Phytopharmacovigilance to protect bee health

Currently, 35% of crop species depend to some extent on pollinating insects for reproduction. Of these cultivated plants, some are totally dependent on pollinating insects, e.g. bananas and kiwis, while in other cases, the pollinators contribute to quality (e.g. strawberries) or yield (e.g. coffee). This crop pollination "service" is provided primarily by the honeybee (*Apis mellifera*), the only insect species with the population numbers necessary to pollinate large plots of cultivated land. Wild pollinators are also involved in the pollination of wild flowers, particularly weeds growing alongside crops on the same plot.

However, insect populations are plummeting, particularly in agricultural areas. The development of intensive farming techniques, for example, has impacted the population of pollinating insects through agricultural practices such as ploughing, or through landscape fragmentation, which has destroyed or scattered many of the secondary habitats (hedges, copses) used as insect refuges. Among the factors studied, plant protection products have also been cited. It is therefore essential to take account of the need to protect pollinating insects when assessing plant protection products, before placing them on the market, and also to monitor any adverse effects when these products are used. This is the role of phytopharmacovigilance.

Reminders relating to phytopharmacovigilance, partners and data mobilised for bee studies

Phytopharmacovigilance was created by the Act on the future of agriculture, food and forestry (2014). Its role is to identify and monitor adverse effects arising from the use of plant protection products[1].

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) has identified the health of the honeybee as a key priority in phytopharmacovigilance. To this end, ANSES has decided to consolidate the reporting and analysis of data collected, to establish the characteristics of the studies to be carried out to supplement the information available and to step up surveillance of the adverse effects of plant protection products on the health of honeybee colonies. The initial standpoint of ANSES was that protecting honeybees would also have a positive impact on wild pollinators,

for which far fewer data are available on exposure and effects.

ANSES is currently mobilising two types of complementary data to monitor the effects of plant protection products on bees.

To study the exposure of bee colonies, it is collecting data on hive contamination by plant protection products. This contamination is identified by looking for plant protection products in the food resources stored by bees in the hive (pollen, honey, bee bread), in bee wax or in the bees themselves, whether found dead or still alive.

These data are produced primarily by the ITSAP (Technical and Scientific Institute of Beekeeping and Pollination[2]) and its partners from the UMT PrADE research unit[3], within the Observatory for Pesticide Residues in the Bee Environment[4].

1. See Vigil'Anses n°3, p8 (https://vigilanses.anses.fr/sites/default/files/Vigil%27Anses-N3_Octobre2017VF_0.pdf)

2. Institut technique et scientifique de l'apiculture et de la pollinisation, http://itsap.asso.fr/

3. Joint technological unit for the protection of bees in their environment), bringing together the ITSAP – Beekeeping Institute, the Bees and Environment unit of the INRA (Agricultural Research Institute)-association for the development of beekeeping in Provence, the Terres Inovia research institute and Acta – federation of agricultural institutes

4. See the annual report of the ITSAP – Beekeeping Institute 2016-2017, p41-43 (http://blog-itsap.fr/wp-content/uploads/2017/05/

Additional data may be obtained from other sources such as the Résabeilles[5] programme set up to monitor bee mortality.

As part of the research programmes conducted since 2012, the ITSAP-Beekeeping Institute and its partners have taken samples from bee matrices, from inside the hive (the bees themselves, dead or alive, trap pollen, bee bread, honey, wax) and from the outside (pollen and flower nectar). At the same time, levels of plant protection products have been quantified using multi-residue methods of analysis. These analyses make it possible to check for the presence of several hundred substances in a single sample. The ITSAP-Beekeeping Institute has made available to ANSES the sample analysis results presented in Table I.

Table I : Results of samples taken from various bee matrices

Matrix	Number of samples	Years of samples	Number of hives sampled
Trap pollen (sampled at the entrance to the hive)	1,007	2014 – 2018	181 hives in 61 apiaries (Aquitaine, Provence – Rhone-Alps, Occitanie, Centre, Corsica)
Bee bread (a mixture of pollen and nectar, stored in the hive)	356	2012 – 2014	356 in 48 apiaries (Aquitaine, Provence, Occitanie)
Honey (stored in the hive)	109	2014 – 2016	27 in 27 apiaries (Centre, Aquitaine)

However, screening a large number of molecules is detrimental to sensitivity, since the analyses may not pick up certain substances present in low concentrations. In some cases, therefore, these analyses may target specific classes of substance, in order to increase sensitivity. For example, neonicotinoid insecticides are known to have sublethal effects at very low doses. Targeted tests were carried out to quantify the presence of these substances, even in low concentrations, in the nectar and pollen of the flowers consumed by bees.

ANSES is also reviewing data on the acute mass mortality of adult bees based on assumed poisoning by products and practices implemented for plant production, biocidal or medication purposes. These data are taken from statements made by beekeepers to their *Direction départementale de la cohésion sociale et de la protection des populations* (DDCSPP, Departmental Directorate for Social Cohesion and Population Protection) after observing deaths in their colonies. Investigations are then implemented and coordinated by the DDCSPP, which appoints a veterinarian to establish the cause of the deaths observed. In the event of suspected poisoning by plant protection products, the investigation seeks to identify plant protection practices around the apiaries concerned and to test for the presence of plant protection products in the dead bees or in samples taken from other matrices in the colonies affected. Based on the criteria set out in the technical instruction, a diagnosis of poisoning is made when a substance is found in dead bees in concentrations equal to or greater than the lethal dose required to kill 50% of the population, or when the expert appraisal concludes that the substance(s) quantified is/are responsible for bee mortality. In this case, beekeepers may request compensation.

The procedure put in place in 2002 is governed by a technical instruction regularly updated by the Directorate General for Food[6], which also centralises data at national level. These data have highlighted the acute effects of certain plant protection substances on bee colonies, leading to high mortality levels and even depopulation (when the population decreases without bees being found dead).

5. The Résabeilles programme is the French component of the European Epilobee surveillance system, coordinated by the ANSES laboratory at Sophia-Antipolis (https://www.anses.fr/fr/content/le-programme-europ%C3%A9en-epilobee)

6. Technical instruction DGAL/SDQPV/2018-444

Example of the use of phytopharmacovigilance data in updating regulatory provisions to protect honeybees and wild pollinating insects

Recently, the data collected for purposes of phytopharmacovigilance were used to assess the regulatory provisions in place to protect honeybees and wild pollinating insects, on the request of the French Ministry of Agriculture. A ministerial order of 2003[7] currently bans the use of insecticides and acaricides on forest stands (hedges, copses, woods, forests) or on crops visited by these insects during flowering or the production of exudates (plant transpiration that may represent a significant source of water for bees, and that can be contaminated by plant protection products). A small number of products receive specific *bee* labelling with their marketing authorisation and can be applied during these periods, but only at certain times of day. Products that have received this special waiver can only be applied early in the morning or in the evening, when the bees have returned to their hive for the night and are no longer active. In 2013, ANSES even recommended that these products be applied only after sunset and not in the morning, in order to limit the risk of bee exposure still further.

Today, data collected through the monitoring of mass mortality show that acute poisoning is not caused solely by insecticides or acaricides. A number of fungicides (azoxystrobin, cyprodinil, fludioxonil, pyrimethanil, trifloxystrobin, fenhexamid, carbendazim, tebuconazole, prothioconazole, difenoconazole), and herbicides (2,4-D, fluazifop, glyphosate), which in principle have low toxicity for bees, have been implicated in cases of mortality, at levels compatible with substance poisoning. Fungicidal and herbicidal substances have also been found in bee matrices. Data provided by the Observatory for Pesticide Residues and the ITSAP-Beekeeping Institute on bee matrix contamination show many co-occurrences of plant protection substances in samples of trap pollen. For example, in a study of 893 samples collected from 156 bee colonies in 34 apiaries between 2014 and 2017, 75.7% samples were contaminated by at least one plant protection substance.
Overall, 143 substances were detected or quantified: 50 insecticides, 24 herbicides, 59 fungicides, 7 acaricides and 3 growth regulators. Of the 12 substances found most frequently, 10 are fungicides (e.g. tebuconazole, boscalid, difenoconazole, captan, cyprodinil, dodine) and two are insecticides (chlorpyrifos-ethyl and tau-fluvalinate). Of the 15 substances present in the highest concentrations, eight are fungicides (e.g. tetrahydrophthalimide, captan, dodine and boscalid), six are insecticides/acaricides (e.g. phosmet, dieldrin and thiacloprid) and one is a herbicide (metribuzin). On average, 4.3 different substances were found in a single sample, but co-exposure could increase the total to 22.

Data from the Résabeilles study showed that, in the apiaries monitored, 86.2% of the honey and 94.6% of the bee bread was contaminated by at least one of the 60 substances screened for by this study.

It therefore appears that bees and their larva are exposed to a significant number of plant protection substances, particularly fungicides. Fungicidal substances belonging to the imidazole-triazole groups are also frequently found, even though their harmful synergistic effect with insecticides has been described in the literature.

In view of this information, ANSES is recommending an extension of the regulatory framework, which currently concerns only insecticides and acaricides, with a view to banning the application of all categories of plant protection products (herbicides, fungicides, insecticide, acaricides) during the period of flowering or exudate production of the crops treated, to avoid the presence of residues in the pollen and nectar collected by the bees.

7. Interministerial order of 28 November 2003 on the conditions of use of insecticides and acaricides for agricultural usage with a view to protecting bees and other pollinating insects

Conclusions and outlook

The conclusions of ANSES highlight the practical importance of having access to data collected after the placing on the market of plant protection products based on synthetic active substances or micro-organisms (bacteria or fungi) in real field conditions. To monitor the adverse effects of plant protection products on bee health, phytopharmacovigilance draws upon data from in-situ monitoring of apiaries as well as on vigilance data, primarily as provided by the scheme for surveillance of the acute mass mortality of bees, put in place by the Directorate General for Food. The role of these complementary data sets is to provide a basis on which to monitor the adverse effects of plant protection products observed in real conditions of use and also to prepare for them, based on hive exposure to plant protection products, as shown by the contamination of bee matrices.

The programme currently implemented by the Directorate General for Food whereby beekeepers are able to declare acute mass mortality of their bees to the DDCSPP, will be incorporated into a broader observatory: the Observatory for Honeybee Mortality and Weakening (OMAA). This new scheme makes it possible to declare disorders other than mass mortality, relating, for example to depopulation or bee brood. Also managed at departmental level, the OMAA[8] is able to undertake several investigative procedures based on any disorders observed in bee colonies, but all data will be centralised by the Directorate General for Food. The first test phase took place in 2018 in two regions (Bretagne and Pays de la Loire). The programme was extended in 2019 to other candidate regions. This observatory will extend the surveillance of acute effects (mortality) by monitoring sublethal effects illustrated by other disorders.

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8. See the web page on the ANSES epidemiological surveillance platform for animal health website : https://www.plateforme-esa.fr/page/observatoire-des-mortalites-et-des-affaiblissements-de-l-abeille-mellifere-omaa