Toxicopathological Determination of Safe Dose Ranges of Neonicotinoids for Honey Bee Colonies

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Neonicotinoid insecticides are used as a seed treatment for a variety of crops worldwide, including canola. Those treated crops contain low residues of neonicotinoids in nectar and pollen, however the true impact on honey bee health is disputed and controversial. In a recent three-year project, researchers at the University of Saskatchewan determined safe dose ranges of neonicotinoids on honey bees. The results showed that honey bee colonies appear to tolerate environmental neonicotinoid contamination of nectar and pollen up to 5-10 ng/g; with no demonstrable effects of concentrations similar to those reported worldwide. However, chronic exposure to high environmental doses of 20 ng/g of neonicotinoids (recently reported in Saskatchewan honey and pollen) had negative effects on honey harvest and population of bees in exposed colonies.

Neonicotinoid insecticides are used as a seed treatment for a variety of crops worldwide and on the Canadian Prairies, including greater than 95 per cent of canola crops. Crops treated by systemic neonicotinoids contain low residues of neonicotinoids in nectar and pollen, however the true impact on honey bee health is disputed and controversial. In addition, reported levels of neonicotinoid residues in honey and pollen vary in different studies, crops, and geographic regions.

Researchers at the University of Saskatchewan initiated a three-year project to evaluate the effects of environmental doses of neonicotinoids on honey bee (*Apis mellifera*) colonies and individual honey bee castes. The purpose of this study was to determine the safe dose ranges of neonicotinoids on honey bees by toxicopathological evaluation. These specific tests are used for detection of sublethal toxic effects to determine if low residues of neonicotinoids (stored in honey and pollen) have potentially toxic effects on honey bees. The project included laboratory *in vitro* and field-based studies to determine the safe dose-range of neonicotinoids exposure in different life stages and castes of the honey bees, reproductive fitness, and on overwinter survival. Various experimental protocols were used to quantify effects on worker, drone, and queen honey bees exposed to neonicotinoids.

For the project, researchers included incremental doses that reflected both residue levels reported worldwide, and a recent Saskatchewan study based on a limited number of apiaries in the Saskatoon region that reported levels of neonicotinoid contamination of honey and pollen 5-10 times higher compared to the worldwide average of 1.8 ng/g (7.2 nM). It isn't clear why there is a discrepancy with the Saskatchewan study results and worldwide levels. The majority of honey bee colonies in Saskatchewan are exposed to vast

canola fields; they flourish and rapidly expand during canola blooming period and generate the highest honey harvest per colony in Canada. Accordingly, the accuracy of the recent study that reported the very high concentrations of neonicotinoids in Saskatchewan honey and pollen is put to question by the results of this three-year investigation conducted at the Western College of Veterinary Medicine, University of Saskatchewan. The project addressed three main objectives.

Objective 1. To determine effects of the incremental dose of the three most common neonicotinoids (imidacloprid, clothianidin and thiamethoxam) on development defects (teratogenicity) in larvae/pupae of workers, queens and drones and to determine their safe dose range.

A total of 68 honey bee colonies established from New Zealand packaged bees received weekly feedings of sugar syrup and pollen patties containing 0 nM, 20 nM (~5 ng/g) (~median Saskatchewan dose and high worldwide dose), or 80 nM (20 ng/g) (high environmental dose in Saskatchewan) of one of the three neonicotinoids. Colony weight gain, brood area and population size were collected and used as measures of colony performance. The study results showed that chronic exposure of honey bees to high environmental doses of neonicotinoids had negative effects on honey production and bee population. However, brood area appeared to be less sensitive to detect sublethal or teratogenic effects of neonicotinoids at the colony level exposure.

Objective 2. To evaluate the survival of adult worker honey bees, and the survival of worker brood when chronically exposed to the neonicotinoid insecticide thiamethoxam, or a combination of thiamethoxam and a fungicide prothioconazole.

For the study, larvae and pupae were reared in vitro (from hatching of early larvae to imago/adult stages of worker honey bees) and adult worker bees were kept in cages under laboratory conditions. Then, in vitro reared larvae/pupae and adult worker bees were exposed to the incremental doses of thiamethoxam alone and in combination with prothioconazole. This procedure allowed individual exposure of developing larva to an exact dose of neonicotinoid and quantification of any effects on development and survival. In addition, these procedures also allowed researchers to evaluate potential negative effects of combination of pesticides. Researchers assessed the combination of thiamethoxam with the fungicide prothioconazole because it has been suspected that fungicides (e.g. prothioconazole) present in nectar and pollen may block the bee detoxifying pathway (cytochrome P450) and predispose bees to higher mortality due to insecticide such as neonicotinoids (e.g. thiamethoxam) that are inactivated by cytochrome P450 pathway. The results indicated that chronic exposure to field-realistic doses of thiamethoxam and/or prothioconazole are unlikely to affect the survival of adult workers and brood. There were no additive effects observed of simultaneous exposure of worker adults or brood to the combination of neonicotinoid and this fungicide.

Objective 3. To correlate data generated from Objectives 1 and 2 to overwinter survival of honey bee colonies exposed to incremental doses of neonicotinoids and to determine the safe dose range.

Researchers first characterized the histology of the reproductive tract of queens and drones exposed to incremental doses of thiamethoxam to help define the effect these pesticides have on reproductive fitness, but also to establish a safe dose range to be used in the future. Trials were also conducted to determine if honey bee castes (workers, drones, queen) were affected equally by exposure to thiamethoxam. Overwinter survival trials were conducted in field colonies and in the laboratory on winter workers chronically exposed to dosages of thiamethoxam or clothianidin from 0, 5, 10, 20 to 100 ng/g.

The results showed that chronic environmental (5-20 ng/g) neonicotinoid exposure decreases survival of winter workers in the laboratory, but only chronic high dose (100 ng/g) thiamethoxam significantly decreases overwinter survival of colonies in the field. Honey bee castes are not equally susceptible/resistant to thiamethoxam during various age stages. In this study the queens were found to be most sensitive during larval and pupal stages (as per capping rate and survival to emergence), whereas workers and drones displayed negative treatment effects only at emergence or post emergence. The adult drones were consistently most sensitive to thiamethoxam contact exposure whereas adult queens were the most resistant.

Based on these studies, it appears that honey bee colonies can tolerate environmental neonicotinoid contamination of nectar and pollen up to 5-10 ng/g; with no demonstrable effects of concentrations similar to those reported worldwide. However, chronic exposure to high environmental doses of 20 ng/g of neonicotinoids, had negative effects on honey harvest and bee population in exposed colonies. The major recommendation of this study is to perform a large and sound scientific study that will document accurately the levels of neonicotinoid residues in honey and pollen collected from Saskatchewan canola fields. These evidence-based data will be useful for policy makers, canola producers, beekeepers, and consumers. Several scientific publications from this study have been published or are in development.

Scientific Publications

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