Original article

Urinary neonicotinoids in rural population of the Philippines and comparison with paired hair samples

Kumiko Taira[1], Alexandre Aebi[2], Gaétan Glauser[3], Edward A. D. Mitchell[2], Yoshinori Ikenaka[4,5], Francisco Sanchez-Bayo[6], Andrea Tapparo[7], Elizabeth Lumawig-Heitzmann[8], Jean-Marc Bonmatin[9], Anton Safer[10], Maarten Bijleveld van Lexmond[11], Florencia Claveria[12], Yoshiko Aoyama[13], Yuichi Miyabara[14] and Makiko Komori[1]

1) Adachi Medical Center, Tokyo Women's Medical University, Japan
2) Laboratory of Soil Biodiversity, University of Neuchâtel, Switzerland
3) Neuchâtel Platform of Analytical Chemistry, University of Neuchâtel, Switzerland
4) Hokkaido University, Japan
5) Northwest University, South Africa
6) The University of Sydney, Australia
7) Department of Chemical Sciences, University of Padua, Italy
8) Marinduque Biological Field Station, Philippines
9) CNRS Center for Molecular Biophysics, France
10) Heidelberg University, Germany
11) Task Force on Systemic Pesticides, Switzerland
12) De La Salle University-Manila, Philippines
13) Aoyama Allergy Clinic, Japan
14) Institute of Mountain Science, Shinshu University, Japan

Abstract

Neonicotinoid insecticides, the most widely used category of insecticides for 20 years, have been detected in various human tissues including blood, cerebrospinal fluid, urine, and hair. We collected spot urine from 99 volunteers in three islands of the Philippines, and compared their neonicotinoid concentrations with those obtained in paired hair samples from our previous study. Geometric means of concentration in urine (μg/L) were 0.048 for imidacloprid, 0.030 for clothianidin, 0.044 for thiamethoxam, 0.007 for acetamiprid, 0.005 for thiacloprid, 0.036 for dinotefuran, 0.026 for nitenpyram, and 0.006 for *N*-desmethyl acetamiprid. The total concentrations of the former five neonicotinoids in urine

受付：2023年９月25日　採用：2024年２月20日
責任著者：平　久美子
東京女子医科大学附属足立医療センター麻酔科
〒123-8558　東京都足立区江北4-33-1
VFG03077@nifty.com

samples were lower in children (6-16 years old, n=28) than in adults (18-39 yo, n=42) and seniors (40-83 yo, n=29) (p=0.030), but not in hair. Imidacloprid concentrations were lower in hair (p=0.014) in adult women with dyed hair (n=9) as compared to women with un-dyed hair (n=18), but not in urine (p=0.71). We also analyzed strong ions in urine by ion chromatography and found an increase in urinary strong ion difference (SIDu) in five urine samples, suggesting possible renal metabolic acidosis. Our results suggest that urine is an appropriate matrix to investigate human exposure to neonicotinoids, while hair may be better suited to identify chronic exposure especially in children.

(Jpn J Clin Ecol 32 : 59－76, 2023)

《**Keywords**》neonicotinoids, hair, urine, Philippines, children

要旨

ネオニコチノイドは世界で最も多く使われている殺虫剤で、ヒトの尿および毛髪から検出される。2017年にフィリピンの三つの島の99人のボランティア（成人男性44人、成人女性27人、6-16歳の子供28人の随時尿と毛髪を採取しネオニコチノイドを分析した。同時に尿中電解質の測定も行った。各ネオニコチノイドの尿中濃度（μg/L）と毛髪中濃度（μg/kg）の幾何平均は、イミダクロプリド0.048 vs 0.076、クロチアニジン0.030 vs 0.036、チアメトキサム0.044 vs 0.021、アセタミプリド0.007 vs 0.005、チアクロプリド0.005 vs 0.005だった。成人女性の毛髪中イミダクロプリド濃度は、染髪（n＝9）で染髪なし（n＝18）と比べ有意に低かった（p＝0.014）。尿中 strong ion differences（SIDu＝Na＋K－CI）は5検体で異常高値を示した。

（臨床環境 32：59－76，2023）

《**キーワード**》ネオニコチノイド、尿、毛髪、フィリピン、子供

Introduction

Neonicotinoids have been the most widely used class of insecticides for 20 years[1]. Five neonicotinoids are registered as pesticides worldwide (imidacloprid, acetamiprid, thiacloprid, thiamethoxam, clothianidin), and other neonicotinoids and related insecticides (e.g. nitenpyram, dinotefuran, cycloxaprid, paichongding, imidaclothiz, flupyradifurone, sulfoxaflor, triflumezopyrim, and flupyrimin) are also frequently used in some countries[2,3]. Being water soluble, systemic, and long-acting, neonicotinoids are widely used for agriculture, forestry, horticulture, turf, termite control for wooden building materials, and for protection against parasites in domestic animals[4]. Their strong and persistent nicotinic acetylcholine receptor (nAChR) competitive modulator action is identified as a causal factor for the decline of honeybees and pollinators in general[5,6], and is of toxicological concern for humans[7-11]. The European community has restricted the use of three major compounds i.e., imidacloprid, clothianidin, and thiamethoxam[12], the registration of thiacloprid has recently expired[13], and the revision of their registration is underway in the USA and Australia[14,15].

Documenting the levels of neonicotinoid contamination in the general population has become an important issue of public health, especially given the suspected risks these pesticides pose on neurodevelopment[11], as well as the documented subacute and chronic health effects, which include neuronal, reproductive, musculoskeletal, cardiovascular, gastrointestinal, liver and renal toxicity in addition to acute health effects[7-10,16-20]. As the human urinary excretion half-lives of some neonicotinoids, i.e. imidacloprid and *N*-desmethyl-acetamiprid (DMAP), the phase 1 metabolite of acetamiprid, exceed 24 hours[21], continuous environmental exposure to neonicotinoids through food[22,23], beverages[24,25], and/or air[26] may cause an increase of tissues concentration in the human body.

Neonicotinoids and their metabolites have been detected in urine (Supplemental Table 1), hair, whole blood, serum, plasma, cerebrospinal fluids, saliva, semen, breast milk, and teeth (Supplemental Table 2) from the general population. A recent study showed very high within- and between-individual variability in urinary concentrations of neonicotinoids and their metabolites in consecutive collection for up to 44 days except for DMAP[27]. Another study suggested there exists a positive relationship between the neonicotinoid concentration in urine, blood and cerebrospinal fluid[28]. Urinary concentration can be an appropriate biomarker of short-term brain neonicotinoid exposure, but the negative prediction value for chronic exposure is unknown. Bio-distribution of neonicotinoids and their metabolites into hair after oral intake has been reported[29], and residues in hair could thus be better indicators of chronic exposure, when occupational direct contamination or the effect of hair dye are eliminated.

Neonicotinoids exposure may cause metabolic acidosis by renal tubular dysfunction[19,20]. Based on urinary biomarkers such as L-FABP and Cystatin C, we have indeed found a correlation between neonicotinoids exposure and renal tubular dysfunction in Sri Lanka where chronic kidney disease with unknown etiology was prevalent[10]. Previous reports suggested an adequate response to non-renal metabolic acidosis should be a negative urinary strong ion difference (SIDu = NaU + KU - ClU)[31,32], while renal metabolic acidosis by acute kidney injury caused high SIDu value above 40 mEq/L[31,32].

In an earlier study, we detected five neonicotinoids (thiamethoxam, clothianidin, imidacloprid, acetamiprid, and thiacloprid) in the hair of the Philippine general population[30]. At the same time, we collected paired spot urine samples with the goal to understand the relationship between neonicotinoid concentrations in urine and hair for surveying human exposure. In urine, neonicotinoids and their metabolites have been detected in the general population including pregnant women, in Japan, China, Sri Lanka, US, Korea, Vietnam, India, Kuwait, Saudi Arabia, Greece, Thailand, Ghana, Switzerland, and Germany (Supplemental Table 1), but not yet in the Philippines.

The aim of the present study was to analyze urinary neonicotinoid concentrations by HPLC-MS/MS in Philippine populations, and to compare them with concentrations in paired hair samples[30]. Additionally, we analyzed strong ions (e.g., sodium, potassium, and chloride) in urine, and calculated SIDu[31,32], in order to screen for renal tubular acidosis which may be caused by renal tubular dysfunction.

Methods

Study design, ethics approval and sample collection

This study was carried out as an academic project of the Task Force on Systemic Pesticides Public Health Working Group (Chair. K. Taira). After obtaining the ethic committee approval of Tokyo Women's Medical University (No.4521) with that of Marinduque Biological Field Station in Philippines (No.1), the written informed consent for sampling from each participant, spot urine samples (10 mL) and hair samples (ca. 100 mg) were collected at three locations on three different Philippine islands from randomly chosen local volunteers: children (more than 5 years old), adult men and women (no less than 18

Figure 1. Geographic data of the three sampling places. Modified from Fig. 1 in our previous paper[30] based on Google map（https://www.google.co.jp/maps）on 23 December 2023.

years old). Geographic data of the three sampling place are shown in Figure 1. Samples were collected between September and November 2017 before the application of insecticides to the crops in the respective areas. The sample-set size was determined by the carrying capacity of urine samples from Philippines to Japan. All participants who consented to participate in this research, were informed about the purpose, benefits, risks, and other options in a written information sheet, which they signed if they agreed with the rules. When volunteers were minors or children, written informed consent was obtained from the next of kin, caretakers, or guardians on their behalf. The participants' age, sex, occupation, and whether their hair was dyed or not were recorded at the same time. No participant had a history of chronic renal failure. All methods were performed in accordance with the relevant guidelines and regulations.

Urine samples were frozen at -20 ˚C immediately after collection, transferred to Hokkaido University, and stored in a freezer at -20 ˚C until they were analyzed. Hair samples were kept in zip locked bags, transported in a cooler to the University of Neuchâtel and stored in a freezer at -20 ˚C until analysis. The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Chemicals and reagents

For the urine samples analyzed at Hokkaido University, acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram thiacloprid, thiamethoxam, *N* - desmethyl-acetamiprid, and *N* - desmethyl-acetamiprid-d3 were purchased from Hayashi Junyaku (Osaka, Japan). PL Pesticides surrogate mix VII (Neonicotinoid-II 7mix), 10 μg/ml in acetonitrile, mixture of acetamiprid - d6, nitenpyram - d3, clothianidin - d3, dinotefuran - d3, imidacloprid - d4, thiacloprid - d4, thiamethoxam - d4, were also purchased from Hayashi Junyaku (Osaka, Japan). Acetonitrile, dichloromethane formic acid, ammonium acetate and distilled water were of HPLC grade and purchased from Kanto Chemical Co. Inc. (Tokyo, Japan).

For the hair samples analyzed at the University of Neuchâtel, analytical standards (99.6 to 99.9% purity) of the five neonicotinoids (acetamiprid, clothianidin, imidacloprid, thiacloprid and thiamethoxam) were purchased from Sigma-Aldrich (Germany). Isotopically labelled neonicotinoids were purchased from CDN Isotopes (Canada) and used as internal standards. Chromatography grade solvents (acetonitrile, ethyl

Table 1. Compound-specific technical data of urine and hair analyses by HPLC-MS/MS.

	Urine					Hair		
	Log P_{OW}	LOQ[a] (μg/L)	Recovery rate (%)	RSD[b] (%)	MRM[c]	Polarity for ESI[d]	LOQ (μg/kg)	RSD (%)	Accuracy (%)
Thiamethoxam	-0.13	0.05	96	4	291.9>211.0	+	0.01	1	99
Clothianidin	0.7	0.05	92	4	250.0>132.1	+	0.02	2	103
Imidacloprid	0.57	0.05	98	5	256.0>209.1	+	0.025	1	101
Acetamiprid	0.8	0.01	102	7	223.0>126.0	+	0.008	1	100
Thiacloprid	1.26	0.01	100	5	252.9>126.1	+	0.01	1	99
Nitenpyram	-0.66	0.05	98	5	271.0>126.1	+	N.A.	N.A.	N.A.
Dinotefuran	-0.55	0.05	100	5	203.0>129.1	+	N.A.	N.A.	N.A.
DMAP[e]	N.A.[f]	0.01	101	9	208.9>126.1	+	N.A.	N.A.	N.A.

[a] LOQ, limit of quantification；[b] RSD, relative standard deviation；[c] MRM, multiple reaction monitoring；[d] ESI, electrospray ionization；[e] DMAP, *N*-desmethyl-acetamiprid；[f] N.A., not available.

acetate, methanol) were purchased from Fisher Scientific (UK). Magnesium sulphate, sodium chloride, trisodium citrate and sodium hydrogen citrate sesquihydrate were purchased from Sigma-Aldrich. Isolute PSA and ZeoPrep 90 C18 bulk phases were purchased at Biotage (Sweden) and ZeoChem (Switzerland), respectively. Ultrapure water was obtained from a Milli-Q system (Millipore, France) and solvents (acetonitrile) and additives (formic acid, ammonium formate) for LC-MS/MS were obtained from VWR (Austria).

Sample analysis

Urine analysis was performed according to a previously established method[33]. Details are shown in Supplemental Text 1 in additional file 1. Seven neonicotinoids (thiamethoxam, thiacloprid, acetamiprid, imidacloprid, clothianidin, nitenpyram, and dinotefuran) and a metabolite, DMAP, were analyzed by LC-MS/MS. For mass spectrometry, multiple reaction monitoring (MRM) was programmed. The precursor and product ions are shown in Table 1. Limits of quantitation (LOQs) of each analyte were calculated as the lowest points on standard curves (Table 1) with relative standard deviations less than 15% (n=5) and signal-to-noise ratios of 10 : 1.

Major ions, i.e. Na^+, K^+, Ca^{2+}, Mg^{2+}, NH_4^+, Cl^-, NO_3^-, SO_4^{2-}, and PO_4^{3-} were analyzed with an Ion Chromatograph (Dionex® ICS-1500). Details are shown in Supplemental Text 2 in additional file 1. SIDu and Na/K concentration ratio were calculated by urinary Na^+ (unit: mEq/L, NaU), K^+ (unit: mEq/L, KU), and Cl^- (unit: mEq/L, ClU) as follows:

$$\text{SIDu} = \text{NaU} + \text{KU} - \text{ClU}$$

$$\text{Na/K concentration ratio} = \text{NaU} / \text{KU}$$

Hair samples were analysed according to the method of Bonmatin et al. (2021)[30]. Details are shown in Supplemental Text 3. Five neonicotinoids (thiamethoxam, thiacloprid, acetamiprid, imidacloprid, clothianidin) were targeted. Limits of quantitation (LOQs) were defined as the concentrations which gave S/N ratios (signal to noise ratio) of 10 in hair samples (Table 1). Precision and accuracy were calculated from a blank hair sample (n=5) spiked at 1 μg/kg. As the blank hair sample, we used a hair sample from the Philippine study which contained no detectable trace of neonicotinoids[30]. The hair data were published alongside with results of soil and water neonicotinoid analyses of the corresponding area[30].

Statistical Analyses

All sample analytical results were sent to Department of Anesthesiology, Adachi Medical Center, Tokyo Women's Medical University for data analysis. Relevant information about age, sex, occupation, pesticides use, and hair dye were combined with the sample analytical results for statistical analysis. As previous reports suggested residue data for urine and hair followed a gamma distribution[30,34], they were log-transformed for statistical analysis. The average concentrations are, therefore, represented by the geometric mean. In order to enable calculation of geometric means of the neonicotinoid concentrations, <LOQ measurements was substituted by half of LOQ for consistency with our previous report[30]. The detection threshold for the analysis was set at 50%. For comparison of geometric mean of two groups, a t-test was used. One-way ANOVA was used to compare hair and urine residue levels among people of the three age groups. The correlation between individual hair and urine concentration was tested by a Spearman's rank correlation test when an analyte was detected no less than 50% both in urine and in hair. All statistical analyses were performed in StatPlus version 7.3.32 (AnalystSoft Inc.2020).

Results

Demographic data

A total of 99 sets of urine and hair samples were collected from 99 volunteers in the three Philippine islands i.e., Luzon, Marinduque and Mindanao, between August 23th and November 10th 2017 (Table 2). All participants resided in rural agricultural areas. While all adult men had an occupation related to potential pesticide exposure (hereafter "pesticide-exposed"), such as farmers, gardeners, and farmworkers in fruit plantation and citrus orchards, this proportion was much lower in adult women (44/44 = 100% vs. 8/27 = 29.6%; Chi-square test $p < 0.0001$; Table2).

Analyses of urine and hair – general results

In urine, at least one of the seven neonicotinoids or DMAP could be quantified in 78.8% (78/99) of samples. Of these positive samples, imidacloprid could be quantified in 60.2% of the samples (47/78, geometric mean = 0.048 μg/L), followed by thiamethoxam (39.7%), dinotefuran (20.5%), clothianidin (17.9%), acetamiprid (12.8%), DMAP (7.7%), nitenpyram (2.6%), and thiacloprid (1.3%) (Table 3). In 77 of 99 hair samples (77.8%), at least one of five neonicotinoids was detected above quantification limits. Of these positive samples, imidacloprid was present in 76.6% of the samples (59/77, geometric mean = 0.076 μg/kg), followed by clothianidin (58.7%), thiamethoxam (39.0%), acetamiprid (16.8%), and thiacloprid (11.7%) (Table 3).

Overall, the total concentrations of five neonicotinoids in urine and in hair were not correlated (r=-0.008, p=0.94). Of the 99 participants, cases with both urinary and hair detections above the LOQ in the same person were found in 25.3% of people for imidacloprid, 11.1% for thiamethoxam, 9.1% for clothianidin, and 53.3% for the total concentration of five neonicotinoids. The relationships between the geometric mean of each neonicotinoid concentration in urine and hair are shown in Figure 2.

Urinary ion analysis was performed in 93 samples (27 of 28 samples in children, 43 of 44

Table 2. Demographic data of the participants.

	Sampling place			
Islands	Luzon	Marinduque	Mindanao	Total
Address	Kabuyao, the municipality of Tuba, province of Benguet	Cawit, the municipality of Boac, province of Marinduque	Tala Mana and Pagatpat Mana, the municipality of Malita, province of Davao Occidental	
Crops: planting season (planting duration)	Sweet peas: November to January [a] (55-60 days)	Rice: December or May [b] (76-92 days)	Banana: all year [c] (3 years), Citrus: all year [d] (several years)	
Sampling date	10-Nov-2017	2-Sep-2017	23-Aug-2017	
N (Male/Female)	31 (16/15)	31 (15/16)	37 (25/12)	99 (56/43)
Age (mean±SD)	27.2±19.6	27.2±15.1	33.6±14.6	29.7±16.6
Age range (years)	7-83	6-50	7-64	7-83
Category (Male/Female)				
Children: 6-16 y.o	5/5	4/6	3/5	12/16
Adults: 18-39 y.o	7/7	7/6	9/5	23/18
Seniors: 40- y.o	4/3	4/4	13/2	21/9
Occupation of adults and seniors (Male/Female)				
Pesticide-exposed workers [e]	11/4	11/3	22/1	44/8
Not pesticide-exposed workers [f]	0/6	0/7	0/6	0/19
Hair dye of adults and seniors (Male/Female)				
Hair dyed	3/4	1/4	1/1	5/9
Hair un-dyed	8/6	10/6	11/6	29/18

[a] https://www.facebook.com/dacentralphilippines/posts/planting-calendar-heres-your-easy-guide-on-when-is-the-best-time-of-the-year-to-/3133675680047184/ [b] M.A. Gutierrez et al. The Rice Planting Window In The Philippines: An Analysis Using Multi-Temporal Sar Imagery. The International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences, Volume XLII-4/W19, 2019. [c] Department of Agriculture, Bureau of Plant Industry, Davao National Crop Research, Development and Production Support Center, Bago Oshiro, Davao City. ‘Lakatan’ production. https://library.buplant.da.gov.ph/images/1640927353Lakatan%20Production%20Guide.pdf [d] Agricultural Training Institute, Production and Processing Calamansi. https://ati2.da.gov.ph/ati-4b/content/sites/default/files/2022-12/calamansi_final.pdf [e] Farmers, gardeners, farmworkers and drivers in fruit plantation and citrus orchards. [f] Students, housewife, and house keepers.

Table 3. Results of neonicotinoid detection in urine (μg/L) and hair (μg/kg).

Neonicotinoid	>LOQ	Mean[a]	Min	25th percentile	50th percentile	75th percentile	90th percentile	Max
Urine (n=99)		(μg/L)						
Thiamethoxam	31.3%	0.044	<LOQ	<LOQ	<LOQ	0.117	0.148	0.446
Clothianidin	14.1%	0.030	<LOQ	<LOQ	<LOQ	<LOQ	0.069	0.302
Imidacloprid	47.5%	0.048	<LOQ	<LOQ	<LOQ	0.086	0.141	0.555
Acetamiprid	10.1%	0.007	<LOQ	<LOQ	<LOQ	<LOQ	0.015	0.128
Thiacloprid	1.0%	0.005	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.124
Total 5	69.7%	0.164	<LOQ	<LOQ	0.166	0.233	0.353	0.810
Dinotefuran	16.2%	0.036	<LOQ	<LOQ	<LOQ	<LOQ	0.215	0.954
Nitenpyram	2.0%	0.026	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.252
DMAP	6.1%	0.006	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.274
Total 8	78.8%	0.262	<LOQ	0.168	0.248	0.356	0.535	1.19
Hair (n=99)		(μg/kg)						
Thiamethoxam	30.3%	0.021	<LOQ	<LOQ	<LOQ	0.074	0.822	129
Clothianidin	45.5%	0.036	<LOQ	<LOQ	<LOQ	0.103	0.525	6.72
Imidacloprid	59.6%	0.076	<LOQ	<LOQ	0.066	0.177	0.570	341
Acetamiprid	13.1%	0.005	<LOQ	<LOQ	<LOQ	<LOQ	0.009	0.069
Thiacloprid	9.1%	0.005	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.022
Total 5	77.8%	0.245	<LOQ	0.043	0.175	0.437	2.43	351

[a] geometric mean.

in adult men, and 22 of 27 in adult women) due to the too low volume of six samples. All tested urinary ions were positive (Table 4). Urine Na concentrations were lower than 15mEq/L in two participants from Mindanao. SIDu concentrations were higher than 40 mEq/L in five participants, two of which were adult men from Luzon and Mindanao, two were adult women from Luzon and one was a child from Marinduque (further details are shown in Supplemental Table 3). No significant correlation was observed between SIDu and the concentration of neonicotinoids in urine or hair. The Na/K concentration ratio was 5.19 ± 3.31 in adult men, 5.04 ± 2.06 in adult women, and 7.06 ± 4.35 in children (mean ± SD).

Difference among islands and age groups

In Luzon and Mindanao Islands, imidacloprid was the most frequently detected neonicotinoid in both urine and hair samples, while in Marinduque Island the most frequently detected compounds were thiamethoxam in urine and clothianidin in hair (Figure 3, Supplemental Table 4). The total concentration of five neonicotinoids in hair was significantly higher in Mindanao Island than in the other two islands ($p<0.001$, one way ANOVA). However, no such difference was observed for urine (Supplemental Table 4).

The total concentrations of neonicotinoids in urine samples were lower in children (6 to 16 years old, n = 28) than in adults (17 to 39 years

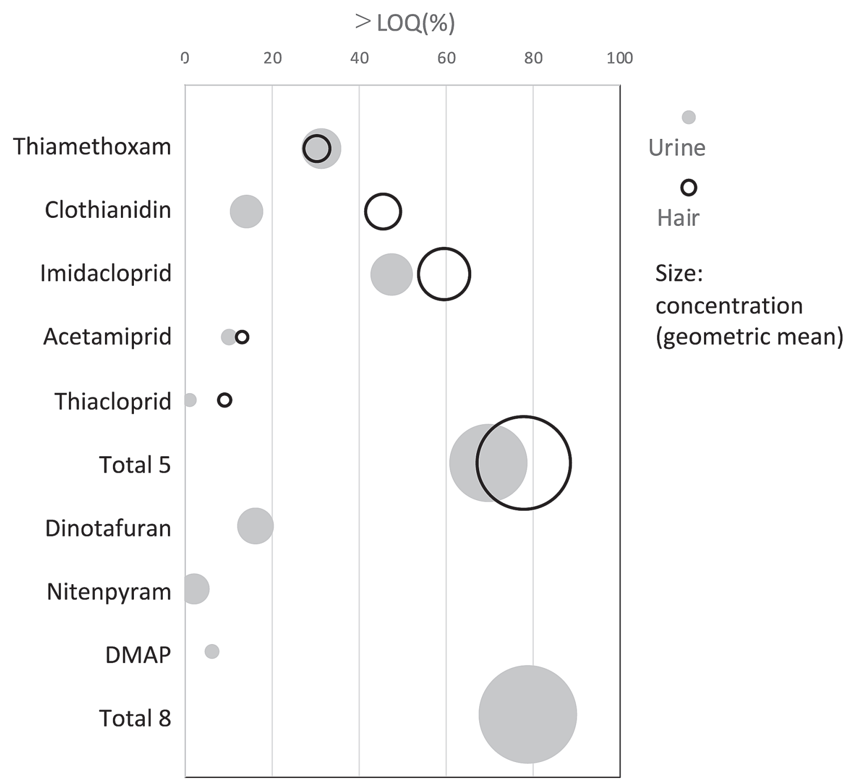

Figure 2. Comparison of neonicotinoid concentrations in urine and hair. The diameter of bubble represents the geometric mean of each neonicotinoid concentration. The center of bubble is placed on the value of > LOQ detection of each neonicotinoid (%).

Table 4. Results of urinary ion level in participants (n=93)

Urinary ion (mEq/L)	Min	25^{th} percentile	50^{th} percentile	75^{th} percentile	97.5^{th} percentile	Max
Na^+	11.8	57.7	80.7	125.2	213.7	257.4
K^+	2.0	9.7	19.6	26.9	61.5	88.4
Ca^{2+}	0.19	1.7	3.0	4.9	10.3	14.6
Mg^{2+}	0.37	3.2	5.8	8.4	14.4	16.9
NH_4^+	2.0	16.5	25.4	35.0	65.2	140.1
Cl^-	13.6	61.2	89.9	158.2	296.8	337.9
NO_3^-	0.01	0.12	0.17	0.35	1.02	1.57
PO_4^{3-}	1.0	8.6	15.4	22.8	56.9	70.6
SO_4^{2-}	3.2	17.3	24.9	35.5	63.4	73.0
SIDu	-95.8	-11.1	4.1	17.0	45.0	84.4
Na^+/K^+	1.02	3.47	4.77	6.68	13.1	24.0

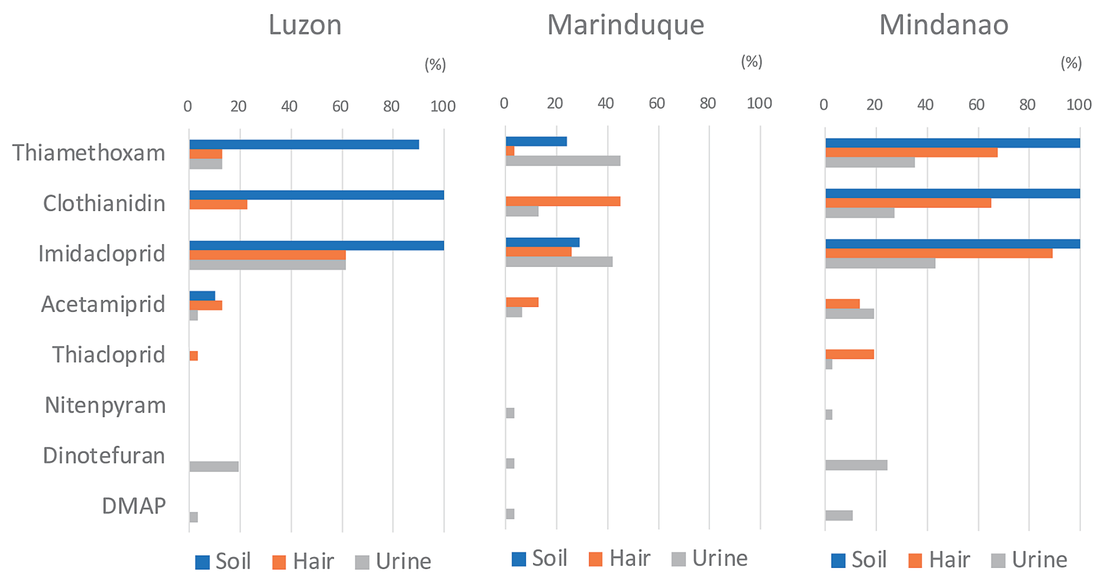

Figure 3. Comparison of neonicotinoid detection in soil, hair, and urine in the three islands. The data of soil and hair were reposted from a previous study [Ref.30]. The number of soil samples and participants were 20 and 31 in Luzon, 21 and 31 in Marinduque, and 17 and 37 in Mindanao, respectively.

old, n = 42) and seniors (40 to 83 year old, n = 29) (p=0.030, p=0.044, respectively, one way ANOVA). By contrast, no significant difference was found for imidacloprid and for the total concentrations of five neonicotinoids in hair (Table 5). The SIDu and Na/K concentration ratio did not differ between children and either adults or seniors (p=0.92, p=0.20, respectively, one way ANOVA).

Differences between exposure categories

The total concentration of the eight analyzed neonicotinoids in urine was significantly higher in pesticide-exposed workers (n=52) than in others (n=47, not pesticide-exposed workers and children) (t-tests, p=0.030) (Table 6). The concentration of imidacloprid and of the total concentration of five neonicotinoids in hair did not differ between pesticide-exposed workers and others despite the fact that the maximum concentrations were far higher in pesticide-exposed workers than in others. The SIDu and Na/K concentration ratio did not differ between pesticide-exposed workers (n=42) and others (n=42) (t-test, p=0.88, p=0.76, respectively).

Imidacloprid concentrations were lower in hair (p=0.014) in adult women with dyed hair (n=9) as compared to women with un-dyed hair (n=18). The total concentration of five neonicotinoids in hair was also lower in women with dyed hair than with un-dyed hair (p=0.017) (Supplemental Table 5).

According to the result above, we classified all participants into four categories, i.e., Category A (children, n=28), Category B (adults and seniors with neither hair dye nor pesticide exposure, n=13, M/F=1/12), Category C (adults and seniors with no hair dye but pesticide-exposed workers, n=44, M/F=39/5), and Category D (adults and seniors with dyed hair, n=14, M/F=5/9). Assuming that hair neonicotinoid detection provides evidence for chronic exposure, sensitivity and specificity of urinary neonicotinoid detection for hair neonicotinoid detection was examined (Supplemental Table 6). Only in Category A, positive likelihood ratio of urinary DMAP detection for

Table 5. Comparison of urine and hair analysis of each age group

	6-16 y.o. (n=28)			17-39 y.0. (n=41)			40- y.o. (n=30)			
Neonicotinoid	>LOQ (%)	Mean[a] (ppb)	Max (ppb)	>LOQ (%)	Mean[a] (ppb)	Max (ppb)	>LOQ (%)	Mean[a] (ppb)	Max (ppb)	p value[b]
Urine										
Thiamethoxam	21.4	0.035	0.147	39.0	0.052	0.446	30.0	0.045	0.366	N.C.
Clothianidin	0	<LOQ	<LOQ	19.5	0.034	0.302	20.0	0.031	0.009	N.C.
Imidacloprid	42.9	0.040	0.127	46.3	0.047	0.296	53.3	0.060	0.555	N.C.
Acetamiprid	14.3	0.007	0.086	7.3	0.006	0.106	10.0	0.007	0.128	N.C.
Thiacloprid	0	<LOQ	<LOQ	0	<LOQ	<LOQ	3.3	0.006	0.124	N.C.
Total 5	57.1	0.126	0.288	78.0	0.181	0.810	70.0	0.183	0.654	**0.030**
Dinotefuran	14.3	0.032	0.366	17.1	0.039	0.954	16.7	0.035	0.282	N.C.
Nitenpyram	0	<LOQ	<LOQ	2.4	0.026	0.252	3.3	0.026	0.098	N.C.
DMAP	7.1	0.006	0.206	2.4	0.005	0.062	10.0	0.007	0.274	N.C.
Total 8	71.4	0.211	0.511	80.5	0.291	1.19	76.7	0.280	1.05	**0.044**
Hair										
Thiamethoxam	28.6	0.015	1.03	36.6	0.029	129	23.3	0.018	34.7	N.C.
Clothianidin	64.3	0.064	0.80	31.7	0.024	3.84	46.7	0.035	6.72	N.C.
Imidacloprid	60.7	0.081	1.69	51.2	0.056	341	70.0	0.111	82.1	0.58
Acetamiprid	7.1	0.004	0.009	17.1	0.005	0.065	13.3	0.005	0.069	N.C.
Thiacloprid	0	<LOQ	<LOQ	17.1	0.006	0.022	6.6	0.005	0.011	N.C.
Total 5	82.1	0.242	3.03	75.6	0.228	351	76.7	0.273	124	0.32

[a] geometric mean; [b] one way ANOVA test.

hair acetamiprid detection was more than 10 (13.0). In all categories, negative likelihood ratios were no less than 0.1. Likelihood ratios above 10 and below 0.1 are considered to provide strong evidence to rule in or rule out diagnoses respectively in most circumstances[35].

Discussion

This is the first analysis of human urinary neonicotinoid contamination in the Philippines, which reveals that it is ubiquitous in the Philippines rural area. Our results suggest an effect of age and occupational pesticide use on the concentrations in urine, and that an effect of hair dye on the neonicotinoid concentration in hair can not be ruled out. Additional strong ion analysis in urine raises the hypothesis that substantial renal tubular dysfunction might be prevalent in Philippines.

In Philippine rural area, most men work in agricultural settings, e.g. rice, banana, and horticultural fruits and vegetables. Neonicotinoids are routinely used as spray applications, coated seeds, granular and drench formulations[36]. Clean drinking water supply is insufficient, especially in low-income class[37]. Even though the

Table 6. Comparison of neonicotinoids and DMAP concentration between pesticide related workers and others.

Neonicotinoid	Pesticide-exposed workers (n=52)			Others (n=47)			
	>LOQ	Mean[a]	Max	>LOQ	Mean[a]	Max	p value[b]
Urine		(μg/L)	(μg/L)		(μg/L)	(μg/L)	
Thiamethoxam	30.8%	0.045	0.446	31.9%	0.044	0.366	N.C.
Clothianidin	21.2%	0.034	0.302	6.4%	0.027	0.091	N.C.
Imidacloprid	55.8%	0.057	0.555	38.3%	0.040	0.296	N.C.
Acetamiprid	11.5%	0.007	0.128	8.51%	0.006	0.086	N.C.
Thiacloprid	1.9%	0.005	0.124	0	<LOQ	<LOQ	N.C.[c]
Total 5	73.1%	0.184	0.810	66.0%	0.145	0.607	0.050
Dinotefuran	17.3%	0.038	0.954	14.9%	0.034	0.366	N.C.
Nitenpyram	3.8%	0.027	0.252	0	<LOQ	<LOQ	N.C.
DMAP	7.7%	0.006	0.274	4.3%	0.006	0.206	N.C
Total 8	76.9%	0.293	1.19	76.6%	0.232	0.858	**0.030**
Hair		(μg/kg)	(μg/kg)		(μg/kg)	(μg/kg)	
Thiamethoxam	32.7%	0.033	129.3	27.7%	0.013	1.03	N.C.
Clothianidin	42.3%	0.033	6.72	48.9%	0.040	0.80	N.C.
Imidacloprid	57.7%	0.084	341.4	61.7%	0.069	1.69	0.64
Acetamiprid	19.2%	0.006	0.0069	6.4%	0.004	0.009	N.C.
Thiacloprid	11.5%	0.006	0.022	6.4%	0.005	0.018	N.C.
Total 5	80.8%	0.324	351	74.4%	0.180	3.03	0.14

[a] geometric mean; [b] comparison of neonicotinoid or metabolite concentration between pesticide related workers and others (t-test, two-tailed); [c] N.C., not calculable due to a low sample number.

information about the type of products and quantities used in particular crops was not available, the lower frequency of detection and median value of urinary DMAP in this study (6.1%, <0.01 μg/L) as compared to previous studies conducted in Japan (100%, 0.46 μg/L)[38], Ghana (94.7%, 0.41 μg/L)[39], and Sri Lanka (92.4%, 0.52 μg/L)[10] by a laboratory with the same method may suggest that the use of acetamiprid in the three studied islands in the Philippines was uncommon in the season when samples were collected. Products containing thiamethoxam, clothianidin, imidacloprid, acetamiprid and dinotefuran are registered as insecticides in the Philippines[40], but not thiacloprid, and shipment volume data for this substance were not available. Nevertheless, thiacloprid was also detected in human samples in this study.

Being less intrusive and easy to collect, urine may be the preferable matrix for screening populations highly or chronically exposed to neonicotinoids and other pollutants, and urinary concentrations can be good biomarkers of brain neonicotinoid exposure for a short time. The detection rate and median concentrations or geometric mean may be useful to compare the degree of neonicotinoid exposure between groups. Lower urinary neonicotinoid concentrations in children than in adults and seniors suggest that the age of participants seems to be an important factor when evaluating urinary neonicotinoid concentration.

Equal or higher levels of neonicotinoid concentrations in hair than in urine, and the unsatisfactory positive and negative likelihood ratio of urinary neonicotinoid detection for hair detection in this study suggests that neonicotinoids residue analysis in hair should be preferred when aiming to assess chronic human exposure to pesticides, especially in children, in accordance with previous studies[41-44]. Residues in hair seems to be better indicators of chronic environmental exposure, when occupational direct contamination or the effect of hair dye were excluded (i.e., excluding people with dyed hair from the analysis). Contamination during hair sampling is deemed unlikely in this study because we observed no significant difference in imidacloprid concentration between the hair of pesticide-exposed workers and others, although neonicotinoid concentrations in urine were much higher in pesticide-exposed workers. In real life monitoring surveys using hair, non-dietary contamination of hair (e.g., adsorption from particles suspended in the air or in the rain) should nevertheless be considered. To clarify the significance of such differences and the possible bias caused by non-dietary contamination, controlled toxicological studies in experimental animals with biomonitoring of hair, plasma and tissue samples including brain could be useful to determine the benefits of hair neonicotinoid analysis to assess brain exposure to neonicotinoids.

An additional advantage of hair sampling is it is fast and easy, and samples are smaller and easier to preserve and transport than urine samples. However, the preparation for analysis of hair samples is more laborious than for urine. Although the stability of pesticide residues in hair is not known, it is presumed to be stable for many days if not months, based on the physicochemical characteristics of neonicotinoids and their documented long-term persistence in the environment[2].

To explain the high neonicotinoid detection in hair, two factors can be considered, i.e., lipophilicity and melanin binding. This is consistent with the binding properties of 75 chemicals to keratin[45]. Lipophilic substances are more likely to bind to keratin and other proteins. Although the lipophilicity of neonicotinoids is not very high (Table 1), neonicotinoid binding to high affinity proteins such as melanin may explain their accumulation in hair. Melanin is a natural pigment found in the eyes, skin, nails and hair, and reportedly binds to a variety of drugs and chemicals[46-52]. The structural characteristics of drugs and chemicals for melanin binding are presumably related to basicity, hydrophobicity, and charge-transfer[49-59]. Reilly et al.[49] studied chemical structures contributing to melanin binding in silico and found that the most contributing structural characteristics were the number of rigid bonds and rings, which is related to pi-pi interactions between the aromatic melanin structure and drugs. Neonicotinoids have rigid structures such as the chloro-pyridyl ring, the thiazole ring and the tetrahydrofuran ring, suggesting they can likely form melanin-drug complexes.

Taken together, these results suggest that neonicotinoid detections in spot urine is preferable to detect acute exposure in the general population, while the analysis of hair is more appropriate for studies of chronic exposure. However, in both cases the results will not yet allow researchers to assess personal exposure levels precisely. Recently, Loser et al.[60] showed that imidacloprid, acetamiprid, clothianidin and thiacloprid have agonistic action on a model of human developing neurons, i.e., dopaminergic neurons generated from LUHMES neuronal precursor cells and that SH-SY5Y neuroblastoma cells can be used as an established model of nAChR signaling. They also showed that one of the imidacloprid metabolites, desnitro-imidacloprid has strong binding affinity and efficacy on the human developing neurons, which was comparable to nicotine[61]. Nimako et al.[62] showed in mice, that continuous low dose imidacloprid exposure caused higher desnitro-imidacloprid concentration in liver, brain, testis, lung and kidney than in blood, but no such pattern was visible for imidacloprid itself. Wang et al.[63] quantified desnitro-imidacloprid in human urine, while Wrobel et al.[64] did not identify the urinary excretion of desnitro-imidacloprid after micro dose of imidacloprid intake. Continuous exposure to neonicotinoids and their metabolites might therefore cause accumulation in tissue as well as in hair, even in the case of relatively low urinary concentration, and therefore might induce negative effects for neurodevelopment.

Considerable increases of SIDu were observed in approximately 5% of Philippine participants. If environmental exposure of neonicotinoids due to occupational use, drift inhalation, drinking water and food contamination, represents risk of chronic kidney disease, a global investigation is urgently needed. Abnormal decreases of urine Na^+ concentration were observed in two participants, but the clinical significance of this observation is unknown. Interestingly, the urine ion analysis of spot urine revealed that the Na/K ratio among Philippine people (5.19 ± 3.31 in adult men, 5.05 ± 2.06 in adult women, and 7.06 ± 4.35 in children; mean ± SD), were higher than the result of the 24h urine in the INTERMAP study from Japan, China, UK, and US (3.89 ± 2.11; mean ± SD; N = 4,680, age 49.17 ± 5.47 yrs)[65]. This result might be caused by the comparatively higher amount of water and salt intake to adapt to the hot climate in the Philippine. Further investigation is needed to better understand and interpret such patterns.

A limitation of this study is that two recent neonicotinoids, dinotefuran and nitenpyram, and DMAP, one of major neonicotinoid metabolites detected in urine were not analyzed in hair. Our results may therefore have under-estimated the total neonicotinoid exposure. Furthermore, blood was not analyzed. Besides, the relatively small number of participants in this study limits the power of statistical tests.

Conclusion

We reported urinary neonicotinoids concentration in the Philippine general population for the first time. The frequency of detection of neonicotinoid of hair and urine were comparable, but the positive and negative likelihood ratio was unsatisfactory. Hair can be an essential matrix to screen chronic neonicotinoid exposure, especially for children. Future analytical and epidemiological studies are required to clarify the potential links between chronic exposure to neonicotinoids and potential human health problems.

Acknowledgements

We would like to thank Dr. Kazutoshi Fujioka at Albany College of Pharmacy and Health Sciences, USA, who gave us many insightful suggestions to finalize this study.

Funding

This study was supported by the Triodos Foundation's Support Fund for Independent Research on neonicotinoids and humans, a scientific investigation into the neurotoxicological effects of neonicotinoid insecticides, awarded to the Public Health Working Group of the Task Force on Systemic Pesticide (Chair Dr. K. Taira), and Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT) awarded to Dr. K. Taira (No.15K00559). The funders had no role in the study design, data collection or analysis.

Competing interests

Authors have no competing interest to disclose.

References

1) Simon-Delso N, Amaral-Rogers V, et al. Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. Environ Sci Pollut Res Int. 22: 5-34, 2015
2) Giorio C, Safer A, et al. An update of the Worldwide Integrated Assessment (WIA) on systemic insecticides. Part 1: new molecules, metabolism, fate, and transport. Environ Sci Pollut Res Int. 28: 11716-11748, 2021
3) Onozaki Y, Horikoshi R, et al. A Novel Insecticide Acting at the Nicotinic Acetylcholine Receptors. J Agric Food Chem. 65: 7865-7873, 2017
4) Bonmatin JM, Giorio C, et al. Environmental fate and exposure; neonicotinoids and fipronil: Environ Sci Pollut Res Int. 22: 35-67, 2015
5) Pisa L, Goulson D, et al. An update of the Worldwide Integrated Assessment (WIA) on systemic insecticides. Part 2: impacts on organisms and ecosystems.: Environ Sci Pollut Res Int. 28, 11749-11797, 2021
6) Goulson D. Pesticides, Corporate Irresponsibility, and the Fate of Our Planet. One Earth 2, 2020. https://doi.org/10.1016/j.oneear.2020.03.004
7) Taira K, Aoyama Y, et al. Detection of chloropyridinyl neonicotinoid insecticide metabolite 6-chloronicotinic acid in the urine: six cases with subacute nicotinic symptoms. Chudoku Kenkyu. 24: 222-30, 2011, Japanese
8) Taira K, Fujioka K, et al. Qualitative profiling and quantification of neonicotinoid metabolites in human urine by liquid chromatography coupled with mass spectrometry. PLoS One. 8: e80332, 2013
9) Marfo JT, Fujioka K, et al. Relationship between Urinary N-Desmethyl-Acetamiprid and Typical Symptoms including Neurological Findings: A Prevalence Case-Control Study. PLoS One. 10: e0142172, 2015
10) Taira K, Kawakami T, et al. Urinary concentrations of neonicotinoid insecticides were related to renal tubular dysfunction and neuropsychological complaints in Dry-zone of Sri Lanka. Sci Rep. 11: 22484, 2021
11) Cimino AM, Boyles AL, et al. Effects of Neonicotinoid Pesticide Exposure on Human Health: A Systematic Review. Environ Health Perspect. 125: 155-162, 2017
12) Stokstad E. European Union expands ban of three neonicotinoid pesticides. Science. 360, 2018
13) European Food Safety Authority (EFSA) et al. Peer review of the pesticide risk assessment of the active substance thiacloprid. EFSA Journal. 17: 5595, 2019
14) United States Environmental Protection Agency (USEPA). Proposed Interim Registration Review Decision for Neonicotinoids. Available from: https://www.epa.gov/pollinator-protection/proposed-interim-registration-review-decision-neonicotinoids
15) Australian Pesticides and Veterinary Medicines Authority (APVMA). Neonicotinoids chemical review - in progress. Available from: https://apvma.gov.au/node/57031
16) Yue M, Liu Q, et al. Urinary neonicotinoid concentrations and pubertal development in Chinese adolescents: A cross-sectional study. Environ Int. 163: 107186, 2022
17) Wang A, Wan Y, et al. Neonicotinoid insecticide metabolites in seminal plasma: Associations with semen quality: Sci Total Environ. 811: 151407, 2022
18) Zhang H, Zhang R, et al. Exposure to neonicotinoid insecticides and their characteristic metabolites: Association with human liver cancer. Environ Res. 208: 112703, 2022
19) Taira K. Health effects of neonicotinoid insecticides -Part 1: Physicochemical Characteristics and Case Reports- Jpn J Clin Ecol. 21: 24-34, 2012. in Japanese
20) Lin PC, Lin HJ, et al. Acute poisoning with neonicotinoid insecticides: a case report and literature review. Basic Clin Pharmacol Toxicol. 112: 282-6, 2013

21) Harada KH, Tanaka K, et al. Biological Monitoring of Human Exposure to Neonicotinoids Using Urine Samples, and Neonicotinoid Excretion Kinetics. PLoS One 11, e0146335, 2016
22) Chang C-H, MacIntosh D, et al. Characterization of daily dietary intake and the health risk of neonicotinoid insecticides for the U.S. Population. J Agric Food Chem. 66: 10097-105, 2018
23) Chen D, Zhang Y, et al. Dietary exposure to neonicotinoid insecticides and health risks in the Chinese general population through two consecutive total diet studies. Environ Int. 135: 105399, 2020
24) Ikenaka Y, Fujioka K, et al. Contamination by neonicotinoid insecticides and their metabolites in Sri Lankan black tea leaves and Japanese green tea leaves. Toxicol Rep. 5: 744-749, 2018
25) Nimako C, Hirai A, et al. Neonicotinoid residues in commercial Japanese tea leaves produced by organic and conventional farming methods. Toxicol Rep. 8: 1657-1664, 2021
26) Wang L, Liu T, et al. Occurrence and Profile Characteristics of the Pesticide Imidacloprid, Preservative Parabens, and Their Metabolites in Human Urine from Rural and Urban China. Environ Sci Technol. 49: 14633-14640, 2015
27) Li AJ, Martinez-Moral MP, et al. Variability in urinary neonicotinoid concentrations in single-spot and first-morning void and its association with oxidative stress markers. Environ Int. 135: 105415, 2020
28) Laubscher B, Diezi M, et al. Multiple neonicotinoids in children's cerebrospinal fluid, plasma, and urine. Environ Health 21: 10, 2022
29) Kavvalaskis MP, Tzatzarakis MN, et al. Development and application of LC–APCI–MS method for biomonitoring of animal and human exposure to imidacloprid. Chemosphere 93: 2612-2620, 2013
30) Bonmatin JM, Mitchell EAD, et al. Residues of neonicotinoids in soil, water and people's hair: A case study from three agricultural regions of the Philippines. Sci Total Environ. 757: 143822, 2021
31) Kellum JA. Determinants of blood pH in health and disease. Crit Care. 4: 6-14, 2000
32) Balsorano P, Romagnoli S, et al. Urinary Strong Ion Difference as a Marker of Renal Dysfunction. A Retrospective Analysis. PLoS One. 11: e0156941, 2016
33) Ichikawa G, Kuribayashi R, et al. LC-ESI/MS/MS analysis of neonicotinoids in urine of very low birth weight infants at birth. PLoS ONE 14: e0219208, 2019
34) Taira K, Hasegawa H, et al. Neonicotinoid insecticides and organic diets. Jpn J Clin Ecol 32: 1-17, 2023
35) Jaeschke R, Guyatt G, et al. Users' guides to the medical literature. Chicago: AMA Press, 121-40, 2002
36) Perez, I.C.J., Gooc, C.M., Cabili, J.R., Rico, M.J.P., Ebasan, M.S., Zaragoza, M.J.G., et al., 2015. Pesticide use among farmers in Mindanao, Southern Philippines. AES Bioflux 7, 90-108.
37) National Economic and Development Authority. Philippine Water Supply and Sanitation Master Plan 2019-2030.
https://neda.gov.ph/wp-content/uploads/2021/09/120921_PWSSMP_Main-Report.pdf
38) Ikenaka Y, Miyabara Y, et al. Exposures of children to neonicotinoids in pine wilt disease control areas: Environ Toxicol Chem. 38: 71-79, 2019
39) Nimako C, Ikenaka Y, et al. Human Exposures to Neonicotinoids in Kumasi, Ghana. Environ Toxicol Chem. 40: 2306-2318, 2021
40) Republic of the Philippines department of agriculture fertilizer and pesticide authority. List of registered agricultural pesticides. https://fpa.da.gov.ph/NW/images/FPAfiles/DATA/Regulation/Pesticide/Files-2023/Products/LRAP-20230403.pdf
41) Knipe DW, Jayasumana C, et al. Feasibility of hair sampling to assess levels of organophosphate metabolites in rural areas of Sri Lanka. Environ Res. 147: 207-211, 2016
42) Appenzeller BMR, Hardy EM, et al. Hair analysis for the biomonitoring of pesticide exposure: comparison with blood and urine in a rat model. Arch Toxicol. 91: 2813-2825, 2017
43) Hernández AF, Lozano-Paniagua D, et al. Biomonitoring of common organophosphate metabolites in hair and urine of children from an agricultural community. Environ Int. 131: 104997, 2019
44) Polledri E, Mercadante R, et al. A liquid chromatography tandem mass spectrometry method to assess 41 pesticides in human hair. J Chromatogr B Analyt Technol Biomed Life Sci. 1159: 122389, 2020
45) Hansen S, Selzer D, et al. An extended database of keratin binding. J Pharm Sci. 100: 1712-1726, 2011
46) Larsson BS. Interaction between chemicals and melanin: Pigment Cell Res. 6: 127-133, 1993
47) Karlsson O, Lindquist NG. Melanin and neuromelanin binding of drugs and chemicals: toxicological implications. Arch Toxicol. 90: 1883-1891, 2016
48) Manzanares JA, Rimpelä AK, et al. Interpretation of

Ocular Melanin Drug Binding Assays. Alternatives to the Model of Multiple Classes of Independent Sites: Mol Pharm. 13: 1251-1257, 2016
49) Reilly J, Williams SL, et al. High-Throughput Melanin-Binding Affinity and In Silico Methods to Aid in the Prediction of Drug Exposure in Ocular Tissue. J Pharm Sci. 104: 3997-4001, 2015
50) Haining RL, Jones TM, et al. Saturation Binding of Nicotine to Synthetic Neuromelanin Demonstrated by Fluorescence Spectroscopy. Neurochem Res. 41: 3356-3363, 2016
51) Pragst F, Balikova MA. State of the art in hair analysis for detection of drug and alcohol abuse. Clin Chim Acta. 370: 17-49, 2006
52) Uematsu T, Sato R, et al. Human scalp hair as evidence of individual dosage history of haloperidol: a possible linkage of haloperidol excretion into hair with hair pigment. Arch Dermatol Res. 282: 120-5, 1990
53) Sato H, Uematsu T, et al. Chlorpromazine in human scalp hair as an index of dosage history: comparison with simultaneously measured haloperidol. Eur J Clin Pharmacol. 44: 439-44, 1993
54) Mizuno A, Uematsu T, et al. Analysis of nicotine content of hair for assessing individual cigarette-smoking behavior. Ther Drug Monit. 15: 99-104, 1993
55) Henderson GL, Harkey MR, et al. Incorporation of isotopically labeled cocaine into human hair: race as a factor. J Anal Toxicol. 22: 156-65, 1998
56) Kronstrand R, Förstberg-Peterson S, et al. Codeine concentration in hair after oral administration is dependent on melanine content. Clin Chem. 45: 1485-94, 1999
57) Kronstrand R, Anderson MC, et al. Incorporation of selegiline metabolites into hair after oral selegiline intake. J Anal Toxicol. 25: 594-601, 2001
58) Pötsch L, Skopp G, et al. A comparison of 3H-cocaine binding on melanin granules and human hair in vitro. Int J Leg Med. 110: 55-62, 1997
59) Claffey DJ, Stout PR, et al. 3H-nicotine, 3H-flunitrazepam, 3H- cocaine incorporation into melanin: a model for the examination of drug–melanin interactions. J Anal Toxicol. 25: 607-11, 2001
60) Loser D, Hinojosa MG, et al. Functional alterations by a subgroup of neonicotinoid pesticides in human dopaminergic neurons. Arch Toxicol. 95: 2081-2107, 2021
61) Loser D, Grillberger K, et al. Acute effects of the imidacloprid metabolite desnitro-imidacloprid on human nACh receptors relevant for neuronal signaling. Arch Toxicol. 95: 3695-3716, 2021
62) Nimako C, Ikenaka Y, et al. Simultaneous quantification of imidacloprid and its metabolites in tissues of mice upon chronic low-dose administration of imidacloprid. J Chromatogr A. 1652: 462350, 2021
63) Wang A, Mahai G, et al. Assessment of imidacloprid related exposure using imidacloprid-olefin and desnitro-imidacloprid: Neonicotinoid insecticides in human urine in Wuhan, China. Environ Int. 141: 105785, 2020
64) Wrobel SA, Bury D, et al. Human metabolism and urinary excretion of seven neonicotinoids and neonicotinoid-like compounds after controlled oral dosages. Arch Toxicol. 96, 121-134, 2022
65) Stamler J, Chan Q, et al. Relation of Dietary Sodium (Salt) to Blood Pressure and Its Possible Modulation by Other Dietary Factors: The INTERMAP Study. Hypertension 71: 631-637, 2018

Additional file（http://jsce-ac.umin.jp/200725/files_jjce/32-2/index.html）

Supplemental Text 1. Details of urine analysis.

Supplemental Text 2. Details of ion analysis in urine.

Supplemental Text 3. Details of hair analysis.

Supplemental Table 1. Neonicotinoids and metabolites quantification in urine from the general population.

Supplemental Table 2. Neonicotinoids and metabolites quantification in body fluid except urine and in hair from the general population.

Supplemental Table 3. Profiles of two participants urinary Na were less than 15 mEq/L, and Five Participants Urinary Strong Ion Difference were more than 40 mEq/L.

Supplemental Table 4. Comparison of urine and hair analysis of the three islands.

Supplemental Table 5. Comparison of urine and hair analysis of each age group.

Supplemental Table 6. Comparison of hair neonicotinoid concentration in adult women between dyed and un-dyed hair groups.

Supplemental Table 7. Relationships between neonicotinoids detection in urine and in hair.

Declarations

Ethics approval and consent to participate

This study was reviewed and approved by the ethic committee of Tokyo Women's Medical University (Approval number 4521). Written informed consent to participate in this study was obtained from each participant.

Consent for publication

Not applicable.

Data availability statement

The data that support the findings of this study are available from the corresponding author at VFG 03077@nifty.com, upon reasonable request.

Authors' contribution

KT, EADM, AT, FSB, AS, MBL, JMB, FC, YA and MK contributed to the substantial conception and designed the work.

EL and FC contributed to the sample acquisition.

GG, YM and YI contributed to the sample analysis.

KT, AA, GG, EADM, FSB, AT, JMB, AS contributed to the interpretation of data.

KT, AA, GG, EADM, YI, FSB, JMB, AS, and YM drafted the manuscript and revised it.