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DATE: February 1, 2022

SUBJECT: RESPONSE TO THE EXTERNAL SCIENTIFIC PEER REVIEW COMMENTS ON DPR's NEONICOTINOID RISK DETERMINATION

In February 2020, in accordance with Health and Safety Code 56004, DPR requested external peer review of its methodologies detailed in the California Neonicotinoid Risk Determination, Addendum to the July 2018 California Neonicotinoid Risk Determination, and Additional Information Related to the Department of Pesticide Regulation's (DPR's) 2018 California Neonicotinoid Risk Determination and Addendum. These documents detail the potential effects that neonicotinoid exposure have on honey bees after feeding on nectar and pollen containing neonicotinoid residues.

DPR requested the scientific peer reviewers determine whether the following conclusions and assumptions were based upon sound scientific knowledge, methods, and practices:

1. The assumption that No Observed Effect Concentration (NOEC) values for bee relevant matrices (e.g., pollen and nectar) can be determined based on the described methods.
2. The assumption that the clothianidin pollen NOEC value can be used as a surrogate for the thiamethoxam and dinotefuran pollen NOEC values.
3. The assumption that plant residue studies generated at the highest annual application rates and minimum reapplication intervals in accordance with registered product labels represent worst-case scenarios.
4. The conclusion that 90th percentile values for residues collected from pollen and nectar in plant residue studies are a realistic representation of the residues found in the field.
5. The assumption that a plant residue bridging strategy from one crop to another within a crop group and from one active ingredient (AI) to another AI within a crop group needed to be developed based on initial reevaluation data requirements. Moreover, the assumption that the plant residue bridging strategy is scientifically valid.

6. The conclusion that certain crop groups are low risk to honey bees based on cultivation practices and bee attractiveness rather than plant residue studies.
7. The conclusion that risk to honey bees from neonicotinoid treated crop groups can be based on the comparison of NOEC values from colony feeding studies and measured residue values in bee relevant matrices (e.g., pollen and nectar) from plant residue studies.
8. The conclusion that residues in pollen and nectar sampled directly from flowers of plants will be used in characterizing risk to honey bees instead of residues in pollen and nectar sampled from alternative sources.

In June 2020, DPR received comments from four external peer review scientists.

1. Christian H, Krupke, Ph.D. - Professor, Department of Entomology, Purdue University
2. Philip N. Smith, Ph.D. - Associate Professor, Department of Environmental Toxicology, Texas Tech University
3. Kimberly Hageman - Associate Professor, Department of Chemistry and Biochemistry, Utah State University
4. Reed Johnson - Associate Professor, Department of Entomology, Ohio State University

We sincerely appreciate the time and effort Drs. Krupke, Smith, Hageman, and Johnson spent in thoroughly reviewing and commenting on the eight conclusions and assumptions. This document summarizes the reviewer's comments and DPR's response. In general, the reviewers are supportive of DPR's methodology and commented that DPR's approach is "based upon sound scientific knowledge, and the best available methods and practices." The reviewers commended DPR for taking a significant step forward with the pollinator risk assessment. DPR believes the review process further validates the scientific basis of DPR's assessment of the risk to pollinators from exposure to nitroguanidine-substituted neonicotinoids in an agricultural setting.

1. ***The assumption that No Observed Effect Concentration (NOEC) values for bee relevant matrices (e.g., pollen and nectar) can be determined based on the described methods.***

   Krupke, Comment 1: The peer reviewer generally agrees with DPR's assumption and states, "that using the approach outlined in the report provide[s] the best data at this time." The reviewer brings up two additional points. First, the commenter raises the issues of abraded seed coat dust and states, "The situation has not been meaningfully addressed, beyond the cited document from [U.S.] EPA stating that it would be."

   DPR Response: Addressing issues of abraded seed coat dust is outside the scope of the peer-review.

   Krupke, Comment 2: The reviewer's second point is "… that this dosing regimen provides bees with a constant level, of xx ng/kg of active ingredient, in food. This sort of exposure never occurs in the field, where levels encountered in the environment move up and down over time and the net effect is one where 'pulses' of insecticide are encountered, rather than a steady and consistent dose."

DPR Response: DPR agrees that in the field, bees may encounter pulses of pesticide exposure as opposed to constant levels. However, colony feeding studies where exposures are pulsed are not widely applicable or practical for risk assessment and mitigation purposes. The duration and magnitude of an exposure pulse is affected by many variables such as weather, climate, hive location, and agronomic practices such as the use of fertilizers, and different irrigation regimens.

According to current guidance on testing and evaluating colony feeding studies, "the study design should make every effort to minimize variability between the exposure levels for each of the hives…." (U.S. EPA, 2016). Varied durations and levels of exposure over the feeding period in a colony feeding study result in uncertainty in determining the concentration and exposure duration that prompted an adverse effect. There is no way to tell if lethal or sublethal effects resulted from a short duration exposure to a high dose, or a longer-term exposure to a lower dose. Subsequently the utility and applicability of pulsed exposure data for regulatory and mitigation purposes is limited.

Overall, the peer reviewer agrees "that using the approach outlined in the report provide[s] the best data at this time," and recognizes that studies on pulsed exposure are "currently difficult or impossible to find."

Smith: The reviewer generally supports the assumption.

DPR Response: No response needed.

Hageman: The reviewer generally agrees that DPR used sound science and the best methods currently available for measuring NOEC values for pollen and nectar.

DPR Response: No response needed.

Johnson: The peer reviewer generally agrees, stating "Colony feeding studies are a new approach to assessing colony-level effects of insecticide exposure to honey bees and present a reasonable compromise between field-realism and practicality." The reviewer continues by noting a few limitations of colony feeding studies, including that colonies were only exposed through either nectar or pollen substitute, there is a limited number of colony feeding studies that evaluated the effects of exposure through pollen, and that colony feeding studies do "not capture effects on foragers collecting contaminated pollen and nectar away from the colony." Finally, the reviewer comments that "bees continued to collect untreated nectar and pollen from the landscape while also consuming the weekly supply of contaminated food," The reviewer recognizes, "...this probably simulates real-world exposure."

DPR Response: While DPR agrees with the limitations inherent to the design of colony feeding studies, the studies used in this assessment were conducted according to current guidance (U.S. EPA, 2016; U.S. EPA, PMRA, DPR, 2014). Study design, limitations, and uncertainties of the Tier 2 studies are discussed in Appendix 8 of the Risk

Determination Document and the current guidance documents for conducting and assessing colony feeding studies (U.S. EPA, 2016; U.S. EPA, PMRA, DPR, 2014).

A strength of colony feeding studies that expose colonies through only one of these matrices, is that they provide increased control, and therefore, utility of the studies. Feeding the colonies both treated pollen and nectar concurrently would confound which matrix contributed to potential colony-level effects, making it difficult to compare an endpoint to pollen and nectar residues measured from crops. By feeding colonies through a single source of exposure, a NOEC endpoint can be set for each of the matrices which can then be directly compared to residue samples from the respective matrix.

While the number of pollen colony feeding studies are limited, the way in which the toxicity endpoints were derived and used in the risk evaluation was conservatively protective of honey bee colonies.

DPR agrees with the peer reviewer's statement that "[d]espite the issues noted above, colony feeding studies represent the best practical method currently available for generating whole-colony NOECs. The high level of replication and clear dose-response relationships between insecticide concentration and colony measures make these studies appropriate for determining a NOECs."

2. ***The assumption that the clothianidin pollen NOEC value can be used as a surrogate for the thiamethoxam and dinotefuran pollen NOEC values.***

Krupke: The reviewer agrees that, "In terms of toxicity alone, this assumption is likely to be valid based upon similarities in published oral toxicity values for the compounds listed." However, they also note that due to the greater water solubility of dinotefuran compared to the other three neonicotinoids, they would expect "dinotefuran to be found in more environmental compartments (i.e., pollen/nectar/drinking water) than either of the other two compounds, and therefore more accessible to pollinators and other non-target organisms."

DPR Response: DPR agrees that the physiochemical properties of dinotefuran may affect its availability in various environmental compartments and acknowledges the importance of differential expression in environmental compartments. However, differential availability in environmental compartments relates to exposure in the field, whereas the subject assumption is regarding bridging of toxicity data. Differential availability in environmental compartments is more likely to be accounted for in the crop residue studies. In the Risk Determination and Addendum, DPR evaluated risk to bees from contaminated pollen and nectar sampled in the crop residue studies. Exposure through feeding on contaminated pollen and nectar represents the two likeliest routes of exposure to pollinators. Other exposure routes, such as drinking water from potential offsite movement of neonicotinoids, is expected to result in less exposure when compared to feeding on pollen and nectar of commodities with direct applications of neonicotinoids.

Smith: The reviewer states, "[t]here is a compelling argument to be made for bridging the pollen NOEC for clothianidin to thiamethoxam; less so for dinotefuran." The reviewer continues by discussing the different physiochemical properties of neonicotinoids that may affect bioavailability and uptake of dinotefuran compared to thiamethoxam and clothianidin.

The reviewer also stated that DPR's assertion that dinotefuran is the least toxic of the four neonicotinoids is not supported by data: "other than the nectar NOEC for dinotefuran which is slightly higher than the other neonicotinoids, there are no data or references provided to support the assertion."

In conclusion, the reviewer states: "the lack of an appropriate dinotefuran pollen NOEC for dinotefuran adds uncertainty to the risk assessment. Given that the dinotefuran nectar NOEC is much lower than the bridged pollen NOEC (and thus it drives most risk determinations/categories), the relative importance of extrapolating to the clothianidin pollen NOEC is questionable."

DPR Response: DPR agrees that the differences in physiochemical properties of dinotefuran, clothianidin, and thiamethoxam may affect the bioavailability and uptake of these compounds in plants, and therefore, included AI specific residue data in the assessment. However, bioavailability and uptake affect the routes of exposure, whereas the subject assumption is regarding bridging of toxicity data.

The nectar NOEC endpoints are not the only data to support dinotefuran as the least toxic of the four neonicotinoids. Tier I laboratory data on page 83 of the Risk Determination provide other endpoints that support the assertion.

DPR acknowledges that there is uncertainty associated with bridging the pollen NOEC from clothianidin to dinotefuran. However, the limited toxicity data available and directly comparable suggests that this bridging strategy results in a reasonable and protective estimate of the dinotefuran pollen NOEC. This methodology will therefore be used to assess risks to pollinators and will be reassessed in future work as more data becomes available.

DPR disagrees with the assertion that the relative importance of extrapolating to the clothianidin pollen NOEC is questionable. Dinotefuran's lower nectar NOEC compared to the bridged pollen NOEC does not necessarily drive the risk conclusions because NOECs are compared to their respective matrices (e.g., pollen and nectar); the NOEC derived from nectar would not be used to compare to the pollen residue samples. Establishing a pollen NOEC for every AI through bridging is especially important for assessment of crops that only produce attractive pollen (e.g., corn).

Hageman: The reviewer points out that imidacloprid and clothianidin NOECs are similar for nectar, but 3.8 times different for pollen. The reviewer also comments that, "after arguing that the imidacloprid pollen NOEC is likely too low, the DPR continues to use it for the imidacloprid risk determination, resulting in questionable conclusions about

imidacloprid risks."

DPR Response: DPR agrees that it is not appropriate to use the imidacloprid pollen NOEC from Dively, 2015, to assess imidacloprid risks. Inconsistencies in effects measured for colony survival rates between years, and lack of effects on bee life stages mean that the uncertainties associated with this study are too great and it cannot be considered sufficiently reliable to use for risk assessment. Due to these uncertainties, DPR reviewers concluded that replication of the experiment was required to verify a consistent effect of imidacloprid dosed pollen on the health and survival of bee colonies. A second imidacloprid pollen colony feeding study is not available at this time. However, the clothianidin pollen colony feeding study was found to be scientifically sound and could be used quantitatively to assess risk to honey bee colonies. As it is the only acceptable colony level effects data available for pollen, DPR plans to bridge the clothianidin pollen NOEC to imidacloprid. The below table identifies the final pollen and nectar NOECs that DPR is relying upon to determine risks to honey bee colonies.

Table 1. Pollen and nectar NOECs used to determine risks to honey bee colonies from imidacloprid, thiamethoxam, clothianidin, and dinotefuran.

Active Ingredient	NOEC (µg/Kg)
Nectar – Colony Feeding Studies	
Imidacloprid [a]	23
Thiamethoxam [b]	30
Clothianidin [c]	19
Dinotefuran [d]	71
Pollen – Colony Feeding Studies	
Imidacloprid [e]	372
Thiamethoxam [e]	372
Clothianidin [f]	372
Dinotefuran [e]	372

All toxicity values derived from the following colony feeding studies:

[a] Bocksch, 2014.

[b] Bocksch, 2015.

[c] Louque, 2016.

[d] Bocksch, 2016.

[e] Bridged from the registrant-submitted colony feeding study with clothianidin.

[f] Bocksch and Werner, 2018.

3. ***The assumption that plant residue studies generated at the highest annual application rates and minimum reapplication intervals in accordance with registered product labels represent worst-case scenarios.***

Krupke: The reviewer generally agrees with the assumption.

DPR Response: No response is needed.

Smith: The reviewer generally agrees that the assumption is valid.

DPR Response: No response is needed.

Hageman: The reviewer generally agrees with the assumption by stating, "plant residue studies used for pesticide risk determination should be conducted with applications of the highest allowed annual application rates and minimum reapplication intervals to obtain worst-case scenario data." The reviewer notes that future work and mitigation efforts could incorporate research on lower application rates and states a concern regarding high variability in measured residues in nectar and pollen.

DPR Response: Following the risk determination, DPR evaluated the available studies conducted at lower rates as well as earlier application timings and incorporated this data into the mitigation.

DPR recognizes the variability of measured concentrations inherent to many of the plant residue studies due to weather, season, bloom duration, application method, location, and crop type may influence uptake and expression of residues. To account for the variability and uncertainty in residue concentrations, DPR chose to use the 90th percentile measured concentration to determine risk. The 90th percentile represents both a protective and field-realistic concentration. Use of other statistics, such as the mean or median, may not appropriately reflect the danger posed by concentrations at the high end of measured distributions. The decision to use the 90th percentile is explained further on page 12 of the Risk Determination.

DPR agrees with the reviewer that a consistent sampling strategy employed in all residue trials would be an improvement to the process.

Johnson: The reviewer agrees with the assumption by stating, "Following the pesticide label guidelines for application is a reasonable worst-case scenario for bee exposure and the only logical starting point determining the risk to bee colonies." However, the reviewer makes 2 specific points.

First, the reviewer states, "there may be specific application scenarios where bee exposure could be greater or longer than measured under the residue studies used here," including year-to-year carryover and residues in non-crop blooming plants. The reviewer adds that although the reviewed studies testing multi-year applications did not indicate

carryover of residues, these studies were predominantly tested on orchard crops and future studies should be performed on other crop types.

Second, the reviewer finds the "current risk determinations are based on in-field crops and may not take into account the totality of bee exposure from an application to a particular crop." The reviewer states that the compounds are able to move in water and thus it is possible that pollen and nectar from non-crop blooming plants in the field or in neighboring areas may contain insecticide residues. However, the reviewer acknowledges that this may be outside the scope of the risk determination.

DPR Response: In response to the first point, DPR agrees that there could be very specific application scenarios that may result in higher or longer exposure. The potential for carryover in non-orchard crops is most adequately assessed using crop specific studies designed to test multiple-year applications and such crop-specific multi-year studies may be considered in future work. However, the peer reviewer did recognize that the multi-year studies that were included in the assessment "found that multi-year application does not result in increasing insecticide concentrations in pollen and nectar on the crops tested despite long half-lives in soils."

With regard to the second point, DPR focused on gathering residue data from production agriculture crops because neonicotinoids are directly applied to the crops at fairly high application rates, and thus is more likely to be detrimental to pollinators. DPR did not evaluate risks to non-crop blooming plants due to label mitigation measures regarding nearby non-crop blooming plants, lack of pollinator exposure (i.e., not bee-attractive, grown indoors, lower uses rates), or lack of widespread or registered use.

Additionally, neonicotinoid pesticides are not registered for use on wildflowers and weeds, so any intentional application would be illegal, and any accidental application, such as through drift, is prohibited by Spray Drift Management label requirements. Furthermore, there are currently no standard methods for incorporating all potential routes of exposure into a risk assessment.

4. ***The conclusion that 90th percentile values for residues collected from pollen and nectar in plant residue studies are a realistic representation of the residues found in the field.***

Krupke: The peer reviewer states, "I agree, and commend the authors for tackling this thorny aspect of risk assessment," but also mentions that "bees never encounter neonicotinoids in isolation," and as such asks, "whether it is possible, or advisable, for the authors to include a higher degree of protection?"

DPR Response: DPR agrees that bees are unlikely to encounter neonicotinoids in isolation. However, evaluating and mitigating risk from exposure to co-occurring pesticides is complex and challenging for risk assessment as it increases the number of factors influencing bee health, increasing uncertainty in the results. The controlled plant residue studies used in DPR's assessment, in which only one AI is applied at a time, increases confidence and regulatory applicability of the results. DPR incorporated a high degree of protection in each step of the risk evaluation and mitigation development

process, including the choice of 90th percentile residues from plant residue studies.

Smith: The reviewer states that "One should not conclude that '90th percentile values for residues collected from pollen and nectar in plant residue studies are a realistic representation of the residues found in the field.' But rather, it may be concluded that that 90th percentile values for residues collected from pollen and nectar in plant residue studies are realistic representations of the 90th percentile residues found in the field."

DPR Response: DPR agrees that the 90th percentile of residues collected from the field is realistic of the 90th percentile of residues found in the field. The purpose of choosing a percentile is not to use the most realistic representation of residues in plants, but rather to pick a residue value that is reasonable and appropriate when comparing back to a colony feeding study NOEC, and for risk assessment purposes. If distributional statistics at the lower to middle portion (i.e., 25 or 50 percentiles) of the measured range in concentrations of treated crops are compared to the NOEC values derived from the colony feeding studies, they could underestimate the potential risk to pollinating honey bee colonies. On the other hand, the maximum concentration value would likely be overly protective because the chance for statistical outliers and because residues are compared back to NOEC values generated from multi-week colony feeding studies. Consequently, the 90th percentile value was determined to be a point in the distribution where the value represented a realistic, yet protective approach to determining risk.

5. ***The assumption that a plant residue bridging strategy from one crop to another within a crop group and from one active ingredient (AI) to another AI within a crop group needed to be developed based on initial reevaluation data requirements. Moreover, the assumption that the plant residue bridging strategy is scientifically valid.***

Krupke: The reviewer generally agrees with the assumption.

DPR Response: No response is needed.

Smith: The reviewer comments, "In short, missing data for crops, crop groups, and AI combinations complicate this specific risk determination effort. When critical data are missing, risk assessors are forced to make assumptions and/or extrapolate from available data. Both attempts to mitigate data gaps introduce uncertainty into risk determinations."

The reviewer continues by stating, "it appears DPR utilized the bridging strategy to substitute residue data that would allow for "low risk to honey bee" determinations and/or generate low risk application rates and timing."

DPR Response: A residue bridging strategy was necessary because the number of active ingredient (AI)/crop/application method combinations is sufficiently large that it is unrealistic to test all of them. DPR required 2-year residue studies on 3-8 representative crops for each AI. U.S. EPA also required residue studies on several crops. DPR evaluated residue studies submitted to both agencies to broaden the scope of data

available. The inclusion of additional data allowed DPR to consider different trials at earlier plant stages and lower rates.

When available, DPR used AI-specific data. If a low-risk application rate and timing could not be determined based on AI-specific data, then data was bridged from other AIs applied to crops within the same crop group. DPR did not bridge data from one AI to another when it contradicted identified risk from AI specific data. This bridging approach was used to determine if different mitigation strategies, such as lower application rates and earlier plant stages, could be used to decrease risks to bees to acceptable levels below the respective NOEC values for each AI.

DPR agrees that there is uncertainty associated with bridging. However, conservatisms, including use of 90th percentiles, were employed to compensate for this uncertainty during the risk mitigation process.

Hageman: The reviewer generally agrees that a residue bridging strategy was necessary, but asks if the residue data itself can be more thoroughly analyzed to understand similarities or differences between crop species, and thus, better justify the bridging strategy.

DPR Response: DPR compiled and evaluated a large number of plant residue studies in the process of conducting this risk determination. DPR analyzed all available data and did not bridge in cases where data revealed substantial differences that would result in a high degree of uncertainty. For example, DPR determined that bridging data from one AI to another AI was not appropriate for the oilseed crop group due to high variability in the data. This is consistent with the findings in U.S. EPA's "Residue Bridging Analysis of Foliar and Soil Agricultural Uses of Neonicotinoids" document (EPA-HQ-OPP-2011-0581-0375). DPR's bridging strategy for data was detailed in the 2020 memorandum titled "Additional Information Related to the Department of Pesticide Regulation's (DPR's) 2018 California Neonicotinoid Risk Determination and Addendum." Additionally, in response to these comments, DPR prepared a memo in 2022 titled, "Update to the Identification of Crop Residue Studies for Development of Proposed Pollinator Protection Regulations in Response to the Neonicotinoid Reevaluation." The memo identifies the specific studies DPR relied upon and indicates when data was bridged.

6. ***The conclusion that certain crop groups are low risk to honey bees based on cultivation practices and bee attractiveness rather than plant residue studies.***

Krupke: The reviewer generally agrees the conclusion is valid.

DPR Response: No response is needed.

Smith: The reviewer generally agrees the conclusion is valid.

DPR Response: No response is needed.

Hageman: The reviewer agrees with the assumption that crops with low attractiveness to bees and crops that are harvested before bloom are classified as low risk unless they are grown for seed, but noted that the approach used is unclear.

The reviewer states that clarification is needed for whether low bee-attractive crops, crops harvested before bloom, and crops grown for seed production were excluded from the risk analysis. The reviewer requests an explanation for why root and tuber vegetables and legumes were determined to be low risk.

DPR Response: DPR determined that honey bee-attractive crops that are harvested before bloom and non-honey bee-attractive crops, as defined in *Attractiveness of Agricultural Crops to Pollinating Bees for the Collection of Nectar and/or Pollen* (USDA, 2017), are low risk due to limited on-field exposure to honey bees. As a result, such crops were not included in the residue risk analysis. Crops that are harvested prior to bloom are never given the opportunity to bloom; therefore, produce no attractive pollen and nectar. Honey bees will not be exposed to neonicotinoid residues in pollen or nectar. Similarly, if a crop is not attractive to honey bees, it is unlikely that honey bees will forage on the treated crop and the potential for exposure to pollen or nectar neonicotinoid residues will be low.

The risk of pollinator exposure in crops grown for seed production were not assessed in the determination. This will be addressed in the final mitigation proposal.

The determination of low risk for applications to root and tuber vegetables and legumes was driven by the results of submitted crop-specific residue data and not by cultivation practices or bee-attractiveness of the crops.

Johnson: The reviewer generally agrees the conclusion is valid.

DPR Response: No response is needed

7. ***The conclusion that risk to honey bees from crop groups is based on the comparison of NOEC values from colony feeding studies and measured residue values in bee relevant matrices (e.g., pollen and nectar) from plant residue studies.***

Krupke: The reviewer asks that DPR refer back to caveats pointed out in 1&4 above.

DPR Response: No response is needed.

Smith: The reviewer generally agrees the conclusion is valid.

DPR Response: No response is needed.

Johnson: The reviewer generally agrees that the assumption is the best available approach, but notes that some realism is sacrificed based on the way forager bees collect

pollen and nectar, and how food is distributed throughout the colony. With regards to the colony feeding study, the reviewer states that providing a colony with a uniform concentration of insecticide may produce different effects than if the colony were fed a range of concentrations that would simulate concentrations in pollen and nectar loads collected by forager bees in the field.

The reviewer notes that the colony feeding studies did not provide both treated nectar and pollen to colonies, which would simulate how colonies are exposed in a real-world scenario.

The reviewer agrees that using the 90th percentile nectar residue to compare to the colony feeding NOEC is likely to be protective; however, they disagree with using the 90th percentile for pollen. They state that the maximum concentration for pollen should be used to compare to the colony feeding NOEC in order to take into account that "cohort[s] of young worker bees may routinely be exposed to the maximum pollen residue concentrations when insecticides are applied to crops."

DPR Response: The colony feeding study was designed to expose the colony in a way that is representative of exposure in the field while controlling for variability in feed concentrations. This leads to less uncertainty in the level of exposure that causes observed effects to the colony. It is important for colony-level effects to be related to a known concentration in diet so that an endpoint (i.e., NOEC, LOEC) can be determined and compared to crop specific residues. If colonies were fed a range of concentrations, there would be more uncertainty in setting an endpoint and the applicability of the data for mitigation development would be limited.

While some realism is lost, concurrently feeding the colonies both treated pollen and nectar would confound which matrix contributed to potential colony-level effects. This makes it difficult to compare an endpoint to pollen and nectar residues measured from crops. By feeding colonies through a single source of exposure, a NOEC endpoint can be set for each matrix which can then be directly compared to residue samples from the respective matrix.

The 6-week duration of the colony feeding tests should account for the "young cohort of bees that are routinely exposed" as the colony goes through 2 brood cycles. Young foragers would be exposed to the treatment concentrations throughout their lifespan. A range of treatment levels are tested and colony level effects should be captured in the higher treatments. DPR determined that the 90th percentile for both pollen and nectar residue data was a balance between both a realistic and protective approach for determining risk.

8. ***The conclusion that residues in pollen and nectar sampled directly from flowers of plants will be used in characterizing risk to honey bees instead of residues in pollen and nectar sampled from alternative sources (e.g., pollen and nectar collected by bees and sampled from within the hive).***

Krupke: The peer reviewer comments that residues collected from bees or from within the hive are likely to be more representative of actual residues encountered for two reasons: 1) just because flowers are open does not mean bees will avail themselves of this resource; and 2) honey bees do not consume raw pollen.

DPR Response: DPR agrees that residues collected from bees or from within the hive are representative of residues encountered by bees. However, comparisons of exposure to toxicity were used to estimate risk, and it is important for these comparisons to be based on matrices that are as similar as possible in terms of collection methods. Since the toxicity values were derived from concentrations in the dosed sucrose solution or pollen patties that had not yet been consumed or manipulated by bees, the most appropriate comparison is to nectar and pollen collected from flowers.

The "Guidance for Assessing Pesticide Risks to Bees" (U.S. EPA, PMRA, DPR, 2014) states that colony feeding studies, "can incorporate multiple treatment levels of residues in spiked food to obtain a dose response and a No Observed Adverse Effect Concentration (NOAEC) at the colony level for the specific route of dietary exposure (e.g., pollen, nectar, or both) employed in the study." If residues collected from in-hive matrices, bee honey stomachs, or pollen from pollen traps in the colony feeding studies are used to determine toxicity reference values (e.g., NOEC/NOAEC), then the appropriate comparison would be to respective residues from the crop residue studies. However, this was not the case. Therefore, the most appropriate comparison is to compare toxicity reference values based on measured residues in spiked nectar solution/pollen patties to exposure reference values collected directly from flowers.

Smith: The reviewer generally agrees the conclusion is valid.

DPR Response: No response is needed.

Johnson: The peer reviewer states that residues collected from bees or from within the hive are a biologically appropriate exposure measurement. Further, the peer reviewer also states that "[i]t can be extremely challenging to collect nectar and pollen directly from the flowers of some plant species in sufficient volumes to enable pesticide residue analysis, which is certainly one motivating factor for using the bees themselves to collect pollen and nectar".

DPR Response: The comment regarding residues collected from bees is similar to comments by Krupke. To this point, please refer to DPR's response to Krupke above.

DPR acknowledges that collecting nectar and pollen directly from flowers can be difficult and has identified legumes (soybean) as a crop group where only bee-collected samples are available. In the only available soybean residue study, efforts were made to collect samples from flowers, but due to flower structure and low amounts of matrices products, bees were utilized to collect samples. Due to the inability to collect residues directly from soybean flowers and that no flower-collected residues are available for the entire crop group, DPR will use the bee honey stomach nectar residues from the only available

soybean studies to assess risks for this crop group.

As sufficient nectar could not be collected by other means, the nectar data collected from bee honey stomachs in a soybean study (TK0250070) was used to assess risks for legumes. Available data shows that residues in nectar and pollen collected directly from flowers is not similar to residues in nectar and pollen collected from bees or from inside the colony. These differences were sufficiently large that a conversion factor was used to convert nectar residues collected from bee honey stomachs, in the soybean study (TK0250070), to the equivalent flower collected nectar residue. DPR calculated the conversion factor from a melon study (VP-39242), which included flower collected nectar and bee collected nectar sampled on the same day. Samples from each of these matrices were paired by sampling date, and then a "flower collected" to "bee collected" ratio was calculated for each sampling period (Table 2). The 90th percentile (both discrete and continuous) of the resulting ratios was calculated and resulted in a conversion factor of 11:1 (flower collected: bee collected). As discussed above, the conversion factor was developed as an alternative method to use bee-collected residue for the determination of risk when collecting nectar and pollen residues directly from flower was not possible, as with legume crops. Thus, the risks for the legume crop group were estimated using this conversion factor to compare bee collected nectar data from the soybean study (TK0250070) to the respective NOEC values.

Table 2. Clothianidin melon study (VP-39242): At each sampling date, one nectar sample from flowers and three nectar samples from bee honey stomachs were collected. The flower nectar sample is used to calculate the ratio with each corresponding bee collected sample.

Trial ID	Date Sampled	Days After Last Application	Flower Collected Residue (ug/kg)	Bee Collected Residue (ug/kg)	Flower: Bee Ratio
A - Paso Robles, California	7/29/2016	38	9.19	0.58	15.75
A - Paso Robles, California	7/29/2016	38	9.19	0.41	22.39
A - Paso Robles, California	7/29/2016	38	9.19	1.48	6.23
B - Jeffersonville, Georgia	8/2/2016	33	10.62	2.79	3.81
B - Jeffersonville, Georgia	8/2/2016	33	10.62	5.95	1.78
B - Jeffersonville, Georgia	8/2/2016	33	10.62	2.43	4.38
A - Paso Robles, California	8/3/2016	43	6.66	2.68	2.48

Trial ID	Date Sampled	Days After Last Application	Flower Collected Residue (ug/kg)	Bee Collected Residue (ug/kg)	Flower: Bee Ratio
A - Paso Robles, California	8/3/2016	43	6.66	2.49	2.67
A - Paso Robles, California	8/3/2016	43	6.66	1.34	4.97
C - Mebane, North Carolina	8/4/2016	34	65.49	3.06	21.43
C - Mebane, North Carolina	8/4/2016	34	65.49	6.03	10.87
C - Mebane, North Carolina	8/4/2016	34	65.49	11.54	5.68
B - Jeffersonville, Georgia	8/8/2016	39	4.94	3.80	1.30
B - Jeffersonville, Georgia	8/8/2016	39	4.94	7.55	0.65
B - Jeffersonville, Georgia	8/8/2016	39	4.94	1.01	4.88
A - Paso Robles, California	8/10/2016	50	4.78	2.06	2.32
A - Paso Robles, California	8/10/2016	50	4.78	1.44	3.32
A - Paso Robles, California	8/10/2016	50	4.78	1.39	3.44
C - Mebane, North Carolina	8/10/2016	40	23.25	7.34	3.17
C - Mebane, North Carolina	8/10/2016	40	23.25	6.82	3.41
C - Mebane, North Carolina	8/10/2016	40	23.25	7.41	3.14
B - Jeffersonville, Georgia	8/15/2016	46	4.39	2.27	1.94
B - Jeffersonville, Georgia	8/15/2016	46	4.39	2.66	1.65
B - Jeffersonville, Georgia	8/15/2016	46	4.39	0.90	4.89
C - Mebane, North Carolina	8/16/2016	46	35.72	5.79	6.17
C - Mebane, North Carolina	8/16/2016	46	35.72	6.10	5.86
C - Mebane, North Carolina	8/16/2016	46	35.72	6.39	5.59

Trial ID	Date Sampled	Days After Last Application	Flower Collected Residue (ug/kg)	Bee Collected Residue (ug/kg)	Flower: Bee Ratio
A - Paso Robles, California	8/17/2016	57	2.84	1.55	1.83
A - Paso Robles, California	8/17/2016	57	2.84	1.22	2.34
A - Paso Robles, California	8/17/2016	57	2.84	0.87	3.28
B - Jeffersonville, Georgia	8/22/2016	53	2.98	7.02	0.42
B - Jeffersonville, Georgia	8/22/2016	53	2.98	2.39	1.25
B - Jeffersonville, Georgia	8/22/2016	53	2.98	0.41	7.28
A - Paso Robles, California	8/24/2016	64	2.41	2.48	0.97
A - Paso Robles, California	8/24/2016	64	2.41	0.69	3.52
A - Paso Robles, California	8/24/2016	64	2.41	0.92	2.63
C - Mebane, North Carolina	8/24/2016	54	8.20	2.31	3.55
C - Mebane, North Carolina	8/24/2016	54	8.20	0.72	11.41
C - Mebane, North Carolina	8/24/2016	54	8.20	2.91	2.82
B - Jeffersonville, Georgia	8/29/2016	60	2.08	0.95	2.18
B - Jeffersonville, Georgia	8/29/2016	60	2.08	0.68	3.05
B - Jeffersonville, Georgia	8/29/2016	60	2.08	1.19	1.74
C - Mebane, North Carolina	8/30/2016	60	11.45	3.40	3.37
C - Mebane, North Carolina	8/30/2016	60	11.45	0.46	25.16
C - Mebane, North Carolina	8/30/2016	60	11.45	2.25	5.09
Continuous 90th Percentile of the Flower to Bee Ratio					11.19
Discrete 90th Percentile of the Flower to Bee Ratio					10.87

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4. Tafarella, B., (2020). "Additional Information Related to the Department of Pesticide Regulation's (DPR's) 2018 California Neonicotinoid Risk Determination and Addendum." Pesticide Registration Branch, California Department of Pesticide Regulation. Memorandum dated February 3, 2020.
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