10.61)**	**15.34 (13.38 – 17.55)**	**29.86 (25.49 – 40.12)**	**53.30 (44.65 – 72.81)**

[1] "N" is the total number of samples collected

2 "Total IMI" combines magnitude of residues of imidacloprid plus degradates IMI-olefin and IMI-5-OH

Open Field Study (Section 3):

Table E-7. Summary of imidacloprid residues in hand collected nectar randomly selected within six blocks of citrus surrounding the location of five honey bee hives. Commercial citrus trees were treated in the Fall of 2009 at half (½X) the labelled rate.

Lindcove Research and Extension Center (LREC) Spring 2010 Loam Soil [15-25% clay, 0.5 – 1% organic matter] Mean Residue Concentration (ppb) (minimum – maximum)					
Citrus Variety	Hand Collected Nectar				
	N[1]	IMI	5-OH	Olefin	Total IMI[2]
Valencia Oranges – South East	10	<1.0 (<1.0 – 1.18)	<1.0	<1.0	1.81 (1.15 – 2.48)
Valencia Oranges – South West	5	2.73 (1.53 – 3.55)	<1.0	<1.0 (<1.0 – 2.22)	5.18 (3.17 – 6.21)
Navel Oranges – North East	4	1.79 (<1.0 – 2.48)	<1.0	<1.0 (1.02 – 1.95)	3.51 (2.11 – 4.42)
Navel Oranges – South East	4	3.51 (2.03 – 6.15)	<1.0	<1.0 (<1.0 – 1.73)	4.87 (2.64 – 8.42)
Tangerines	10	6.82 (2.84 – 13.26)	<1.0	1.99 (<1.0 – 3.67)	9.42 (4.03 – 18.28)
Young Oranges	5	2.76 (1.34 – 4.21)	<1.0	<1.0 (<1.0 – 1.11)	3.73 (1.76 – 5.88)
Means		**3.29 (<1.0 – 6.82)**	**<1.0**	**1.19**	**5.01 (1.81 – 9.42)**

[1] "N" is the total number of samples collected
[2] "Total IMI" combines magnitude of residues of imidacloprid plus degradates IMI-olefin and IMI-5-OH

Table E-8. Summary of imidacloprid residues in nectar collected from free-ranging honey bees in commercial citrus trees treated in the fall of 2009 at half the labelled rate.

Lindcove Research and Extension Center (LREC) Spring 2010 Loam Soil [15-25% clay, 0.5 – 1% organic matter] Mean Residue Concentration (ppb) (minimum – maximum)						
Tunnel	Bee Collected Nectar N[1] = varies [one hive per tunnel, mean of up to 20 samples] Sampling occurred 4/23/2010		Uncapped Stored Nectar N[1] = varies [one hive per tunnel, mean of up to 10 samples] Sampling occurred 4/25/2010		Hive Pollen [Each value represents a single measurement for the analysis of the entire pollen retrieved from traps within individual hives] Sampling occurred 4/25/2010	
	IMI	Total IMI[2]	IMI	Total IMI[2]	IMI	Total IMI[2]
1	1.05 (<1.0 – 2.43)	2.11 (<1.0 – 5.07)	6.25 (4.73 – 8.67)	11.63 (9.31 – 15.53)	N/A	
2	3.77 (<1.0 – 9.31)	7.59 (1.16 – 16.02)	N/A		N/A	
3	1.94 (<1.0 – 7.56)	3.59 (<1.0 – 12.16)	N/A		N/A	
4	<1.0 (<1.0 – 2.69)	1.11 (<1.0 – 3.71)	3.23 (2.47 – 4.98)	6.96 (4.47 – 9.25)	6.58	8.57
5	1.94 (<1.0 – 4.2)	3.23 (<1.0 – 7.29)	5.98 (3.81 – 8.18)	10.60 (8.13 – 13.98)	5.84	10.2

Mean for Tunnels 1-5	**1.86 (<1.0 – 3.77)**	**3.49 (1.11 – 7.59)**	**4.99 (3.23 – 6.25)**	**9.56 (6.96 – 11.63)**	**6.21**	**9.39**

[1] "N" is the total number of samples collected

[2] "Total IMI" combines magnitude of residues of imidacloprid plus degradates IMI-olefin and IMI-5-OH

One Year Citrus Nectar Collections (Section 4):

The 1X label rate treatments resulted in an average residue measurement of 19.30 ppb in nectar at the Bakersfield grove and 16.30 ppb at the LREC. While the 2X label rate treatments resulted in an average residue measurement of 47.36 ppb at the Bakersfield grove and 35.83 ppb at the LREC.

Residues of imidacloprid, IMI-5-OH and IMI-olefin were higher in nectar sampled from the trees treated with the 2X label rate compared with the 1X label rate, with the *ca.* twofold difference in residue levels reflecting the twofold difference in application rates. The imidacloprid and total residues were lower at the LREC site. The most likely reason for this difference is the higher tree density at the LREC site. Since the insecticide was applied on a per acre basis (1X = 14 fl oz./acre and 2X = 28 fl oz./acre), the amount of active ingredient per tree would have been higher at the Bakersfield site and this could, therefore, account for the higher residues in the nectar.

Table E-9. Summary of imidacloprid residues in hand collected nectar resulting from a soil treatment at maximum label rate (1X) and twice maximum label rate (2X). Application made by watering can to individual trees in Bakersfield on 9/8/2009 and LREC on 9/3/09.

Spring 2010 Mean Residue Concentration (ppb) (minimum – maximum)					
Location Citrus Variety	Rate	Hand Collected Nectar (N[1] = 10)			
		IMI	5-OH	Olefin	Total IMI[2]
Bakersfield Valencia Oranges (soil type unknown)	1X	12.13 (3.62 – 18.82)	2.53 (1.10 – 3.61)	4.64 (1.58 – 6.93)	19.30 (6.30 – 29.10)
	2X	31.10 (9.70 – 92.05)	5.67 (1.88 – 15.95)	10.60 (3.12 – 31.15)	47.36 (14.70 – 139.15)
LREC Navel Oranges [Loam Soil]	1X	9.51 (3.31 – 15.49)	3.09 (1.18 – 5.39)	3.70 (1.56 – 6.04)	16.30 (6.81 – 26.45)
	2X	22.23 (5.98 – 43.94)	6.70 (2.25 – 11.74)	6.89 (1.70 – 13.06)	35.82 (9.93 – 68.30)

[1] "N" is the total number of samples collected

[2] "Total IMI" combines magnitude of residues of imidacloprid plus degradates IMI-olefin and IMI-5-OH.

Citrus Nectar Collections From Field Sites Treated in Successive Years (Section 5):

Imidacloprid nectar residues in Hemet were lowest in the 1X – 1X label rate treatment with an average residue measurement of 23.84 ppb and highest in the 2X – 2X label rate treatment with an average residue measurement of 58.66 ppb. Following the 1X – 1X label rate treatments in two successive years at Temecula and LREC (supplemental trial), an average residue measurement of the nectar residues were 5.15 ppb and 11.16 ppb, respectively. The maximum mean residues of 21.65 ppb were measured at LREC. The imidacloprid and total residues measured in the nectar sampled from trees treated with the 1X-2X label rate sequence were approximately 2-fold higher than those measured for the 1X- 1X label rate treatments, suggesting that the residue levels reflected the rate of imidacloprid used in the most recent

application. Thus, residues for the 1X- 1X and 2X-1X rate sequences were not significantly different, and data for the 1X-2X and 2X-2X rate sequences were not significantly different. While the means for the 2X treatments exceeded the 1X measurements by more than twofold, the statistical analysis shows that imidacloprid and its metabolites did not accumulate significantly from one year to the next.

Data from six commercial groves in Temecula that were treated in two successive years (2008 and 2009) with the maximum label rate of imidacloprid (1X-1X) were completed by June each year. The highest total residues (9.56 ppb and 15.53 ppb for the two trees sampled) were measured at Site 6, where the youngest trees (seven years of age) used in the sampling program was located. The overall means for the Temecula samples were lower than those measured for the Hemet site where the 1X rate was applied for two successive years.

Data from five blocks at the LREC that were treated in two successive years (2008 and 2009) with the label rate of imidacloprid (1X-1X) had higher residues than those measured at the Temecula site, possibly reflecting the later timing of treatments. Despite the higher values at the LREC site, the residues were still lower than those measured for trees at Hemet. The major difference between the LREC and Hemet sites is the soil type and it seems that the lighter and sandier soil at Hemet allowed better uptake of imidacloprid into the trees resulting in higher residues in nectar the following spring.

Table E-10. Summary of imidacloprid residues in hand collected nectar of Ruby Red grapefruit trees resulting from two successive year soil treatments at maximum label rate (1X) and twice maximum label rate (2X) in Fall 2008/Fall 2009.

Hemet Site Spring 2010 [Sandy Loam] Mean Residue Concentration (ppb) (minimum – maximum)					
Rate	Hand Collected Nectar				
	N	IMI	5-OH	Olefin	Total IMI[2]
1X – 1X	10	16.07 (9.88 – 25.67)	5.05 (3.62 – 6.43)	2.72 (1.57 – 4.00)	23.84 (15.57 – 35.47)
1X – 2X	9	35.13 (13.28 – 63.60)	9.70 (6.70 – 12.33)	6.04 (3.77 – 9.31)	50.87 (27.54 – 85.03)
2X – 1X	7	14.50 (11.77 – 22.66)	4.78 (3.63 – 7.49)	2.44 (1.73 – 3.66)	21.73 (17.62 – 33.81)
2X – 2X	7	41.41 (28.62 – 62.67)	10.78 (7.14 – 13.56)	6.47 (4.38 – 10.48)	58.67 (40.14 – 86.71)

[1] "N" is the total number of samples collected

[2] "Total IMI" combines magnitude of residues of imidacloprid plus degradates IMI-olefin and IMI-5-OH.

Table E-11. Supplement to **Table E-10.** Summary of imidacloprid residues in hand collected nectar resulting from successive year soil treatments at maximum label rate (1X), twice maximum label rate (2X), and no application (0X) in Fall 2008/Fall 2009/Fall 2010.

Hemet Site Spring 2011 [Sandy Loam] Mean Residue Concentration (ppb) (minimum – maximum)	
Rate	Hand Collected Nectar (N[1] = 10)

Hemet Site Spring 2011 [Sandy Loam] Mean Residue Concentration (ppb) (minimum – maximum)				
	IMI	5-OH	Olefin	Total IMI[2]
1X – 1X – 1X	19.68 (12.26 – 33.82)	3.13 (2.02 – 4.31)	1.59 (<1.0 – 4.96)	24.40 (17.56 – 39.86)
1X – 2X – 0X	26.01 (23.05 – 30.00)	3.93 (3.35 – 4.37)	1.73 (1.44 – 1.92)	31.67 (28.97 – 36.27)
2X – 1X – 0X	27.02 (20.01 – 33.45)	3.87 (2.28 – 5.43)	2.08 (1.51 – 3.11)	32.97 (25.12 – 41.99)
2X – 2X – 2X	36.86 (26.67 – 41.92)	5.18 (3.86 – 5.76)	2.77 (2.27 – 3.16)	44.81 (32.80 – 50.35)

[1] "N" is the total number of samples collected

[2] "Total IMI" combines magnitude of residues of imidacloprid plus degradates IMI-olefin and IMI-5-OH

Table E-12. (plus supplement). Information on trees used for hand collected nectar collections during bloom in Spring 2011 after three successive years of imidacloprid application at the label rate 2008, 2009, 2010.

Temecula [Sandy Loam] Spring 2011				
Site	Citrus Variety	2008 Treatment Date	2009 Treatment Date	2010 Treatment Date
1	Star Ruby Grapefruit	6/7/2008	5/27/2009	5/22/10
2	Star Ruby Grapefruit	6/9/2008	4/13/2009	6/11/10
3	Valencia Orange	6/6/2008	4/7/2009	6/12/10
4	Star Ruby Grapefruit	6/9/2008	4/21/2009	5/17/10
5	Star Ruby Grapefruit	6/7/2008	4/10/2009	6/2/10
6	Star Ruby Grapefruit	6/16/2008	4/17/2009	5/14/10
LREC [Loam Soil] Spring 2011				
Site	Citrus Variety	2008 Treatment Date	2009 Treatment Date	2010 Treatment Date
1	Atwood Navel	9/18/2008	9/10/2009	9/13/2010
2	Atwood Navel	9/17/2008	9/10/2009	9/10/2010
3	Caracara Navel	9/18/2008	9/16/2009	9/09/2010
4	Parent Navel	9/18/2008	9/16/2009	9/09/2010
5	Red Valencia	9/17/2008	9/14/2009	9/13/2010

Table E-13. Summary of imidacloprid residues in hand collected nectar sampled in Spring 2010 from citrus trees receiving a soil treatment at maximum label rate (1X) in successive years 2008 and 2009.

Spring 2010 Mean Residue Concentration (ppb) (minimum – maximum)

Sampling occurred 4/2010						
Location	Rate	N[1]	Hand Collected Nectar			
			IMI	5-OH	Olefin	Total IMI[2]
Temecula	1X – 1X	11	3.17 (<1.0 – 10.60)	<1.0 (<1.0 – 1.18)	1.48 (<1.0 – 3.76)	5.15 (<1.0 – 15.54)
LREC	1X – 1X	9	6.51 (<1.0 – 13.65)	1.80 (<1.0 – 5.40)	2.86 (<1.0 – 8.42)	11.17 (1.53 – 21.64)

[1] "N" is the total number of samples collected

[2] "Total IMI" combines magnitude of residues of imidacloprid plus degradates IMI-5-OH and IMI-olefin

Table E-14. Summary of imidacloprid residues in hand collected nectar sampled in Spring 2011 from citrus trees receiving a soil treatment at maximum label rate (1X) in successive years 2008, 2009, and 2010.

Spring 2011 Mean Residue Concentration (ppb) (minimum – maximum)						
Location	Rate	N[1]	Hand Collected Nectar			
			IMI	5-OH	Olefin	Total IMI[2]
Temecula	1X – 1X – 1X	12	1.68 (<1.0 – 3.48)	<1.0	<1.0 (<1.0 – 1.62)	2.58 (1.01 – 5.91)
LREC	1X – 1X – 1X	10	3.50 (<1.0 – 16.87)	<1.0 (<1.0 – 3.88)	<1.0 (<1.0 – 3.20)	4.95 (<1.0 – 23.95)

[1] "N" is the total number of samples collected

[2] "Total IMI" combines magnitude of residues of imidacloprid plus degradates IMI-olefin and IMI-5-OH

Citrus Nectar Collections from Field Sites Treated in Successive Years (Supplemental):

Total mean residues in nectar of imidacloprid from trees treated three years in a row at the Temecula site ranged from 1.02 to 5.91 ppb with an average residue measurement of 2.57 ppb. Mean nectar residues from Tulare County ranged from 0.29 to 4.21 ppb with an average residue measurement of 1.54 ppb. Total mean nectar residues from trees at the LREC with a loam soil ranged from 0.52 to 23.95 ppb with an average residue measurement of 4.55 ppb. Total mean nectar residues from the UCR site with a loam soil ranged from 0.83 to 13.88 ppb with an average residue measurement of 3.68 ppb. Imidacloprid nectar residues from Ventura County sites treated at various times during 2010 were all less than 1 ppb regardless of the application timing. Following three years of treatments at the Hemet site at various application rates, the average residue measurement in nectar for the 1X-1X-1X treatment regimen was 24.40 ppb. The average residue measurement following the third year of treatment for sites treated once per year with 2X the label rate (2X-2X-2X) was 44.81 ppb. For the 1X-2X-0X and 2X-1X-0X treatments the average residue measurement was 31.67 and 32.97 ppb. The residues following three years of applications at 2X the maximum label rate were slightly lower than residues after just two years.

Table E-15. Information on trees used for hand collected nectar collected from six commercial orchards in Tulare County during bloom in Spring 2011 after three successive years of imidacloprid application for control of glassy-winged sharpshooter 2008, 2009, 2010.

Tulare County [Porterville Clay – 40% clay] Spring 2011				
Site	Citrus Variety	2008 Treatment	2009 Treatment	2010 Treatment

		Date	Date	Date
1	Tangelos	Not treated	6/16/2009	5/21/2010
2	Navel Oranges	5/22/2008	6/29/2009	6/18/2010
3	Navel Oranges	7/8/2008	6/18/2009	6/9/2010
4	Navel Oranges	5/17/2008	6/18/2009	6/6/2010
5	Navel Oranges	5/21/2008	6/29/2009	6/15/2010
6	Valencia Oranges	7/3/2008	6/26/2009	6/16/2010

Table E-16. Summary of imidacloprid residues in hand collected nectar sampled in Spring 2011 from citrus trees receiving a soil treatment at maximum labelled rate as part of the area-wide control program for glassy-winged sharpshooter in successive years 2008, 2009, and 2010.

Spring 2011 Mean Residue Concentration (ppb) (minimum – maximum)						
Location	Rate	N[1]	Hand Collected Nectar			
			IMI	4/5-OH	Olefin	Total IMI[2]
Tulare County	1X[3] – 1X – 1X	12	1.29 (<1.0 – 3.31)	<1.0	<1.0	1.70 (<1.0 – 4.21)

[1] "N" is the total number of samples collected

[2] "Total IMI" combines magnitude of residues of imidacloprid plus IMI-olefin and IMI-5-OH

[3] One site did not receive an application in 2008.

Temporal Variability in Residues. Nectar samples were obtained from two locations (citrus blocks in the Temecula region and at LREC) where the 1X soil application rate of imidacloprid had been made in two successive years (2008, 2009) prior to sampling in April 2010. Residue levels at these 11 sites averaged 8 ppb and ranged from 1 to 18 ppb. The application timing (May, July, September, October) appears to be an important factor in determining residue levels in flower nectar the following year particularly for sites planted to coarse soils which consistently yielded the higher imidacloprid residues. Fall (Sept) applications resulted in about twofold higher residue concentrations than spring (April-June) applications.

Spatial Variability in Residues. The six locations for the citrus trials were in relatively close proximity. Soil types reflect sandy loam, loam or clay compositions (20-40% clay) and low organic carbon content (0.35-1.9%). Weather conditions (temperature and precipitation) were similar across the three trials. As a result of the close proximity of trial sites, this study provides very limited information on how differences in environmental conditions across different areas of the U.S. may affect accumulation of total imidacloprid in pollen and nectar.

Pesticide Carryover. The authors speculated that imidacloprid residues at the Hemet site appear to be a function of the rate applied at the most recent application only, with no evidence of carryover from previous years. However, following the third year of application at the Hemet site, residues were higher at the two sites receiving no treatment in 2010 than at the site treated all three years with 1X. This indicates some degree of carryover from previous application years, at least for sites treated with the 2X rate during one of the two years prior to the no treatment year. This was the only site where samples were collected following a year without treatment.

Classification/Utility for Bee Risk Assessment. This study is classified as supplemental for use in risk assessment due to no pollen data available and a potential underestimation of a worst case scenario due to current labels not restricting pre bloom and during bloom applications whereas this study done post bloom. The study results characterize expected imidacloprid residues in citrus nectar from applications in various soils ranging from fine to coarse and following different application rates and application timing in California. These results may not extrapolate directly to expected results in other regions of the U.S.

Concentrations in nectar extracted from the stomachs of free-ranging bees (open field study) were somewhat lower than samples collected directly from flowers of nearby trees. This may reflect a "dilution effect" from bees foraging on other (untreated) flower types. The few pollen samples obtained during the open field study had imidacloprid concentrations roughly equal to the nectar sampled from the same hives. Concentrations in flower nectar samples appear to be linearly related to the application rate, based on *ca.* twofold increases in residue levels with doubling the application rate in the Hemet trials.

Reference

F. J. Byrne, Morse, G. J., Visscher, P. K., Grafton-Cardwell, E. E., and Leimkuehler, W. (2011). Determination of exposure levels of honey bees foraging on flowers of citrus trees previously treated with imidacloprid. Project Number: EBNTL056-7. University of California at Riverside. Riverside CA. 70p. MRID 49090504.

F. J. Byrne, Morse, G. J., Visscher, P. K., Grafton-Cardwell, E. E., Leimkuehler, W., and Fischer, D. (2012). Determination of exposure levels of honey bees foraging on spring 2011 flowers of citrus trees previously treated with imidacloprid. Project Number EBNTL056-7a. University of California at Riverside. Riverside CA. 17p. MRID 4909050.

Blueberry

(MRID 49535602; Gould et al., 2014)

Study Objectives and Design. This study was designed to measure the magnitude of imidacloprid residues in bee-relevant pollen and nectar samples and in/on flowers, leaves from blueberry bushes following one post-harvest banded soil application of Admire Pro® Systemic Protectant (0.50 lbs. ai/A) per year (current maximum U.S. label rate) over two successive years (2012 and 2013). Three separate trials were conducted (NT001 in Penn Yan, NY, NT002 in Marengo, IL and NT003 in Fennville, MI), each with a treated (TRTD) and untreated control (UTC) plot. Each treated plot was divided into 5 sampling subplots, for the construction of bee-tight, ventilated mesh-covered tents (tunnels). Treated plot sizes ranged from 0.7 to 1.4 acres. Soil types (loam, silt loam and sand), and the variety of blueberry (Blue Ray, Duke, and Bluecrop) differed between NT001, NT002, and NT003, respectively. Soils at trial NT001 consisted of 39% sand, 46% silt and 15% clay; trial NT002's soil consisted of 31% sand, 53% silt and 16% clay and trial NT003 soil consisted of 90% sand, 10% silt and 0% clay. The pH was lowest in soils at NT001 and NT003 (4.8 and 4.4, respectively) vs. pH 6.4 for the soil in the treatment site in trial NT002. Overall, the total rainfall was similar between all trials, despite the difference in locations (New York, Illinois and Michigan) although irrigation differed among the trials.

Nature of Pesticide Application. A single soil application at **0.5 lbs. ai/A** of Gaucho® 600 FL Admire Pro Systemic Protectant was applied at each of the three treatment sites 3 days post-harvest of blueberries during two successive years. Applications consisted of an 18-inch band on each side of the row using ground-based equipment. The volume of application was similar across trials and years (data not shown). The interval between the 1st and 2nd applications was approximately one year (360-366 days). All applications were made after blueberry harvest between September 26 and October 4 of each year, when the plants were between BBCH growth stages 92 and 97 (BBCH 92: leaves begin to change color or fall; BBCH 97: plant resting or dormant).

Residue Sampling (Pollen and Nectar). Honey bees were used to collect pollen and nectar samples, and bumble bees were used to aid in the collection of pollen samples in year 2. Samples from sites that were treated in 2012 were collected in 2013 (year 1 of the study), and samples from sites treated in 2013 were collected in 2014 (year 2 of the study). Each trial location was divided into sampling subplots, for the construction of bee-tight, ventilated mesh-covered tents (tunnels), which were used for collection of pollen and nectar. Two tunnels were erected on control plot UTC, and five tunnels were erected on each TRTD plot. Each tunnel enclosed one or two rows of blueberry bushes. The bee tunnels were erected over the same blueberry bushes in 2013 and 2014, except in trial NT002, when the tunnel size was expanded in 2014 to include bushes that were treated but not sampled in 2013. One honey bee hive was placed in each bee tunnel 1 to 5 days before the first nectar/pollen samples were collected. Honey bee hives were equipped with pollen traps, and the honey bees were provided no supplemental feed. In year 2 apparently healthy bumble bees (*Bombus impatiens*, purchased) were used alongside the honey bees to aid in pollen collection. One honey bee hive and one to four bumble bee colonies were placed in each bee tunnel 3 to 6 days before the first nectar/pollen samples were collected.

After 1 to 5 days of bee foraging, nectar was collected from samples deposited in uncapped cells within a newly-placed bee hive using a disposable spoon and sample vial. For honey bees pollen samples were collected via pollen traps affixed to the hives. In year 2 when bumble bees were used to aid in the collection of pollen, pollen was collected from the pollen basket on the bumble bee's leg. Blueberry nectar and pollen were collected at four target sampling periods 228 to 257 days after the last application (DAA) at growth stages of BBCH 60 to 69 (BBCH 60: first flowers open; BBCH 69: end of flowering, fruit set visible) in years 1 and 2. In trial NT001, pollen collected from bumble bees was shipped as separate samples from the honey bee hive-collected pollen; in trials NT003, pollen collected from both bee sources was combined into one pollen sample for the subplot and interval, while in trial NT002, no pollen was collected using honey bees.

Residue Sampling (Other Matrices). Composite samples (separate runs through the plot) of blueberry leaves, and flowers, were collected from plots UTC and TRTD in years 1 and 2 of the study, except in trial NT002, in which no year 2 UTC samples could be collected because the UTC blueberry plants died during the previous winter. Five samples were targeted for collection from TRTD plots and two samples from an UTC untreated control plots at each sampling period, corresponding to one sample per erected bee tunnel (subplot). However, in all trials, not enough plant matrix material was present at every interval to allow for the full number of target samples or sampling intervals to be collected. Blueberry flowers were collected during four target sampling periods 228 to 257 days after the last application (DAA) at growth stages of BBCH 60 to 69 (BBCH 60: first flowers open; and BBCH 69: end of flowering, fruit set visible) in years 1 and 2. Blueberry leaves were collected at six target sampling periods, 228 to 264 DAA when the blueberry plants were at growth stages BBCH 59 to 74 (BBCH 59: first flower petals visible in petalled forms) in years 1 and 2 . Nine soil samples were collected prior to treatment and at the end of growing season in each plot, each year. The exceptions were trial NT001 year 2 (2014), when the soil samples were collected before the spray application and just before the start of the blueberry collection in year 2, (2014) and trial NT003, when the only soil samples collected were after the growing season in year 2 2014 (no year 1 2013 soil samples were collected).

Analytical Methods and QA/QC. The residues of imidacloprid, IMI-5-OH, and IMI-olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labelled internal standards. For total imidacloprid, the LOQ and LOD were 1 ppb and 0.7 ppb in nectar and 1 ppb and 0.5 ppb in pollen, respectively. All samples were stored within the limits of storage stability trials and spike recoveries were within 70%-120% with a standard deviation of less than 20%.

Magnitude of Residues. Summary statistics of the overall magnitude of total IMI (sum of IMI-olefin, IMI-5-OH, and parent imidacloprid) in blueberry nectar and pollen are shown in **Table E-17.** These statistics reflect analysis of individual composite samples (usually 5/sampling date) among all of the sampling times within each trial and year. For calculation purposes, values of each analyte that were reported as less than the limit of detection (<LOD) were assumed equal to ½ the LOD. Values reported as greater than or equal to the LOD (>LOD) but less than the limit of quantitation (<LOQ) assumed equal to ½ the LOQ.

Total IMI residues in uncapped blueberry nectar collected from hives from trial NT001 (year 1) and all but one sample from trial NT002 (both years) are < LOD (0.81 ppb for uncapped nectar and 0.60 ppb for pollen). Mean total IMI residues in nectar from trial NT003 range from **1.8 ppb** (2014) and **7.3 ppb** (2013) with an **overall maximum of 16 ppb**. Except for trial NT001 (2013), mean residues of total IMI in bee-collected blueberry pollen are about 2X to 7X those measured in nectar for the same trial and year (Table E-3). The mean residues of total IMI in pollen among trial/years range from **0.6 to 14 ppb** with an overall maximum reported value of **42 ppb.** The higher concentrations of total IMI in pollen and nectar from trial NT003 may reflect the coarser nature of the soils (90% sand) compared to the other two trials (3`%-39% sand). There were no pollen samples collected by the honey bees in year 1 of trial NT002, therefore no comparison of year on year total residues in blueberry pollen could be made.

Table E-17. Magnitude of total imidacloprid residues in blueberry nectar and pollen

Statistic	Trial NT001 (NY; Loam)		Trial NT002 (IL; Silt Loam)		Trial NT003 (MI; Sand)	
	2013	2014	2013	2014	2013	2014
Total IMI in Blueberry Hive-Collected Nectar (ppb)						
Mean	0.81	1.8	0.81	0.87	7.3	1.8
Min.	0.81	0.81	0.81	0.81	1.2	0.81
Med.	0.81	1.1	0.81	0.81	7.0	1.1
90th	*n.d.*	3.7	0.81	0.81	*n.d.*	3.3
Max.	0.81	5.8	0.81	1.8	16.0	4.7
n	5	20	18	17	9	20
Total IMI in Blueberry Bee-Collected Pollen (ppb)						
Mean	0.60	2.9	*n.d.*	4.5	13.7	14.0
Min.	0.60	0.60	*n.d.*	2.0	11.0	3.1
Med.	0.60	2.0	*n.d.*	4.3	15.0	13.5
90th	*n.d.*	5.6	*n.d.*	6.4	*n.d.*	22.9
Max.	0.60	19.0	*n.d.*	6.8	17.0	42.0
n	3	29	*n.d.*	11	7	18

Source: MRID 49535602.Total IMI = sum of imidacloprid, 5-OH imidacloprid, and IMI-olefin.
Bold values indicate overall maximum values from the study. Values less than the LOD were assumed equal to ½ * LOD. For total IMI, the sum of ½ the individual LODs for olefin, 5-OH and IMI (parent) is 0.81 (nectar) and 0.60 (pollen). *n.d.* = not determined.

The proportion of total IMI occurring as parent and metabolites (IMI-olefin, 5-OH IMI) in blueberry nectar could not be reliably determined because of the high proportion of samples with reported values below analytical detection. With pollen from trial NT003, the mean % of total IMI as IMI-olefin in pollen was 9% and the mean % of total IMI as 5-OH IMI was 4%. Parent IMI was present at 87% of total IMI in pollen on average from that trial.

Temporal Variability in Residues. Plots of the mean concentrations of total IMI vs. sampling time (relative to the last foliar application) are shown in **Figure E-4** for nectar and **Figure E-5** for pollen. Within each trial and year, the duration of sampling times were relatively short, ranging from approximately 3 days to 20 days which apparently reflects the blooming period for these blueberry varieties. With hive-collected nectar, there appears to be a slight increase in mean residues of total IMI over the sampling time (Trials

NT001 and NT003). With pollen, the temporal trend in mean residues appears to be variable or stable over the sampling period. Furthermore, the overall range in reported residues of total IMI in pollen is substantially greater than that observed with nectar. This may reflect differences in collection methods between the two matrices (hive collected for nectar, bee collected for pollen).

Spatial Variability in Residues. The geographic coverage among the three trials was relatively broad (northwestern NY, northern IL, and southwestern MI) although were in the northern portion of the US. A range of soil types are represented (loam, silty loam and sand) which is considered important for evaluating the effect of soil texture on residue accumulation in plant pollen and nectar.

Pesticide Carryover. A qualitative evaluation of the potential for 'year-to-year' accumulation of total IMI residues in nectar and pollen could only be conducted for 1 of the 3 trials (NT003); since a majority of the mean residue data for trial NT001 and NT002 were below levels of detection or quantification. Examination of **Figures E-4 and E-5** (and **Table E-3**) indicates the mean of total IMI residues in nectar from Trial NT003 decreased in year 2 relative to year 1 (7.25 ppb vs 1.8 ppb) while those in pollen remained essentially the same (13.7 vs 14.0 ppb). Thus, there is little evidence of year-to-year carryover of residues in bee-relevant matrices, despite evidence of increases in soil concentrations from the end of the first to the second growing season from two of the trials (151 to 233 ppb in NT001; 151 to 339 ppb in trial NT002).

Classification/Utility for Bee Risk Assessment. This study is classified as acceptable and appropriate for quantitative use in risk assessment.

Reference

Gould, T., Bowers, L., Dyer, D., and Jerkins, E. (2014). *Determination of the Residues of Imidacloprid and its Metabolites IMI-5-OH and IMI-olefin in Bee Relevant Matrices Collected from Blueberries following Soil Application of Imidacloprid over Two Successive Years.* Bayer Crop Science, Research Triangle Park, NC. MRID 49536502.

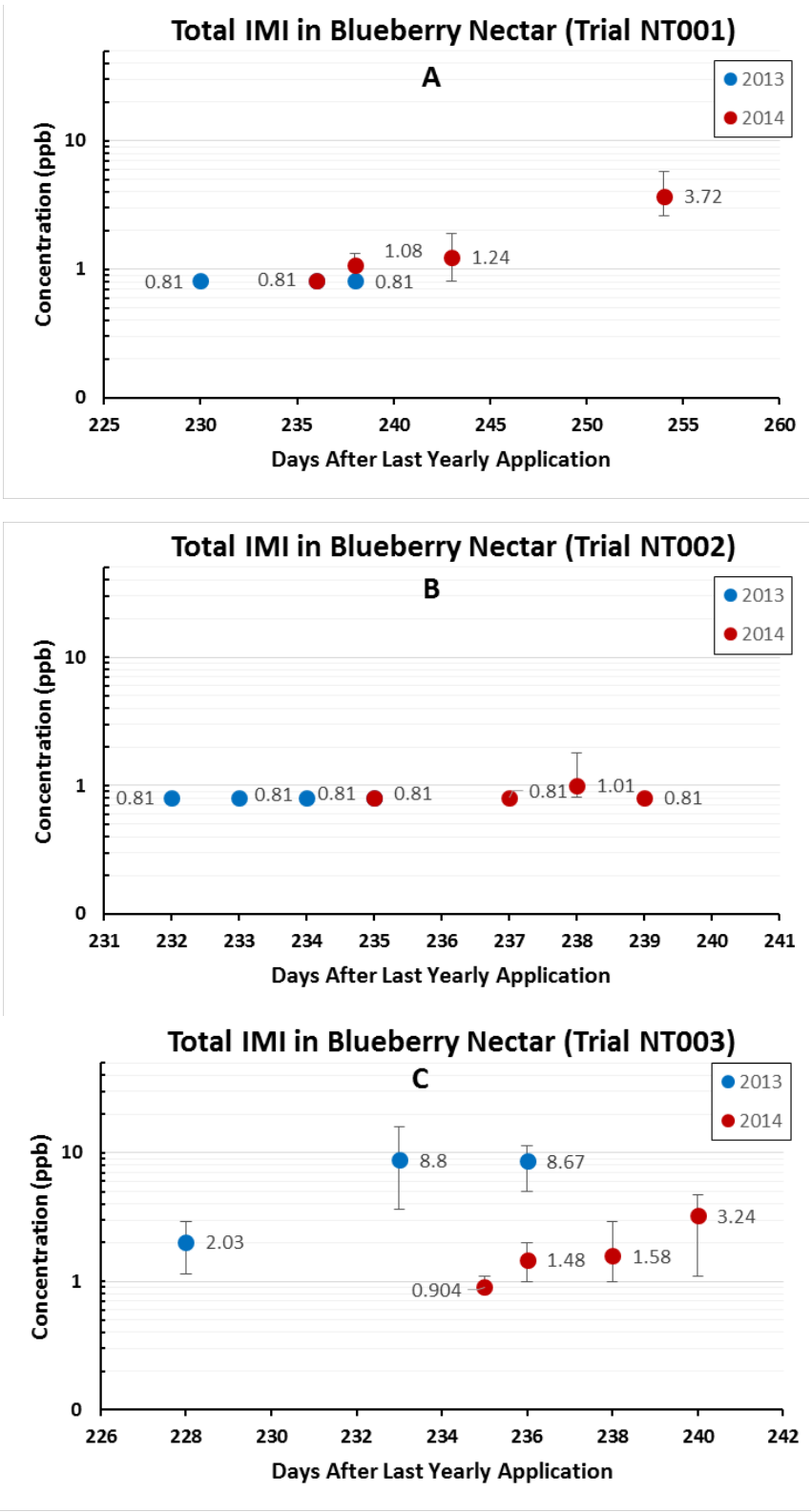


Figure E-4. Occurrence of total IMI in blueberry hive-collected nectar following post-harvest soil applications (1 x 0.5 lbs. ai/A)/yr. of imidacloprid (error bars = range).

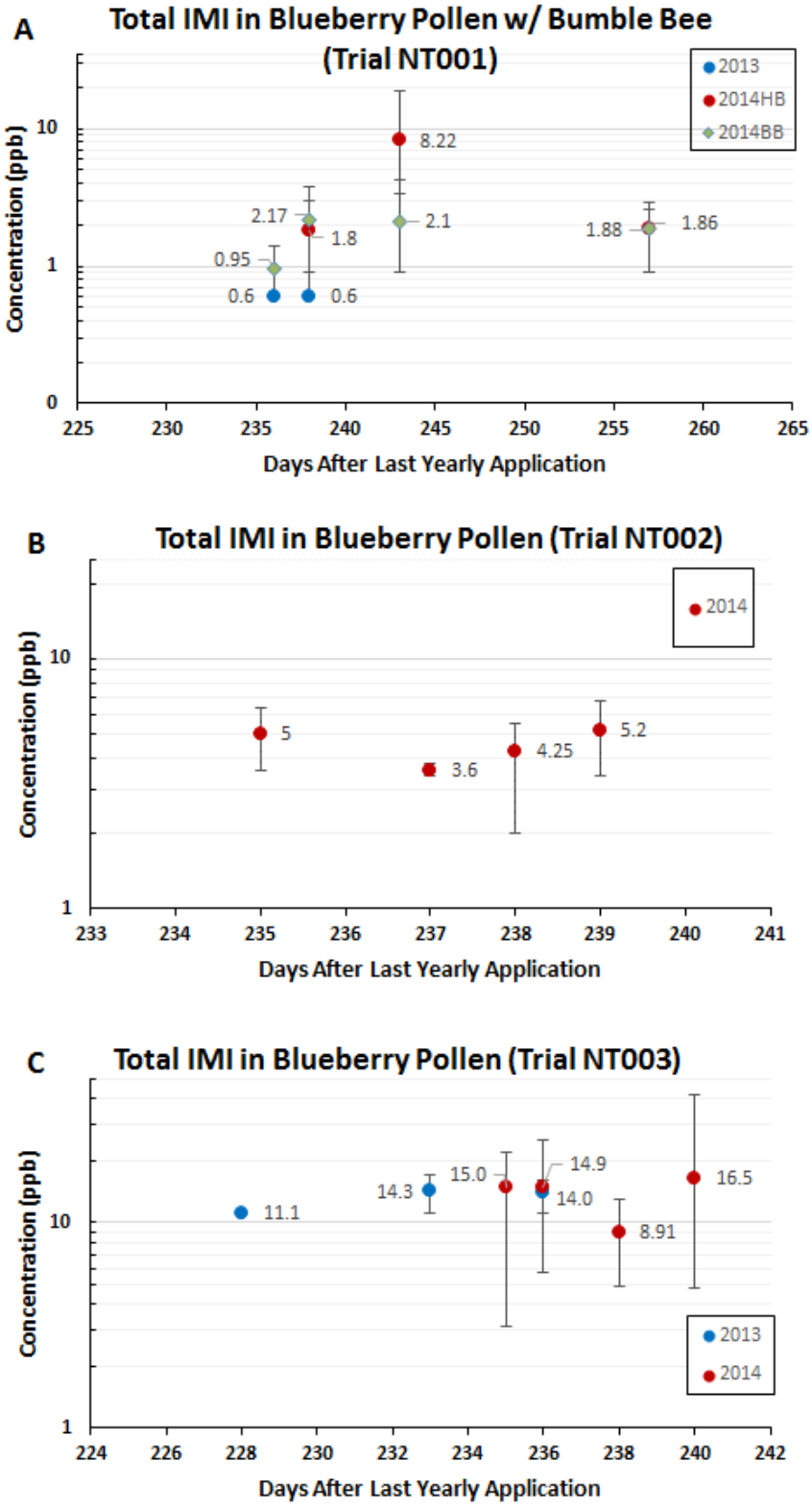


Figure E-5. Occurrence of total IMI in blueberry trapped and bumble-bee collected pollen following post-harvest soil applications (1 x 0.5 lbs. ai/A)/yr. of imidacloprid (error bars = range).

Strawberry

(MRID 49090502; Gould et al 2012)

Study Objectives and Design. This study was designed to measure the magnitude of imidacloprid and its metabolites, IMI-5-OH and IMI-olefin, in blossoms, anthers, pollen and leaves collected from strawberries treated with imidacloprid once per year for two successive years. Samples were collected from seven treated field sites (three coarse-textured sandy soils, four medium-textured loamy soils). All soils had received previous application(s) of either Alias 4F or Admire Pro at a rate of 0.5 lb a.i/A in 2011, as well as an application of imidacloprid in 2010. All seven field sites were located in Santa Maria, CA, and temperature and rainfall was similar across sites. Untreated (control) blossom and leaf samples were collected from a nearby farm thought to have no recent imidacloprid application in order to validate analytical methods and for transit stability analysis.

Nature of Pesticide Application. In 2011, one application of either Alias 4F or Admire Pro was made to the soil at a rate of 0.5 lb a.i/A in each of the seven field sites. In 2010, one soil application of an imidacloprid product (exact product was not confirmed for four out of seven of the sites) was made at a rate of 0.5 lb a.i/A in each of the seven field sites. Method, timing or growth stage, and spray volume of these applications were not reported by the study authors. Thus, it is uncertain when the sampling was conducted in relation to the imidacloprid applications.

Residue Sampling. Duplicate samples of strawberry blossoms, strawberry blossoms for anther samples, strawberry blossoms for pollen samples, and strawberry leaves were collected in 2011 from the treated sites during flowering (BBCH 61-69).

Analytical Methods and QA/QC. Samples of anthers, blossoms, leaf, and pollen were analyzed for residues of imidacloprid, IMI-olefin, and imidacloprid IMI-5-OH, using high performance liquid chromatography/tandem mass spectrometry (LC/MS/MS) and stable isotopically labeled internal standards. The total imidacloprid residue is the sum of the individual measured residue values of imidacloprid, IMI-5-OH, and IMI-olefin in parent equivalents.

This method of analysis was validated by measuring the recovery of all analytes (imidacloprid, IMI-olefin, and IMI-5-OH) from control matrices (blossoms, anthers, pollen and leaves) fortified at their respective LOQs. Acceptable recoveries (86-102%) measured concurrently with each set of samples ensured the integrity of the sample extracts during the period of time between extraction and analysis. The limits of detection (LODs) were determined from this method validation data. For total imidacloprid, the LOQ and LOD were 5 ppb and 3.9 ppb in blossoms, 5 ppb and 3.9 ppb in anthers, 10 ppb and 2.6 ppb in pollen, and 10 ppb and 4.6 ppb in leaves, respectively.

In addition, quadruplicate, one gram transit stability samples (control pollen) were fortified with 20 ng each of imidacloprid, IMI-5-OH, and IMI-olefin (20 ppb). These transit stability samples were prepared to monitor the stability of the analytes during sample collection, transit, and storage. The overall mean values (n=4) of the field fortification recoveries for pollen ranged from 93% to 104%, and the relative

standard deviation (RSD) values were below 20%. Demonstrated freezer stability in the transit stability samples from this study are representative of the freezer stability of imidacloprid residues to be expected in the blossoms, anthers, pollen, and leaves collected in this study. The residue data were not corrected for any in-storage decomposition.

Magnitude of Residues. Summary statistics for concentration of total imidacloprid in strawberry blossoms, anthers, pollen and leaves are contained in **Table E-18**. The individual analyte residues were summed to give a total imidacloprid residue. The study authors stated that when individual analyte residue values were less than the LOD, the residues were assigned a finite value of half the value of the respective LOD.

In coarse-textured soils, mean residues of total imidacloprid were 360, 180, 190, and 2200 ppb in blossoms, anthers, pollen, and leaves, respectively and, maximum residues were 530, 300, 320, and 2800 ppb in blossoms, anthers, pollen, and leaves respectively. In medium-textured soils, mean residues of total imidacloprid were 9.4, 18, <0.010 (LOQ), and 11 ppb in blossoms, anthers, pollen, and leaves, respectively, and maximum residues were 31, 33, <0.010 (LOQ), and 18 ppb in blossoms, anthers, pollen, and leaves respectively. Thus, in all matrices (blossoms, anthers, pollen, and leaves) mean concentrations of total imidacloprid were approximately ten times higher in strawberries grown in coarse-textured soil than in medium-textured soil.

Temporal Variability in Residues. This study was not designed to measure temporal variability in residues. All samples were taken in 2011, within five days of each other. Thus, this study was designed to analyze imidacloprid residues at a single time point. In addition, time of sampling in relation to the imidacloprid applications, is unknown.

Spatial Variability in Residues. All seven sites for this strawberry study were located in Santa Maria, CA. As expected, reported weather conditions (temperature and precipitation) were the same across all seven sites. As a result of the close proximity of trial sites, this study provides very limited information on how differences in environmental conditions across different areas of the US may affect accumulation of total IMI in bee-relevant matrices. However, because there are different soil types represented in this study (3 sand soil sites and 4 loam soil sites), this study may offer insight to how soil type may affect accumulation of total IMI in bee-relevant matrices. In all matrices (blossoms, anthers, pollen, and leaves) mean concentrations of total imidacloprid were higher in strawberries grown in sandy soil than in loam soil.

Pesticide Carryover. This study was not designed to measure pesticide carryover. All samples were taken in 2011, within five days of each other. This this study is designed to analyze imidacloprid residues at a single time point.

Table E-18. Magnitude of total imidacloprid residues in strawberry blossoms, anthers, pollen, and leaves.

Commodity	Soil Texture Category[a]	Total Imidacloprid Residue Levels (ppb)[b]						
		N	Min	Max	Highest Avg. Site Residue	Median	Mean	Standard Deviation
Strawberry Blossoms	Coarse	6	210	530	500	380	360	130

Commodity	Soil Texture Category[a]	Total Imidacloprid Residue Levels (ppb)[b]						
		N	Min	Max	Highest Avg. Site Residue	Median	Mean	Standard Deviation
Strawberry Anthers	Coarse	6	81	300	250	200	180	82
Strawberry Pollen	Coarse	6	78	320	280	210	190	95
Strawberry Leaves	Coarse	6	1700	2800	2400	2100	2200	410
Strawberry Blossoms	Medium	8	<0.0050	31	18	6.4	9.4	9.1
Strawberry Anthers	Medium	8	11	33	23	13	18	7.9
Strawberry Pollen	Medium	8	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Strawberry Leaves	Medium	8	<0.010	18	17	11	11	<0.010

[a] Classification of the soils was obtained from the Soil Survey Geographic (SSURGO) Database provided by the Natural Resources Conservation Service and converted to soil texture categories.

[b] Abbreviations used are as follows: Min is the lowest treated residue value; Max is the highest treated residue value; Median is the geometric median of the treated residue values; Mean is the mathematical average of the treated residue values; Standard Deviation is the standard deviation for a small population of "N" samples.

Classification/Utility for Bee Risk Assessment. The study is supplemental for considering the level of residues of imidacloprid in pollen after soil application in strawberry. The level of imidacloprid residues appeared to be much higher in strawberries grown in coarse-textured sandy soil than in medium-textured loamy soil. In sandy soil, soil application at 0.5 lbs. ai/A of imidacloprid for two consecutive years resulted in maximum total imidacloprid residues of 320 ppb (mean ± SEM, 190 ± 95), 300 ppb (180±82), 530 ppb (360±130) and 2800 ppb (2200±410) respectively in strawberry pollen, anthers, blossoms and leaves. Residues in strawberry nectar, another bee-relevant matrix, were not measured. Another concern is that the date of the last soil application was not provided, thus the duration from the soil application to the collection of the samples could not be estimated.

Reference

Gould, T.; Dallstream, K.; Beedle, E. (2012) Determination of the Residues of Imidacloprid and its Metabolites IMI-5-OH and IMI-olefin in Bee Relevant Matrices Collected from Strawberries, Grown at Locations Treated with Imidacloprid at Least Once Per Year During Two Successive Years. Bayer CropScience, Research Triangle Park, NC. Bayer Report No. EBNTL056-04. 186p. MRID 49090502.

Seed Treatment Applications

Corn

(MRID 49511701; Miller et al., 2014)

Study Objectives and Design. This study was designed to measure the magnitude of imidacloprid residues in bee-relevant pollen samples, anthers and leaves from corn planted from seeds treated with Gaucho® 600 Flowable seed treatment over two successive years (2012 and 2013). In addition, a second series of trials was conducted to measure the same residues in/on bee-relevant white clover pollen and nectar samples and in blossoms, leaves, and soil from white clover plants grown at locations where seed treated corn plants were grown the previous year. Trials were conducted at three sites: NT010 in Edgerton, KS, NT011 in Springfield, NE and NT012 in York, NE. At each of three study sites, two treated trials were conducted (TRTDA and TRTDB). The TRTDA trials consisted of seed treatment at a rate of 1.34 mg ai/seed (equivalent to a soil loading rate of 0.011 – 0.12 lbs. ai/A) over two successive years. The TRTDB trials consisted of seed treatment of 1.34 mg ai/seed (equivalent to a soil loading rate of 0.12 lbs. ai/A) during year 1 followed by planting of white clover and residue sampling of clover during year 2. All plots were tilled or disked at least once prior to the year 2 planting of corn or clover. In addition to the treated crop study sites, two control sites were included for each trial (UTCA and UTCB).

Treated plots were approximately 1 ac in area and had similar row and plant spacing. Soils at trial NT010 were classified as silty clay (16% sand, 58% silt, 28% clay); soils at trial NT011 were classified as silt loam (28% sand, 50% silt, 22% clay) and soils at trial NT012 were classified as loam (36% sand, 44% silt, 20% clay). Temperatures were similar among sites, but considerably more rainfall occurred at site NT010 (55.7 in) vs the other two treated sites (33 and 37 in). With the exception of the spring 2012 and winter 2013 at site NT010, monthly precipitation patterns follow similar temporal patterns among the sites. Plots at sites NT010 and NT011 were irrigated during summer (2-3 months; 1.5-2 in/month) while plots at NT012 were irrigated 4 months during late spring/summer (0.75-3.75 in/month).

Nature of Pesticide Application. For plot TRTSTA, the seed planting rate ranged from 36,440 to 41,480 seeds/A across both years. Soil application rates due to seed treatment for TRTSTA ranged from 0.115 to 0.122 Ib a.i./A) in year 1 and from 0.108 to 0.121 Ib a.i./A in year 2. For plot TRTSTB, the treated seed planting rate ranged from 38,820 to 41,330 seeds/A in year 1 and soil application rates due to seed treatment ranged from 0.115 to 0.122 Ib a.i./A in year 1.

Residue Sampling (Corn). Corn sample collection began 58-78 days after planting seed treated corn (DAA) each year. Samples of corn pollen and tassels were collected at four sampling intervals per year of collection. Sampling of corn pollen and tassels targeted growth stages BBCH 63, 65, 67, and 69. For corn, BBCH 63 (male) represents the beginning of pollen shedding; BBCH 63 (female) represents tips of stigmata visible; and BBCH 69 represents the end of flowering, stigmata completely dry. Corn leaves were sampled at six times from BBCH 59-71. Five composite samples of corn leaves (and tassels) were collected by hand from at least 12 different areas of the treated plot (avoiding the edges) at each six sampling intervals. Two composite samples were taken from the control plots in the same manner.

Residue Sampling (Clover). Samples of white clover leaves, blossoms, nectar (hive-collected), and pollen (hive collected) were collected at four sampling periods in study year 2. Sample of clover flowers targeted growth stages of BBCH 63, 65, 67 and 69 (clover BBCH 63 corresponds to ~ 30% flowering and BBCH 69 is the end of flowering). For pollen and nectar collection, two bee-tight, ventilated mesh-covered tents (tunnel) were constructed on plot UTCB. Five tunnels were constructed on plot TRTDB at the beginning of clover blooming. Normally developed, apparently healthy and queen-right honey bees, *Apis mellifera* (purchased new from Heartland Honey in Orland, GA), were used to collect the clover pollen and nectar samples. One bee colony housed in a standard 10 frame Langstroth hive was placed in each tent 1-2 days prior to sample collection. The colonies were equipped with pollen traps. Colonies and tents were removed after the end of pollen and nectar sampling. The bee colonies were reportedly in good condition, with no visible signs of infection or infestation, throughout their use in the study.

All clover blossom and leaf samples were collected by hand into cloth sample bags from at least 12 different areas of the tent, avoiding the edges. Each sample contained a target of 100 g collected from all areas of the plant. All clover pollen and nectar samples were collected from the tented bee colonies, which were used as a sampling device for nectar (hive-deposited) and pollen (from pollen traps). After 1-2 days of bee foraging, nectar was sampled from uncapped cells within a newly placed bee hive frame using a disposable spoon and sample vial. Pollen from the blossoms in the tent was sampled from the pollen traps affixed to the colonies.

Residue Sampling (Soil). Nine soil samples were collected from all plots using a soil sampling device prior to corn planting and at the end of the growing season each year. Soil samples were collected from the surface of the plots (6 inches [15 cm] deep) by bulb planter or soil probe and placed in a plastic bag. In each sampling period, sample locations were randomly selected from the inner half of the plots (e.g., the inner 100 ft x 200 ft [30 x 60 m] area of a 200 ft x 400 ft [60 x 120 m] plot), and individual sample locations were separated by at least 10 feet (3 m).

Analytical Methods and QA/QC. The residues of imidacloprid, IMI-5-OH, and IMI-olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labelled internal standards. For total imidacloprid, the LOQ and LOD were 1 ppb and 0.5 ppb in pollen, respectively. All samples were stored within the limits of storage stability trials and spike recoveries were within 70%-120% with a standard deviation of less than 20%.

Magnitude of Residues (Corn Pollen & Tassels). Summary statistics of the overall magnitude of total IMI (sum of imidacloprid-olefin, 5-OH imidacloprid, and parent imidacloprid) in corn pollen and tassels are shown in **Table E-19.** These statistics reflect analysis of individual composite samples (usually 5/sampling date) among all of the sampling times within each trial and year. For calculation purposes, values of each analyte that were reported as less than the limit of detection (<LOD) were assumed equal to ½ the LOD. Values reported as greater than or equal to the LOD (>LOD) but less than the limit of quantitation (<LOQ) assumed equal to ½ the LOQ. As a result, residue values of total IMI at or below 1.2 ppb essentially reflect these assumptions regarding <LOD and <LOQ.

Across all three trials, yearly mean residues of total IMI in corn pollen range from approximately **2.1 – 7.5 ppb** while yearly maxima range from **4.8– 39.7 ppb**. Based on yearly means, results for corn tassels are about 2X – 3X the mean residues reported for corn pollen, with yearly average total IMI residues ranging from 4.8 to 13.7 ppb. Not surprisingly, overall trends in mean residues of total IMI in tassels across trials follow those seen in pollen. Notably, the highest mean (and maximum) values of total IMI in corn pollen and tassels occurred in trial NT012. Precipitation patterns are comparable among the three trials (especially during year 2). The percent sand in soils from trial NT012 (36%) is about 2X that of trial NT010 (16%) and 30% greater than trial NT011 (28%). This suggests that the greater accumulation in pollen and tassels n trials NT012 may be due its greater fraction of sand in soil.

Table E-19. Magnitude of total imidacloprid residues in corn pollen and tassel

Corn Pollen (ppb)									
	Trial NT010			Trial NT011			Trial NT012		
	TRTDA		TRTDB	TRTDA		TRTDB	TRTDA		TRTDB
	2012	2013	2012	2012	2013	2012	2012	2013	2012
Mean	2.9	2.6	3.2	3.2	2.1	2.7	7.5	7.4	7.0
Min.	0.9	0.60	0.9	0.9	0.9	0.9	0.6	0.6	3.30
Med.	2.7	2.5	2.7	2.8	1.8	3.0	7.2	2.8	6.9
90th	4.9	4.7	5.7	5.1	4.6	4.2	12.1	16.4	9.5
Max.	6.5	4.8	8.6	9.3	5.3	6.1	16.4	**39.7**	15.4
Corn Tassel (ppb)									
Mean	8.8	10.3	10.8	9.6	4.8	7.9	13.7	10.5	13.3
Min.	3.2	4.5	5.3	4.3	2.6	4.4	4.9	3.2	4.3
Med.	8.1	11.0	9.9	8.7	3.8	7.1	10.7	9.0	14.0
90th	13.6	14.5	17.6	14.3	8.5	11.0	25.2	18.4	25.2
Max.	20.1	17.2	23.0	22.3	9.4	15.2	**26.1**	19.8	25.7

Source: MRID 49511701.Total IMI = sum of imidacloprid, 5-OH imidacloprid, and IMI-olefin.
Bold values indicate overall maximum values from the study. Values less than the LOD were assumed equal to ½ the LOD. *n.d.* = not determined.

Magnitude of Residues (Rotational White Clover). The concentrations of total IMI measured in white clover nectar and pollen planted following planting and harvesting of seed treated corn the previous year were near or below the combined limits of detection for total IMI (1.24 ppb for pollen; 1.33 ppb for nectar) in the majority of samples analyzed (detection frequency = 28% for clover pollen and 0% for clover nectar). The maximum concentrations of total IMI measured in clover pollen in three trials is **3.8 ppb.**

Imidacloprid Metabolites. The proportion of total IMI occurring as parent and metabolites (IMI-olefin, 5-OH IMI) in corn pollen and white clover (pollen and nectar) could not be reliably determined because of the high proportion of samples with reported values below analytical detection.

Temporal Variability in Residues. Plots of the daily average residue values of total IMI (and daily ranges) in corn pollen are shown in **Figure E-6**. Among all trials, the maximum duration of the sampling period during bloom is approximately 8 days. Temporal trends in daily average total IMI in pollen were variable or declining with time in 2012 while interestingly in 2013, concentrations increased with time in each of the three trials. The maximum daily average concentration of total IMI (22.3 ppb) from DAA 84 in trial

NT012 is much greater than the previous sampling event for that trial (4.0 ppb) and is also greater than daily means from all other trials. Inspection of the raw data indicates this high value is not a result of a single outlier among the 5 replicates. The reason for the increasing concentrations of total IMI during the second year of sampling is not clear but may reflect greater desiccation of pollen over time. This explanation, however, does not explain why such increasing trends were not consistently observed during year 1 (2012).

With corn tassels, the daily average of total IMI residues generally followed a similar trend as that described previously with pollen (**Figure E-7**). This is expected since tassels are the pollen bearing portion of the corn plant. This also suggests that the increase in concentration over time consistently observed in year 2 (and in year 1 of trial NT012) is not limited to the pollen matrix alone, but rather reflects the pattern in the entire corn tassel.

Spatial Variability in Residues. The three trials were located in the midwestern U.S. with a maximum distance of approximately 270 miles. Examination of the monthly precipitation records suggests a similar magnitude and temporal pattern over time. Thus from a climate perspective, these trials are relatively similar. A range of soil types are represented (silty clay, silt loam, loam) although none were predominately composed of sand.

Pesticide Carryover. Means of total IMI in corn pollen and tassels are comparable from years 1 and 2 of TRTSTA and suggest no obvious year-to-year carry over in total IMI residues in corn pollen and tassels. It is clear that IMI residues in soil measured prior to planting in year 2 (**9-80 ppb**) are elevated compared to those measured prior to planting in year 1 **(2-4 ppb**). Within each trial/plot, concentrations of IMI increased in soil by 2-13X prior to planting in year 1 vs. year 2. Comparison of soil concentrations measured at the end of each growing season in plot TRTSTA also suggest year-to-year carry over in soil, with year 2 IMI concentrations increasing by 4X to 5X relative to year 1. It is important to recognize that these trends reflect parent IMI only and do not include possible contribution of toxic degradates (IMI-olefin, 5-OH IMI).

Classification/Utility for Bee Risk Assessment. This study is classified as acceptable and appropriate for quantitative use in risk assessment.

Reference

Miller, A., Bowers, L., Dyer, D., and Jerkins, E. (2014). *Determination of the Residues of Imidacloprid and its Metabolites IMI-5-OH and IMI-olefin in Bee Relevant Matrices Collected from Seed Treated Seed Treated Field Corn During Two Successive Years and in White Clover Planted after Seed Treated Field Corn.* Study ID EBNTY009. Bayer Crop Science, Research Triangle Park, NC. MRID 49511701

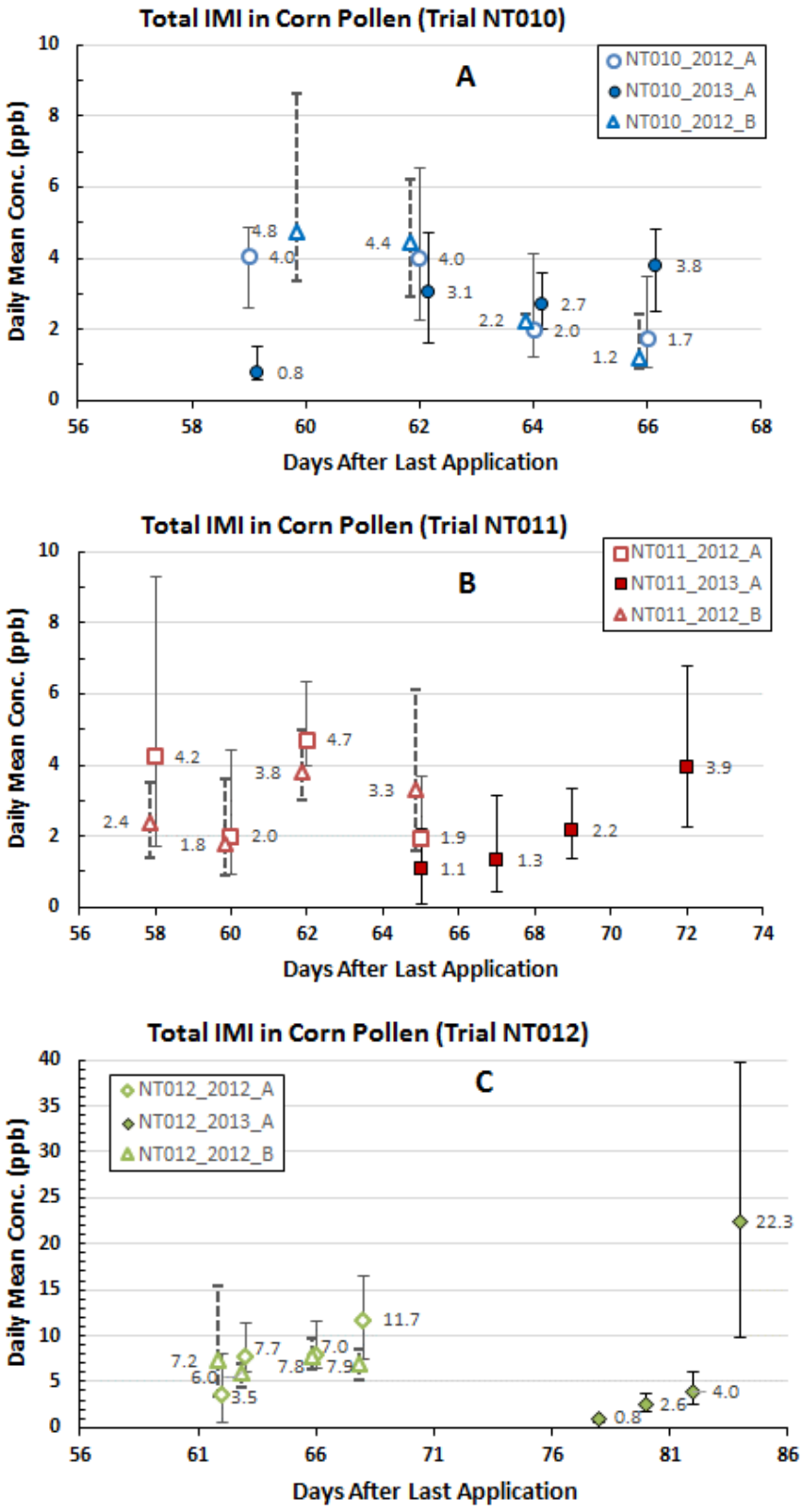


Figure E-6. Total imidacloprid residues measured in corn pollen from trials NT010, NT011 and NT012.

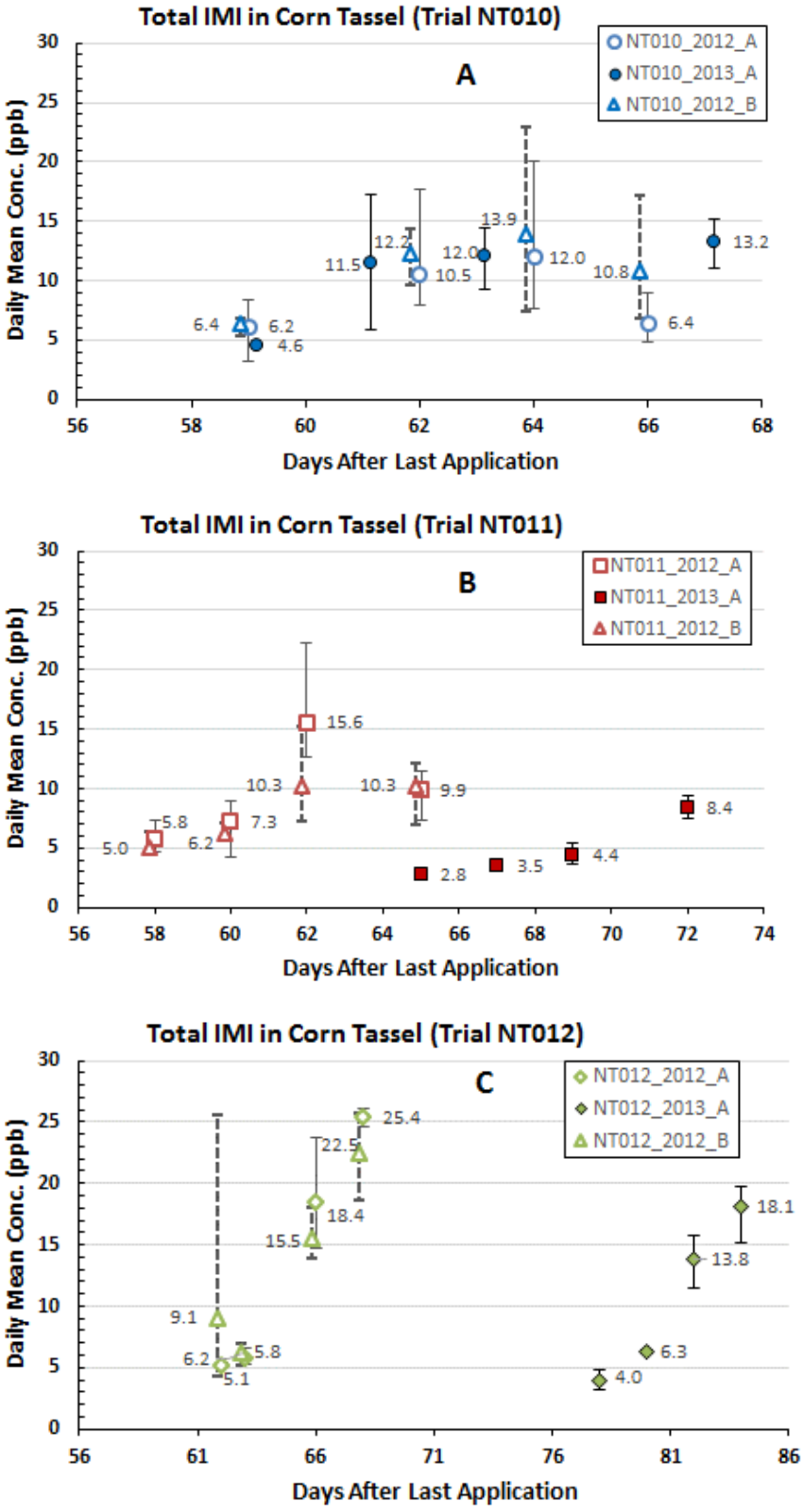


Figure E-7. Total imidacloprid residues measured in corn tassels from trials NT010, NT011 and NT012.

Combined Application Methods (Soil+Foliar)

Tomato

(MRID 49665201; Gould and Jerkins 2015)

Study Objectives and Design. A total of nine field trials were conducted to measure the magnitude of imidacloprid residues in transplanted tomato pollen and in/on transplanted tomato leaves following three applications of Admire Pro Systemic Protectant in each of two successive years. Total seasonal application rates were 0.50 lbs. ai/A (0.56 kg/ha). All nine field trials were located in central California (cities of Porterville [2], Fresno, Guadalupe, Sanger [2], San Luis Obispo, San Joaquin, and Kerman). Each of the nine field trials contained a treated (TRTD) and untreated control (UTC) plot. One bee tunnel was erected on untreated plot UTC, and 2 bee tunnels were erected on treated plot TRTD, except in trials NT013-13ZA, NT040-13ZA, and NT041-13ZA, when only one TRTD tunnel was erected. Bumble bee (*Bombus impatiens*) colonies (1 to 3 per tunnel) were placed in each tunnel for the collection of pollen. The tunnels were 100 to 210 feet long and 20 to 40 feet wide, and each tunnel enclosed four to eight rows of tomato plants. To date, Bayer CropScience has collected at least one year of residue data from all nine trial sites with five located in coarse texture, one in medium-textured, and three in fine-textured soils. Two years of data collection are complete at four sites two of which are coarse-textured, one medium-textured, and one fine-textured soils. Bayer CropScience continues work on a second year of data collection on five sites in the 2015 growing which is anticipated in January 2016.

Nature of Pesticide Application. Plot TRTD received one soil (in-furrow) drip/drench application of Admire Pro five to seven days after tomato transplantation followed by two equivalent Admire Pro foliar applications per planting season. Individual soil application rates ranged from 0.37 to 0.38 lbs. ai/A per application (0.42 to 0.43 kg/ha). The interval between the soil and first foliar applications was 48 to 78 days. Individual foliar application rates ranged from 0.058 to 0.062 lbs. ai/A/application (0.066 to 0.070 kg/ha). All foliar applications were made to flowering tomato plants, after the first two sampling events were complete. The interval between foliar applications was four to five days. The foliar spray volumes ranged from 50 to 101 gal/A, with the exception of the second foliar spray in 2013 to trial NT018-13ZA (48 gal/A). Total seasonal application rates were 0.50 lbs. ai/A (0.56 kg/ha).

Residue Sampling. Tomato leaf and pollen samples were collected at four sampling intervals: two samples were collected after the soil application, approximately 14 days apart (31 to 68 and 45 to 77 days after the soil application, respectively), and two samples were collected after the last foliar application, approximately 14 days apart (2 to 8 and 16 to 22 days after the last foliar application, respectively). At each interval, fresh bumble bee colonies were placed in each bee tunnel, and the bumble bees were allowed to forage from the tomato flowers for several days. Then, bumble bees carrying pollen were collected from the tunnels, and the pollen was removed from the bees. During the described sampling intervals, composite samples of tomato leaves were collected from within the tunnels of the treated plots. Composite samples of tomato pollen and leaves were collected from the control plot tunnel of each trial on the same days and using the same methods as samples collected from the treated plots. One sample was collected per bee tunnel, yielding two TRTD samples and one UTC sample at each sampling interval,

except in trials NT013-13ZA, NT040-13ZA, and NT041-13ZA, when two replicate samples were collected from the single erected TRTD tunnel.

Analytical Methods and QA/QC. The residues of imidacloprid, IMI-5-OH, and IMI-olefin were quantified by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labelled internal standards. For total imidacloprid, the LOQ and LOD were 1 ppb and 0.7 ppb in nectar, and 1 ppb and 0.5 ppb in pollen, respectively. All samples were stored within the limits of storage stability trials and spike recoveries were within 70%-120% with a standard deviation of less than 20%.

The residues of Admire Pro Systemic Protectant (imidacloprid, IMI-5-OH, and IMI-olefin) were quantified by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labelled internal standards. The individual analyte residues were summed to give a total imidacloprid residue.

The methods for determining imidacloprid, IMI-5-OH, and IMI-olefin residues in/on tomato leaves and pollen were validated by measuring the recovery of these analytes from control matrices fortified at their respective LOQs. Additional recoveries at higher fortification levels validated the method for the highest residues observed in individual matrices. Concurrent recoveries of imidacloprid, IMI-5-OH, and IMI-olefin from fortified samples were measured with each set of samples to verify method performance. All recoveries were corrected for any interference in corresponding controls. The overall means of the recoveries for each matrix at each fortification level were within the acceptable range of 70 to 120%, and the standard deviation values were below 20%.

Storage stability studies indicate that the imidacloprid residues would have been stable during frozen storage for at least for at least 1,080 days (36 months) in tomato leaves prior to analysis. Transit stability samples showed that imidacloprid residues were stable in pollen for the duration of the study. The maximum storage period of frozen samples in this study for Admire Pro Systemic Protectant was 561 days for tomato leaves and 560 days for tomato pollen.

Magnitude of Residues. Summary statistics of the concentration for imidacloprid and its metabolites in tomato pollen and leaves are contained in **Table E-20** and **Table E-21**. Comparison of overall statistics for total imidacloprid residue indicated much greater concentrations in all plant parts at the third sampling interval than at the other three sampling intervals. Mean total imidacloprid residues in pollen, which is relevant to potential bee exposure, was 66 and 39 ppb for sampling intervals 1 and 2, respectively (**Table E-20**). After the second sampling interval plants received two additional foliar applications at approximately 5 day intervals. Concentrations in tomato pollen at the third sampling interval that occurred around 6 days after the second foliar application increased nearly tenfold to a mean of 493 ppb with the 90th percentile value at 1,003 ppb and the maximum value at 1268 ppb. The fourth and final sampling interval occurred around 19 days after the second foliar application, when the mean total imidacloprid residue concentration was found to drop back down to 88 ppb. For leaves the same pattern was measured as for pollen where mean total imidacloprid values were 163, 143, 1527, and 131 ppb for intervals 1, 2, 3, and 4, respectively (**Table E-21**).

Table E-20. Magnitude of imidacloprid and related metabolites in tomato pollen (ppb).

	Interval 1				Interval 2				Interval 3				Interval 4			
	Olefin	**5-OH**	**IMI**	**Total**	**Olefin**	**5-OH**	**IMI**	**Total**	**Olefin**	**5-OH**	**IMI**	**Total**	**Olefin**	**5-OH**	**IMI**	**Total**
N	12	12	12	12	10	10	10	10	10	10	10	10	13	13	13	13
Mean	1.1	2.7	62.1	66	0.4	1.5	37.1	39	6.0	22.5	464.3	492.9	1.8	7.0	78.7	88
SD	1.4	2.7	54.0	58.0	0.4	1.2	29.5	30.8	1.8	12.2	291.8	302.7	1.6	10.1	75.9	86.0
CV (%)	127%	103%	87%	88%	107%	94%	80%	79%	31%	54%	63%	61%	86%	144%	96%	99%
Min.	0.2	0.3	6.6	7.0	0.2	0.3	2.6	3.0	3.0	9.7	232.9	249.1	0.2	1.0	21.8	22.9
Median	0.5	1.4	38.6	40.9	0.2	1.2	29.4	30.8	6.4	16.8	364.8	387.1	0.9	4.4	60.6	68.4
75th	1.7	4.3	102.3	108.3	0.4	2.1	48.2	51.6	7.9	30.2	426.1	458.9	3.6	6.9	79.1	87.3
90th	2.4	6.6	121.9	130.8	1.0	4.0	83.4	87.6	8.0	40.0	955.9	1003	3.9	9.7	119.6	132.9
Max.	4.6	8.7	185.1	198.4	1.4	4.8	103.3	106.6	8.1	49.3	1212.3	1268.4	4.4	39.4	312.2	353.9
% of Total	1.7%	4.1%	94.2%		0.9%	3.8%	95.1%		1.2%	4.6%	94.2%		2.1%	8.0%	89.8%	

NOTES: IMI= imidacloprid, olefin = IMI-olefin, 5-OH = 5 hydroxy imidacloprid, Total = sum of IMI, olefin and 5-OH (in bold). SD=standard deviation, CV = coefficient of variation.

Table E-21. Magnitude of imidacloprid and related metabolites in tomato leaves (ppb).

	Interval 1				Interval 2				Interval 3				Interval 4			
	Olefin	**5-OH**	**IMI**	**Total**	**Olefin**	**5-OH**	**IMI**	**Total**	**Olefin**	**5-OH**	**IMI**	**Total**	**Olefin**	**5-OH**	**IMI**	**Total**
N	13	13	13	**13**	13	13	13	**13**	13	13	13	**13**	13	13	13	**13**
Mean	7.2	8.1	147.6	**163**	6.2	5.7	131.1	**143**	38.1	71.0	1417.5	**1527**	7.2	8.6	115.1	**131**
SD	7.0	11.3	148.6	**165.7**	4.6	5.3	100.2	**109.4**	21.8	46.8	1213.3	**1276.1**	4.2	5.9	86.8	**95.8**
CV (%)	97%	141%	101%	**102%**	74%	92%	76%	**76%**	57%	66%	86%	**84%**	58%	68%	75%	**73%**
Min.	0.5	0.4	1.7	**2.5**	0.8	0.4	18.6	**20.7**	7.6	12.3	221.9	**241.8**	2.3	1.7	39.1	**43.1**
Median	4.5	4.4	111.5	**121.1**	6.3	3.4	102.2	**114.6**	29.6	56.2	863.6	**942.8**	6.2	7.4	67.5	**82.3**
75th	9.6	6.3	159.2	**173.5**	9.0	9.0	231.9	**253.7**	54.0	107.7	2794.2	**2968.2**	9.5	11.3	129.1	**150.0**
90th	16.4	26.5	388.1	**445.8**	13.3	13.3	266.4	**293.4**	69.2	121.3	3311.5	**3502.3**	9.7	16.3	268.6	**292.1**
Max.	24.7	38.3	517.2	**568.4**	13.5	16.5	284.0	**306.6**	78.0	163.6	3348.9	**3566.5**	18.5	22.0	324.7	**365.2**
% of Total	4.4%	5.0%	90.7%		4.3%	4.0%	91.6%		2.5%	4.7%	92.8%		5.5%	6.6%	87.9%	

NOTES: IMI= imidacloprid, olefin = IMI-olefin, 5-OH = 5 hydroxy imidacloprid, Total = sum of IMI, olefin and 5-OH (in bold). SD=standard deviation, CV = coefficient of variation.

Temporal Variability in Residues. The general pattern of mean concentrations rising nearly tenfold at the third sampling interval was observed at all sites. A sharp decline in concentration between intervals 3 and 4 was also predominant except for the San Luis Obispo site where there was only a slight decrease at interval 4.

Spatial Variability in Residues. There were no analyses of effects of the effects of soil type on imidacloprid residues in plant matrices in this study. However, preliminary analysis indicate that levels measured in pollen from fine-textured soil sites approximate levels measured in coarse-textured soils, especially for sampling interval 3. Thus, data at interval 3 provide an initial indication that foliar applications greatly increased levels in all plant parts irrespective of soil type.

Pesticide Carryover. Comparisons of imidacloprid levels measured between 2013 and 2014 are available for only one site, the Sanger site, for pollen, and for 4 sites, the Sanger, Porterville, and Fresno sites for leaves. For leaves, the values in 2013 are either equal to or greater than the values measured in 2014. For pollen the values are similar between the two years. Although the data are sparse there is no indication of a carry-over effect of residues between each year.

Classification/Utility for Bee Risk Assessment. The data from this study provide an expected distribution of the concentration of imidacloprid residues that bees are exposed to in pollen of tomato plants grown under actual agronomic practices in California. The study is considered scientifically sound and useful for risk assessment purposes. The study is classified as ACCEPTABLE for quantitative use in risk assessment

Reference

Gould, T., and Jerkins, E. (2015) Determination of the Residues of Imidacloprid, IMI-5-OH, and IMI-olefin in Bee Relevant Matrices Collected from Tomatoes Following Application of Imidacloprid Over Two Successive Years: Final Report. Project Number: EBNTN012. Unpublished study prepared by Bayer CropScience 466p. MRID 49665201.

Cotton

(MRID 49665202; Fischer and Jerkins 2015)

Study Objectives and Design. This study, conducted in 2013 in California's Central Valley, measured the concentrations of imidacloprid and its metabolites (IMI-5-OH and IMI-olefin) in cotton leaves, extrafloral nectar, floral nectar, and pollen. The cotton was grown in nine locations (sites) treated with one soil application of imidacloprid in 2013 followed by three foliar applications starting 75 to 99 days after the soil application. One soil application of imidacloprid in 2013 was followed by three foliar applications starting 75 to 99 days after the soil application. Imidacloprid was applied to soil at a rate of 0.33 to 0.34 lbs. ai/A and the foliar applications were conducted at rates ranging from 0.056 to 0.060 lbs. ai/A. The interval between the three foliar applications was six to seven days. Of the nine sites used in the study there were four sites with coarse soil, three sites with medium soil, and two sites with fine soil.

Nature of Pesticide Application. All nine sites had one soil application (0.33 to 0.34 lbs. ai/A), followed 75 to 99 days later by three foliar applications (0.056 to 0.060 lbs. ai/A) with six or seven day retreatment intervals. The end-product used was Admire Pro Systemic Protectant (550 g/L) (Imidacloprid SC 550 G). The adjuvant Dyne-Amic (0.25% v/v) was used during the three foliar applications. The total seasonal application rates used in the study were 0.50 to 0.51 lbs. a.i/A.

Residue Sampling. Samples were collected four to five days before the first foliar application, four to five days after the third (and final) foliar application, and 12 to 14 days after the third foliar application. Each composite flower sample weighed at least 125 grams and contained at least 250 flowers. Each composite leaf sample weighed at least 100 grams. Extrafloral nectar was collected from sub bracteal and inner bracteal nectaries using a micropipette. Floral nectar was collected from floral nectaries. Pollen was collected using either vacuum aspiration or by tapping the pollen from the blossoms onto wax paper.

Analytical Methods and QA/QC. Residues of imidacloprid, IMI-5-OH, and IMI-olefin were analyzed using high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) using stable, isotopically labelled internal standards. The Limit of Quantification (LOQ) for of imidacloprid, IMI-5-OH, IMI-olefin, and total imidacloprid was 5 ppb for leaves and 1 ppb for extrafloral nectar, floral nectar, and pollen. The Limit of Detection (LOD) values varied according to the matrix and the analyte. Samples were frozen at ≤ -11°C before analysis for a maximum of 569 days. Storage stability studies show that cotton leaves are stable (<30% decomposition) for at least 1080 days (36 months) during frozen storage. The transit stability samples, fortified with approximately 100 ppb each of imidacloprid and its metabolites IMI-5-OH and IMI-olefin, accompanied the respective study sample types throughout the sampling, shipping, and storage events of the field phase of the study. The average transit spike stability data for imidacloprid, IMI-olefin, and IMI-5-OH in nectar and pollen were ≥ 70% for the entire study so the residue data were not corrected for any in-storage decomposition.

Magnitude of Residues. Total imidacloprid was calculated as the sum of imidacloprid, IMI-5-OH, and IMI-olefin in parent equivalents. Summary statistics for the distribution of the concentration of total imidacloprid in cotton nectar, extra floral nectar, pollen, and leaves are presented in the Tables. In cases where individual analyte residues were less than the LOD, a value of half the LOD was used to calculate total imidacloprid. For all matrices the highest residue values were observed four to five days after the third (and final) foliar application.

Floral Nectar: The first soil application occurred at planting. Sampling for the first interval occurred at a mean of 81 days after the soil application with a range of values from 70 to 95 days. Mean total imidacloprid residue was 23.1 ppb in floral nectar (**Table E-22**). The second sampling interval was conducted after 3 foliar applications of imidacloprid and was approximately 23 days after the first interval. Concentrations in floral nectar at the second sampling interval increased to a mean of 83.9 ppb. The third sampling interval occurred approximately 9 days after the second interval with total imidacloprid residue concentration dropping to a mean of 38.7 ppb. **Table E-22** compares the total imidacloprid residues in floral nectar between soil types. At interval 1, coarse mean values appear higher. At interval 2, the fine mean value is higher than for coarse soil. At interval 3, coarse and medium values are similar.

Table E-22. Magnitude of imidacloprid and related metabolites in cotton floral nectar (ppb).

	Interval 1				Interval 2				Interval 3			
	Olefin	**5-OH**	**IMI**	**Total**	**Olefin**	**5-OH**	**IMI**	**Total**	**Olefin**	**5-OH**	**IMI**	**Total**
N	15	15	15	**15**	15	15	15	**15**	15	15	15	**15**
Mean	0.7	0.7	21.8	**23.1**	1.4	2.4	80.2	**83.9**	1.2	1.2	36.4	**38.7**
SD	0.5	0.4	27.0	**27.7**	0.8	1.1	38.9	**40.5**	0.7	0.7	25.8	**26.9**
CV (%)	70%	60%	124%	**120%**	56%	45%	49%	**48%**	61%	57%	71%	**69%**
Min.	0.3	0.4	0.4	**1.0**	0.5	1.0	19.2	**20.8**	0.3	0.6	9.3	**10.5**
Median	0.5	0.5	5.8	**6.7**	1.3	2.2	80.1	**83.6**	0.8	0.8	23.5	**25.1**
75th	1.1	0.9	41.4	**43.1**	1.9	3.5	107.6	**113.4**	1.7	1.6	54.1	**57.4**
90th	1.5	1.5	71.0	**74.1**	3.0	3.9	139.0	**144.0**	1.9	2.4	75.8	**78.8**
Max.	1.6	1.5	80.9	**83.1**	3.2	4.1	146.9	**153.3**	2.9	2.9	99.3	**103.9**
% of Total	2.9%	2.9%	94.4%		1.7%	2.8%	95.5%		3.0%	3.2%	93.9%	

NOTES: IMI= imidacloprid, olefin = IMI-olefin, 5-OH = 5 hydroxy imidacloprid, Total = sum of IMI, olefin and 5-OH (in bold). SD=standard deviation, CV = coefficient of variation.

Table E-23 Comparison of total imidacloprid residues between soil types at each sampling period for floral nectar (ppb).

	Interval 1			Interval 2			Interval 3		
	Coarse	**Medium**	**Fine**	**Coarse**	**Medium**	**Fine**	**Coarse**	**Medium**	**Fine**
N	10	3	2	10	3	2	10	3	2
Mean	32	9	2	88	51	114	45	30	20
SD	31	8	0.4	39	32	43	31	9	2
CV (%)	96	88	19	45	62	38	69	31	12
Min.	1	3	2	32	21	84	11	24	18
Median	24	6	2	86	48	114	40	25	20
75th	53	18	2	113	84	144	64	41	22
90th	79	18	2	144	84	144	91	41	22
Max.	83	18	2	153	84	144	104	41	22

Extrafloral Nectar. Although a similar increase in concentrations were observed at sampling interval 2 for extrafloral nectar, the increase in concentration measured was much larger than observed for floral nectar (**Table E-24**). The mean value was 438 ppb, which was approximately 500% greater than the value measured for floral nectar. There were a few high values measured in the extrafloral nectar which tend to bias the mean value. Comparison of the median values, which puts less weight on the large values, still indicated a large increase in residues in the extrafloral nectar where the median was 296 ppb as compared to 84 ppb for the floral nectar. Comparison of median values still indicated a substantial increase in concentration in extrafloral compared to floral nectar of approximately 350%. **Table E-25** compares the total imidacloprid residues in extrafloral nectar between soil types. At interval 1, coarse mean values similar to medium mean values. At interval 2, coarse mean value higher than other two types but all values are extremely high. At interval 3, mean values are similar.

Table E-24. Magnitude of imidacloprid and related metabolites in cotton extrafloral nectar (ppb).

	Interval 1				Interval 2				Interval 3			
	Olefin	**5-OH**	**IMI**	**Total**	**Olefin**	**5-OH**	**IMI**	**Total**	**Olefin**	**5-OH**	**IMI**	**Total**
N	14	14	14	**14**	15	15	15	**15**	15	15	15	**15**
Mean	0.9	0.5	7.3	**8.4**	2.5	12.1	423.8	**438.3**	0.6	1.5	30.8	**32.9**
SD	1.3	0.3	9.5	**9.7**	2.7	12.1	461.0	**475.2**	0.7	0.8	19.5	**20.2**
CV (%)	142%	65%	129%	**116%**	112%	97%	109%	**108%**	116%	57%	63%	**61%**
Min.	0.3	0.4	0.5	**1.2**	0.9	2.9	50.5	**54.3**	0.3	0.5	5.2	**7.6**
Median	0.4	0.4	4.5	**5.4**	1.4	8.2	284.7	**296.6**	0.3	1.5	26.0	**26.8**
75th	0.8	0.5	9.1	**9.7**	2.5	13.2	524.3	**538.8**	0.5	2.0	57.8	**60.7**
90th	1.8	1.0	18.1	**19.3**	4.8	26.0	904.0	**932.5**	0.9	2.6	59.0	**63.1**
Max.	5.0	1.4	34.1	**35.9**	11.6	48.9	1891.0	**1951.5**	3.1	3.6	64.8	**66.7**
% of Total	10.5%	6.3%	87.1%		0.6%	2.8%	96.7%		1.8%	4.5%	93.6%	

NOTES: IMI= imidacloprid, olefin = IMI-olefin, 5-OH = 5 hydroxy imidacloprid, Total = sum of IMI, olefin and 5-OH (in bold). SD=standard deviation, CV = coefficient of variation.

Table E-25. Comparison of total imidacloprid residues between soil types at each sampling period for extrafloral nectar (ppb).

	Interval 1			Interval 2			Interval 3		
	Coarse	**Medium**	**Fine**	**Coarse**	**Medium**	**Fine**	**Coarse**	**Medium**	**Fine**
N	10	3	2	10	3	2	10	3	2
Mean	10	5	1	567	216	135	31	32	41
SD	11	4	0	540	140	9	19	25	31
CV (%)	101	96	8	96	65	6	61	79	76
Min.	1	5	1	179	54	129	8	12	19
Median	7	8	1	373	297	135	28	24	41
75th	16	8	1	624	297	141	35	61	63
90th	28	8	1	1442	297	141	64	61	63
Max.	36	8	1	1952	297	141	67	61	63

SD=standard deviation, CV = coefficient of variation

Pollen. Mean residues of total imidacloprid in cotton pollen also followed the pattern noted in floral nectar where concentrations were greater at sampling interval 2 than at the other two sampling intervals (**Table E-26**). The mean values appeared similar to those measured for floral nectar but the maximum value at sampling interval 2 was twice as large as measured for floral nectar.

Table E-26. Magnitude of imidacloprid and related metabolites in cotton pollen (ppb).

	Interval 1				Interval 2				Interval 3			
	Olefin	**5-OH**	**IMI**	**Total**	**Olefin**	**5-OH**	**IMI**	**Total**	**Olefin**	**5-OH**	**IMI**	**Total**
N	15	15	15	**15**	15	15	15	**15**	15	15	15	**15**
Mean	0.2	0.3	5.6	**6.1**	0.6	1.2	85.0	**86.9**	0.5	0.8	39.7	**41.0**
SD	0.1	0.1	10.7	**10.8**	0.8	1.3	102.3	**104.1**	0.5	1.0	48.7	**50.0**
CV (%)	52%	46%	190%	**178%**	125%	104%	120%	**120%**	99%	113%	123%	**122%**
Min.	0.2	0.3	0.2	**0.6**	0.2	0.3	3.8	**4.2**	0.2	0.3	2.8	**3.2**
Median	0.2	0.3	0.6	**1.0**	0.2	0.8	36.3	**39.9**	0.2	0.4	12.0	**12.6**
75th	0.2	0.3	10.4	**10.8**	0.8	2.0	154.1	**156.7**	0.8	0.8	85.2	**87.5**
90th	0.2	0.25	13.9	**14.3**	2.0	3.3	280.0	**285.2**	1.5	2.7	123.7	**126.9**
Max.	0.5	0.8	40.2	**41.1**	2.4	4.3	317.7	**324.4**	1.5	2.9	127.2	**131.6**
% of Total	2.8%	4.6%	92.6%		0.7%	1.4%	97.8%		1.3%	2.0%	96.8%	

NOTES: IMI= imidacloprid, olefin = IMI-olefin, 5-OH = 5 hydroxy imidacloprid, Total = sum of IMI, olefin and 5-OH (in bold). SD=standard deviation, CV = coefficient of variation.

Leaves. As expected, direct foliar application of imidacloprid to plants between the first and second sampling interval greatly increased the magnitude of residues of total imidacloprid in leaves measured at the second sampling interval (**Table E-27**). At around 80 days after the soil application mean total residues in leaves was at 49 ppb. The concentration at sampling interval 2 was 30 times greater at 1621 ppb. At interval 3 the levels in leaves were greatly decreased measured at 327 ppb, which was still greater than measured at the first interval.

Table E-27. Magnitude of imidacloprid and related metabolites in cotton leaves (ppb).

	Interval 1				Interval 2				Interval 3			
	Olefin	**5-OH**	**IMI**	**Total**	**Olefin**	**5-OH**	**IMI**	**Total**	**Olefin**	**5-OH**	**IMI**	**Total**
N	15	15	15	**15**	15	15	15	**15**	15	15	15	**15**
Mean	2.7	3.7	42.1	**48.5**	42.7	105.9	1472.7	**1621.2**	26.5	41.8	258.5	**326.7**
SD	4.3	4.3	49.5	**56.8**	17.3	35.9	678.9	**711.4**	10.5	15.7	140.4	**156.6**
CV (%)	124%	114%	118%	**117%**	41%	34%	46%	**44%**	40%	38%	54%	**48%**
Min.	0.4	0.4	0.6	**1.3**	14.9	62.5	437.8	**524.5**	4.8	16.4	40.2	**61.4**
Median	0.7	1.5	25.5	**27.4**	43.8	97.9	1371.5	**1509.6**	28.3	39.1	233.6	**305.0**
75th	4.4	5.3	55.4	**62.2**	47.7	121.3	1780.7	**2043.7**	34.7	52.2	333.5	**416.1**
90th	9.5	11.6	118.0	**140.1**	65.2	159.1	2653.6	**2860.3**	36.3	57.9	418.0	**503.0**
Max.	10.5	13.7	169.7	**192.9**	78.4	184.6	2956.7	**3098.2**	46.2	79.6	606.0	**698.7**
% of Total	5.6%	7.7%	86.8%		2.6%	6.5%	90.8%		8.1%	12.8%	79.1%	

NOTES: IMI= imidacloprid, olefin = IMI-olefin, 5-OH = 5 hydroxy imidacloprid, Total = sum of IMI, olefin and 5-OH (in bold). SD=standard deviation, CV = coefficient of variation.

Temporal Variability in Residues. Samples were collected four to five days before the first foliar application, four to five days after the third (and final) foliar application, and 12 to 14 days after the third foliar application. The highest concentrations were recorded four to five days after the third foliar application.

Spatial Variability in Residues. All nine sites were in California's Central Valley, in the cities of Davis, Fresno, Kerman, Sanger, Wheatland, and Yuba City. Of the nine sites used in the study there were four sites with coarse soil, three sites with medium soil, and two sites with fine soil.

Pesticide Carryover. The extent to which prior year applications of imidacloprid contributed to year-to-year carryover was not analyzed by the study authors.

Classification/Utility for Bee Risk Assessment. This study is classified as ACCEPTABLE for quantitative use in the risk assessment.

Reference

Fischer, D.R., and Jerkins, E. (2015) Determination of the Residues of Imidacloprid, IMI-5-OH, and IMI-olefin in Bee Relevant Matrices Collected from Cotton During Two Successive Years: Final Report. Project Number: EBNTN011. Unpublished study prepared by Bayer CropScience, Research Triangle Park, NC. 632p. MRID 49665202.

Combined Application Methods (Seed + Foliar)

Cotton

(MRID 49511702; Gould et al., 2014)

Study Objectives and Design. This study was designed to measure the magnitude of imidacloprid residues in bee-relevant pollen and nectar (floral and extrafloral) samples and in/on flowers, leaves, and soil in cotton grown from imidacloprid-treated seeds (Gaucho 600 Flowable) and pre-bloom, foliar applications of imidacloprid (Admire Pro Systemic Protectant) over two successive years. In addition, this study examined the occurrence of imidacloprid residues in bee relevant matrices of white clover planted after foliar application to seed treated cotton harvested the previous year. Six separate trials were conducted in three study sites (NT013 in Malden, MO; NT014 in Glennonville, MO; and NT015 in Fisk, MO). Three of the trials focused on residues in cotton over two years (TRTDA) and the other half focused on residues in cotton after 1 year and rotationally-planted white clover during the second year (TRTDB).

Treated plots were approximately 1.5 ac in area and had similar row and plant spacing. Soils at trial NT013 were classified as sandy loam (66% sand, 30% silt, 4% clay); soils at trial NT014 were classified as silt loam (20% sand, 61% silt, 19% clay) and soils at trial NT015 were classified as sand (89% sand, 10% silt, 1% clay). Temperatures and total precipitation were similar among sites. Monthly precipitation patterns follow similar temporal patterns which is expected given the relatively close proximity of study sites. All three sites were irrigated to some extent during the summer months (0.5 – 3 in/month).

Nature of Pesticide Application. The TRTDA trials consisted of seed treatment (0.375 mg a.i/seed or 0.047 lbs. a.i/A) and 5 x 0.06 lbs. a.i/A foliar applications (5-8 days apart) each year over two successive years (total application rate = 0.35 lbs. a.i/A). The TRTDB trials consisted of seed treatment and 5 x 0.06 lbs. a.i/A foliar applications (5-8 days apart) during year 1 followed by planting of white clover and residue sampling of clover during year 2.). All applications were made with adjuvant (Dyne-Amic, 0.25%) using a ground-based equipment. Plot seeding rates ranged from 57,088 to 60,002 seeds/A. In addition to the treated crop study sites, two control sites were included for each trial (UTCA and UTCB). The year 1 foliar applications (TRTDA and TRTDB were made between BBCH growth stage 19 (nine or more leaves unfolded) and BBCH 60 (first flowers open). The year 2 foliar applications were made between BBCH growth stage 24 (four side shoots detectable) and BBCH 59 (first petals visible and many flower buds closed).

Residue Sampling (Cotton). Cotton sample collection began 13 to 15 days after the last application (DAA) each year. Samples were collected at five sampling intervals per year of collection. The sampling intervals were targeted for growth stages of BBCH 60, 61, 65, 67, and 69 (cotton BBCH 60: first flowers opened, sporadically within the population; BBCH 69: end of flowering). Five composite samples of cotton blossoms for direct analysis, cotton blossoms for pollen and nectar processing, and cotton leaves were collected by hand from at least 12 different areas of the treated plot (avoiding the edges) at each sampling interval. Two composite samples were taken from the control plots in the same manner.

Residue Sampling (Clover). Samples of white clover leaves, blossoms, nectar (hive-collected), and pollen (hive collected) were collected at four sampling periods in study year 2. Sample collection was targeted for growth stages of BBCH 61, 63, 65, and 67 (clover BBCH 61: flowers open on first raceme; BBCH 67: flowering declining). For trials NT014-12HA and NT015-12ZA, two bee-tight, ventilated mesh-covered tents (tunnel) were constructed on plot UTCB. Five tunnels were constructed on plot TRTDB. The tents were constructed after the last test substance application (to plot TRTDA) in year 2 and around the start of clover blooming. Normally developed, apparently healthy and queen-right honey bees, *Apis mellifera* (purchased from local sources), were used to collect the clover pollen and nectar samples. One bee colony housed in a standard 10 frame Langstroth hive was placed in each tent 3-4 days prior to sample collection. The colonies were equipped with pollen traps. Colonies and tents were removed after the end of pollen and nectar sampling. The bee colonies were reportedly in good condition, with no visible signs of infection or infestation, throughout their use in the study.

All clover blossom and leaf samples were collected by hand into cloth sample bags from at least 12 different areas of the tent, avoiding the edges. Each sample contained a target of 100 g collected from all areas of the plant. All clover pollen and nectar samples were collected from the tented bee colonies, which were used as a sampling device for nectar (hive-deposited) and pollen (from pollen traps). After 3-4 days of bee foraging, nectar was sampled from uncapped cells within a newly placed bee hive frame using a disposable spoon and sample vial. Pollen from the blossoms in the tent was sampled from the pollen traps affixed to the colonies.

Residue Sampling (Soil). Nine soil samples were collected from all plots using a soil sampling device before cotton planting in year 1, before cotton planting in year 2, and after all sampling was complete in year 2. Soil samples were collected from the surface of the plots (6 inches [15 cm] deep) by bulb planter or soil probe and placed in a plastic bag. In each sampling period, sample locations were randomly selected from the inner half of the plots (e.g., the inner 100 ft x 200 ft [30 x 60 m] area of a 200 ft x 400 ft [60 x 120 m] plot), and individual sample locations were separated by at least 10 feet (3 m).

Analytical Methods and QA/QC. The residues of imidacloprid, IMI-5-OH, and IMI-olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labelled internal standards. For total imidacloprid, the LOQ and LOD were 1 ppb and 0.5 ppb in pollen, respectively. All samples were stored within the limits of storage stability trials and spike recoveries were within 70%-120% with a standard deviation of less than 20%.

Magnitude of Residues (Cotton Floral Nectar, Extrafloral Nectar, Pollen). Summary statistics of the overall magnitude of total IMI (sum of IMI-olefin, IMI-5-OH, and parent imidacloprid) in cotton pollen and nectar are shown in **Table E-28.** These statistics reflect analysis of individual composite samples (usually 5/sampling date) among all of the sampling times within each trial and year. For calculation purposes, values of each analyte that were reported as less than the limit of detection (<LOD) were assumed equal to ½ the LOD. Values reported as greater than or equal to the LOD (>LOD) but less than the limit of quantitation (<LOQ) assumed equal to ½ the LOQ. As a result, residue values of 1.2 ppb total IMI in pollen at 0.9 ppb in nectar reflect these assumptions of ½ the LOD.

Across all three trials, yearly mean residues of total IMI in cotton extrafloral nectar range from approximately **3.4 – 11 ppb** while yearly maxima range from **21– 30 ppb**. Results for floral nectar are somewhat greater than those for extrafloral nectar, with yearly average total IMI residues ranging from **4.3 to 16.2 ppb** and yearly maxima ranging from **10.7 to 39.5 ppb.** Notably, the highest mean (and maximum) values of total IMI in floral and extrafloral nectar occurred in trials NT013 and NT015 (year 2 of TRTDA). The percent sand in soils from trials NT013 and NT015 (66% and 89%) are 3X and 4X that found in trial NT014 (19%). **This suggests that the greater accumulation in nectar in trials NT013 and NT015 may be due to their coarser soils.**

Yearly mean residues of total IMI pollen vary from **1.5 – 6.9 ppb** while maxima range from **3.9 – 56.7 ppb**. As observed with floral nectar, the highest yearly mean (and maximum) values of total IMI in cotton pollen occurred in year 2 of the TRTDA trials, which were 1.5 – 3X the respective yearly means from year 1. The relationship between total IMI residues in pollen and soil type (% sand vs. % silt) was not as evident as that observed with floral and extrafloral nectar. Specifically, the lowest mean and maxima residues of total IMI occurred with trial NT015 (89% sand) while mean and maxima residues in trials NT013 and NT014 are similar despite their large difference in soil coarseness (66% and 19% sand, respectively).

Table E-28. Magnitude of total imidacloprid residues in cotton extrafloral nectar, floral nectar and pollen

	Trial NT013			Trial NT014			Trial NT015		
	TRTDA		TRTDB	TRTDA		TRTDB	TRTDA		TRTDB
	2012	2013	2012	2012	2013	2012	2012	2013	2012
Extrafloral Nectar (ppb)									
Mean	4.1	11	3.4	4.2	5.1	4.2	4.2	7.1	2.5
Min.	1	2.8	1.8	1.2	1.2	1.2	1.2	2.2	1.2
Med.	3.2	12	3.1	2.4	2.6	1.8	4.2	5.1	2.5
90th	7.5	19	7.2	12	14	11	9.3	11	4.9
Max.	9.1	24	8.9	16	21	16	14	**30**	6.2
n	*20*	*25*	*14*	*21*	*25*	*24*	*18*	***25***	*20*
Floral Nectar (ppb)									
Mean	5.2	14.4	9.0	5.2	7.4	4.3	9.8	16.2	6.6
Min.	2.4	5.9	2.3	2.0	1.9	1.8	2.7	4.2	3.3
Med.	4.6	11.0	5.0	3.3	2.6	2.6	9.6	11.7	6.0
90th	8.8	26.3	19.9	12.9	18.0	10.1	14.3	29.8	9.9
Max.	11.6	28.9	38.4	15.7	21.9	17.7	19.8	**39.5**	10.7
n	*18*	*25*	*18*	*25*	*25*	*24*	*25*	***25***	*25*
Concentration in Pollen (ppb)									
Mean	2.4	6.9	2.7	4.0	6.1	5.1	1.5	3.0	1.5
Min.	0.9	0.6	0.9	0.6	0.6	0.6	0.6	0.6	0.6
Med.	2.1	2.8	1.7	1.7	0.6	1.9	0.9	0.9	0.9
90th	3.9	16.8	6.3	12.2	19.4	5.2	3.2	6.2	3.5
Max.	5.3	36.0	11.7	17.4	47.4	**56.7**	3.9	19.4	4.4
n	*19*	*25*	*18*	*25*	*25*	***25***	*25*	*25*	*24*

Source: MRID 49511702.Total IMI = sum of imidacloprid, 5-OH imidacloprid, and IMI-olefin.
Bold values indicate overall maximum values from the study. Values less than the LOD were assumed equal to ½ the LOD.

Magnitude of Residues (Rotational White Clover). The concentrations of total IMI measured in white clover nectar and pollen planted following foliar application to seed treated cotton harvested the previous year (trials NT014 and NT015) were near or below the level of detection (0.7 ppb) in the majority of samples analyzed (detection frequency = 38% for clover nectar and 53% for clover pollen). The maximum concentrations of total IMI measured in clover nectar in trials NT014 and NT015 are **1.6 and 2.7 ppb**, respectively. The maximum concentrations of total IMI measured in clover pollen in trials NT014 and NT015 are **8 and 8.6 ppb**, respectively.

Imidacloprid Metabolites. Very few samples contained concentrations of IMI-olefin or 5-OH-IMI above the limits of quantitation in pollen and nectar (0.7 ppb). Therefore a quantitative analysis of the fraction of total IMI represented by these two metabolites was not conducted.

Temporal Variability in Residues. Plots of the daily average residue values of total IMI (and daily ranges) in cotton floral nectar, extrafloral nectar and pollen **Figures E-8, E-9 and E-10**, respectively. Total IMI residues in the three matrices generally declined over the duration of the 30-40 day sampling period (except when residues were in the low ppb range which are likely influenced by assumptions regarding non-detects. For residues of total IMI in cotton floral nectar, DT_{50} values vary from **19-68 days** while those for extrafloral nectar vary from **27 – 373 days**. With floral and extrafloral nectar, 14/16 of the DT_{50} values of total IMI are between **19 and 51 days**. With pollen, most of the trials contained insufficient data for reliable determination of DT_{50} values (<4 sampling points with detectable residues of total IMI). Three DT_{50} values for total IMI in pollen varied from **14 to 58 days**.

Spatial Variability in Residues. The three trials were located in the midwestern U.S. within the same general vicinity. Examination of the monthly precipitation records suggests a similar magnitude and temporal pattern over time. Thus from a climate perspective, these trials are relatively similar. A range of soil types are represented (sandy loam, silt loam, sand).

Pesticide Carryover. Based on yearly mean values of total IMI in floral and extrafloral nectar, year 2 means increase by **1.2X to 2.7X** over year 1 means. With cotton pollen, yearly averages of total IMI increase by **1.5X to 2.9X** from year 1 to year 2. The majority of increase in year 2 residues of total IMI occurred sooner after application (13-20 days) across the trials. Interestingly, the two trials with the greatest % sand in soils (NT013 and NT015) show the greatest relative increase in yearly average total IMI from year 1 to year 2 in nectar and pollen (1.7X to 2.9X) compared to NT014 which contained mostly silt (1.2-1.5X). It is not certain whether this differential increase is related to differences in soil composition, but all three trials had similar amounts of IMI in soil prior to the 2nd year planting (24-42 ppb).

Residues of total IMI in soil measured prior to planting in year 2 (**24-45 ppb**) are elevated compared to those measured prior to planting in year 1 (**0.3-12 ppb**). This could explain some of the higher residues of total IMI in pollen and nectar observed in year 2. Unfortunately, soil samples at the end of the 1st growing season were not taken; therefore, it is not known whether post application residues in soil increase from year to year or remain similar from year to year. Other factors (weather) may also contribute to these observed differences.

Classification/Utility for Bee Risk Assessment. This study is classified as acceptable and appropriate for quantitative use in risk assessment.

Reference

Gould, T., Bowers, L., Dyer, D., and Jerkins, E. (2014). Determination of the Residues of Imidacloprid and its Metabolites IMI-5-OH and IMI-olefin in Bee Relevant Matrices Collected from Treated Cotton During Two Successive Years and in White Clover Planted after Treated Cotton. Study ID EBNTY010. Bayer Crop Science, Research Triangle Park, NC. MRID 49511702.

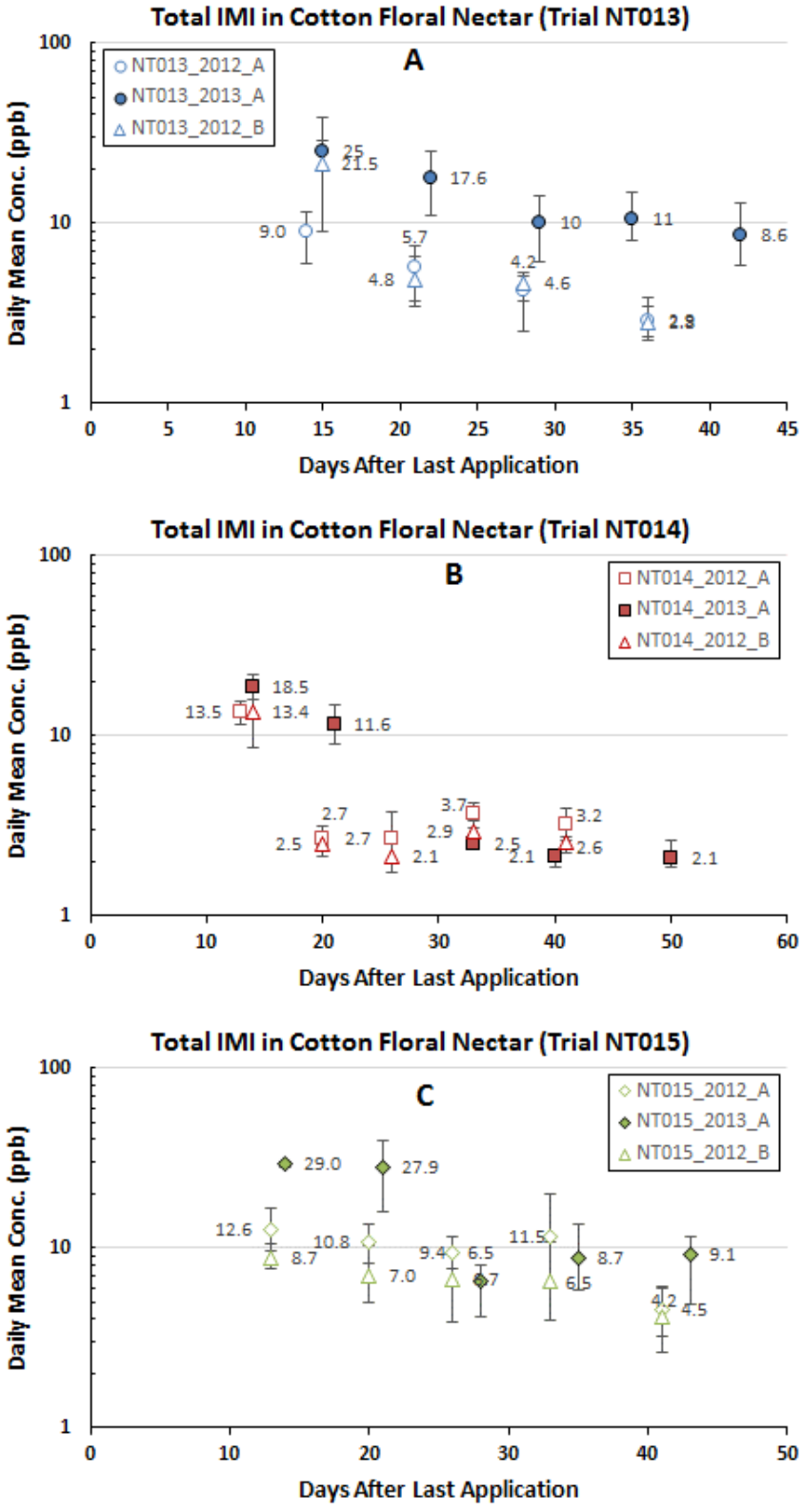


Figure E-8. Total imidacloprid residues measured in cotton floral nectar for trials NT013, NT014 and NT015.

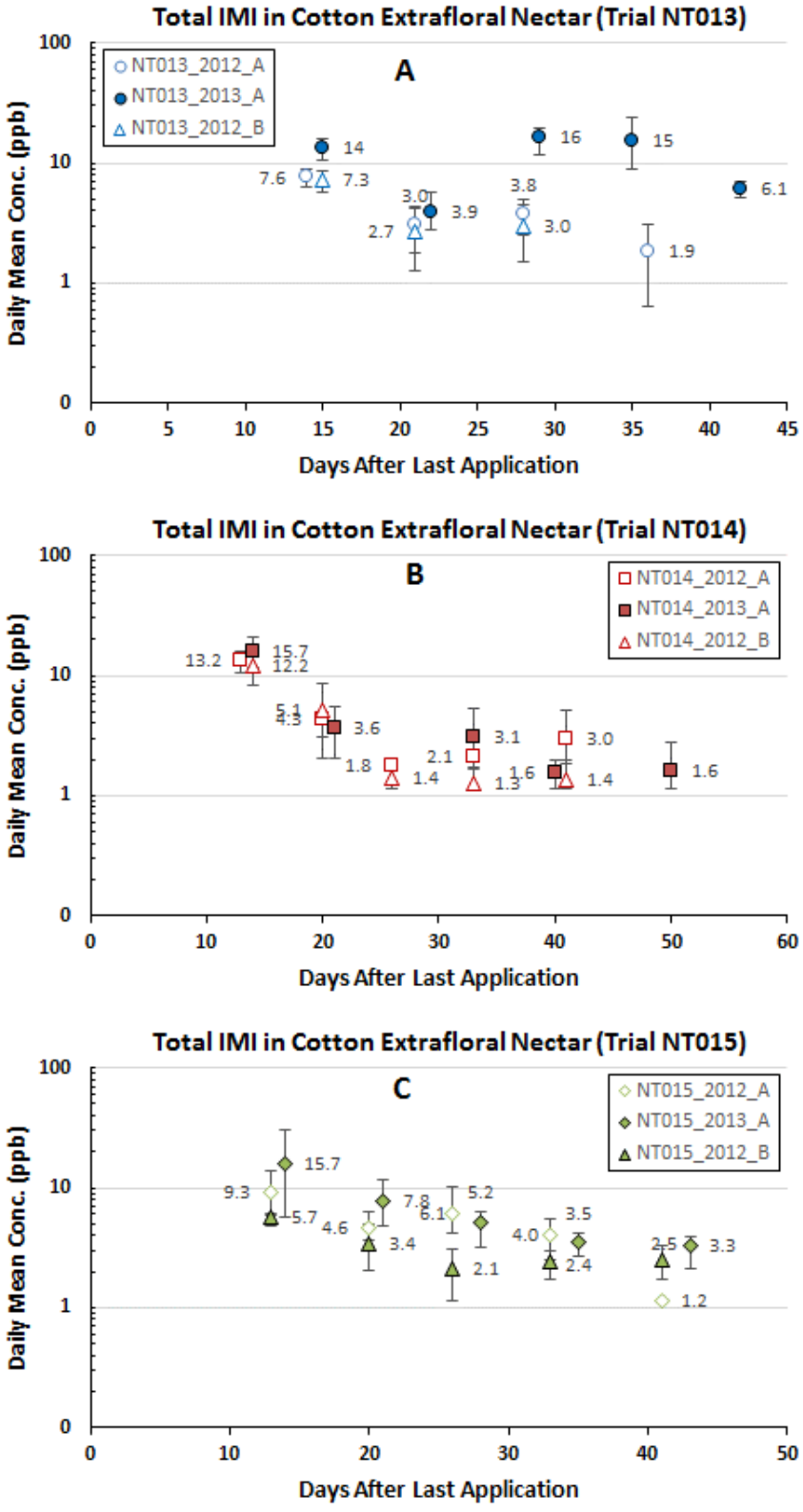


Figure E-9. Total imidacloprid residues measured in cotton extrafloral nectar for trials NT013, NT014 and NT015.

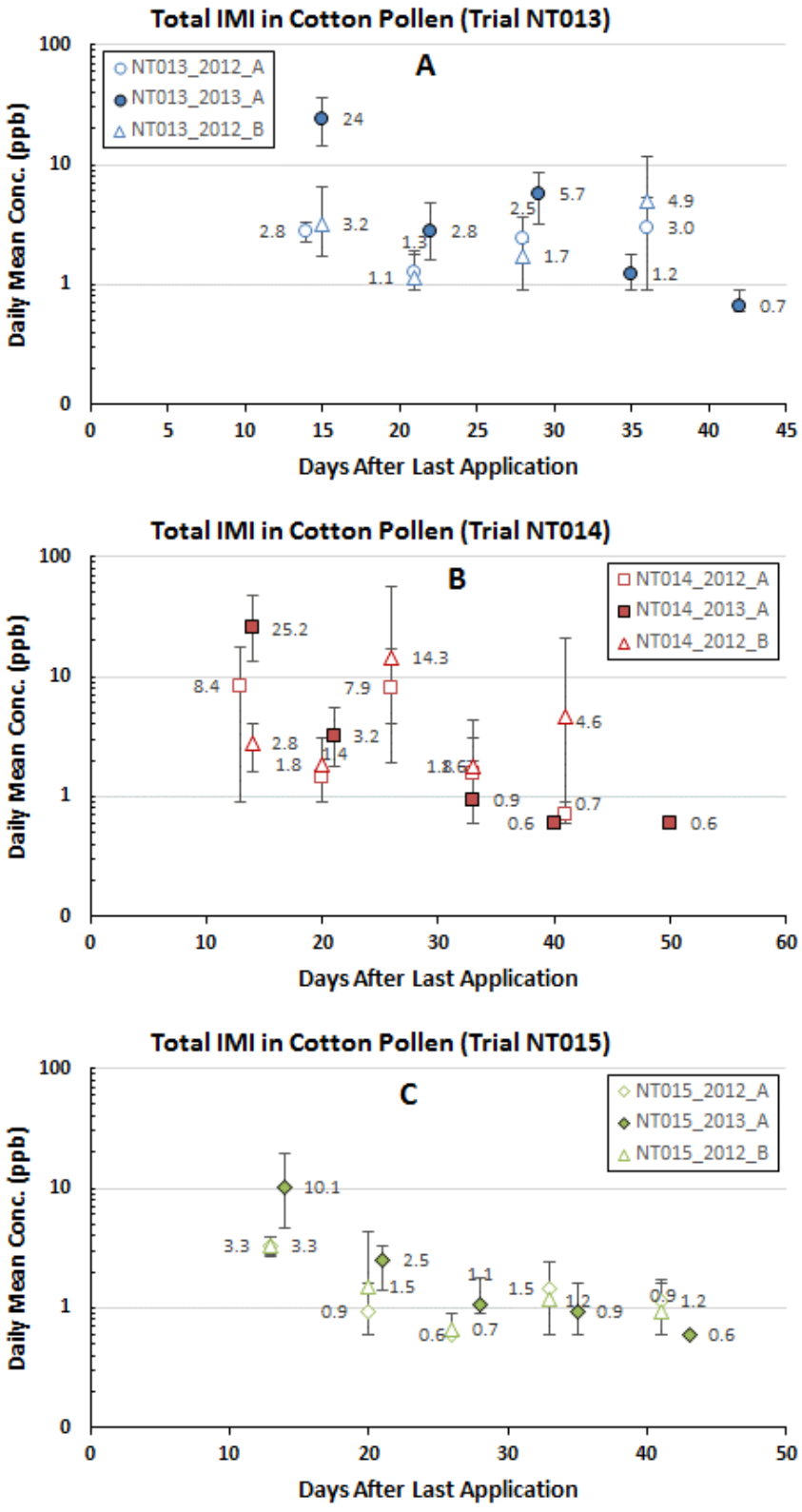


Figure E-10. Total imidacloprid residues measured in cotton pollen for trials NT013, NT014 and NT015.

Appendix F. Reported Pollinator Incident Summaries (I023737-005 and I024127)

In a study by Bortolotti et al., 2009 (EIIS No. I023737-005), a survey was conducted amongst beekeepers across Italy in the spring of 2008. The survey was initiated following several reports of bee losses within Italy and across Europe that were thought to be due to dust dispersion from the sowing of imidacloprid treated seeds. **Table F-1** below shows the results of the survey broken down by region. Dead bee sample analysis was also conducted.

Table F-1. Results of 2008 hive losses in five Italian regions amongst beekeepers (I023737-005)

Region	**Number of Affected Hives**	**Number of Affected Beekeepers**
Lombardy	1513	40
Piedmont	1167	8
Emilia-Romagna	187	7
Veneto and Trentino	1000	20
Friuli Venezia Giulia	2461	110
Total	**6328**	**185**
Dead Bee Sample Analysis		
Number tested	**Percentage Positive**	
105	**25.7%**	**1.01 – 241**

After any reporting of losses, samples of dead bees and pollen were collected from affected hives and sent for residue analysis in Bologna for imidacloprid, thiamethoxam, and clothianidin. This effort took place only in Northern Italy in the regions of Lombardy and Veneto. A total of 105 dead bee samples were analyzed (65 from Lombardy and 40 from Veneto) as well as 4 samples of pollen from surrounding areas in Lombardy.

It was determined that 25.7% of the 105 samples total dead bee samples were positive for imidacloprid. Three out of the four total pollen samples also tested positive for imidacloprid. Concentrations in the dead bee samples ranged from 1.01 – 240.6 ppb and were as high as 311 ppb in pollen. It is worth nothing that the study did not report a limit of detection and limit of quantitation. Additionally, questionnaires were sent to beekeepers in the 65 apiaries of the Lombardy region (corresponding to 1513 hives). All reports were stated to have come from cultivated areas where the main surrounding crop was corn and in 96.2% of the cases of affected hives, the damage occurred during or after sowing. While thiamethoxam (2.8% of dead bee samples) and clothianidin (25.7% of dead bee samples) were also detected, it is noted that half of the dead bee samples were not positive for any analyzed neonicotinoid. Despite this, the study authors suggest that this does not exonerate these chemicals as the primary cause of the losses observed as part of the survey for this study.

In a report from the Austrian government (I24127) entitled "Investigations on the occurrence of bee losses in corn and canola-growing regions of Austria and possible associations with bee diseases and the use of pesticides." The research spanned 3 years (2009-2011) which sought to identify possible correlations between the incidence of honey bee losses in corn and canola growing areas. The methodology used in this study was very similar to that described above in the Bortolotti et al study in which beekeepers would provide the incident after which samples would be collected for residue analysis as well as on-site inspection of the apiaries.

The residue screen of chemicals included the neonicotinoids imidacloprid, thiamethoxam, and clothianidin, as well as fipronil. Analysis of all samples (dead bees, beebread, honey, and plants) was conducted using LC/MS/MS with an LOD and LOQ of 0.2 and 1 ppb, respectively. Samples of honey bees and brood were analyzed for parasites and pathogens including *Varroa*, *Nosema apis* and *Nosema ceranae*, deformed wing virus, acute bee paralysis virus, chronic bee paralysis virus, black queen cell virus, sac brood virus, Kashmir bee virus, and Israeli acute paralysis virus. What follows is a year-by-year of the findings.

2009: Incidents of suspected poisoning were reported from 33 beekeeping operations concerning 36 bee yards and a total of 676 affected hives. It was reported that no total colony loss was found but temporary loss of foraging force, as well as reduced honey crop were observed. The appearance of bees showing symptoms of poisoning (included increased mortality, observations of flightless bees, trembling bees, and decrease in colony strength) coincided with the corn sowing period to a high degree. Examination of the apiaries did not reveal signs of correlation between the symptoms described above and disease presence. Although insecticides presence was detected in 83% of the dead bee samples and 65% of the beebread samples, there were no detections of imidacloprid (clothianidin was detected in 64% of the samples).

2010: Samples from hives suspected of bee poisoning were sent shortly after the overwintering inspection as well as throughout the spring and summer. The samples taken just after the overwintering period originated from four beekeeping operations for which analysis of residues in dead bee samples and beebread showed no presence of imidacloprid. Instead, it was determined that varroatosis was the primary cause for the loss of these colonies. The spring and summer samples were provided by 76 beekeeping operations originating from 95 bee yards. It was noted, as it was in 2009, the coincidence of these losses being reported with the timing of corn being sowed in these areas. Also, as with 2009, there was no correlation between disease presence and the finding of pesticide residues. In 89 samples analyzed for residues, imidacloprid was not detected (clothianidin was detected in 51%, thiamethoxam in 23%). Similarly, imidacloprid was not detected in any of the 62 samples of extracted honey. Additionally 17 plant samples from fields surrounding the corn fields showed imidacloprid presence in 18% of the samples (residue level not provided), as well as clothianidin in 35% and thiamethoxam in 18% of the samples. Finally, analysis of 34 pooled samples of beebread sampled after corn sowing and 19 samples collected from bee yards after corn flowering determined imidacloprid presence in 3 and 11% of these samples, respectively (residue level not provided).

2011: Six beekeeping operations provided samples originating from 9 bee yards. Beebread samples from two of these operations showed trace amounts of imidacloprid below the limit of quantification. Samples from the other operations were not subjected to residue analysis as there were clear indications of varroatosis.

Table F-2. Summary of reported pollinator incident reports

Incident Record	Date	Use Pattern	Product	Location	Legality	App. Method	Comments
I010775-001	1999	Sunflower	Gaucho	France	Registered Use	Seed treatment	Incident is a report from a news article implicating a possible link between seed treated imidacloprid honey bee colony loss of approximately 1/3 from 1996 levels to 1999 levels and is not verified by any site specific reports or residue analysis
I019743-002	1995	Canola/ rapeseed	NR	ND	Not determined	NR	Report is from an online news article (CommonDreams.org) on the bans of certain neonicotinoid chemicals allegedly linked to honey bee colony loss. This incident refers to a report of a group of North Dakota beekeepers suing Bayer Crop Science after losing "thousands" of honey bee colonies in 1995 during a period which oilseed rape in the area had been treated with imidacloprid.
I020700-001	06-2008	Ornamental	Merit 2F	DE	Registered use	Soil injection	Submitted under FIFRA 6(a)(2). Linden trees (*Tilia cordata*) on a commercial golf course were treated for Japanese beetle control using Merit 2F soil injection treatment. It was stated in the report that some months after treatment, the trees bloomed, and dead bumble bees (*Bombus*) and carpenter bees (*Xylocopa*) were found at the base of the tree. It was estimated that 2000-4000 individuals were affected (11 trees treated). A follow up residue analysis (August 2008) conducted by Bayer confirmed imidacloprid presence in the leaves of parent imidacloprid (ranging from 2.6 – 11.7 ppm), IMI-5-OH (1.6 – 2.2 ppm), and IMI-olefin (0.59 – 1.8 ppm). Residues of these products in dead bee samples were 0.146, 0.016, 0.138 ppm, respectively (composite samples).
I021017-001	03-2009	Ornamental	Xytect 75 WSP	PA	Undetermined	NR	Submitted under FIFRA 6(a)(2). Product applied to control aphids in 6 linden trees that were reported to 8-10 inches. The application took place March 30, 2009. During blooming, it was discovered that an unspecified number of bees were killed and that the bee deaths ceased when blooming ended. It was unspecified of what species of bee was affected.

Incident Record	Date	Use Pattern	Product	Location	Legality	App. Method	Comments
I022340-001	04-2010	NR	NR	IN	Undetermined	NR	Summary report from bee kill incidents at the Purdue University Department of Entomology. There were reports of hives with dead bees out in front with observations of seed treated corn being planted in the fields adjacent to the university lab. A residue analysis determined that sampled pollen from affected hives (composite sample) had imidacloprid at levels of 2.8 ppb. All other sampled matrices (live and dead bees) did not return any detectable residues (LOD and LOQ not reported). Clothianidin was detected in pollen at 21 ppb.
I023051-001	07-2011	Pepper, sweet/bell	Bayer Advance	MD	Registered use	Soil treatment	Homeowner made soil application of Bayer Advance on sweet pepper bushes in her backyard in April 201 to treat for Japanese beetles. Six bumble bees were observed to be dead on the ground or in the flower heads on the weekend of July 16, 2011. No follow up residue analysis was conducted.
I023225-006	04-2011	Corn, field	NR	Slovenia	Undetermined	NR	From report (Ministry of Agriculture, Forestry, and Food) on bee declines Mura region of Slovenia from mid-April to late May 2011. Samples of dead bees were taken from 3 beekeepers, in addition to two samples of pollen, 3 samples of oilseed rape, and 2 samples of corn seed from the field. Imidacloprid was one of 5 chemicals detected (in addition to clothianidin), although residues were not provided. Imidacloprid was not detected in the pollen samples and was one of 3 chemicals detected in the corn seed samples (no residues provided)
I023702-001	2006	Canola, rapeseed	Gaucho	ND	Undetermined	2006	Part of an April 2009 report from the Nebraska Beekeepers Association. Seven beekeepers in North Dakota and Minnesota initiated legal action against Bayer Crop Science when they suspected Gaucho (used as a seed treatment on neighboring canola fields) were responsible for their bee losses. Laboratory analysis of the wax comb and honey found imidacloprid in all samples with residues ranging from 22 – 671 ppb. Carbofuran, dichlotvos, and coumaphos were also screened for (no results provided).

Incident Record	Date	Use Pattern	Product	Location	Legality	App. Method	Comments
I023702-002	2006	Cotton	Gaucho, Admire	Possible	Undetermined	Aerial, ground	Part of an April 2009 report from the Nebraska Beekeepers Association. It was reported that in the summer of 2006 that 500 beehives to the cotton fields of west Texas. Ground and aerial applications of Gaucho and Admire were made to control for aphids were sustained exposure to imidacloprid was reported with no ill effects to the colonies. The hives were relocated back to central Texas in the fall appearing strong heading into overwinter. By January 2007, it was reported that a significant proportion of the total hive operation was failing with CCD-like symptoms. It was discovered that all colonies experiencing these symptoms had been in the cotton field exposed to imidacloprid the previous summer. No residue information to confirm exposure
I023702-003	2004	Apple	Admire	NR	Undetermined	NR	Part of an April 2009 report from the Nebraska Beekeepers Association. Hives involved in apple pollination (location not provided) were stated to have experience 75-80% loss in the fall after the summer pollination period. The number of colonies affected as well as confirmatory residue analysis were not provide.
I023702-004	2007	Watermelon	Admire	CA	Undetermined	Ground	Part of an April 2009 report from the Nebraska Beekeepers Association. A beekeeper in California stated that 1000 colonies of his bees were used to pollinate watermelons in the summer of 2007. After approximately 50% of the colonies died the following winter (compared to 18% colony loss that did not pollinate watermelons), the beekeeper contacted the grower to discover the watermelons had been chemigated with Admire. Other colonies that did not experience such losses were exposed to other pesticides but not to imidacloprid.

Incident Record	Date	Use Pattern	Product	Location	Legality	App. Method	Comments
I023702-005	2007	Citrus	Admire	FL	Undetermined	NR	Part of an April 2009 report from the Nebraska Beekeepers Association. A beekeeper maintaining 7500 hives for honey production and crop pollination provided 18 hives to a research project organized by Penn State that would monitor the hives to investigate causes for mortality. The beekeeper stated that while he provided 18 hives for the study, he only received 4 back with only 1 hives in a state sufficient to produce honey. The first samples taken were from when the bees were pollinating Florida citrus where imidacloprid residues ranging from 14-17 ppb were detected in the pollen. Follow up with the grove manager revealed that Admire Pro had been used as a ground application as the trees began to bloom.
I024004-001	05-2012	Corn	NR	IA	Undetermined	NR	Incident reported by Iowa beekeeper who maintains approximately 550 colonies for breeding purposes with Russian bees (reported to be more resistant to *varroa* and tracheal mites) and honey production. In May 2012, it was discovered that 30 of these colonies had dead or dying bees with approximately 25% loss of each colony. The fields were reported to be adjacent to corn fields where corn was reported to planted before the discovery of the hive loss. A follow up residue analysis confirmed that pollen and dead bee samples were negative for imidacloprid.
I025013-001	03-2013	NR	NR	FL	Undetermined	NR	Reported to EFED from Florida beekeeper Barry Hart who had observed increasing numbers of dead bees in front of colonies close to orange orchards with a cluster of 5-6 bee yards that appeared worse than other bee yards. Empty containers of imidacloprid (Montana 2F) were found on burn piles in the vicinity of these colonies. No other information (*i.e.* residue) to link imidacloprid to the losses was provided other than the empty containers.

Incident Record	Date	Use Pattern	Product	Location	Legality	App. Method	Comments
I025019-001	03-2013	Orchard (orange)	Montana 2F	FL	Undetermined	NR	Incident reported via phone call to EFED (March 21, 2013). The caller was a beekeeper who manages 1600 colonies in orange orchards in Polk County, Florida. The colonies were within 12 miles of each other and he has been bringing his been to this location for 25 years. The beekeeper indicated that approximately 400 colonies had been affected with observations of dead bees outside the hive entrances. The affected colonies were reported to have lost at 25-40% of their forage force. The beekeeper associated the bee kills with the use of Montana 2F on orange groves adjacent to the colonies. He described having colonies in other areas treated with "softer chemistries" and reported these bees were not affected by losses. A residue analysis was not available with the report.
I025023-001	03-2013	Orange grove	Montana 2F	FL	Undetermined	NR	Incident reported via phone call to EFEFD (March 27, 2013). The caller was a beekeeper ion Polk County, Florida who maintains 80 colonies in orange orchards. The colonies were reported to have been brought to this location for the past 7 years. At a hive inspection reported to be approximately a week before the call to EFED was made, a high number of dead bees were observed in front of the hive entrances. It was estimated that each hive had its adult population reduced by 60%, effectively eliminating the foraging force. All colonies were reported to be queen right and the queens were actively laying eggs. It was noted however that the loss of foraging forced would prevent obtaining a honey crop off of the orange trees. Based on other reports in the area of bee kill events resulting from the use of Montana 2F, the caller attributed the losses to imidacloprid. A residue analysis was not available with the report.
I025027-001	03-2013	Orange orchard	NR	FL	Undetermined	NR	Incident reported via phone call to EFED (March 22, 2013). The caller indicated that 100 of his honey bee colonies have been affected by application of pesticides. The colonies are located in a single bee yard surrounded by orange orchards in Indian River County, Florida. The colonies have been at that location for 4 weeks and the beekeepers had been reported to coming to the same location for the prior three years. After an inspection of the colonies revealed that approximately 10% were dead and the remainder had piles of dead bees at the entrance to the hives. The colonies that were still alive had substantial declines in

Incident Record	Date	Use Pattern	Product	Location	Legality	App. Method	Comments
							foraging force and brood (as evidenced by expelled larvae/pupae in the piles of dead bees in front of the hives). It was also reported that approximately 50% of the colonies were without a queen and had not attempted to replace the queen through supersedure. The beekeepers believes that the bee kill was the result of Admire use based on other losses reported in the area from other beekeepers and growers attempts to control the Asian citrus Psyllid. The beekeeper had contacted the grower who confirmed he had used insecticides but not Admire. Prior to this incident, the colonies were reported to have been in good health with monthly thymol treatments to control varroa. A residue analysis was not available with the report.
I025052-001	03-2013	Agricultural area	Montana 2F	FL	Undetermined	Aerial	Incident reported via phone call to EFED (March 21, 2013). The caller indicated he had 600 colonies in orange orchards of which 300 are affected. The colonies were reported to have been in orchards in Polk County, Florida and had been there since mid-February 2013. Roughly one month later, upon inspection of the bee yards, dead bees were laying everywhere and those that were still alive were exhibiting a jerking movement. He reported the colonies were queen right and noted that the foreman of the orange grove notified him he would be applying another application of Montana 2F via aerial application on April 1 (roughly ten days after this incident was reported).
I025067-001	04-2012	Holly tree	Bonide Tree and Shrub Insect Control	NC	Registered Use	Ground	Submitted as report to the National Pesticide Information Center (NPIC). A homeowner reported she applied Bonide Tree and Shrub Insect Control product to six holly trees in her yard. Three weeks later, she started seeing dead bumble bees on the sidewalk next to the trees on a daily basis. No other information provided in the report.
I025512-001	08-2013	Soybean	Leverage 360	MO	Undetermined	Ground	Submitted by Bayer Crop Science under FIFRA 6(a)(2). A soybean farm was being sprayed by Leverage 360 (imidacloprid and beta-cyfluthrin) which was adjacent to neighbor who had 11 honey bee hives. The neighbor had reported that he had "piles of dead honey bees," back on his property. There was no further confirmatory residue information provided in the report.

Incident Record	Date	Use Pattern	Product	Location	Legality	App. Method	Comments
I025560-001	2013	Garden	Bayer Advance	NE	Registered Use		Reported by homeowner using Bayer Advance Rose and Flower Care in her garden. Plants were reported not to be in bloom, pesticide was applied per label instructions as proper dilution and as soil drench. Product was believed to have been applied in early May with blooming taking place later that month. Trifluralin was also applied. The gardener only made the observational statement that in previous years where she had used no insecticides, that she noticed more honey and bumble bees in her plants than the year she started treating roses with imidacloprid (and did subsequent research on the product). The report contains no other information directly linking imidacloprid use to the death of the bees such as residue analysis.
I025610-001	05-2013	Parking lot	Quali-Pro	OR	Misuse	Soil drench	Submitted under FIFRA § 6(a)(2) by Makhteshim Agan of North America (MANA) involving soil drench of linden trees at a golf club in Portland, Oregon. Oregon Department of Agriculture investigated and conducted residue analysis and determined presence of imidacloprid but there were no residues presented in the report. It was reported that while this use represented one that was permitted by the label, the pest control operator (PCO) did not have the necessary licenses to make this application.
I025610-002	2013	Urban	Quali-Pro	OR	Misuse	Soil drench	Submitted under FIFRA § 6(a)(2) by Makhteshim Agan of North America (MANA) involving soil drench of linden trees at 200 Market Street in Portland, Oregon. Oregon Department of Agriculture investigated and conducted residue analysis and determined presence of imidacloprid but there were no residues presented in the report. It was reported that while this use represented one that was permitted by the label, the pest control operator (PCO) did not have the necessary licenses to make this application.

Incident Record	Date	Use Pattern	Product	Location	Legality	App. Method	Comments
I025980-001	12-2013	Citrus Orchard	Admire Pro	FL	Undetermined	Surface	Submitted by Bayer Crop Science as part of FIFRA §6(a)(2). Admire Pro application was made on orange tree orchard in which bees had been placed. The hive yards ranged from 1550 – 5500 feet away from the treated grove. The application method was described as "surface" application. A follow up investigation conducted by Bayer found that the apiaries had small hive beetle in some hives, no varroa present, small amount of early stage European foulbrood, adequate honey stores, and all stages of brood present in the queen yard (1550 yards from grove). In the yard 5500 yards from grove, there fewer dead bees than in the queen yard and evidence of hive robbing and heavily infested with all stages of small hive beetle larvae. Residue analysis of dead bees from the various yards returned total residues of imidacloprid (parent + olefin+ 5-OH) yielded results of 2.5 – 2456 ppb. Live bee residue analysis had total residues ranging from 1.1 – 5.1 ppb.
I026288-002	04-2013	Residential	Merit 2F	CA	Registered Use	NR	Reported by the Santa Barbara Beekeepers Association. The California Department of Food and Agriculture (CDFA) treated for Asian Psyllid on citrus trees in April 2013 (in an unspecified land use are according ot the report). The state had an exemption to allow for application during bloom of the trees. Only one colony of the 12 that was within 300 yards of the area survived. The beekeepers expressed concern that imidacloprid or beta-cyfluthrin were among the chemicals used but no confirmatory residue analysis accompanied the report.
I026301-001	08-2013	Residential	Merit 2F	CA	Undetermined	Tree injection	Submitted as part of FIFRA 6(a)(2) by Bayer Crop Science. A pest control operator (PCO) applied Merit 2F (imidacloprid) as tree injection to Arbutus and Laurel trees on residential property. The observation of dead bees (number not specified) occurred shortly after the trees were treated. No other confirmatory residue analysis provided in the report.

Incident Record	Date	Use Pattern	Product	Location	Legality	App. Method	Comments
I026563-001	06-2014	Residential	NR	OR	Misuse	NR	Sidewalks were reported to be littered with dead and dying bumble bees in Eugene, Oregon. The bees were collected the following day by the Oregon Department of Agriculture for testing. Imidacloprid and acephate were detected at 0.05 and 0.30 µg a.i/bee, respectively. An investigation prompted a suspension of the pest control operator company who sprayed linden trees while in bloom, which is a violation of the label restrictions.
I026593-001	06-2014	Residential	Ima-Jeet	OR	Registered Use	Tree injection	Beaverton, OR incident involving bumble bees and honey bees being discovered underneath linden trees in a neighborhood. The trees were treated to control aphids. An investigation led to the discovery that the same pesticides (imidacloprid, dinotefuran) were used here as in a related incident involving linden trees in a parking lot (I025610-001). Follow on investigation took bee, flower, and leaf samples where analysis determined residue levels of 0.050 µg a.i/bee, 0.49 ppm, and 2.2 ppm, respectively.
I026607-001	NR	NR	NR	MA	Undetermined	NR	From article called "Why are Concord's Bee Dying?" posed to www.apinews.com. Account describes the yield of honey from 4 hives near Concord, MA previous to suspected use of imidacloprid in the area and post introduction, after which the colonies were stated to decline. No other information part of the report directly linking imidacloprid as the cause of the decline as well as absence of confirmatory residue analysis.
I026789-001	08-2014	Soybean	Leverage 360	IL	Registered Use	Ground	Submitted by Bayer Crop Science under FIFRA 6(a)(2). Four hives adjacent to soybean fields were reported to be implicated, with at least 300 in one hive and 100 from the other 3 hives. The bees were within ½ mile from the field which had been reported to have made applications of Leverage (imidacloprid, beta cyfluthrin) and Stratego (trifloxystrobin, propiconazole). There was no residue analysis of the bee or any other in hive matrices to confirm exposure to imidacloprid or any other pesticide applied.

Incident Record	Date	Use Pattern	Product	Location	Legality	App. Method	Comments
I026904-001	08-2014	Oilseed rape	NR	United Kingdom	Undetermined	Seed treatment	From news article of Smallholder (United Kingdom-based news service). The incident was reported to have occurred in Havering, East London, next to a field of oilseed rape that was thought to have been planted with imidacloprid-treated seeds the previous fall. Hundreds of dead bees were scattered all over the ground with queens from at least 3 species being identified among dead bees. Results of residue analysis of the dead bees determined imidacloprid at levels of 6 ppb as well as two fungicides (one being flusilazole, the other not being reported).
I026927-001	09-2014	Residential	Bayer Advance	CT	Undetermined	Ground	A gardener called the National Pesticide Information Center (NPIC) seeking information about imidacloprid and clothianidin. She noticed that there was a beehive on the exterior of the second floor of one of her clients. It was described there was little bee activity around the hive and that all throughout the yard were soil injection sites of what was later discovered (in conversations with the pest control company) to be Bayer Advance (containing imidacloprid and clothianidin). No other confirmatory information in the narrative such as residue analysis provided in the report.
I027383-003	05-2014	Unknown	NR	IN	Undetermined	NR	The Office of the Indiana State Chemist (OISC) received a complaint from a local beekeeper that noticed dead and dying bees outside of one of his hives in May, 2014. A sample of 30-50 bees were collected and sent for residue analysis. A follow up investigation revealed that the field across from the yard that the bees were in was not recently planted or had even prepared something for planting. A residue analysis showed a detection of clothianidin at 0.5 ppb but returned no detection of imidacloprid.
I027663-001	05-2014	Commercial flowers	Criterion 75 WSP	MO	Undetermined	NR	Report from the curator of the GT Butterfly House and Bug Zoo in Michigan. The facility inquired about neonicotinoid use on the commercial flowers they wanted to purchase in time for their butterflies to arrive in early spring. They settled on Beroske Farms in Ohio that confirmed that no neonicotinoids were used on their flowers. After delivery of flowers, subsequent planting, and the beginning of the observation of the foraging of the butterflies on the flowers, it was discovered that 4 nectar feeding butterflies appeared comatose and then later died. No deaths were reported among the fruit eating butterflies. A call with Beroske farms confirmed that the product Criterion 75 WSP (75%

Incident Record	Date	Use Pattern	Product	Location	Legality	App. Method	Comments
							imidacloprid) was used among 6 other pesticides on the flowers before delivery to the museum. It was later confirmed that commercial flowers represented an unlabeled use of this product. A follow on report (I027748) summarized the residue analysis results of geranium (<LOQ), butterfly bush (1.5 ppb), coneflowers (<LOQ), livewire grass inside butterfly area (0.51 ppb), potting medium (0.12 ppb), and dead butterflies (no analysis conducted).
I028034-001	06-2015	Urban	NR	OR	NR	Soil drench	Reported by Oregon Department of Agriculture to have occurred near Portland State University in Portland, Oregon. According to ODA, the preliminary investigation revealed that the linden trees in the reported incident location had been treated with imidacloprid via soil drench in 2013 and with clothianidin via soil drench in 2014 to control for aphids. Samples of dead bumble bees, linden flowers and leaves were collected for residue analyses. Samples of dead bumble bees, linden flowers and leaves were collected for residue analyses and indicate residues of imidacloprid, its degradates (5-hydroxy, desnitro and olefin) and chlorothalonil in leaves and flowers, while the samples of dead bumble bees contained the parent imidacloprid (0.0009 µg a.i/bee) and chlorothalonil (0.029 µg a.i/bee) alone.
I028034-002	06-2015	Urban	NR	OR	NR	Soil drench	Reported by Oregon Department of Agriculture to have occurred near linden trees at 200 & 100 Market Street, Portland, Oregon. The ODA investigation indicated that linden trees had been treated with imidacloprid by drench application in 2013 to control for aphids and that plants in close proximity to the trees had been recently treated with chlorothalonil. These were the same trees involved in a previous incident (I025610-002). Residues of imidacloprid (0.0095 µg a.i/bee, IMI-olefin (0.0010 µg a.i/bee), desnitroimidacloprid HCl (0.0037 µg a.i/bee) and clothianidin 0.0026 µg a.i/bee) were detected in bumble bee samples and in linden leaves, while linden flowers contained parent imidacloprid and clothianidin alone (imidacloprid, 0.028 ppm; clothianidin, 0.065 ppm). It was confirmed by ODA that these trees were the same as those involved in an earlier incident (I025610-002)

NR: Not reported

Appendix G . Supplemental Information for the Tier II Colony Feeding Study (MRID 49510001)

Study Course

Table G-1 below shows the chronology of key events of this study including the start of exposure, dates of colony condition assessments, and dates of in hive residue collection. The hives were established within their assigned apiaries based on colony strength for roughly one month prior of exposure beginning (exposure started June 26, 2013). During the 6-week exposure phase, the colonies were fed twice weekly (1 L sugar solution per colony) where it was also determined the amount of food that was not consumed. It was reported that all colonies across all treatment groups consumed most or all of the provided sugar solution with a slightly lower, but not significant ($p>0.05$) reduction at the top two treatment groups (100 and 200 µg a.i/L groups). Just before the exposure phase began, as well as after the exposure phase in late August and in the following spring, the colonies were assessed for the incidence and frequency of *Varroa* mites and *Nosema* fungal spores. Apiary C was vandalized during the 5th week of the exposure phase and therefore all hives at this site were removed from the subsequent statistical analysis. Additionally, roughly one month after the exposure phase ended, hives in the 100 and 200 µg a.i/L treatment groups were relocated out of their respective apiaries to another location. These groups experienced a marked decline in colony strength that was observed during the CCAs in the exposure phase and the relocation was conducted to prevent hive robbing by bees from other colonies within the apiary.

Table G-2. Chronological list of key dates and activities in a honey bee colony feeding study. Abbreviations: CCA=Colony Condition Assessment; UTC=Untreated Control

Week	Date	Activity	Week	Date	Activity
-7	12 May 2013	CCA1	6	07 Aug 2013	1st sample shipment
-4	30 May 2013	Hive samples (uncapped nectar, bee bread)	6	08 Aug 2013	Measurement of remaining food
-4	30 May – 13 Jun 2013	CCA2	7	12 Aug 2013	Recording of hive weights
-1	21 Jun 2013	Recording of hive weights	7	14 Aug 2013	Hive samples (uncapped nectar, bee bread)
-1	21 – 25 Jun 2013	CCA3	7	14/15 Aug 2013	CCA5
-1	21 – 23 Jun 2013	Hive bee sampling for *Varroa* and *Nosema* assessment	7	14/15 Aug 2013	Hive bee sampling for *Varroa* and *Nosema* assessment
0	26 Jun 2013	Feeding	8	21 Aug 2013	*Varroa* counts CCA5
0	28 Jun 2013	Feeding; Measurement of remaining food	10	05/06 Sep 2013	Removal of 100 and 200 ppb hives to separate apiary
0	28 Jun 2013	Pollen samples from pollen trap	11	10/11 Sep 2013	CCA6 (UTC, 12.5 ppb, 25 ppb, 50 ppb)
1	01 Jul 2013	Feeding; Measurement of remaining food	12	17 Sep 2013	Recording of hive weights
1	03 Jul 2013	Stability samples	12	18/20 Sep 2013	CCA6 (100 ppb, 200 ppb)
1	03 Jul 2013	Feeding; Measurement of remaining food	15	08 Oct 2013	Recording of hive weights
1	03 Jul 2013	Pollen samples from pollen trap	16	16/17 Oct 2013	CCA7 (UTC, 12.5 ppb, 25 ppb, 50 ppb)
2	09 Jul 2013	Feeding; Measurement of remaining food	16	17 Oct 2013	Pollen samples from pollen trap
2	12 Jul 2013	Feeding; Measurement of remaining food	16/17	18/23 Oct 2013	CCA7 (100 ppb, 200 ppb)
2	12 Jul 2013	Stability samples	--	13 Dec 2013	Feeding 1 L 2:1 sugar syrup per hive
2	12 Jul 2013	Pollen samples from pollen trap	--	19 Dec 2013	Feeding 1 L 2:1 sugar syrup per hive
3	16 Jul 2013	New stock solution	--	13 Jan 2014	Feeding 1 L 2:1 sugar syrup per hive
3	17 Jul 2013	Feeding; Measurement of remaining food	--	20 Jan 2014	Feeding 1 L 2:1 sugar syrup per hive
3	17/18 Jul 2013	CCA4	--	27 Jan 2014	Feeding 1 L 2:1 sugar syrup per hive
3	18 Jul 2013	Hive samples (uncapped nectar, bee bread)	--	07 Feb 2014	Feeding 1 L 2:1 sugar syrup per hive
3	19 Jul 2013	Feeding; Measurement of remaining food	--	18 Feb 2014	Feeding 1 L 2:1 sugar syrup per hive
3	19 Jul 2013	Pollen samples from pollen trap	--	02 Mar 2014	Feeding 1 L 2:1 sugar syrup per hive
4	22 Jul 2013	Recording of hive weights	--	11 Mar 2014	Feeding 1 L 2:1 sugar syrup per hive
4	24 Jul 2013	Feeding; Measurement of remaining food	After over-wintering		
4	26 Jul 2013	Feeding; Measurement of remaining food	--	22 Mar 2014	CCA8
5	27/28 Jul 2013	Apiary C vandalized	--	22 Mar 2014	Hive bee sampling for *Varroa* and *Nosema* assessment
5	31 Jul 2013	Feeding; Measurement of remaining food	--	22 Mar 2014	Hive samples (capped honey, bee bread)
5	02 Aug 2013	Feeding; Measurement of remaining food	--	24 Mar 2014	Recording of hive weights
5	02 Aug 2013	Stability samples	--	15 Apr 2014	2nd sample shipment
5	02 Aug 2013	Pollen samples from pollen trap			

Residue Analysis

Residue analysis was conducted on the feeding solutions to determine the stability of imidacloprid during the course of the study as well as on the in hive stored pollen and nectar samples. Additionally, the major metabolites of imidacloprid, IMI-olefin and IMI-5-OH were screened. The limits of detection within the difference matrices assessed are summarized in **Table G-3** below.

Table G-3. LOD for imidacloprid and its metabolites

Matrix	IMI-Olefin	IMI-5-OH	Parent Imidacloprid
Dosing/Stability Solutions	2.07 ppb	2.22 ppb	0.38 ppb
Hive Collected Nectar	1.38 ppb	1.43 ppb	1.43 ppb
Pollen	0.74 ppb	0.18 ppb	0.36 ppb

Table G-4 below shows that imidacloprid remained relatively stable throughout the exposure period. The stability of imidacloprid at 200 µg/L in the feeding solution was not provided, but a significant reduction is not expected based on the reported data for all other concentrations. It is noted that imidacloprid was detected at 0.56 ppb in one of the control solution for one control hive (Apiary H, colony 4) sampled on 12 July 2014. There were no residues of IMI-olefin or IMI-5-OH detected in any of the samples (LOD of 2.07 ppb and 2.22 ppb, respectively).

Table G-4. The stability of imidacloprid in feeding solutions during the course of the study on 3 Jul, 12 Jul, and 2 Aug, 2013.

Nominal concentration (µg/L)	Average of measured concentrations (ppb)	Number of samples measured	Measured imidacloprid concentrations (ppb)		
			03 Jul, 2013	12 Jul, 2013	02 Aug, 2013
Control	<LOD†	20	0.56 ppb in one out of 20 samples. <LOD in 19 samples		
12.5	11.4	12	11.74	11.86	10.65
25	23.2	10	23.65	23.40	22.89
50	47.4	10	46.62	46.09	51.78
100	93.6	12	95.77	92.09	92.98
200	N/A*	N/A	N/A		

†: LOD: 0.38 ppb for imidacloprid;
*: N/A: data not available.

The analysis of residues in stored honey and pollen within the hives was conducted one month before exposure, during the exposure phase, just after the exposure phase, and once after the overwintering period. For the analysis conducted before the initiation of the exposure phase, six samples pollen and nectar samples were collected from different apiary sites. Imidacloprid was detected in 2 of the 6 pollen residue samples at 0.43 and 1.19 ppb, which are approximately at the level of and 3 fold the level of the LOD (0.38 ppb), respectively. All nectar samples were below the LOD (1.43 ppb) as was also the case for IMI-olefin and IMI-5-OH in all sampled pollen and nectar samples.

During the first sampling period of (CCA4) exposure phase, the concentration in hive pollen (beebread) showed a dose-response correlation between the average concentrations measured but were noted to be variable within each treatment group. The mean of the measured concentrations in bee bread within each treatment group of 12.5, 25, 50 and 100 ug/L was 2.86 (range: 0.77-5.34), 5.37 (range: 1.45-9.41),

10.84 (range: 4.2-19.41), and 17.89 ppb (range: 2.66-35.1), respectively. No information was provided for the 200 µg a.i/L treatment group. The lower concentrations in bee bread is somewhat expected due to the dilution since bee bread is a mixture of nectar and pollen from various sources. Similarly, for uncapped hive nectar, the results showed a dose-response correlation between the average concentrations measured and the concentrations in the feeding solution but varied within each treatment group. The mean of the measured concentrations in uncapped hive nectar within each treatment group of 12.5, 25, 50, 100, and 200 ug/L was 6.31 (range: 0.88-9.42), 13.24 (range: 1.19-20.53), 27.66 (range: 2.31-40.59), 46.87 (range: 2.1-80.15), and 109.14 ppb (range: 0.89-152.94) respectively. The results showed that after 3 weeks of feeding, imidacloprid concentrations in hive nectar appeared lower than that in the feeding solutions, which indicated that the foraging bees also foraged on nectar sources other than the provided sucrose, diluting the level of treatment. As for pollen, this result is expected, as bees were allowed to freely forage, and also, under natural conditions bees typically forage on multiple plant pollen and nectar sources.

Residue analysis one week after the exposure phase indicated similar results as those during the exposure phase for beebread and uncapped nectar samples. For both beebread and uncapped nectar, there was a dose-response correlation between the average concentrations of imidacloprid measured and the concentrations in the feeding solution, again with marked variability. In bee bread, the mean of the measured concentrations for 12.5, 25, 50 and 100 ug/L was 4.22 (range: 3.26-5.25), 5.74 (range: 4.89-6.4), 16.44 (range: 14.37-18.00), and 22.89 ppb (no range, only one measurement), respectively and the mean of the measured concentrations within each treatment group of 12.5, 25, 50, 100, and 200 ug/L was 5.88 (range: 3.36- 7.28), 7.18 (range: 0.89-10.68), 27.46 (range: 22.93-33.39), 54.98 (range:5.79-79.79), and 127.93 ppb (range:103.32-144.27) respectively. There was no beebread residues reported for the 200 µg/L.

After the overwintering period, only surviving hives in four apiaries were sampled (Apiaries E, I, J, and L). In bee bread, imidacloprid was not detected in the control, 12.5, 25 and 100 ug/L group, but was detected in three samples in the 50 µg a.i/L group with 2 samples at 0.52 ppb and one at 0.40 ppb. It is noted that these samples are either at or approximately 1.5 fold the LOD (0.38 ppb). No measurements were provided for treatment at 200 ug/L. In honey, no imidacloprid residues were detected in all measured hives except for one sample at 13.53 ppb at treatment of the 100 ug/L treatment group. It is noted that the average concentration of imidacloprid in hives after overwintering (CCA8) is considered to be uncertain, especially for hives at 100 and 200 ug/L due the effects on overwintering mortality where only one and two hives survived, in these groups, respectively.

It is noted that despite the reduced amount of imidacloprid present in beebread and uncapped nectar samples relative to the concentration in feeding solutions, demonstration of exposure was adequately characterized as the study authors noted that colonies consumed most or all of the feeding solution when replenishment occurred twice weekly during the exposure phase. It is also noted that the variability that was determined within treatment groups for the beebread and uncapped nectar samples during and just after the exposure phase are likely the result of the nature of the sampling methodology of these measurements. Specifically, these measurements represent one sample of one area of the comb on one

side of the frame to represent the beebread or uncapped nectar residues of an entire hive and therefore may not reflect the true nature of the residues across all portions of a given hive.

Statistical analysis

A joint review of this study was conducted by the EPA, PMRA, and CDPR. As part of that effort, a separate statistical analyses were conducted by each regulatory entity as an independent verification of the results from the analysis provided by the registrant. These analyses were distinct in approach but generally yielded similar statistical results. When weighing the statistical results as well as biological concerns, particularly as they relate to honey bee biology at the colony level, EPA, PMRA, and CDPR arrived at the same conclusion and are therefore harmonized in terms of the overall NOAEC and LOAEC yielded by this study.

Results

As mentioned previously, this study examined a variety of colony health parameters including overwintering survival, the percentage of frame coverage by various life stages (adults, eggs, larvae, and pupae), and the percentage of frame coverage by food stores (pollen and nectar). Additionally, hive weight data were collected. While data were collected for these response variables at every CCA, the figures depicting response variables begin at CCA3 as this was the first CCA after the colonies were established in the test apiaries. The data at first CCA mainly serve to indicate that there were no significant differences among the colonies for the various response variables before the exposure phase. What follows is a summary of the results of each response variable. It is worth noting that the figures presented with indications of statistical significance refer to the EPA analysis. The figures show the trends of each response variable over the course of the study with the data summarized as a proportion of frame coverage. Indications of statistical significance for all response variables except overwintering survival are denoted by the red ($p<0.05$) and black ($p<0.1$) dots.

<u>Hive overwintering survival and disease presence:</u>

After overwintering, only one hive survived (Apiary L) at the 100 ppb, and two hives survived (Apiary D and F) at the 200 ppb treatment groups. The hive mortality showed a non-monotonic response as depicted in **Figure G-1**. The percent mortality decreased from 36% in control to 9% in the 25 µg/L treatment, and then increased to 36% in the 50 µg/L treatment and 91% in the 100 µg/L treatment and 82% in the 200 µg/L treatment. The results presented in **Table G-5** and **Figure G-1** below show that control mortality after overwintering was higher than it was for the 12.5 and 25 µg/L groups. For this reason, the ability to detect treatment-related effects on overwintering colony survival may be masked by the magnitude of control colony loss.

Table G-5. Overwintering hive mortality based on the final colony condition assessment (CCA8) for colonies treated with varying levels of imidacloprid.

Treatment (µg/L)	Control	12.5	25	50	100	200
Number of deceased colonies /total colonies	8/22	2/11	1/11	4/11	10/11	9/11

Colony mortality (%)	36	18	9	36	91	82
Colony survival (%)	64	82	91	64	9	18

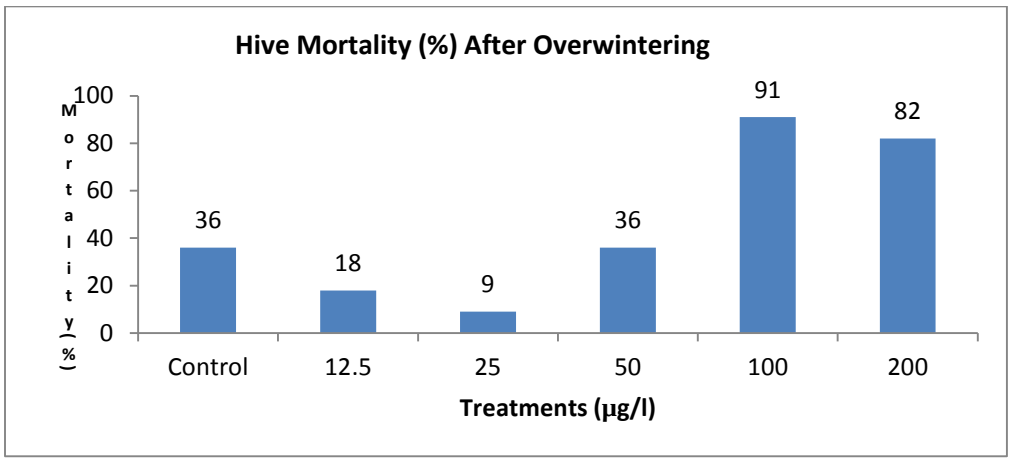


Figure G-1. Hive mortality across imidacloprid-treated colonies after overwintering.

Varroa mite presence in the colonies was assessed the week before and after the exposure phase, as well as after over-wintering (CCA3, CCA5 and CCA8). The number of mites per 100 bees was calculated (depicted below in **Figure G-2**). Hives were treated with one application of Apiguard® (active ingredient: thymol) following typical apicultural practice for the region immediately after the September 10th-11th CCA (CCA6) to prevent high mite loads. After over-wintering, the colonies of all treatment groups, except the 100 µg/L group, had similar *Varroa* infestation levels.

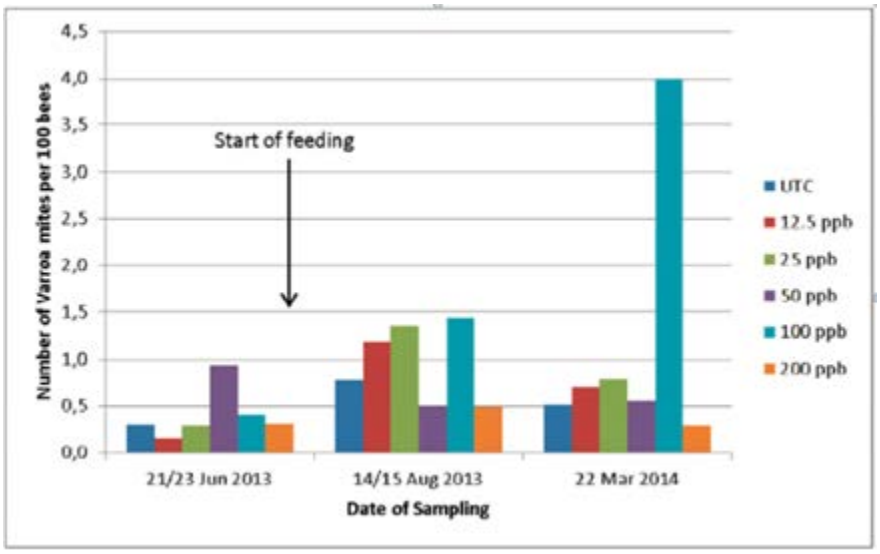


Figure G-2. Mean number of *Varroa* mites per 100 bees

The number of *Nosema* spores per bee was determined at the same time points as those for *Varroa* (*i.e* CCA3, CCA5 and CCA8). After the overwintering period, there appeared to be a great incidence of *Nosema* across the control and all treatment groups, particularly at 100 µg/L treatment group.

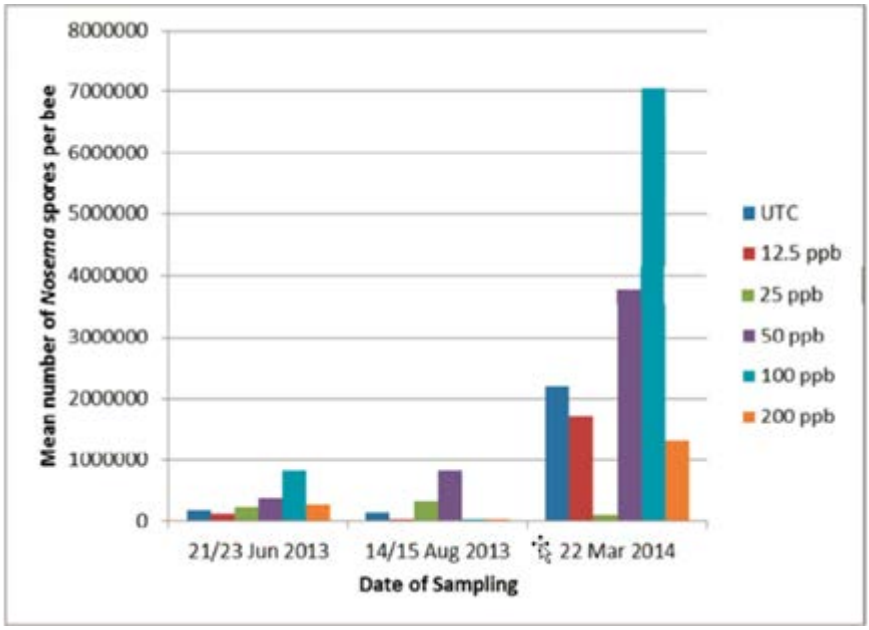


Figure G-3. Mean number of *Nosema* spores per bee.

Proportion of Adults:

Figure G-4 below shows the proportion of adult honey bees across CCAs and treatment groups. Compared with the control, no differences in the number of adults in hives ($p>0.1$) during the exposure period (CCA4) were apparent in any of the treatment groups. Additionally, the total number of adults in the 12.5 and 25 µg/L treatments was not reduced in any of the CCAs. However, the numbers of the adults in the 100 and 200 µg/L treatments were significant lower than controls ($p<0.05$) at CCA5, CCA6, and CCA7 with reductions ranging from 24.4 – 59.4%. An exception is that the EPA and CDPR analyses did not detect significant reductions ($p>0.05$) at the 200 µg/L treatment level, relative to controls, at CCA5 while the PMRA analysis did. However, it is apparent from all analyses that there were clear impacts to adults at the 200 µg/L group during the course of the study. The number of adults in the 50 µg/L treatment was also significantly reduced at CCA5 ($0.05 < p < 0.1$) but at $p<0.05$ at CCA6 and CCA8. The PMRA analysis also determined a significant reduction relative to controls at CCA7 while the EPA and CDPR analyses did not, but like the 100 and 200 µg/L treatment groups, the persistent nature of significant reductions to adults at the 50 µg/L group is evident.

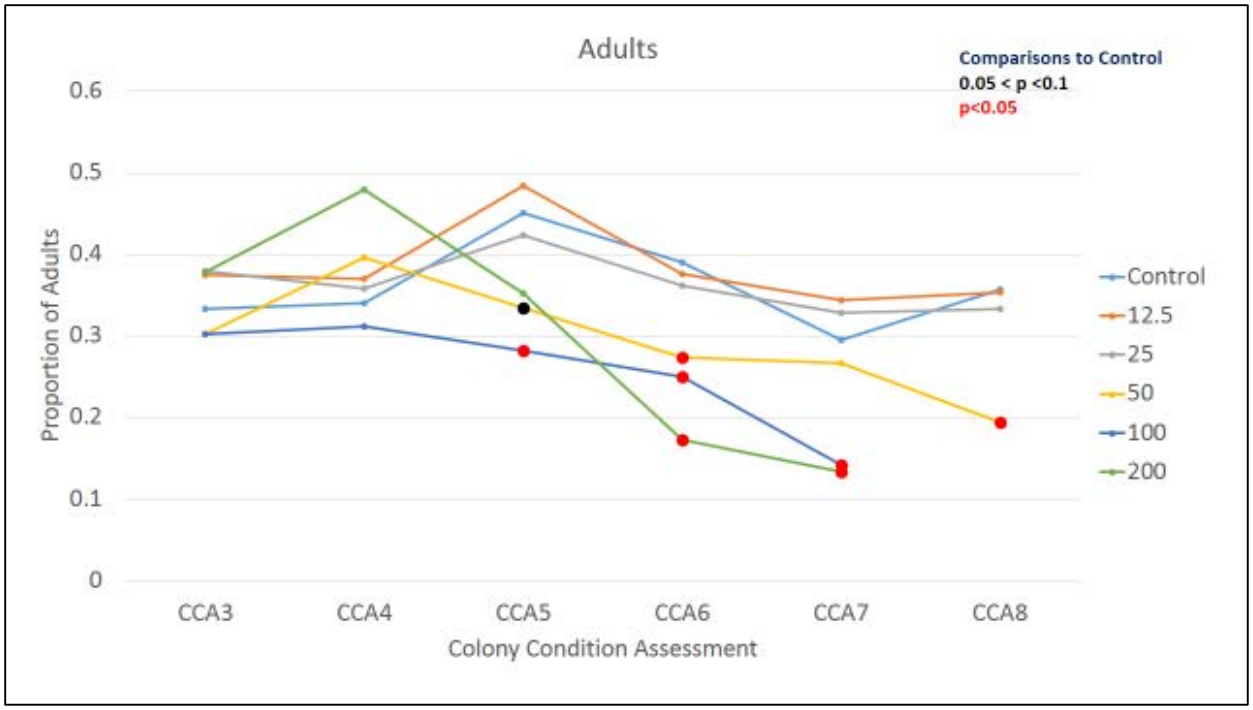


Figure G-4. Proportion of adults following exposure of honey bee colonies to varying dietary concentrations of imidacloprid across Colony Condition Assessments (CCA3 – CCA8).

Proportion of Eggs:

There were consistently lower numbers of eggs in treatments at 100 and 200 µg/L ($p<0.05$) following exposure (**Figure G-5**). While there were minor differences in the statistical findings of the three analyses not only at a given CCA and treatment group but also at what alpha level an effect was statistical significance was determined, these differences do not have an impact on the determination that there was not only an early onset but also a persistent reduction in the numbers of eggs that was not confined to one CCA. At the 100 and 200 µ/L treatment groups, all analyses determined significant reductions ($p<0.1$) in eggs for the 100 and 200 µg/L treatment groups for at least two CCAs (percent reductions ranged from 38 – 138% for the 100 µg/L group and 14.5 – 153.2 µg/L group, depending on the CCA, based on raw counts of eggs. The sole finding of significant reduction in the number of eggs at the 50 µg/L group was at CCA8 at the 0.05 alpha level.

For the 12.5 µg/L the PMRA and EPA analyses only determined a significant reduction in the number of eggs at CCA8 ($p<0.1$); a similar finding was made at CCA4 in the PMRA analysis only. However, there were no significant reductions ($p>0.1$) at both CCA4 and CCA8 in the 25 µg/L group, indicating a lack of dose responsiveness within these time points. The biological significance of this finding at 12.5 µg/L is therefore considered to be low. Similarly, there was a significant reduction in eggs determined at CCA6 in the 25 µg/L group (PMRA analysis only). However, a similar finding of statistical significance at this CCA was not determined for the 50 µg/L group. Finally, this effect was not observed before CCA6 or in the subsequent CCAs (CCAs 7 and 8) indicating this effect was isolated to this time point. At the 50 µg/L treatment level, all analyses determined a significant reduction ($p<0.05$) at CCA8 (78.2%, based on raw counts). While this

effect was isolated to just CCA8 for this treatment group, there is uncertainty as to whether hives could have compensated for this reduction as spring progressed should an additional CCA had been conducted.

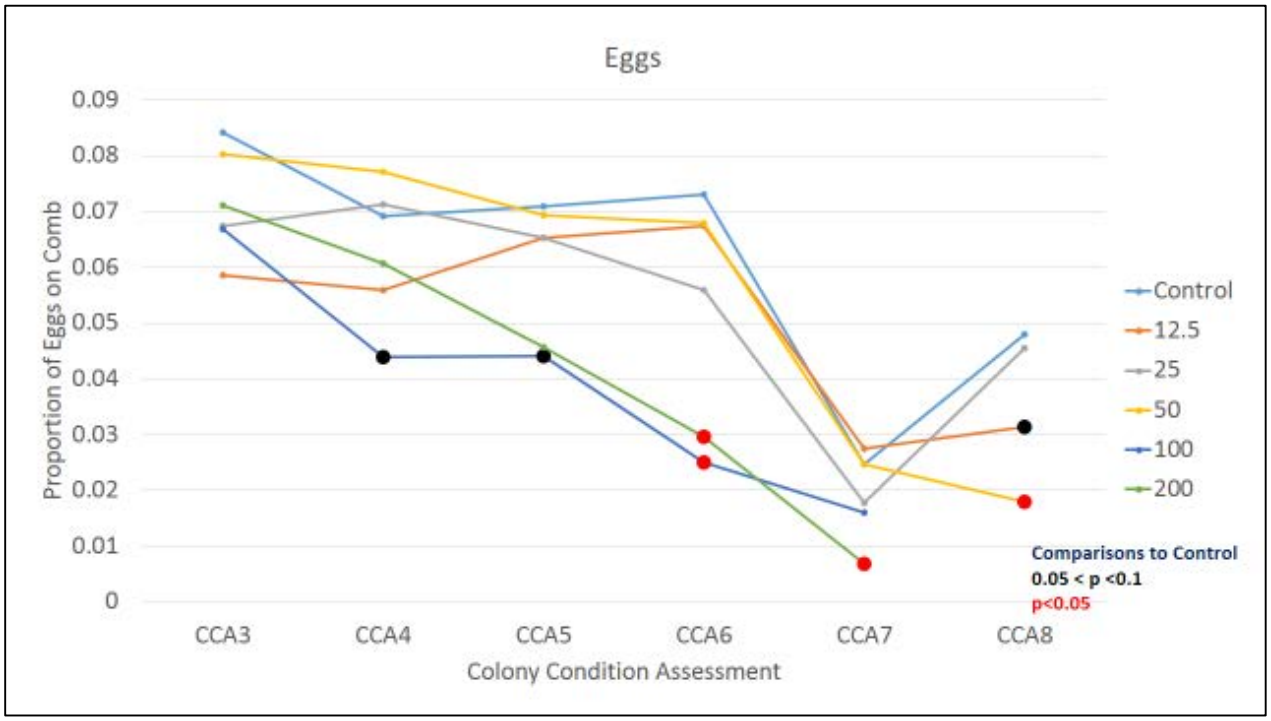


Figure G-5. Proportion of eggs following exposure of honey bee colonies to varying dietary concentrations of imidacloprid across Colony Condition Assessments (CCA3 – CCA8).

Percentage of Larval (Open; Uncapped) cells

There were consistently and significantly lower ($p<0.05$) numbers of larvae in the 100 and 200 µg/L groups as compared to control beginning at CCA4 and persisting throughout the remainder of the study leading up to and after overwintering at CCA8 (data from CCA8 in these groups excluded from EPA analysis). There were no significant reductions from control at any CCA for the 12.5, 25, and 50 µg/L treatment groups based on the EPA analysis.

In the PMRA analysis at CCA6, a significant reduction from control was determined at 25 µg/L ($p<0.1$), but not at 12.5 µg/L and 50 µg/L ($p>0.1$ for both treatments). Additionally, the effect was not determined at CCA4, CCA5, and CCA7. Similarly, the CDPR analysis determined a significant difference ($p<0.05$) at CCA7 at 12.5 µg/L, but this finding was not determined at the 25 and 50 µg/L treatment groups ($p>0.1$). Also in the PMRA analysis only, at CCA8, a statistical reduction was determined at 50 µg/L treatment group ($p<0.05$). This effect was not determined to be significantly reduced from control from CCA4 to CCA7 ($p>0.1$). Although this difference was not detected in the EPA and CDPR analyses, the percent reduction in larval cells at CCA8 for this group was 43% (based on raw counts).
Figure G-6 below shows the trends of the control and all treatment groups for larval cells across all CCAs assessed. A clear divergence in the 100 and 200 µg/L groups is evident beginning at CCA4 where the

numbers of larvae in these groups undergo a marked decline (percent reduction of 37.1 and 64%, respectively, based on raw counts) while the other treatment groups generally trend with control.

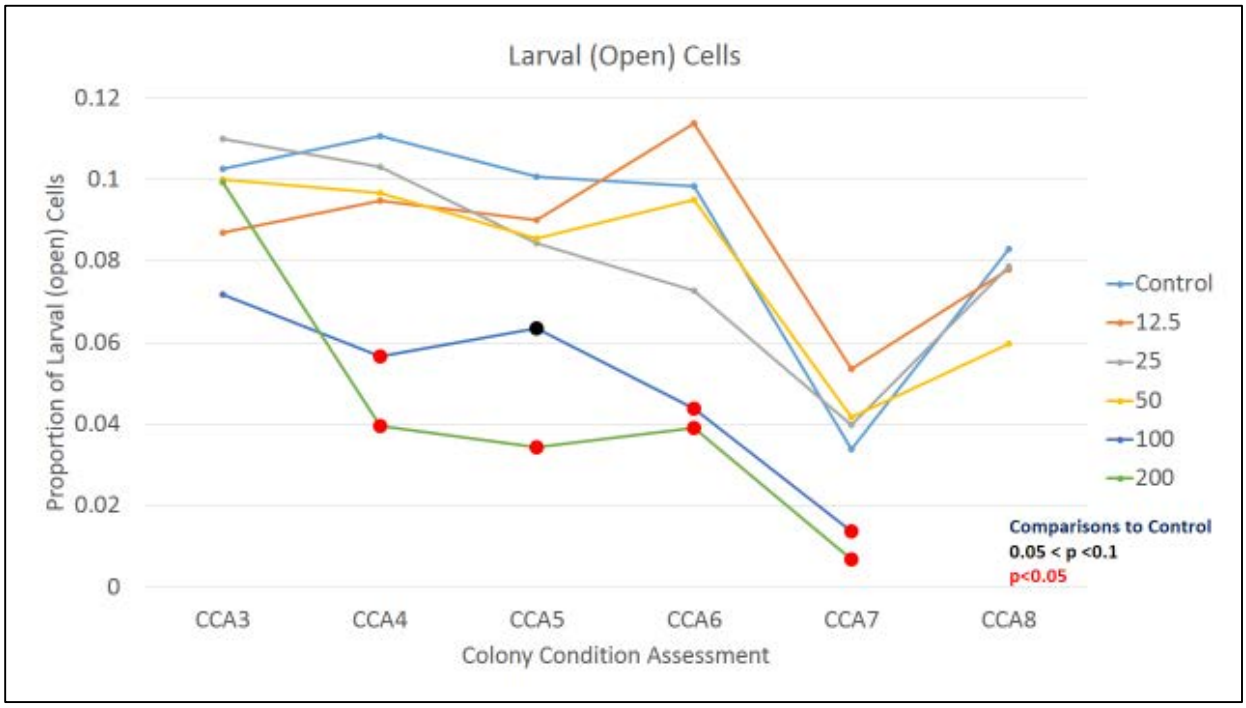


Figure G-6. Proportion of larval cells following exposure of honey bee colonies to varying dietary concentrations of imidacloprid across Colony Condition Assessments (CCA3 – CCA8).

Percentage of Pupal (Capped) cells:

At the 50, 100 and 200 µg/L treatment groups, there were significant reductions from control ($p<0.05$) that persisted through most of the study (EPA findings at the 50 µg/L were significant at two CCAs, CDPR at 3 CCAs and the PMRA analysis determined significant reductions from control at 5 CCAs). The percent reductions from control based on the raw counts of pupal cells in the 100 and 200 µg/L groups ranged from 49.7 – 93.5% during CCA4 – CCA7.

At the 12.5 µg/L treatment group, there were significant reductions determined at CCA6 for the EPA and PMRA analyses. While findings of significance were not determined at the 25 µg/L treatment group at CCA6 for the EPA and CDPR analyses, they were for the approach used by PMRA. It is noted that significant reductions in pupal cells were not determined by any analysis at 12.5 and 25 µg/L in any CCA preceding or subsequent to CCA6, thus the significant effect for the 12.5 and 25 µg/L treatments was isolated to the CCA6 timepoint. Additionally, although PMRA determined significant reductions at CCA6 for all treatment groups ($p<0.05$ for 12.5, 25, 100, and 200; $p<0.1$ for 50 µg/L groups), the effects did not demonstrate a dose response at the lowest three doses with the percent reductions from control (based on raw counts) at 22.3, 18.3, 12.5, 49.7 and 75.5% for the 12.5, 25, 50, 100 and 200 µg/L groups, respectively. Finally, after overwintering at CCA8, the proportion of pupae in the surviving hives at the 12.5 and 25 µg/L treatment groups were actually above the level of control (based on raw counts of the data) by 1.3 and 10.8%, respectively. The percent reduction from control at CCA8 in the 50 µg/L group was 70.6%.

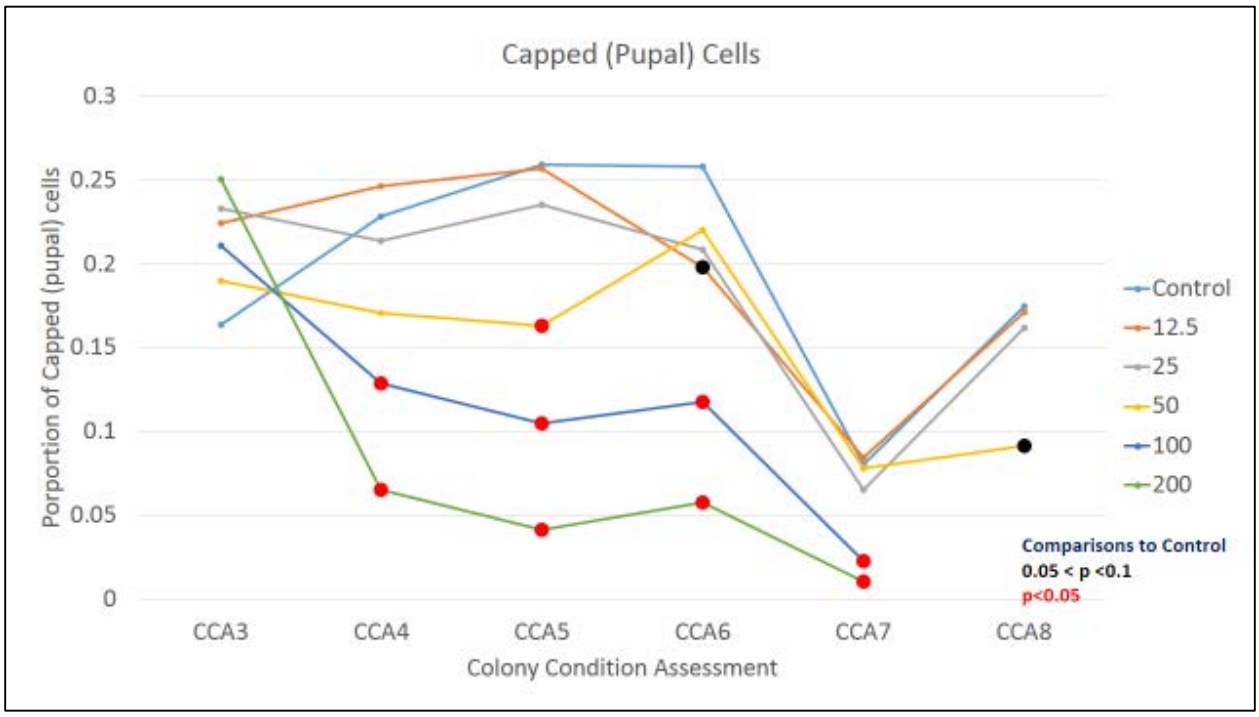


Figure G-7. Proportion of pupal (capped) cells following exposure of honey bee colonies to varying dietary concentrations of imidacloprid across Colony Condition Assessments (CCA3 – CCA8).

In summarizing the information provided by the different analyses for this response variable, a few points can be made:

- All analyses find significant differences at the 100 and 200 µg/L levels starting at CCA4 and persisting until CCA7 (data from CCA8 excluded from EPA analysis at these two treatment groups)
- PMRA analysis determined significant difference at the 50 µg/L group (at either 0.1 or 0.05 alpha level) for all CCAs assessed except CCA7 (for CDPR, same findings except no significant findings at CCA6 and CCA7)
- EPA analysis determined significant differences at 50 µg/L group (at either 0.1 or 0.05 alpha level) at CCA5 and CCA8
- PMRA determined significant differences at all treatment groups at CCA6 while EPA determined significant effects for the 12.5, 100 and 200 µg/L groups, and CDPR only at 100 and 200 µg/L.

In further exploring this last point, the difference in findings can potentially be explained by the statistical model selections employed for each analyses. The discussion below focuses on additional lines of evidence to further characterize the findings, particularly the potential impact at and below 50 µg/L.

It is noted that the average proportion of comb area as pupal cells in controls is similar after overwintering at CCA8 (17%) as compared to CCA3 (16%) when the hives were initially placed in the test sites. In the 12.5 µg/L group, unlike the control group, where the average proportion of pupal cells remained stable between the time of CCA5 and CCA6 (26%), there was an apparent decrease in the 12.5 µg/L group from CCA5 (26%) to CCA6 (20%) based on average proportions. This decrease continued for the CCA6 to CCA7

interval to an equivalent level as controls (approximately 8%). After the overwintering period in the 12.5 µg/L group, the proportions of the various life stages (*i.e.*, eggs, larvae, capped pupae) were similar to CCA8 for the control group in that the proportion of pupal cells 17% at CCA8 (as compared to 17% in the control).

In the 25 µg/L treatment group, there were again no significant differences in the proportions of all life stages at CCA3 before the start of exposure ($p>0.05$). As opposed to the steady buildup that was observed in the control and 12.5 µg/L treatment groups from CCA3 to CCA5, the numbers of pupal cells remained similar from CCA3 to CCA5; they were decreased slightly at CCA4 (21%) as compared to CCA3 (23%) but at CCA5 (24%) were again to the level of CCA3. As with the other life stages, a decline in numbers was observed between CCA6 and CCA7 as the hives prepared for overwintering. The average proportion of pupal cells at CCA8 for the 25 µg/L treatment group were similar to the proportions in CCA8 of the control group, that is, 16% and 17% frame coverage at CCA8 for the 25 µg/L group and control group, respectively.

As distinguished from the control and 12.5 and 25 µg/L groups, there was a steady decline in the number of pupal cells at the 50 µg/L group beginning at CCA4 and continuing through CCA5 (average proportion at CCA3 was 19% compared to 16% at CCA5). This is also evidenced by the average proportion of pupal cells at CCA5 at the 50 µg/L group which was 16% of the comb areas as compared to 26, 26, and 24% at CCA5 for the control, 12.5, and 25 µg/L groups, respectively. This finding is also statistically significant for all analyses conducted as indicated above. An examination of the proportions at CCA8 also suggest the persistent nature of these effects at this treatment group. The average proportion of pupal cells at CCA8 for the 50 µg/L group was 9% as compared to 17, 17, and 16% for the control, 12.5, and 25 µg/L groups, respectively. This finding was significant at $\alpha = 0.1$ in the EPA analysis and at $\alpha = 0.05$ for the PMRA analysis (with a 71 % reduction compared to control).

Regarding the statistical analyses, all methods found significant differences at 100 and 200 µg/L that were apparent at early CCAs and persisted throughout the study. Additionally, effects were noted at multiple CCAs for 50 µg/L, and the effects continued following overwintering. While all analyses found a significant effect at the 12.5 µg/L treatment level at CCA6, and the PMRA analysis also found significant effect at the 25 µg/L treatment level, these effects were considered to be transient. This is because effects at 12.5 and 25µg/L were isolated to CCA6 with levels returning to those similar to control after overwintering, and at CCA6 the effects lacked a clear dose-response relationship and were similar among all three lower treatment levels (12.5, 25, 50 µg/L; 22.2, 18.3, 12.5 % reduction compared to control based on raw data, respectively). Additionally, the discussion presented above indicates that the average proportions of pupal cells in the 12.5 and 25 µg/L group at different CCAs resemble the responses found in the control group in terms of their level before, during, and after exposure and overwintering. The effects at the 50 µg/L however, appear earlier, persist longer, when compared to the control.

<u>Percentage of Total Individuals (Adults, Egg, Larval, and Pupal Cells:</u>

When evaluating the proportion of frame coverage of total individuals, the pattern of effects has some similarity to the proportion of frame coverage of adults and pupae, as these two life stages made up the largest components of the hive population throughout the course of the study. In the 100 and 200 µg/L treatment groups, total individuals were significantly reduced ($p<0.05$) from the level of control from

CCA4 to CCA7. The EPA analysis did not find a significant difference (p>0.1) at the 200 µg/L group at CCA4 but the reduction relative to control at this treatment level is evident at other CCAs.

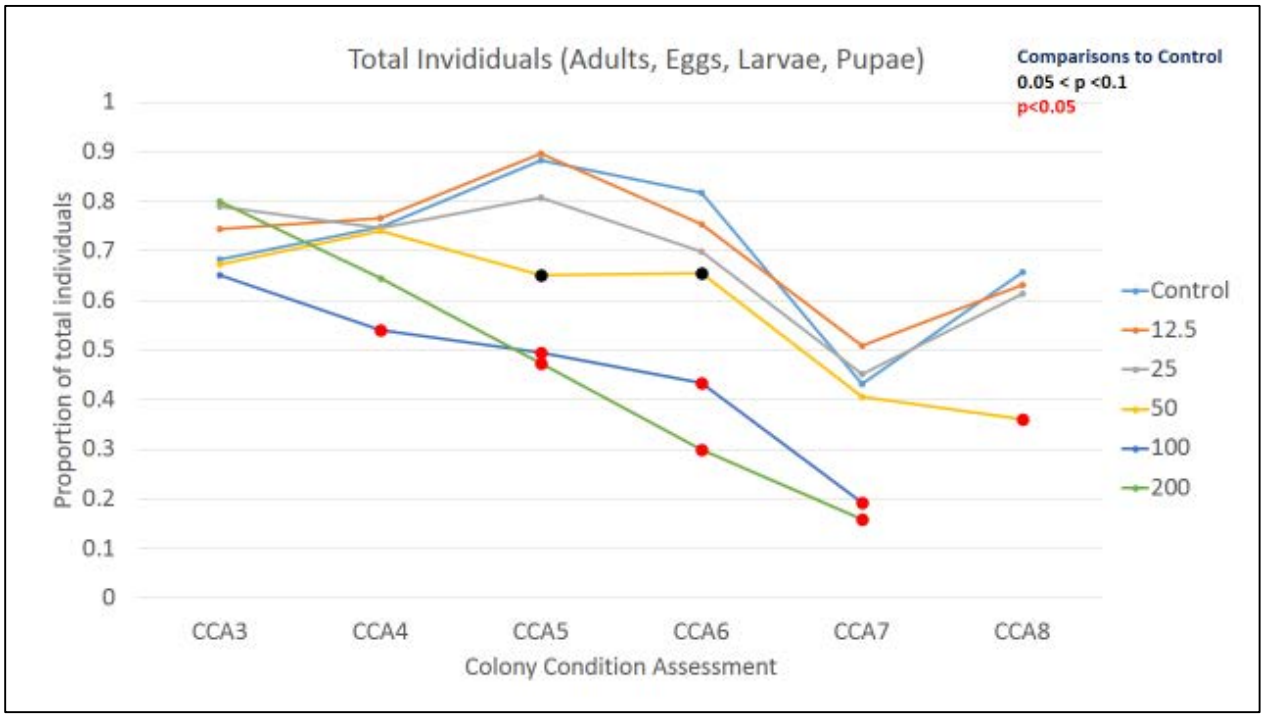


Figure G-8. Proportion of total individuals (adult, eggs, larvae, pupae) following exposure of honey bee colonies to varying dietary concentrations of imidacloprid across Colony Condition Assessments (CCA3 – CCA8).

For the 50 ppb treatment group, reductions relative to the control at CCA5 and CCA6 were significant at α=0.10 and reductions relative to the control at CCA8 were significant at α=0.05 (CCA6 result was significant at α=0.05 in the PMRA analysis). For the EPA analysis, there were no further findings of statistical significance which includes all CCAs at the 12.5 and 25 µg/L treatment groups. In the PMRA analysis, total individuals were significantly reduced at 12.5 (p<0.1) and at the 25 µg/L treatment groups (p<0.05). It is noted here, as it has been previously for other response variables, the effects determined at 12.5 and 25 µg/L are isolated to CCA6, with no determinations of statistical significance before and after this CCA, indicating this may be a transient effect. After overwintering at CCA8, the surviving hives in the 12.5 and 25 µg/L groups were actually above the level of control by 2.6 and 11.4% respectively (based on the raw counts of total individuals) while the 49% reduction from control at CCA8 in the 50 µg/L group was significantly reduced at a 0.05 alpha level.

Figure G-9 below shows the trends of the various life stages from CCA3 to CCA8 in the control, 12.5, 25, and 50 µg/L treatment groups. As indicated previously, there were clear impacts on every life stage during and persisting until after the exposure phase ended for colonies exposed to dietary concentration of imidacloprid at 100 and 200 µg/L groups for 6 consecutive weeks. **Figure 5-11** and the discussion below illustrate the divergence of effects observed at the 50 µg/L as compared to the 12.5 and 25 µg/L treatment groups, which largely follow a similar trend as the control across all life stages and CCAs of the study.

Based on **Figure G-9** the average proportions of adults began to decline to 39% by CCA6, which represents a time when the colony as a whole starts to prepare for overwintering. During this time, the numbers of adults and particularly brood (*i.e*., eggs, larvae and pupae) steadily declines and are clearly decreased from levels during hive build up (CCA4 and CCA5) by the time of CCA7, even in untreated control colonies. During this pre-overwintering phase, adult foragers naturally die and their numbers are not replenished at a rate similar to that during hive expansion due to reduced egg laying by the queen. After the overwintering period the following spring, the hive will undergo another expansion period. This trend is also illustrated from the data in this study as the average proportion of adults is similar after overwintering at CCA8 (35%) as compared to before exposure at CCA3 (33%) when the hives were initially placed in the test sites. The response of the 12.5 and 25 µg/L treatment groups generally follow the response of the control group with the proportions of adults generally increasing through CCA5(to build up a foraging worker bee force for pollen and nectar collection) before beginning to decline. This response is distinguished from that of the 50 µg/L group where the numbers of adults began to decline as early as CCA4. This is further evidence by the average proportion of adults at CCA5 in the 50 µg/L group which was 33% as compared to 45, 48, and 42% for the control, 12.5 and 25 µg/L groups, respectively (percent reductions ranging from 24 – 37% based off the proportions of frame coverage).

For the proportion of eggs, by CCA8, the average number in the control group increased relative to CCA7, but the average proportion at CCA8 (4.8%) was approximately half the proportion initially recorded at CCA3 (8.4%). It is noted that the 50 µg/L group was the only group among the control and three lowest treatment levels that underwent a downward trend from CCA7 to CCA8.

The combined statistical analyses did not identify a significant reduction in the proportion of larval cells as compared to the control in the 12.5, 25 and 50 µg/L groups. One exception is the significant ($p<0.5$) reduction in the 50 µg/L group at CCA8 that was determined in the PMRA analysis.

For pupal cells, several effects were determined at the 50 µg/L group as well as at the 12.5 µg/L in the EPA analysis and all treatment groups for the PMRA analysis at CCA6 only. In examining the response of the control for this variables, it is noted that the proportion of capped (pupal) cells in controls is similar after overwintering at CCA8 (17%) as compared to CCA3 (16%) when the hives were initially placed in the test sites. Also notably, the average proportion of pupal cells in the 12.5 and 25 µg/L groups (22%) is higher than it was for the control group (16%), a finding that was not related to imidacloprid treatment, given that exposure had not yet occurred. There was a steady increase in average numbers of capped cells from CCA3 (16%) through CCA5 (26%), where the proportion remained stable through CCA6 (25%).

By contrast, in the 12.5 µg/L group, while there was a steady increase in the proportion of pupal cells from CCA to CCA5 as with the control group, there was an apparent decrease in the 12.5 µg/L group from CCA5 (26%) to CCA6 (20%) based on average proportions. This decrease continued for the CCA6 to CCA7 interval to an equivalent level as controls (approximately 8%). As opposed to the steady increase observed in the control and 12.5 µg/L treatment groups from CCA3 to CCA5, the numbers of pupal cells remained similar from CCA3 to CCA5 in the 25 µg/L group. As with the other life stages, a decline in numbers was observed between CCA6 and CCA7 as the hives prepared for overwintering. The average proportion of pupal cells at CCA8 for the 25 µg/L treatment group were similar to the proportions in CCA8 of the control group,

that is, 16% and 17% frame coverage at CCA8 for the 25 µg/L group and control group, respectively. The nature of these responses in the 12.5 and 25 µg/L groups suggest that while divergent from the trend exhibited by the control group at variable times leading up to CCA6 depending on the treatment group, that their response showed recovery to the level of the control by CCA7 and after overwintering at CCA8.

The trends at the 12.5 and 25 µg/L treatment groups are distinguished from that of the 50 µg/L group where instead of a buildup, there a steady decline in the number of pupal cells at the 50 µg/L group beginning at CCA4 and continuing through CCA5 (average proportion at CCA3 was 19% compared to 16% at CCA5). This is also evidenced by the average proportion of pupal cells at CCA5 at the 50 µg/L group which was 16% of the comb areas as compared to 26, 26, and 24% at CCA5 for the control, 12.5, and 25 µg/L groups, respectively, a finding that was also statistically significant for all analyses conducted as indicated above. An examination of the proportions at CCA8 also suggest the persistent nature of these effects through multiple CCA at this treatment group. The average proportion of pupal cells at CCA8 for the 50 µg/L group was 9% as compared to 17, 17, and 16% for the control, 12.5, and 25 µg/L groups, respectively. This finding was significant at $\alpha = 0.1$ in the EPA analysis and at $\alpha = 0.05$ for the PMRA analysis (with 70.6 % reduction compared to control)

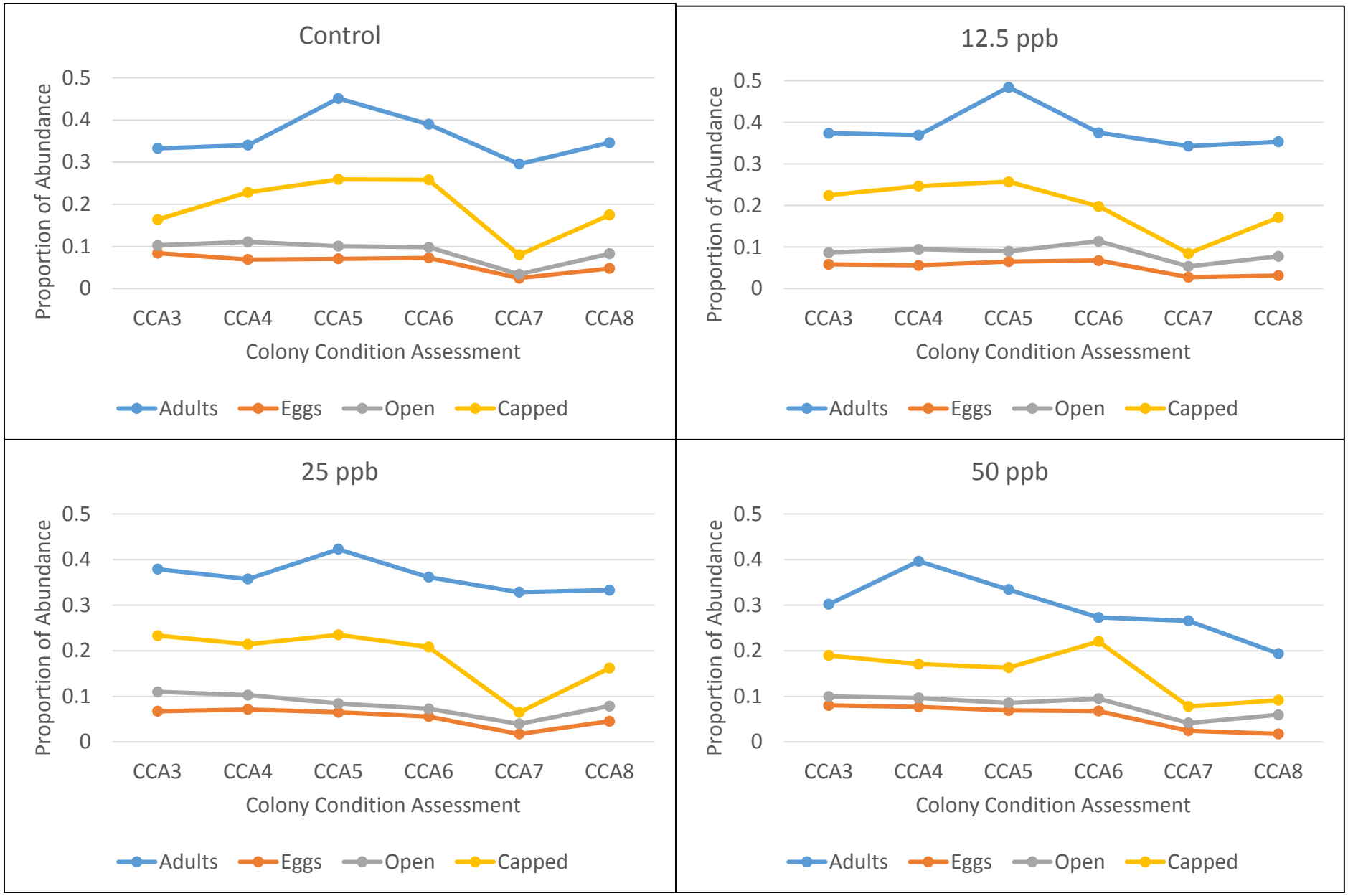


Figure G-9. Proportion of abundance of various life stages in the control, 12.5, 25, and 50 µg a.i/L treatment groups across the study duration

Pollen store abundance:

Pollen stores were significantly reduced ($p<0.05$) in the 200 µg/L treatment group during from CCA4 to CCA7, with the CDPR analysis not finding a significant difference at CCA7 only. Pollen stores were significantly reduced ($p<0.05$) at 100 µg/L treatment group at CCA4 andCCA5. Similarly, pollen stores were significantly reduced at the 50 µg/L treatment group at the 0.05 alpha level at CCA4 and CCA5, but not CCA6 and CCA7 ($p>0.1$). A significant reduction at CCA7 ($0.05 < p < 0.1$) for the 50 µg/L was determined in the PMRA analysis but not in the EPA or CDPR analyses. The reduction of pollen stores was not determined in the 12.5 and 25 µg/L treatment groups for any CCA assessed. The reduced pollen store was most pronounced during and just after the exposure phase (CCA4 and CCA5) for the treatment levels exhibiting the effect.

At CCA8, in the hives that survived overwintering, the total amount of pollen store was reduced in the 50 µg/L group (↓64% based off raw counts). The PMRA analysis also determined a significant reduction ($p<0.05$) in the 100 µg/L group but not in the 200 µg/L group ($p>0.1$) (these data excluded from EPA's analysis). It is noted however, that the lack of statistical significance for pollen stores in the 200 µg/L group may be an artifact as there were only two hives surviving overwinter and there was a wide confidence interval.

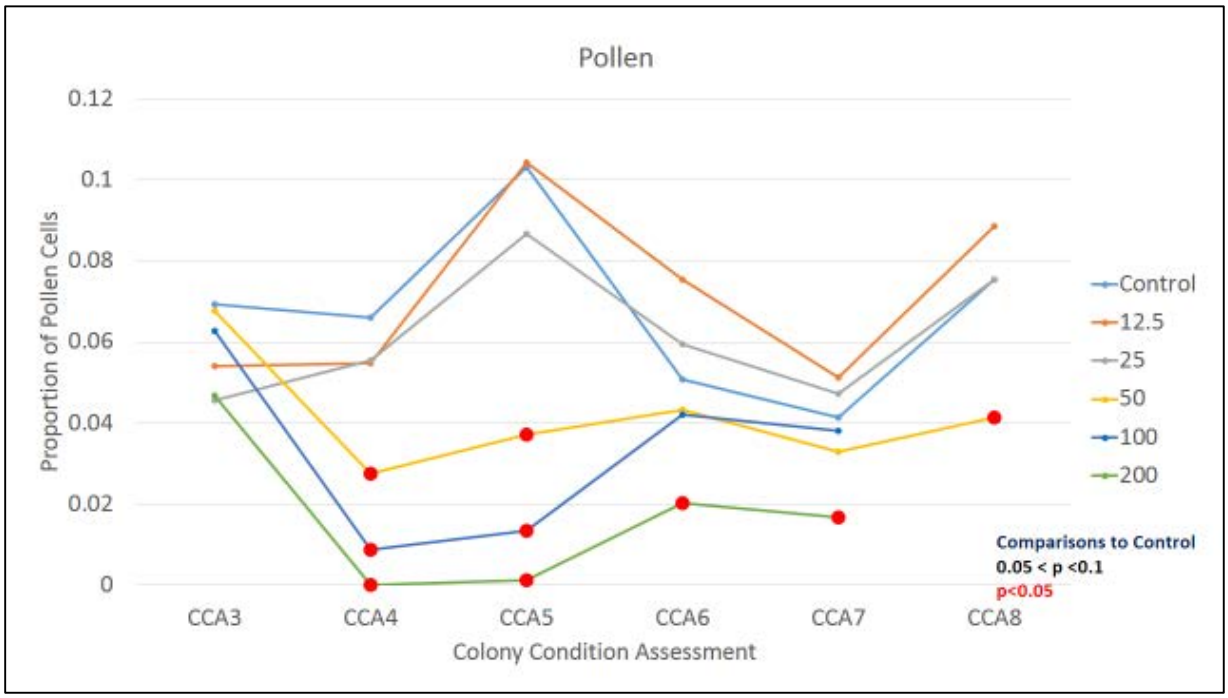


Figure G-10. Proportion of honey stores following exposure of honey bee colonies to varying dietary concentrations of imidacloprid across Colony Condition Assessments CCA3 – CCA8.

Nectar (honey) store abundance:

There was a consistent and significantly ($p<0.1$) a lower amount of honey stored in treatment hives at 50, 100 and 200 µg/L than in the control at CCA6 and thereafter (**Figure 5-13** below). One exception is the

absence of a determination of significance at CCA6 for the 100 µg/L group which PMRA determined as significant but EPA did not. All other findings after CCA6 at the 50, 100 and 200 µg/L (EPA excluded data at CCA8 for the 100 and 200 µg/L groups) were in general agreement with slight variations of the alpha level at which the effect was significant. No reduction of the honey stores was determined at the 12.5 and 25 µg/L during the study, with the exception of the PMRA finding of a reduction at CCA6 at the 12.5 µg/L treatment group ($p<0.1$) at CCA6. This statistical difference at 12.5 µg/L was unlikely to be treatment related, as there were no reductions before or after the CCA6 at the same concentration, nor at the higher concentration of 25 µg/L at CCA6. While there were no significant findings of impact at CCA8 in the 200 µg/L group (EPA excluded these data), the lack of significance is considered to be questionable as there were only 2 surviving hives at this treatment group.

Figure G-11 below for the honey store trends in the control, 12.5, 25, and 50 µg/L groups only show a marked divergence at the 50 µg/L treatment group beginning at CCA6 and persisting up to and after overwintering at CCA8.

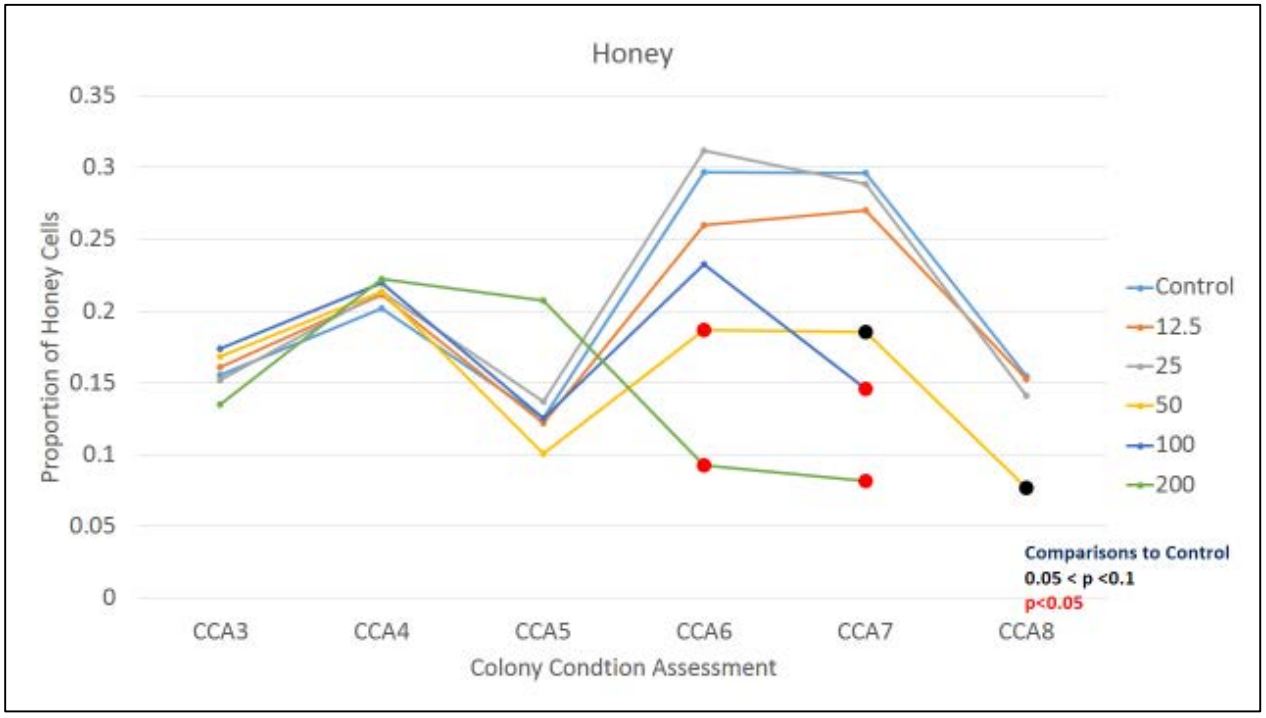


Figure G-11. Proportion of honey stores following exposure of honey bees to varying concentrations of imidacloprid in the diet across CCA3 – CCA8.

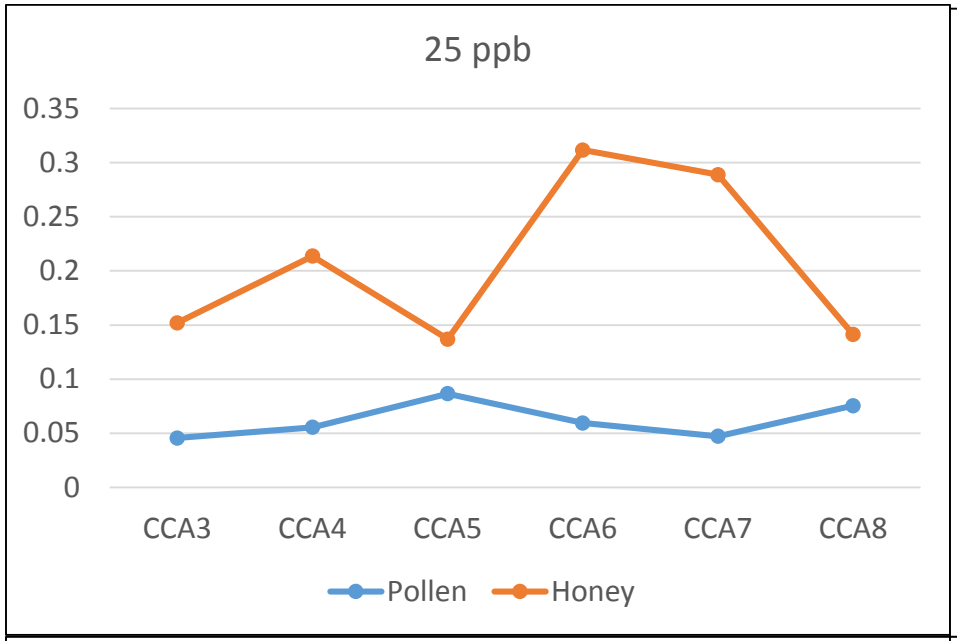
Similar to **Figure G-9** above with regard to the abundance of various life stages, **Figure 5-14** shows the trends food stores (pollen and nectar) from CCA3 to CCA8 in the control, 12.5, 25, and 50 µg a.i/L treatment groups.

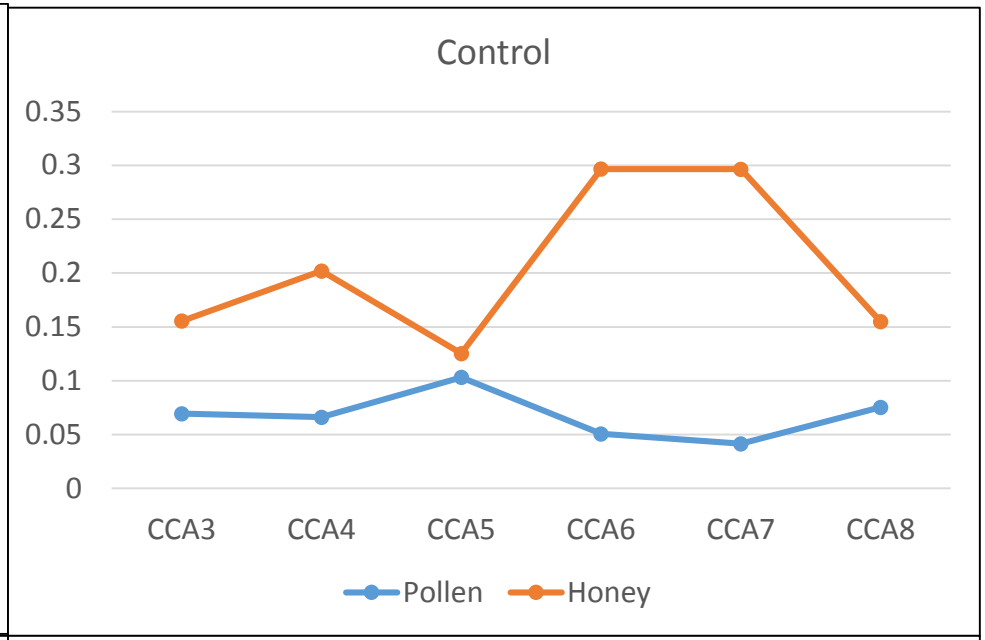
In examining the trends of pollen stores in the control, there was a buildup in stores that occurred from CCA3 (7% of frame coverage area) to CCA5 (10%). This increase in protein stores supports the queen in her effort to produce brood during the late spring and early summer months that eventually recruit into foragers and build sufficient honey stores for the hive. Pollen stores experienced a decline in numbers

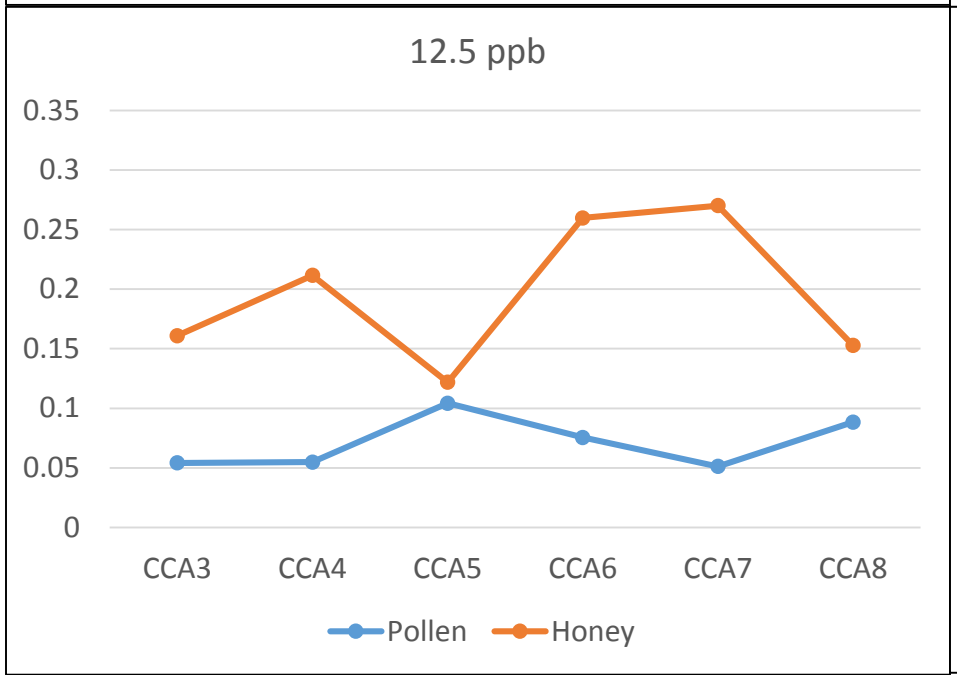
from CCA5 (10%) to CCA7 (4%) before showing an upward trend from CCA7 to CCA8 (8%). This downward trend reflects the fact that up to overwintering, brood production will slow as the hive prepares for winter and therefore there is a reduced need for pollen within the hive. As depicted in the **Figure 5-14** below, the trends of the proportions in the 12.5 and 25 µg/L groups track very similarly with the control. Pollen stores at the 50 µg/L group, in contrast to the control, 12.5 and 25 µg/L groups, began a decline in stores earlier than the other groups as well as having an average proportion of approximately 50% of the stores in the control, 12.5, and 25 µg/L groups, after overwintering in CCA8 among the surviving hives.

Honey stores in the control group underwent an upward trend from CCA3 (16% of the frame coverage) to CCA4 (20%), before a subsequent decline in average proportion from CCA4 to CCA5 (13%). This was followed by an approximately 140% increase in stores from CCA5 to CCA6 (average proportion of 30%) that remained stable until CCA7 (30%). This buildup of honey stores took place ahead of CCA7 that represented the last time point before overwintering. The honey stores declined markedly from CCA7 to CCA8 (16%) which is expected given lack of foraging and utilization of these reserves during the overwintering period. It is noted that the proportion of comb cells containing honey stores at CCA3 and at CCA8 were approximately the same at 16%.

In the 12.5 µg/L treatment group, honey stores also underwent an initial build up from CCA3 (16%) to CCA4 (21%) before a subsequent decline from CCA4 to CCA5 (12%), similar to that of the control. This was followed by a large buildup of honey stores from CCA5 to CCA7 (27%). Honey stores declined during overwintering and represented 15% of the brood area at CCA8, as compared to 16% at CCA3, which a similar finding to that in the control group. The proportion of honey stores at CCA8 was comparable to that of the control group (15% for both 12.5 µg/L and control). A similar picture was found for the 25 µg/L treatment group, in that honey stores underwent an initial build from CCA3 (15%) to CCA4 (21%) before a decline at CCA5 (14%). The subsequent build up reached 29% at CCA7 before a decline through overwintering to CCA8 (14%). At CCA8 the percentage of cells with nectar and pollen were comparable to that of the control at CCA8 (honey: 14% and 15%; 25 µg/L and control groups, respectively).


25 ppb
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
CCA3
CCA4
CCA5
CCA6
CCA7
CCA8
Pollen
Honey


Control
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
CCA3
CCA4
CCA5
CCA6
CCA7
CCA8
Pollen
Honey


12.5 ppb
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
CCA3
CCA4
CCA5
CCA6
CCA7
CCA8
Pollen
Honey

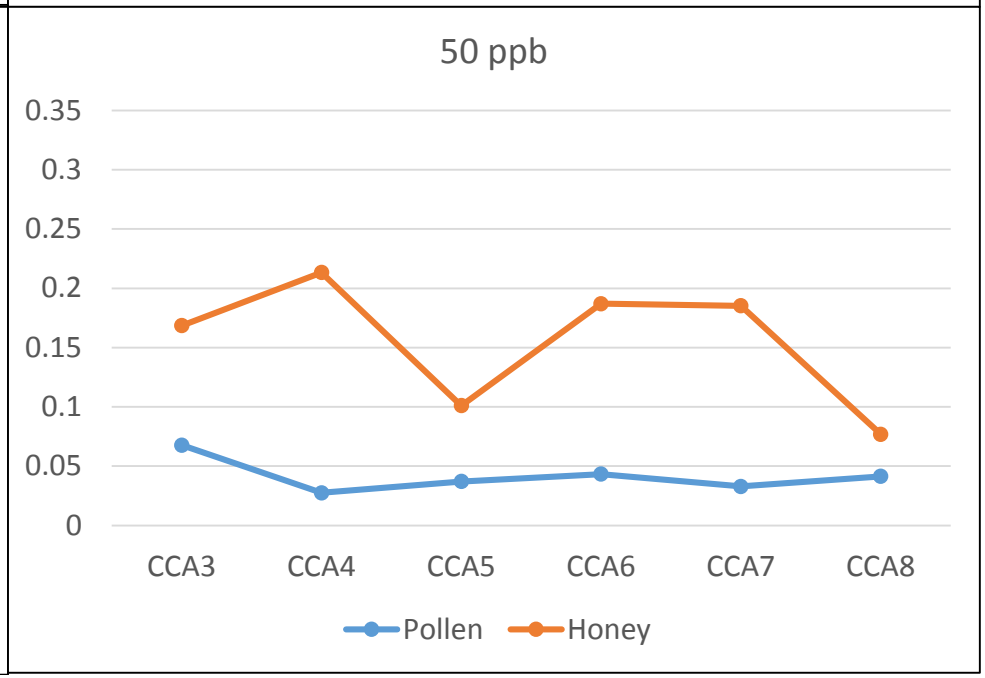

50 ppb
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
CCA3
CCA4
CCA5
CCA6
CCA7
CCA8
Pollen
Honey

Figure G-12. Proportion of abundance of food stores in the control, 12.5, 25, and 50 µg a.i/L treatment groups across the study duration

Hive weight:

There were significant reductions from control observed at the two highest dose levels (100 and 200 µg/L) beginning at CCA4 and persisting until CCA8 ($p<0.05$, data from CCA8 excluded from EPA analysis). Additionally, there were no significant reductions from control in the 12.5 and 25 µg/L treatment groups determined for all CCAs assessed ($p>0.1$). For the 50 µg/L group, there were significant reductions at both the 0.05 and 0.1 alpha level starting as early as CCA4 (CDPR and PMRA analyses). The PMRA analysis determined significant reductions at the 0.1 alpha level for CCA4 and CCA6 and at the 0.05 alpha level for CCAs 5, 7, and 8. The EPA analysis determined significant reductions from the control at the 0.1 alpha level at CCAs 7 and 8 only. Despite these differences in statistical findings, there is an apparent effect on hive weight at the 50 µg/L level that is supported by both analyses indicating significant reductions at multiple CCAs.

While there was no difference ($p>0.1$) in the hive weight at the 200 µg/L treatment groups (data not included in EPA analysis), the lack of statistical difference is questionable as there were only two hives surviving overwintering.

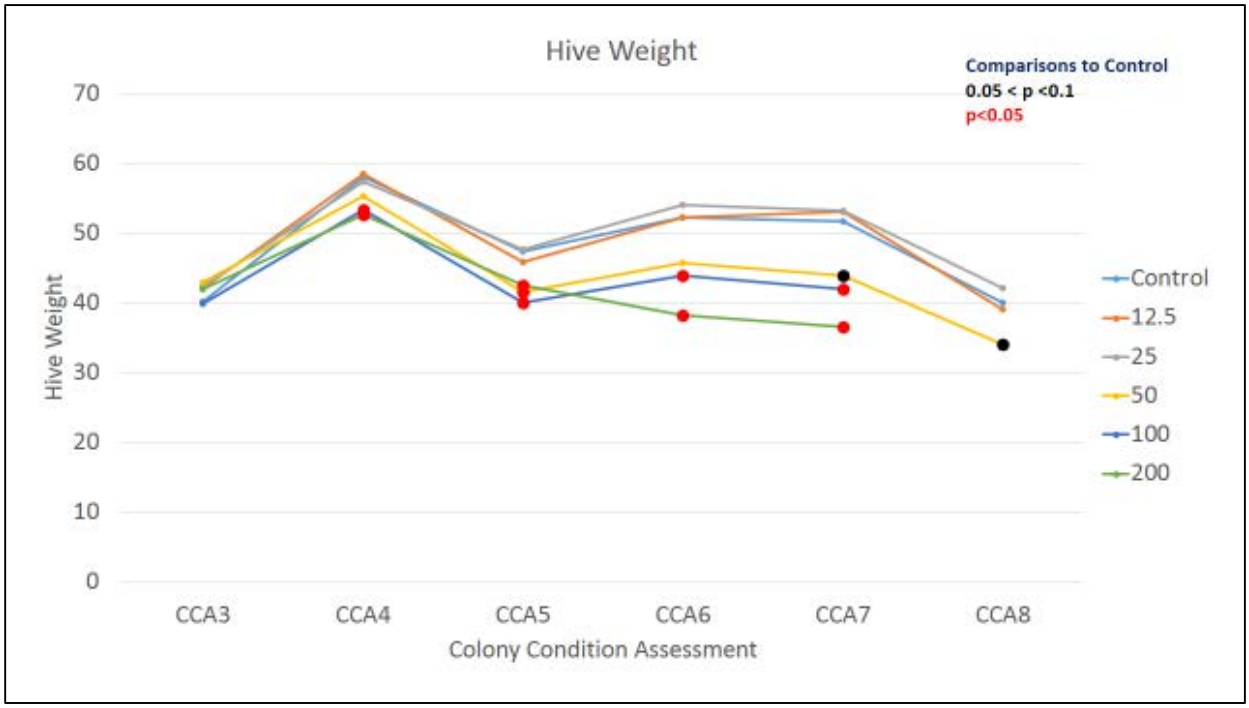


Figure G-13. Hive weight (in pounds) following exposure of honey bee colonies to varying dietary concentrations of imidacloprid across Colony Condition Assessments CCA3 – CCA8

These trends in the control and two lower treatment groups are distinguished from the response at the 50 µg/L group. While honey stores underwent an initial buildup and then decline from CCA 3 (17%) to CCA4 (21%), the subsequent larger buildup leading up to CCA7 that took place in the control and lower treatment groups was much less pronounced with the 50 µg/L group. Specifically, the proportion of honey stores from CCA3 to CCA7 roughly doubled from 15 to 30% of the brood comb in the control, 12.5 and 25 µg/L treatment groups. This is distinguished from the 50 µg/L group were the buildup that occurred from

CCA5 to CCA7 reached a marginally higher level than the starting proportion at CCA3 (18% and 17% respectively). That is to say, that the amount of honey stores at CCA7 (before the overwintering period) in the 50 µg/L group was approximately half of that in the control, 12.5, and 25 µg/L treatment groups (18% for 50 µg/L as compared to 30, 27, and 29% at CCA7 for the control, 12.5 and 25 µg/L treatment groups, respectively). Notably, it is also the only group out of these 4 in which the proportion of honey stores at CCA8 was markedly lower than that of CCA3 (8% at CCA8 and 17% at CCA3).

It is noted that the feeding solutions (sugar solutions) provided during the exposure period might have affected natural honey storage patterns; however, effects on honey storage are still able to be considered as all treatments were compared to control hives (which also received feeding solutions).

Appendix H . Additional Environmental Fate Information

Soil Residues of Imidacloprid

Table H-1 Imidacloprid residues in three types of soil following two spray band application to blueberries

Item	*Parameter*	*Site 1, NY (Loam)*	*Site 2, IL (Silt Loam)*	*Site 3, MI (Sand)*
	Date	Sep 29, 2012	Oct 01, 2012	Oct 12, 2012
	Time from 1st application (days)	**Zero**	**Zero**	**Zero**
Application 1	**EEC ppb** (Just after the 1st application)[1]	244.7	246.9	244.1
	Date	Jun 01, 2013	Jul 03, 2013	
	Time from 1st application (days)	**245 & 361**	**275 & 357**	
	Parent: **Range (ppb)** [2]	32-563	26-380	
	Parent: **Average in ppb** [2]	171	166	
	Parent: **Average in % Of Applied Parent** [3]	70%	67%	
	Olefin: **Range (ppb)** [2]	0-25	2-35	
	Olefin: **Average in ppb** [2]	6	11	
	Olefin: **Average in % Of Applied Parent** [3]	2%	5%	
	5-Hydroxy: Was not detected			
	Stressor: **Range (ppb)** [2]	32-588	29-398	
	Stressor: **Average in ppb** [2]	177	177	
	Stressor: **Average in % Of Applied Parent** [3]	72%	72%	No Sampling
	Date	Sep 30, 2013	Sep 26, 2013	Oct 13, 2013
	Time from 1st application (days)	**366**	**360**	**366**
	Added Amount/2nd Application (ppb) [1]	244.6	246.6	245.0
Application 2	*No sampling to obtain the Concentrations (ppb) just after the second application*			
	Date	May 10, 2014	Jun 02, 2014	Jun 12, 2014
	Time from 1st application (days)	**588**	**611**	**608**
	Parent: **Range (ppb)** [2]	93-558	69-1,246	9-1,025
	Parent: **Average in ppb** [2]	233	339	232
	Parent: **Average in % Of Applied Parent** [4]	48%	69%	48%
	Olefin: **Range (ppb)** [2]	3-33	3-120	0-53
	Olefin: **Average in ppb** [2]	10	26	11
	Olefin: **Average in % Of Applied Parent** [4]	2%	5%	2%
	5-Hydroxy: Only in 8 out of 27 samples with concentrations range of 1-5 ppb (<0.4% of applied parent)			
	Stressor: **Range (ppb)** [2]	96-580	72-1,296	9- 1,082
	Stressor: **Average in ppb** [2]	245	365	244
	Stressor: **Average in % Of Applied Parent** [4]	50%	69%	48%

Observed Concentrations At Sampling 1 *(Each Site: n=9)* **And Sampling 2** *(Each Site: n= 9)*

Observed Concentrations At Sampling 3 *(Each Site: n= 9)*

[1] **EEC** (Just after 1st application and added amount/2nd application were estimated for the top 6" of the soil using the application rate and bulk density of 1.5 g/cm^3;

[2] **Range or Average (ppb)**: Observed Concentration range and average for samples from top 6" of the soil;

[3] **Average in % of applied** for sampling 1 and sampling 2= average in ppb as % of EEC in ppb for the first application;

[4] **Average in % of applied** for sampling 3= average in ppb as % of EEC in ppb for the first application + the second application

Plant Up-take and Metabolism

Plant Up-take

Several studies were evaluated in order to understand root and foliage up-take. These studies were not specifically designed for this purpose but rather for determining the nature of imidacloprid residues in varied raw agricultural commodities (apples, corn, tomatoes, potatoes and eggplants). In this section, plant up-take will be examined for imidacloprid applied to soil (including seed treatment) as well as that applied to foliage.

Soil and Seed Treatment Applications

In four studies, radio-labeled compound (^{14}C-imidacloprid) was soil applied to cotton, potatoes, corn and eggplant:

- In cotton (MRID: 425561-05): imidacloprid was applied as a seed treatment at a rate of 0.46 mg a.i/seed corresponding to 460 g a.i/100 kg of seed (0.46 lbs/100 lbs of seed) assuming that the weight of one seed= 100 mg.

- In potatoes (MRID: 425561-06): It was applied at planting as granules at a rate of 0.05 g a.i/running meter (0.00003 lbs a.i/ft)

- In corn (MRID: 425561-11): It was applied as a seed treatment at a rate of 721g a.i/100 kg seed (0.73 lbs/100 lbs of seed); and

- In eggplants (MRID: 425561-10): It was applied as granules to eggplant plantlets (transplanted at the 8 leaves stage) at a rate of 18.8 mg a.i/plant (2 g/plant of granules containing 0.94% a.i).

Plant up-take (through roots) was measured in various plant parts at crop maturity in cotton and potatoes and at varied growth stages for corn and eggplant. Experiments were executed in the green house using individual plants or more than one plant grown in pots. Reasonable mass balance was achieved in potatoes and eggplant as applied radioactivity was measured in both plants and soil. For example, in the study with eggplants, measured radioactivity in soil plus plant material were: 82% of the applied radioactivity at Day 14, 77% of the applied radioactivity at Day 35 and 80% of the applied radioactivity at Day 69 from transplanting/application. It is important to note that normal agricultural practices were employed including irrigation which contributed to loss of radioactivity via leaching.

Results from these four studies are summarized in **Table H-2**. In this **Table H-2**, observed up-take in percent of applied and resultant concentrations are included. It is noted, that data for foliage were combined from stems and leaves but most of the radioactivity assigned for foliage in **Table H-2** is present in leaves rather than stems.

Table H-2. Root up-take/distribution and resultant concentrations of imidacloprid in cotton, potatoes, corn and eggplant (%= up-take in % of the applied radioactivity and numbers in brackets are resultant concentrations in mg/kg)

	Timing [1]	***211 days***					
	Type [2]	***Foliage***	***Seeds***				
Cotton	***Data***	**4.7% (0.11)**	**0.2% (0.007)**				
	Timing [1]	***129 days***					
	Type [2]	***Foliage***		***Tubers***			
	Plant	**2.2% (5.76)**		**0.3% (0.091)**			
Potatoes	***Soil***	**98.4% (0-20 cm: 0.98-0.47; 20-50 cm: 0.007-0.002)**					
	Timing [1]	***33 days***	***61 days***	***134 days***			
	Type [2]	***Foliage***	***Foliage***	***Foliage***	***Husks***	***Cobs***	***Grain***
Corn	***Plant***	**4.2% (5.84)**	**10.2% (1.52)**	**19.7% (3.08)**	**0.12% (0.21)**	**0.15% (0.12)**	**0.14% (0.04)**
	Timing [1]	***14 days***	***35 days***		***69 days***		
	Type [2]	***Foliage***	***Foliage***	***F/FC/IMMF***	***Foliage***	***F/FC/IMMF***	***Calyx***	***Fruits***
	Plant	**2.7% (5.89)**	**2.7% (3.63)**	**0.03% (0.73)**	**1.6% (1.47)**	**0.04% (0.74)**	**0.01% (0.17)**	**0.03% (0.04)**
Eggplant	***Soil***	**79% (1.67)**	**74% (1.43)**		**78% (1.60)**			

[1] **Timing:** Timing in days from imidacloprid application which coincides with planting time noting that it was transplanting time for eggplant plantlets which were transplanted at the eight leaves stage

[2] **Type:** Type of sample noting that ***Foliage***= Stems and leaves/vines; ***F/FC/IMMF***= Flowers, flower clusters and immature fruits

A shown in **Table H-2**, root up-take from soil applied imidacloprid appears to be very low in cotton, potatoes and eggplant (totals range from 1.7 to 4.8% of the applied) and appears to occur early after application reaching an almost constant amount (<2% of the applied parent) throughout the plant growth up to maturity. Root up-take in corn appears to be an exception as up-take increased steadily with plant growth from 4.2% at Day 14 from planting/application through 10.2% at Day 61 into 20.1% at Day 134 (corn maturity). This may be taken as an indication that plant physiology plays a role in the amount of imidacloprid up-take through the roots. Distribution of radioactivity within the plant appears to be similar in all crops examined. Most of the material appears to arrive at and stays in the plant foliage (mainly the leaves/vines) with relatively low amounts reaching the productive parts of the plant (*i.e.*, seeds, tubers, flowers, grains and fruits). Almost all of the radioactivity that entered corn through the roots was associated with the foliage up to maturity with <0.4% of the applied reached and distributed in other parts of the plants.

Additionally, **Table H-2** shows resultant concentrations observed in leaves/vines which were two to three orders of magnitude higher than the other plant parts. Concentrations in foliage appear to decrease with maturity. Concentrations in immature corn/eggplant foliage decreased from nearly 6 mg/kg to nearly 2 to 3 mg/kg which is probably related to dilution by increased plant growth. Concentrations in cotton

leaves, seed, and lint and gin trash were 0.11, 0.0049, 0.0019 and 0.0050 mg/kg, respectively (data not shown in **Table H-2**) compared to the 460 mg/kg applied to the cotton seed.

Radioactivity left in the soil was measured in only two studies in eggplant at 14, 35 and 69 days showing that radioactivity left in the soil were almost constant (74 to 79% of the applied). The same was observed in the soil planted with potatoes in which 98.4% of the applied radioactivity left in the soil. Loss of radioactivity may be related to leaching, in addition to expected analytical errors. Transformation of imidacloprid was observed in the soil planted in eggplant as observed parent concentrations were between **62** and **82%** with not more than 2% of the metabolites IMI-5-OH and nitrosimine compounds and 6-CNA.

As stated above, the concentration of radio-labeled imidacloprid in cotton seed study was relatively low. Therefore, additional soil drench of imidacloprid was applied to some of the cotton plants (60 X of the seed treatment amount applied in the main experiment). The aim of this additional experiment was to support the results of the metabolism investigation in the main experiment. In response to this higher treatment rate the resultant concentrations in cotton seed, lint and gin trash increased from 0.0049 to 9.35 mg/kg for seeds, from 0.0019 to 0.72 mg/kg for lint and from 0.0050 to 3.5 mg/kg for gin trash, representing levels that were 1,908- , 379- and 700-fold higher, respectfully. This indicates that concentration of imidacloprid in the soil is important in determining root up-take. Presence of higher concentration of imidacloprid in the soil appears to result in higher root up-take.

<u>Applications to Foliage and Fruits</u>

In three studies, ^{14}C-imidacloprid was applied as formulated liquid spray to the foliage of the potato plants and to the fruits of apple and tomato plants planted in the greenhouse:

- In potatoes: It was applied as a spray to potato foliage at a rate equivalent to 134 g/ha (0.12 lbs. a.i/A; MRID: 425561-07). Potato plants were sprayed 83 days after planting and foliage (vines)/tubers were sampled at 7 days after application or 90 days after planting (abbreviated here 7/90 d) and at 28/111 days and 64/147 days (maturity). Although imidacloprid was applied to the plant foliage (canopy), nearly half of applied material reached the underlying soil

- In tomatoes: ^{14}C-imidacloprid was applied as a surface spray to immature fruits and samples were taken from fruits that matured after 4, 7, 14 and 21 days after the application (MRID: 425561-09). Radioactive residues were tracked on fruit surfaces and inside the fruit (*i.e*., the pulp). Summation of recovered radioactivity indicated that 12% of the applied radioactivity was unaccounted for; however, data were normalized to 100% and no explanation was provided for the lack of mass balance (radioactivity loss).

- In apples: ^{14}C-imidacloprid was applied only to surfaces of immature apple fruits while on the trees to investigate movement from the fruit surface into the peel and pulp of the fruits by monitoring radioactive residues in mature fruits harvested at 0 and 14 days after the last application (MRID: 425561-08). Previously, the three applications were made to the immature fruits as follows: the 1st

application at -56 days, 2nd application at -28 days and the 3rd application at 0 day (28-day intervals). The amount applied was approximately equivalent to 375 g/ha (0.33 lbs/A). Approximately 20-21% of the applied radioactivity was unaccounted for; however, data were normalized to 100% and no explanation was given to this loss. Data obtained from the three studies for radioactivity distribution and resultant concentrations are summarized in **Table H-3**.

Table H-3. Imidacloprid up-take/distribution and resultant concentrations in various parts of the potato plants and only in the fruits of apples and tomatoes (%= up-take in % of the applied radioactivity and numbers in brackets are resultant concentrations in mg/kg).

Potatoes	***Timing [1]***	***7/90 days***		***28/111 days***		***64/147 days***	
	Type [2]	***Vines***	***Tubers***	***Vines***	***Tubers***	***Vines***	***Tubers***
	Plant	**40.1% (2.51)**	**0.02% (0.01)**	**48.5% (1.97)**	**0.02% (0.01)**	**49.0% (1.35)**	**0.20% (0.01)**
	Soil	**50.75% (0-15 cm: 0.006-0.004; 15-55 cm: 0.001-<0.001); Samples for 64/147 days only and the depth of sampling, in cm, is indicated**					
Tomatoes	***Timing [1]***	***4 days***		***7 days***		***14-21 days***	
	Type [2]	***Fruit Surfaces***	***Fruit Pulp***	***Fruit surfaces***	***Fruit Pulp***	***Fruit surfaces***	***Fruit Pulp***
	Fruits	**88% (0.89)**	**12% (0.12)**	**77% (0.64)**	**23% (0.19)**	**76-60% (0.65-0.39)**	**24-40% (0.2-0.25)**
Apples	***Timing [1]***	***Zero Day (Just After) the Last of 3 Applications***			***14 Days After the Last of 3 Application***		
	Type [2]	***Fruit Surfaces***	***Fruit Peel***	***Fruit Pulp***	***Fruit Surfaces***	***Fruit Peel***	***Fruit Pulp***
	Fruits	**74.2% (1.31)**	**15.9% (0.28)**	**9.9% (0.17)**	**64.9% (0.94)**	**21.1% (0.31)**	**14.0% (0.2)**

[1] **Timing:** Timing in days from imidacloprid application; **For p**otato 7/90 days mean that (vines)/tubers were sampled 7 days after application on plants at age of 90 days)

[2] **Type: Column:** Type of sample noting that ***Potato Vines***= Stems and leaves

In the potato study, imidacloprid was applied directly into the plant foliage and the total foliage was analyzed, therefore presence of radioactivity may not be used as an indication that the chemical entered into the plant through the foliage up-take. Evidence of up-take is based mainly on its movement from foliage surfaces into other plant parts and the measured plant metabolism; which will be discussed later. In the tomato and apple fruit studies, movement of the chemical from the surface of the fruits into the peel and pulp as well as the measured metabolism in peels and pulp are indications for fruit up-take. In the case of the chemical that is found in potato tubers, radioactivity reaching tubers may not be specifically attributed to foliar up-take followed by translocation into the tubers because root or direct tuber up-take, from the soil cannot be discounted. In both potato experiments (soil/foliage applied) radioactivity was present in the soil due to direct application in case of soil application or due to chemical reaching the soil indirectly upon foliage spray.

Data in **Table H-3** suggest the following:

- Applied radioactivity to potato foliage was, upon application, evenly distributed between foliage (targeted direct application) and soil (not-targeted indirect contamination). An increase in the radioactivity was observed in the foliage as it increased from 40%, 7 days after application to 49%, 28

and 64 days after application. This suggests that root up-take of radioactivity, from the soil, may have occurred. Radioactivity reaching the tubers was relatively low (0.02 to 0.2% of applied); however, radioactive residues were one order of magnitude higher in tubers harvested at 64 days (0.20% of applied) compared to those harvested 7 and 28 days after application (0.02% of applied). This increase in up-take may be attributed to direct tuber up-take from the soil;

- In fruits, most of the applied radioactivity stayed on the fruit surfaces (maximum of 88% on tomatoes 4-days after application and 74% on apples 0-days after the last application). This surface applied radioactivity decreased from the observed maximum of 88% to 60% within 17 days on tomatoes and from 74% to 65% within 14 days on apples. Part of the applied radioactivity entered into the fruit (tomato pulp and apple peel and pulp). This radioactivity increased from 12 to 40% in tomato pulp, from 16 to 21% in apple peel and from 10 to 14% in apple pulp. This suggest the occurrence of fruit peel and pulp chemical up-take from the surface of the fruits where it was applied.

Translocation within the Plant

In the apple study, translocation of radioactivity between leaves and fruits was investigated in two replicates. For each replicate, five apple leaves were chosen at a distance of nearly 10 cm from one apple. The ^{14}C-imidacloprid was applied three times to the five leaves with the nearby apple protected (by plastic cover) from direct spray contamination. Timing of ^{14}C-imidacloprid application coincided with times specified previously in the main apple study. However, the two fruit samples were obtained at 14 days following the last application and radioactivity was distributed as follows:

Replicate one: 55.88% in the leaves and 0.06% in the whole fruit with an unexplained 44.06% loss; and

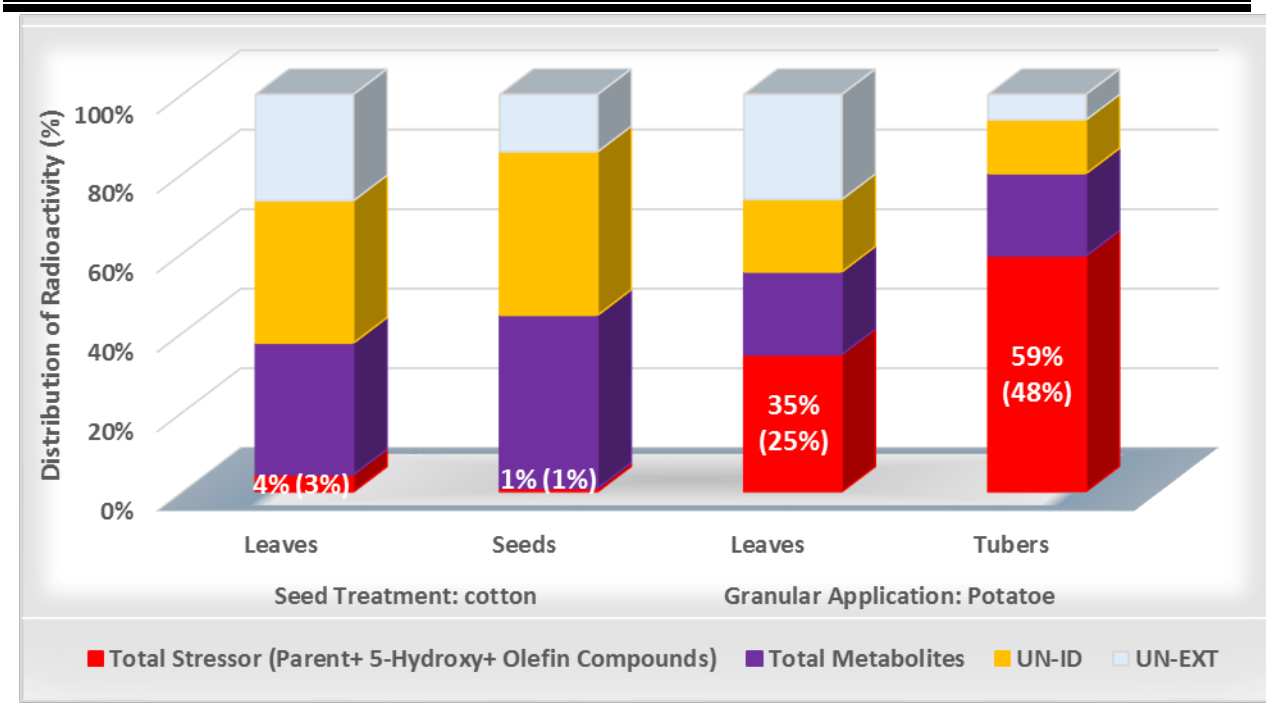
Replicate two: 52.16% in the leaves and 0.07% in the whole fruit with an un-explained 47.77% lost. If the unexplained loss is considered as an important uncertainty, it could be postulated that the low percentage of radioactivity found in the fruits (0.06 to 0.07%) may be taken to suggest insignificant translocation between leaves and fruits.

In the tomato experiment, only tomato plant leaves were sprayed with ^{14}C-imidacloprid. Fruits, which were protected from the spray, on the same plant were harvested after 14 days of the application. Data obtained from this experiment show no significant transport of radioactivity from the treated leaves into the fruits. Radioactivity reaching the fruits ranged from 0.09 to 0.13% of the leaf applied radioactivity with a resultant concentration ranging from 0.003 to 0.006 mg/kg.

Plant Metabolism of Imidacloprid

In the experiments discussed in the preceding section, radioactivity that was applied and/entered into the plant, as parent, was examined to obtain data on imidacloprid plant metabolism during the period from initial exposure (application time) to fruit maturity.

Metabolism for imidacloprid in cotton (seed treatment) and potatoes (granular soil treatment) is summarized in **Figure H-1**. Observed concentrations of the various metabolites are included, as major/minor, at the bottom of **Figure H-1**.

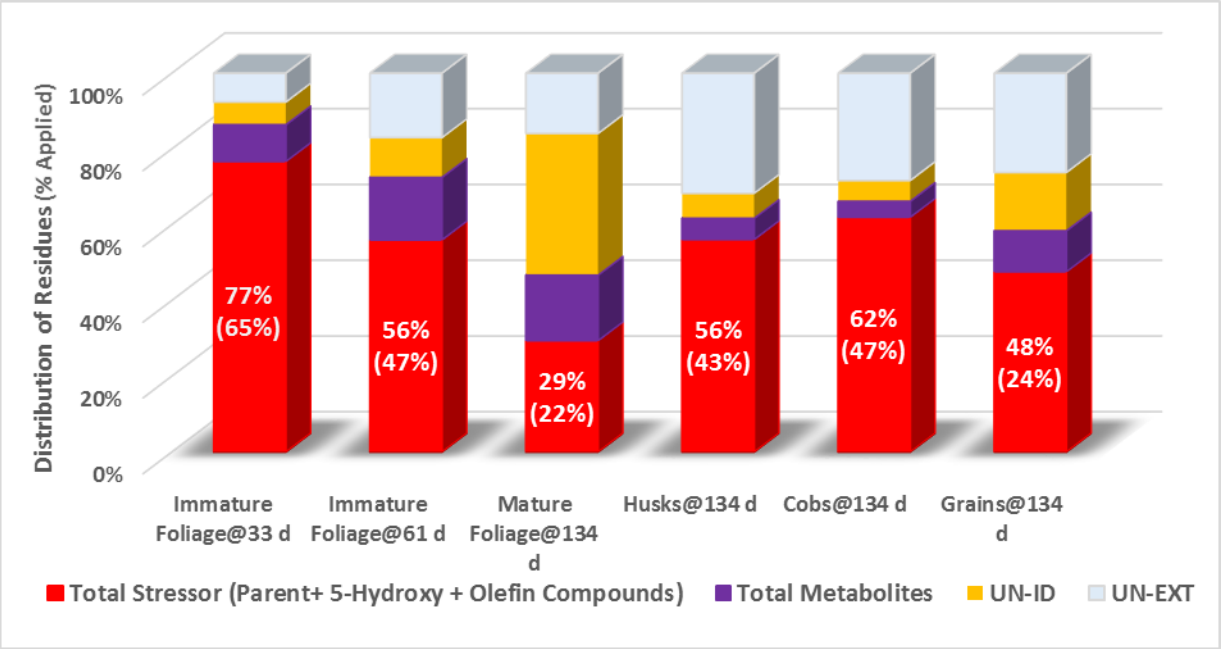


In Cotton: ***Major Metabolites:*** Guanidine, Glucoside in leaves and 6-CNA in seeds;
Minor Metabolites: Nitrosimine, IMI-olefin and 6-CNA and 6-CPA in leaves only
In Potatoes: ***Major Metabolites:*** 5-Hydroxy, Guanidine and 6-CNA;
Minor Metabolites: Nitrosimine, IMI-olefin and 6-CPA
Total Metabolites: All of the observed metabolites excluding IMI-5-OH and IMI-olefin compounds which was added to parent to give the Total Stressor (Value in brackets = Parent% only)

Figure H-1. Imidacloprid metabolism in cotton and potato sampled at maturity (@ 221 days in cotton and 129 days in potatoes). Distribution in % as parent equivalent. **Abbreviations:** 6-CNA (6-chloronicotinic acid); 6-CPA (6-chloropicolyl alcohol); IMI-5-OH (5-hydroxy imidacloprid); UN-ID (unidentified); UN-EXT (un-extracted).

Data in **Figure H-1** suggest that plant metabolism for imidacloprid appears to differ from one species of plant to another. In cotton, parent was transformed into mainly guanidine and glucoside in the leaves and 6-CNA in seeds. In contrast, a comparatively high percentage of parent persisted in both leaves and tubers in potatoes (25 and 48% of parent alone excluding IMI-5-OH and IMI-olefin compounds). Transformation in potatoes produced the major metabolites IMI-5-OH, guanidine and 6-CNA and the minor metabolites nitrosimine, IMI-olefin and 6-CPA.

Metabolism for imidacloprid in corn (seed treatment) is summarized in **Figure H-2**. Observed concentrations of the various metabolites are included, as major/minor, at the bottom of **Figure H-2**.



Days After Planting	*Plant Sample*	*Metabolites*	
		Major	*Minor*
33	**Immature Foliage**	**IMI-5-OH**	**Guanidine, IMI-olefin, 4,5-Hydroxy, Nitrosimine, 6-CNA, 6-CPA and Open Ring Guanidine**
61	**Immature Foliage**	**Guanidine**	**4,5 & IMI-5-OH, IMI-olefin, Nitrosimine, 6-CNA, 6-CPA and Open Ring Guanidine**
	Mature Foliage		
134	**Husks**	**IMI-5-OH**	**Guanidine, IMI-olefin, 4,5-Hydroxy & Nitrosimine, 6-CNA, 6-CPA and Open Ring Guanidine**
	Cobs		
	Grains	**IMI-5-OH and IMI-olefin**	**Guanidine, 6-CPA and 6-CNA**

Total Metabolites: All of the observed metabolites excluding IMI-5-OH and IMI-olefin compounds which was added to parent to give the Total Stressor (Value in brackets = Parent% only)

Figure H-2. Imidacloprid metabolism in corn sampled at different stages of growth (% as parent equivalent). Abbreviations: 6-CNA (6-chloronicotinic acid); 6-CPA (6-chloropicolyl alcohol); IMI-5-OH (5-hydroxy imidacloprid); UN-ID (unidentified); UN-EXT (un-extracted).

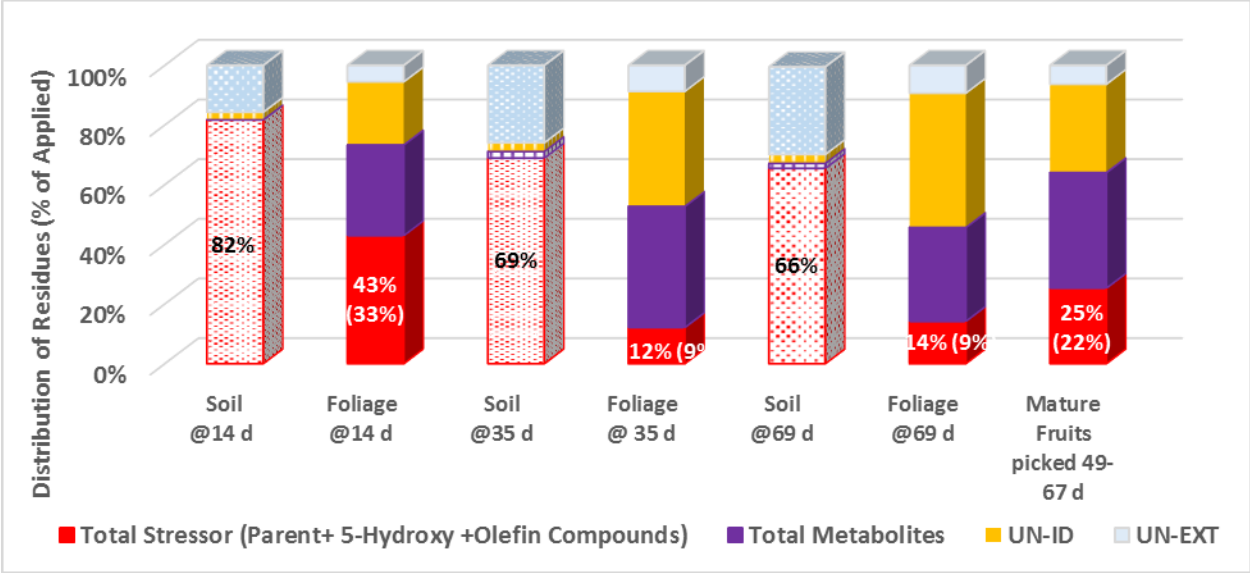
Data in **Figure H-2** indicate the following:

- Imidacloprid entering corn plant from seed treatment appears to be transformed primarily into 5-OH IMI and guanidine with minor amounts of IMI-olefin, 4, IMI-5-OH, nitrosimine, 6-CNA, 6-CPA and open ring guanidine;

- Relatively high percentages (65 to 47%) of imidacloprid parent appear to persist in the whole plant throughout the plant growth stages up to maturity as it decreases to 22%. High concentrations were observed in corn husks and cobs (43 to 47%) with lower concentrations in grains (24%); and

- A higher percentage of unidentified and un-extracted imidacloprid residues appear to form as the corn plant matures, possibly due to increased plant metabolism and association of residues with natural plant compounds forming compounds that are difficult to identify.

Metabolism for soil applied granular imidacloprid in eggplant is summarized in **Figure H-3**. The relative percentage of applied radioactivity for the various metabolites are included, as major/minor, at the bottom of **Figure H-3**.



Days After transplant [2]	*Plant Sample*	*Metabolites*	
		Major	*Minor*
	Soil	**None**	**None**
14	**Immature Foliage**	**Guanidine**	**4,5 & IMI-5-OH, IMI-olefin, Nitrosimine, Urea, Glucoside, 6-CNA & 6-CPA**
	Soil	**None**	**IMI-5-OH & Nitrosimine, 6-CNA**
35	**Immature Foliage**	**Guanidine**	**4,5 & IMI-5-OH, IMI-olefin, Nitrosimine, Urea, Glucoside, 6-CNA**
	Soil	**None**	**IMI-5-OH & Nitrosimine, 6-CNA**
69	**Mature Foliage**	**Guanidine**	**IMI-5-OH, IMI-olefin, Nitrosimine, Glucoside, 6-CNA**
49-67	**Fruits**	**Guanidine, Glucoside, 6-CNA**	**IMI-5-OH**

[1] Soil metabolites were IMI-5-OH and nitrosimine compounds and 6-CNA with maximums of nearly 1% each
[2] Transplant of 8-leaf plantlets
Total Metabolites: All of the observed metabolites excluding IMI-5-OH and IMI-olefin compounds which was added to parent to give the Total Stressor (Value in brackets = Parent% only)

Figure H-3. Imidacloprid metabolism in eggplant sampled at different stages of growth (% as parent equivalent; Column for soil 1 data are with pattern fill). **Abbreviations:** 6-CNA (6-chloronicotinic acid); 6-CPA (6-chloropicolyl alcohol); IMI-5-OH (5-hydroxy imidacloprid); UN-ID (unidentified); UN-EXT (un-extracted).

Data in **Figure H-3** indicate the following:

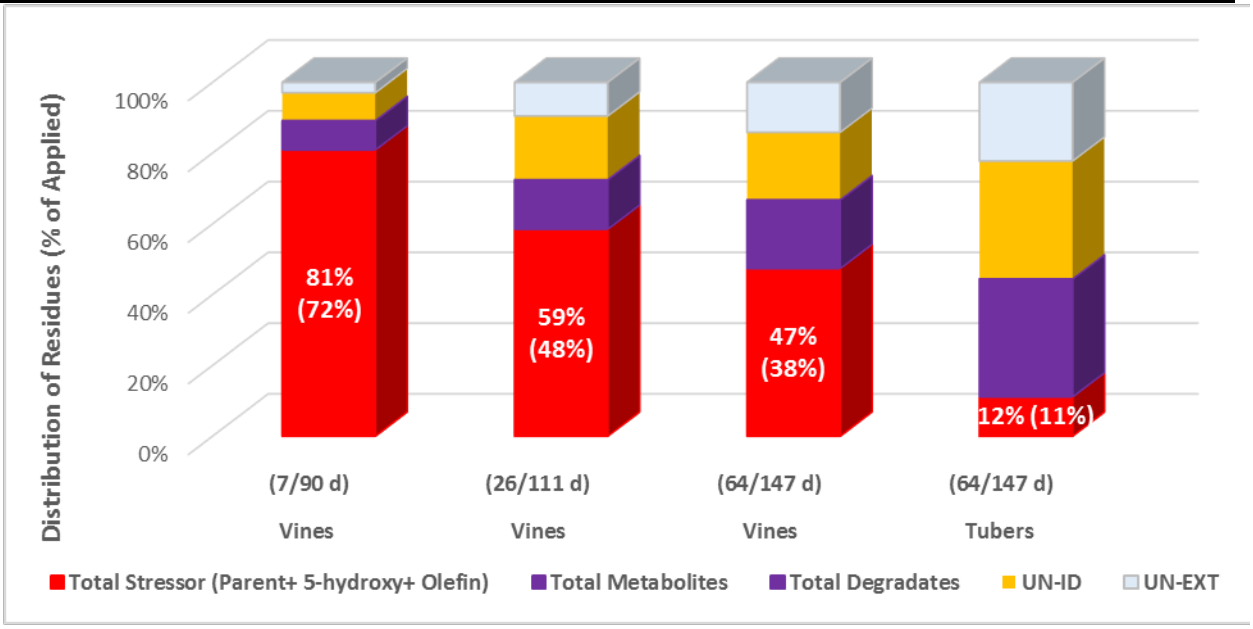
- Imidacloprid entering eggplant, from the soil, appears to be transformed primarily into the major metabolite guanidine with minor amounts of IMI-olefin, IMI-5-OH, nitrosimine, glucoside and 6-CNA. However, glucoside and 6-CNA were the major metabolites in fruits;

- Imidacloprid parent dominated (82 to 66%) residues detected in the soil at all times throughout the growth stages of the plant. Only minor metabolites including 5-OH IMI, nitrosimine and 6-CNA were observed in the soil;

- Imidacloprid parent appears to decrease as the plant matures (33 to 9% of the residues); however, imidacloprid parent represented 22% of the residues in fruits.

- A higher percentage of unidentified residues appear to form as eggplant matures, possibly due to increased plant metabolism and association of residues with natural plant compounds forming compounds that are difficult to identify.

Figure H-4 represent effects of plant metabolism on parent entering potato vines sampled at age of 90, 111 and 147 days and 7, 26 and 64 days after treatment. Parent imidacloprid dominated the percentage of radioactive residues in the vines at Day 90 but declined as the plant matured, suggesting the occurrence of plant metabolism. Residues in young and immature vines consisted primarily of the metabolites guanidine, 5, 4—OH-imidacloprid, IMI-5-OH, and minor amounts nitrosamine, IMI-olefin and glucoside. Residues in the tubers were primarily 6-CNA with a large percentage of unidentified or un-extracted residues possibly due to incorporation into the plant natural constituents.



In vines (all plant growth stages): *Major Metabolites*: Guanidine & IMI-5-OH;

Minor Metabolites: Nitrosimine, IMI-olefin and Glucoside;

In the tubers at maturity: ***Major Metabolites:*** 6-CNA only (33%)

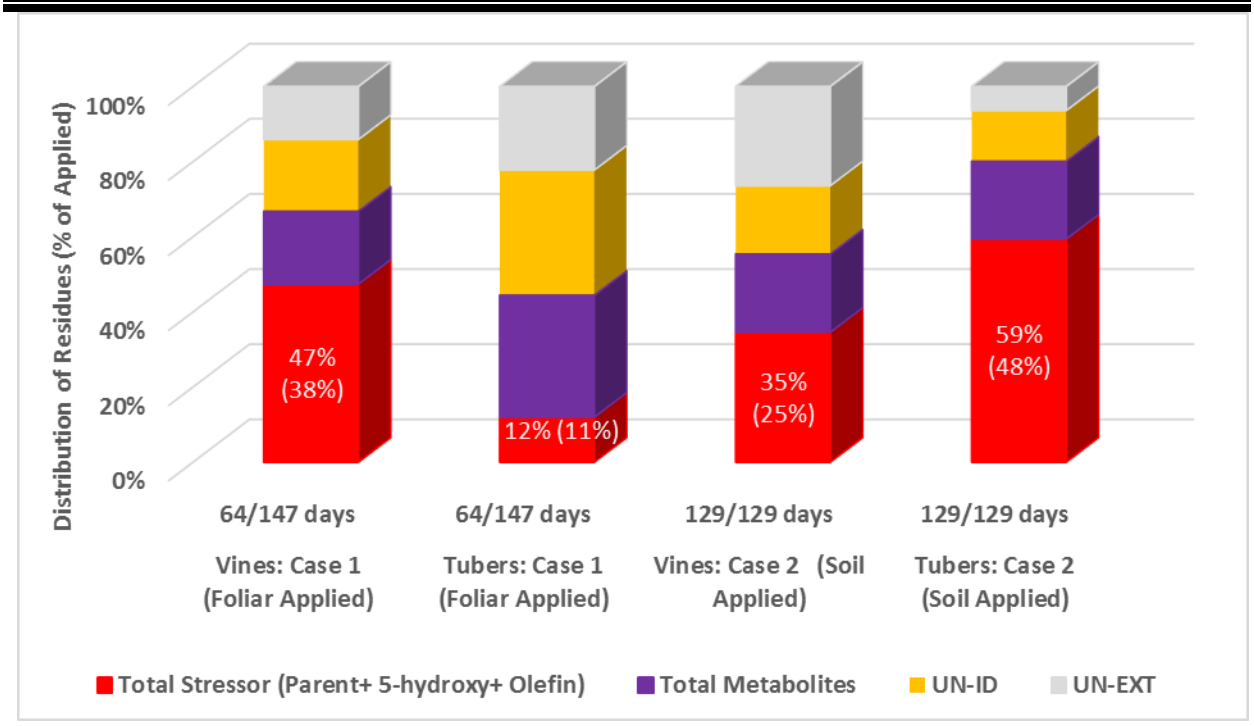
Minor Metabolites: None identified

Note: Distribution of radioactivity entering in immature tubers at 7/90 and 28/111 d are not shown in the graph because it constitutes only 10-11% of that present in mature tubers (shown in the graph, above). In these tubers, radioactive residues were mostly un-identified/un-extractable and the rest were not identified

Total Metabolites: All of the observed metabolites excluding IMI-5-OH and IMI-olefin compounds which was added to parent to give the Total Stressor (Value in brackets = Parent% only)

Figure H-4. Distribution of radioactivity (% as parent equivalent) in immature (Age: 90 and 111 days), mature potato vines/tubers (Age 147 days) after 7, 28, and 64 days from soil application). **Abbreviations:** 6-CNA (6-chloronicotinic acid; IMI-5-OH (5-hydroxy imidacloprid); UN-ID (unidentified); UN-EXT (un-extracted).

Figure H-5 compares data on plant metabolism to imidacloprid for two cases: ***Case 1*** in which the chemical entered potato plant through the leaves from foliar application, and ***Case 2*** in which the chemical entered the leaves from soil through root up-take following a soil application; noting that (a) Root up-take may not be discounted in the ***Case 1*** due to the fact that imidacloprid was present in the soil from indirect contamination during application and (b) Resident time in the plant was 64 days in ***Case 1*** while it was 129 days in ***Case 2***.



Total Metabolites: All of the observed metabolites excluding IMI-5-OH and IMI-olefin compounds which was added to parent to give the Total Stressor (Value in brackets = Parent% only)

Figure H-5. Distribution of radioactivity entering potatoes % as parent equivalent following two separate routes of application, *i.e.*, foliar and soil. **Abbreviations:** UN-ID (unidentified); UN-EXT (un-extracted).

In order to interpret data in **Figure H-5** the following should be noted:

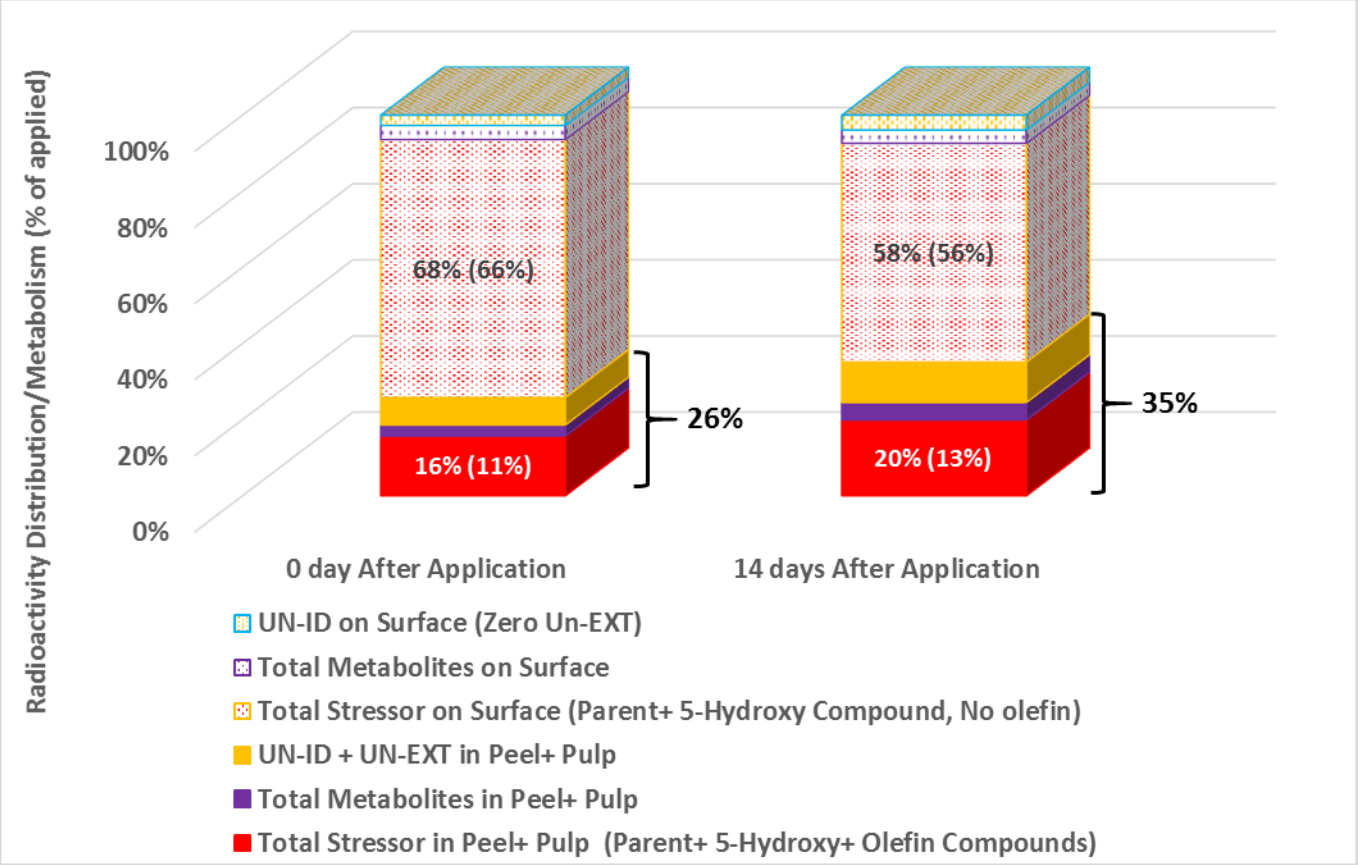
- Vines in ***Case 1*** received direct application of parent into the surfaces of the vine with 38% persisting as parent and 62% metabolizing. Parent metabolism here is an indication that the chemical entered into the leaves, especially if no photolysis occurring on the surfaces of the vine;
- Vines in ***Case 2*** the chemical entered into the plant vines through root up-take with 25% persisting as parent and 75% metabolizing by plant metabolism;

Data suggest that plant transformation of imidacloprid in the leaves following foliar application in ***Case 1*** is less pronounced than in ***Case 2*** in which imidacloprid was applied as a soil treatment. This might be resulting from the longer resident time of imidacloprid in the plant in ***Case 2*** compared to ***Case 1*** (129 days compared to 64 days) giving more time for metabolism to occur.

Data indicate that chemical residues reaching the tubers in ***Case 1*** (0.2% of the applied, refer to **Figure H-5**) contains 11% as parent and those reaching the tubers in ***Case 2*** (0.3% of the applied, refer to **Figure H-5**) contains 48% as parent. Residues in both cases are at least partly translocated from other parts of the plant, therefore, no conclusions can be drawn on possible imidacloprid transformation in the tubers. The high amounts of parent in tubers in Case 2 compared to Case 1 (48% compared to 11%) appear to suggest the tubers are affected by direct up-take of parent from the soil. It is noted however, that imidacloprid parent was available, in the soil, for tuber up-take in both cases.

In the foliar application, imidacloprid is applied directly to foliage, flowers and/or fruits depending on the crop (indeterminate/determinate blooming crop) and timing of application As stated earlier, imidacloprid reaching fruits surfaces appears to enter inside the fruits immediately as shown in apples in which 16% and 10% of the applied a radioactivity entered the peel and pulp, respectively just after application. The same was shown in tomatoes in which 12% of the applied radioactivity entered the pup in 4 days. Data also show increase in radioactivity that enters the fruits by increasing residence time on the fruit surfaces as up to 40% of the radioactivity entered inside the fruits after a resident time of 21 days in tomatoes and 35% in apples after a resident time of 14 days.

Figure H-6 contains the distribution of radioactivity in mature apple fruits harvested just after (0 day) and 14 days after the third application (Radioactive parent was previously applied three times to the surface of these fruits at their immature stage.

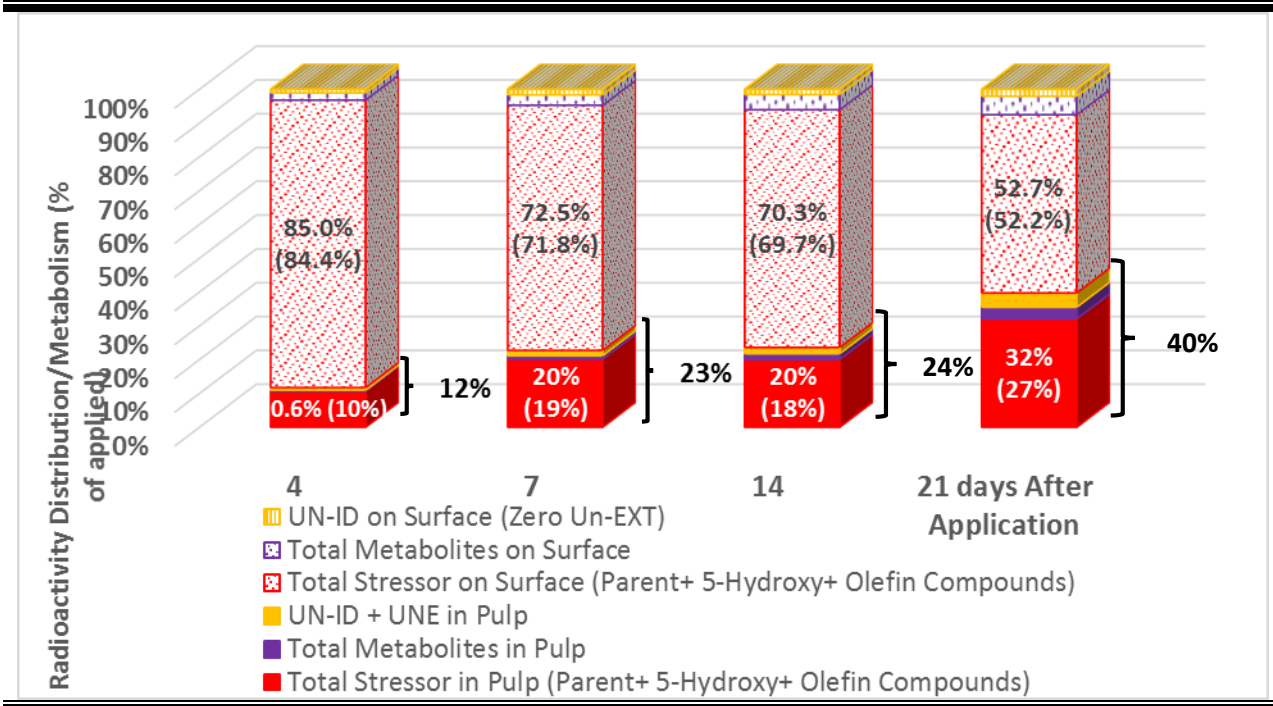


Notes: On surface: ***Major Metabolites:*** None and ***Minor Metabolites:*** Guanidine, 4-5-Hydroxy, Urea and Nitrosimine**;**
Peel+ Pulp: ***Major Metabolites:*** None and ***Minor Metabolites:*** Guanidine, 4-5 & IMI-5-OH, IMI-olefin and Glucoside
Total Metabolites: All of the observed metabolites excluding IMI-5-OH and IMI-olefin compounds which was added to parent to give the Total Stressor (Value in brackets = Parent% only)

Figure H-6. Distribution and metabolism of imidacloprid entering apple fruits following foliar application.

Data in **Figure B-6** show that imidacloprid applied to immature apple fruits moves inside the fruits and that the amount slightly increases with exposure level time (26% moved immediately following application compared to 35% in 14 days). Parent imidacloprid dominates residues that remained on the surface of the fruits with only minor transformation into guanidine, 4-5-hydroxy, urea and nitrosimine compounds. Although photolysis was expected to occur, the lack of moisture may have limited the extent to which this degradation pathway occurred given that plants were irrigated through the soil. Residues found inside the fruits were predominately parent; however, a higher percentage of degradates were detected within the fruit compared to what remained on the fruit surface.

In tomatoes, ^{14}C-imidacloprid was applied to the surfaces of immature fruits to investigate movement/transformation from the fruit surface into the pulp inside the fruits by monitoring radioactive residues in mature fruits harvested at 4, 7, 14 and 21 days after the application. **Figure B-7** contains a summary of the results for the distribution of the total radioactivity between the fruit surface (site of application, hatched part of the graph) and the pulp/inner part of the fruits (solid part of the graph).



On fruit surface: ***Major Metabolites:*** None; ***Minor Metabolites:*** Guanidine, IMI-5-OH, Urea and Nitrosimine**;**
Inside the fruit (pulp): ***Major Metabolites:*** None; ***Minor Metabolites:*** Guanidine, IMI-5-OH, Nitrosimine, IMI-olefin & Glucoside
Total Metabolites: All of the observed metabolites excluding IMI-5-OH and IMI-olefin compounds which was added to parent to give the Total Stressor (Value in brackets = Parent% only)

Figure H-7. Distribution of radioactivity in mature tomato fruits harvested at 4, 7, 14 and 21 days after the application (C^{14}-imidacloprid was previously applied to the surface of these fruits at their immature stage)

Data in **Figure H-7** show that imidacloprid applied to immature tomato fruits moves inside the fruits and the amount that moves increases with the increase in exposure time. In this experiment the chemical was applied to immature fruits at different stages of development, the amount of chemical penetrating into fruit that matured 4 days after application was lower than in fruits that matured 14 and 21 days after application (12% compared to 40%). This suggests the likelihood of an increase in residues in fruits sprayed at younger age compared to those sprayed at older age. Data also indicate that most of the applied radioactivity remained on the surface of the fruits (88 to 60%).

Parent dominates residues remaining on the surface of the fruits with only minor transformation into guanidine, 4-5-hydroxy, urea and nitrosimine compounds. The authors suggested minimal abiotic transformation (assume photolysis). Residues within the fruits were primarily parent; however, a larger percentage of degradates were measured within the fruit than were measured on the fruit surface.