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Biomarker study shows health effects of fungicide on honeybees, including DNA damage

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New research finds that a common agricultural fungicide can have toxic effects on honeybees at standard concentrations. This study uses a biomarker method to identify cell and chemical changes in honeybees after exposure to one fungicide, one toxic metal and one toxin known to cause genetic damage; with such changes indicating stress on their biological functions. This method has not previously been used to show these effects in honeybees after exposure to fungicide and highlights the potential for further research using biomarkers.

Many issues, such as pesticide use and habitat loss, are endangering honeybees worldwide. The effect of insecticides on honeybees (Apis mellifera) has been widely studied and in 2013 the European Commission restricted the use of products containing three damaging insecticides (neonicotinoids) to protect honeybee health. However, honeybees are also exposed to herbicides, fungicides and heavy metals, with a 2019 study in France, for example, finding that half of the pesticides present in pollen were fungicides[1].

Little is known about the sublethal health effects of fungicides on honeybees; however, it is important to investigate toxicity of substances that they may encounter in the environment, which may cause long-lasting changes in population health. To address this, the study identifies if, and how, honeybee chemical activity and cells change after exposure to contaminants, focusing on the common fungicide Amistar® Xtra[2], the metal cadmium, and the toxin Ethylmethane sulfonate (EMS). Although Amistar® Xtra does not kill honeybees at typically used concentrations, its health effects — including DNA damage — could still be significant.

The researchers developed a study method using biomarkers — indicators of an organism's health and biological state — that have been little used in insects. They collected 300 honeybees from hives in Pisa, Italy, and split them into six groups of 50. Two groups were directly exposed to the fungicide Amistar® Xtra at concentrations recommended for use on cereal crops (100 g per litre of water and 200 g/L). Cadmium was also applied to two groups at concentrations that might be found in the environment (0.1g/L and 2.5g/L). Thirdly, EMS, a known genotoxic compound, was used as a positive control for 50

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Biomarker study shows health effects of fungicide on honeybees, including DNA damage (continued)

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bees (at 12.4 g/L, 24.8 µg per bee). The final group received no treatment except a tiny dose of acetone, which was used in a small amount in the other treatments to aid absorption.

After five days, no bees had died, confirming that the doses were sublethal. The researchers then looked for certain chemicals, cells and changes in each bee's head, gut and haemolymph (invertebrate equivalent of blood) that serve as biomarkers, indicating a range of biological responses. They aimed to test a range of biomarkers to develop a method that could be replicated in further research.

Acetylcholinesterase (AChE), an enzyme involved in neural transmission, was significantly inhibited at the higher fungicide dose and at both cadmium doses. This is the first study to show the neurotoxic effect of a fungicide on AChE in honeybees, say the researchers, and confirms previous studies showing that metals may inhibit AChE.

Meanwhile, treatments increased the activity of chemicals involved in detoxification processes; the enzyme glutathione-S-transferase (GST), for example, was significantly raised at the higher dose of fungicide. The digestive enzyme alkaline phosphatase (ALP) was inhibited in all treatment groups, indicating metabolic toxicity, with the strongest decrease seen under cadmium treatments. Cadmium may form insoluble clusters with proteins, suggest the researchers, or bind to the enzyme. Lysozyme, an enzyme that plays an important role in immune function, was also inhibited by treatment, especially cadmium and EMS; while plasmocyte immune cells (which have not been studied before in honeybees exposed to these contaminants) showed slight decreases in bees receiving the highest doses of cadmium and fungicide.

After all treatments, the researchers observed a general increase in nuclear abnormalities, indicating DNA damage. The testing method is novel for honeybees and is validated by EMS showing the strongest effect, highlight the researchers. While the genotoxic potential of cadmium was known, the fungicide results are new. The higher fungicide dose (of 200g/L) is used on cereal crops and sunflowers, note the researchers, and the results show that this level of exposure could be genotoxic, causing irreversible changes.

The researchers quantified the combined effects of each compound with a biomarker response index[3], visualising the induced/inhibited values (compared to control values) in spider graphs. EMS showed the greatest overall effect — a result that was not unexpected (as it was used as a positive control) — followed by cadmium and fungicide, with lower doses giving lower overall values.

The researchers emphasise the effectiveness of the biomarker approach for investigating the effect of plant protection products on non-target organisms such as honeybees. They call for further investigation of the effects of agricultural fungicides on non-target organisms, recommending that research could use the set of biomarkers tested in this study.

1. Prado, A., Pioz, M., Vidau, C., Requier, F., Jury, M., Crauser, D., Brunet, J.-L., Le Conte, Y., Alaux, C. (2019) Exposure to pollen-bound pesticide mixtures induces longer-lived but less efficient honey bees. *Sci. Total Environ.* 650: 1250–1260.

2. Mainly composed of azoxystrobin and cyproconazole; among the most frequently applied types of fungicide globally (e.g. Bartlett, D.W., Clough, J.M., Godwin, J.R., Hall, A.A., Hamer, M., Parr-Dobrzanski, B. (2002) The strobilurin fungicides. *Pest Manag. Sci.* 58: 649–662.)

3. Sanchez, W., Burgeot, T., Porcher, J.-M. (2013) A novel "Integrated Biomarker Response" calculation based on reference deviation concept. *Environ. Sci. Pollut. Res.* 20: 2721–2725.