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Determination and dietary risk assessment of neonicotinoid and insect growth regulators in honey

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Abstract

Aim: The aim of this study was to determine the presence, concentrations, dietary intake, and risk of residues of neonicotinoids (NEO) and insect growth regulators (IGR) in commercially available honey in South Africa.

Methods: Sample preparation for honey was based on the "dilute and shoot" principle, followed by analysis using an internally validated ultra-high-performance liquid chromatographic coupled to tandem mass spectrometric method. Estimated daily intake and acute and chronic hazard quotients were determined to measure human exposure and health risk to NEO and IGR as well as the risk posed to honeybee.

Results: NEO and IGR were detected in 50% and 21% of the 115 honey samples, respectively. The average concentration ranged 0.062-6.50 μgkg^{-1} and 0.479-1.644 μgkg^{-1} for NEO and IGR, respectively. While acetamiprid was the most detected (24.35%) NEO, imidacloprid presented the highest concentration (16.945 $\mu g\ kg^{-1}$) in a sample. IGR co-occurred at variable concentrations with NEO in honey samples. The estimated daily intakes (EDI) of NEO and IGR ranged from 9.35×10^{-7} to 4.93×10^{-6} mg kg^{-1} bwd^{-1}. The chronic hazard quotient (HQc) and acute hazard quotient (HQa) for NEO and IGR were considerably < 1, indicating negligible risk to human health and honeybee population.

Conclusion: A UHPLC-MS/MS method was validated for the simultaneous determination of neonicotinoids and insect growth regulators in honey. Overall, the result of the present study confirms the widespread occurrence of NEO and IGR in honey consumed in South Africa. The EDIs, HQc, and HQa indicate that exposure to all target NEO and IGR via honey consumption constitutes negligible human health risk; however, the consequences of multiple routes of exposure to NEO and IGR cannot be overemphasized.

Keywords: Neonicotinoids, insect growth regulator, honey, dietary risk

INTRODUCTION

Neonicotinoid insecticides are a class of pesticides that were introduced in the 1990s as replacement for organophosphate pesticides[1-3]. Due to their high efficacy for insect control and ease of application, neonicotinoids have quickly become the most widely used insecticides in agriculture, veterinary, and residential environments[4]. Based on the actual insecticide consumption, neonicotinoid have a share of approximately 30% of the global market for insecticides[5,6]. They have a similar chemical structure to nicotine, and as such are classified into N-nitroguanidines (i.e., imidacloprid, thiamethoxam, clothianidin, and dinotefuran) and N-cyanoamidines (i.e., acetamiprid and thiacloprid)[4,7]. They are characterized by high water solubility [from 185 (thiacloprid) to 4100 mg/L (thiamethoxam)], which makes them to be readily absorbed by plants either via roots or leaves before being transported throughout the plant tissue[4,8].

The high water solubility of neonicotinoids provides advantage in pest control as they protect the whole plant effectively against boring insects and root-feeding insects[9]. The mode of action of these insecticides is to bind to the nicotine acetylcholine receptor agonists, which causes paralysis and death in insects[1,10]. Thus, neonicotinoid insecticides use gained application covering many crops from cereals and vegetables to various fruit cultures[11].

Despite their high efficacy, selectivity, and versatile application[4,11,12], there are growing concerns regarding toxicity of neonicotinoid not only to non-target organisms - especially pollinators such as honeybees and wild bees[8,13] as well as other terrestrial and aquatic invertebrates[14] - but also to vertebrates, including humans[15,16]. Neonicotinoids toxic effects include mainly reproductive toxicology, neurotoxicity, hepatotoxicity/hepatocarcinogenicity, immunotoxicity, and genetic toxicity[17,18]. Studies also showed that neonicotinoids can adversely affect the developing brain especially for children[17-19].

Research shows that neonicotinoids residues can accumulate in pollen and nectar of treated plants, thereby presenting potential risk to pollinators[20]. For their survival, bees rely on pollen and nectar sources[21]. Nectar is transformed into honey and stored in the hive for daily adult bees and human consumption, which make bees distinctive sentinels of environmental quality[13,21]. Thus, the residue concentrations of insecticides in honey can be extrapolated as a measure of contamination in the surrounding environment[22]. Due to its high nutritional value, palatable flavor, and medicinal properties, the use of honey has substantially grown and adopted into human consumption habits[23].

In South Africa, about 5000 tons of honey is consumed annually[24]. Only 40% of the total honey consumption is produced locally with China accounting for 80% of total imported honey for the Country[24]. Honey can easily be obtained from a broad range of geographical localities, and studies have reported the presence of neonicotinoid insecticides in honey[10,13,23,25]. To protect human and environmental health, the European Union has set maximum residue levels for neonicotinoids in honey (European Data Base, 2019). However, there are few data on the levels of neonicotinoids in honey from the African

continent[10]. To close this gap, this study reports an ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (MS/MS)-based method for the simultaneous determination of neonicotinoid and insect growth regulators in honey. The method was developed, validated, and applied to quantify eight NEO, acetamiprid, clothianidin, dinotefuran, imidacloprid, imidaclothiz, nitenpyram, thiacloprid, and thiamethoxam, and four IGR, buprofezin, cyromazine, fenoxycarb, and triflumuron, in commercially available honey from South Africa. The dietary intake of NEO and IGRs and the risk posed to humans and bees were elucidated.

EXPERIMENTAL

Chemicals

Pure standards of eight neonicotinoid insecticides, acetamiprid, clothianidin, dinotefuran, imidacloprid, imidaclothiz, nitenpyram, thiacloprid, and thiamethoxam, and four IGR, buprofezin, cyromazine, fenoxycarb, and triflumuron, as well as two deuterated internal standards, thiamethoxam-D4 and imidacloprid-D4, were products of Dr. Ehrenstorfer GmbH obtained from Industrial Analytical (Pty) Ltd, Johannesburg, South Africa. Microsep (Pty) Ltd, South Africa, supplied mass spectrometry-grade (99.9%) water and methanol. Ammonium acetate and sodium hydroxide were obtained from Sigma Aldrich, South Africa.

Sampling

In total, 115 commercially available honey samples were obtained between 2017 and 2020. The samples included both locally produced and imported honey. These samples were collected directly from various establishments under the framework of the national residue-monitoring program. All samples were stored at 4 °C in the dark prior to analysis. None of the honey samples crystallized before analysis.

Extraction

Dilute and shoot extraction

Honey samples (1.0 ± 0.1 g) were accurately weighed in a 50 mL polypropylene tube. The sample was spiked with 2 ng mL^{-1} of the mixture of internal standards. This was followed by the addition of 2 mL each of 10 mM NaOH and water and 5 mL of methanol. The mixture was vortex-mixed for 1 min and centrifuged at 5000 rpm for 5 min in a refrigerated centrifuge kept at 4 °C. Then, a 1 mL aliquot of the mixture was filtered through 0.22 µm PTFE syringe filter into an HPLC vial.

QuEChERS extraction

One gram of honey was weighed in a 50 mL centrifuge tube. The sample was spiked with 2 ng mL^{-1} of the mixture of internal standards and vortexed for 30 s. This was followed by the addition of 10 mL each of ultrapure water and acetonitrile. The QuEChERS extraction packs comprising of 4 g $MgSO_4$ and 1 g NaCl were added and dissolved in the mixture by vigorous agitation. The mixture was vortex-mixed for 1 min and centrifuged at 5000 rpm for 5 min in a refrigerated centrifuge kept at 4 °C. Approximately 2 mL of the supernatant were evaporated to insipient dryness using a stream of nitrogen at 30 °C. The residue was reconstituted in 1 mL of the initial gradient of the mobile phase composition and filtered through a 0.22 µm syringe filter into an autosampler vial for ultra-high-performance liquid chromatography (UHPLC)-MS/MS analysis.

Instrumental method

The chromatographic separation of NEO and IGR was achieved with a Perkin Elmer LX-50 UHPLC system equipped with a Kinetex® C_{18} 1.7 µm: 2.1 × 100 mm column. The column oven temperature was kept at 50 °C. Separation was achieved with a gradient consisting of 10 mM ammonium acetate in water (A) and methanol (B), at a constant flow rate of 0.4 mL min^{-1}. The LC gradient condition is presented in

Supplementary Table 1. Ten microliters of sample was injected onto the LC.

Mass spectrometric identification and confirmation of neonicotinoids was achieved using a PerkinElmer® QSight™ 220 triple quadruple mass spectrometer (MS/MS) operated in the positive electrospray ionization mode, with an electrospray voltage set at 4000 V. Nitrogen was used as drying and nebulizer gas, set at 140 and 400, respectively. The optimized hot surface-induced desolvation temperature was set at 320 °C, while the ion source temperature was set at 350 °C. The acquisition of neonicotinoids was achieved using the time-managed multiple reaction monitoring (MRM) mode. Data were acquired by using Simplicity™ 3Q software (version 1.4.1806.29651).

The MS/MS analysis of NEO and IGRs involved the selection of a minimum of two MRM transitions corresponding to the precursor ion or pseudomolecular ion, together with two daughter ions (obtained through direct MS/MS infusion of native standards of individual NEO and IGR). These ions were used to unequivocally confirm and quantify the occurrence of NEO and IGRs. The most intense MRM was selected as the quantitative ion pair, while the second and third MRMs (where available) were utilized as qualitative ion pairs.

Human health risk assessment

Estimated daily intake and chronic and acute hazard quotients of NEO and IGR to human

The health risk associated with pesticide residues in food usually combines chemical occurrence data with food consumption. In this study, an average body weight of 70 kg for an adult South African[26] and per capita consumption of 0.21 g honey per day, based on average annual consumption of 5000 tons of honey by the South African population, were considered[24].

The estimated daily intake (EDI) of each NEO and IGR was calculated following Equation (1):

$$\text{EDI (mg kg}^{-1}\text{ bw d}^{-1}) = \frac{STMR \times FIR}{bw} \quad (1)$$

where STMR is the supervised trial mean residue concentrations for each NEO and IGR (mg/kg) in this study, FIR is the average daily consumption of honey in South Africa (0.00021 kg), and bw is the body weight in kg.

The long-term risk to each NEO and IGR was determined by expressing their respective EDIs as a fraction of their corresponding acceptable daily intake (ADI) using Equation (2) and expressed as chronic hazard quotient (HQc).

$$HQc = \frac{EDI}{ADI} \quad (2)$$

To determine the acute dietary exposure risk to human, the estimated short term intake (mg/kg) of NEO and IGR residues was applied following the method reported by Wang *et al.*[27], as shown in Equation (3). The acute exposure risk of each NEO and IGR was determined as a fraction of the corresponding acute reference dose (ARfD) and expressed as acute hazard quotient (HQa) [Equation (4)]:

$$ESTI = \frac{LP \times HR}{bw} \quad (3)$$

$$HQa = \frac{ESTI}{ARfD} \quad (4)$$

where LP is the large portion in kg, HR is the maximum residue concentration (mg/kg), and bw is the body weight in kg. For the estimation of HQa, the LP value of 0.100 kg honey for the Chinese population was used[27]. To the authors' knowledge, there are currently no records of LP for the South African population.

An HQc or HQa > 1 indicates an unacceptable health threat. The lower are the HQc and HQa, the lesser is the risk of chronic and acute exposures.

Risk of neonicotinoids posed to bees

The hazard assessment of dietary exposure of honeybee foragers and worker nurse bees for the oral exposure to NEO and IGR in honey was estimated. Foragers consume 80 mg of honey while brood nurse bees consume 40 mg of honey per day[10,28]. Risk was estimated by incorporating frequency of detection of individual insecticides based on dose[29] and using the average and maximum concentrations of NEO and IGR detected in the honey samples according to Equation (5).

$$\text{Risk} = \frac{Detection\ frequency\ (\%) \times residue\ concentration\ (\boldsymbol{ng})}{LD_{50}\ (ng\ bee{-}1)} \quad (5)$$

The LD_{50} values adopted in this study were those reported by Codling *et al.*[10] and are presented in Table 1.

Statistical analysis

Descriptive statistics such as mean, median, and standard deviations and parametric tests such as the analysis of variance were carried out using Microsoft® Excel 2016. Non-parametric statistical analysis was performed using XLSTAT 2019. Only samples with concentration ≥ LOQ were considered for the analysis of dietary intake and risk assessment since none of the target NEO and IGR were detected above LOQ in more than 50% of the samples.

Quality control and quality assurance

Honey samples that previously tested negative for the target NEO and IGR were used as matrix blanks [Supplementary Figure 1] for the validation experiments and matrix matched calibration curves used for the quantification of target NEO and IGR. Since the use of isotope-labeled internal standards for each of the target neonicotinoids and IGR was not economically feasible, two deuterated (D4) compounds were utilized. The choice of internal standards was based on the relative recovery and relative standard deviation (n = 21) obtained for each NEO and IGR. For each batch of analyses, a check standard was analyzed at the beginning and the end of the batch to monitor any drift in retention times of target analytes. Spiked quality control samples (n = 3) were analyzed for each batch of analyses to monitor the method performance. Similarly, solvent blanks were analyzed after each sample run to monitor possible carryover. Method blanks (n = 3), treated as an unknown samples, were analyzed for each batch of analyses to monitor possible contamination through the analysis. None of the target neonicotinoids and IGRs were detected above 1% of the concentrations of the least contaminated sample, hence samples were not blank corrected.

Table 1. Average LD_{50} (ng bee^{-1}) values based on oral exposure of bees reported in the literature[10]

Compound	LD_{50} ng bee^{-1}
Cyromazine	5000
Dinotefuran	26.8
Acetamiprid	10,140
Clothianidin	18
Imidacloprid	120
Nitenpyrum	138
Thiamethoxam	11.8
Fenoxycarb	
Triflumuron	200,000
Buprofezin	
Imidaclothiz	

RESULTS

Method development

Honey is one of the most difficult matrices among foods of animal origin, comprising complex sugars such as monosaccharides, essential oils, dyes, residues of wax and bee pollen, and organic acids. In the analysis of honey, careful sample preparations are invaluable for the determination of pesticide residues such as neonicotinoids and IGRs. The optimized sample preparation method in this study involved the treatment of honey with 10 mM NaOH solution followed by the addition of water and methanol. Following vortex mixing and centrifugation, 1 mL of the mixture was filtered through a 0.22 μm syringe filter and directly injected onto the UHPLC-MS/MS. The obtained extracts were very clean, resulting in clear chromatographic separation of each of the target analytes [Figure 1].

The suitability of this simple sample preparation method was compared with a QuEChERS method. As shown in Figure 2, this method resulted in better extraction recovery of NEO and IGR compared to QuEChERS.

At least two MRM transitions corresponding to a minimum of four identification points were monitored for each target analyte [Table 2]. The collision energies, entrance voltages, and cell exit potential (CCL2) were individually optimized for each analyte through a flow injection analysis of individual neonicotinoid standard solution [Table 2].

The optimized chromatographic separation of NEO and IGR was achieved by using a Kinetex® C_{18} (1.7 μm: 2.1 mm × 100 mm) column, which resulted in well-resolved chromatographic peak shape for all target compounds simultaneously [Figure 2]. A mobile phase composition containing 10 mM ammonium acetate in water (aqueous phase) and methanol (organic phase) at a flow rate of 0.4 mL min^{-1} and injection volume of 10 μL was found sufficient for the simultaneous determination of NEO and IGR in honey. The retention times [Table 2] were very stable for all target analyte with %RSD ranging 0.13%-1.15% (n = 21).

Method validation

The analytical method utilized in this study was validated for recovery, precision (repeatability and within-laboratory reproducibility), linear range, limit of detection (LOD), and limit of quantitation (LOQ). The LOD and LOQ were determined as the lowest detectable concentrations with signal to noise ratio greater than 3 and 10, respectively. The LOQs were verified in spiked matrices and formed part of the concentrations used in the matrix-matched calibration employed for the quantitation of unknown samples.

Table 2. Optimized MS/MS conditions and retention time of neonicotinoids

Name	ESI polarity	Precursor (m/z)	Products (m/z)	Retention time (min)	Collision energy	Entrance voltage	Cell exit potential
Acetamiprid	Positive	223.2	126.1	4.37	-30	25	-49
			99.1		-56	25	-73
Clothianidin	Positive	250	169.1	3.99	-16	25	-39
			132		-26	25	-48
Cyromazine	Positive	167.2	68	2.02	-30	25	-44
			85		-30	25	-44
			108		-29	25	-43
Dinotefuran	Positive	203.1	114.1	2.34	-20	25	-38
			129		-16	25	-35
Imidacloprid	Positive	256.2	209	3.97	-18	25	-42
			175.2		-26	25	-49
Thiacloprid	Positive	253.1	126.1	4.79	-26	25	-49
			99.1		-60	25	-79
Fenoxycarb	Positive	302.2	88	7.64	-34	25	-61
			116		-15	25	-44
Nitenpyram	Positive	271.2	126.1	2.78	-35	25	-59
			237.2		-25	25	-50
Thiamethoxam	Positive	292	211	3.28	-18	25	-45
			181		-28	25	-54
Triflumuron	Positive	359	156.2	8.4	-24	25	-58
			139.1		-35	25	-67
			285.2		-20	25	-54
Buprofezin	Positive	306.1	201.1	11.02	-18	25	-47
			116.2		-24	25	-52
Imidaclothiz	Positive	262.1	181.2	4.18	-16	25	-45
	Positive		122.2		-16	25	-45
Thiamethoxam-D4	Positive	295.7	214.7	3.26	-18	25	-45
Imidacloprid-D4	Positive	260	179	3.95	-26	25	-49

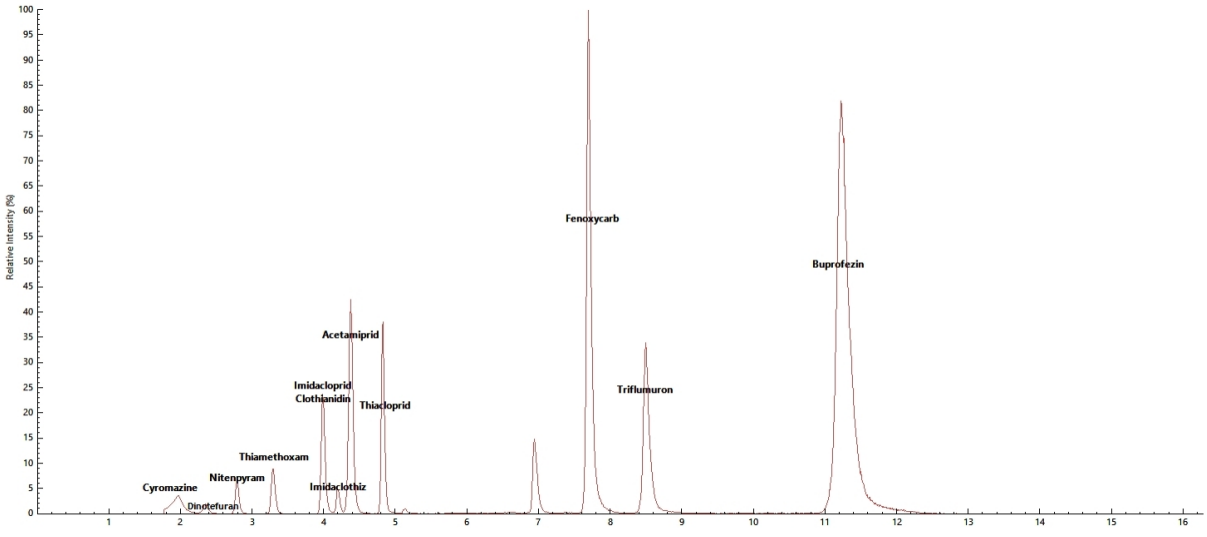

Figure 1. Total ion chromatogram of neonicotinoids and insect growth regulators.

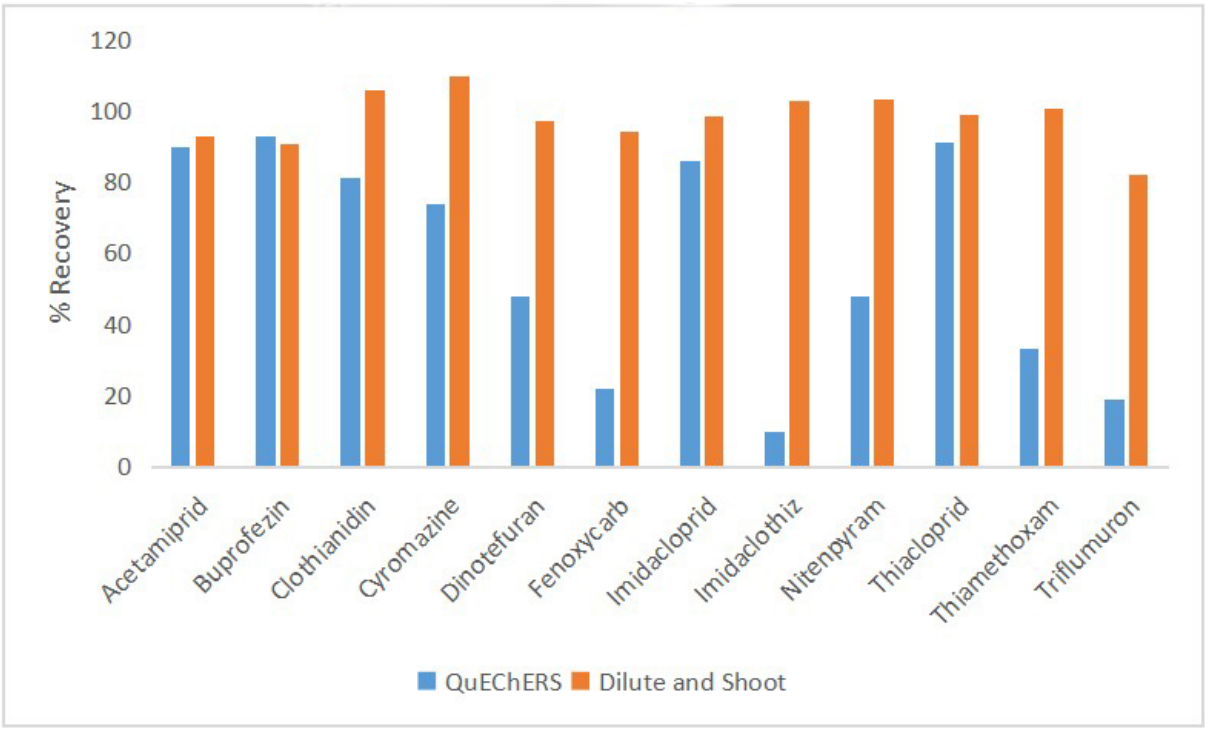

Figure 2. Recovery of neonicotinoids and insect growth regulators using direct extraction method and QuEChERS.

Recoveries and precisions were determined by analysis of replicate samples (n = 21) spiked at three different concentrations (0.5, 5, and 10 μg kg^{-1}) over a period of three days. The linearity of the method was determined through regression analysis of an eight-point matrix-matched calibration curve in the concentration range of 0.01-10 μg kg^{-1} for each analyte. Table 3 summarizes the method performance.

The recoveries of NEO and IGR at the three validation levels ranged 74%-108% with precision (%RSD) < 30% for all target analytes [Figure 3]. The LODs and LOQs of NEO and IGR ranged 0.01-0.03 and 0.01-0.09 μg kg^{-1}, respectively. The method was linear over the working range of the calibration curve with coefficient of determination (r^2) > 0.996 for all target analytes.

Occurrence and concentrations of NEO and IGR in honey

In total, 115 honey samples were analyzed for the presence of neonicotinoid, as presented in Supplementary Table 2. The descriptive statistics for individual NEO and IGR are presented in Table 4. Approximately 50% of the samples contained at least one NEO; 8.7% and 3.5% of samples contained a combination of two and three NEO each, respectively, while two samples contained a combination of ≥ 4 NEO. Acetamiprid was the most detected NEO with detection frequency of 24.4%, while thiacloprid was the least detected (0.87%). Imidacloprid presented the highest concentration of NEO in any sample (16.945 μg kg^{-1}). The detection frequency of NEO was in the order: acetamiprid > clothianidin > dinotefuran > thiomethoxam > imidacloprid = imidaclothiz > nitenpyram > thiacloprid. The mean concentrations (μg kg^{-1}) were in the order: clothianidin (0.315) > imidacloprid (0.283) > thiomethoxam (0.20) > dinotefuran (0.114) > nitenpyram (0.10) > acetamiprid (0.05) > imidaclothiz (0.01) > thiacloprid (< LOQ) [Table 4]. The concentrations of clothianidin and imidacloprid accounted for 29.3% and 26.3% of the total NEO in all samples. Four samples contained NEO concentrations > 10 μg kg^{-1}, while 15% of the samples had NEO concentrations of 1-10 μg kg^{-1}.

Table 3. Summary of validation performance characteristics for neonicotinoids in honey

Neonic	IS	Recovery/%	RSD/%	LOD/μg kg^{-1}	LOQ/μg kg^{-1}	R^2
Acetamiprid	Imidcloprid-D4	93	14.9	0.01	0.01	0.9997
Buprofezin	Imidcloprid-D4	91	6.2	0.01	0.05	0.9999
Clothianidin	Thiomethoxam-D4	111	13.9	0.01	0.01	0.9998
Cyromazine	Thiomethoxam-D4	110	15	0.01	0.05	0.9997
Dinetofuran	Thiomethoxam-D4	97	16.4	0.03	0.05	0.9972
Fenoxycarb	Imidcloprid-D4	94	9.4	0.01	0.05	0.9996
Imidaclothiz	Thiomethoxam-D4	103	10.3	0.03	0.09	0.9975
Imidacloprid	Imidcloprid-D4	98	8.9	0.01	0.05	0.9999
Nitenpyram	Thiomethoxam-D4	103	7	0.03	0.09	0.9996
Thiacloprid	Imidcloprid-D4	99	14.6	0.01	0.05	0.9998
Thiamethoxam	Thiamethoxam-D4	100	10.9	0.01	0.05	0.9998
Triflumuron	Imidcloprid-D4	81	12.7	0.03	0.09	0.9996

Insect growth regulators were detected and quantified in 21% of the 115 honey samples in this study. While cyromazine (~14%) was the most frequently detected IGR, triflumuron was detected at higher concentrations. The relative abundance of IGR was in the order: cyromazine > triflumuron > buprofezin > fenoxycarb. The concentrations ranged <LOQ-0.801, <LOQ-1.456, <LOQ-1.524, and <LOQ-11.304 μg kg^{-1}, respectively, for buprofezin, cyromazine, fenoxycarb, and triflumuron. Only one honey sample contained IGR concentration of > 10 μg kg^{-1} for triflumuron. Four honey samples contained all four IGR, one sample contained three IGR, and three honey samples had at least two IGR.

The EDIs of NEO and IGR ranged from 1.86×10^{-10} to 1.95×10^{-8} and 9.35×10^{-10} to 4.93×10^{-9} mg kg^{-1} bwd^{-1}, respectively [Table 5]. While imidacloprid and triflumuron were the highest contributors to the total EDIs of NEO and IGR, thiacloprid and buprofezin made the least contributions to the total EDIs.

The HQc of the target NEO and IGR in this study ranged from 9.93×10^{-6} to 1.07×10^{-4} and 2.71×10^{-5} to 3.52×10^{-4}, respectively [Table 5]. The acute exposures of NEO and IGR were estimated as a fraction of their respective ARfDs using the maximum concentrations of each compound measured and the consumption of honey[27]. The HQa of NEO and IGR ranged from 2.95×10^{-6} to 6.05×10^{-5} and 1.09×10^{-6} to 2.1×10^{-5}, respectively.

Dietary risk to bees

The dietary risk of NEO and IGR to forager and nurse bees is presented in Table 6. Clothianidin, imidacloprid, thiamethoxam, and dinotefuran presented more risk to forager and nurse bees than the other target NEO and IGRs in this study.

DISCUSSION

Occurrence of NEO and IGR in honey

Five active ingredients of NEO, namely acetamiprid, clothianidin, imidacloprid, thiacloprid, and thiamethoxam, are currently registered under over 100 trade names in South Africa. These active ingredients are used on several crops including maize, oats, peaches, apples, barley, cotton seed, canola, citrus, grapes, cucurbits, sunflower seeds, sorghum, wheat, and tomatoes[30].

The average detection frequency of NEO in the honey samples in this study (49.6%) was generally lower than the global average of 75%[21] and the American average of 90%[1]; however, this frequency of detection

Table 4. Descriptive statistics of NEO and IGR in honey

Descriptive statistics	Acetamiprid	Buprofezin	Clothianidin	Cyromazine	Dinotefuran	Fenoxycarb	Imidacloprid	Imidaclothiz	Nitenpyram	Thiacloprid	Thiamethoxam	Triflumuron
Sum	6.487	2.181	36.196	9.517	11.440	2.396	32.503	1.025	11.299	0.062	22.913	21.368
Mean	< LOQ	< LOQ	0.002	0.001	0.001	< LOQ	0.007	< LOQ	0.003	< LOQ	0.003	0.002
Median	0.075	0.054	0.197	0.504	0.890	0.067	0.491	0.069	2.365	0.062	1.817	0.223
Minimum	0.008	0.033	0.032	0.424	0.384	0.050	0.310	0.021	0.637	0.062	0.386	0.026
Maximum	1.975	0.801	14.020	1.456	3.094	1.524	16.945	0.706	5.933	0.062	8.500	11.379
Detection frequency (%)	24.4	6.1	18.3	14	9.0	4.3	4.3	4.3	3.5	0.9	7.0	11.3
95 percentile	0.815	0.782	12.532	1.023	2.529	1.359	16.436	0.606	5.422	0.062	7.478	9.218

exceeded the 40.8% recently reported in China[27]. The profile of NEO recorded in this study is in tandem with the high volume use of five active ingredients of NEO, which are currently registered in over 130 products available for use in South Africa[31]. Interestingly, the profiles of NEO in this study were similar to those reported in China, whereby acetamiprid and imidacloprid had the highest detection frequency and highest detection concentration in sample, respectively[27]. This observation is not surprising as > 60% of honey consumed in South Africa originates from China[24]. Only two honey samples exceeded the maximum residue limit (MRL) for clothianidin (i.e., MRL = 10 μg kg^{-1}). This is a concern considering the toxicity of clothianidin to honeybees. Both thiamethoxam and imidacloprid are used in seed dressing of maize to control the black maize beetle *Heteronychus arator* in South Africa[31].

The profile of NEO detected in these samples were different from those reported across the European Union, in which thiacloprid and thiamethoxam were the dominant NEO found in honey[11]. Whereas only about 4.3% of the samples in this study contained imidacloprid, a recent study found imidacloprid in 27% of Irish honey samples. Prevailing landscape, land use, bee species, pesticide governance, and floral origin are some of the factors responsible for the residue profile of NEO in honey from different countries and geographical regions[25].

The use of both imidacloprid and clothianidin has been restricted in the European Union since 2013 (EC, 2018). Studies conducted around the globe showed low detection frequency of insecticides in honey[23]. A study by Codling *et al.*[10] reported detection frequencies of 19%, 8%, 3%, and 38% for acetamiprid, imidacloprid, thiamethoxam, and dinotefuran, respectively, in honey from Egypt. In Australia, Ligor *et al.*[25] reported concentrations of neonicotinoids in excess of 1350 ng g^{-1} for thiamethoxam in three honey samples.

The levels of IGR detected in these samples were considerably lower than concentrations reported in honey from China[32]. It was observed that certain NEO, for example, acetamiprid, clothianidin, dinotefuran, and imidacloprid, occurred frequently with IGR in the same honey samples. IGR have been rarely reported in honey; however, they have been measured in cabbage[33], citrus[34], Chinese traditional herbs[35], and animal tissues[36]. Triflumuron is a registered IGR for use in mangoes, peaches, apples, and pears in South Africa; hence, the high concentration of this IGR in honey could be associated with its widespread

Table 5. Summary of daily intake and risk associated with human exposure to NEO and IGR

NEO/IGR	ADI/ mg kg^{-1} bwd^{-1}	ARfD/ mg kg^{-1} bw	EDI/mg kg^{-1} bwd^{-1}	ESTI/mg kg^{-1}	HQc	HQa
Acetamiprid	0.07	0.1	6.95036E-07	2.82×10^{-6}	9.92908E-06	2.82143E-05
Buprofezin	0.01	0.5	9.34714E-07	1.14×10^{-6}	9.34714E-05	2.28857E-06
Clothianidin	0.1	0.6	5.17086E-06	2.0×10^{-5}	5.17086E-05	3.3381E-05
Cyromazine	0.06	0.1	1.78444E-06	2.08×10^{-6}	2.97406E-05	2.08E-05
Dinotefuran	0.2	1	3.43E-06	4.42×10^{-6}	1.76E-05	4.42E-06
Fenoxycarb	0.053	2	1.4376E-06	2.18×10^{-6}	2.71245E-05	1.08857E-06
Imidacloprid	0.06	0.4	1.95018E-05	2.42×10^{-5}	3.25E-04	6.05179E-05
Imidaclothiz	0.025		6.15E-07	1.0×10^{-6}	2.46E-05	
Nitenpyram	0.53		8.47425E-06	8.48×10^{-6}	1.59892E-05	
Thiacloprid	0.01	0.03	1.86E-07	8.86×10^{-8}	1.86E-05	2.95238E-06
Thiamethoxam	0.08	1	8.59238E-06	1.21×10^{-5}	1.07E-04	1.21429E-05
Triflumuron	0.014		4.93108E-06	1.63×10^{-5}	3.52E-04	

Table 6. Dietary risk of neonicotinoid to bees (ng/bee) via honey ingestion

Compound	Foragers Average	Maximum	Nurse Average	Maximum
Cyromazine	1.98E-08	3.5E-06	9.9E-09	1.75E-06
Dinotefuran	2.7E-5	7.5E-04	1.35E-05	3.75E-04
Acetamiprid	3.9E-08	7.8E-05	1.95E-08	3.9E-05
Clothianidin	1.2E-05	3.5E-04	6E-06	1.75E-04
Imidacloprid	1.3E-05	7.9E-04	6.5E-06	3.95E04
Nitenpyrum	2.5E-06	8.6E-05	1.25E-06	4.3E-05
Thiamethoxam	2E-05	7.9E-04	1E-05	3.95E-04
Triflumuron	4E-09	4E-07	2E-09	2E-07

use. Overall, the result of the present studies confirms the widespread occurrence of neonicotinoid and insect growth regulators in honey consumed in South Africa. The synergistic effects of these co-occurring compounds in honey could pose a risk to non-target organisms such as bees and human health.

Dietary intakes of NEO and IGR via honey consumption

The EDIs of each NEO and IGR were several orders of magnitude lower than their respective ADIs. Recent studies have mostly employed the use of the mean concentrations of pesticide residues rather than the median concentrations, because it was found to be mostly higher and could represent the worst-case scenario[27].

Generally, the HQc and HQa reported in this study were several orders of magnitude lower than values reported in honey and other food commodities in China and other parts of the world[27,37].

Overall, the EDIs, HQc, and HQa of NEO and IGR in honey consumed in South Africa indicate that the consumption of honey does not currently pose any risk of adverse effects of NEO and IGR to human in South Africa.

The dietary risk of NEO and IGR to forager and nurse bees is presented in Table 5. The obtained result shows negligible risks of neonicotinoids and IGR toward bees (risk < 0.01) for all the analytes for both the mean concentration and the highest concentration detected. Thiamethoxam, acetamiprid, and dinotefuran

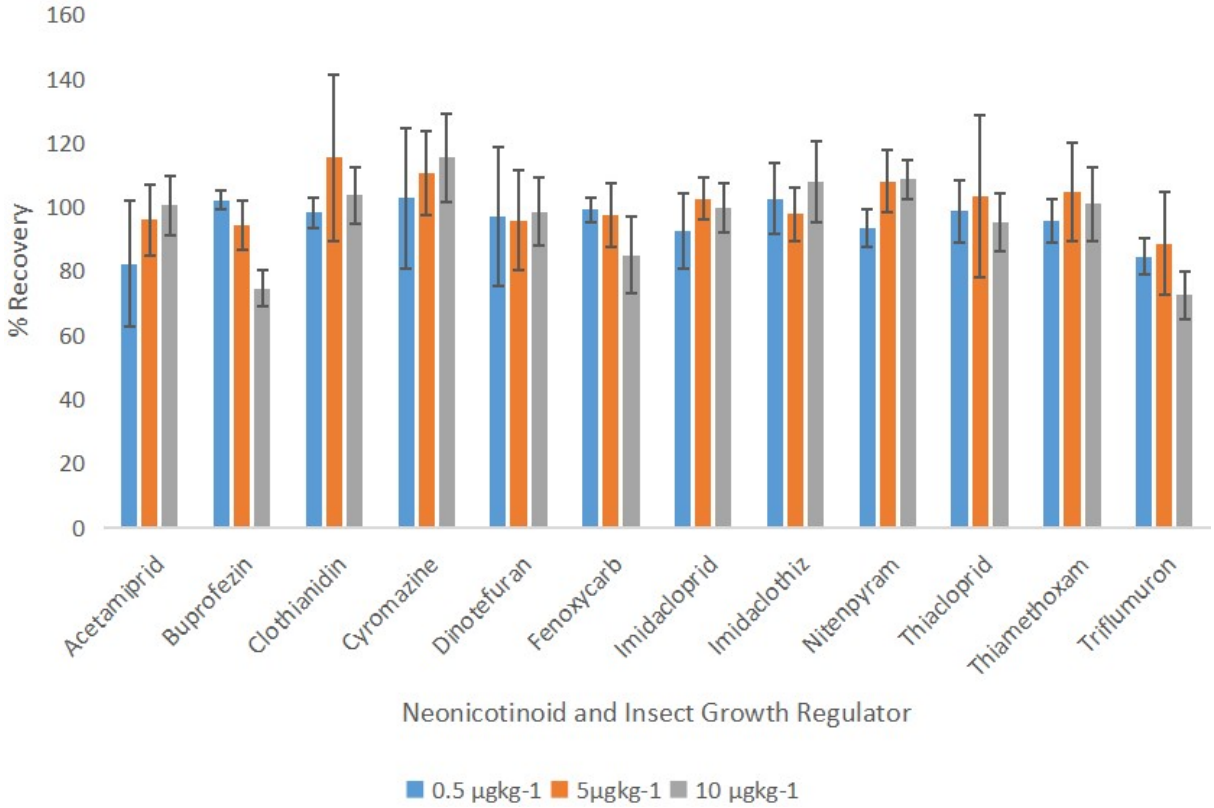

Figure 3. Recovery and within-laboratory reproducibility of neonicotinoids and insect growth regulator.

showed similar risk at maximum concentrations for both the foragers and nurse bees, albeit at a negligible risk category (risk < 0.01).

The congeners of NEO with reported residues were in tandem with their bee toxicities. Acetamiprid was the most frequently detected NEO in the samples, while imidacloprid was detected at the highest concentration for any single honey sample in this study. These two NEO have relatively low toxicities to bees, as shown by their acute oral LD_{50} of 10,140 and 120 ng/bee, for acetamiprid and imidacloprid, respectively [Table 1]. Hence, it can be deduced that honeybees are not exposed to lethal doses of these NEO during foraging, thus able to transport acetamiprid and imidacloprid into beehives. In contrast, three of the frequently detected NEO in the honey samples - clothianidin, dinotefuran and thiamethoxam - are highly toxic to bees with their reported oral LD_{50} of 18, 26.8, and 11.8 ng/bee, respectively[10]. The exposure of foraging honeybees to small doses of these highly toxic NEO could lead to the death of bees before they can transfer these compounds into honey[11]. Bee colony collapse has been reported at various times in South Africa. A study by Pirk *et al.*[38] reported an average bee colony loss of 29% between 2009 and 2010. This was attributed to small hive beetles, absconding, varroa mite, and chalkbrood disease[38]. However, a recent BBC report associated the loss of over one million bees in South Africa in 2018 to the insecticide Fiprinol, used by wine farmers[39]. Imidacloprid poisoning in granivorous Cape spurfowl (*Pternistis capensis*) was recently reported in South Africa following the ingestion of imidacloprid-treated wheat and barley seeds sown in a field in South Africa[40]. This poisoning resulted in severe neurological abnormalities and eventual deaths of several non-target organisms such as *Pternistis capensis* and *Francolinus africanus* (Greywing francolin)[40].

CONCLUSION

A simple analytical method was developed and validated in this study. The method satisfied regulatory requirements for the monitoring of NEO and IGR in honey (SANCO/10684/2009).

This study provides background data on the occurrence of neonicotinoid and insect growth regulators in commonly consumed honey in South Africa. The concentrations of NEO and IGR found in honey indicate negligible risk to human and bee health.

Future studies focusing on the identification of the exact sources of NEO and IGR as well as the risk posed to the honeybee population in South Africa are encouraged; studies on the human health risk associated with multiple dietary and non-dietary exposure routes of mixtures of these compounds cannot be overemphasized.

DECLARATIONS

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Authors' contributions

Made substantial contributions to conception and design of the study, funding, and performed data analysis and interpretation: Abafe OA

Performed data acquisition, as well as provided administrative, technical, and material support: Abafe OA, Chokwe T

Availability of data and materials

Additional data for this study are presented in the supplementary information.

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Conflicts of interest

Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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