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A response to a recent review of publications on neonicotinoids and pollinators prepared by Heintzelman, Kelly, Fischer and Maus (dated 23 May 2012), focusing on work of Krupke et al.(1). Comments of Heintzelman et al., appear in *italics*, response from lead author of the study (C. Krupke) below each comment.

"Krupke et al. (2012) report the findings of a study on potential routes of exposures for honeybees to pesticides, especially to neonicotinoids, conducted in a corn growing region in USA in 2010. The study was initiated in response to reports of bee kills at Indiana apiaries in spring of 2010 which coincided with the corn planting period in the area and which were believed to be related to neonicotinoid seed treatment products".

The study was conducted in both 2010 and 2011 – there were reports of bee kills both years. There are currently a number of similar reports in 2012 from Indiana, Minnesota, Ohio, and the provinces of Ontario and Quebec in Canada. The investigations into the causes of these bee kills are ongoing.

All dying bees exhibited the convulsions, uncoordinated movements and tremors that are typical of neurotoxic insecticide exposure. An analysis of the bees using LC-MS/MS revealed that the neonicotinoid clothianidin was present in each case (range of 3.8 – 13.3 ppb); these were the only insecticides found in those samples. Note that thiamethoxam is rapidly metabolized in insects to clothianidin; so it is not possible to identify which of these compounds honey bees were initially exposed to. Apparently healthy bees collected from the same apiary contained no traces of any insecticides. These data suggest that there is a strong likelihood that the neonicotinoids thiamethoxam and/or clothianidin were responsible for the bee kills we investigated.

Corn and soybean seed treatments are the only major use of clothianidin and thiamethoxam in our study area. All reports of bee kills occurred when corn was being planted nearby. Therefore, the remainder of our study focused upon itemizing the potential routes of honeybee exposure to these compounds and quantifying the concentrations of a range of pesticides in each potential exposure route.

"This study does not provide any fundamentally new evidence about honeybee exposure to neonicotinoid seed treatment products. The exposure levels reported in soil, pollen and nectar are generally consistent with previous research and were not high enough to represent a significant risk for honey bees."

While anecdotal reports have persisted for years, there was no documentation prior to our study (including, but not limited to, peer-reviewed publications) of the potential causes of bee deaths in association with the planting of neonicotinoid treated seeds in North America.

Our study took the approach of quantifying pesticide levels in a variety of materials that honey bees may be exposed to near plantings of treated seed. We found seed treatment insecticides at a wide range of concentrations in virtually every sample type we investigated (field soils, dandelions near fields, bee-collected pollen stored in cells and collected from pollen traps, maize pollen, and exhaust talc from planters). The only sample type that showed no trace of neonicotinoids was nectar collected from within the hive.

The concentrations found in soil samples varied (2.1-9.6 ppb), and these results reflect the persistence of these compounds in field soils (2); we found neonicotinoids 2 full growing seasons after the treated seed was planted.

The spurious claim that the concentrations of neonicotinoids we reported in stored pollen (2.9-10.7 ppb clothianidin and 6.2-20.4 ppb thiamethoxam, found co-occurring) were *"not high enough to represent a significant risk for honey bees"* is not supported by data. Sublethal effects of these compounds remain an active area of research and what constitutes a "significant risk" is far from well-defined. Using figures from our study provide a case in point: a bee fed pollen containing 20.4 ppb of thiamethoxam for the 10 d period it spends as a nurse bee would be exposed to an equivalent of ca. 50% of the oral LD50 of 2.8 ng/bee (3). While the effects of such levels of dietary exposure level on individual bees (and colonies) remain unclear, dismissing these results out of hand and without supporting documentation reflects a gross oversimplification of a complex problem.

"Higher concentrations found in waste talc collected from inside pneumatic equipment post-planting represent an intrinsic hazard to honey bees; however actual exposure of bees to this material was not demonstrated and would appear to be preventable."

The concentrations found in waste talc were extremely high (up to 15,030 ppm clothianidin, up to 13,240 ppm thiamethoxam). These concentrations reflect that the treatments applied to the seed are partially removed by and become part of the used talc/seed debris that constitutes planter exhaust material. The material we removed from the planter for our analysis is the same material that ultimately enters the environment. Most of this material is exhausted while planting: either with the seed and into the seed furrow or into the air behind and above the planter via exhaust fans. The remaining material in planters is removed during routine cleaning operations. Thus, this material represents a hazard to honey bees and other non-target insects that encounter it, because all of it (by design) leaves the planter at some point.

While neonicotinoids were found on dead bees, stored pollen, dandelions etc., our methods did not allow us to determine how the materials came to be there. However, given the timing and location of bee kills (near corn fields during corn

planting) and the extremely high toxicity and potential mobility of waste talc, our findings lead us to conclude that this planter exhaust has a role in distributing seed treatment pesticides to areas where foraging bees will encounter them. The findings of Tapparo et al. (4), published shortly after our work, provides direct support for this hypothesis. This research group manipulated honey bee forager flight paths so that they flew behind corn planters sowing neonicotinoid treated seeds. The foragers returning to the hive were found to be coated with insecticides found in the seed treatments, significantly above the published lethal levels of 22-44 ng/bee (5,6).

Clearly, this exposure route may be preventable. However, the current situation is that there are no recommended methods for minimizing or preventing exposure of honey bees to planter exhaust. No recommendations exist for disposal of used talc. In short, growers planting treated seeds using currently recommended practices for planting and cleaning of equipment will result in large volumes of exhaust talc being distributed to the outside environment.

"*In their field experiment, low exposure levels and no adverse effects were observed for bee colonies placed "in harm's way" around the perimeter of a field as it was planted with treated corn seeds. Overall, the publication represents an interesting case study, but it does not provide any significant new insights into exposure of honeybees to neonicotinoid insecticides."*

The first part of this statement is misleading, as it implies that we designed and conducted this field study to document adverse effects of planting upon colonies. This was never an intended part of the study. There was no measure of colony health at any time (before, during or after the study).

Simply put, we used bee colonies placed near planted fields as pollen collectors. We placed pollen traps in each hive and analyzed the bee-collected pollen to determine 1) what proportion of pollen collected by foraging bees was corn pollen, and 2) whether pollen collected by honey bees in these environments was contaminated with pesticides, and whether that level of contamination increased following planting of treated seed and associated planter exhaust.

This portion of our study demonstrated that foraging bees collected large amounts of corn pollen while it was present (experiment-wide mean of ca. 44%), and that neonicotinoids were found in 10 of 20 samples analyzed (ranging from 1.2-7.4 ppb thiamethoxam and from 1.1-88 ppb clothianidin). These levels of neonicotinoids are up to 10-fold higher than those reported by previous studies of bees foraging near treated crops (4).

Our study and others have shown that seed treatment pesticides are found in the pollen of corn and other plants grown from treated seeds (7,8). Corn pollen is abundant across large areas of the continental US during late June and early July each year, so it is important to determine whether bees use this food

resource. The collection of this pollen by foraging honey bees reflects that dietary exposure to pesticides occurs later in summer as well, and not solely during the spring planting period.

These linkages between honey bees and seed treatment insecticides were not known to our fellow researchers and, more importantly, by the agricultural stakeholders who either use seed treatment insecticides or live and work in areas where they are used. The response from growers and beekeepers alike has overwhelmingly demonstrated that they feel this work identifies critical, previously unexplored links between planting of treated seed and honey bees.

The primary contribution of our studies is two-fold: 1) we document that bee-kills were associated with the presence of seed treatment insecticides in each sample tested; and 2) evaluate and provide an initial "ranking" (using concentrations of neonicotinoids as a guideline) for several honey bee exposure routes. These types of data are a key step in determining when and where risks to pollinators occur and can be readily mitigated. For example, the extremely high levels of seed treatment pesticides found in talc exhausted from planters stands out as a potential source of acute exposure and a previously unidentified target for remedial action. The lower concentrations found in many other sample types are certainly worthy of further study as well, and may act as important routes for sublethal exposure.

References

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