A worldwide survey of neonicotinoids in honey

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Growing evidence for global pollinator decline is causing concern for biodiversity conservation and ecosystem services maintenance. Neonicotinoid pesticides have been identified or suspected as a key factor responsible for this decline. We assessed the global exposure of pollinators to neonicotinoids by analyzing 198 honey samples from across the world. We found at least one of five tested compounds (acetamiprid, clothianidin, imidacloprid, thiacloprid, and thiamethoxam) in 75% of all samples, 45% of samples contained two or more of these compounds, and 10% contained four or five. Our results confirm the exposure of bees to neonicotinoids in their food throughout the world. The coexistence of neonicotinoids and other pesticides may increase harm to pollinators. However, the concentrations detected are below the maximum residue level authorized for human consumption (average ± standard error for positive samples: 1.8 ± 0.56 nanograms per gram).

Neonicotinoids are currently the most widely used class of insecticides worldwide (1). These pesticides are increasingly prevalent in terrestrial and aquatic environments (2, 3). Neonicotinoids are taken up by plants and transported to all organs, including flowers, thus contaminating pollen and nectar as well as any fluid produced by the plant (3). There are increasing concerns about the impact of these systemic pesticides, not only on nontarget organisms—especially pollinators such as honey bees (4–6) and wild bees (7, 8), as well as in other terrestrial and aquatic invertebrates (9, 10)—but also on vertebrates (11–14), including humans (15, 16). Impacts on such a broad range of organisms ultimately also affect ecosystem functioning (17). As a result, the pertinence of use of these pesticides is currently being questioned in many countries (18), with a ban now implemented in France, and alternatives proposed (19). However, despite increasing research efforts to understand the patterns of neonicotinoid uses and their effects on living organisms, we lack a global view of the worldwide distribution of neonicotinoid contamination in the environment (18) to evaluate the risk posed to living organisms. To build such a map, we measured neonicotinoid concentrations in 198 honey samples from different regions of the world.

Bees rely on nectar and pollen sources for their survival. Nectar is transformed into honey and stored in the hive for daily adult consumption and is essential for winter survival. A mature colony can be populated by up to 60,000 adult bees and therefore needs vast amounts of food. Individuals harvest nectar and pollen less than 4 km from the hive, on average, but may travel up to 12.5 km away (20, 21), which makes bees distinctive sentinels of environment quality. Indeed, the residue level of pesticides in honey from a hive is a measure of the contamination in the surrounding landscape (22). Honey samples are easy to obtain from a very broad range of geographical localities, thus enabling a worldwide analysis. Analytical protocols have been developed to analyze neonicotinoid concentrations in honey (23), and several studies have quantified the concentration of neonicotinoids in honey (24–26). However, the amount of data is limited, quantification thresholds vary among studies, and a global picture of neonicotinoid contamination in honey is lacking.

Here we present a global survey of neonicotinoid contamination in honey samples from all continents (except Antarctica), as well as numerous isolated islands. We measured the concentrations of five commonly used neonicotinoids—acetamiprid, clothianidin, imidacloprid, thiacloprid, and thiamethoxam—in 198 samples (tables S1 to S3) collected through a citizen science project (described in details in the supplementary materials). Overall, 75% of all honey samples contained quantifiable amounts of at least one neonicotinoid. This proportion varied considerably among regions, being highest in North American (86%), Asian (80%), and European (79%) samples and lowest in South American samples (57%) (Fig. 1, figs. S1 and S2, and tables S1 and S4). Thirty percent of all samples contained a single neonicotinoid, 45% contained between two and five, and 10% contained four or five. Multiple contaminations were most frequent in North America, Asia, and Europe and least frequent in South America and Oceania (table S4 and Fig. 1). Frequency of occurrence was highest for imidacloprid (51% of samples) and lowest for clothianidin (16%). Maximum and average concentrations among positive samples were highest for acetamiprid and thiacloprid (table S5).

The frequency of occurrence of individual neonicotinoid in honey samples and their relative contribution to the overall neonicotinoid concentration varied among the regions (Fig. 1). Imidacloprid dominated overall concentrations in Africa and South America, thiacloprid in Europe, acetamiprid in Asia, and thiamethoxam in Oceania and North America (Fig. 1), reflecting regional differences in usage of specific pesticide types. In all regions, at least one neonicotinoid was recorded in at least 25% of samples, and three neonicotinoids (thiamethoxam, imidacloprid, and clothianidin) were recorded in at least 50% of samples in North America (table S6).

The total concentration of the five measured neonicotinoids was, on average, 1.8 ng/g in positive (i.e., contaminated) samples and reached a maximum of 56 ng/g over all positive samples (table S4). This average concentration lies within the bioactive range (27, 28), causing deficits in learning (29, 30), behavior (31), and colony performances (8, 32) in honey bees (table S8). As for the percentage of positive samples, maximum, median, and average concentrations were highest in European, North American, and Asian samples (figs. S3 to S8 and table S4). Maximum residue levels (MRLs) authorized in food and feed products in the European Union (EU MRLs: 50 ng/g for acetamiprid, imidacloprid, and thiacloprid and 10 ng/g for clothianidin and thiamethoxam) were not reached for any tested neonicotinoid. The sum of percentages of EU MRLs for the five neonicotinoids reached 3.6%, on average, for all positive samples, exceeded 10% in eight samples, and surpassed 100% in two European samples (table S1).

Our global survey showed that 75% of all analyzed honey samples contained at least one neonicotinoid in quantifiable amounts and that these pesticides are found in honey samples from all continents and regions. Previous studies conducted at smaller scales (regional to national) reported a broad range of frequency of occurrence and concentrations of neonicotinoids in honey, depending on the compound, distance to neonicotinoid-treated agricultural field, and limits of detection. The percentage of positive samples is, to some extent, correlated with the detection limits (table S7). For example, in a British study (26), 16 out of 22 samples were positive for clothianidin, but for all of these samples the measured concentrations (0.02 to 0.82 ng/g) were below the detection limit of a Serbian study (1.0 ng/g) in which no sample tested positive (33). With the improvement of analytical methods, we can therefore expect that the proportion of positive samples will increase. Differences in methods and especially in limits of quantification (LOQ) render comparisons among studies of little relevance. Thus, to some extent,
our results illustrate that the ever-increasing analytical sensitivity allows detecting traces of pesticides where they previously were not detectable. But given the increasing use of neonicotinoid pesticides in the different regions of the world, despite partial bans such as the one implemented in the EU, it is also reasonable to expect contamination to have increased over time. Total bans, such as the one soon to be implemented in France, may reverse this trend in the future.

Although 75% of samples tested positive for at least one neonicotinoid, concentrations were, in all cases, below the admissible limits for human consumption according to current EU and U.S. regulations (i.e., MRLs). On the basis of our current knowledge, consumption of honey is therefore not thought to harm human health. However, recent evidence for impacts of neonicotinoids on vertebrates ([12, 13]), including humans ([15, 16, 34]), and especially evidence for up-regulation of nicotinic a4b2 AChRs receptors in the mammal brain during chronic exposure and for higher affinity of metabolites versus the parent neonicotinoid (imidacloprid) ([14]), could lead to reevaluating MRLs. Although the impact of the measured concentrations of neonicotinoids in honey on vertebrates, including humans, is considered negligible, a significant detrimental effect on bees is likely for a substantial proportion of the analyzed samples, as adult bees rely on honey for food, including during periods of overwintering or seasons without blossoming flowers. The increasingly documented sublethal effects of neonicotinoid pesticides at environmentally relevant concentrations on bees and other nontarget organisms include growth disorders, reduced efficiency of the immune system, neurological and cognitive disorders, respiratory and reproductive function, queen survival, foraging efficiency, and homing capacity at concentrations as low as 0.10 ng/g (table S8).

One of the challenges of assessing the risks associated with the use of pesticides is to evaluate their impact at field-realistic exposure concentrations. A total concentration of 0.10 ng/g, corresponding to the lowest concentration at which marked detrimental effects were observed on nontarget insects ([27] (table S8), was exceeded in 48% of our honey samples (table S1). Therefore, our results, combined with the growing body of evidence for detrimental effects on bees and other nontarget invertebrates, suggest that a substantial proportion of world pollinators are probably affected by several neonicotinoids. Another challenge is to evaluate the influence of chronic exposure to some neonicotinoids on nontarget insects’ sensitivity to other neonicotinoids. Recent studies showed an increased

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**Fig. 1. Worldwide contamination of honey by neonicotinoids.**

(A) Worldwide distribution of honey contamination by neonicotinoids. White symbols, concentration below quantification levels (<LOQ) for all tested neonicotinoids; colored symbols, >LOQ for at least one neonicotinoid; shading indicates the total neonicotinoid concentration (nanograms per gram). Pie chart insets: Relative proportion of overall concentration of each neonicotinoid by continent (legend in bottom inset). (B) Overall percentage of samples with quantifiable amounts of 0, 1, or a cocktail of 2, 3, 4, or 5 individual neonicotinoids. (C) Proportion of samples with 0, 1, 2, 3, 4, and 5 individual neonicotinoids in each continent. (D) Rank-concentration distribution of total neonicotinoids in all of the 149 samples in which quantifiable amounts of neonicotinoids were measured.
sensitivity to neonicotinoids after frequent or long-term exposure (37, 32).

Defining the thresholds below which neonicotinoids would not even have a sublethal effect under chronic exposure is much more difficult than assessing levels corresponding to short-term acute toxicity. Therefore, the proportion of samples that may affect bees cannot be ascertained based on current knowledge, but this study shows that pollinators are globally exposed to neonicotinoids, partly at concentrations shown to be harmful to bees. The fact that 45% of our samples showed multiple contaminations is worrying and indicates that bee populations throughout the world are exposed to a cocktail of neonicotinoids. The effects of exposure to multiple pesticides, which have only recently started to be explored (35), are suspected to be stronger than the sum of individual effects (38). This worldwide description of the situation should be useful for decision-makers to reconsider the risks and benefits of using neonicotinoids and provides scientists an inventory of the most frequent combinations of neonicotinoids found in honey (table S9). We urge national agriculture authorities to make the quantities of neonicotinoids and other pesticides used on their territories publicly available and also professionally available to epidemiologists at a much higher geographical resolution to enable correlative studies between local events and pesticide load.

REFERENCES AND NOTES

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SUPPLEMENTARY MATERIALS
www.sciencemag.org/content/358/6359/109/suppl/DC1
Materials and Methods
Supplementary Text
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References (36–79)