EXPOSURES OF CHILDREN TO NEONICOTINOIDS IN PINE WILT DISEASE CONTROL AREAS

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1. INTRODUCTION

Agrochemicals, including insecticides and herbicides, have been used to protect agricultural plants and forests from various kinds of pests, with general understanding on the balance of costs and benefits in society. However, exposures to agrochemicals can induce acute and chronic poisoning in sensitive populations, such as fetuses and infants as well as chemically sensitive population at risk. In children, pesticide exposures reportedly occur via multiple routes, including diet, drinking water, inhalation, and skin absorption (Chensheng et al. 2000). Moreover, children living with parents who work with pesticides or who live in the proximity of pesticide-treated farmlands are more susceptible to higher pesticide exposures than others living in the same communities (Chensheng et al. 2000*).*

Children may be highly susceptible to the toxic effects of pesticides, with potential developmental, dietary, and physiologic consequences (Roberts et al. 2012) that are exacerbated by their rapid growth rates and high energy demands (caloric and oxygen requirements). Compared with adults, children drink more water, eat more food, and breathe more air relative to their body weights, likely facilitating the accumulation of high doses of pesticides in their bodies (NRC 1993). In addition, blood–brain barriers of fetuses and neonates are immature during brain development, allowing the passage and accumulation of various chemicals,

including pesticides, into fetal brains (Zheng et al. 2001). This may also result in higher circulating levels and enhanced toxicity of pesticides in children (Weiss et al. 2004). Taken together, the high susceptibility of children to pesticide exposures and the potential impacts of these compounds on children's health are considered to be serious public and academic concerns (CEH 2012).

Various insecticides, including organochlorines, organophosphates, and pyrethroids, have been developed and widely used for pest management. However, insecticides that are less toxic to humans relative to targeted species are highly sought, and since their introduction in the 1992, neonicotinoids have been increasingly marketed. In 2008, neonicotinoids comprised 24% of all marketed agrochemicals (total volume of €6.330 billion), mainly replacing organophosphates (13.6%) and carbamates (10.8%) (Jeschke et al. 2011). Global neonicotinoid use has continued to increase, and neonicotinoids have been registered for use in more than 120 countries, with a total production volume of US$2.5 billion across the globe (Akash et al. 2016). Neonicotinoids were designed as specific agonist of insect nicotine-like receptors (nAChRs). However, recent studies have shown that neonicotinoids can bind to not only insect nAChRs but also mammalian nAChRs, with non-negligible dissociation constants (Kimura-Kuroda et al. 2012). These receptors are of critical importance to human brain function, especially during the development *(*Kandel et al. 2012) of memory, cognition, and behavior (Chen et al. 2014).

In Japan, pine trees are considered symbolic of the beauty of the natural environment and are appreciated in mountain and coastal environments and in gardens of historic sites. However, since the beginning of the 20th century, pine trees have been threatened by pine wilt disease in Japan (Proença et al. 2017), and the nematode *Bursaphelenchus xylophilus* was identified as the

main cause. Because these worms are transmitted by the beetle species *Monochamus alternatus* (Mamiya et al. 1988), pine trees have been protected from pine wilt by spraying insecticides over large areas using helicopters or jet-spray machines, although effectiveness of such spraying practices has remained to be proven.

Inhabitants of communities in such spraying zones are seriously concerned by these practices, and complaints of health problems and symptoms that are related to pesticide toxicity have been widely recorded. In previous studies, neonicotinoids were detected in urine samples from Japanese women and children, and neonicotinoid concentrations in 3-year-old children living in Aichi Prefecture of Japan ranged from the limit of detection (LOD) to 370 μg/g of creatinine, with a geometric mean of 4.16 μg/g of creatinine (Ueyama et al. 2015; Osaka et al. 2016*)*. However, despite the ubiquitous use of neonicotinoids in pine wilt control areas, exposures and health impacts of neonicotinoids in these areas have not been formerly reported. Thus, to assess neonicotinoid exposure levels of children (3 to 6 years old) living in Nagano Prefecture of Japan, we determined concentrations of thiacloprid and other six neonicotinoids in urine specimens and estimated daily exposure levels with regard to acceptable daily intake (ADI) values.

2. MATERIALS AND METHODS

This study was approved by the Ethics Screening Committee of Hokkaido University (No. 28-1), and written informed consent was granted by all primary guardians prior to inclusion of children in the study.

2.1.Study areas

Subjects were selected from communities in Nagano Prefecture, which is located in the central part of Honshu Island, Japan. This prefecture has numerous valleys, high mountains and forests, and the Japanese red pine (*Pinus densiflora*) is a predominant plant species in the local forests, which are periodically sprayed with insecticides to control pine wilt disease.

2.2.Study subjects

Children of 3 to 6 years of age were recruited by advertising in city papers and in a local newspaper, and a total of 46 (23 males and 23 females) subjects were included. Mean (range: min. to max.) ages of male and female subjects were 4.8 (3 to 6) and 4.9 (3 to 6) years, respectively. Early morning urine samples (before breakfast) were collected by guardians before (May 26, 2016), during (June 23, 2016), and after (July 21, 2016) insecticide spraying events using pre-distributed paper cups and were transferred into plastic 15 mL centrifuge tubes. Tubes were then placed in zip lock bags and stored in household freezers until transfer to the Department of Toxicology, Faculty of Veterinary Science, Hokkaido University, for analysis.

2.3.Air Sample Collection

Air samples were collected at two sites (A and B) in the proximity of the residences of the studied subjects, and at a reference site in Suwa City, Nagano Prefecture, where neonicotinoids have never been used to control pine wilt disease. Air samples (particulate matter) were collected using low-volume air samplers equipped with quartz filters (2500QAT-UP 55 mm, Pall life Sciences, Ann Arbor, USA). Air sampling was performed in affected areas from May 19 to 26, 2016, (2) from June 17 to 24, 2016, and (3) from July 14 to 22, 2016, and was performed in the reference area on May 30, 2016, and June 6, 2016.

107 *2.4.Analysis of neonicotinoid levels in urine specimens*

108 Urine was thawed, stirred, and allowed to stand for 1 h. Neonicotinoids and their metabolites
109 were then extracted and purified using solid phase extraction methods. In these procedures,
110 Presep RPP cartridges (60 mg; Wako Pure Chemical Industries, Ltd., Osaka, Japan) were
111 conditioned with 2 mL of methanol and 2 mL of distilled water, and 1.0 mL aliquots of urine
112 were then thoroughly mixed with 5 ng (50 μL of 100 ppb solution) of internal standards and
113 loaded into the cartridge. ENVIcarb/PSA (500 mg/300 mg; Sigma-Aldrich, Japan) cartridges
114 were then conditioned with 10 mL of acetone and connected in series with Presep RPP
115 cartridges and were then eluted with 8 mL of dichloromethane:acetonitrile (2:8,v/v) solution.
116 After concentrating and dry-solidifying using a centrifugal concentrator (CVE-200D with UT-
117 2000, EYELA, Tokyo, Japan), extracts were reconstituted with 100 μL of 3% (v/v) methanol
118 solution and were transferred into vials for analysis.

119 The LC-ESI/MS/MS instrument (Shimadzu 20 A series with LCMS8040, Shimadzu Co.,
120 Kyoto, Japan) was equipped with a RSpak DE-213 (2 mm ID × 150 mm) column (Showa
121 Denko, Tokyo, Japan) for sample analyses. HPLC solvents A and B comprised 0.1% (v/v)
122 formic acid and 10 mM acetic acid in water and 0.1% (v/v) formic acid and 10 mM acetic acid
123 in methanol, respectively, and were applied with the following gradient: t = 0 to 2 min, 20%
124 Solvent B; t = 11 min, 95% Solvent B; t = 11 to 13 min, 95% Solvent B. The column oven
125 temperature and flow rate were 45 °C and 0.4 mL/min, respectively. Multiple reaction
126 monitoring for mass spectrometry was programed as described in Table 1, and six
127 neonicotinoids and an acetamiprid metabolite, *N*-dm-acetamiprid, were detected in the range of
128 96% to 102%. Precision of analysis of all seven neonicotinoids was confirmed by multiple

analysis, with a relative standard deviation of 10% (Table 1). Analytes were quantitated using internal standard methods, and calibration curves were generated for each analyte by mixing compounds with blank urine specimens to final concentrations of 0.05, 0.1, 0.2, 0.5, 1.25, 2.5, 3.75, and 5 ng/mL. During the preparatory stage of our study, internal standards for nitenpyram and N-dm-acetamiprid were not commercially available. Hence Nitenpyram was quantified using the Dinotefuran internal standard; dindotefuran-d3. Our choice of Dinotefuran-d3 for Nitenpyram quantification based on the similarity in the retention times Nitenpyram and Dinotefuran, as well thee similarities in physicochemical properties of both compounds. In the absence of an internal standard, N-dm-Acetamiprid was also quantified using Acetamipride-d6. Undetectable neonicotinoid levels were confirmed in urine specimens from a volunteer who mainly eats organic food, and these were then used as blank urine. Extraction and purification of each calibration point was performed using the method described above, and linearity exceeds $r^2 = 0.9$ in all calibration curves. Limits of quantitation (LOQs) were calculated as the lowest points on standard curves (Table 1) with relative standard deviations of less than 15% ($n = 5$) and signal-to-noise ratios of 5:1.

2.5.Measurements of urinary creatinine concentrations

Urinary creatinine concentrations were determined using Urinary Creatinine 10-Plate Detection Kits (Arbor Assays, City, MI, USA) according to the manufacturer's instructions.

2.6.Air sample preparation and analysis

Particulate matter from study and reference areas were prepared as described previously (Takenouchi et al. 2016). Briefly, shredded filters were put into 10 mL aliquots of ethyl acetate: acetone (9:1,v/v) solutions and were sonicated for 5 min. Extracts were dried under a gentle

stream of nitrogen gas and were then dissolved in 1 mL of distilled water:acetone (4:1,v/v) solution. Finally, extracts were filtered through 0.45 µm pore membrane filters (DISMIC-25cs, Advantech, Tokyo, Japan) and were transferred into HPLC vials for LC-ESI/MS/MS analyses.

2.7.Calculation of EDI from urinary neonicotinoid concentrations

Estimated daily intake (EDI) of neonicotinoids was calculated from urinary neonicotinoid and creatinine concentrations using the following formula:

EDI (µg/day) = urine neonicotinoids concentration (µg/g cre) × 0.3 (g cre/day) × 1/r (urinary excretion correction coefficient) *(1)*

Excreted creatinine levels were assumed to be 0.3 g per day, as shown previously in children (Sakurabayashi et al. 1999). Because kinetics parameters for thiacloprid have not been established in humans, these were adopted from rat experiments (r = 0.05) in which 1.8% to 5.9% of orally ingested thiacloprid was excreted through urine (Pfeil et al. 2006). Kinetic parameters for acetamiprid, clothianidin, dinotefuran, and imidacloprid in humans are reportedly r = 0.586, 0.596, 0.899, and 0.133, respectively (Harada et al. 2016). In the absence of established correction coefficients for excretions of nitenpyram and thiamethoxam in humans, we applied r = 0.8 and 0.6, respectively, for these neonicotinoids (JMPR 2010; FSC 2016). Finally, daily exposures to thiacloprid and other neonicotinoids were calculated by applying these coefficients to equation (1). According to a previously reported kinetic study (Harada et al. 2016), most of acetamiprid, once absorbed into the body, is rapidly metabolized and excreted to urine as N-dm-acetamiprid. In the estimation of EDI values for acetamiprid, we incorporated N-dm-acetamiprid data into acetamiprid data.

Atmospheric contributions to EDIs of neonicotinoids in children from study areas were calculated using equation (2), with the assumption that the daily breathing volume of 1 to 12-year-old children is 8.7 m^3 (Kawahara et al. 2010):

Neonicotinoid intake from the atmosphere (ng/day) = Atmospheric neonicotinoid concentration (pg/m^3) × Daily breathing volume of children (8.7 m^3/day) (2)

2.8.Statistical analysis

Statistical analyses were performed using JMP 12 (SAS Institute Inc., Cary, NC, USA). Non-parametric Steel–Dwass tests were performed to compare seasonal variations in urinary neonicotinoid concentrations. Differences among groups were considered significant when $p < 0.05$ in all analyses.

3. RESULTS AND DISCUSSION

3.1.Neonicotinoids in children's urine

Concentrations, detection frequencies, and percentiles of urinary neonicotinoids in children living around target areas of thiacloprid (main ingredient of EcoOne-3 Flowable insecticide) spraying are summarized in Tables 2, 3 and 4, and were calculated before (May), during (June), and after (July) insecticide spraying was conducted. Frequencies of thiacloprid contents more than LOQ in early-morning urine samples were 28%, 30%, and 33% before, during, and after insecticide spraying, respectively, and did not differ between sampling months (Tables 2, 3 and

4; Steel–Dwass test, $p > 0.05$). Moreover, no significant changes were observed in plots of urinary thiacloprid concentrations against sampling times relative to insecticide spraying events (Fig. 1). These analyses suggest that spraying of EcoOne-3-Flowable insecticide in target areas of the pine wilt disease prevention program did not cause excessive thiacloprid exposures in children. Alternatively, the days between insecticide spraying and urine sample collection may have been sufficient for clearance of neonicotinoids. Accordingly, a previous study showed that the first and terminal elimination phase half-lives of thiacloprid were 2.2 and 19.0 h in male rats and 3.3 and 44.5 h in female rats, respectively (Pfeil et al. 2006). EcoOne-3-Flowable insecticide was sprayed in the study area on the June 1 to 3, 9, 22, and 29 and urine samples were collected on June 23, 2016, only 1 day after spraying. However, urinary thiacloprid concentrations did not differ significantly before and after spraying, further suggesting that thiacloprid is rapidly metabolized and eliminated. These observations indicate that exposures to thiacloprid through EcoOne-3-Flowable insecticide spraying exercises are not commensurate with absorbed levels in children.

In a similar recent study, Osaka and associates (Osaka et al. 2016) analyzed neonicotinoid levels in urine samples from 3-year-old children (108 boys and 115 girls) living in Aichi Prefecture, Japan, but did not detect thiacloprid in any of their samples. These discrepancies with the present data may reflect their reported LOQ for thiacloprid (0.32 ng/mL), which was much higher than our present LOQ of 0.05 ng/mL. Alternatively, Ueyama et al. (2015) observed a steady increase in urinary thiacloprid detection frequencies in specimens that were collected from adult women from 2003 to 2011, suggesting that the present data reflect an increasing trend of neonicotinoid use in Japan.

In addition to thiacloprid, six other neonicotinoid compounds that are not constituents of EcoOne-3-Flowable insecticides were detected in the children of this study (Tables 2, 3 and 4). Although acetamiprid was detected in only 8.5% to 12.8% of subjects, its major metabolite *N*-dm-acetamiprid was present in 86.6% to 93.5% of our urine specimens. Detection frequencies of the other neonicotinoids were 28.3% to 46.6% for thiamethoxam, 43.4% to 54.3% for dinotefuran, and 41.3 to 52.2% for clothianidin. These detection frequencies in children were far higher than those of thiacloprid (Tables 2, 3 and 4), and absolute urinary concentrations of these neonicotinoids were higher than those of thiacloprid (Table 2, 3 and 4; Fig. 2).

Urinary concentrations of nitenpyram increased from May (below LOQ) to June (10.3 μg/L; Tables 2 and 3, Fig. 1), suggesting that nitenpyram exposures follow the consumption of agricultural products. Although pesticide use in rice, fruit, and vegetables from the study area usually increase during June, the precise contributions of domestic and international agricultural products to nitenpyram exposures in children remain unknown. In contrast, urinary concentrations of dinotefuran, thiacloprid, N-dm-acetamiprid, and clothianidin did not differ significantly during sampling periods (Tables 2, 3 and 4; Fig. 1), indicating that exposures of the children to neonicotinoids occur at the present study areas irrespective of the timing of spraying of thiacloprid. Hence further studies are needed to identify exact exposure sources of neonicotinoids in the present study area.

Children seemed to be exposed to multiple kinds of neonicotinoids in June and July compared to May, the observation of which may reflect a more active pest control activity in summer in this area. Generally, the majority (more than 80%) of the children were found to be exposed to

multiple kinds of neonicotinoids in the study areas (Fig. 2), warranting investigations of synergistic effects of neonicotinoids in these children.

3.2.Atmospheric neonicotinoids

Neonicotinoid concentrations in atmospheric particulate matter from sites A and B are presented in Table 5. Among these, atmospheric thiacloprid concentrations were 67.9, 25.8, and 35 pg/m^3 before, during, and after spraying of EcoOne-3-Flowable insecticide (Table 5), respectively. The comparatively high levels before spraying preclude associations with EcoOne-3-Flowable insecticide spraying activities in this study, although our air sampling was performed 14 days after spraying of the EcoOne-3-Flowable insecticide, allowing residual thiacloprid to diffuse away before the sampling period. In contrast, atmospheric concentrations of thiacloprid in site B were 90 pg/m^3 during the spraying exercise, were only 32 pg/m^3 before spraying, and were 45 pg/m^3 after spraying. Hence, the elevated levels of thiacloprid observed in June likely follow variations in atmospheric thiacloprid concentrations. Similarly, Takenochi et al. (2016) collected air samples during a similar spraying period at the Togura region in Chikuma City of Nagano Prefecture in 2013. Their determinations of thiacloprid concentrations in atmospheric particulate matter showed increased thiacloprid concentrations from below LOQ (<35 pg/m^3) to 1,900 pg/m^3 immediately after spraying exercises and restoration of baseline thiacloprid levels below the LOQ after only 1 day. These observations confirm that thiacloprid has a short atmospheric residence time, presumably due to its low vapor pressure (Table S2). The disparity between the trends of atmospheric thiacloprid concentrations observed in the report by Takenochi et al. (2016) and that of the present study may be due to differences in

sampling spots. Whereas Takenochi. et al. (2016) collected atmospheric particulate matter beside the sprayed spots, samples in the present study were collected in the residential area a few kilometer far from the sprayed spots. Apparently, a greater proportion of the thiacloprid concentrations in the atmosphere might have either drifted away or settled on the ground as at the time of sampling for the present study.

3.3.EDI of neonicotinoids

EDI of thiacloprid in children from the study areas were maximal at 2.15 µg/day, and 75th percentile amounts of intake were 0.287, 0.310, and 0.367 µg/day in May, June, and July, respectively (Table 6,7 and 8). These amounts are within the ADI of 12 µg/kg/day, which is equivalent to 180 µg/15 kg/day in children (Table S1).

In addition, we compiled EDI values of other neonicotinoids besides thiacloprid in children during the EcoOne-3-Flowable insecticide spraying exercise in June (Table 7). At an EDI of 15.2 µg/kg/day, daily intake of acetamiprid was about 1.4% of its acceptable daily intake (ADI), whereas dinotefuran (31.4 µg/kg/day) was consumed at 1.0% of its ADI, nitenpyram (8.92 µg/kg/day) was consumed at 0.1% of its ADI, and thiamethoxam (0.813 µg/kg/day) was consumed at 0.3% of its ADI (Table 7). Although these EDIs are relatively low compared to ADIs, the maximal EDI of 51.6 µg/kg/day during the pesticide spraying season suggests that the EcoOne-3-Flowable spraying coincided with other agricultural activities that increased exposures of children to these neonicotinoids. The maximum EDI of imidacloprid in children was 11.1 µg/day (1.3% of its ADI) before the EcoOne-3-Flowable insecticide spraying exercise (Table 6) but was 1.23 µg/kg/day in June (0.1% of ADI; Table7) and 3.60 µg/kg/day (0.4% of

ADI; Table 8) in July, indicating limited effects of the spraying on imidacloprid exposure levels among our study subjects. Moreover, whereas peak EDI for acetamiprid and imidacloprid were greater than those of all the other detected neonicotinoids, these did not exceed 2% of ADI (Tables 6, 7 and 8).

Finally, we determined contributions of neonicotinoid inhalation from the atmosphere and showed that inhaled thiacloprid amounts were between 0.22 and 0.78 ng/day in the study area (Site A and B, Table 9), which are less than 1% of the EDI of thiacloprid (maximum 0.516 µg/day). We also found that inhalation from the atmosphere contributed very little to other neonicotinoid exposures (Table 9). Generally, neonicotinoids have very low vapor pressure (Raina-Fulton 2016; Table S2) meaning that most neonicotinoid compounds, especially imidacloprid and thiacloprid have limited volatility and short residence time in the atmosphere. Hence it is possible that thiacloprid which was used for the aerial spraying exercise in the present study area, quickly settled on soil and/or water immediately after spraying exercise, and thence limited inhalation among the children. Collectively, these data suggest that ingestion of neonicotinoids from foods and drinks contributes predominantly to total intakes by children and that inhaled neonicotinoid exposures are very limited in the present study areas.

EDIs of all detected neonicotinoids in the children of the present study were far lower than ADI values. However, a recent study indicates that exposures to no obvious adverse effect levels (NOAELs) of neonicotinoids may induce adverse effects in animals. Specifically, whereas the NOAEL of clothianidin has been set at 9.7 mg/kg (NRDC 2016), 5 mg/kg clothianidin reportedly induced anxiety-related behaviors in mice (Hirano et al. 2018), suggesting that the accepted NOAEL for clothianidin should be revised to a lower threshold. In the absence of

301 robust evidence, a clothianidin NOAEL value of 0.5 mg/kg (one-tenth of 5 mg/kg) would
302 correspond with an ADI value of 0.005 mg/kg per day (using 100 as an uncertainty factor and
303 15 kg as the child's body weight). Under these conditions, the present clothianidin EDI of
304 7.6 μg/kg/day represents about 10% of the ADI. In another study, Sun et al. (2016*)* reported that
305 exposures to daily imidacloprid doses of 0.06 mg/kg promoted high-fat-induced adiposity and
306 insulin resistance in male mice. These observations also imply that at 1% of the NOAEL
307 (5.7 mg/kg), imidacloprid may affect energy metabolism via the AMP-activated protein kinase-
308 α pathway. In the present study, the NOAEL for imidacloprid that we used was 0.006 mg/kg,
309 which was one-tenth of 0.06 mg/kg in Sun et al.'s study; therefore, on comparing the results, the
310 EDI of imidacloprid in the present children was two times higher than the ADI (currently 1.3%
311 of the ADI). Hence, further studies are warranted to precisely assess the toxicity of
312 neonicotinoids in humans and adjust ADI values accordingly.

313

314 **4. CONCLUSIONS**

315 In this study, concentrations of the neonicotinoids acetamiprid, clothianidin, dinotefuran,
316 imidacloprid, nitenpyram, thiacloprid, and thiamethoxam were determined in urine samples
317 from children living in areas where thiacloprid was used to control pine wilt disease. Subsequent
318 analyses showed very limited neonicotinoid inhalation among children. However, the presence
319 of six other neonicotinoids reflected high intakes of agricultural products by these children,
320 although estimated intake levels of neonicotinoids were less than 2% of ADI values. Finally,
321 whereas current exposure levels of the compounds detected in this study were far below the
322 doses that induce acute toxicity, sufficient caution should be taken to avoid the potential

cumulative impacts of these compounds in sensitive populations, especially among children and chemically sensitive individuals.

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Conflicts of interest

The authors declare no conflicts of interest.

Data Accessibility

Data, associated metadata, and calculation tools are available by contacting the corresponding author (y_ikenaka@vetmed.hokudai.ac.jp; Tel: +81-11-706-5102; Fax: +81-11-706-5105).

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413 **Figure Captions**

414 Fig.1: Time course of Thiacloprid and other neonicotinoid concentrations in urine (μg/g Cre);

415 Time course plots did not include urinary neonicotinoid concentrations below the limits of

416 quantification.

417 Fig.2**:** Multiple exposure evaluation of neonicotinoids among the children; most children were

418 found to be exposed to multiple neonicotinoid compounds.

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432 Table 1**:** Selected neonicotinoids and their metabolites

Target Neonicotinoids	LOQ* (ppb)	Recovery rate (%)	RSD** (%)	MRM***	Polarity for ESI

Acetamiprid	0.05	102	7	223.0 > 126.0	+
Clothianidin	0.1	92	4	249.0 > 132.1	+
Dinotefuran	0.1	100	8	203.0 > 129.1	+
Imidacloprid	0.2	100	5	256.0 > 209.1	+
Nitenpyram	0.1	98	5	271.0 > 126.1	+
Thiacloprid	0.05	100	5	252.9 > 126.1	+
Thiamethoxam	0.1	96	4	291.9 > 211.0	+
N-dm- Acetamiprid	0.05	101	9	208.9 > 126.1	+
Internal Standards					
Acetamiprid d6	-	-	4	229.0 > 126.0	+
Clothianidin d3	-	-	4	249.0 > 132.1	+
Dinotefuran d3	-	-	7	206.0 > 132.3	+
Imidacloprid d4	-	-	10	259.7 > 179.2	+
Thiacloprid d4	-	-	6	256.9 > 126.1	+
Thiamethoxam d4	-	-	4	295.7 > 215.1	+

433 * LOQ = limit of quantification, ** RSD = relative standard deviation; *** MRM = multiple
434 reaction monitoring; ESI = electrospray ionization

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Table 2：Urinary neonicotinoids (μg/L) in children before the insecticide spraying in May 26, 2016.

Neonicotinoid	Frequency (%)	Selected percentile				
		25th	50th	75th	95th	Max
Acetamiprid	9	<LOD	<LOD	<LOD	0.21	0.53
Clothianidin	41	<LOD	<LOD	0.50	1.32	3.60
Dinotefuran	43	<LOD	<LOD	0.55	10.25	15.60
Imidacloprid	13	<LOD	<LOD	<LOD	0.35	4.70
Nitenpyram	0	<LOD	<LOD	<LOD	<LOQ	<LOQ
Thiacloprid	28	<LOD	<LOD	0.06	0.06	0.10
Thiamethoxam	28	<LOD	<LOD	0.10	0.26	0.85
N-dm-Acetamiprid	91	0.27	0.39	0.71	3.48	11.16
ΣNEO	98	0.59	1.34	2.58	12.38	16.06

Max = Maximum; LOD = limit of detection, NEO = neonicotinoid.

Table 3：Urinary neonicotinoids (μg/L) in children during the insecticide spraying exercise (June 23, 2016).

Neonicotinoid	Frequency (%)	Selected percentile				
		25th	50th	75th	95th	Max
Acetamiprid	11	<LOQ	<LOQ	<LOQ	0.27	0.54
Clothianidin	52	<LOQ	0.14	0.59	3.18	4.58
Dinotefuran	54	<LOQ	0.14	0.68	5.13	72.31
Imidacloprid	15	<LOQ	<LOQ	<LOQ	0.36	0.64
Nitenpyram	30	<LOQ	<LOQ	0.20	2.23	10.83
Thiacloprid	30	<LOQ	<LOQ	0.06	0.06	0.13
Thiamethoxam	37	<LOQ	<LOQ	0.12	0.67	1.71
N-dm-Acetamiprid	93	0.25	0.34	0.66	5.09	7.17
ΣNEO	100	0.59	1.79	4.13	10.85	75.09

All abbreviations have been defined in Table 2.

Table 4：Urinary neonicotinoids (μg/L) in children after the insecticide spraying exercise (July 21, 2016).

Neonicotinoid	Frequency (%)	Selected percentile				
		25th	50th	75th	95th	Max
Acetamiprid	11	<LOQ	<LOQ	<LOQ	0.23	1.34
Clothianidin	49	<LOQ	<LOQ	0.64	3.24	6.02
Dinotefuran	49	<LOQ	<LOQ	0.21	1.54	8.58
Imidacloprid	18	<LOQ	<LOQ	<LOQ	0.39	1.48
Nitenpyram	27	<LOQ	<LOQ	0.19	0.26	0.63
Thiacloprid	33	<LOQ	<LOQ	0.06	0.06	0.10
Thiamethoxam	47	<LOQ	<LOQ	0.13	0.47	1.92
N-dm-Acetamiprid	87	0.24	0.46	0.98	6.42	18.72
ΣNEO	100	0.59	1.14	2.68	11.79	19.33

All abbreviations have been defined in Table 2.

Table 5: Atmospheric neonicotinoid concentrations (pg/m^3) in dust from sites A and B and the control site (EcoOne-3-Flowable Non-Spraying Area)

Neonicotinoid	Site A			Site B			Control Site
	5/19-5/26	6/17-6/24	7/14-7/22	5/19-5/26	6/17-6/24	7/14-7/22	5/30-6/6
Acetamiprid	54.2	77.5	35.5	58.3	60.6	47.5	43.6
Clothianidin	26.1	<LOQ	<LOQ	50.2	<LOQ	5.1	<LOQ
Dinotefuran	<LOQ	<LOQ	128.9	76.5	71.3	216.7	<LOQ
Imidacloprid	98.8	76.0	56.1	143.1	100.5	52.5	64.4
Nitenpyram	<LOQ	<LOQ	<LOQ	0.2	1.6	<LOQ	<LOQ
Thiacloprid	67.9	25.8	35.0	32.1	90.0	45.3	18.9
Thiamethoxam	64.2	54.2	44.7	<LOQ	<LOQ	44.2	<LOQ
N-dm-Acetamiprid	7.9	7.0	6.1	8.3	5.8	6.3	6.8

<LOQ = below the limit of detection.

Table 6: Estimated daily intake (EDI) of neonicotinoids in children before insecticide spraying exercise (26th May)

Neonicotinoid	EDI percentile (µg/day)					
	25th	50th	75th	95th	Max	%ADI
Acetamiprid	0.145	0.393	0.568	5.37	9.35	0.9
Clothianidin	-	-	0.298	1.02	1.99	0.1
Dinotefuran	-	-	0.230	9.22	15.90	0.5
Imidacloprid	-	-	-	1.05	11.0	1.3
Nitenpyram	-	-	-	-	-	-
Thiacloprid	-	-	0.287	1.29	2.15	1.2
Thiamethoxam	-	-	0.030	0.201	0.407	0.2
ΣNEO	0.618	1.02	2.40	12.5	19.1	0.1

Max = maximum EDI of neonicotinoids among subjects; %ADI = percent of acceptable daily intakes. EDI values below limits of quantification are indicated by "-".Estimated exposures were calculated assuming that creatinine excretion in children is 0.3 g per day. The excretion coefficients "r" of Acetamiprid, Clothianidin, Imidacloprid and Dinotefuran were retrieved from Harada et al. 2016 (r = 0.586, 0.596, 0.133, and 0.899, respectively). Excretion coefficients "r" of Nitenpyram, Thiacloprid, and Thiamethoxam were inferred from animal experiments (thus; 0.8, 0.05 and 0.6 for Nitenpyram, Thiacloprid, and Thiamethoxam respectively).

Table 7: Estimated daily intake (EDI) of neonicotinoids in children during insecticide spraying exercise (23rd June)

Neonicotinoid	EDI percentile (µg/day					
	25th	50th	75th	95th	Max	%ADI
Acetamiprid	0.410	0.240	0.521	5.17	15.2	1.4
Clothianidin	-	0.086	0.318	1.25	3.64	0.2
Dinotefuran	-	0.033	0.240	1.80	31.4	1.0
Imidacloprid	-	-	-	0.961	1.23	0.1
Nitenpyram	-	-	0.094	1.95	8.92	0.1
Thiacloprid	-	-	0.310	0.796	1.77	1.0
Thiamethoxam	-	-	0.065	0.408	0.813	0.3

ΣNEO	0.486	1.26	3.08	10.3	51.6	0.3

All abbreviations have been defined in Table 6.

Table 8: Estimated daily intake (EDI) of neonicotinoids in children after insecticide spraying exercise (21st July)

Neonicotinoid	EDI percentile (µg/day)					
	25th	50th	75th	95th	Max	%ADI
Acetamiprid	0.153	0.307	0.904	3.05	13.7	1.3
Clothianidin	-	-	0.418	1.98	7.59	0.5
Dinotefuran	-	-	0.136	0.611	3.16	0.1
Imidacloprid	-	-	-	1.87	3.60	0.4
Nitenpyram	-	-	0.057	0.208	0.468	0.0
Thiacloprid	-	-	0.367	0.811	1.22	0.7
Thiamethoxam	-	-	0.060	0.376	1.01	0.4
ΣNEO	0.565	1.13	2.97	8.29	19.4	0.1

All abbreviations have been defined in Table 6.

Table 9: Daily atmospheric neonicotinoid exposure estimates (ng/day) in children

Neonicotinoid	Site A			Site B		
	May	June	July	May	June	July
Acetamiprid	0.47	0.67	0.31	0.51	0.53	0.41
Clothianidin	0.23	-	-	0.44	-	0.04
Dinotefuran	-	-	1.12	0.67	0.62	1.88
Imidacloprid	0.86	0.66	0.49	1.24	0.87	0.46
Nitenpyram	-	-	-	0.00	0.01	-
Thiacloprid	0.59	0.22	0.30	0.28	0.78	0.39
Thiamethoxam	0.56	0.47	0.39	-	-	0.38
N-dm-Acetamiprid	0.07	0.06	0.05	0.07	0.05	0.05

Neonicotinoid intake from the atmosphere (ng/day) = Atmospheric neonicotinoid concentration (pg/m^3) × Child's daily breathing volume 8.7 m^3/day. The daily breathing volume in children of 1–12 years of age was assumed as 8.7 m^3 *(*Koenig et al. 2000). Daily atmospheric exposures below limits of quantification are indicated by "-".

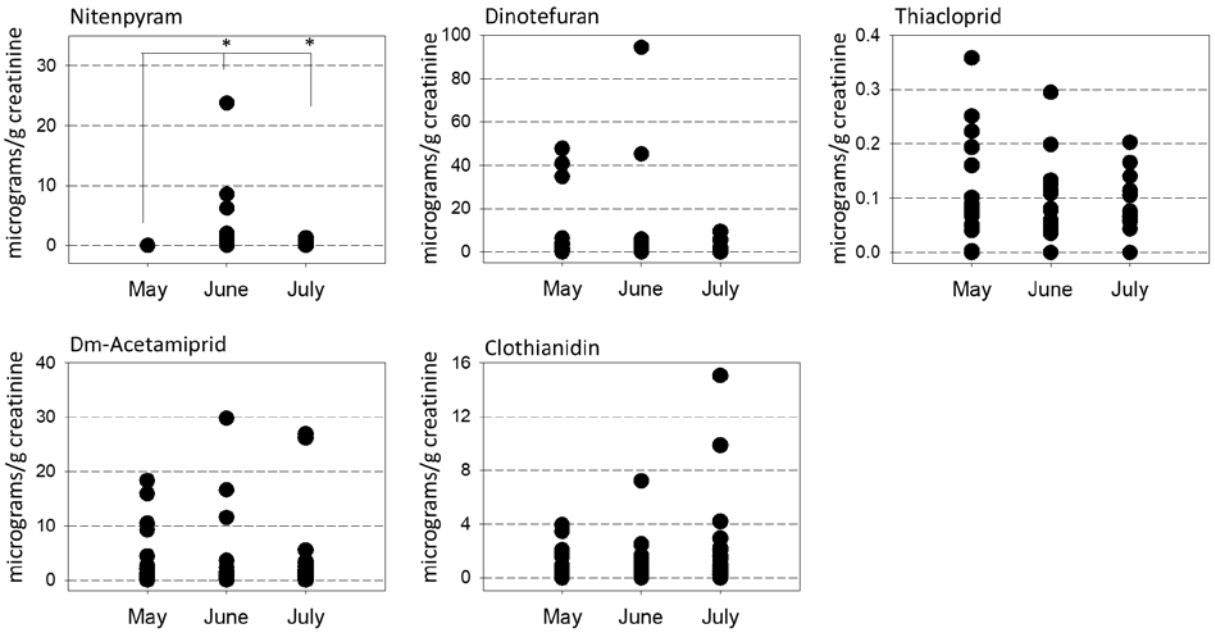

Fig.1: Time course of Thiacloprid and other neonicotinoid concentrations in urine (μg/g Cre); Time course plots did not include urinary neonicotinoid concentrations below the limits of quantification.

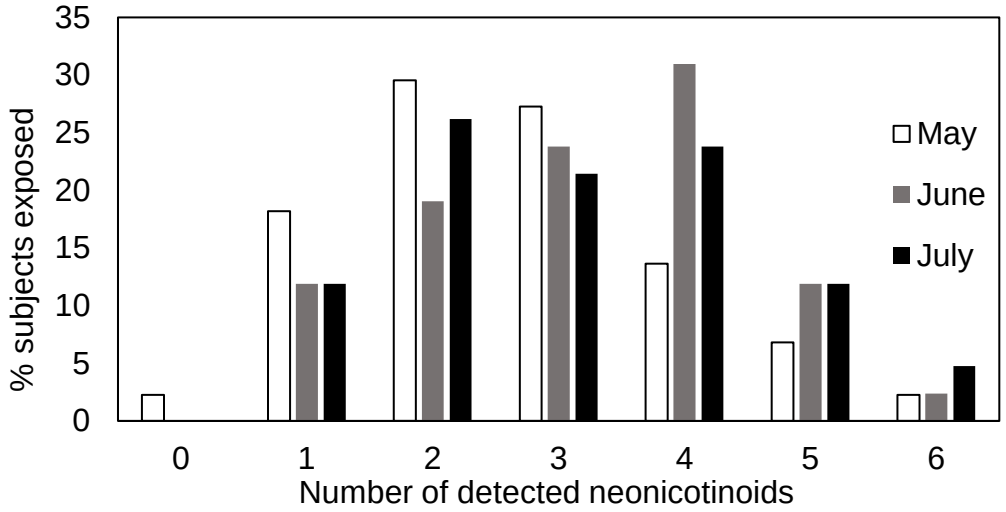

Fig.2**:** Multiple exposure evaluation of neonicotinoids among the children; most children were found to be exposed to multiple neonicotinoid compounds.

Reference data

Table S1: Maximum residual limits (MRL) and acceptable daily intakes (ADI) of neonicotinoids

Neonicotinoid	MRL for Tea*mg/kg	MRL for Spinach* mg/kg	MRL for Strawberry* mg/kg	ADI mg/kg/day
Acetamiprid	30	3	3	0.071
Clothianidin	50	40	0.7	0.097
Imidacloprid	10	15	0.5	0.057
Nitenpyram	10	5	5	0.53
Thiacloprid	30	-	5	0.012
Thiamethoxam	20	10	2	0.018
Dinotefuran	25	15	2	0.22

* The Japan Food Chemical Research Foundation (2016)

Table S2: Physicochemical properties of neonicotinoids

Neonicotinoid	Water Solubility (mg/L)	Vapor Pressure (Pa)	Log Kow	Soil Half-Life (days)
Acetamiprid	4,250 (25°C)	1×10^{-6} (25°C)	0.80	3 (4–7)
Clothianidin	327 (20°C)	1.3×10^{-10} (25°C)	0.91	545 (13–1,386)
Imidacloprid	610 (20°C)	4×10^{-10} (25°C)	0.57	191 (104–228)
Nitenpyram	600,000 (20°C)	$<1 \times 10^{-4}$ (50°C)	-0.64	-
Thiacloprid	185 (20°C)	8×10^{-10} (25°C)	1.26	15.5 (9–27)
Thiamethoxam	4,100 (25°C)	2.7×10^{-9} (20°C) 6.6×10^{-9} (25°C)	−0.13	50 (7–72)
Dinotefuran	39,830 (25°C)	$<1.7 \times 10^{-6}$ (30°C)	−0.55	82

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