CENTER FOR FOOD SAFETY

May 4, 2020

OPP Docket
Environmental Protection Agency Docket Center (28221T)
1200 Pennsylvania Ave. NW.
Washington, DC 20460-0001

RE: Center for Food Safety's comments to EPA on the Proposed Interim Registration Review Decisions for Several Neonicotinoid Pesticides: Imidacloprid, Clothianidin, Thiamethoxam, Acetamiprid and Dinotefuran

Docket IDs:
EPA-HQ-OPP-2008-0844: Imidacloprid
EPA-HQ-OPP-2011-0865: Clothianidin
EPA-HQ-OPP-2011-0581: Thiamethoxam
EPA-HQ-OPP-2011-0920: Dinotefuran
EPA-HQ-OPP-2012-0329: Acetamiprid

Center for Food Safety appreciates the opportunity to comment on EPA's proposed interim registration review decisions for the above-named neonicotinoid insecticides.

HUMAN HEALTH ASSESSMENT

Common Mechanism of Toxicity Demands Cumulative Risk Assessment

These five neonicotinoids operate by disrupting neural transmission in the central nervous system of invertebrates. By binding to nicotinic acetylcholine receptors (nAChRs) in the brain, neonicotinoids continuously stimulate neurons, resulting in death as well as sublethal effects (Simon-Delso et al. 2015). Neonicotinoids are more highly toxic to invertebrates than vertebrates because the former have have a larger number of nAChRs with high affinity to these insecticides. Neonicotinoids target primarily the nAChR subtype $\alpha 4\beta 2$ in insects and mammals, and mammalian toxicity correlates with agonist action and binding affinity at these receptors, their primary target in the brain (Tomizawa and Casida 2005).

This shared mechanism of toxicity demands cumulative risk assessment of these neonicotinoids, as required under the Food Quality Protection Act. EPA provides no explanation for its failure to conduct a cumulative assessment, beyond noting that it has not made an official finding as to the fact that neonicotinoids share a common mechanism of toxicity to humans (e.g. EPA Imidacloprid 2020, p. 17). EPA refused to make this finding despite abundant evidence, even in registrant-sponsored animal feeding studies conducted for the human health assessment, that neurotoxicity is the most prominent and consistent class of

adverse effects of all five neonicotinoids. For instance, imidacloprid via oral administration induces tremors, decreased motor activity and similar effects in multiple studies on rats and dogs (EPA 6/22/17, p. 3). Clothianidin induces decreased arousal, motor activity and acoustic startle response; tremors; and other neurotoxic effects in various animal studies (EPA 9/7/17, p. 13). Thiamethoxam triggers developmental neuorological effects in rats, including reduced brain size and weight (EPA 12/5/17, pp. 5-6). Neurotoxic effects induced by acetamiprid include decreases in locomotor activity, alertness, reactivity, spontaneous activity, rearing, muscle tone and grip strength; tremors; and depressed reflexes in rat, mouse and/or rabbit studies (EPA 12/15/17, pp. 17-18). Dinotefuran likewise induced declines in motor activity, grip strength, and brain weight in animal studies (EPA 9/12/17, p. 5).

EPA refuses to officially affirm a common mechanism of human toxicity between any of these neonicotinoids despite acknowledging the fact. EPA states that neurotoxicity is among the classes of adverse effects "commonly observed in mammalian toxicity studies of neonicotinoids" (EPA 9/7/17, p. 12). Still more explicitly, EPA affirms that neonicotinoids have a neurotoxic mode of action both for insect pests and humans: "Dinotefuran is a neonicotinoid and has a pesticidal and mammalian neurotoxic mode of action. Consistent with this mode of action, changes in motor activity were seen in acute neurotoxicity (ACN) and subchronic neurotoxicity (SCN) studies" (EPA 9/12/17, p. 20). EPA also notes that dinotefuran induced "changes in motor activity which are consistent with effects on the nicotinic cholinergic nervous system [nicotinyl acetylcholine receptors, as noted above] seen after repeat dosing" (EPA 9/12/17, p. 5).

Four of the five neonicotinoids belong to a common subclass – the nitroguanidines – while the fifth, acetamiprid, is a closely related cyanoamidine-substituted neonicotinoid (Tomizawa and Casida 2005, Figure 1). EPA "made a programmatic decision to align the registration review schedule for all four nitroguanidine-substituted neonicotinoids (clothianidin, dinotefuran, imidacloprid and thiamethoxam)" (EPA 1/16/20), and subsequently added acetamiprid to the group. This decision makes no sense if, as EPA tacitly assumes, entirely separate risk assessments for each of them is adequate to the task of ensuring human and environmental safety.

Independent scientists have assessed cumulative dietary exposure to neonicotinoids on the basis of their common mechanism of toxicity, employing relative potency factors to permit expression of the cumulative toxicity in imidacloprid-equivalent units (Lu et al. 2018; Zhang et al. 2019). EPA has used this method to assess the toxicity of related groups of compounds, such as dioxins (Staskal et al. 2010). Because cumulative exposure to neonicotinoids would be considerably higher than exposure to any single compound of its class, EPA has underestimated both human exposure to and the health risks of neonicotinoids. To take one example, EPA's estimated dietary exposure to imidacloprid alone is nearly equal to the acute safety threshold (population-adjusted dose, or aPAD) for infants (84%) and toddlers (93%) (EPA 6/22/17, p. 23, Table 5.4.4). Cumulative exposure to all five neonicotinoids would almost certainly exceed the acute safety threshold for these vulnerable groups.

EPA should abstain from any final registration review decision until it has completed a thorough cumulative risk assessment of neonicotinoids.

Safety Factor to Protect Infants and Children

EPA is required by the Food Quality Protection Act (FQPA) to apply "an additional tenfold margin of safety" to account for "the special susceptibility of infants and children," and in particular the "potential for pre- and postnatal toxicity…," and reduce or eliminate it only if "reliable data" demonstrate it is not needed. According to EPA policy, the 10x FQPA safety factor is to be applied when the young exhibit increased susceptibility to a pesticide (i.e. effects not seen in adult animals) or increased sensitivity (the effects occur at lower doses or increased severity in the young) (FQPA 2002, p. 30).

Based purely on registrant studies, EPA found increased susceptibility or sensitivity to neurotoxic harms in young test animals versus adult animals for four of the five neonicotinoids at issue here: imidacloprid ("evidence of an increased quantitative susceptility" in the rat," EPA 6/22/17, p. 14); clothianidin (same, EPA 9/7/17, p. 13); thiamethoxam (same, EPA 12/5/17, p. 6); and acetamiprid ("increased qualitative susceptibility," EPA 12/15/17, p. 17-18).

Despite these findings, the clear mandate of the Food Quality Protection Act, and EPA's policy prescriptions regarding implementation of the FQPA, EPA rejected the default 10x safety factor for all five neonicotinoids.

EPA should abstain from any final registration review decision until it has correctly applied the FQPA 10x safety factor to arrive at reference doses that reflect the increased toxicity of these insecticides to the young.

Independent Studies Reveal Greater Mammalian Sensitivity to Neonicotinoids Than Registrant Studies

Kara et al. (2015) administered via gavage 0.5, 2 or 8 mg/kg/day imidacloprid to infant and adult Wistar rats for 3 months. Learning activities were diminished significantly at 2 and 8 mg/kg/day doses in infant rats, but only at 8 mg/kg/day in adult rats. This study's NOAEL for infant rats of 0.5 mg/kg/day is 16-fold lower than the 8.0 mg/kg/day NOAEL (acute and chronic) based on a subchronic dog study conducted by Bayer AG in 1990.[1] This study supports an oral reference dose of 0.005 mg/kg/day (vs. EPA's 0.08 mg/kg/day), and also provides further support for retaining the 10x FQPA safety factor, given the greater sensitivity of infant vs. adult rats.

Burke et al. (2018) infused 0.5 mg/kg/day imidacloprid into pregnant CD-1 mice via an implanted osmotic minipump from gestation day (GD) 4 to post-natal (PN) day 21. Imidacloprid accumulated in livers and brains of maternal mice, and was found in trace levels in offspring. Offspring exhibited a number of neurobehavioral impacts: elevated motor activity, enhanced

[1] Ruf J. 1990. NTN 33893 Technical: Subchronic Toxicity Study on Dogs in Oral Administration (Thirteen-Week Feeding Study). Lab Project Number: 18732: 100176. Unpublished study prepared by Bayer AG. 305 p. MRID 42256328.

social dominance, reduced depressive behavior, and a diminution in social aggression compared to controls. Adult male offspring had reduced weight. Maternal animals had significantly reduced fecundity (roughly 8 vs. 13 pups per mother for treatment vs. control groups). Transient exposure to imidacloprid over the developmental period induced long-lasting changes in behavior and brain function in mice. Based on Burke et al. (2018), the LOAEL for imidacloprid is 0.5 mg/kg/day. This study also supports application of the FQPA 10x safety factory.

ENVIRONMENTAL ASSESSMENT

Cumulative Toxicity

As with human health, EPA must assess neonicotinoids cumulatively, in view of their common mechanism of toxicity to insects and other non-target organisms (Xerces 2016), and their frequent co-occurrence (e.g. Krupke et al. 2012). Maloney et al. (2018) reported roughly concentration-additive toxicity of various neonicotinoid mixtures to the aquatic insect *Chironomus dilutus*, with mild synergism for thiamethoxam-imidacloprid. EPA must also assess the additive or synergistic toxicity of neonicotinoids together with co-occurring formulation additives ad other pesticides (Xerces 2016). To give an idea of the scope of the problem, Sanchez-Bayo and Goka (2014) report that in various studies, a total of 161 pesticides have been found in bee hives: 124 in pollen, 95 in wax and 77 in honey or nectar.

For instance, neonicotinoids are strongly synergized by inhibitors of CPY450 detoxification enzymes, such as piperonyl butoxide, a common "inert ingredient" in over 2,500 pesticide formulations (Tomizawa and Casida 2005; Cross et al. 2017). Imidacloprid exhibits synergy in concert with the adjuvant nonylphenyl polyethoxylate, R-11, towards the crustacean *Ceriodaphnia dubia* (Chen et al. 2010). A wide range of other formulation additives and surfactants, such as organosilicone surfactants, make pesticides more toxic and can also be toxic in their own rights (Mullin 2015, Chen et al. 2018). This is problematic, because regulatory toxicity tests on the active ingredient alone will often underestimate real-world formulation toxicity. For this reason, Zhu et al. (2017) tested the toxicity to honey bee of the imidacloprid formulation Advise 2FL in binary combinations with seven other pesticides they commonly encounter, and found synergistic toxicity between imidacloprid/Advise and Domark/tetraconazole, Transform/sulfoxaflor, and Vydate/oxamyl, with mortality significantly increased by 20%, 15% and 26%, respectively. Tsevtkov et al. (2017) found that both clothianidin and thiamethoxam were twice as acutely toxic to honey bee workers with co-exposure to field-realistic levels of the fungicide boscalid.

Neonicotinoids have frequently been found to synergize with ergosterol biosynthesis inhibitor (EIB) fungicides (reviewed in Wood and Goulson 2017). Thompson et al. (2014) exposed honey bees to sprayed fungicides at realistic, worst-case scenario concentrations and various neonicotinoids. They found mild synergism on a contact basis between thiamethoxam and tebuconazole (synergism ratio of 2.6) and on an oral basis between clothianidin and tebuconazole (synergism ratio of 1.9), with synergism ratio equivalent to the LD_{50} of the

neonicotinoid divided by that of the neonicotinoids plus fungicide mixture. Similarly, Sgolastra et al. (2016) found synergism in three bee species (*A. millifera* [honey bee], *B. terrestris* [buff tailed bumble] and *O. bicornis* [red mason bee]) exposed to LD_{10} doses of clothinadin and a non-lethal dose of the fungicide propiconazole, in the form of increased mortality for the mixture.

These are just a few of many studies that have arrived at similar findings, though because most assess only binary mixtures and pollinators are exposed to far more complex combinations of multiple pesticides, the reported results are likely to substantially underestimate the degree to which neonicotinoids are synergized by co-exposure to other pesticides. Yet EPA makes no attempt to assess the increased risks posed by neonicotinoids upon co-exposure with other pesticides.

Declines in Insect Populations Worldwide Coincide with Rise of Neonicotinoids

Massive declines in insect and pollinator populations worldwide

There have been many reports of declines in various insect species over the years (Dirzo et al. 2014), for instance the over 80% reduction in the migratory monarch butterfly populations since the mid-1990s in North America (Pleasants 2015). However, recently there has been great interest in charting trends in overall insect abundance as a more relevant marker of ecosystem health. For instance, researchers in Gerrmany documented an astounding 76% decline in flying insect biomass in 63 German nature reserves from 1989 to 2016 (Hallmann et al. 2017). They posit agricultural intensification, including pesticide use, as one potential cause, noting that most of the preserves are surrounded by cropland that may serve as an ecological traps or sinks for insects whose origins are in the natural areas. Sanchez-Bayo and Wyckhuys (2019) review 73 historical reports of insect declines around the world, and find that *Lepidoptera* (moths and butterflies), *Hymenoptera* (bees and wasps) and dung beetles have been most impacted among terrestrial insects. They predict extinction of 40% of remaining insect species in the next few decades, and regard habitat loss to agriculture and urbanization as well as pollution, particularly from pesticides and fertilizers, as major drivers. A recent meta-analysis of studies across the world finds a roughly 9% reduction in terrestrial insect abundance per decade, a trend driven largely by findings in North America and parts of Europe (van Klink et al. 2020).

Rise in insecticidal toxicity due to neonicotinoid seed treatments

In the U.S., the toxicity of insecticide use in agriculture has increased dramatically over the past two decades. Researchers found that insect toxic load – a metric that adjusts the amount of insecticides used by their acute potency to honey bees – has increased nine-fold on an oral basis since just 1997 (Douglas et al. 2020). The main driver of this trend is the seed industry's massive deployment of neonicotinoid seed coatings on the seed of field crops (e.g. corn and soybeans) that had previously not been extensively treated with insecticides of any sort (Douglas and Tooker 2015). Because of their extremely high potency as well as extent of usage, by 2012 neonicotinoids alone comprised 98% of oral insect toxic load, equivalent to 16 billion honey bee oral LD_{50} doses per treated hectare (Douglas et al. 2020). The most dramatic increases occurred in the Heartland (121-fold increase) and the Northern Great Plains (53-fold increase), where the majority of corn and soybeans, nearly all (corn) or are grown (Ibid.).

While neonicotinoids are deployed as foliar and soil-applied sprays, seed treatments employing imidacloprid, clothianidin or thiamethoxam comprise roughly three-fourths of total agricultural use of the five neonicotinoid compounds on a weight basis. This is based on EPA's screening level usage analyses for each of the five: 3 million lbs. seed treatment vs. just over 1 million lbs. for foliar and soil-applied sprays, annually, though this is a substantial underestimate thanks in part to lack of data on seed treatments since 2015.[2] Yet EPA has enacted little if any mitigation for this predominant use of neonicotinoids.

Neonicotinoid Exposure Routes

Neonicotinoid dust from treated seeds kills honey bees and other insects

Seeds treated with neonicotinoids (clothianidin, thiamethoxam or imidacloprid) and other pesticides (often fungicides) can stick together, causing uneven plant spacing. Talc or some other lubricant is added to seed boxes to reduce friction and ensure the smooth flow of seed during planting. Some portion of the seed coating is abraded in the seed box and contaminates the talc with high levels of the neonicotinoid. The talc is expelled either with the seed or behind the planter via exhaust fan (Krupke et al. 2012). This seed dust, broadcast across the landscape, has been implicated in numerous bee mortality events since 1999 in Italy, France, Slovenia, German and Canada as well as the U.S.: "[i]n all cases, a great number of dead and dying bees were found near the hive entrance" (Bonmatin et al. 2015).

One study examined the threat of neonicotinoid-laced seed dust to honey bees in Indiana, and found that over 94% of honey bee foragers in the State of Indiana are at risk of exposure to varying levels of neonicotinoid insecticides, including in some cases lethal levels during the planting of corn. They also found that deposition of neonicotinoid residues on non-target lands and waterways occurs on over 42% of the state of Indiana, and that risks to pollinators could be dramatically reduced, with no yield loss, by limiting use of seed treatments to situations where they are actually needed (Krupke et al. 2017).

EPA has not proposed any mitigation to address lethal or sublethal exposure to neonicotinoid-laced seed dust.

Other exposure routes

A major pathway of pollinator exposure to neonicotinoids is the pollen and nectar of crops from treated seed. In a review of 20 studies, Godfray et al. (2014) estimate ***average*** maximum levels of neonicotinoids of 1.9 ppb in the nectar of 6.1 ppb in the pollen of seed-treated crops, values in line with those found in an update to that review (Godfray et al. 2015). Wood and Goulson (2017) report expected residues in several crops (corn, sunflower, rape, cotton) as calculated by

[2] First, the seed treatment figures for each relevant crop that comprise the total are long-term averages (e.g. 2005 to 2013 for thiamethoxam, EPA 1/26/16), and the averages understate usage because the proportion of crop seed, and in the case of corn the rate applied, have increased steadily over that period (Douglas and Tooker 2015). Second, the private sector firm that EPA relies upon for seed treatment usage data stopped collecting it after 2014; and usage of neonicotinoids was trending steadily upward for all major crops (corn, soybeans, cotton and wheat) up until that time, and thanks to inaction on the part of EPA has almost certainly continued to increase since then (Hitaj et al. 2020).

the European Food Safety Authority based on outdoor studies and seed treatment rates authorized in the Europesn Union. Maximum expected residues in pollen ranged up to 37 ppm in corn (clothianidin); 19 ppm in oilseed rape (clothianidin and thiamethoxam); and 4 ppm in sunflower (imidacloprid). See Table 1 below. Guttation droplets (small water droplets exuded by plants) of treated plants contain four to five orders of magnitude (10,000 to 100,000 times) higher neonicotinoid concentrations than those found in nector (Girolami et al. 2009, Wood and Goulson 2017). While the potential for exposure (pollinator visitation of guttation droplets) is uncertain, a honeybee would only need to consume 0.005 ul to receive an LD_{50} dose (Wood and Goulson 2017). Thus, even infrequent visitation could cause considerable harm.

Neonicotinoids are relatively persistent in soil, and the planting of many fields every year to treated seeds (e.g. as in the common corn-soybean in the U.S., with treated seed comprising a majority of each crop) ensures a continual presence in soil (e.g. Xu et al. 2016). Various studies find single digit to 50 ppb concentrations of imidacloprid, clothianidin and/or thiamethoxam in crop fields, with detections even in fields that had not received any treatment in the previous three years (reviewed in Wood and Goulson 2017). EPA does not pay sufficient consideration to this exposure pathway, in part because it is of lesser significance for honey bees, the surrogate for terrestrial invertebrates in EPA's ecotoxicity regulatory scheme. Yet soil contact and/or ingestion is an important exposure pathway for ground-nesting bumblebees and many other terrestrial invertebrates that reside in the soil.

Table 1 Summary of expected residues in pollen and nectar of various neonicotinoid-treated flowering crops calculated by EFSA from the review of outdoor field trials

Crop	Pesticide	Application rates (g a.s./ha)	Residues in pollen (ng/g)		Residues in nectar (ng/g)	
			Minimum	Maximum	Minimum	Maximum
Oilseed rape	Clothianidin	25–80	5.95	19.04	5	16
Sunflower	Clothianidin	27		3.29		0.324
Maize	Clothianidin	25–125	7.38	36.88	*n/a*	*n/a*
Oilseed rape	Imidacloprid	10–52.5	1.56	8.19	1.59	8.35
Sunflower	Imidacloprid	24–35		3.9		1.9
Maize	Imidacloprid	54–268	3.02	15.01	*n/a*	*n/a*
Cotton	Imidacloprid	75–100	3.45	4.6	3.45	4.6
Oilseed rape	Thiamethoxam	8–33.6	4.592	19.29	0.648	2.72
Sunflower	Thiamethoxam	16.4–20.8	2.378	3.02	0.59	0.75
Maize	Thiamethoxam	63–101	13.419	21.513	*n/a*	*n/a*

No nectar values are available for maize as this plant does not produce nectar. Blanks are where no minimum values were stated

Source: Wood and Goulson (2017).

Neonicotinoids have also been detected in the tissues of off-field wild plants. For instance, Krupke et al. (2012) found thiamethoxam (up to 2.9 ppb) and clothianidin (up to 9.4 ppb) in dandelions near a treated corn seed field, while Pecenka and Lundgren (2015) found clothianidin in the leaves of milkweed plants adjacent to treated corn fields. In a field study conducted in the U.K., Botías et al. (2015) placed honey bee colonies near oilseed rape and wheat fields that originated from treated seed. Based on pollen collected in June and August from honey bee foragers returning to the hives, 97% of the total neonicotinoids present in pollen were of wildflower origin, from plants growing in hedges along the field margins.

Remarkably, direct measurements of the neonicotinoid content of pollen and nectar of these wildflowers showed concentrations of the same order as and even greater than that found in treated crops pollen and nectar. Indeed, others have made similar findings. In a review of studies published since 2013, Wood and Goulson (2017) found:

> "… average levels of neonicotinoids in wild plants range from 1.0 to 7.2 ng/g in whole flower samples, 0.4 to 13.5 ng/g in foliage samples, <0.1 to 1.5 ng/g in nectar samples and <0.04 to 14.8 ng/g in pollen samples. Due to the limited number of studies available, it is difficult to make a comparison with levels in directly treated crop plants. However, they are broadly comparable to the levels found in the treated crop itself."

Neonicotinoids are highly water-soluble and are also frequently found in water bodies, another avenue of exposure to these long-lived compounds (Morrissey et al. 2015, Bonmatin et al. 2015, Wood and Goulson 2017).

Neonicotinoid Effects on Pollinators

A major weakness of EPA's assessment is the failure to evaluate the sublethal effects of neonicotinoids and their interactions with other factors such as disease and pest pressure.

Impacts on growth and reproduction

Whitehorn et al. (2012) simulated exposure of bumblebee colonies to concentrations of imidacloprid in pollen and sugar water realistic for seed treatment use of this neonicotinoid, and found significantly reduced growth rate in the colonies and an 85% reduction in the production of new queens compared to controls. Laycock et al. (2012) found that queenless microcolonies of worker bumble bees subjected to a range of imidacloprid doses delivered in sugar syrup exhibited a dose-dependent decline in fecundity, with realistic doses in the range of 1 ppb reducing brood production by a third. Williams et al. (2015) found that exposure of honey bee queens to field-realistic concentrations of neonicotinoids (bee-collected pollen supplements spiked with 3 ppb thiomethoxam + 1 ppb clothianidin) during development resulted in comprised ovaries and reduced queen success. Tsetkov et al. (2017) quantified the duration and magnitude of exposure to neonicotinoids over four months in Canada's corn-growing region, and then conducted realistic experiments in which honey bee colonies were exposed to clothianidin in an artificial pollen supplement with the concentration time course matching that previously observed. They found increased worker mortality, declines in social immunity (reduced hygienic behavior) and increased queenless over time. James (2019) found that monarch adults feed a field realistic rate of imidacloprid for 22 days suffered nearly 80% mortality by day 22, compared to 20% in untreated controls.

Weakened immunity

There is a large and growing literature demonstrating that neonicotinoid exposure weakens pollinators' defenses against disease pathogens and pests. Alaux et al. (2010) found that honeybees exposed to imidacloprid and the parasitic microsporidia Nosema suffered higher mortality and energetic stress than untreated bees or those exposed to only imidacloprid (IMI) or Nosema. They also found that the IMI-Nosema group had significantly reduced glucose

oxidase activity, which enables bees to sterilize colony and brood food, and hypothesize that IMI and Nosema synergize to render honeybee colonies more susceptible to infection by pathogens. Pettis et al. (2012) exposed honey bee colonies over three brood generations to sublethal doses of imidacloprid, then challenged with Nosema, which produced significantly increased infections versus controls not exposed to imidacloprid.

> "The finding that individual bees with undetectable levels of the target pesticide, after being reared in a sub-lethal pesticide environment within the colony, had higher Nosema infections is significant. Interactions between pesticides and pathogens could be a major contributor to increased mortality of honey bee colonies, including colony collapse disorder, and other pollinator declines worldwide."

Neonicotinoid exposure has also been associated with increased susceptibility to viral disease. For instance, Di Prisco et al. (2013) found that clothianidin negatively modulates NF-xB immune signaling in insects and adversely affects honey bee antiviral defenses controlled by this transcription factor. Clothianidin enhances the transcription of a gene encoding a protein that inhibits activation of NF-xB. Imidacloprid was also found to have this effect. The antiviral suppression led to proliferation of dwarf wing virus.

> "Collectively, our data demonstrate that two neonicotinoid insecticides, each representing one of two alternative structure types in the group of nitroguanidines, actively promote DWV [dwarf wing virus] replication."

A recent study on honey bees collected from a winter apiary in France tested the effects of co-exposure to thiamethoxam and the chronic bee paralysis virus (CBPV). The researchers found that co-exposure did not affect bee survival or their ability to metabolize the thiamethoxam to clothianidin; however they found that co-exposure increased CBPV loads, which reached levels usually found in overt infections, and was associated with down-regulation of vitellogenin and dorsal-1a gene transcription, both of which are involved in immune system pathways.

Sanchez-Bayo et al. (2016) review additional studies on the subject of neonicotinoid exposure and bee diseases. There is also evidence that neonicotinoids weaken plant defenses, for instance to spider mites, by suppressing the expression of plant defense compounds and altering the levels of phytohormones involved in plant defense in cotton, corn and tomato (Szczepaniec et al. 2013).

<u>Other sublethal effects</u>

Neonicotinoid exposure has also been associated with impaired learning, memory and foraging behaviors in various bee species, sublethal effects that are likely contributing to bee declines (reviewed in Wood and Goulson 2017; Godfray et al. 2014, 2015). For one of many examples, Tosi et al. (2017) found that an acute, sublethal dose of thiamethoxam (1.34 ng/bee) triggered excitation and significantly increased flight duration among foragers, while chronic exposure reduced flight duration, distance and velocity.

Neonicotinoid Effects on Other Invertebrates

Douglas et al. (2015) found that slugs feeding on neonicotinoid-treated soybean seeds/seedlings accumulated neonicotinoids in their tissues; and that ground beetles attacking these neonic-laced slugs experienced nervous system impairment, with substantial mortality. They also showed that neonicotinoids suppressed slug predation by ground beetles, and was associated with a significant yield loss relative to an untreated soybean field control. Similarly, Szczepaniec et al. (2011) found that application of imidacloprid to elm trees caused an outbreak of spider mites, an effect mediated by a reduction in the density of the mites' predators due to imidacloprid-induced mortality. Such tritrophic impacts of neonicotinoid use could well be quite common, yet are missed entirely by EPA's regulatory guideline tests. Douglas et al. (2015) also detected neonicotinoid concentrations of 54 and 279 ppb in two earthworms from a thiamethoxam-treated soybean field. While not evidently affected themselves, earthworm predators might take up neonicotinoid residues with their prey, with potential adverse effects.

Neonicotinoid impacts on vertebrates

Neonicotinoids pose a severe acute risk of mortality to birds which consume treated seeds. EPA notes that:

> "The highest risk was identified for small size birds which would need to consume less than a single treated sorghum and wheat seed to exceed the acute level of concern, while with small or medium size birds consuming cotton, sorghum, and wheat seed, a bird would only need to consume 1-4 seeds [two (cotton) or four (sorghum and wheat)] to exceed the acute level of concern." (EPA PIRRD Imidacloprid, p. 23).

Insecticides so toxic that consumption of just one or several treated seeds is sufficient to kill obviously have no place in agriculture. Birds may also be at risk through consumption of neonicotinoid-containing prey, such as slugs or earthworms. Sublethal effects must also be considered. Eng et al. (2017) found that migratory white-crowned sparrows exposed to sublethal doses of imidacloprid suffered significant declines in body fat and mass, and failed to orient properly. A follow-up experiment on the same species revealed similar imidacloprid effects: reduced food consumption, mass, fat and altered likelihood of departure when exposed at a migratory stopover (Eng et al. 2019). A recent study found that the echolocation system of Insectivorous bats might be impaired by exposure to imidacloprid (Wu et al. 2019).

Endocrine-disrupting potential of neonicotinoids

EPA has not yet made any findings regarding the endocrine disruption potential of these five neonicotinoids. Before making any determinations, EPA should consult independent studies on the subject. For instance, three recent studies suggest imidacloprid is an endocrine disruptor, with implications for both human health and wildlife (Yuan et al. 2020, Mikolic et al. 2018, Pandey and Mohanty 2015).

COSTS AND BENEFITS OF NEONICOTINOID USE

EPA as usuals conducts a "benefits" rather than a "cost-benefit" assessment of neonicotinoids. Examples of costs not accounted for are the soybean yield reductions attributable to predation of treated soybean seedlings by slugs, whose populations increase thanks to release from control by ground beetles, which are poisoned when they attempt to attack them (Douglas et al. 2015). Growing resistance to neonicotinoids in thrips and other insects is predictable, given their prophylactic use, every year, across hundreds of millions of acres of cropland, and is already leading to a dramatic increase in insecticide use in cotton (Huseth et al. 2018). EPA fails to account for the follow-on costs of this resistance, both increased expenditures on insecticides and environmental harms, which are a direct result of the Agency's blanket approvals for virtually unlimited seed treatment use of neonicotinoid insecticides.

EPA also counts "benefits" in situations where it fails to consider less chemical-intensive and more beneficial alternatives. For instance, a beneficial fungus, *Hirsutella citriformis*, naturally infests and kills the psyllid vector; even better, the dead pysllids remain on citrus leaves for extended periods, spreading the fungus to other psyllids (O'Brian 2013). Another promising biocontrol predator is *Tamarixia radiata*, a parasitic wasp that specializes in killing psyllids (Lopez 2013). For both fungus and wasp, pesticide use for other purposes is an obstacle to their effectiveness. Another neonicotinoid use is for control of the glass-winged sharpshooter, an insect that pierces plants and feeds on their xylem fluids, but which also vectors a plant pathogenic bacterium, Xylella fastidiosa, that infests grapes and other valuable crops in California. Biocontrol options also exist for this pest, but will likely not be pursued diligently as long as there is the easy option of neonicotinoid application (Irvin undated). This failure to develop biocontrol solutions is a clear cost of the neonicotinoid registrations.

On the other hand, the predominant seed treatment use of neonicotinoids provide little or no benefit in terms of yield. EPA itself came to this conclusion for soybeans (EPA 10/15/14), which was recently confirmed by a long list of agronomists from universities across the country (Mourtzinis et al. 2019). A study in Indiana found the same "no yield benefit" of neonicotinoid seed treatments for corn (Krupke et al. 2017).

OTHER REGULATORS SEE AND ACT ON RISKS THAT EPA DISCOUNTS

Canada's Pest Management Regulatory Agency (PMRA) – hardly an enemy of pesticide use – has worked jointly with EPA on assessing neonicotinoids (EPA 1/6/16). On the basis of much the same evidence as EPA, PMRA decided the risks were too great, especially to aquatic invertebrates, and possible mitigation measures ineffective. Despite delays, PMRA is still officially committed to a phase-out. In 2018, the European Food Safety Authority expanded a pre-existing restriction on neonicotinoids to cover all filed crops (Stokstad 2018). EPA is thus alone in denying the overwhelming evidence of harm caused by neonicotinoid insecticides to pollinators and other wildlife.

CONCLUSION

EPA is urged to cancel the registrations of the five neonicotinoid insecticides discussed in these comments. At the very least, suspend the use of imidacloprid, thiamethoxam and imidacloprid as seed treatments, particularly for high acreage crops like corn and soybeans.

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