JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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J. Agric. Food Chem., **Just Accepted Manuscript** • Publication Date (Web): 16 Jun 2014

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Quantitative analysis of neonicotinoid insecticide residues in foods: implication for dietary exposures

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Abstract: In this study, we 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 5 out of 7 pollen from Massachusetts detected positive for imidacloprid. These results show the prevalent presence 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 the new reports of toxicological effects in mammals, our results strengthen the importance to assess dietary neonicotinoid intakes and the potential human health effects.

Keywords: *neonicotinoid insecticides; dietary exposure; pollen; honey*

1. 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 publics, neonicotinoids are the most commonly used insecticides in the world, which includes acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam. Additionally, flonicamid has been categorized as a neonicotinoid insecticide[2], although the mode of action is different from 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 the broad spectrum of insecticidal activity, the high receptors 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), 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 flea control[4,7].

Because of neonicotinoids' wide uses and their extreme toxicity to bees, neonicotinoids have been implicated to cause the steep decline of 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 crops[10-12], and substantially high levels of resides 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 hours 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 (either after foliar application or seed/soil treatment) may potentially be subject to human consumption, and subsequently dietary intake.

In order to estimate dietary exposure to neonicotinoids in humans, it is important to monitor neonicotinoid residues in and on foods that people consume. Although US Department of Agriculture (USDA) publishes Pesticide Data Program (PDP) reports annually, usually less 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, 53% of grapes, as reported by USDA PDP[17]. Imidacloprid is not only widely detectable in fruits and vegetables, but can also be 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[19]. These results raise the concerns of potential health risks from chronic exposure to the consumers by dietary intake of residues with food. Since half-lives for most neonicotinoids in aerobic soil can last from months to years[20], 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 uptakes over a long period of time, and therefore contaminate the 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[21-22]. 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 ≤ 6 neonicotinoids were simultaneously monitored [22-28], and 8 neonicotinoids was monitored in only one study and in one commodity[2]. In recent years, QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) sample preparation procedure has been widely used for extraction of wide variety of pesticides from various food matrices[29-31]. In this study, we used a sensitive and modified LC-MS/MS method along with the QuEChERS procedure to simultaneously measure 8 neonicotinoid residues in various foods incluing fruits, vegetables and honey, and in pollen[30]. The results provided an initial assessment of the potential dietary exposure of neonicotinoids, and will contribute to future epidemiological research linking neonicotinoid insecticide exposure to potential human health effects.

2. Experimental Section

2.1 Materials

Acetamiprid, flonicamid, thiacloprid, thiamethoxam, and nitenpyram standard solutions were purchased from Accustandard (New Haven, CT, USA) with purity higher than or equal to 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 isotope labeled internal standards (IS) for imidacloprid-d_4 (99.2%), clothianidin-d_3 (98.9%), and thiamethoxam-d_3 (99.8%) were purchased from C/D/N Isotopes, Inc. (Quebec, Canada). LC-MS/MS grade formic acid and ammonium formate were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade reagents, including acetonitrile and water were purchased from JT Baker (Center Valley, PA, USA). 1.5 mL vials with 0.2-µm nylon filter was purchased from VWR international (Radnor, PA, USA). QuEChERS extraction salt package which includes 4 g $MgSO_4$, 1 g NaCl, 1 g Sodium citrate, and 500 mg of disodium citrate sesquihydrate in each salt pack, and 2 mL of QuEChERS dispersive SPE containing 50 mg PSA+50 mg C18+150 mg $MgSO_4$; or 50 mg PSA+50 mg Graphitized Carbon Black (GCB)+150 mg $MgSO_4$; or 25 mg PSA+7.5 mg GCB+150 mg $MgSO_4$; ceramic homogenizer were purchased from Agilent Technologies (Santa Clara, CA, USA).

2.2 Liquid chromatography/mass spectrometry (LC-MS/MS) instrumentation

The HPLC system consists of a Shimadzu LC SCL-10AVP solvent delivery unit, an on-line solvent degasser, a gradient mixer, and a system controller (Shimadzu Scientific, Columbia, MD, USA), coupled with a CTC-PAL auto-sampler (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 ShaQer 1500 from SPEX SamplePrep (Metuchen, NJ, USA) was used for mixing samples in the QuEChERS extraction procedure.

2.3 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[30].

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 YMC ODS-AQ column (100 mm × 2.1 mm, 3 μm particle size, YMC, Allentown, PA, USA) with the mobile phase, consisted of water with 5mM ammonium formate and 0.1% formic acid (mobile phase A) and acetonitrile/water (95:5 v/v) with 5mm 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; linear increased to 100% B from 1.3.0 to 2.3 min, and then maintained at 100% B from 2.3 to 7.5 min, went 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 223/126, 250/169, 203/129, 230/203, 256/209, 271/225, 253/126 and 292/211 for acetamiprid, clothianidin, dinotefuran, flonicamid, imidacloprid, nitenpyram, thiacloprid and thiamethoxam, respectively. A second transition was used for each analyte for identification purpose. The *m/z* of the internal standard (IS) of imidacloprid-d_4, clothianidine-d_3 and thiamethoxam-d_3 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 vs 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 of accepting or rejecting the run (within 20% of accuracy and precision).

2.4 Sample collection

Twenty-nine fresh fruit and vegetable samples were purchased from several neighborhood grocery stores in Boston Massachusetts in 2012. Honey samples were collected directly from hives located in urban and sub-urban areas in Massachusetts (n=3), or purchased from a local grocery store in Boston Massachusetts (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 flowing for two days in 2011. The pollen samples were then separated into kiwifruit pollen and others (non-kiwifruit) pollen based on colour of the pollen pellets. Another seven pollen samples were collected from hives located in 7 different locations in the central Massachusetts area in July 2012.

2.5 Sample preparation

Calibration and QC samples: A prior analysis of neonicotinoid-free pollen, honey, 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, blanks matrix samples were also injected immediately after high-level standards to check on carry-over of the instrument. No carry-over was observed during the analysis. All calibration, QCs and blanks were generated in 2g blank matrix for pollen, 5g for honey and 10 g for fruits/vegetables. The calibration curves for 8 neonicotinoids at seven levels ranged from 0.1 to 100 ng/g (except 0.1 to 50 ng/g for honey) were prepared by adding aliquots of intermediate standard solutions (preparation steps can be found in the previous study[30]) to blank sample matrix. The QC samples at two concentration levels 5 (QCL) and 50ng/g (QCH) (except 2 (QCL) and 40ng/g (QCH) for honey) were prepared the same way in blank sample matrix. The standards and QCs were stored at -20^{o}C.

Food samples: The fruit and vegetable samples were washed under cold water for 15s, and allowed to drain for at least 2 minutes on paper towel. 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, leafy top of strawberries, were also removed. Then the samples were chopped into small pieces and blended in Magic Bullet until homogeneous paste achieved. If the sample was homogenized in portions, all portions shall be mixed together in a clean container to assure 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[30]. Ten grams of homogenized fruit and vegetable samples were weighted in a 50 mL centrifuge tube, 10 mL of acetonitrile, 20 μL of IS solutions were added. For calibration standard and QCs, 10 g of homogenized organic samples were 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 seconds in ShaQer at 1500 strokes per minute. Then one pack of QuEChERS salt and one ceramic homogenizer were added. Additional 5 mL of *n*-hexane were added to all olive samples. The tubes were shaken vigorously for 40s in the shaker, and centrifuged for 4 min at 4000x*g*. 1 mL from the acetonitrile layer was transferred to a 2-mL QuEChERS dispersive SPE vial. Pollen, honey and olive samples were added to d-SPE vials containing 50mg PSA, 50mg C18 and 150mg MgSO4, spinach sample was added to d-SPE containing 50mg PSA, 50mg GCB and 150mg MgSO4, and all the rest fruits and vegetables were added to d-SPE containing 25mg PSA, 7.5mg GCB and150mg MgSO4. The next d-SPE extraction, sample drying and transfer steps are the same as presented before[30].

3. 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 8 neonicotinoids in various sample matrix. LOQ was calculated as ten 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 and fruits and vegetables, 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 were 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 only found in fruits. Nitenpyram was the only neonicotinoids that is not detectable in any fruit or vegetable samples. Imidacloprid was detected not only with the highest concentration in a green pepper sample (7.2 ng/g), but also the most frequently detected neonicotinoids 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 multiple neonicotinoids are also often detected in a single sample, especially in apples. Seventy two percent of fruits and forty five percent of vegetables were found to have multiple neonicotinoids. These percentages included five out of eight apples which were detected with three different neonicotinoids, and six out of ten fruits and five out of eleven vegetables were positive for two neonicotinoids. Since most commercially available products don’t contain multiple neonicotinoids as the active ingredients, except for clothianidin, which could be the break down 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 neonicotinoids residues accumulated in soil. The persistence of neonicotinoids in aerobic soil is highly likely due to the accumulation of repeated applications of neonicotinoids, especially ones with long half-lives[20].

In addition to fruits and vegetables, we analyzed ten domestic and foreign honey samples collected from urban, sub-urban, 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 nine out of ten honey samples, including two 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 samples. The USDA organic regulations allowed residues of prohibited pesticides up to 5 percent of the tolerance set by United States Environmental Protection Agency (US EPA) in organic products, but there is 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 neonicotinoids, especially imidacloprid, in these samples indicates the wide usage of the neonicotinoids in the environment where honeybees often foraged.

We also tested thirteen pollen samples, and seven of them were from central Massachusetts and six from New Zealand. As shown in Tables 4, imidacloprid continued to be the most commonly detected neonicotinoids with detection frequency of 77% in all thirteen pollen samples. Among seven pollen samples from Massachusetts, five 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 those collected from central Massachusetts areas, as shown in Table 4. All six samples contained multiple neonicotinoids, and five out of those six samples contained three neonicotinoids. It is also noticeable that non-kiwifruit (others) pollen samples contained higher concentrations of imidacloprid and clothianidin than kiwifruit pollen. Those non-kiwi pollen samples were likely from nearby dandelion, clover and other weed flowers, based on the color of the pollen pellets. Since kiwifruit has little nectar, honeybees need to forage widely (as far as 2km) 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 than kiwifruit pollen. This finding is consistent to the systemic property of neonicotinoids since upon application (such as spray), neonicotinoid residues can penetrate and translocate through the plant/weed, including nectar and pollen, located near the adjacent area. Since pollen is honey bees' 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 being used as soil treatment for insect controls because of their long half-lives in aerobic soil (half-lives for imidacloprid and clothianidin are 26 to 229 days, and 148 to 1,155 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 the clothianidin-treated agriculture area[34]. Another study has shown that untreated sunflowers grown in fields previously treated with imidacloprid one year ago can still uptake imidacloprid from the soil[35]. Therefore, some neonicotinoids can become persistent for several years and accumulated in the environment after repetitive applications, and therefore causes concern for prolonged exposure.

This result from pollen samples is consistent with that from the fruits and vegetable samples in this study since most of these types of fruits and vegetables tested positive were pollinated by honeybees. Our study also raises the concern of pollen contaminated with neonicotinoids because not only pollen is 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, dinotefuran, flonicamid and thiacloprid (two types of food), and nitenpyram (only in 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 7 out of 8 apple samples in this study, as well as in apples/applesauces/apple juice samples reported by PDP from 2004 to 2010 (Table 6). Along with imidacloprid, apples/applesauce/apple juice has 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 with the increasing trend of use from 4.7% in 2007, 9% in 2009 to 12.6% in 2010. Although clothianidin was not detectable in apples or apple juice in PDP from 2007-2009, clothianidin was found in 3 out of 8 apples in the current study conducted in 2012. Consistent with results from PDP, the current study have 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 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 limit of detection ≥ 1ng/g. Smaller sample sizes in our study could be another reason for higher frequency of detection; however, the commodities tested 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 (13.2ng/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 dinotefuran in our study was found in a cantaloupe sample which was also reported by PDP in 2010 and 2011 with the highest detection rate 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 the link to adverse health effects in honeybees as the results of sub-lethal 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 number of evidences have 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 sub-lethal 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 thalamus[42]. 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 AD, PD, schizophrenia and depression[43-44]. 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[45-46]. 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[18,47]. Neonicotinoids and some of their metabolites are also shown to be able to pass through the blood-brain barrier in mouse, and some metabolites having enhanced potency to nAChR are even more toxic than their parent compounds[48]. Therefore, there is an inevitable question if neonicotinoids could pose potential health risk 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 the 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 US EPA, the determination of these tolerances is based on the best field practice and studies conducted in test animals which exposed at acute and chronic toxic levels, and therefore does not take into sufficient account the protection of human health on long-term low dose exposure. In light of the extensive use of neonicotinoids on fruits and vegetables crops and their widespread present in foods along with the new information of 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.

Acknowledgments

This project was supported by the Harvard-NIEHS Center for Environmental Health (P30ES000002) Pilot Project Program. Authors would like to thank Dr. Christine Austin (University of Sydney, Sydney Australia), Erin Collin, Michaela Kapp (Harvard School of Public Health), and Barry Foster (New Zealand) for their assistance in sample collection, analytical method development, and sample preparation in the lab.

Author Contributions

Main text paragraph.

Conflicts of Interest

All authors declare no conflict of interest.

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Figure Captions

Figure1. Sample extraction procedures in different matrix.

Table 1. The limit of quantitation (LOQ) (ng/g) for 8 neonicotinoid insecticides in various sample matrices

LOQ		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	0.5	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

Table 2. Concentrations of neonicotinoids measured in conventional fruits and vegetables purchased from a local grocery store in Boston Massachusetts

Types of Foods		Analyte Concentrations (ng/g)							
		Acetamiprid	Clothianidin	Dinotefuran	Flonicamid	Imidacloprid	Nitenpyram	Thiacloprid	Thiamethoxam
Fruits	Apple (Cortland)	-	-	-	-	-	-	0.4	-
	Apple (Granny Smith)	40.7	-	-	0.1	0.1	-	-	-
	Apple (Fuji)	-	-	-	0.1	0.1	-	-	-
	Apple (Red delicious)	-	-	-	0.1	4.2	-	-	-
	Apple (Golden delicious)	0.3	0.2	-	-	0.6	-	-	-
	Apple (Gala)	-	0.1	-	-	0.1	-	18.3	-
	Apple (Honey crispy)	100.7	-	-	0.2	0.1	-	-	-
	Apple (Macintosh)	-	1.9	-	-	0.1	-	4.7	-
	Nectarine	-	-	-	-	-	-	-	-
	Orange	-	-	-	0.2	0.9	-	-	-
		-	-	-	0.2	1.1	-	-	-
	Strawberry	-	-	-	-	0.2	-	-	-
		19.5	-	-	-	-	-	-	-
	Watermelon	-	-	-	-	0.1	-	-	0.2
		-	-	-	-	0.2	-	-	2.4
	Cantaloupe	-	-	34.8	-	3.0	-	-	-
	Honey dew	-	-	-	-	2.8	-	-	-
	Olive	-	-	-	-	0.1	-	-	0.4
Vegetables	Spinach	-	-	-	0.4	6.5	-	-	-
	Tomato	-	-	-	-	-	-	-	-
	Yellow pepper	-	-	0.1	-	-	-	-	-
	green Pepper	-	-	-	-	7.2	-	-	-
	Potato	-	0.7	-	-	-	-	-	0.3
	Eggplant	-	-	-	-	1.6	-	-	-
	Cucumber	-	0.6	-	-	-	-	-	13.2
		-	-	-	-	2.76	-	-	-
	Pumpkin	-	0.7	-	-	-	-	-	0.4
	Zucchini	-	-	-	-	0.4	-	-	0.5
	Summer squash	-	-	-	-	2.8	-	-	-

- below limit of quantitation

Table 3. **Concentrations of neonicotinoids measured in honey samples**

Analytes	Concentration (ng/g) of honey Samples from different sources									
	Urban	**Sub-urban 1**	**Sub-urban 2**	**Rural**	**Foreign 1**	**Foreign 2**	**Raw honey 1**	**Raw honey 2**	**Organic-foreign**	**Organic-domestic**
Acetamiprid	-	-	-	-	-	0.2	-	-	-	-
Clothianidin	-	0.1	-	-	-	-	-	-	0.5	-
Dinotefuran	-	-	-	-	-	-	-	-	-	-
Flonicamid	-	-	-	-	-	-	-	-	-	-
Imidacloprid	0.1	0.3	0.2	-	0.7	0.8	0.2	0.1	0.2	1.3
Nitenpyram	-	-	-	-	-	-	-	0.2	-	-
Thiacloprid	-	-	-	-	-	-	-	-	-	-
Thiamethoxam	-	-	-	-	-	-	-	-	-	0.4

- below limit of quantitation

Table 4. Concentrations of neonicotinoids measured in pollen samples collected from central Massachusetts USA and New Zealand

Pollen Samples		Acetamiprid	Clothianidin	Dinotefuran	Flonicamid	Imidacloprid	Nitenpyram	Thiacloprid	Thiamethoxam
MA, USA[1]	**1**	-	-	-	-	2.3	-	-	-
	2	-	-	-	-	0.4	-	-	-
	3	-	-	-	-	2.2	-	-	-
	4	-	-	-	-	0.7	-	-	-
	5	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-
	7	-	-	-	-	0.6	-	-	-
New Zealand[2]	**kiwi-A[3]**	-	0.2	-	-	0.2	-	1.7	-
	others-A[4]	-	1.9	-	-	1.2	-	3.3	-
	kiwi-B[3]	-	0.5	-	-	-	-	1.3	-
	others-B[4]	-	2.6	-	-	0.5	-	0.1	-
	kiwi-C[3]	-	0.6	-	-	0.2	-	1.4	-
	others-C[4]	-	2.2	-	-	0.4	-	1.1	-

1. Pollen samples were collected from honeybees in the hives located in 7 different locations of central Massachusetts.
2. Pollen samples were collected from two day collections from three hives in a kiwifruit orchard at the beginning of their pollination assignment in New Zealand in 2011.
3. Pollen samples were sorted by color, kiwifruit pollen was the dominant pollen.
4. Other pollens collected were a range of color, according to their host plants, such as clovers, dandelions, in the proximity of kiwi orchard.

\- below limit of quantitation

Table 5. Summary of neonicotinoids concentrations in foods.

Analytes	Food Types	Total samples collected	No. of samples>LOQ	Freq. of detection (%)	Conc. Rang (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	Mass.
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

Table 6. Neonicotinoids residues measured in fruits and vegetables reported by the USDA Pesticide Data Program (PDP) from 2004 to 2011.

Analytes	Year	Total samples collected	No. of samples>LOQ	Freq. of detection (%)	Max conc. (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					
	2005	123	0	0.0		watermelon (0%) (no apple data)
	2006	3482	29	1		summer squash (5.6%) (no apple data)
	2007	6381	28	0.4		summer squash (2.8%) (apple juice 0%)
	2008	7744	65	1		potatoes (5.3%) (apple juice 0%)
	2009	8447	104	1		grapes (4.8%) (apple 0%)
	2010	8739	172	2		hot peppers (11.3%) (no apple data)
	2011	9337	218	2		cherry tomatoes (14.1%) (no apple data)
	Overall	44253	616	1		
Flonicamid	2004					
	2005					
	2006	132	1	1		summer squash (0.8%)
	2007	2094	6	0		summer squash (1.1%) (apple 0%)
	2008	4661	73	2		spinach (13.9%) (apple 0%)
	2009	5990	58	1		cucumbers (6%) (apple 0.5%)
	2010	7910	114	1		cucumbers (6.9%) (apple 1.6%)
	2011	8162	139	2		lettuce (7.4%) (no apple data)
	Overall	28949	391	1		
Thiacloprid	2004					
	2005	480	4	1		apples (3%)
	2006	1853	96	5		apple sauce (12.8%)
	2007	1891	5	0		apple juice (4.7%)
	2008	3343	6	0		apple juice (4.6%)
	2009	7467	102	1		apples (9%)
	2010	7628	163	2		apples (12.6%)
	2011	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)
	2011	10477	348	3		sweet bell peppers (17.1%)(no apple data)
	Overall	59778	1409	2		

*Freq. of detection (%) in apples or applesauce or apple juice as a comparison.

No	Extraction Procedures	Pollen	Honey	Fruits and Vegetables		
				Olive	Spinach	Others
1	Weigh Xg of homogenized sample into a 50mL centrifuge tube	2g	5g	10g		
2	Add IS solution+ XmL of water	8mL	10mL	No water added		
3	Shake to dissolve	by hand	in water bath at 50 °C for 20 min	by hand		
3	Add 10mL of acetonitrile + XmL of n-hexane and shake for 30s	3mL	no hexane	5mL	no hexane	
4	Add one QuEChERS citrate salt package + one ceramic homogenizer, and shake for 40s and centrifuge					
5	Transfer 1mL of supernatant into a 2 mL d-SPE, and vortex 30s and then centrifuge	50mg PSA+50mg C18+150mg MgSO4			50mg PSA+50mg GCB+150mg MgSO4	25mg PSA+7.5mg GCB+150mg MgSO$_4$
7	Dispense 600µL of supernatant into a glass test tube, and dry under N$_2$ in water bath at 40 °C					
8	Reconstitute residues using 200µL of 15% Acetonitrile in water, transfer 150µL filtered solution into HPLC vials and analyze by LCMS/MS					

X - the weight of samples or volume of the solvents.

Figure 1

TOC graphic