Assessment of the report:
Relative contribution of the Varroa destructor mite, selected Agricultural pesticides and other risk factors to Dutch honey bee losses for winter 2011-2012 by Romee van der Zee, Alison Gray, Jaap Kerkvliet, Lennard Pisa and Theo de Rijk

vanderZee

The study in question was a one season monitoring study (winter 2011-2012) in the Netherlands. Data were modelled to determine potential relationships between a variety of factors and colony loss. Data used included information from bee keeper surveys in 2010/2011 on colony losses in the winter of 2010-2011 (used in "random effects" modelling), samples collected at the end of july 2011, august 2011 and October 2011, and data from bee keeper surveys in 2011/2012 on colony losses in the winter of 2011-2012. In this type of study only correlations can be determined, as there are no positive or negative controls and a wide variety of variables cannot be controlled. A very large number of replicates and a longer timeframe would be more useful for drawing conclusions from such a study. For example, the study Genersch, et. al. 2010, was a similar study in Germany, but conducted over 4 years and with 1200 colonies. In contrast, the present study focuses on one year (2011-2012) and uses 86 colonies. This makes it more difficult to control for such variables as extreme weather, yearly changes in land-use patterns, yearly fluctuations in natural parasite levels and yearly fluctuations in bee colony strengths themselves. These variables are in addition to the wide variety of uncontrolled variables inherent to such a study, i.e. varying beekeeper policies, self-reporting of colony losses (survey data), and implicit bias due to factors looked for and which can be observed. As a result, it is difficult to make concrete decisions based on such a study, particularly the one in question, which is relatively small and short-term.

The authors state that *The statistical analysis was limited to the group of neonicotinoids rather than all the pesticides, because only this group was present in a sufficient number of samples (table 6).* However, Table 6 shows that a number of varroacides were found in pollen samples at high enough levels (it is not clear how many is "sufficient", however acetamiprid was found in 3 pollen samples and 1 bee, so it is assumed that at least 3 is enough). These varroacides were found mainly in pollen, which is not surprising considering their tendency to move to the pollen/bees wax and lack of water solubility. In addition, the insecticide piperonyl-butoxide (mainly used non-professionally) was found in 6 pollen samples, and the fungicide propioconazole was found in 2 samples. The varroacides and propioconazole were found at higher levels than than acetamiprid. The neonicotinoid insecticides appear to have relatively lower limits of detection than most of the other tested substances. Taken together, it seems that the reasoning behind only analyzing the correlation with imidacloprid/acetamiprid/thiacloprid and colony loss should be further supported.

The authors point out that the summer of 2011 (particularly July and August) was particularly bad for foraging. This is evidenced by the number of colonies where pollen presence was "nearly absent" or "absent" in august of 2011 (40). However, the authors found no correlation between this lack of pollen presence and colony loss in the winter of 2011-2012. This is probably because, as the authors themselves note, most of the bee keepers had begun supplementing the hives with alternative food sources. Interestingly, the authors later propose that correlation between the presence of acetamiprid and thiacloprid and colony losses may be exacerbated due to starvation of the bees, who then foraged on treated crops they might otherwise avoid, at which point toxic levels of acetamiprid and thiacloprid were reached (referring to the laboratory studies by Laurino, 2011). This conclusion is confounded by the fact that the majority of bee keepers will (and did) provide an alternative (and closer) food source for their bees under starvation circumstances. Additionally, the type of supplementary diet provided by the bee keepers has been shown to have an effect on bee

colony health (Brodschneider and Crailscheim, 2010), adding yet another variable to the study. In addition, long non-foraging periods and insufficient and untimely feeding of carbohydrates after harvesting honey make starvation one of the most common reasons for colony winter mortality (Brodschneider et. al., 2010; vanEnglesdorp et. al., 2010 – as from Brodschneider and Crailscheim, 2010), and it is unclear if these variables are controlled for (or investigated) in the study at hand. Finally, although the authors mentioned that the late summer of 2011 was particularly poor for foraging, they do not mention that the winter of 2011-2012 was also quite a severe one, nor do they look for a correlation between areas with highest rainfall or lowest temperatures and colony collapse (or at least this is not reported).

Although a total of 86 colonies were sampled for this study, only 37 of them contained imidacloprid, acetamiprid or thiacloprid. It is unclear the number that contained acetamiprid and/or thiacloprid. The small sample number (and only one sampling time) further impedes drawing conclusions as to the relevance of the presence of acetamiprid or thiacloprid in colony survival. The presence of imidacloprid or it's metabolites was not correlated with colony collapse.

The highest correlation between colony loss and a single factor was found for the presence of *Varroa destuctor* in October 2011. This is in line with other similar studies (Grenscher et al, 2011), unlike the correlation between pesticides and colony loss, which has not been shown previously. The authors report that there was a correlation between *Varroa* infestation and colony loss and that *Varroa* infestation rates were calculated for 81 of the 86 colonies. Figure 2 seems to suggest that the presence of *Varroa* in October is the only certain corollary, while the other three have rather large confidence intervals, making the conclusion of correlation less certain. However, the figure is inadequately explained, making interpretation difficult.

Interestingly, a correlation between colony loss and the presence of *Brassicaceae* in bee bread was determined in the study. The authors do not offer a conclusion as to why this might be the case, but do note that the neonicotiniods tested in the study were not authorized for use in brassicaceae at the time of the study. This would seem to suggest other factors at play or a lack of robustness of the model. The lack of information on land use patterns is a severe limitation here, as no real conclusions can be drawn about quality and type of honey bee forage in the areas of greatest colony loss.

The authors report that there was no cross-correlation between the four factors that correlated with bee colony collapse (varroa, acetamiprid/thiacloprid, brassica and "random effect values at a post code level"). This seems strange since all evidence to date implies that colony collapse is a multi-faceted problem. A better description of the data analysis might assist in interpreting this result.

To summarize, although it is agreed that real-world monitoring is important to understanding population-level effects on desirable species, this study does not offer any information to suggest that re-assessment of the registrations of acetamiprid and thiacloprid in the Netherlands is warranted. It is doubtful whether such a reassessment would result in any change in the registration of these plant protection products in the Netherlands. Certainly the current study does not offer any new information to support a change in registration status. On the contrary, it supports the idea that a wide variety of factors can significantly affect honey bee populations and that the problem of colony collapse is a highly complex one and that natural variables such as bee nutrition and forage quality are particularly important, but difficult to assess or control.

Brodschneider R and Crailsheim K (2010). *Nutrition and health in honey bees*. Apidologie 41: 278-294.

Brodschneider R, Moosbeckhofer R, Crailsheim K (2010). *Surveys as a tool to record winter losses of honey bee colonies – a 2-year case study in Australia and South Tyrol, Tyrol.* J Apic Res 49:23-30.

vanEngelsdorp D, Hayes J, Underwood RM, Pettis JS (2010). A survey of honey bee colony losses in the United States, fall 2008 to spring 2009. J Apic Res 49: 7-14.

Assessment of the report:
Pesticide Residues and Bees – A Risk Assessment
by Francisco Sanchez-Bayo and Koichi Goka, published in April 2014 in PLOS One, 9(4).


PlosOne2014.pdf

The authors present a risk assessment for honey and bumble bees based on measured residues as reported for the most part in the public literature and toxicity data from both public literature and regulatory databases. The risk assessment takes into account residue levels and prevalence of residues (number of times measured) to account for exposure. An acute risk assessment is then performed for forager and nurse adult bees and for larvae based on time to reach the oral LD50 (so-called T50 in days) compared to lifespan. The authors also perform a cumulative risk assessment for imidacloprid and thamethoxam in honey, only, and suggest that such a risk assessment should be performed for all substances which have cumulative toxicity. Additionally, they assess the risk from some mixtures by adjusting the LD50 of the insecticide by a "synergistic factor" and proceeding with the risk assessment as outlined above.

The synergistic factors in question are derived from the ratio of the decrease in the LD50 as reported by Iwasa et. al. 2004 and Pilling et. al. 1993. Thus, they focus solely on EBI fungicides and insecticides (neonicotinoid and pyrethroid, respectively). The authors do not perform any assessments for herbicides since "they are non-toxic to bees, i.e. LD50 values above 100 or 200 µg/bee" (p. 3). Additionally, they do not perform a risk assessment for combinations of insecticides since "the effects of insecticide mixtures are additive, not synergistic" (p.13).

As regards the risk assessment methodology pertaining to single substances, it is noted that many of the "new" aspects of the risk assessment are also included in the 2013 Bee Guidance, including assessment of nurse and forager adult bees and larvae and an assessment of potential cumulative toxicity. In addition, chronic toxicity and sublethal effects that can be clearly linked to colony-level effects (hypopharengael gland changes) are to be assessed in the new Guidance. In addition, the majority of the potential refinements in the Guidance focus on refining the exposure via measurement of residues in pollen, nectar and guttation water, as well as, potentially in the future, refinements based on landscape-level use and residues analysis. Thus, many aspects discussed in this paper will be a part of the regulatory risk assessment at some point in the future. It should be noted, however, that looking at the "prevalence" of residues in a world-wide or EU-wide scale and adjusting the exposure based on both residues and prevalence would be both difficult to implement (no residues prevalence for new substances, for example), and possibly not within the purview of the regulatory authorities, legally-speaking. Despite the focus on the importance of the residue prevalence in ascertaining the risk to bees, the authors themselves note that the toxicity of the substance drives the risk in the majority of cases.

As regards the methodology used to assess the "mixture toxicity" from EGI fungicides and insecticides (only neonicotiniods and pyrethroids), this is not feasible in a regulatory setting. It depends on the so-called "synergism factor", as reported in two published studies comparing toxicity of insecticides with and without simultaneous EGI fungicide exposure. These studies themselves have been discussed in our previous report on synergism and will not be discussed at length here. It is of note that the levels required for synergism to occur are not accounted for in this risk assessment. Thus the levels used in the studies quoted are accepted as being appropriate, and/or it is assumed that synergism will occur at any level of exposure (of both compounds). This would be unacceptable in a regulatory setting as a range of concentrations would be necessary to show at what level(s) synergism was

important and set an appropriate ratio or “synergism factor” based on these data (just as a toxicity test requires a variety of concentrations to find the appropriate LD50). It would also not be feasible, as the derivation of the “synergism factor” requires testing all compounds for synergism in order to derive such ratios for use in regulatory risk assessment.

As it stands, the only synergistic effects found have been between the EGI fungicides and insecticides. The publication in question gives a “risk” from these combinations, but this is based on a wide variety of less certain (and non-standard) data, thus the actual risk remains unknown. Adding an additive risk assessment to the standard risk assessment would be the most feasible manner in which to add the most “wins” as far as combinational toxicity is concerned, as opposed to attempting to assess synergism for each and every combination of substances.