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Exposure of Male Farmers and Nonfarmers to Neonicotinoid Pesticides in the South-West and Littoral Regions of Cameroon: A Comparative Study

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Abstract: Pesticides, especially the newly developed neonicotinoids, are increasingly used in many countries around the world, including Cameroon, to control pests involved in crop destruction or disease transmission. Unfortunately, the pesticides also pose tremendous environmental problems because a predominant amount of their residues enter environmental matrices to affect other nontargeted species including humans. This therefore calls for continuous biomonitoring of these insecticides in human populations. The present study sought to assess the neonicotinoid insecticide exposures in two agrarian regions of Cameroon, the South-West region and Littoral region. The study involved 188 men, including 125 farmers and 63 nonfarmers. Spot urine samples were obtained from these subjects and subjected to liquid chromatographic–tandem mass spectrometric analysis for concentrations of neonicotinoid compounds, including acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, nitenpyram, thiamethoxam, and *N*-dm-acetamiprid. Neonicotinoid compounds were detected in all study participants, and residues of all the screened pesticides were detected among participants. *N*-dm-Acetamiprid and imidacloprid were the most prevalent among the subjects (100.0% and 93.1%, respectively), whereas nitenpyram was less common (3.2%). The median values of imidacloprid and total urinary neonicotinoid concentrations were elevated among farmers (0.258 vs. 0.126 µg/L and 0.829 vs. 0.312 µg/L, respectively). Altogether the findings showed that both the farmer and nonfarmer study populations of Cameroon were exposed to multiple residues of neonicotinoids, with relatively higher levels of pesticides generally recorded among farmers. Although exposure levels of the neonicotinoids were generally lower than their respective reference doses, these results warrant further research on the health risk evaluation of multiple residues of the pesticides and reinforcement of control measures to minimize the exposure risks, especially among farmers. *Environ Toxicol Chem* 2024;43:952–964. © 2024 SETAC

Keywords: Agriculture; Biomonitoring; Farmer; Neonicotinoid; Occupational exposure

INTRODUCTION

Pesticides are natural or synthetic chemicals used to control, regulate, or repel pests interfering with crops or involved in

disease transmission. Synthetic pesticides, including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematocides, insect/plant growth regulators, and so forth, are increasingly used worldwide because of their significant roles in control of vector-borne diseases and improvement of agriculture. Substantial amounts of pesticides are used in agriculture, and this stands as one of the main sectors triggering the economy in many developing countries such as Cameroon (Ahtar et al., 2009). Pesticide imports in Cameroon increased

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from 15,304 tons in 2014 to 20,338 tons in 2018, to boost agriculture, an activity that currently employs over 70% of the country's workforce and 90% of the rural workforce (TRADESTAT, 2019; World Food Programme, 2023).

Despite the benefits related to pesticides, they generally pose tremendous environmental problems because only <0.1% reaches the targeted pests (Pimentel & Burgess, 2012), whereas the major portion, that is, >99.9%, enters environmental matrices to affect nontargeted species, including humans. This has been of particular concern for persistent chemicals, such as organochlorine insecticides, which were banned or restricted because of their persistence and toxicity in nontargeted organisms (Aktar et al., 2009). Therefore, more attention has been directed toward developing safer chemicals, and neonicotinoids were thought to provide satisfaction.

Neonicotinoid pesticides entered usage around the world in the early 1990s (Harada et al., 2016) as replacements for the highly toxic pesticides. Neonicotinoids are believed to be selectively toxic to arthropods and relatively nontoxic to vertebrates and can be used in smaller doses relative to previously used insecticidal agents (Harada et al., 2016). Over the past two decades, the demand for neonicotinoids has increased tremendously; and now, they figure as the most widely used insecticides worldwide for agricultural, domestic, and veterinary use (Network of African Science Academies, 2019). There is already evidence of neonicotinoid use in Cameroon for agriculture. Several formulations containing acetamiprid, imidacloprid, or thiamethoxam have been homologated within the country (Manfo et al., 2020; Ministry of Agriculture and Rural Development [MINADER], 2021). The latter formulations containing acetamiprid, imidacloprid, or thiamethoxam are currently applied on crops by farmers, and this may result in contamination of foodstuff and environmental matrices. For instance, a total diet study revealed higher acetamiprid concentrations in leafy vegetables from Cameroon (94–241 µg/kg; Ingenbleek et al., 2019). Contamination of food items by neonicotinoids has been recorded in other African countries, with residues being found in honey, soils, and water, sometimes at very high levels when compared with the levels found in European studies (Borsuah et al., 2020; Network of African Science Academies, 2019). The presence of neonicotinoid residues in foods suggests that dietary exposure among humans is plausible.

The adverse effects of neonicotinoids are of particular concern given that their increasing use may lead to contamination of foodstuffs and water, thereby potentiating oral exposures in the general human population. Humans may also be exposed to the chemicals occupationally, following inappropriate handling or improper self-protection; and previous studies in Cameroon showed that a substantial number of farmers applied pesticides without proper protective measures and hence suffer exposure/application symptoms (Manfo et al., 2020). The potential toxicity of neonicotinoids urges assessment of exposure levels among the population in Cameroon where there has been a continuous use of neonicotinoids since their introduction in the country (Academy of Science of South Africa [ASSAf], 2019; Manfo et al., 2012, 2020; Ministry of Agriculture and Rural Development, 2013). Nevertheless, there is limited evidence of human

exposure to neonicotinoids through biomonitoring studies, and this constitutes the motivation of the present study, which is the first of its kind within Cameroon and across central Africa. Screening of the pesticides and their related residues in biological samples represents the most reliable way for assessing human exposure to the chemicals. Neonicotinoid residues have been found to be excreted in urine with short biological half-lives in animal experiments and in humans (Harada et al., 2016), making urine a reliable biological sample for assessing exposure to this class of insecticides. Screening of urinary neonicotinoid levels has revealed human exposure to neonicotinoids in several countries including China, Japan, and Sri Lanka, whereas the African continent is currently represented by Ghana only (Harada et al., 2016; Ikenaka et al., 2019; Nimako et al., 2021; Song et al., 2020; Taira et al., 2021). The objective of the present study was to assess exposure levels of neonicotinoid and its related metabolites among men from two agrarian areas in Cameroon, including the Littoral and South-West regions. Farmers using agro pesticides and a reference population who do not use agro pesticides ("nonfarmers") were involved in the study to delineate the impact of occupational use of the agrochemicals on neonicotinoid exposure levels. Such data are significant for risk assessment, especially in the context where an effective remedy to neonicotinoid-induced alterations remains unavailable (Vinod et al., 2015).

MATERIALS AND METHODS

Investigated/sampling areas

Two regions, Littoral (Loum, Njombe-Penja, Bonaberi/Douala) and South-West (Maumu, Muea, Bolifamba, Molyko, Great Soppo, and Wotutu), in Cameroon were selected as sampling areas based on the involvement of their populations in agricultural activities. Though previously characterized by the production of cash crops such as coffee and cocoa, there has been a remarkable shift toward the cultivation of market gardening crops and fruits in these regions. Of note, agriculture is practiced by either local farmers or industrialized companies such as the Cameroon Development Corporation and Plantations du Haut Penja.

Study population and urine sample collection

A total of 188 healthy men, aged 26 to 61 years, and resident in the South-West and Littoral regions volunteered to participate in the present study. They were contacted face to face and given a detailed explanation of the study protocol and objectives, after which they freely provided written informed consent. Questionnaires were used to collect demographic characteristics and confounding factors such as age, height, body weight, residential location, and occupation, with emphasis on agro pesticide use. The body mass index (BMI) of each participant was calculated as weight in kilograms divided by height in meters squared. The participants were either farmers using agro pesticides (125 agro pesticide users) or nonfarmers involved in other activities unrelated to

agrochemical use (reference population, 63 participants). Ethics approval for the present study was granted by the institutional review boards of the University of Buea (ref. 2017/005/UB/SG/IRB/FHS) and the University of Douala in Cameroon (ref. 1716 IEC-UD/02/2019/M). Administrative authorizations were obtained from the delegations of public health for the South-West (ref. R11/MINSANTE/SWR/RDPH/PS/186/720) and Littoral (ref. 1070/AR/MINSANTE/DRSPL/BCASS/CN°1) regions in Cameroon. Each participant was provided a 50-mL clean plastic vial for collection of the first-morning void urine sample. Tubes containing urine samples were maintained at 4 °C during transportation to the University of Buea, where they were stored at –80 °C. Thereafter, the samples were shipped on dry ice to the Laboratory of Toxicology at the Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Japan, for neonicotinoid analysis. The interview and urine collection processes were all carried out from June 2017 to May 2019.

Determination of neonicotinoid levels in urine specimens

Urine samples were analyzed for seven neonicotinoid parent compounds (acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam) and a metabolite (*N*-desmethyl-acetamiprid). The analytes were extracted and purified using a combination of the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method and solid-phase extraction (SPE), described previously by Shinya et al. (2022). Briefly, 2 mL of urine sample was spiked with 50 µL of 100 ng/mL neonicotinoid internal standard master mix containing deuterated labeled standards for acetamiprid (acetamiprid-d3), *N*-desmethyl-acetamiprid (dm-acetamiprid-d3), imidacloprid (imidacloprid-d4), clothianidin (clothianidin-d3), thiacloprid (thiacloprid-d4), thiamethoxam (thiamethoxam-d4), nitenpyram (nitenpyram-d3), and dinotefuran (dinotefuran-d3) and vortex-mixed. Then, 2 mL of 16% sodium acetate buffer (0.5 g/3 mL double-distilled water) and 4 mL of 1% formic acid in acetonitrile were added to the sample, and the mixture was vortexed for 5 min. The mixtures were centrifuged at 10,000 *g* for 5 min to separate the organic (top) and aqueous phase (bottom), and the aqueous phase was frozen overnight at –20 °C. The organic phase or supernatant (400 µL) was collected, subjected to SPE using an ISOLUTE® HYDRO DME⁺ 400 mg/3 mL cartridge (Biotage; Suwannarin et al., 2020), and eluted with 600 µL of acetonitrile. After dry-solidifying using a centrifugal evaporator (CVE-200D with UT-2000; EYELA), extracts were redissolved in 200 µL of 20% (v/v) methanol and transferred into vials for instrumental analysis.

Urine extracts were analyzed using a liquid chromatograph triple quadrupole mass spectrometer (LC-MS/MS; Agilent 6495B; Agilent). Multiple reaction monitoring (MRM) for the mass spectrometry was programmed as indicated in Supporting Information, Table S1.

Analytes were quantified using the internal standard method. Calibration curves were plotted using eight-point

matrix-matched calibration points ranging from 0.01 to 20 ng/mL. Extraction and purification of each calibration point were performed using the method described previously (Shinya et al., 2022), and linearity ($r^2 \geq 0.99$) was obtained in all calibration curves. Limits of quantitation (LOQs) ranged from 0.01 to 0.05 ng/mL for all target compounds, and they were estimated as the lowest points on the standard curves with relative standard deviations of <10% ($n = 5$) and signal-to-noise ratios of 9:1 (Supporting Information, Table S1). Undetectable neonicotinoid concentrations were confirmed by running a blank analysis with urine specimens from a volunteer who mainly eats organic food. Method accuracy (percentage recovery) was also determined by replicate analysis of matrix-matched working standard solutions of the neonicotinoids, and recoveries were found within the range of 96% to 108%. The precision of the analytical procedure was confirmed by multiple intralaboratory analysis, and the relative standard deviations recorded for all the neonicotinoids were $\leq 10\%$ (Supporting Information, Table S1).

Determination of urinary creatinine levels

Urine samples were first diluted 100 times in distilled water; then, a 10-µL diluted sample was transferred into a vial containing 990 µL creatinine-d3 (200 ng/mL in acetonitrile). The sample was finally analyzed for creatinine using an LC-MS/MS (Shimadzu 20A series with LCMS8040; Shimadzu) equipped with an Shodex Asahipak NH2P-40 2D (2 × 150 mm inner diameter) column (Showa Denko America). Solvents A (50 mM ammonium formate in distilled water) and B (100% acetonitrile) were used as mobile phases for LC and applied with the following gradients: 0.04 and 0.36 mL/min for A and B, respectively. The column oven temperature was 30 °C. Detection was done using positive electrospray ionization. Two ion transition pairs were used under MRM mode for the detection and quantification of creatinine and creatinine-d3. These were 113.9/86.25 and 113.9/43 for creatinine and 116.9/89.1 and 116.9/47.1 for creatinine-d3. Calibration curves were generated at five concentrations (20, 50, 100, 200, and 500 ng/mL), and urinary creatinine concentration was calculated using the internal standard method.

Estimation of daily neonicotinoid exposures

The daily neonicotinoid exposure (DNE) of each participant was estimated using the following formula:

$$\text{DNE}(\mu\text{g/day}) = \frac{\text{UrNN} \times 1.5}{r}$$

In this equation, UrNN and r represent the creatinine-adjusted urinary neonicotinoid concentration (micrograms per gram creatinine) and the urinary excretion correction coefficient, respectively. The excreted creatinine level in an individual was assumed to be 1.5 g over 24 h (Ikenaka et al., 2019; Tanaka et al., 2002). Acetamiprid was shown to be mainly metabolized to dm-acetamiprid in rats (World

Health Organization, 2011). Also, a neonicotinoid excretion kinetics study showed that most acetamiprid ingested by humans was metabolized to desmethyl-acetamiprid and excreted in urine (Harada et al., 2016). Therefore, data for the latter metabolite and parent compound were summed up to represent the overall acetamiprid exposure levels. Dm-Acetamiprid has also been suggested as a marker for exposure to acetamiprid (Harada et al., 2016; Ikenaka et al., 2019; Song et al., 2020). The values of r were 0.586, 0.596, 0.899, 0.133, 0.800, 0.600, and 0.050 for acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiamethoxam, and thiocloprid, respectively (Food and Agriculture Organization of the United Nations & World Health Organization, 2010; Food Safety Committee, 2016; Harada et al., 2016; Ikenaka et al., 2019; Pfeil & Tasheva, 2006). The residue intake was also expressed in milligrams per kilogram body weight per day by dividing the DNE by the body weight (kilograms) of the participant.

Statistical analyses

Concentrations of the target neonicotinoids below the LOQ were either set to zero when estimating ΣNN or assigned a value equal to the LOQ divided by the square root of 2 during statistical significance analysis (Hornung & Reed, 1990). Comparison of pesticide exposures was done for the following pairs: nonfarmers from South-West region versus farmers from South-West region, nonfarmers from Littoral region versus farmers from Littoral region, all participants from South-West region versus all participants from Littoral region, and all nonfarmers versus all farmers. Frequencies and medians were compared between two study groups using either the χ^2 test or Fisher's exact test. The concentrations of the target agrochemicals and other continuous variables were tested for normality using the Kolmogorov-Smirnov test. The Student t test was used to analyze the differences between mean values for normally distributed data; otherwise, the Welch test was applied. Data analysis was performed using MedCalc (Ver 14.2) software, and differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Demographic and anthropometric characteristics of the study participants

The present study was designed to investigate neonicotinoid exposures among farmers using agro pesticides and a reference population of nonfarmers in the South-West and Littoral regions of Cameroon, central Africa. The groups of farmers and nonfarmers were similar in terms of age in each region (Table 1). However, the reference populations generally had elevated BMI, with higher proportions of preobese and obese participants ($p < 0.023$, χ^2 test [Supporting Information, Table S2]). The population of farmers was mainly composed of residents from rural areas ($p < 0.023$, χ^2 test [Table 1]), and a study in Cameroon earlier reported decreased BMI in residents from rural areas when compared with city dwellers. Urban

populations are generally more prone to a sedentary lifestyle and have a lower incidence of poverty when compared with rural populations; both sedentary lifestyle and improved living standards have been suggested as factors favoring overweight or obesity (Mbanya et al., 2015; Sobngwi et al., 2002). The decreased BMI in farmers may also be attributed to a difference in energy expenditure from occupational physical activity among farmers and nonfarmers. Farming generally requires a lot of physical exercise by small-scale farmers and may have contributed to their decreased body weight (Sobngwi et al., 2002). In fact, a cross-sectional survey in southeastern Australia revealed higher occupational physical activity levels in agricultural workers when compared with nonfarmers (Davis-Lameloise et al., 2013).

Neonicotinoids in participants' urine

Neonicotinoids were analyzed in urine samples from study participants, and results on occurrence and levels of the pesticides are presented in Table 2. The overall study population was exposed to neonicotinoids as suggested by the relative detection frequencies of the cumulated urinary neonicotinoids, which was 100.0%. This was also reflected by the number of residues detected per individual, which was ≥ 1 for all study participants as depicted in Figure 1. All eight assessed neonicotinoids were detected in urine, though at different frequencies. Among the neonicotinoids, *N*-dm-acetamiprid showed the highest detection frequency, occurring in all samples (100.0%). This was followed by imidacloprid, thiamethoxam, clothianidin, acetamiprid, thiocloprid, and dinotefuran—detected in 93.1%, 47.9%, 43.1%, 29.8%, 14.9%, and 7.4% of the study population, respectively—whereas nitenpyran was detected exclusively in farmers from Littoral region (9.1% farmers from Littoral, corresponding to 3.2% of the study population). *N*-dm-Acetamiprid showed a higher detection frequency (100.0% vs. 29.8%) and an elevated median value (0.263 vs. 0.066 $\mu\text{g/L}$) when compared with acetamiprid. This corroborates a previous report of *N*-dm-acetamiprid as the main metabolite of acetamiprid (Harada et al., 2016). Nimako et al. (2021) also reported a higher detection frequency and median levels of acetamiprid and *N*-dm-acetamiprid (94.7% vs. 2.7% and 0.41 $\mu\text{g/L}$ vs. <LOQ) in human urine samples from Kumasi, Ghana.

Occurrence of pesticide residues in urine may be related to dietary exposure following use of the agrochemicals for improvement of crop production. Indeed, some residues of the chemicals may remain in the harvested crops, which are thereafter consumed by humans, with residues excreted into urine. Higher acetamiprid concentrations occurred in leafy vegetables from Cameroon (94–241 $\mu\text{g/kg}$; Ingenbleek et al., 2019). Contamination of foodstuff, tea, and water samples by neonicotinoid residues has been also reported previously in other countries where neonicotinoid insecticides are used (Ikenaka et al., 2018; Ingenbleek et al., 2019; Lu et al., 2018). Noteworthy, the biomonitoring study conducted in Kumasi, Ghana, reported lower prevalence of the parent compound

TABLE 1: Age and residential location of the study population

Study area	Parameter	Range	Nonfarmers	Farmers	<i>p</i> (χ^2 test)
			Mean $\pm$ SD (n) n%	Mean $\pm$ SD (n) n%	
South-West region	Age (years)	<35	29.86 $\pm$ 2.67 (7) 17.5%	31.71 $\pm$ 2.98 (7) 11.9%	0.703
		35–45	39.56 $\pm$ 2.86 (18) 45.0%	40.00 $\pm$ 2.78 (30) 50.8%	
		45–55	46.27 $\pm$ 1.49 (15) 37.5%	47.46 $\pm$ 2.02 (22) 37.3%	
		≥ 55	—	—	
		≥ 55	(0) 0.0%	(0) 0.0%	
Littoral region	Residential location	Urban	5 (12.5%)	0 (0.0%)	<0.0001*
		Periurban	34 (85.0%)	29 (49.2%)	
		Rural	1 (2.5%)	30 (50.8%)	
	Age (years)	<35	29.50 $\pm$ 2.68 (10) 43.5%	29.50 $\pm$ 2.51 (12) 18.2%	0.09
		35–45	39.20 $\pm$ 2.59 (5) 21.7%	39.92 $\pm$ 3.33 (25) 37.9%	
		45–55	47.83 $\pm$ 2.48 (6) 26.1%	49.83 $\pm$ 2.57 (18) 27.3%	
		≥ 55	58.00 $\pm$ 0.00 (2) 8.7%	57.64 $\pm$ 1.91 (11) 16.7%	
		≥ 55	(2) 8.7%	(11) 16.7%	
	Residential location	Urban	20 (87.0%)	0 (0.0%)	<0.0001*
		Periurban	0 (0.0%)	0 (0.0%)	
		Rural	3 (13.0%)	66 (100.0%)	
South-West and Littoral regions	Age (years)	<35	29.65 $\pm$ 2.60 (17) 27.0%	30.32 $\pm$ 2.83 (19) 15.2%	0.136
		35–45	39.48 $\pm$ 2.74 (23) 36.5%	39.96 $\pm$ 3.01 (55) 44.0%	
		45–55	46.71 $\pm$ 1.90 (21) 33.3%	48.53 $\pm$ 2.55 (40) 32.0%	
		≥ 55	58.00 $\pm$ 0.00 (2) 3.2%	57.64 $\pm$ 1.91 (11) 8.8%	
		≥ 55	(2) 3.2%	(11) 8.8%	
	Residential location	Urban	25 (39.7%)	0 (0.0%)	<0.0001*
		Periurban	34 (54.0%)	29 (23.2%)	
		Rural	4 (6.3%)	96 (76.8%)	

Mean $\pm$ SD represents mean $\pm$ standard deviation, (n) represents absolute frequencies, and n% represents relative frequency. Not determined values are indicated by “—.”

*Significant difference between the two study populations at $p < 0.05$ (χ^2 test).

acetamiprid, 2.7%, when compared with the present study, 29.8% (Nimako et al., 2021). This may be attributed to the difference in sampling approach in the two studies. Participants in the Kumasi study were both men and women, whereas the present data were generated in men exclusively.

Data from the present study were dichotomized based on agro pesticide use in farming activities or study area, for better appreciation of the impact of occupational use of the agro-chemicals and residence on neonicotinoid exposure. It was observed that the neonicotinoids generally occurred with similar frequency in the farmers and nonfarmers not using agro pesticides (Table 2). However, the farmers showed a significantly higher median value for the total neonicotinoids, which was over two times elevated (0.829 vs. 0.312 $\mu\text{g/L}$) when compared with the median value of the nonfarmers ($p < 0.001$, Fisher's exact test; Table 2). The average urinary concentration of the total neonicotinoids was also over four times higher in the farmers, though this was not statistically significant, as shown in Supporting Information, Table S3. Looking at the individual neonicotinoids, there were higher detection frequencies of clothianidin, dinotefuran, imidacloprid, nitenpyram, and thiamethoxam among inhabitants from the Littoral region than

those from the South-West region of Cameroon. Likewise, median concentrations and/or mean values of imidacloprid and thiamethoxam were significantly higher in participants from the Littoral region (Table 2). These observations suggest higher exposure levels among Littoral dwellers; however, both the Littoral and South-West are agrarian regions, with agriculture practiced by either local farmers or industrialized companies. Cash crops and staple food from these regions are also similar; hence, the observed difference suggests differences in preferences/choice of pesticide formulations applied to crops. It may also result from possible discrepancies in knowledge, attitudes, and practices (KAP) of pesticide use among agricultural workers from the two regions because KAP may influence residue levels in crops harvested for human consumption. A comparative KAP study between the two regions, which was not within the scope of the present study, is therefore warranted.

The highest neonicotinoid concentration recorded was that of imidacloprid, 216.592 $\mu\text{g/L}$, and occurred in a farmer from the Littoral region. The median exposure level for imidacloprid was elevated among farmers when compared with nonfarmers. Also, acetamiprid was more frequently detected in all farmers in general (40.0% vs. 9.5%; $p < 0.0001$, Fisher's exact test), and

TABLE 2: Occurrence and levels of the neonicotinoid pesticides in urine samples among study participants

Neonicotinoid	Group	Frequency (n)%	Selected percentiles (µg/L)					
			P5	P25	P50	P75	P95	P100
Acetamiprid	Nonfarmers from SW	(3) 7.5%	–	–	0.1143	–	–	0.114
	Farmers from SW	(35) 59.3%**	<LOQ	0.0497	0.06	0.092	–	0.193
	Nonfarmers from LT	(3) 13.0%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Farmers from LT	(15) 22.7%	<LOQ	0.073	0.134	0.445	–	0.549
	All participants from SW	(38) 38.4%	<LOQ	0.051	0.063	0.108	–	0.193
	All participants from LT	(18) 20.2%**	–	0.073	0.134	0.445	–	0.549
	All nonfarmers, SW and LT	(6) 9.5%	<LOQ	<LOQ	0.1143	–	–	0.114
	All farmers, SW and LT	(50) 40.0%**	0.047	0.052	0.063	0.134	0.549	0.549
N-dm-Acetamiprid	Total population	(56) 29.8%	0.047	0.052	0.066	0.13	0.531	0.549
	Nonfarmers from SW	(40) 100.0%	0.082	0.112	0.128	0.212	0.667	0.855
	Farmers from SW	(59) 100.0%	0.085	0.132	0.209*	0.569	3.644	12.168
	Nonfarmers from LT	(23) 100.0%	0.194	0.369	0.678	1.402	8.793	9.197
	Farmers from LT	(66) 100.0%	0.149	0.245	0.452	0.845	3.687	13.261
	All participants from SW	(99) 100.0%	0.084	0.121	0.546	0.343	2.151	12.168
	All participants from LT	(89) 100.0%	0.16	0.252	0.498**	0.961	4.395	13.261
	All nonfarmers, SW and LT	(63) 100.0%	0.086	0.12	0.203	0.588	2.666	9.197
Clothianidin	All farmers, SW and LT	(125) 100.0%	0.0978	0.169	0.301	0.797	3.717	13.261
	Total population	(188) 100.0%	0.09	0.157	0.263	0.741	3.463	13.261
	Nonfarmers from SW	(15) 37.5%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Farmers from SW	(16) 27.1%	–	–	0.082	–	–	0.082
	Nonfarmers from LT	(16) 69.6%	–	0.119	0.145	0.273	–	0.312
	Farmers from LT	(34) 51.5%	0.073	0.084	0.128	0.409	3.677	3.914
	All participants from SW	(31) 31.3%	–	–	0.082**	–	–	0.082
	All participants from LT	(50) 56.2%**	0.073	0.094	0.129	0.273	2.967	3.914
Dinotefuran	All nonfarmers, SW and LT	(31) 49.2%	–	0.119	0.147	0.273	–	0.312
	All farmers, SW and LT	(50) 40.0%	0.073	0.08	0.127	0.305	3.559	3.914
	Total population	(81) 43.1%	0.073	0.085	0.128	0.255	2.847	3.914
	Nonfarmers from SW	(2) 5.0%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Farmers from SW	(1) 1.7%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Nonfarmers from LT	(3) 13.0%	<LOQ	<LOQ	0.323	–	–	0.323
	Farmers from LT	(8) 12.1%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	All participants from SW	(3) 3.0%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Imidacloprid	All participants from LT	(11) 12.4%*	<LOQ	<LOQ	0.323	–	–	0.323
	All nonfarmers, SW and LT	(5) 7.9%	<LOQ	<LOQ	0.323	–	–	0.323
	All farmers, SW and LT	(9) 7.2%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Total population	(14) 7.4%	<LOQ	<LOQ	0.323	–	–	0.323
	Nonfarmers from SW	(33) 82.5%	0.097	0.113	0.122	0.163	0.193	0.675
	Farmers from SW	(53) 89.8%	0.109	0.118	0.163**	0.305	0.78	1.363
	Nonfarmers from LT	(23) 100.0%	0.077	0.099	0.146	0.227	0.618	0.997
	Farmers from LT	(66) 100.0%	0.123	0.208	0.549**	1.266	6.891	216.592
Nitenpyram	All participants from SW	(86) 86.9%	0.1	0.115	0.141	0.21	0.693	1.363
	All participants from LT	(89) 100.0%**	0.088	0.16	0.281**	0.752	2.458	216.592
	All nonfarmers, SW and LT	(56) 88.9%	0.086	0.112	0.126	0.175	0.245	0.997
	All farmers, SW and LT	(119) 95.2%	0.111	0.147	0.258**	0.686	1.736	216.592
	Total population	(175) 93.1%	0.096	0.122	0.177	0.515	2.504	216.592
	Nonfarmers from SW	(0) 0.0%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Farmers from SW	(0) 0.0%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Nonfarmers from LT	(0) 0.0%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Thiacloprid	Farmers from LT	(6) 9.1%	–	0.488	0.705	1.402	–	3.812
	All participants from SW	(0) 0.0%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	All participants from LT	(6) 6.7%*	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	All nonfarmers, SW and LT	(0) 0.0%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	All farmers, SW and LT	(6) 4.8%	–	0.488	0.705	1.402	–	3.812
	Total population	(6) 3.2%	–	0.488	0.705	1.402	–	3.812
	Nonfarmers from SW	(8) 20.0%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Farmers from SW	(10) 16.9%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Nonfarmers from LT	(0) 0.0%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Farmers from LT	(10) 15.2%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	All participants from SW	(18) 18.2%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	All participants from LT	(10) 11.2%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	All nonfarmers, SW and LT	(8) 12.7%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	All farmers, SW and LT	(20) 16.0%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Total population	(28) 14.9%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

(Continued)

TABLE 2: (Continued)

Neonicotinoid	Group	Frequency (n)%	Selected percentiles (µg/L)					
			P5	P25	P50	P75	P95	P100
Thiamethoxam	Nonfarmers from SW	(11) 27.5%	–	0.069	0.071	0.074	–	0.074
	Farmers from SW	(12) 20.3%	–	0.065	0.072	0.11	–	0.196
	Nonfarmers from LT	(22) 95.7%	–	0.249	0.3312	0.534	–	0.68
	Farmers from LT	(45) 68.2%**	0.069	0.099	0.185	0.433	3.537	4.957
	All participants from SW	(23) 23.2%	–	0.066	0.072	0.08	–	0.196
	All participants from LT	(67) 75.3%**	0.069	0.107	0.250**	0.533	2.686	4.957
	All nonfarmers, SW and LT	(33) 52.4%	–	0.075	0.26	0.463	–	0.68
	All farmers, SW and LT	(57) 45.6%	0.065	0.081	0.126	0.287	2.827	4.957
	Total population	(90) 47.9%	0.066	0.079	0.127	0.348	1.902	4.957
	Sum of all NN							
	Nonfarmers from SW	(40) 100.0%	0.113	0.218	0.249	0.311	0.883	0.985
	Farmers from SW	(59) 100.0%	0.128	0.274	0.416**	0.99	3.821	12.479
	Nonfarmers from LT	(23) 100.0%	0.357	0.685	1.004	1.838	9.659	10.156
	Farmers from LT	(66) 100.0%	0.402	0.728	1.474	3.405	14.07	221.599
	All participants from SW	(99) 100.0%	0.1217	0.23	0.315	0.693	2.486	12.479
	All participants from LT	(89) 100.0%	0.405	0.702	1.203**	2.568	13.176	221.59
	All nonfarmers, SW and LT	(63) 100.0%	0.12	0.233	0.312	0.952	2.78	10.156
	All farmers, SW and LT	(125) 100.0%	0.215	0.407	0.829**	2.029	10.486	221.599
	Total population	(188) 100.0%	0.189	0.304	0.681	1.597	8.36	221.599

Except for detection frequencies (%), all other values are expressed as micrograms per liter. Not determined averages are indicated by “–.”
P = percentile; LT, Littoral region; SW = South-West region; LOQ = limit of quantitation; NN = neonicotinoid.
Significant difference between two groups (farmers vs. nonfarmers or SW vs. LT) at * $p < 0.05$ or ** $p < 0.01$ (Welch test for the frequencies or Fisher's exact test for the mean and median values).

farmers from the South-West region in particular (7.5% vs. 59.3%; $p < 0.01$, Fisher's exact test; Table 2). This suggests higher occupational exposure among farmers, that is, uptake of acetamiprid and imidacloprid by farmers during application, as a result of inappropriate personal protective equipment (Manfo et al., 2020, 2021). Higher exposure to imidacloprid and acetamiprid corroborated with consumption rates of these agrochemicals for farming activities in Cameroon. Imidacloprid was the first discovered and commercialized neonicotinoid and figures among the bestselling insecticides worldwide (Borsuah et al., 2020). Pesticide formulations containing imidacloprid (Gongfut 50EC, Parastar, Gamalin 80 EC, Iron 30 SC, Lamida Gold, Mamira super 90EC, Dinacacao, and Imida30 EC) but also acetamiprid (K optimal, Pacha 25 EC, and Supercot 35EC) were used by farmers from the Littoral and South-West regions, where samples analyzed in the present study were collected (Manfo

et al., 2020, 2021). Twenty formulations of acetamiprid were registered for application in Cameroon (ASSAf, 2019), and this may suggest the presence of the pesticides and/or related residues in food commodities in Cameroon. In addition to imidacloprid and acetamiprid formulations, several pesticides containing thiacloprid and thiamethoxam have been registered in Cameroon (ASSAf, 2019; MINADER, 2021). Thiamethoxam occurs in several pesticide formulations homologated in Cameroon (MINADER, 2021) such as Actara, which was used by 2% of farmers in Buea subdivision to improve crop production (Manfo et al., 2021). However, thiamethoxam occurred more frequently among nonfarmers from the Littoral region (Table 2) and therefore represents more likely dietary exposure.
Nitenpyram has not yet been authorized in Cameroon, though it was detected in six urinary samples (3.2% of the study population). Likewise, agricultural use of clothianidin

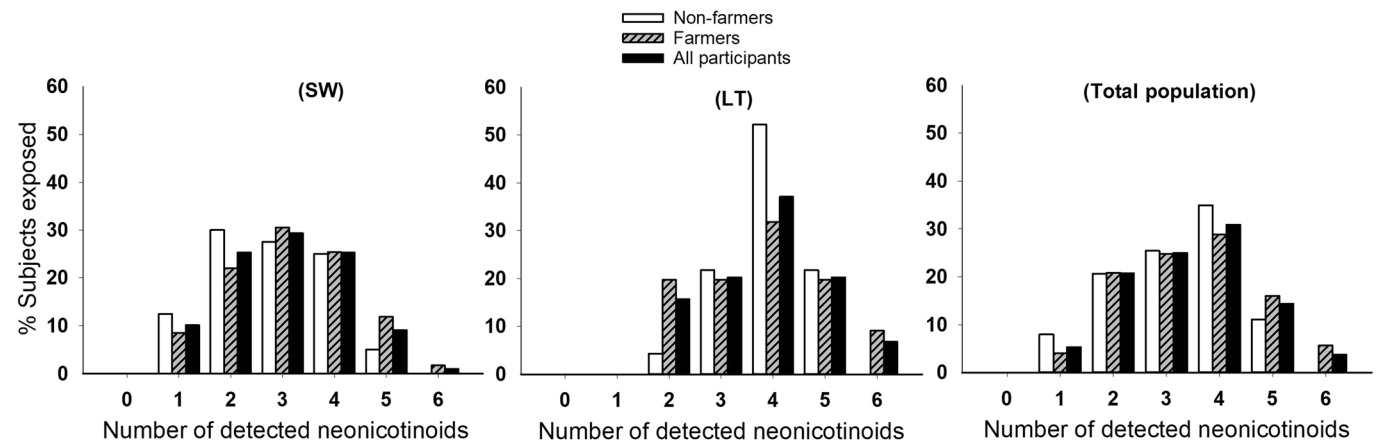


FIGURE 1: Exposure to multiple neonicotinoid pesticides among nonfarmers and farmers from Littoral and South-West regions, as well as in the overall study population. SW = South-West; LT = Littoral.

and dinotefuran was not reported in the study areas, to the best of our knowledge. Therefore, the occurrence of these neonicotinoids in urine samples could also reflect dietary exposure through consumption of imported food commodities like rice, tea, and fruits (Ikenaka et al., 2018; Network of African Science Academies, 2019) from countries where the chemicals are used in crop production. Clothianidin also results from biotransformation of the homologated pesticide thiamethoxam in mammalian species, including humans (Shi et al., 2009).

The median value of total neonicotinoids among farmers was more than two times higher when compared with the reference population of nonfarmers (Table 2). Among study participants from the South-West region, farmers also showed a higher median value when compared with the corresponding group of nonfarmers (Table 2). Looking at individual pesticides, median values for *N*-dm-acetamiprid and imidacloprid were all elevated among farmers from the South-West region (Table 2). Likewise, the median urinary imidacloprid level was more than three times higher in farmers from the Littoral region (Table 2). These data further stress that the current farming population of Cameroon may be more susceptible to occupational exposure to various neonicotinoid active compounds in their daily activities. This observation was consistent with the use of acetamiprid and imidacloprid by farmers from the South-West region to enable increased crop production (Manfo et al., 2020, 2021). The occupational exposure of neonicotinoids observed in the present study population was validated by the fact that a higher median value for total neonicotinoids was generally observed among farmers when compared with the reference population (Table 2). Similarly, a study carried out in farmers and nonfarmers in Iowa, United States, revealed significantly higher urinary levels of agro pesticide metabolites in the farmers who reported applying the pesticides on crops (Curwin et al., 2005). Moreover, the farmers from Cameroon used agrochemicals without appropriate protective measures (Manfo et al., 2020, 2021), and this unsafe practice could potentiate their exposure to the pesticides. Pesticide formulations containing thiacloprid have been homologated in Cameroon (MINADER, 2021), hence their detection in the urinary samples from humans. Of note, nitenpyram occurred exclusively among farmers from the Littoral region. This pesticide has not been homologated in Cameroon, and its detection among farmers could be attributed to its illegal use and dietary exposure from imported food.

Exposure of farmers and nonfarmers to multiple residues of neonicotinoids

The number of neonicotinoids detected in each urine sample was cumulated, and the data are presented in Figure 1. All participants, including farmers and nonfarmers, showed at least a single type of pesticide residue. Up to six different pesticide residues were recorded among farmers, while a maximum of five neonicotinoids per individual was recorded in

nonfarmers, though statistical analyses revealed an insignificant difference between the two study groups. Dichotomizing the data set per region also did not reveal any significant difference in terms of exposure to multiple pesticide residues. However, occurrence of multiple residues within the studied urine samples is indicative of the exposure of the populations of the Littoral and South-West regions to many types of neonicotinoids. Neonicotinoid demand has been increasing worldwide since their introduction in the early 1990s (Harada et al., 2016), and these chemicals are presently among the most widely used insecticides. Neonicotinoid compounds have various applications including agricultural, domestic, and veterinary uses (Network of African Science Academies, 2019). The extensive application of neonicotinoids may explain the widespread presence of their multiple residues within food commodities, which represent a substantial exposure source within human populations. Neonicotinoid use leads to pollution of environmental matrices, which then become exposure sources for the general population (Anderson & Meade, 2014). Multiple neonicotinoid residues occurred in 94.7% of the overall population (Figure 1), which corroborates with a study from Kumasi, Ghana, where up to 92% of analyzed urine samples were found to contain multiple neonicotinoids (Nimako et al., 2021). This may be related to the importation of neonicotinoid-based insecticides for cocoa farming in the latter countries, all of which figure among the top five world's largest cocoa producers (Maciel et al., 2018).

It was observed that the number of residues of the neonicotinoids occurring in the urine did not vary significantly between nonfarmers and farmers who used agrochemicals occupationally (Figure 1). This may imply that some residues of the agrochemicals used in farming activities remain in harvested crops that are consumed by both farmers and nonfarmers, leading to the exposures among the two groups. Dietary exposure has been known as the main human exposure route for many chemicals including pesticides. Further appreciation of occupational exposure came through assessment of DNE levels in the study participants.

Estimated daily exposure of neonicotinoids among farmers and nonfarmers and health implications

Based on the urinary levels of the pesticide residues, human DNE for each detected neonicotinoid was derived, and the results are presented in Table 3. Four samples had very low (undetected) creatinine levels and hence were not considered for DNE determination. The DNE values were compared with acceptable daily intakes (ADI) reported elsewhere (Ikenaka et al., 2018) for further assessment of the risks of adverse health effects associated with pesticide residues. In fact, neonicotinoids such as dinotefuran, clothianidin, and imidacloprid were excreted in urine with short biological half-lives in animal experiments, suggesting that urinary concentrations are well suited for assessment of daily exposure/intake (Harada et al., 2016).

TABLE 3: Estimated daily exposure of neonicotinoids in the study population

Neonicotinoid	Group	DNE (µg/kg/day) P100 (%RfD)	DNE selected percentiles (µg/day)					
			P5	P25	P50	P75	P95	P100
Acetamiprid	Nonfarmers from SW	0.055 (0.076)	0.305	0.697	0.96	1.786	3.134	4.093
	Farmers from SW	1.821 (2.565)	0.275	0.509	1.056	1.798	13.047	142.037
	Nonfarmers from LT	0.074 (0.104)	0.262	0.352	1.004	1.325	7.148	7.684
	Farmers from LT	0.181 (0.255)	0.172	0.367	0.806	1.434	3.142	15.394
	All participants from SW	1.821(2.565)	0.295	0.582	0.992*	1.782	8.21	142.037
	All participants from LT	0.181 (0.255)	0.198	0.364	0.839	1.38	6.901	15.394
	All nonfarmers, SW and LT	0.074 (0.104)	0.269	0.594	0.966	1.535	3.748	7.684
	All farmers, SW and LT	1.821 (2.5655)	0.218	0.42	0.847	1.685	8.311	142.037
	Total population	1.821 (2.565)	0.247	0.462	0.915	1.677	7.755	142.037
Clothianidin	Nonfarmers from SW	–	–	–	–	–	–	–
	Farmers from SW	0.002 (0.002)	–	–	0.126	–	–	0.126
	Nonfarmers from LT	0.003 (0.004)	–	0.098	0.177	0.245	–	0.263
	Farmers from LT	0.102 (0.112)	0.076	0.088	0.142	1.804	6.108	6.428
	All participants from SW	0.002 (0.002)	–	–	0.126	–	–	0.126
	All participants from LT	0.102 (0.112)	0.079	0.091	0.142**	0.263	5.146	6.428
	All nonfarmers, SW and LT	0.003 (0.004)	–	0.098	0.177	0.245	–	0.263
	All farmers, SW and LT	0.102 (0.112)	0.076	0.09	0.126	1.164	5.947	6.428
	Total population	0.102 (0.112)	0.079	0.093	0.126	0.258	4.986	6.428
Dinotefuran	Nonfarmers from SW	–	–	–	–	–	–	–
	Farmers from SW	–	–	–	–	–	–	–
	Nonfarmers from LT	0.001 (0.001)	–	–	0.149	–	–	0.149
	Farmers from LT	–	–	–	–	–	–	–
	All participants from SW	–	–	–	–	–	–	–
	All participants from LT	0.001 (0.001)	–	–	0.149	–	–	0.149
	All nonfarmers, SW and LT	0.001 (0.001)	–	–	0.149	–	–	0.149
	All farmers, SW and LT	–	–	–	–	–	–	–
	Total population	0.001 (0.001)	–	–	0.149	–	–	0.149
Imidacloprid	Nonfarmers from SW	0.174 (0.306)	1.302	2.245	4.319	4.976	10.618	12.54
	Farmers from SW	0.234 (0.411)	0.924	2.144	3.566	5.74	14.03	15.687
	Nonfarmers from LT	0.110 (0.194)	0.322	0.414	0.822	1.123	4.454	7.5
	Farmers from LT	13.279 (23.296)	0.527	1.418	3.449**	7.265	56.259	1208.353
	All participants from SW	0.234 (0.411)	1.008	2.189	4.028*	5.455	12.923	15.687
	All participants from LT	13.279 (23.296)	0.365	0.839	2.131	6.56	52.739	1208.353
	All nonfarmers, SW and LT	0.174 (0.306)	0.349	0.844	1.935	4.431	10.236	12.54
	All farmers, SW and LT	13.279 (23.296)	0.712	1.631	3.547	6.652	33.137	1208.353
	Total population	13.279 (23.296)	0.428	1.285	13.23	5.939	28.625	1208.353
Nitenpyram	Nonfarmers from SW	–	–	–	–	–	–	–
	Farmers from SW	–	–	–	–	–	–	–
	Nonfarmers from LT	–	–	–	–	–	–	–
	Farmers from LT	0.039 (0.008)	–	0.55	0.73	0.789	–	3.535
	All participants from SW	–	–	–	–	–	–	–
	All participants from LT	0.039 (0.007)	–	0.55	0.73	0.789	–	3.535
	All nonfarmers, SW and LT	–	–	–	–	–	–	–
	All farmers, SW and LT	0.039 (0.008)	–	0.55	0.723	0.789	–	3.535
	Total population	0.039 (0.007)	–	0.55	0.73	0.79	–	3.535
Thiacloprid	Nonfarmers from SW	–	–	–	–	–	–	–
	Farmers from SW	–	–	–	–	–	–	–
	Nonfarmers from LT	–	–	–	–	–	–	–
	Farmers from LT	–	–	–	–	–	–	–
	All participants from SW	–	–	–	–	–	–	–
	All participants from LT	–	–	–	–	–	–	–
	All nonfarmers, SW and LT	–	–	–	–	–	–	–
	All farmers, SW and LT	–	–	–	–	–	–	–
	Total population	–	–	–	–	–	–	–
Thiamethoxam	Nonfarmers from SW	0.002 (0.012)	–	0.102	0.143	0.184	–	0.184
	Farmers from SW	0.004 (0.024)	–	0.101	0.164	0.237	–	0.297
	Nonfarmers from LT	0.009 (0.048)	–	0.212	0.368	0.555	–	0.603
	Farmers from LT	0.128 (0.713)	0.063	0.099	0.241	0.628	6.24	8.085
	All participants from SW	0.004 (0.024)	–	0.102	0.164	0.209	–	0.3
	All participants from LT	0.128 (0.713)	0.057	0.101	0.288**	0.603	5.133	8.085
	All nonfarmers, SW and LT	0.009 (0.048)	–	0.143	0.278	0.474	–	0.603
	All farmers, SW and LT	0.128 (0.713)	0.066	0.1	0.218	0.482	5.317	8.085
	Total population	0.128 (0.713)	0.06	0.101	0.218	0.469	4.185	8.085

(Continued)

TABLE 3: (Continued)

Neonicotinoid	Group	DNE ($\mu\text{g/kg/day}$) P100 (%RfD)	DNE selected percentiles ($\mu\text{g/day}$)					
			P5	P25	P50	P75	P95	P100
Sum of all NN	Nonfarmers from SW	0.221 (0.028)	0.428	2.199	5.012	6.154	12.695	15.944
	Farmers from SW	1.898 (0.237)	0.855	2.595	4.609*	8.983	18.7	148.031
	Nonfarmers from LT	0.116 (0.015)	0.733	1.353	2.289	2.755	8.415	8.925
	Farmers from LT	13.334 (1.665)	0.971	2.562	5.278**	11.388	58.706	1213.391
	All participants from SW	1.898 (0.237)	0.586	2.338	4.796**	7.706	17.179	148.031
	All participants from LT	13.334 (1.665)	0.803	2.077	3.658	9.619	55.29	1213.391
	All nonfarmers, SW and LT	0.221 (0.028)	0.572	1.798	3.031	5.628	12.423	15.944
	All farmers, SW and LT	13.334 (1.665)	0.898	2.579	4.943	10.672	42.551	1213.391
	Total population	13.334 (1.665)	0.794	2.141	4.439	8.75	30.296	1213.391

Not determined values were indicated by “–.” *N*-dm-Acetamidrid data were incorporated into acetamidrid data because most acetamidrid is rapidly metabolized into *N*-dm-acetamidrid and excreted into urine (Harada et al., 2016). Four samples with undetected creatinine levels were not considered for DNE determination.

DNE = daily neonicotinoid exposure; P = percentile; RfD = reference dose; SW = South-West region; LT = Littoral region; NN = neonicotinoids.

Significant difference between two groups (farmers vs. nonfarmers or SW vs. LT) at * $p < 0.05$ or ** $p < 0.01$ (Welch test for the frequencies or Fisher's exact test for median values).

All exposure levels for the six detected neonicotinoids (with data for acetamidrid and its metabolite *N*-dm-acetamidrid cumulated) were $\leq 13.279 \mu\text{g/kg/day}$ and remained far below the respective ADIs, that is, from $12 \mu\text{g/kg}$ for thiacloprid to $220 \mu\text{g/kg}$ for dinotefuran. The most frequently detected neonicotinoids that occurred in $\geq 50\%$ participants were imidacloprid and acetamidrid. Their highest DNEs were 13.279 and $1.821 \mu\text{g/kg/day}$, respectively; and these were all below the corresponding ADIs (23.296% and 2.565% of the corresponding ADI, respectively). Similarly, a study by Ikenaka et al. (2019) reported neonicotinoid exposure below ADIs in children from Nagano prefecture in Japan, where neonicotinoids were sprayed to control pine wilt disease. Nimako et al. (2021) also reported daily intakes below ADIs among men from Kumasi, Ghana. The maximum estimated daily intake of imidacloprid for men in the latter study ($13.430 \mu\text{g/kg/day}$) was similar to the maximum intakes reported in the present study, suggesting similar exposure among men in Kumasi and the study areas in Cameroon. However, the maximum DNE of acetamidrid for men in Kumasi was $5.480 \mu\text{g/kg/day}$, that is, three times higher than the maximum DNE reported in Cameroon, and could imply a relatively high health risk associated with acetamidrid in the Ghana study population (Nimako et al., 2021).

Because DNEs in the present study were generally lower than corresponding ADIs ($\leq 13.279\%$ ADI), the results seem to indicate that exposure levels to the neonicotinoids may present minimal adverse health outcomes in the populations of the South-West and Littoral regions of Cameroon. This class of agricultural pesticides is relatively new in Cameroon, although their usage is now gaining popularity in the country. Other alternative insecticides/agrochemicals are also available for agricultural applications (Manfo et al., 2020; MINADER, 2021), and this may explain the relatively low exposure levels. Neonicotinoids are short-lived chemicals and less likely to bioaccumulate, and this may also account for the lower exposure levels. It is worth mentioning that some experimental studies have suggested adverse effects of neonicotinoids at doses similar to or below the current ADIs. For instance, Sun et al. (2017) reported alteration of high-fat diet-induced body weight gain and adiposity in male

C57BL/6J mice fed 0.06 mg/kg imidacloprid and a high-fat diet. The neonicotinoids thiacloprid and thiamethoxam alter promoter-specific cytochrome P450 family 19 messenger RNA expression and catalytic activity in human adrenocortical carcinoma (H295R) cells at lower concentrations only, and this biphasic endocrine-disrupting effect further stresses the toxicity potential of the pesticides at lower doses (Caron-Beaudoin et al., 2016). Moreover, although human/mammal nicotinic acetylcholine receptors (nAChRs) are less sensitive than those of the insects to original neonicotinoids, some metabolites of neonicotinoids appear to be more toxic to humans than insects. An example is the imidacloprid metabolite desnitro-imidacloprid, which has a high affinity for mammalian nAChRs and can be produced in mammalian systems during metabolism or in the environment (Chao & Casida, 1997; Koshlukova, 2006).

Farmers and nonfarmers are continuously exposed to multiple residues of neonicotinoids, and this chronic exposure might result in adverse health outcomes. The associated health risk appears to be elevated in farmers. In fact, when DNEs for all six detected neonicotinoids were cumulated in each participant and the data dichotomized based on agro pesticide use in farming activities and residence, the farmers from each region showed elevated median daily exposure to the total neonicotinoids when compared with the corresponding nonfarmers (6.154 vs. $8.983 \mu\text{g/day}$ and 1.7334 vs. $3.2581 \mu\text{g/day}$; $p = 0.021$ and $p < 0.0001$, respectively; Table 3). The average daily exposure (0.3402 vs. $0.0157 \mu\text{g/kg/day}$; $p = 0.008$, Welch test; Table 3) as well as median value (3.449 vs. $0.822 \mu\text{g/day}$; $p < 0.0001$, Fisher's exact test; Table 3) for imidacloprid were also higher among farmers from the Littoral region when compared with nonfarmers of the same region. Imidacloprid was most frequently used by farmers in Cameroon (Manfo et al., 2020, 2021), hence the elevated median exposure levels among farmers in the Littoral region. Imidacloprid has been homologated in Cameroon for over 5 years. It figures among the most used insecticide active ingredients within the country, and hence is more likely used by a majority of the farmers who take up some residues of the agrochemical into their system during application on crops (Manfo et al., 2021; MINADER, 2021). Consistent with

the observations for imidacloprid, the median value of the overall DNE for the neonicotinoids was four times higher among nonfarmers in the Littoral region (11.388 vs. 2.755 $\mu\text{g}/\text{day}$; Table 3), further stressing the importance of occupational exposure to agro pesticides among farmers. Neonicotinoid use in agriculture has been implicated in the colony collapse disorder of bees reported over recent decades (Tsvetkov et al., 2017). This has led to a ban of the outdoor use of imidacloprid, clothianidin, and thiamethoxam in the European Union (European Food Safety Authority, 2020), though these chemicals are still authorized for outdoor spraying in several developing countries including Cameroon (MINADER, 2021).

Comparison between the two regions revealed higher overall DNEs in the Littoral region (Table 3). This was consistent with the higher detection frequencies of some neonicotinoids in the latter region, such as clothianidin, dinotefuran, imidacloprid, nitenpyram, and thiamethoxam (Table 2). Median DNE values for clothianidin and thiamethoxan were also higher in the Littoral population. However, this contrasted with the DNEs of imidacloprid and acetamiprid, for which higher median values were reported among South-West participants (Table 3). Despite the lower DNE values, which were generally below ADIs, the latter inconsistency further stresses the possible discrepancies in neonicotinoid preferences and KAP of pesticide use among agricultural workers from the two regions.

The concomitant occurrence of multiple residues of neonicotinoids in humans is of concern because current established safety levels of these pesticides do not take into consideration the potential adverse effects that may arise from exposure to their multiple residues. Continuous biomonitoring of neonicotinoids is therefore needed. Neonicotinoids are relatively new; hence, they are not well investigated for their potential health hazard in humans and other nontargeted species.

Despite the pertinence of our results, some shortcomings occur and may be considered for further improvement of the work. These include nonincorporation of all metabolites of the studied pesticides in the analytical process, which would have enabled exhaustive estimation of the exposure levels among participants. Sampling did not include women and children, for a more exhaustive assessment of the exposure of the general population to neonicotinoids. The sample size was also relatively small, and single-spot urine samples were used instead of longitudinal data coupled with a thorough assessment of participants' health status, which would have enabled a better appreciation of the impact of pesticides on human health.

CONCLUSION

Findings from the present study showed that both farmers and nonfarmers in the study areas in Cameroon were exposed to multiple residues of neonicotinoids, with higher levels of the pesticides generally recorded among farmers (median values 0.829 vs. 0.312 $\mu\text{g}/\text{L}$; $p < 0.01$). Acetamiprid and imidacloprid were the most commonly occurring neonicotinoids, with detection frequencies of 100.0% and 93.1%, respectively. These

results, which represent the first evidence of neonicotinoid exposure among humans in Cameroon and across central Africa, warrant further research on the health risk evaluation of multiple residues of the pesticides and reinforcement of the control of exposure risks.

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Ishizuka: Conceptualization; Funding acquisition; Resources; Supervision; Validation. **Yoshinori Ikenaka:** Conceptualization; Funding acquisition; Investigation; Methodology; Resources; Supervision; Validation; Writing—review & editing.

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