Neonicotinoids in Honey
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Background

Neonicotinoids are a novel class of synthetic insecticides that have become one of the most commonly used insecticides in the United States (US). This new class of insecticide is being used to manage a variety of devastating crop pests that have developed resistance to other pesticides [6]. New classes of pesticides that have unique modes of action are very useful because cross-resistance is not a concern. Cross-resistance is when a new insecticide, for example, has a similar mode of action to an older insecticide, allowing insects, that have developed resistance to the old insecticide, to have resistance to the new insecticide. Neonicotinoids have a novel mode of action compared to all other pesticides except for nicotine, which was only briefly used due to human health concerns.

Most pesticides have been traditionally sprayed on plants or over whole plots of land, and neonicotinoids can be applied that same way, but they are more commonly applied as a seed treatment in the agricultural sector. Seed treatments are when the pesticides are applied directly to the seeds to make it easier for farmers to apply the correct amount of pesticides to their seeds and as a preemptive attempt to prevent pest damage. However, when tracking the amounts of pesticides used, seed treatments are hard track, causing underestimates of the usage of neonicotinoids in crops and the quantity of neonicotinoids, potentially, in the environment [4]. There are many surveys done to try to estimate the quantities of pesticides used in agriculture and potentially going into the environment. The National Agricultural Statistics Service does not include seed treatments when they are surveying the use of insecticides [4]. This is a major flaw in their surveys because they may be seeing decreases in pesticide use because of increases in using pesticides in seed treatments instead, so the net use of pesticides may not be changing. Luckily, the United States Geographical Survey include seed treatments in their insecticide usage data until 2014 [4, 3]. This data set previously provided a more complete understanding of how many chemicals are being used than the previous survey. However, since it's discontinuation in 2014, the USDA has taken over collecting this same data but has not released any of it publicly [3]. This gap in knowledge prevents any legislative action from being taken, as they are not receiving any pressure from the public because this data has not been publicized. In the US, the use of neonicotinoid seed treatments has increased and, in response to that, so has the toxicity load of pollinators [3]. What this means is pollinators are exposed to and accumulate an increased amount of neonicotinoids from seed treatments than they previously have from conventional application. A different study, that did a survey of neonicotinoids in honey around the world, found that the most commonly used neonicotinoid in the world was imidacloprid and found that, in the US, the top three neonicotinoids detected were imidacloprid, thiamethoxam, and clothianidin [10]. This study, unlike the previous surveys, did not look at the world-wide pesticides used directly, but the contamination of honey from the use of these chemicals, which will be covered in more detail later.

Neonicotinoids are highly toxic to insects and not toxic to mammals because of their target site and mode of action. The target site of neonicotinoids are nicotinic acetylcholine receptors and their mode of action is nicotinic acetylcholine receptor agonists [6]. Neonicotinoids bind to the nicotinic acetylcholine receptor, the target site, which causes that receptor to keep sending signals to the nerve to fire, the mode of action, eventually leading to paralysis and death. Insect acetylcholine receptors are located only in their central nervous system and those are the receptors neonicotinoids target [6]. All insects have these receptors, making neonicotinoids a broad-spectrum insecticide, meaning they have potential to indiscriminately harm any and all susceptible insects that come into contact with them. As eluded to before, they are also systemic, spreading throughout the plants. Neonicotinoids are able to come into contact with a variety of insects because they are systemic. Systemic pesticides translocate throughout the plant using the xylem and/or phloem to go from the roots to all of the other parts of the plant. This allows the applied neonicotinoid to be present throughout the plant, affecting any pests that may come to feed on it, regardless of their feeding method. Neonicotinoids have become one of the most commonly used insecticides because they are a novel class of insecticide, are very effective on insect pests, systemic, and not harmful to mammals.

Movement

As the use of neonicotinoids increase, so does the research looking into the intended and unintended movement of these insecticides away from the plants they were applied to. Neonicotinoids can move throughout the plant and beyond in a variety of ways. The obvious intended movement is throughout the plant from the neonicotinoid applied to the seed coat or plant. Seed coatings are used on a variety of agricultural crops in the US, including corn and soybeans [5]. Seed coatings are an effective way to apply neonicotinoids because neonicotinoids are systemic, meaning that the insecticide is applied to the seed and is taken up into the plant even as it begins to grow. In the US, corn, soybeans, wheat, and cotton all have seed treatments applied to them that contain neonicotinoids [1]. These crops are all grown heavily throughout the Midwest and Central US, encompassing a huge area, and the organisms living within it, are all at risk of unintended exposure. However, only about 5% of neonicotinoid in the seed coating is absorbed by the plant that grows from that seed, while the other 95% of the insecticide goes somewhere else [5]. In the future, decreasing the percentage loss of neonicotinoid on seed treatments would greatly benefit the environment, but chemical companies that produce these seed treatments will not change their formulations unless they are required to by law, before spending millions of dollars trying to solve this problem or creating an entirely new insecticide. The movement of neonicotinoids from seed treatments into the environment has become a heavily studies topic. Before getting into off-site risks of neonicotinoids, all systemic pesticides carry risks when translocating throughout the plant that are often accepted as a worthwhile to fend off pests.

Although movement throughout the plant is what neonicotinoids are designed to do and sounds great to the consumer, this ability allows the pesticide into the flowers of the plant as well. Neonicotinoids have been found in all parts of the plant, roots, stems, leaves, pollen, and nectar, putting pollinators and other beneficial insects at risk [5]. These risks will be covered in significantly more detail in the next section, but it is worth noting how these movements affect non-target organisms. The understanding that agricultural workers and the general public have about the systemic abilities of neonicotinoids is that they will provide protection from pests all season long. Neonicotinoids have been advertised showing that they protect the plants and peace of mind for farmers, however a recent study showed this was not true. This study showed that the amount of neonicotinoid in the seed treatment does translocate into the plant, but after about a month, this chemical is nearly undetectable in most roots, shoots, and seeds [1]. Without this chemical dispersed throughout the plant, and potentially not in the plant anymore, does not protect that plant from damage from pest. Neonicotinoids are a systemic pesticide that are advertised to provide season long protection from pests, but may cause more harm than good when applied as seed treatments.

Neonicotinoids are water soluble, which is what allows them to translocate within the plant, but this also means they can easily move away from the application site with water. Neonicotinoids have been found in a variety of water sources, including rivers, groundwater, and wetlands [5]. All organisms on this planet need water to survive, including wild plants, animals, and people. Finding neonicotinoids in many of those water sources shows that no matter how much effort is put into protecting non-target organisms, like people and wildlife, from direct neonicotinoid exposure, anything can be exposed to these chemicals when they drink. Neonicotinoids move into water either by leaching, movement of the soluble pesticide from irrigation or rain into the soil, or runoff, movement of the soluble pesticide by rain or irrigation into a water source [1, 5]. This movement can happen from seed treatments or any other application method of neonicotinoids. Water acts like a highway for soluble pesticides, spreading a pesticide, potentially, very far from the application site and potentially contaminating water in sensitive places. One study showed that neonicotinoids can bind to particles in the soil, which would reduce their ability to leach further into the soil and potentially prevent it from contaminating groundwater sources [2]. Different soil types and mixes will influence how well neonicotinoids can leach through the soil or bind to soil particles to prevent the contamination of groundwater. However, this does not address the contamination of water sources due to runoff, since runoff happens at surface level. Non-target organisms are at risk for unintended exposure from the water sources they drink from or live in, but there is another way these organisms can also be exposed.

Neonicotinoids can drift off-site through two main mechanisms, dust from a planter or spray from direct application. Contaminated dust is another, direct, way of unintended exposure to non-target organisms [2]. Clouds of dust form behind seed drills, machines commonly used in agricultural settings to plant seeds on a large scale. This dust is a mixture of the soil released from the ground when the seed drill drills the seeds into the ground, the talc or graphite in the seed hoppers used to lubricate the seeds, and any pesticides that did not stick to the coating during planting. Spraying neonicotinoids can also cause them to drift off-site if proper protocols are not followed. This can be caused by over spraying, carelessly applying, and wind dispersal [2]. All of these involve the particles of the pesticide to be small enough, and light enough, to be lifted into the air and transported elsewhere by the wind. This can also lead to contamination of water sources and can carry pesticides far from their application site, causing even more non-target organisms to be exposed to these chemicals. Careful application and taking all of the necessary precautions can reduce the movement of neonicotinoids in the environment, but researchers need to show, and have shown, that this is a problem and provide an idea for a potential solution.

Bee Exposure and Products

Neonicotinoids, as previously stated, are found in the pollen and nectar of plants because they are systemic and travel throughout the plant. Bees collect pollen and nectar from the flowers to feed the entire hive. The oral LD50, the lethal dose that kills 50% of the tested population, of neonicotinoids for bees is 1 to 5 ng/bee [5]. This means that bees only have to ingest 1-5 ng of neonicotinoid to die. 1-5 ng of neonicotinoid is a very small amount, but since bees gather pollen and nectar from a variety of flowers and then bring them back to the hive, the neonicotinoids in the nectar and pollen will collect in the hive. Honey bees have become the model organism for all bees, as they are easier to keep and experiment on than native bees because of their behavior and colony structure.

Although the lethal effects are important to understand, the sublethal affects can also be detrimental to bees and their colonies. Neonicotinoid concentration can be as low as 0.1 ng/g to have sublethal effects on the whole hive [10]. Sublethal effects are the negative effects that happen because of exposure to a small amount of pesticide, but that do not cause death directly. Sublethal effects for honey bees "include growth disorders, reduced efficiency of the immune system, neurological and cognitive disorders, respiratory and reproductive function, queen survival, foraging efficiency, and homing capacity" [10]. Sublethal effects do not kill the bees, but can cause them to grow incorrectly, be more susceptible to diseases, miscommunicate with other bees, decrease foraging abilities, and not get back to the hive, which can all result in other causes of death and can be detrimental to the survival of the hive as a whole.

Bees can also be exposed to neonicotinoids from sources other than their food, from agricultural dust and guttation. Bees forage by flying and will fly into and across agricultural fields which can expose them to agricultural dust produced by seed drills. Multiple studies have shown that bees can come into contact with dust from these planters, which contain higher concentrations of neonicotinoids than are found in pollen or nectar directly from the plants [7, 8]. Bees groom themselves regularly, cleaning all of the dust off of them with their legs and then cleaning their legs off with their tongue, ingesting any chemicals mix in with that dust. Bees also need water, juts like any other organism, and although it is not clear where bees collect their water from, they could be collecting guttation from plants [9]. Guttation is the formation of water droplets at the tips of the leaves due to the water pressure coming up from the roots. These water droplets could be contaminated from the systemic nature of neonicotinoids or from neonicotinoid-contaminated dust [9]. Bees may collect these water droplets to drink, prepare food, or cool down their nests, further risking contaminating their nests with neonicotinoids [2].

Neonicotinoids have been found in all bee products, including pollen, honey that people would consume, and resin/propolis. The world-wide honey survey mentioned before found 75% of all honey samples they collected from around the world are contaminated from neonicotinoid [10]. These honey samples are the same samples that bees and people would be eating, ingesting the neonicotinoids unknowingly. Studies have also shown that neonicotinoids are found in pollen samples in the field and in the pollen the bees collect [2, 5, 8, 9]. Pollen is the main protein source for bees and their larvae, so pollen is regularly ingested all year round, which can lead to continuous ingestion of neonicotinoids. Lastly, neonicotinoids were also found in resin, which bees use to seal holes or otherwise fix up the hive [2]. This integrates neonicotinoids into the hive, so regardless of what nectar and pollen they collect, bees can still be exposed to neonicotinoids in their hives. This continuous exposure increases the concentration of neonicotinoids within the bee and the colony, increasing the chances they will suffer from sublethal effects.

Neonicotinoids are a new class of broad-spectrum insecticide that has replaced many older insecticides and is an easy tool to keep in everyone's tool box. However, taking precautions to limit the exposure of neonicotinoids to the environment and non-target organisms is vital for the continued use of this chemical. Studies have shown how neonicotinoids can contaminate the environment and get into waterways and non-target plants. Neonicotinoid exposure can harm or kill all susceptible insects that encounter these chemicals because they are a broad-spectrum insecticide. The insects at risk include pollinators like honey bees, which have been used as a model organism for other pollinators. Neonicotinoids are found in many of the products made and consumed by honey bees and can cause lethal or sublethal effects to the whole hives of bees. More studies need to be done to continue looking into these effects and also to look into potential risks to other organisms as well.

Due to almost continuous personal situations and then the COVID-19 pandemic starting, we were unable to process honey samples and determine the neonicotinoid content within them. Our original plan was to look to see if there was a difference in the neonicotinoid content between store bought and farmers market honey in Indiana. By content, we would be looking into the total quantity and individual quantities of imidacloprid, clothianidin, and thiamethoxam, specifically. We chose specific neonicotinoid chemicals to limit the chemicals we are looking for and those three chemicals are the ones most often detected in honey samples in the US [10]. We thought it would be interesting to see if there were any differences between store bought and farmers market honey. We did hit some road bumps in finding where store-bought honey was coming from specifically. The labels most often listed a variety of different countries their honey was sourced from, so we looked specifically for honey only sourced from the United States. If the labels only listed the source of the honey as from the US, as was seen on some of the Walmart brands we collected, I email the company to see if they could tell me where in the US the honey was sourced from. However, I never got a response and it has been 4 months since I sent that email. The honey we got from Payless and the farmers markets had specific information about where that honey was sourced from. We thought there may be a difference between the honey bought at farmers markets verses that bought at stores because stores need to mass collect honey all year round, while farmers markets are typically only open in the summer to fall and do not feel the need to take as much honey as they can from their hives. We planned on running an analysis on these samples to show how much of each neonicotinoid had contaminated all of our samples so we could compare the neonicotinoid load in store-bought and farmers market honey.

We planned to follow the protocol used by Mitchell et al. in their study looking at the neonicotinoids in honey collected from around the world [10]. The first thing they did was prepared the honey samples using a QuEChERS protocol they adapted from another paper, measuring out 2.5g of honey into a 15mL polypropylene tube and then added 9mL of H_2O:CAN and 20µl of internal standard was added containing all 5 labelled neonicotinoids [10]. Then, they dissolved the honey using manual agitation and ultrasonication for 5 minutes and transferred it to a 15mL vial with extraction salts [10]. Next, they shook the mixtures by hand and then centrifuged the tubes at 4,000g for 10 minutes [10]. They collected the top 4.5-4.8mL of that solution into another tube with purification salts, shook it for a minute, and centrifuged again [10]. They then took the supernatant, the liquid at the top of the tube after centrifuging and put it into a glass tube to evaporate in a CentriVap [10]. Then they re-suspended the residue in 0.5mL of MeOH 25%, vortexed, ultrasonicated for a minute, and finally filtered through a 20mm syringe filter into a new vial with 250µL conical inserts [10]. From here, they ran a UHPLC-MS/MS analysis to determine which and how much neonicotinoid products had contaminated their honey products. If we had been able to run this analysis, we hoped to see a significant difference between farmers market honey and store-bought honey. However, we were also skeptical that we would see any difference because of the different ways neonicotinoids can move in the environment and the different ways bees can be exposed to them.

References

1. Alford, A., & Krupke, C. H. (2017). Translocation of the neonicotinoid seed treatment clothianidin in maize. *PLOS ONE*, *12*(3), e0173836. https://doi.org/10.1371/journal.pone.0173836
2. Bonmatin, J.-M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D. P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E. A. D., Noome, D. A., Simon-Delso, N., & Tapparo, A. (2014). Environmental fate and exposure; neonicotinoids and fipronil. *Environmental Science and Pollution Research*, *22*(1), 35–67. https://doi.org/10.1007/s11356-014-3332-7
3. Douglas, M. R., Sponsler, D. B., Lonsdorf, E. V., & Grozinger, C. M. (2020). County-level analysis reveals a rapidly shifting landscape of insecticide hazard to honey bees (Apis mellifera) on US farmland. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-019-57225-w
4. Douglas, M. R., & Tooker, J. F. (2015). Large-Scale Deployment of Seed Treatments Has Driven Rapid Increase in Use of Neonicotinoid Insecticides and Preemptive Pest Management in U.S. Field Crops. *Environmental Science & Technology*, *49*(8), 5088–5097. https://doi.org/10.1021/es506141g
5. Hladik, M. L., Main, A. R., & Goulson, D. (2018). Environmental Risks and Challenges Associated with Neonicotinoid Insecticides. *Environmental Science & Technology*, *52*(6), 3329–3335. https://doi.org/10.1021/acs.est.7b06388
6. Jeschke, P., & Nauen, R. (2008). Neonicotinoids-from zero to hero in insecticide chemistry. *Pest Management Science*, *64*(11), 1084–1098. https://doi.org/10.1002/ps.1631

7. Krupke, C. H., Holland, J. D., Long, E. Y., & Eitzer, B. D. (2017). Planting of neonicotinoid-treated maize poses risks for honey bees and other non-target organisms over a wide area without consistent crop yield benefit. *Journal of Applied Ecology*, *54*(5), 1449–1458. https://doi.org/10.1111/1365-2664.12924
8. Krupke, C. H., Hunt, G. J., Eitzer, B. D., Andino, G., & Given, K. (2012). Multiple Routes of Pesticide Exposure for Honey Bees Living Near Agricultural Fields. *PLoS ONE*, *7*(1), e29268. https://doi.org/10.1371/journal.pone.0029268
9. Krupke, C. H., & Long, E. Y. (2015). Intersections between neonicotinoid seed treatments and honey bees. *Current Opinion in Insect Science*, *10*, 8–13. https://doi.org/10.1016/j.cois.2015.04.005
10. Mitchell, E. A. D., Mulhauser, B., Mulot, M., Mutabazi, A., Glauser, G., & Aebi, A. (2017). A worldwide survey of neonicotinoids in honey. *Science*, *358*(6359), 109–111. https://doi.org/10.1126/science.aan3684