NATURAL RESOURCES DEFENSE COUNCIL

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Comments from the Natural Resources Defense Council on the EPA Preliminary Pollinator Assessment to Support the Registration Review of Imidacloprid

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The following comments are being submitted on behalf of the Natural Resources Defense Council (NRDC). NRDC uses law, science, and the support of more than 1.2 million members and online activists nationwide to protect the planet's wildlife and wild places and to ensure a safe and healthy environment for all living things. NRDC has no direct or indirect financial or fiduciary interest in the manufacture or sale of any chemical that would be the subject of the deliberations of this Committee.

These comments are supported by the following environmental protection groups:

American Bird Conservancy
Cynthia Palmer, Director,
Pesticides Science and Regulation

Beyond Toxics
Lisa Arkin, Executive Director

Central Maryland Beekeepers Association
Bonnie Raindrop, Chair, Legislative Committee

Environment America
Margie Alt

Friends of the Earth US
Tiffany Finck-Haynes

People and Pollinators Action Network
Joan Gregerson

Pollinator Minnesota
Erin Rupp

BACKGROUND:

In early 2016 the U.S. EPA issued for public comment a preliminary pollinator risk assessment for the neonicotinoid insecticide, imidacloprid. The assessment was prepared in coordination with California's Department of Pesticide Regulation and Canada's Pest Management Regulatory Agency.

The assessment uses a complicated tiered approach and purports in its title to consider threats to "pollinators" generally. In actuality, however, the scope of the assessment is far more narrow: it "evaluates the risk of the registered agricultures uses of imidacloprid to bees alone" (underline in original, EPA p. 13).

The assessment bases its final conclusions solely on its final estimated risk to honey bees, and only to honey bees at the whole-colony level – the third and final tier of its assessment (it does a cursory review of some non-honey bee studies, but only qualitatively, EPA p. 17). EPA concludes, "*The preliminary risk assessment identified a residue level for imidacloprid of 25 ppb, which sets a threshold above which effects on pollinator hives are likely to be seen, and at that level and below which effects are unlikely. These effects include decreases in pollinators as well as less honey produced.*"[1]

The imidacloprid assessment is the first of four preliminary pollinator risk assessments for the neonicotinoid insecticides. Preliminary pollinator risk assessments for three other neonicotinoids, clothianidin, thiamethoxam, and dinotefuran, are scheduled to be released for public comment in December 2016. A preliminary risk assessment of all ecological effects for imidacloprid, including a revised pollinator assessment and impacts on other species such as aquatic and terrestrial animals and plants will also be released in December 2016.[2]

OVERVIEW OF COMMENTS:

In 2015, EPA proposed to prohibit the use of pesticides that are toxic to bees, including the neonicotinoids, when crops are in bloom and bees are under contract for pollination services. At that time the Agency temporarily halted the approval of new outdoor neonicotinoid pesticide uses until new bee data is submitted and pollinator risk assessments are complete.

Here EPA has issued an assessment based on three tiers of risk estimates. Tier I identifies uses at which the acute and chronic risk Level of Concern (LOC) values are exceeded for individual honey bees. At the Tier II level a No- and Lowest- Observed Adverse Effect Concentration (NOAEC and LOAEC) of 25 and 50 ppb of total imidacloprid in sucrose was determined from a registrant-sponsored field study of a honey-bee colony. Where pollen residue data are available, levels above 100 ppb overwintering LOAEC are considered for qualitative purposes only, as a way to put risk estimates into context (Dively 2015, qualitative). The Tier III portion of the assessment is an attempt to characterize the potential effects of imidacloprid on bee colonies under actual conditions of use (EPA p. 154); here EPA has organized the studies by source (whether registrant-submitted or open literature, although we note below that much of the open literature honeybee studies are sponsored by the registrants).

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[1] *EPA Releases the First of Four Preliminary Risk Assessments for Insecticides Potentially Harmful to Bees*. EPA News Release, 01/06/2016. https://yosemite.epa.gov/opa/admpress.nsf/0/63E7FB0E47B1AA3685257F320050A7E3

[2] *EPA Releases the First of Four Preliminary Risk Assessments for Insecticides Potentially Harmful to Bees*. EPA News Release, 01/06/2016. https://yosemite.epa.gov/opa/admpress.nsf/0/63E7FB0E47B1AA3685257F320050A7E3

Unfortunately now EPA has issued an assessment that will allow almost all uses of imidacloprid to continue. This is because the assessment was limited to impacts on honey bee colonies. Although EPA's assessment identifies citrus and cotton (p. 222) uses that may have residues of the pesticide in pollen and nectar above the threshold level, it gives a pass to most other crops including corn and leafy vegetables, either because they do not produce nectar or because EPA estimated them to have residues below the EPA identified trigger level of 25 ppb for whole colony honey bee risks.

Failure to use appropriate uncertainty factors:

The study used by EPA to set the trigger level (point of departure) reported statistically significant effects at both 25 ppb and 12.5 ppb, the lowest doses tested. The study did not, therefore, identify a true 'no observable adverse effect concentration' (NOAEC). This means that harmful effects are likely to be found at doses even below 12.5 ppb. Nonetheless, EPA used 25 ppb as a NOAEC, thereby leaving bees at risk of harmful exposures below that level. In addition, numerous study limitations and weaknesses make it unlikely that it would identify adverse effects (bias the study towards the null), making the observed effects at the low doses all the more relevant.

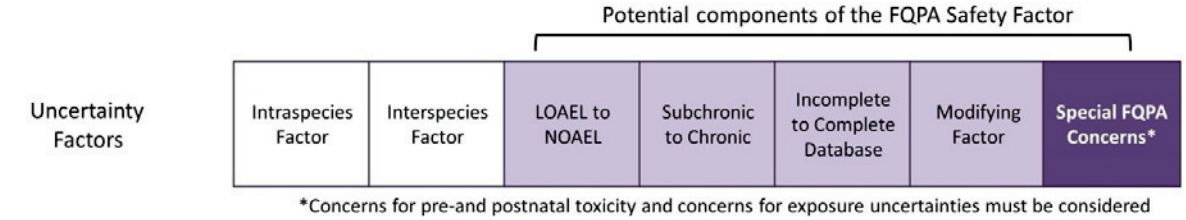
In summary, if EPA is going to rely on the field study to set a point of departure:

- Use the lowest dose of 12.5 ppb as a LOAEC, instead of 25 ppb as a NOAEC (2X more protective);
- Add an additional Uncertainty Factor of at least 3X for use of a LOAEC in the absence of a NOAEC (3X more protective);
- Add an Uncertainty Factor of at least 10X for extensive problems with the main study that EPA relies on for its assessment (10X more protective);
- Add an additional Database Uncertainty Factor of at least 10X for data gaps with the exposure and risk estimate methodology as a whole (10X more protective).

This would provide a 600-fold margin of safety, and render all uses currently identified in the draft assessment as unacceptably high risk.

EPA has clear guidance within the Food Quality Protection Act (FQPA) context, which lays out the potential components that can contribute to the overall safety factor, as shown in Figure A below. These components encompass several data deficiency uncertainty factors. The data deficiency uncertainty factors derive from FQPA's "completeness of the data" clause and are employed when there is: extrapolation from a Lowest Observed Adverse Effects Level (LOAEL) to a No Observed Adverse Effects Level (NOAEL), extrapolation from a subchronic study to a chronic outcome, or key data missing from a chemical's database (such as dose-response). "Special" FQPA concerns refer to potential pre- or post-natal toxicity and exposure uncertainties with respect to infants and children, and are not normally applied to pollinator risks.

Figure A. The FQPA Safety Factor encompasses data completeness, toxicity and exposure considerations (EPA 2002). Light shaded boxes show the data deficiency uncertainty factors.



There is much precedent for EPA OPP using the above uncertainty factors directly relevant to the limitations of the study used by EPA for this pollinator assessment. For example:

Chemical	Barcode	FQPA	FQPA Rationale
1,2,4-Triazole	D322215	30*	UF: Use of a LOAEL & data gaps (DNT & other studies)
Alpha-chlorohydrin	D326010	30*	UF: Use of a LOAEL & data gap (developmental studies)
Bifenthrin	D372550	30*	10X for data gap (inhalation study); Add'l. 3X is applicable to children < 6 years old only.
Carbendazim (MBC)	D275774	30*	UF: Use of a LOAEL; FQPA: susceptibility concerns
Cycloate	0052131	30*	UF: Use of LOAEL and data gap
Demiditraz	D378783	30*	Add'l 10X UFDB for lack of DNT and species sensitivity (dog is most sensitive); Add'l 3X UFL for lack of NOAEL.
Molinate	D271384	30*	UF: Use of a LOAEL; FQPA: susceptibility concerns
Phostebupirim (Tebupirimphos)	D368530	30*	UF: Use of a LOAEL and Data gap (CCA)
Pirimiphos-methyl	D256633	30*	UF: use of a LOAEL & severity of the effects at the LOAEL; FQPA: susceptibility concerns
Surfonic AGM 550	0014604	30*	UF: Data gap (multiple studies) and use of a subchronic study
Tridemorph	D322126	30*	UF: Use of a subchronic study & data gap
Triphenyltin hydroxide (TPTH)	D259257	30*	UF: deficiency of test material characterization and datag gap (DNT)

The EPA Integrated Risk Information System aggregates chemical assessments from across the EPA, including pesticides but also many industrial chemicals. It has a public database that is searchable by the Uncertainty Factor Value (along with other parameters of interest), including use of a LOAEL in absence of a NOAEL, and Database Incomplete UFs. [3] In that database, chemicals adding a 3X UF for use of a LOAEL include acephate, aroclor 1254, benzene, furfural, linuron, pentachlorophenol. Zineb has a 5X UF for use of a LOAEL. Chemicals with a 10X UF for use of a LOAEL include aldrin, antimony, asulam, baygon, benzidine, bromodichloromethane, captafol, chloral hydrate, and chloramben. There are thirty chemicals listed that have a 10X UF for incomplete database (missing or limited studies), including the following pesticides: bidrin, mepiquat chloride, methoxychlor, and thiram.

In conclusion, it is our assertion that EPA has placed an undeserved and unearned confidence in this assessment – which doesn't even meet the bar of a sophisticated guesstimate – by disregarding

[3] https://cfpub.epa.gov/ncea/iris/search/index.cfm?keyword=uncertainty+factors

statistically significant effects at low doses in the key study it uses to set exposure limits, and failing to add uncertainty factors for data gaps, study bias, inconsistencies in pathogen infection rates in the key study, lack of data to address native species, and many other serious flaws discussed in more detail below.

Major Gaps and Uncertainties Supporting the Use of Uncertainty Factors

Failure to consider genetic variability within the colony

Genetic variability within a colony can strongly influence the ability of a colony to withstand disease resistance, pathogen infestations, temperature fluctuations, and other challenges to colony health and survival (Tarpy 2003). Because this assessment relies on data from only a double-digit number of colonies, and only honey bees, it cannot reasonably represent the important impact of genetic variation that makes colonies more or less vulnerable to pesticide harm. EPA and the study authors should provide a statistical power calculation to clarify what the chances are of the study actually detecting an adverse effect. This is likely to be a very significant – but unquantified – uncertainty with EPA's assessment that would bias towards the null by failing to capture the most sensitive colonies. This supports the use of a Database Uncertainty Factor.

Failure to consider impact of pesticides on pathogen infection

The spread of infections among bees of all types is a very serious concern. Combinations of viruses and mites can kill a hive, whereas viruses alone can be harmful but do not seem to be as deadly (Dainet et al 2012; de Miranda et al 2010; Siede et al 2008). The synergistic increased harm from virus/mite combinations appears to be a result of a compromised immune response in affected bees (Martin et al 2012; Nazzi et al 2012; Ryabov et al 2014). This means that any factors that impair immunity – including pesticides and other non-chemical stressors – will increase the risk of mortality from pathogens (Nazzi and Pennacchio 2014). *Nosema* infection has been shown to suppress immunity in honey bees (Antunez et al 2009), alter honeybee worker behavior, reduce homing ability of foragers (Wolf et al 2014), and shorten the lifespan of adult honey bees (Eiri et al 2015; Goblirsch et al 2013). Sublethal exposure of honeybees to imidacloprid leads to a dose-dependent increase in *Nosema* infection, demonstrating that the pesticide can promote infections (Pettis et al 2012), by suppressing immunity and leading to higher bee deaths (Aufauvre et al 2014). In fact bees are more likely to die from pathogen infection if they have also been exposed to pesticides (Alaux 2010; Pettis et al 2013; Wu et al 2012).

EPA failed to explain the dramatic differences in *Nosema* infection rates across treatment groups in the key study that EPA used to set the colony risk estimate (see discussion below). Failure to address the impacts of combinations of pathogens with pesticides is a significant uncertainty with this assessment, particularly with regards to bumblebees, solitary bees, and other vulnerable native species that have a smaller colony size and may therefore have less ability to survive a pathogen-pesticide combined assault. These real-world scenarios are not represented in EPA's assessment. This supports the use of a Database Uncertainty Factor.

Failure to consider synergistic toxicity with fungicides and other tank mix pesticides

A recent study reported a significant correlation between fungicide residues and the presence of viruses in honeybee colonies (Simon-Delso et al 2014). Other studies have reported that fungicides may

increase the toxicity of the neonicotinoids acetamiprid and thiacloprid (Iwasa et al 2014) and other pesticides including those used to treat pathogens in honeybee hives (Johnson et al 2013). It is clear now that systemic pesticides like imidacloprid and other neonicotinoids together with fungicides increase the spread and virulence of Nosema infections among bees (Sanchez-Bayo et al, 2016). EPA's assessment fails to account for these synergistic effects, and therefore underestimates the impact of imidacloprid for both honeybees and native species.

EPA expressly omits consideration of synergistic toxicity with fungicides, and states that the assessment cannot adequately assess risk to bees without taking this into account. The assessment says, "There are reports in the literature that these chemicals [i.e., fungicides] may exhibit a greater than additive (e.g., synergistic) effect on toxicity when bees are exposed simultaneously with neonicotinoid chemicals like imidacloprid. . . . [T]he extent of this relationship is beyond the scope of this assessment . . . ." (p. 100). Failure to include these toxicity data adds to the overall uncertainty of the assessment, and casts further doubt on its ability to identify adverse effects when they occur. EPA should request data on the impacts of tank mixtures and formulations from the registrant, and in the meantime this represents a database gap that justifies a Database Uncertainty factor.

<u>Honey bee colony poor and inaccurate proxy for bumble bees and native species</u>

In February 2016 the Government Accountability Office issued a report on Bee Health that emphasized the importance of native bees to agriculture:

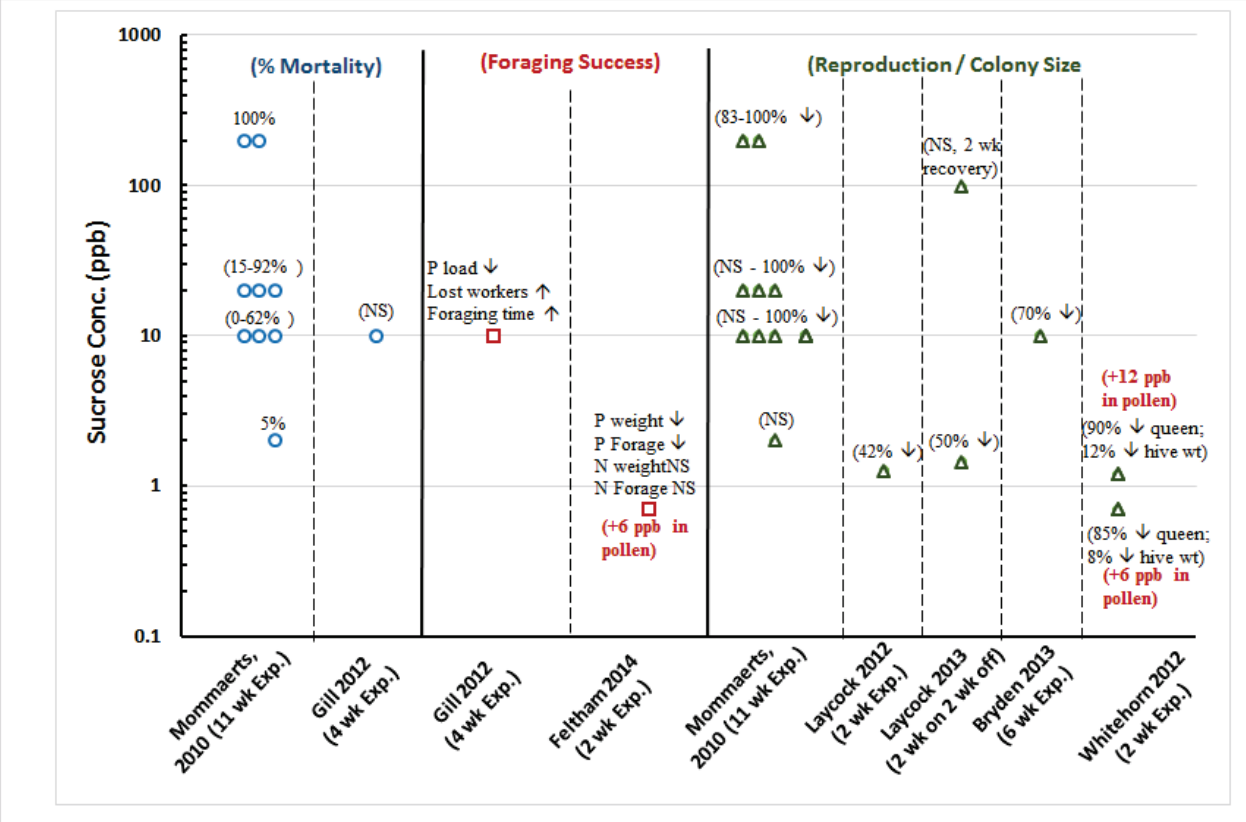
> In addition to honey bees, certain managed bees and wild, native bees also provide valuable pollination services. Whereas honey bees comprise an estimated 98 percent of managed bees in the United States, other managed bee species—including bumble bees, alfalfa leafcutting bees, and orchard mason bees—comprise the remaining 2 percent, according to a representative of the Pollinator Stewardship Council. These other managed bees pollinate alfalfa, almonds, apples, cherries, and tomatoes. Wild, native bee species may also pollinate agricultural crops. In 2009, crops directly and indirectly dependent on pollination by other managed bees; wild, native bees; and other insects were valued at almost $10 billion according to the 2012 study of the value of pollinators to U.S. food and agriculture. In addition, a 2007 National Research Council study found that wild, native bees provide most of the pollination in natural plant communities, which contributes to valuable ecosystem services, including water filtration and erosion control. (GAO 2016)

Both the GAO (2016) and an earlier report by the National Research Council (NRC 2007) noted that wild, native bees are arguably the most important and least studied pollinators in the country, and noted that declines have already been reported in the US and Europe, although much more data is needed to fully understand the declining trend.

The honeybee colony is unlike the bumble bee colony or any other native bee species, and is a poor representative for the potential risk of pesticides on the approximately four hundred native species that pollinate trees, wildflowers, and agriculturally important cover crops like clover (Koh et al, 2015; Sanchez-Bayo et al, 2016). For example, honeybee colonies have 10 thousand to 80 thousand bees, whereas a bumblebee colony may only have a few hundred and native bees can be solitary. And, honeybees overwinter about ten thousand bees including the queen, whereas among bumble bees only the queen overwinters and the rest of the colony dies off in the fall. That means in the spring the honeybee queen has many workers to help her get the colony started, whereas the bumble bee queen

has no help until she lays her larvae and grows them up into workers. Overall, these and other differences allow a honeybee colony to withstand much more assault and individual deaths than any other bee species, making honeybees a poor and unprotective proxy for other bee species.

In Tier II of the assessment, EPA reviews some bumble bee (Bombus terrestris) studies from the open literature, and provides a summary which demonstrates a number of adverse individual and colony-level impacts at exposure levels below 25 ppb (EPA, Figure 6-36, p. 283):



EPA (p. 283) Figure 6-36. Comparison of effect levels from qualitative Tier II feeding studies on B. terrestris obtained from the open literature (numbers in parentheses refer to the magnitude of effects and/or additional exposure to pollen)

EPA's assessment says: "Although data were very limited for non-*Apis* bees, results suggest oral exposure and effects of imidacloprid on the honey bee are reasonably representative (protective) or available data on adult non-*Apis* bees (primarily bumble bees)" (p. 17). However, EPA has failed to provide evidence that this is so, and the evidence available demonstrates otherwise (EPA Figure 6-36, p. 283). It does not appear that EPA has made any serious quantitative effort to assess risks to bumblebees or other native bee species.

EPA's assessment fails to adequately address the risk to 4000 wild bee species, justifying a Database Uncertainty Factor.

Failure to address risks to non-*Apis* bees from soil or foliar contact

EPA acknowledges the economic value of native bee species (GAO 2016), but nonetheless has failed to address risks to bumble bees and other native species from off-target residues in soil and non-target plants (p. 38). EPA acknowledges that "due to the limitations in available data, the current risk assessment process for honey bees does not address exposure via soil or foliar contact exposure which are likely more important for some non-*Apis* bees (p. 279). This represents an important gap in EPA's pollinator assessment, justifying the use of a Database Uncertainty Factor.

Failure to address exposure from guttation and surface water

EPA acknowledges that it lacks reliable methods for quantifying exposures to bees from guttation and surface water (p.37). Guttation fluid is the water droplets that are given off by plants in the early morning, as droplets around the leaf edges. Honey bees and other pollinators can collect this fluid for drinking. Unfortunately, extremely high levels of neonicotinoids (several hundred ppb) have been reported in guttation water of treated plants (Girolami et al 2009; Tapparo et al 2011). Imidacloprid has been reported in guttation fluid from melons (Hoffmann et al 2012). EPA should request data from the registrant, and in the meantime this represents a database gap that justifies a Database Uncertainty factor.

No data on impact to colony from pollen route of exposure

EPA acknowledges having no data on the impact to the colony from the pollen route of exposure (p. 272). Note in Figure 6-36 reproduced above and in EPA's assessment (EPA p. 283) that there are significant adverse effects reported in bumble bees at exposures below 25 ppb in pollen. How is EPA addressing this serious data gap?

Methodology Flaws:

Cumulative risks remain unaddressed:

The most significant problem with OPP's entire approach to the neonicotinoid pesticides – particularly related to pollinator impacts - is that it will not address the true cumulative risk posed by pesticide exposures. Agents that act on different pathways leading to adverse impacts may increase risks qualitatively (such as additional target organs) and/or quantitatively (increased potency) to levels greater than that of individual agents. These synergistic effects are not addressed in the single-mechanism risk assessments that the pesticide office continues to do.

We urge the pesticide office to consider the EPA Framework for Cumulative Risk Assessment (2003) that defined a cumulative risk assessment as "an analysis, characterization, and possible quantification of the combined risks to health or the environment from multiple agents or stressors" (EPA 2003, p. 6). EPA 2003 highlighted that "*[s]everal key points arise from this definition of cumulative risk. First, cumulative risk involves multiple agents or stressors, which means that assessments involving a single chemical or stressor are not 'cumulative risk assessment' under this definition. Second, there is no limitation that the 'agents or stressors' be only chemicals. 'Agents or stressors' may be chemicals, of course, but they may also be biological or physical agents or even the absence of a necessity such as habitat. Third, this definition requires that the risks from multiple agents or stressors be combined*" (EPA 2003, p. 7). This is

particularly relevant to pollinators, that experience the additive and synergistic impacts of multiple pesticides – even within the same tank mix - and non-chemical stressors including pathogens, climate change and habitat loss (Goulson et al 2015).

Other offices in the EPA are moving forward with efforts to modernize risk assessment by expanding cumulative risk groups beyond a shared common mechanism of toxicity (Burke and Bahadoori, 2015, personal communication), as well as state Agencies (Dunn and Alexeeff, 2010). The pesticide office is out of step with current scientific discourse and practice, instead following a cumulative risk assessment framework that is so backwards that it even lags behind the EPA 2003 Cumulative Risk Assessment framework of a dozen years ago.

The assessment fails to include critical elements of a systematic review process for evaluating and integrating multiple streams of data.

Government agencies and academic institutions in the US and world-wide are developing coordinated systematic and transparent methods of research synthesis in environmental health, using methods developed in clinical sciences as a model. Systematic reviews integrate information from human epidemiologic data, *in vivo* toxicologic data, *in vitro* cellular and mechanistic data, and *in silico* computational information. Systematic reviews methods for chemical assessments have been developed by the National Toxicology Program (NTP) Office of Health Assessment and Translation (OHAT), the EPA Integrated Risk Information System (IRIS) program, the University of California Navigation Guide method (Woodruff and Sutton 2014), and others. Various resources and guides are available, particularly from the NIEHS NTP:

> *Systematic review and evidence integration for literature-based environmental health science assessments.* Rooney AA, Boyles AL, Wolfe MS, Bucher JR, Thayer KA. Environ Health Perspect. 2014 Jul;122(7):711-8. doi: 10.1289/ehp.1307972.
>
> *Intersection of Systematic Review Methodology with the NIH Reproducibility Initiative.* Thayer KA, Wolfe MS, Rooney AA, Boyles AL, Bucher JR, Birnbaum LS. Environ Health Perspect. 2014 Jul 1;122(7):A176-7.
>
> *Implementing systematic review at the National Toxicology Program: status and next steps.* Birnbaum LS, Thayer KA, Bucher JR, Wolfe MS. Environ Health Perspect. 2013 Apr;121(4):A108-9.

The "NavGuide" from the University of California is a systemic review method consistent with the OHAT approach:

> Woodruff TJ, Sutton P. *The Navigation Guide systematic review methodology: a rigorous and transparent method for translating environmental health science into better health outcomes*. Environ Health Perspect. 2014 Oct;122(10):1007-14.

The National Research Council in its 2014 report complimented the EPA IRIS program on its development of systematic review methods for its chemical evaluations. The NRC recommended that a systematic evidence-integration process be developed that considers all lines of evidence (i.e., human, animal, and mechanistic), systematically determine the strength of evidence (not weight of evidence) considering such aspects as consistency of exposure, evaluates study bias, and treats cancer and non-cancer outcomes more uniformly (that is, does not suppose that non-cancer effects have thresholds below which the risk is negligible) (NRC 2014, Section 6). The IRIS program responded very positively

with public workshops that included government and academic experts from the US and Europe, as well as industry representatives (for example, see EPA IRIS workshop October 2014).

Unfortunately, in stark contrast to the chemical assessment programs at EPA IRIS and NIEHS, the pesticide office has not conducted a systematic review process for integrating multiple data streams. It is critical that OPP do this in a rigorous and transparent manner. We recommend that the pesticide office fall into step with IRIS and the National Toxicology Program, adopting their systematic review process since it has already undergone significant inter-agency and public review and is now being successfully implemented. These agencies have moved beyond the unrealistically simplistic MOA/AOP cumulative assessment of the pesticide office.

The literature search was not systemic, did not include clear exclusion criteria, and failed to identify sponsorship

Early in a systematic review process the risk assessor is required to conduct a comprehensive literature search, gathering relevant information from the published, unpublished, and "grey" literature (publicly available government reports, etc.) as part of the literature search (NIEHS NTP 2015). The pesticide office has no systematic or transparent way of doing this, and it often seems as if the only studies that OPP reviews are the ones that the pesticide industry sponsors supply in support of the registration of their pesticide product. This leads to both a deficit in scientific quality and public trust.

In this assessment, the pesticide office did not establish clear and consistent criteria for excluding studies. OPP did attempt to divide studies by whether or not they were "registrant submitted" or from the "open literature." This is both meaningless and – worse - misleading. It seems as if the pesticide office is addressing legitimate concerns raised by NRDC and others that all of its assessments rely exclusively on industry-sponsored data, by being transparent about which studies are from industry and which are not. But, this is not so. In fact, the pesticide office's division of registrant-submitted and open literature does no such thing, since many of the studies from the open literature are industry-sponsored. OPP is fully aware that the public, including NRDC, is concerned about whether or not there is financial bias, that is, the sponsorship of the study. This is a legitimate concern, and financial bias is a well-recognized form of study bias that can affect study outcome (Mandrioli and Silbergeld, 2016; Barnes and Bero 1998; Bero et al 2007; Bero 2013; Lundh et al 2012) including in pesticides (Bero et al 2015).

By failing to identify financial bias properly, the EPA assessment gives the misimpression that because the results of the 'registrant-submitted' studies are within the mid-range of the results of the 'open literature' studies, then the industry-sponsored and non-industry studies produced similar results. They did not. The industry-sponsored studies are much less sensitive at identifying adverse effects, and therefore report much less protective lethal-dose (LD50) acute toxicity values. For example, see Figure 5-1 the way EPA has reported it (page 109) and when studies are identified by financial sponsorship:

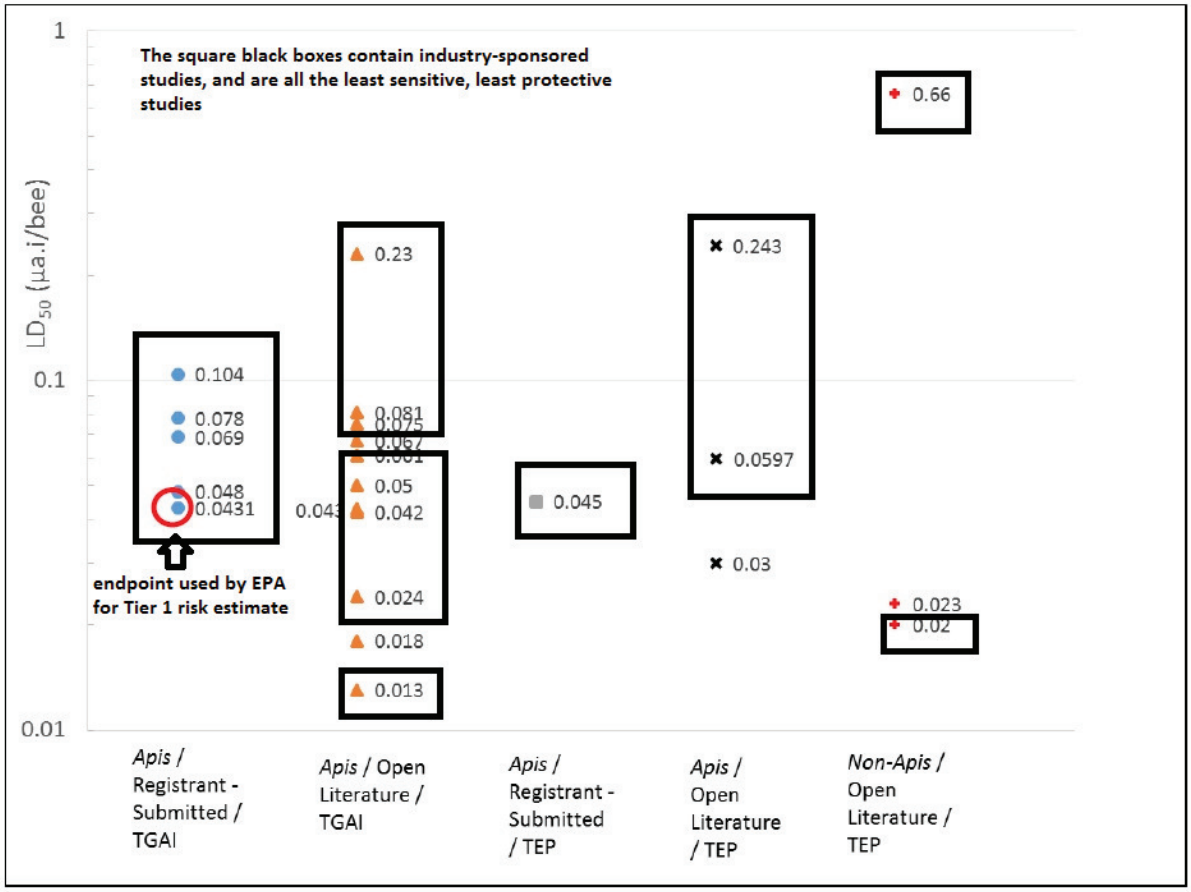


Figure 5-1. Scatterplot of adult acute contact toxicity of *Apis* and non-*Apis* bees from registrant-submitted and open literature sources conducted with technical grade active ingredient (TGAI) and formulated typical end product (TEP) imidacloprid. Red circle denotes endpoint used for Tier I risk estimation purposes.

Looking at the above figure, it is clear that by not quantitatively including non-industry studies, EPA has also disregarded studies with the most sensitive endpoints. The pesticide office is continuing to generate risk assessments that fail to reflect the best available science, instead relying almost exclusively on industry-sponsored data. Without a systematic review framework that includes study inclusion and exclusion criteria, the pesticide office simply cannot defend its study selections.

The pesticide office should exclude or downgrade studies that fail to find an effect (null association studies) that are statistically underpowered if they are inconsistent with the whole body of literature. The National Toxicology Program systematic review framework identifies some study aspects that would lead to downgrading the confidence rating in that study: risk of bias, unexplained inconsistency, indirectness in the relationship between a measured outcome and a health effect, imprecision, and publication bias serious enough to significantly decrease confidence in the body of evidence.

Ultimately, conclusions should be based on the whole body of literature, particularly where data is so sparse to begin with, and all the non-industry studies offer important information that can either be used quantitatively to calculate risk estimates, or qualitatively to support the use of additional Uncertainty Factors. Instead, in this assessment the pesticide office does the opposite, largely ignoring

the whole body of literature, and drawing its industry-favorable conclusions from industry-sponsored studies that are less sensitive and underpowered (see discussion below regarding the Tier II colony feeding study), leading to biased assessments that are inadequately protective and put pollinator health at risk.

Bias in the Tier II Colony Feeding Study Justifies Additional Uncertainty Factors and Consideration of Effects at Lower Doses

The EPA NOAEC for whole colony honey bee risks is 25 ppb, derived from an industry-sponsored long term feeding study (Tier II Colony Feeding Study MRID 49510001).[4] The problem is that all bee field trials are difficult to analyze unless the data are "fully crossed with factorial designs and mathematical models that consider the effects of multiple stressors together" (Sanchez-Bayo et al 2016), including pathogens, other insecticides and fungicides, and other non-chemical stressors including loss of habitat, climate change and food sources – and these analyses are, understandably, very difficult to do. The industry-sponsored study didn't do these analyses. Use of this study introduces significant and unquantified uncertainty, which EPA has failed to either discuss or address with uncertainty factors.

In addition to the limitations identified in the previous paragraph, there are a number of deficiencies in this study that all make it harder to detect an effect of the treatment, particularly at the low doses (the study is biased towards the null). If EPA persists in relying on this study for deriving risk estimates and setting exposure limits, then EPA should not dismiss effects at the lower doses, including the 25 ppb dose, and must add more uncertainty factors to account for its serious limitations.

The Tier II Colony Feeding Study MRID 49510001 reported colony-level effects following exposure to a sugar solution spiked with imidacloprid in concentrations of 12.5, 25, 50, 100, and 200 ug a.i./L for six weeks continuously.[5] There was a total of 84 hives, spread among 12 test areas in North Carolina. In each treatment area there was one hive per treatment group and two control hives, although data was not collected from all treatment areas, so statistical power was reduced (Appendix page 179). In addition to overwintering survival, the study reported on the percentage of frame coverage by various life stages (adults, eggs, larvae, and pupae), and the percentage of frame coverage by food stores (pollen and nectar). Additionally, hive weight data were collected. Effects in the highest two doses were so severe that the hives were removed from the experimental area to prevent other hives from robbing them (Appendix G, page 176).

There are significant problems with the study that introduces exposure misclassification, which bias the results towards the null (make it harder to detect an effect): [6]

- *Contamination of controls*: EPA reported that imidacloprid was detected at 0.56 ppb in one of the control solution for one control hive (Apiary H, colony 4) sampled on 12 July 2014 (Appendix G page 178). Any cross-contamination leads to exposure misclassification, which weakens the ability to detect differences between groups (i.e reduces the statistical power of the

[4] As reported in Appendix G. Supplemental Information for the Tier II Colony Feeding Study (MRID 49510001). US EPA. Imidacloprid Preliminary Pollinator Assessment Table of Appendices. EPA-HQ-OPP-2008-0844-0139
[5] As reported in Appendix G. Supplemental Information for the Tier II Colony Feeding Study (MRID 49510001). US EPA. Imidacloprid Preliminary Pollinator Assessment Table of Appendices. EPA-HQ-OPP-2008-0844-0139
[6] As reported in Appendix G. Supplemental Information for the Tier II Colony Feeding Study (MRID 49510001). US EPA. Imidacloprid Preliminary Pollinator Assessment Table of Appendices. EPA-HQ-OPP-2008-0844-0139

experiment). Contamination of control hives reduces the differences between control and treated hives and makes it harder to detect an effect, particularly at the lower doses.

- *Overlapping levels in pollen across treatment groups blurs differences between control and exposed groups*: EPA reported that the first sampling period of the exposure phase demonstrated a very wide range of imidacloprid concentrations in hive pollen (beebread) that overlapped to such a large degree that it would have made the different treatment groups almost indistinguishable from each other. The mean of the measured concentrations in bee bread within each treatment group of 12.5, 25, 50 and 100 ug/L was 2.86 (range: 0.77-5.34), 5.37 (range: 1.45-9.41), 10.84 (range: 4.2-19.41), and 17.89 ppb (range: 2.66-35.1), respectively. No information was provided for the 200 µg a.i/L treatment group. Why was no information provided for the highest treatment group? Note that pesticide levels in all groups overlap, reducing the statistical ability to reliably identify differences between each group, and certainly absolutely impossible to identify a clear and reliable dose-response relationship across groups – after all, in some cases there was no difference in exposure levels across treatment groups.
- *Overlapping levels in nectar across treatment groups blurs differences between groups*: This is the same problem as identified for pollen above. The mean of the measured concentrations in uncapped hive nectar within each treatment group of 12.5, 25, 50, 100, and 200 ug/L was 6.31 (range: 0.88-9.42), 13.24 (range: 1.19-20.53), 27.66 (range: 2.31-40.59), 46.87 (range: 2.1-80.15), and 109.14 ppb (range: 0.89-152.94) respectively.

Since EPA failed to report the statistical power of the experiment, it is unknown how big the effect would have to be at the lower doses before it would be statistically significant – certainly the effect would have to be quite large given the amount of exposure misclassification. EPA should report the statistical power of the experiment, and, importantly, EPA should NOT disregard effects at the lower doses. Unfortunately, and without scientific justification, EPA disregarded statistically significant effects at the two lowest doses for the following reasons: lack of strong dose-responsiveness, did not persist across multiple assessment times, or did not persist after overwintering (Appendix G, p 125, 187). Given the exposure misclassification bias (cross-contamination among dose groups) and other limitations in the study it is doubtful that the study had enough statistical power to detect distinct differences across exposure groups; neither EPA nor the study authors have provided any power calculations to suggest that they could have detected a dose-response, if one were to exist. Moreover, EPA has not provided any scientific data to support its expectation that there would be a dose-response. Adverse effects include immune suppression and susceptibility to pathogen infection – these multi-stressor effects are highly complex and poorly understood in bees. Dismissing statistically significant effects at lower doses is simply unjustified and unsupported by evidence.

After the overwintering period, only surviving hives in four apiaries were sampled (Apiaries E, I, J, and L; Appendix G page 179). EPA did not report on why the rest of the data was not collected. Why would data from overwintering not be collected on hives from half of the experimental groups? In addition, no measurements were provided for treatments at 200 ug/L (Appendix G page 179). Why not? How does EPA explain why these data – on half the experimental apiaries – was not collected? Would the additional data have altered the outcome? How did the study's industry sponsorship bias the design and conduct of the study? Studies conducted on animals that do not collect data are unethical; "Do not conduct unnecessary or poorly designed animal experiments" (Resnick, 2015).

The EPA reports that there "appeared to be a great incidence of Nosema across the control and all treatment groups, particularly at 100 ug/L treatment group" (Appendix G, page 181) But, EPA fails to discuss at all the dramatic lack of Nosema in the 25 ppb group (Appendix G Fig. G-3) – how does EPA

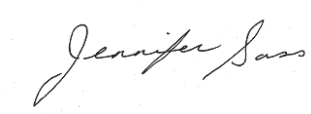
explain this? The level of Nosema is not independent of the level of pesticide, as demonstrated by the apparent dose-response in the other treatment groups, and also by the scientific reports linking bee pathogens and diseases with pesticides (Sanchez-Bayo et al, 2016; Goulson et al 2015). Failure to explain such dramatic outliers that are relevant to the test material puts into question the whole reliability of the study.

EPA failed to consider risk of bias in its review of this study. Unfortunately, this study suffers from a number of biases including financial bias (sponsored by the industry that profits from the test material), design bias (exposure bias as detailed above), reporting bias (failed to report on half the overwintering apiaries and the hives at the highest dose), and detection bias (no blinding or masking of outcome assessors; failure to report selected data). EPA OPP has no systematic pre-stated criteria for scoring study bias, making its study selections for calculating risk estimates, identifying MOA/AOPs, and reducing default protective factors - all based on industry studies - scientifically indefensible and arbitrary.

Despite all these problems with the study, EPA reported significant effects at the lowest dose (12.5 ug/L) and second lowest dose (25 ug/L). The 25 ppb treatment is clearly not a no-effect level, even by EPA's own analysis. EPA dismissed the significant effects at 25 ppb with the excuse that the effects didn't last over the winter, or lacked a clear dose-response relationship. It is unclear when either of those criteria were defined as a requirement for an adverse effect under the study conditions. In fact, because the study was designed and conducted in such a way that effects at the lower doses would be almost impossible to detect, it is simply irresponsible and unjustifiable to then disregard statistically significant effects when they are detected. It is as if the study is reaching into a haystack looking for needles, with the lower doses being fewer reaches (fewer chances of finding needles), and the higher doses being more reaches (more chances of finding needles). The study reported several significant needles that were found at the two lowest doses (12.5 and 25 ppb), but EPA disregards them because they didn't report a linear increase in the number of needles found at each increasing dose. EPA is re-calculating reality, but unfortunately bees live in the real world, not the one that EPA calculates. According to an international review of over a thousand scientific studies, bees are being harmed by neonicotinoid pesticides as used in the real world (WIA 2015).

If EPA is going to rely on this study, it should consider the lowest dose to be a LOAEC since significant adverse effects were reported at that doses, and since the study therefore did not have a true no-effect level. EPA should then add an additional uncertainty factor of at least 3X for failure to identify a true no-effect level, and an additional uncertainty factor of at least 10X for overall Uncertainties with the study, as detailed above.

Respectfully Submitted,



Jennifer Sass, Ph.D.
Natural Resources Defense Council

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