Quantitative Analysis of Neonicotinoid Insecticide Residues in Foods: Implication for Dietary Exposures

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ABSTRACT: This study quantitatively measured neonicotinoids in various foods that are common to human consumption. All fruit and vegetable samples (except nectarine and tomato) and 90% of honey samples were detected positive for at least one neonicotinoid; 72% of fruits, 45% of vegetables, and 50% of honey samples contained at least two different neonicotinoids in one sample, with imidacloprid having the highest detection rate among all samples. All pollen samples from New Zealand contained multiple neonicotinoids, and five of seven pollens from Massachusetts detected positive for imidacloprid. These results show the prevalence of low-level neonicotinoid residues in fruits, vegetables, and honey that are readily available in the market for human consumption and in the environment where honeybees forage. In light of new reports of toxicological effects in mammals, the results strengthen the importance of assessing dietary neonicotinoid intakes and the potential human health effects.

KEYWORDS: neonicotinoid insecticides, dietary exposure, pollen, honey

INTRODUCTION

A growing body of research shows that fruits and vegetables are critical to promoting good health. Fruits and vegetables are major contributors of nutrients, such as folate, magnesium, dietary fiber, and vitamins A, C, and K, and essential for disease prevention. Diets rich in fruits and vegetables also help adults and children to achieve and maintain a healthy weight.1 However, because of the wide usage of pesticides in agriculture, diet also becomes an important source of exposure to pesticides. Although relatively unknown to the public, neonicotinoids are the most commonly used insecticides in the world, which include acetamiprid, clothianidin, dinofeturan, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam. Additionally, flonicamid has been categorized as a neonicotinoid insecticide,2 although the mode of action is different from that of other neonicotinoids.3 These insecticides act as nicotinic acetylcholine receptor (nAChR) agonists, which cause insect paralysis to death. Advantages of neonicotinoids in pest control, including a broad spectrum of insecticidal activity, high receptor specificity for insects relative to mammals, and versatility in application methods, have led to the replacement of organophosphates, carbamates, and synthetic pyrethroids.4 They are now registered globally in more than 120 countries and extensively used in seed treatment (such as seed dressing or film coating) and soil treatment (by broadcast application, mechanical incorporation, soil drench, or soil injection) and are also directly applied to plant foliage for crop protection.5,6 Additionally, neonicotinoids have been used as insect control on pets or companion animals, such as termite and fleas.1,4,7

Because of neonicotinoids’ wide uses and their extreme toxicity to bees, neonicotinoids have been implicated in causing the steep decline of the global honeybee population and specifically colony collapse disorder (CCD).8,9 Neonicotinoids are systemic insecticides and water-soluble, which means that they have superb plant-systemic activity.4 When applied into the soil or as seed treatment, they are taken up by the roots and translocated through the entire plant.10,11 Residue studies have detected low levels of neonicotinoids in the pollen of treated crops10–12 and substantially high levels of residues in corn grown from imidacloprid-treated corn seeds.13 When applied to the top surface of leaves and fruits, they penetrate into the plant tissues and afford long-term protection from piercing–sucking insects.4 For example, substantial portions of thiacloprid and clothianidin residues and radiolabeled neonicotinoids were found to penetrate in and beyond the outer flesh regions of apples 24 h after topical application.14,15 In another study, thiamethoxam and acetamiprid were detected in cherry leaves and the fruit’s interior tissue 14 days after application.16 Translocation of neonicotinoids into plant tissues (after either foliar application or seed/soil treatment) may potentially be subject to human consumption and subsequently dietary intake.

To estimate dietary exposure to neonicotinoids in humans, it is important to monitor neonicotinoid residues in and on foods that people consume. Although the U.S. Department of Agriculture (USDA) publishes Pesticide Data Program (PDP) reports annually, usually fewer than 15 fresh fruits and vegetables are included each year. Nevertheless, imidacloprid, the most commonly used insecticide in the world, had been detected in 81% of sweet bell peppers, 81% of broccoli, and 53% of grapes, as reported by the USDA PDP.17 Imidacloprid is not only widely detectable in fruits and vegetables but also absorbed with high efficiency, as shown in a human intestinal cell model.18 Imidacloprid and acetamiprid have also shown excitatory effects on cultured cerebellar neurons from neonatal...
rats, suggesting possible neurotoxicity in developing mammalian brains. These results raise the concerns of potential health risks from chronic exposure to consumers by dietary intake of residues with food. Because half-lives for most neonicotinoids in aerobic soil can range from months to years, neonicotinoids can become persistent in the environment for several years after repeated applications. Consequently, the persistence of neonicotinoids in soil would have created a reservoir of residues for plant to take up over a long period of time and, therefore, contaminate crops for human consumption. In addition, because of their systemic characteristics, neonicotinoids often occur as residues in the plant flesh and could not be washed off easily.

To the best of our knowledge, this is the first study aiming to demonstrate the presence of neonicotinoids in foods that people commonly consume. Only specific neonicotinoids (such as imidacloprid) in fruits and vegetables have been monitored and reported. Most data on neonicotinoid residues in fruits and vegetables were from brief reports of the application of the newly developed analytical methods. In addition, in most of these brief reports, only up to six neonicotinoids were simultaneously monitored and eight neonicotinoids were monitored in only one study and in one commodity. In recent years, the QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation procedure has been widely used for extraction of a wide variety of pesticides from various food matrices.

In this study, we used a sensitive and modified LC-MS/MS method along with the QuEChERS procedure to simultaneously measure eight neonicotinoid residues in various foods including fruits, vegetables, and honey and in pollen. The exposure of neonicotinoids and will contribute to future epidemiological research linking neonicotinoid insecticide exposure to potential human health effects.

### Experimental Procedures

#### Materials

Acetamiprid, flonicamid, thiacloprid, thiamethoxam, and nitenpyram standard solutions were purchased from Accustandard (New Haven, CT, USA) with purity ≥99.7%. Imidacloprid and clothianidin were purchased from Sigma-Aldrich (St. Louis, MO, USA) with purity of 99.9%. Dinotefuran was purchased from Chem Service (West Chester, PA, USA) with purity of 99.2%. The isotopically-labeled internal standards (IS) for imidacloprid-d₄, clothianidin-d₄, and thiamethoxam-d₄ (99.8%) were purchased from C/D/N Isotopes, Inc. (Quebec, Canada). LC-MS/MS grade formic acid and ammonium formate were purchased from VWR international (Radnor, PA, USA). QuEChERS extraction salt packages, which include 4 g of MgSO₄, 1 g of NaCl, 1 g of sodium citrate, and 500 mg of disodium citrate sesquihydrate in each salt pack, and 2 mL of QuEChERS dispersive SPE containing 50 mg of PSA + 50 mg of C18 + 150 mg of MgSO₄ or 50 mg of PSA + 50 mg of graphitized carbon black (GCB) + 150 mg of MgSO₄ or 25 mg of PSA + 7.5 mg of GCB + 150 mg of MgSO₄, were purchased from Agilent Technologies (Santa Clara, CA, USA).

#### Liquid Chromatography–Mass Spectrometry (LC-MS/MS) Instrumentation

The HPLC system consists of a Shimadzu LC-SCL-10AVP solvent delivery unit, an online solvent degasser, a gradient mixer, and a system controller (Shimadzu Scientific, Columbia, MD, USA), coupled with a CTC-PAL autosampler (LEAP Technologies, Carrboro, NC, USA) for injecting samples. The mass spectrometer is an API 4000 LC-MS/MS system (AB SCIEX, Framingham, MA, USA) equipped with a Turbo V IonSpray ionization source. The ShAsQer 1500 from SPEX SamplePrep (Metuchen, NJ, USA) was used for mixing samples in the QuEChERS extraction procedure.

#### LC-MS/MS Conditions

Neonicotinoids in pollen and honey were analyzed by the LC-MS/MS method that was developed previously for pollen and high-fructose corn syrup (HFCS) samples. Analysis of neonicotinoids in fruits and vegetables was conducted by using the modified method for pollen and HFCS. Briefly, the chromatographic separation was performed on a YMC ODS-AQ column (100 mm × 2.1 mm, 3 μm particle size, YMC, Allentown, PA, USA) with the mobile phase, consisting of water with 5 mM ammonium formate and 0.1% formic acid (mobile phase A) and acetonitrile/water (95:5 v/v) with 5 mM ammonium formate and 0.1% formic acid (mobile phase B), running gradient at 170 μL/min in 11 min for each analysis. The mobile phase gradient was as follows: 0% B for 1.3 min; linearly increased to 100% B from 1.30 to 2.3 min, and then maintained at 100% B from 2.3 to 7.5 min, back to 0% B from 7.5 to 8.0 min, and maintained at this proportion from 8.0 to 11.0 min. Injection volume into the LC-MS/MS is 10 μL. The mass spectrometer equipped with an electrospray was operated in the positive ionization mode with multiple reaction monitoring (MRM). The mass/charge (m/z) ratios monitored were 225/126, 250/169, 203/129, 230/203, 256/209, 271/225, 253/126, and 292/211 for acetamiprid, clothianidin, dinoterifuran, flonicamid, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam, respectively. A second transition was used for each analyte for identification purposes. The m/z of the internal standards (IS) of imidacloprid-d₄, clothianidin-d₄, and thiamethoxam-d₄ were 260/213, 253/172, and 294/214, respectively. The quantification of neonicotinoids was made from matrix-matched standard calibration curves using peak-area ratio of analyte versus IS. The calibration curves were constructed using weighted (1/x) linear least-squares regression. A calibration standard curve and two concentrations of QCs (QC low and QC high) in duplicate were incorporated into each analytical run. The QCs provide the basis for accepting or rejecting the run (within 20% of accuracy and precision).

#### Sample Collection

Twenty-nine fresh fruit and vegetable samples were purchased from several neighborhood grocery stores in Boston in 2012. Honey samples were collected directly from hives located in urban and suburban areas in Massachusetts (n = 3) or purchased from a local grocery store in Boston (n = 5) and from a store in Israel in 2012 (n = 2). Six pollen samples from New Zealand were collected by using pollen collectors set out under three hives located near a kiwifruit orchard during peak flowering for 2 days in 2011. The pollen samples were then separated into kiwifruit pollen and others (non-kiwifruit) pollen on the basis of the color of the pollen pellets. Another seven pollen samples were collected from hives located in seven different locations in the central Massachusetts area in July 2012.

#### Sample Preparation

**Calibration and QC Samples.** A prior analysis of neonicotinoid-free pollen, honey, and organic fruits and vegetable samples, which were used as blank matrix samples, was performed and confirmed to have no contamination of neonicotinoids. These blank samples were used for calibration, QCs, and blanks for the analysis. In addition, as a background check for any possible interference, blank matrix samples were also injected immediately after high-level standards to check the carry-over of the instrument. No carry-over was observed during the analysis. All calibration, QCs, and blanks were generated in 2 g blank matrix for pollen, 5 g for honey, and 10 g for fruits/vegetables. Calibration curves for eight neonicotinoids at seven levels ranging from 0.1 to 100 ng/g (except 0.1–50 ng/g for honey) were prepared by adding aliquots of intermediate standard solutions (preparation steps can be found in the previous study) to blank sample matrix. The QC samples at two concentration levels, 5 (QCL) and 50 ng/g (QCH) (except 2 (QCL) and 40 ng/g (QCH for honey), were prepared the same way as the blank sample matrix. The standards and QCs were stored at −20 °C.

**Food Samples.** The fruit and vegetable samples were washed under cold water for 15 s and allowed to drain for at least 2 min on paper toweling. Oranges were peeled, and the rinds of pumpkin and watermelon were removed. Anything that would not normally be consumed, such as apple core, pepper core, orange seeds, and the leafy tops of strawberries, were also removed. Then the samples were
chopped into small pieces and blended in a Magic Bullet until a homogeneous paste was achieved. If the sample was homogenized in portions, all portions were mixed together in a clean container to ensure an evenly mixed sample.

The overall sample preparation procedure is shown in Figure 1. The sample extraction procedures for fruits and vegetables were modified from the method that we developed previously for pollen and HFCS samples, and the procedure for honey samples was the same as that for HFCS. Ten grams of homogenized fruit and vegetable samples was weighed in a 50 mL centrifuge tube, and 10 mL of acetonitrile and 20 μL of IS solutions were added. For calibration standard and QCs, 10 g of homogenized organic samples was fortified with appropriate levels of working standard solutions. Double-blanks and blanks were also prepared in parallel with and without IS added, respectively. The tube was subsequently shaken for 30 s in a ShaQer at 1500 strokes per minute. Then one pack of QuEChERS salt and one ceramic homogenizer were added. An additional 5 mL of n-hexane was added to all olive samples. The tubes were shaken vigorously for 40 s in the shaker and centrifuged for 4 min at 4000g. One milliliter from the supernatant was transferred to a 2 mL d-SPE vial. Pollen, honey, and olive samples were added to d-SPE vials containing 50 mg of PSA, 50 mg of C18, and 150 mg of MgSO4; spinach sample was added to d-SPE containing 50 mg of PSA, 50 mg of GCB, and 150 mg of MgSO4; all the rest of the fruits and vegetables were added to d-SPE containing 25 mg of PSA, 7.5 mg of GCB, and 150 mg of MgSO4. The next d-SPE extraction, sample drying, and transfer steps were the same as presented before.

## RESULTS AND DISCUSSION

In this study, we analyzed various types of fruits, including oil fruits, stone fruits, pome fruits, citrus fruits, and berries, and vegetables, including leafy vegetables and root vegetables, as well as honey and pollen. Table 1 shows the performance of this method including the limit of quantification (LOQ) and the average recoveries (and the variations) of eight neonicotinoids in various sample matrices. LOQ was calculated as 10 times the signal-to-noise ratio of the quantitative ion transition in the matrix and was 0.1 ng/g for neonicotinoids in pollen, honey, fruits, and vegetables with the exceptions of 0.5 ng/g for flonicamid and 0.5 ng/g for nitenpyram in pollen and dinotefuran in honey. A sample was considered positive when residue levels were above the LOQ. As shown in Table 2, all fruit and vegetable samples in our study tested positive for one or more neonicotinoids, except nectarine and tomato. Five neonicotinoids, including clothianidin, dinotefuran, flonicamid, imidacloprid, and thiamethoxam, were detected in both fruits and vegetables; however, acetamiprid and thiacloprid were found only in fruits. Nitenpyram was the only neonicotinoid that is not detectable in any fruit or vegetable samples. Imidacloprid not only was detected with the highest concentration in a green pepper sample (7.2 ng/g), but also was the most frequently detected neonicotinoid in both fruits and vegetables with the overall detection rate of 70%. The results in Table 2 show that not only are neonicotinoids widely found in fruits and vegetables but also multiple neonicotinoids are often detected in a single sample, especially in apples. Seventy-two percent of fruits and 45% of vegetables were found to have multiple neonicotinoids. These percentages included 5 of 8 apples, which were detected with three different neonicotinoids, and 6 of 10 fruits and 5 of 11 vegetables were positive for two neonicotinoids. Because most commercially available products do not contain multiple neonicotinoids as the active ingredients, except for clothianidin, which could be the breakdown product of thiamethoxam, this result indicates that these fruit and vegetable plants were likely treated multiple times during their lifespan with different neonicotinoids or absorbed neonicotinoid residues accumulated in the soil. The persistence of neonicotinoids in aerobic soil is highly likely due

### Table 1. Limit of Quantitation (LOQ) for Eight Neonicotinoid Insecticides in Various Sample Matrices

| LOQ (ng/g) | acetamiprid | clothianidin | dinotefuran | flonicamid | imidacloprid | nitenpyram | thiacloprid | thiamethoxam |
|------------|-------------|--------------|-------------|-----------|--------------|------------|-------------|--------------|
| matrix     |             |              |             |           |              |            |             |              |
| fruits/vegetables (n = 20) | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 |
| honey (n = 5) | 0.1 | 0.1 | 0.5 | 0.5 | 0.1 | 0.1 | 0.1 | 0.1 |
| pollen (n = 5) | 0.1 | 0.1 | 0.1 | 0.5 | 0.1 | 0.1 | 0.1 | 0.1 |
| recovery (%) | 110.4 | 97.5 | 96.1 | 95.6 | 101.6 | 97.4 | 99.6 | 101.5 |
| RSD (%) | 13 | 14 | 20 | 14 | 12 | 18 | 17 | 10 |
to the accumulation of repeated applications of neonicotinoids, especially ones with long half-lives.20

In addition to fruits and vegetables, we analyzed 10 domestic and foreign honey samples collected from urban, suburban, and rural areas. Table 3 shows that five neonicotinoids, including acetamiprid, clothianidin, imidacloprid, nitenpyram, and thiamethoxam, were found in these honey samples. Similar to fruits and vegetables, imidacloprid was found in 9 of 10 honey samples, including 2 organic honey samples purchased from a local grocery store. The highest concentration of imidacloprid of 1.3 ng/g was found in a domestic organic honey sample. The USDA organic regulations allowed residues of prohibited pesticides up to 5% of the tolerance set by the U.S. Environmental Protection Agency (EPA) in organic products, but there are no defined tolerances for most neonicotinoids (including imidacloprid) in honey.32 Five honey samples contained multiple neonicotinoids, including two organic honey samples. Only one honey sample collected from a hive located in the rural area was free from neonicotinoid contamination. The high frequency of detections of neon-

### Table 2. Concentrations of Neonicotinoids Measured in Conventional Fruits and Vegetables Purchased from a Local Grocery Store in Boston, MA, USA

| analyte concentrations (ng/g) | types of foods | acetamiprid | clothianidin | dinotefuran | flonicamid | imidacloprid | nitenpyram | thiacloprid | thiamethoxam |
|-----------------------------|----------------|-------------|-------------|-------------|------------|--------------|------------|-------------|--------------|
| fruits                      |                |             |             |             |            |              |            |              |              |
| apple (Cortland)            | 40.7           | .01         | .01         | 0.2         | 0.4        | 100.7        | 1.2        | 10.7        | 20.7         |
| apple (Granny Smith)        | 1.9            | .01         | 11.2        | 11.2        | 11.2       | 11.2         | 11.2       | 11.2        | 11.2         |
| apple (Fuji)                | 0.1            | 0.1         | 0.1         | 0.1         | 0.1        | 0.1          | 0.1        | 0.1         | 0.1          |
| apple (Red Delicious)       | 0.1            | 0.1         | 0.1         | 0.1         | 0.1        | 0.1          | 0.1        | 0.1         | 0.1          |
| apple (Golden Delicious)    | 19.5           | 0.2         | 0.2         | 0.2         | 0.2        | 0.2          | 0.2        | 0.2         | 0.2          |
| apple (Gala)                | 1.2            | 0.2         | 0.2         | 0.2         | 0.2        | 0.2          | 0.2        | 0.2         | 0.2          |
| apple (Honey Crisp)         | 22.0           | 0.2         | 0.2         | 0.2         | 0.2        | 0.2          | 0.2        | 0.2         | 0.2          |
| Apple (Macintosh)           | 0.1            | .01         | .01         | .01         | .01        | .01          | .01        | .01         | .01          |
| nectarine                   | 0.1            | 0.1         | 0.1         | 0.1         | 0.1        | 0.1          | 0.1        | 0.1         | 0.1          |
| orange                      | 0.1            | 0.1         | 0.1         | 0.1         | 0.1        | 0.1          | 0.1        | 0.1         | 0.1          |
| strawberry                  | 0.1            | .01         | .01         | .01         | .01        | .01          | .01        | .01         | .01          |
| watermelon                  | 0.1            | .01         | .01         | .01         | .01        | .01          | .01        | .01         | .01          |
| cantaloupe                  | 0.1            | .01         | .01         | .01         | .01        | .01          | .01        | .01         | .01          |
| honeydew                    | 0.1            | .01         | .01         | .01         | .01        | .01          | .01        | .01         | .01          |
| olive                       | 0.1            | .01         | .01         | .01         | .01        | .01          | .01        | .01         | .01          |
| vegetables                  |                |             |             |             |            |              |            |              |              |
| spinach                     | 0.1            | .01         | .01         | .01         | .01        | .01          | .01        | .01         | .01          |
| tomato                      | 0.1            | .01         | .01         | .01         | .01        | .01          | .01        | .01         | .01          |
| yellow pepper               | 0.1            | .01         | .01         | .01         | .01        | .01          | .01        | .01         | .01          |
| green pepper                | 0.1            | .01         | .01         | .01         | .01        | .01          | .01        | .01         | .01          |
| potato                      | 0.1            | .01         | .01         | .01         | .01        | .01          | .01        | .01         | .01          |
| eggplant                    | 0.1            | .01         | .01         | .01         | .01        | .01          | .01        | .01         | .01          |
| cucumber                    | 0.1            | .01         | .01         | .01         | .01        | .01          | .01        | .01         | .01          |
| pumpkin                     | 0.1            | .01         | .01         | .01         | .01        | .01          | .01        | .01         | .01          |
| zucchini                    | 0.1            | .01         | .01         | .01         | .01        | .01          | .01        | .01         | .01          |
| summer squash               | 0.1            | .01         | .01         | .01         | .01        | .01          | .01        | .01         | .01          |

### Table 3. Concentrations of Neonicotinoids Measured in Honey Samples

| analyte | concentration (ng/g) of honey samples from different sources |
|---------|-------------------------------------------------------------|
|         | suburban 1 | suburban 2 | rural | foreign 1 | foreign 2 | raw honey 1 | raw honey 2 | organic foreign | organic domestic |
| acetamiprid | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| clothianidin | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| dinotefuran | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| flonicamid | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| imidacloprid | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| nitenpyram | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| thiacloprid | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| thiamethoxam | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |

"—", below the limit of quantitation.
The wide usage of neonicotinoids in the environment where honeybees often foraged indicates the presence of these pesticides in pollen samples. We tested 13 pollen samples; 7 of them were from central Massachusetts and 6 from New Zealand. As shown in Table 4, imidacloprid continued to be the most commonly detected neonicotinoid with a detection frequency of 77% in all 13 pollen samples. Among the seven pollen samples from Massachusetts, the five that tested positive were collected from central Massachusetts within the so-called “quarantine area”, where trees were injected with imidacloprid to combat the Asian Longhorn beetle problem in 2011−2012. The other two pollen samples containing no neonicotinoids were collected outside the “quarantine area”, where neonicotinoid uses have not been observed or reported. The degree of neonicotinoid contamination in pollen samples collected in New Zealand is more extensive than that of samples collected from central Massachusetts areas, as shown in Table 4. All six samples contained multiple neonicotinoids, and five of those six samples contained three neonicotinoids. It is also noticeable that Table 4. Concentrations of Neonicotinoids Measured in Pollen Samples Collected from Central Massachusetts, USA, and New Zealand

| Analyte      | Food Type | Total Samples Collected | No. of Samples > LOQ | Freq of Detection (%) | Conc. Range (ppb) | Commodity with Max Conc. |
|--------------|-----------|-------------------------|----------------------|-----------------------|-------------------|--------------------------|
| Imidacloprid | Fruits    | 17                      | 15                   | 82                    | 0.1−4.2           | Apple                    |
|              | Vegetables| 12                      | 7                    | 58                    | 0.4−7.2           | Green pepper             |
|              | Honey     | 10                      | 9                    | 90                    | 0.1−1.3           | Organic, Domestic        |
|              | Pollen    | 13                      | 10                   | 77                    | 0.2−2.3           | MA                       |
| Clothianidin | Fruits    | 17                      | 3                    | 18                    | 0.1−1.9           | Apple                    |
|              | Vegetables| 12                      | 3                    | 25                    | 0.6−0.7           | Potato, Pumpkin          |
|              | Honey     | 10                      | 2                    | 20                    | 0.1−0.5           | Organic, Foreign         |
|              | Pollen    | 13                      | 6                    | 46                    | 0.2−2.6           | New Zealand, Other       |
| Thiamethoxam | Fruits    | 17                      | 3                    | 18                    | 0.2−2.4           | Watermelon               |
|              | Vegetables| 12                      | 4                    | 33                    | 0.3−13.2          | Cucumber                 |
|              | Honey     | 10                      | 1                    | 10                    | 0.4               | Organic, Domestic        |
| Acetamiprid  | Fruits    | 17                      | 4                    | 24                    | 0.3−100.7         | Apple                    |
|              | Honey     | 10                      | 1                    | 10                    | 0.2               | Foreign                  |
| Dinotefuran  | Fruits    | 17                      | 1                    | 6                     | 34.8              | Cantaloupe               |
|              | Vegetables| 12                      | 1                    | 8                     | 0.1               | Yellow Pepper            |
| Flonicamid   | Fruits    | 17                      | 6                    | 35                    | 0.1−0.2           | Apple, Orange            |
|              | Vegetables| 12                      | 1                    | 8                     | 0.4               | Spinach                  |
| Thiacloprid  | Fruits    | 17                      | 3                    | 18                    | 0.4−18.3          | Apple                    |
|              | Pollen    | 13                      | 6                    | 46                    | 0.1−3.3           | New Zealand, Other       |
| Nitenpyram   | Honey     | 10                      | 1                    | 10                    | 0.2               | Raw                      |

协同发展, especially imidacloprid, in these samples indicates the wide usage of neonicotinoids in the environment where honeybees often foraged.

We also tested 13 pollen samples; 7 of them were from central Massachusetts and 6 from New Zealand. As shown in Table 4, imidacloprid continued to be the most commonly detected neonicotinoid with a detection frequency of 77% in all 13 pollen samples. Among the seven pollen samples from Massachusetts, the five that tested positive were collected from central Massachusetts within the so-called “quarantine area”, where trees were injected with imidacloprid to combat the Asian Longhorn beetle problem in 2011−2012. The other two pollen samples containing no neonicotinoids were collected outside the “quarantine area”, where neonicotinoid uses have not been observed or reported. The degree of neonicotinoid contamination in pollen samples collected in New Zealand is more extensive than that of samples collected from central Massachusetts areas, as shown in Table 4. All six samples contained multiple neonicotinoids, and five of those six samples contained three neonicotinoids. It is also noticeable that

Table 5. Summary of Neonicotinoids Concentrations in Foods

| Analyte      | Food Type | Total Samples Collected | No. of Samples > LOQ | Freq of Detection (%) | Conc. Range (ppb) | Commodity with Max Conc. |
|--------------|-----------|-------------------------|----------------------|-----------------------|-------------------|--------------------------|
| Imidacloprid | Fruits    | 17                      | 15                   | 82                    | 0.1−4.2           | Apple                    |
|              | Vegetables| 12                      | 7                    | 58                    | 0.4−7.2           | Green pepper             |
|              | Honey     | 10                      | 9                    | 90                    | 0.1−1.3           | Organic, Domestic        |
|              | Pollen    | 13                      | 10                   | 77                    | 0.2−2.3           | MA                       |
| Clothianidin | Fruits    | 17                      | 3                    | 18                    | 0.1−1.9           | Apple                    |
|              | Vegetables| 12                      | 3                    | 25                    | 0.6−0.7           | Potato, Pumpkin          |
|              | Honey     | 10                      | 2                    | 20                    | 0.1−0.5           | Organic, Foreign         |
|              | Pollen    | 13                      | 6                    | 46                    | 0.2−2.6           | New Zealand, Other       |
| Thiamethoxam | Fruits    | 17                      | 3                    | 18                    | 0.2−2.4           | Watermelon               |
|              | Vegetables| 12                      | 4                    | 33                    | 0.3−13.2          | Cucumber                 |
|              | Honey     | 10                      | 1                    | 10                    | 0.4               | Organic, Domestic        |
| Acetamiprid  | Fruits    | 17                      | 4                    | 24                    | 0.3−100.7         | Apple                    |
|              | Honey     | 10                      | 1                    | 10                    | 0.2               | Foreign                  |
| Dinotefuran  | Fruits    | 17                      | 1                    | 6                     | 34.8              | Cantaloupe               |
|              | Vegetables| 12                      | 1                    | 8                     | 0.1               | Yellow Pepper            |
| Flonicamid   | Fruits    | 17                      | 6                    | 35                    | 0.1−0.2           | Apple, Orange            |
|              | Vegetables| 12                      | 1                    | 8                     | 0.4               | Spinach                  |
| Thiacloprid  | Fruits    | 17                      | 3                    | 18                    | 0.4−18.3          | Apple                    |
|              | Pollen    | 13                      | 6                    | 46                    | 0.1−3.3           | New Zealand, Other       |
| Nitenpyram   | Honey     | 10                      | 1                    | 10                    | 0.2               | Raw                      |
| analyte      | year | total samples collected | no. of samples with detection | freq of detection (%) | max concn (ppb) | commodity with highest freq of detection (freq of detection, %)
|--------------|------|-------------------------|-------------------------------|-----------------------|-----------------|---------------------------------------------------------------|
|             |      |                         |                               |                       |                 |                                                                 |
| imidacloprid| 2004 | 5920                    | 1510                          | 26                    | 780             | sweet bell peppers (81%) (apple 30.2%)                       |
|             | 2005 | 6956                    | 1567                          | 23                    | 470             | cauliflower (85%) (apple 26.6%)                              |
|             | 2006 | 6930                    | 1405                          | 20                    | 520             | broccoli (81%) (applesauce 17.5%)                            |
|             | 2007 | 7107                    | 1654                          | 23                    | 1000            | broccoli (72%) (apple juice 0%)                              |
|             | 2008 | 8176                    | 1389                          | 17                    | 1000            | broccoli (67%) (apple juice 0%)                              |
|             | 2009 | 8981                    | 1267                          | 14                    | 1100            | grapes (53%) (apple 16.9%)                                  |
|             | 2010 | 10322                   | 1473                          | 14                    | 1100            | grapes (48%) (apple 20.3%)                                  |
|             | 2011 | 10480                   | 1104                          | 11                    | 750             | cauliflower/lettuce (36%) (no apple data)                    |
| overall     |      | 64872                   | 11369                         | 18                    |                 |                                                               |
| acetamiprid | 2004 | 412                     | 79                            | 19                    |                 | apples (100%)                                                |
|             | 2005 | 1528                    | 272                           | 18                    |                 | apples (70%)                                                  |
|             | 2006 | 3298                    | 453                           | 14                    |                 | apple sauce (51.5%)                                          |
|             | 2007 | 5284                    | 350                           | 7                     |                 | summer squash (100%) (apple juice 34%)                       |
|             | 2008 | 8261                    | 416                           | 5                     |                 | apple juice (33.3%)                                          |
|             | 2009 | 9194                    | 817                           | 9                     |                 | pears (41.1%) (apple 33%)                                    |
|             | 2010 | 10323                   | 552                           | 5                     |                 | apples (28.8%)                                               |
|             | 2011 | 10096                   | 295                           | 3                     |                 | baby food, pears (26.3%) (no apple data)                     |
| overall     |      | 48396                   | 3234                          | 10                    |                 |                                                               |
| clothianidin| 2004 | 123                     | 0                             | 0.0                   |                 | watermelon (0%) (no apple data)                               |
|             | 2005 | 3482                    | 29                            | 1                     |                 | summer squash (5.6%) (no apple data)                         |
|             | 2006 | 6381                    | 28                            | 0.4                   |                 | summer squash (2.8%) (apple juice 0%)                        |
|             | 2007 | 7744                    | 65                            | 1                     |                 | potatoes (5.3%) (apple juice 0%)                             |
|             | 2008 | 8447                    | 104                           | 1                     |                 | grapes (4.8%) (apple 0%)                                    |
|             | 2009 | 8739                    | 172                           | 2                     |                 | hot peppers (11.3%) (no apple data)                          |
|             | 2010 | 9337                    | 218                           | 2                     |                 | cherry tomatoes (14.1%) (no apple data)                       |
| overall     |      | 44253                   | 616                           | 1                     |                 |                                                               |
| flonicamid  | 2004 | 132                     | 1                             | 1                     |                 | summer squash (0.8%)                                         |
|             | 2005 |                         |                               |                       |                 |                                                               |
|             | 2006 | 2094                    | 6                             | 0                     |                 | summer squash (1.1%) (apple 0%)                              |
|             | 2007 | 4661                    | 73                            | 2                     |                 | spinach (13.9%) (apple 0%)                                   |
|             | 2008 | 5990                    | 58                            | 1                     |                 | cucumbers (6%) (apple 0.5%)                                  |
|             | 2009 | 7910                    | 114                           | 1                     |                 | cucumbers (6.9%) (apple 1.6%)                                |
|             | 2010 | 8162                    | 139                           | 2                     |                 | lettuce (7.4%) (no apple data)                                |
| overall     |      | 28949                   | 391                           | 1                     |                 |                                                               |
| thiodiprid  | 2004 | 480                     | 4                             | 1                     |                 | apples (3%)                                                   |
|             | 2005 | 1853                    | 96                            | 5                     |                 | apple sauce (12.8%)                                          |
|             | 2006 | 1891                    | 5                             | 0                     |                 | apple juice (4.7%)                                           |
|             | 2007 | 3343                    | 6                             | 0                     |                 | apple juice (4.6%)                                           |
|             | 2008 | 7467                    | 102                           | 1                     |                 | apples (9%)                                                   |
|             | 2009 | 7628                    | 163                           | 2                     |                 | apples (12.6%)                                               |
|             | 2010 | 6338                    | 106                           | 2                     |                 | baby food, pears (no apple data)                              |
| overall     |      | 29000                   | 482                           | 2                     |                 |                                                               |
| dinotefuran | 2009 | 7323                    | 26                            | 0                     |                 | cucumbers (15%)                                              |
|             | 2010 | 9580                    | 150                           | 2                     |                 | cantaloupe (14.6%)                                           |
|             | 2011 | 9678                    | 153                           | 2                     |                 | cantaloupe (11.9%) (no apple data)                            |
| overall     |      | 40085                   | 472                           | 1                     |                 |                                                               |
| nitenpyram  | overall | NA                     | NA                            | NA                    |                 |                                                               |
| thiamethoxam| 2009 | 10257                   | 196                           | 2                     |                 | cucumbers (11.6%)                                            |
|             | 2010 | 10602                   | 422                           | 4                     |                 | sweet bell peppers (26.6%)                                   |

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Table 6. Neonicotinoid Residues Measured in Fruits and Vegetables Reported by the USDA Pesticide Data Program (PDP) from 2004 to 2011

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nonkiwifruit (others) pollen samples contained higher concentrations of imidacloprid and clothianidin than kiwifruit pollen. Those nonkiwifruit pollen samples were likely from nearby dandelion, clover, and other weed flowers, on the basis of the color of the pollen pellets. Because kiwifruit has little nectar, honeybees need to forage widely (as far as 2 km) to get their nectar feed for energy. In this search, bees could pick up other pollens containing higher levels of neonicotinoids around the kiwifruit orchard than the pollen of kiwifruit; therefore, higher levels of neonicotinoids showed up in these “others” pollen. This finding is consistent with the systemic property of neonicotinoids because upon application (such as spray), neonicotinoid residues can penetrate and translocate through the plant/weed, including nectar and pollen, located near the adjacent area. Because pollen is honeybees’ main source of protein and neonicotinoids have been linked to CCD,8,9 the widespread presence of neonicotinoids in these pollen samples could pose a potential threat to the survival of honeybees.

In addition, neonicotinoids, such as imidacloprid, clothianidin, and thiacloprid, have often been used as soil treatment for insect controls because of their long half-lives in aerobic soil (half-lives for imidacloprid and clothianidin are 26−229 and 148−1155 days, respectively) and water solubility.6,20,33 A recent study has shown that clothianidin was found in both soil and dandelion flowers grown adjacent to a clothianidin-treated agriculture area.34 Another study has shown that untreated sunflowers grown in fields previously treated with imidacloprid a year ago can still take up imidacloprid from the soil.35 Therefore, some neonicotinoids can become persistent for several years and accumulated in the environment after repetitive applications and, therefore, cause concern for prolonged exposure.

This result from pollen samples is consistent with that from the fruit and vegetable samples in this study because most of these types of fruits and vegetables testing positive were pollinated by honeybees. Our study also raises the concern of pollen contaminated with neonicotinoids because not only is pollen the primary protein source for honeybees but also it could be readily available for human exposure via inhalation as well.

Table 5 shows the summary results of neonicotinoid residues detected in all samples that were analyzed in this study. Both imidacloprid and clothianidin were found in all four types of foods followed by thiamethoxam (three types of foods); acetamiprid, dinofeturan, flonicamid, and thiacloprid (two types of food); and nitenpyram (in only one honey sample). Results from this study were generally consistent with what have reported by the USDA PDP during 2004−2011 (Table 6). Consistent with having the highest frequency of detection among all neonicotinoids in samples that we analyzed, imidacloprid was found in seven of eight apple samples in this study, as well as in apples/applesauce/apple juice samples reported by PDP from 2004 to 2010 (Table 6). Along with imidacloprid, apples/applesauce/apple juice have the highest detection frequency of acetamiprid in almost every year from 2004 to 2010, although the frequency of detection for acetamiprid has gradually decreased from 100% in 2004 to approximately 30% in 2007. Similarly, apple, applesauce, or apple juice has consistently been the commodity with the highest frequency of detection for thiacloprid every year from 2004 to 2010 and has an increasing trend of use from 4.7% in 2007 to 9% in 2009 to 12.6% in 2010. Although clothianidin was not detectable in apples or apple juice in PDP from 2007 to 2009, clothianidin was found in three of eight apples in the current study conducted in 2012. Consistent with results from PDP, the current study has confirmed the wide use of imidacloprid, clothianidin, acetamiprid, flonicamid, and thiacloprid on apples, ranging from 38 to 88%. Unfortunately, we were not able to continue monitoring the trend of neonicotinoid residues in apples because they were not included in PDP after 2010. We found a higher frequency of detection of imidacloprid than USDA PDP reported primarily because we used a more sensitive analytical method with lower LOQs for all neonicotinoids in this study than those used by USDA PDP with a limit of detection ≥1 ng/g. Smaller sample sizes in our study could be another reason for higher frequency of detection; however, the commodities testing positive in our study matched the commodities reported by PDP. For instance, the highest concentration of thiamethoxam detected in our study was from a cucumber sample (13.2 ng/g), and cucumber was found with the highest detection rate of 12% in 2009 and an even higher rate of 16% in 2010 by PDP. The highest concentration of dinofeturan in our study was found in a cantaloupe sample, which was also reported by PDP in 2010 and 2011 with the highest detection rates of 15 and 12%, respectively.

This study has demonstrated the widespread presence of neonicotinoids in foods that people commonly consume and in pollen and honey that honeybees bring back to hives from the environment. Although those levels were low, studies have suggested a link to adverse health effects in honeybees as the results of sublethal exposure to neonicotinoids.8,36,37 Neonicotinoid insecticides are known to selectively target insects’ nicotinic acetylcholine receptors (nAChRs) and therefore were previously thought to pose less toxicity in mammals. However, a growing amount of evidence has shown that neonicotinoids are capable of directly activating and/or modulating the activation of mammalian nAChRs. Both in vitro and in vivo studies have shown that imidacloprid can change the membrane properties of mouse neurons,38 significantly impair sensorimotor performance, and elevate glial fibrillary acidic protein expression in the motor cortex and hippocampus of neonatal rats after in utero exposure to a single sublethal dose.39 Most mammalian adverse toxic effects of neonicotinoids are associated with their action on binding to the α4β2 nAChR subtype.40 In an in vitro study, imidacloprid and other neonicotinoids have been shown to directly activate and modulate human α4β2 nAChR subtypes.41 The α4β2 nAChR subtype is the most prominent nAChR subtype in the mammalian brain, with the highest density of receptors in the

Table 6. continued

| analyte year | total samples collected | no. of samples with detection | freq of detection (%) | max concn (ppb) | commodity with highest freq of detection (freq of detection, %) |
|--------------|------------------------|-------------------------------|----------------------|----------------|---------------------------------------------------------------|
| 2011         | 10477                  | 348                           | 3                    | sweet bell peppers (17.1%) (no apple data)                  |
| Overall      | 59778                  | 1409                          | 2                    |                                                             |

“Frequency of detection (%) in apples or applesauce or apple juice as a comparison.
The α4β2 nAChR is involved in various brain functions, such as cognition, memory, and behavior. There is strong evidence for a role of α4β2 nAChR and alteration of the receptor density in CNS disorders, such as Alzheimer’s disease, Parkinson’s disease, schizophrenia, and depression. In the developing brain (such as perinatal stage), α4β2 nAChR subtypes have been implicated in neuronal proliferation, apoptosis, migration, differentiation, synapse formation, and neural-circuit formation. It is likely that neonicotinoids could affect these processes when activating nAChRs. In addition, absorption studies using the human intestinal cell line have shown that neonicotinoids can be absorbed by active transportation. Neonicotinoids and some of their metabolites are also shown to be able to pass through the blood-brain barrier in mice, and some metabolites having enhanced potency to nAChR are even more toxic than their parent compounds. Therefore, there is an inevitable question if neonicotinoids could pose potential health risks to humans as well.

In conclusion, this is the first paper to document the widespread presence of neonicotinoid residues in fruits, vegetables, and honey that are readily available in the market for human consumption. We also reported neonicotinoid residues in pollen collected by honeybees in areas where neonicotinoids are known to be used, and the variation of neonicotinoid levels in pollen likely reflects the amount of neonicotinoids applied. It is important to note here that although all neonicotinoid residues reported in this study were below the maximum residue levels, or tolerances, established by the EPA, the determination of these tolerances is based on the best field practice and studies conducted in test animals exposed at acute and chronic toxic levels, and therefore does not take into sufficient account the protection of human health from long-term low-dose exposure. In light of the extensive use of neonicotinoids on fruit and vegetable crops and their widespread presence in foods along with new information on the toxicological effects of neonicotinoids in mammals, it is therefore warranted to conduct epidemiological studies to assess dietary neonicotinoid intakes in humans and the health effects.

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