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Contamination by neonicotinoid insecticides and their metabolites in Sri Lankan black tea leaves and Japanese green tea leaves

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ABSTRACT

Tea is one of the world's most popular beverages due to health promoting effects. Despite these, there have been concerns about the adverse effects of tea contamination by neonicotinoid insecticides. Only a handful of studies on neonicotinoid insecticides in tea have been carried out and this study was therefore performed to determine the concentrations of seven neonicotinoid insecticides and 20 metabolites in Japanese green tea leaves, and black tea leaves from Sri Lanka; and assess the Maximum Daily Intake (MDI) of neonicotinoid insecticides. From the results, the seven parent compounds were detected in Japanese tea leaves and beverages. Dinotefuran (3004 ng/g) was found at the highest level in green tea leaves. Ten of the 20 metabolites were detected in Japanese tea products. Dinotefuran-urea (92%) and thiacloprid-amide (89%) were most frequently detected in Japanese tea leaves. Clothianidin-urea (100 ng/g) was found at the highest level in green tea leaves. Neonicotinoid insecticides and metabolites were not detected in Sri Lankan black tea leaves. The concentrations and MDI of neonicotinoid insecticides in tea leaves were below the Maximum Residual Levels (MRLs) and Acceptable Daily Intakes (ADIs), respectively.

1. Introduction

Neonicotinoid insecticides are among the most rapidly adopted insecticides introduced to the market since the introduction of pyrethroid insecticides. The current market share of neonicotinoid insecticides in the world is over £600 million per year [1]. Imidacloprid was the biggest selling insecticide worldwide, followed by acetamiprid, nitenpyram, thiacloprid, thiamethoxam, clothianidin, and dinotefuran, and the former six are chlorinated compounds [1]. Neonicotinoid insecticides are used at various stages of cultivation and post-harvest storage. Since 1993, many tons have entered the Japanese market, and they have been widely used on a variety of crops. In 2015, large quantities of various neonicotinoid insecticides, including acetamiprid (51.5 t), thiamethoxam (49.2 t), clothianidin (75.6 t), imidacloprid (66.0 t), thiacloprid (12.7 t), nitenpyram (6.5 t), and dinotefuran (167 t), were shipped to Japan [2].

Tea is one of the most widely consumed non-alcoholic beverages globally [3] and it contains multiple health-promoting compounds, including vitamins, caffeine, catechin, and other polyphenols [4]. Unfermented green tea and semi-fermented tea are commonly consumed in East Asian countries, while fermented black tea is common in the West [5]. Epidemiological studies have shown the beneficial health effects of tea consumption [6]. However, the consumption of tea may represent a potential source of human exposure to toxicants, because of

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the high levels of insecticides in tea cultivation [7]. For example, neonicotinoid insecticides, such as imidacloprid, thiamethoxam, and acetamiprid, are regularly used in tea cultivation to control pests, such as *Empoasca vitis* (false-eye leafhopper) [8–11].

Neonicotinoid insecticides act as modulators at the nicotinic acetylcholine receptors (nAChR) for mammals and insects [12]. In addition, some metabolites of neonicotinoid insecticides, especially *N*-unsubstituted imines, i.e., desnitro- or descyano-metabolites, have greater IC_{50} values on mammalian α4β2 nAChR than *Drosophila* nAChR, e.g., 4.6 nM and 2600 nM for imidacloprid and 1500 nM and 8.2 nM for *N*-desnitro-imidacloprid, respectively [13]. These results indicated that it is necessary to screen for neonicotinoid metabolites as well as their parent compounds. Several studies in Japan have suggested that tea is one of the major sources of human exposure to neonicotinoid insecticides in the Japanese population [14–18]. However, there have been limited studies to assess the levels of parent compounds and metabolites in tea leaves and tea beverages, with the exception of one monitoring survey that indicated residual levels of approximately 0.5 ppm of clothianidin, imidacloprid, and thiamethoxam in domestic tea leaves in 2012 [19].

The present study was performed to quantify neonicotinoid insecticides, i.e., acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam, and 20 neonicotinoid metabolites (Table 1) in tea leaves and tea beverages. As there are only a few standards available for neonicotinoid metabolites, we synthesized most of the neonicotinoid metabolites and developed a quantitative method using solid-phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-ESI/MS/MS). In addition, we compared the intakes of neonicotinoid insecticides through Japanese green tea with the current Acceptable Daily Intake (ADI) in Japan.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Acetamiprid, dinotefuran, imidacloprid, and thiacloprid were purchased from Kanto Chemical (Tokyo, Japan). Clothianidin and *N*-desmethyl-thiamethoxam were purchased from Fluka (Buchs, Switzerland). Nitenpyram, CPMA, CPMF, and CPF were purchased from Wako Pure Chemical Industries (Osaka, Japan). Clothianidin-d3, dinotefuran-d3, imidacloprid-d4, thiacloprid-d4, thiamethoxam-d4, *N*-desmethyl-acetamiprid and *N*-desnitro-imidacloprid were purchased from Sigma-Aldrich (St. Louis, MO). Hydroxy-imidacloprid was purchased from Hypha Discovery (Uxbridge, UK). Acetamiprid-d6 and nitenpyram-d3 were purchased from Hayashi Junyaku (Osaka, Japan). Thiamethoxam was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Other metabolites (*N*-acetyl-acetamiprid, *N*-acetyl-desmethyl-acetamiprid, *N*-descyano-acetamiprid, *N*-desmethyl-clothianidin, *N*-desmethyl-clothianidin urea, *N*-desnitro-clothianidin, clothianidin urea, *N*-desmethyl-desnitro-clothianidin, *N*-desmethyl-dinotefuran, dinotefuran-urea, *N*-desmethyl-nitenpyram, thiacloprid-amide, *N*-descyano-dehydro-thiacloprid) were synthesized at Toho University (Supplement).

2.1.2. Tea leaves and beverages

From January to May 2016, green tea leaves labeled "domestically grown" of different brands and manufacturers were purchased from randomly selected grocery stores in Japan (n = 39), and black tea leaves were purchased from grocery stores in Sri Lanka (n = 30). In addition, bottled green tea beverages (these are ready-to-drink green teas stored in bottles) were purchased from convenience stores in Japan (n = 9). The tea leaves and bottled tea were all produced from the respective countries, but the samples were of different brands and manufacturers, and were produced between 2015 and May 2016. All were stored in a refrigerator keep under 5 centigrade until analysis was performed.

2.2. Methods

2.2.1. Extraction of neonicotinoids from tea leaves

Tea leaves were ground using a mortar and pestle, and 1.5 *g* of finely ground tea leaves were placed in 50-mL centrifuge tubes (Corning Inc., Corning, NY). After adding the internal standards to the leaves (50 μL of 100 ppb solution), water (40 mL) at 25 °C was added. The content was vortex-mixed for 10 min and centrifuged at 10,000 × *g* for 10 min.

Table 1
Target neonicotinoids and their metabolites.

Neonicotinoids		Recovery rate (%)	LOQ (ng/ml-bottled tea beverage)	LOQ (ng/g-tea leaves)
Acetamiprid		80.2 ± 2.9	0.05	1.33
	N-desmethyl-Acetamiprid	87.6 ± 5.4	0.05	1.33
	N-acetyl-Acetamiprid	33.2 ± 23.4	0.125	3.33
	N-acetyl-desmethyl-Acetamiprid	78.2 ± 16.8	1.25	33.33
	N-descyano-Acetamiprid	81.0 ± 3.7	0.05	1.33
Clothianidin		91.8 ± 3.7	0.125	3.33
	N-desmethyl-Clothianidin	79.7 ± 5.2	0.5	13.33
	N-desmethyl-Clothianidin urea	73.6 ± 3.2	0.05	1.33
	N-desnitro-Clothianidin	54.1 ± 2.7	0.05	1.33
	Clothianidin urea	84.0 ± 3.1	0.125	3.33
	N-desmethyl-desnitro-Clothianidin	41.7 ± 2.5	0.05	1.33
Dinotefuran		92.6 ± 2.8	0.125	3.33
	N-desmethyl-Dinotefuran	87.1 ± 6.1	0.125	3.33
	Dinotefuran-urea	75.2 ± 9.3	0.05	1.33
Imidacloprid		87.0 ± 2.7	0.5	13.33
	Hydroxy-Imidacloprid	78.2 ± 9.7	1.25	33.33
	N-desnitro-Imidacloprid	85.7 ± 5.5	1.25	33.33
Nitenpyram		88.6 ± 4.6	0.5	13.33
	N-desmethyl-Nitenpyram	100.6 ± 31.3	0.5	13.33
	CPMA	47.9 ± 10.2	0.5	13.33
	CPMF	84.8 ± 10.3	0.05	1.33
	CPF	32.9 ± 20.3	0.05	1.33
Thiacloprid		92.9 ± 1.8	0.05	1.33
	Thiacloprid-amide	94.0 ± 5.6	0.05	1.33
	N-descyano-dehydro-Thiacloprid	66.7 ± 11.7	0.05	1.33
Thiamethoxam		116.7 ± 7.9	0.125	3.33
	N-desmethyl-Thiamethoxam	70.2 ± 10.3	0.5	13.33