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First insights into the occurrence of pesticide residues in edible insects from sub-Saharan African countries

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Abstract

Pesticide use is a common practice worldwide, especially in Sub-Saharan African (SSA) countries, where ongoing agriculture intensification and the need for disease vector control make it essential. The population can thus be exposed to variable amounts of pesticides through the diet. Edible insects are a highly regarded food source in SSA. However, they are still mostly harvested from the wild, where chemical applications are not necessarily controlled, representing a major cause of concern for consumers. We investigated residues of legacy (OCPs) and current-use pesticides (CUPs) in selected edible insects commonly consumed in Uganda and Nigeria, and evaluated the eventual health risk for the adult population associated with their consumption. Targeted OCPs were < LOQ in all analysed edible insects, except for hexachlorobenzene (up to 0.87 ng/g dw), while several CUPs were present at

notable levels. Cypermethrin showed the highest median concentration (17 ng/g dw), while the Nigerian cricket *Brachytrupes membranaceus* was the most contaminated sample, with concentrations of aldicarb, propoxur, chlorpyrifos, dichlorvos and paclobutrazol reaching 118 ng/g dw, 327 ng/g dw, 156 ng/g dw, 26 ng/dw, and 14 ng/g dw, respectively. The concentrations of pesticides were generally well below the available maximum residue levels (MRLs), and the dietary risk assessment did not indicate health threats for the adult population. However, we suggest that the monitoring of the chemical safety of edible insects in SSA should be further investigated and insects should be integrated into more extensive dietary studies.

Keywords: Legacy pesticides, current-use pesticides, entomophagy, Uganda, Nigeria, human exposure

INTRODUCTION

Pesticides are chemical compounds applied to control disease vectors or household pests and to help increase crop productivity[1]. They are often divided into organochlorine pesticides (OCPs), also called legacy pesticides, and current-use pesticides (CUPs). OCPs were widely used in the past for agricultural purposes, but because of their persistence in the environment and proven toxicity to wildlife and humans[2], they have been included in the list of persistent organic pollutants (POPs) regulated by the Stockholm Convention[3] and banned for agricultural uses. However, they are still used in some developing countries for disease vector control[4]. CUPs emerged mostly as an alternative to OCPs and encompassed several chemical groups of insecticides, fungicides, and herbicides which are thought to be less persistent in the environment and less bioaccumulative than OCPs. Nevertheless, some CUPs (like atrazine, imidacloprid, and chlorpyrifos) are also highly toxic for non-target organisms and have been associated with adverse environmental and public health effects[1].

In many Sub-Saharan African (SSA) countries, ongoing agriculture intensification, driven by population growth and the expansion of local markets, and the crucial need for disease vector control have led to the increasing use of pesticides[5]. Once applied, pesticides can be transported from the application site to non-target areas by wind erosion of particles or volatilization from soils, water, and vegetation, resulting in a complex mixture of compounds potentially entering the environment and related food chains[1,6]. In addition, product usage and handling as prescribed by the manufacturers are often not complied with, leading to the misunderstanding of safety guidelines and the consequent direct and indirect exposure of applicators and individuals and families living adjacent to agricultural sites, respectively[7]. The population can ultimately be extensively exposed to minor quantities of pesticides through food and water, making it imperative to monitor the levels of various pesticides in foods to safeguard consumers' wellbeing[8].

Entomophagy, the practice of eating insects, has been and still is an essential source of nutrients in many developing countries and in certain cultures. In Western countries, insects are recently promoted as an alternative protein source for food and feed because of their high nutritional contents[9] and environmental sustainability[10]. In SSA, insects are included as a regular part of the diet throughout the year or in seasons of occurrence[11], and the type of edible insects consumed is usually community- or area-dependent[12]. Insects are mostly harvested from the wild (e.g., grasslands, bushes, forests and farmlands), where the control of chemical applications is difficult, thus representing a major cause of concern for consumers[11]. The environmental advantages of insect farming over wild harvesting, including the fact that chemical hazards can be controlled to a larger extent, have been recently highlighted[10]. However, insect farming practices in most SSA countries remain limited, mostly due to the lack of clear legislation regarding the rearing, consumption, and commercialization of edible insects[13,14].

Therefore, taking into account, on the one hand, the general lack of knowledge about the environmental occurrence and human exposure to pesticides in SSA countries[5], and on the other hand, the high potential represented by edible insects as a sustainable food source in the interest of food security and wealth creation[11], the chemical food safety of edible insects should receive particular attention.

In this preliminary study, residues of legacy and current-use pesticides were investigated for the first time in selected wild and farmed edible insects commonly sold and consumed in Uganda and Nigeria. Additionally, eventual health risks for the adult population associated with the consumption of such edible insects were also evaluated.

MATERIALS AND METHODS

Sample collection

A total of nine edible insect samples were collected in the frame of this preliminary study [Table 1]. House crickets from Uganda were obtained in 2022 from a cricket farmer in Masaka (UGD-01) and from the cricket rearing laboratory at the Department of Zoology, Makerere University (UGD-02). In addition, between November and December 2018, wild harvested long-horned grasshoppers were obtained from traders from three geographical areas and major harvesting hubs (Masaka - UGD-03, Mubende - UGD-04, Kampala - UGD-05) in Uganda. Samples were packed in zip-sealed plastic bags and delivered to Makerere University on flaked ice. These samples were then washed clean using water, drained, dried in a solar dryer on aluminium foil, milled per species (about 5 g insects) using an electric blender with a plastic body (acrylonitrile butadiene styrene, ABS plastic) and stainless-steel blades, transferred to pre-cleaned polypropylene (PP) tubes, and finally stored at -18 °C.

Four species of wild harvested edible insects, already processed and ready for consumption, were purchased from local markets in North-central Nigeria (NGR-01) in February 2020 and Southwestern Nigeria (NGR-02, -03 and -04) in January 2022. NGR-01 (short-horned grasshopper) and NGR-02 (giant cricket) are usually consumed in the adult life stage after being roasted [Supplementary Figure 1A]. NGR-03 (rhinoceros beetle) and NGR-04 (African palm weevil) are typically consumed in their larval life stage and processed by frying [Supplementary Figure 1B]. Individual insects per sample (about 5 g) were pooled, homogenized using an electric blender with plastic (ABS) body and stainless-steel blades, and stored in pre-cleaned PP tubes at -18 °C pending analyses.

The lipid content of each sample was determined gravimetrically, as detailed in the reference[15].

Sample preparation and instrumental analysis

Detailed information on purchasing chemicals and materials is reported in Supplementary Material, Supplementary Table 1. A total of 12 OCPs and 44 CUPs were analysed in the edible insects [Supplementary Table 2].

Organochlorine pesticides

Analysis of OCPs was done according to the procedure described in[16]. Briefly, 1.0 g of dry insect sample was weighed in pre-cleaned PP tubes, spiked with 50 µL internal standard, IS mix (CB-143, 500 pg/µL and ^{13}C-HCB, 50 pg/µL), and extracted with 10 mL of hexane:acetone (3:1, v/v). The extract was concentrated to a volume of 0.5 mL and cleaned up via an acid silica (AS) cartridge (25 mL cartridge containing 6 g of AS, 44% w/w, prewashed with 15 mL hexane) by elution with 20 mL of hexane and 15 mL of DCM. The final extract was concentrated to near dryness and reconstituted in 50 µL iso-octane and 50 µL of recovery standard (RS, CB-207, 50 pg/µL). The targeted OCPs were analysed by gas chromatography coupled to a

Table 1. Details of edible insect samples collected from Uganda (UGD) and Nigeria (NGR)

ID	Species (order)	Common name	Life stage	Collection site	Wild/farmed	Processing	Lip (%) dw
UGD-01	*Acheta domesticus* (Orthoptera)	House cricket	Adult	Uganda, Masaka district	Farmed	Sun dried	17
UGD-02	*Acheta domesticus* (Orthoptera)	House cricket	Adult	Uganda, Makerere University	Farmed	Sun dried	14
UGD-03	*Ruspolia differens* (Orthoptera)	Long-horned grasshopper	Adult	Uganda, Masaka district	Wild	Sun dried	39
UGD-04	*Ruspolia differens* (Orthoptera)	Long-horned grasshopper	Adult	Uganda, Mubende district	Wild	Sun dried	34
UGD-05	*Ruspolia differens* (Orthoptera)	Long-horned grasshopper	Adult	Uganda, Kampala district	Wild	Sun dried	46
NGR-01	*Cytacanthacris naeruginosus* (Orthoptera)	Short-horned grasshopper	Adult	North Central Nigeria	Wild	Roasted	36
NGR-02	*Brachytrupes membranaceus* (Orthoptera)	Giant cricket	Adult	Southwestern Nigeria	Wild	Roasted	28
NGR-03	*Oryctes boas* (Coleoptera)	Rhinoceros beetle	Larvae	Southwestern Nigeria	Wild	Fried	29
NGR-04	*Rhynchophorus phoenicis* (Coleoptera)	African palm weevil	Larvae	Southwestern Nigeria	Wild	Fried	5

mass spectrometer operated in electron capture negative ionization mode (GC-ECNI/MS), as detailed in[16].

Current-use pesticides

Analysis of CUPs was performed by applying a modified QuEChERS method. Samples (between 0.2 and 0.5 g depending on the lipid content) were weighed in pre-cleaned PP tubes, spiked with 30 µL IS mix (composition reported in Supplementary Table 1), and extracted with 6 mL of acetonitrile acidified with 0.1% formic acid, FA. Salting out of the supernatant was achieved by adding 1 g of $MgSO_4$ and 0.25 g of NaCl to the extract, followed by vortexing for 1 min and centrifugation at 3000 rpm for 5 min. The supernatant was transferred to a clean glass tube and concentrated to 2 mL under a gentle nitrogen stream. The sample clean-up was then performed by dispersive solid phase extraction, SPE (d-SPE), by the addition of 50 mg of PSA and 100 mg of C18 to the extract, followed by vortexing for 1 min and centrifugation at 3000 rpm for 5 min. The supernatant was finally transferred into a clean glass tube, concentrated to dryness, reconstituted in 200 µL MeOH:MilliQ (1:1 v/v), and filtered through a 0.2 µm nylon membrane to an autosampler vial for analysis by LC-MS/MS.

Analysis of CUPs was carried out using an Agilent 1290 Infinity liquid chromatography system coupled to an Agilent 6460 Triple Quadrupole mass spectrometer (LC-MS/MS, Agilent, Santa Clara, USA) equipped with an electrospray ionization (ESI) source. Briefly, separation was achieved using a Kinetex XB-C18 column (4.6 × 100 mm, 2.6 µm, Phenomenex, Torrace, USA) using MilliQ and MeOH, both containing 5 mm of ammonium formate, as mobile phases. The acquisition was carried out in dynamic multiple reaction monitoring (dMRM) mode. Details of the method are described in Supplementary Material (Supplementary Section 1, Supplementary Tables 3-6).

QA/QC

Quality assurance and control of the analytical method for the quantification of OCPs in edible insects was performed through four replicate analyses of SRM 1945 (NIST, organics in whale blubber) with accuracy ranging from 67% to 152% [Supplementary Table 2]. The mean recoveries of IS were 108% ± 22% for CB-143 and 85% ± 20% for ^{13}C-HCB. The reliability of the method for CUPs was assessed using a duplicate analysis of a blank sample spiked with a known mass of native compounds. Accuracies for individual

compounds are reported in Supplementary Table 2, while the recoveries of IS ranged between 80% ± 32% (D5-Atrazine) and 121% ± 15% (^{13}C-Fipronil).

To control potential background contamination, two procedural blanks were run in parallel with the batch of samples. For OCPs, the average blank levels per batch were then subtracted from the sample results, and a value equal to 3×SD of the blank measurement was used as the limit of quantification (LOQ). For CUPs, due to the absence of detectable pesticide concentrations in the procedural blanks, the LOQ was set at the lowest quantifiable calibration concentration. LOQs of all individual compounds are shown in Supplementary Table 2.

Statistical analysis

For descriptive statistics, concentrations < LOQs were treated as LOQ*df, where df is the detection frequency of the compound above LOQs in the samples. The normal distribution of the dataset was tested by the Kolmogorov-Smirnov test (K-S test). Most concentration results (86%) were found to have a skewed distribution. To better understand the correlations and/or patterns of residue concentrations, only compounds with df > 30% were considered in the statistical analysis and discussed in the text. Spearman's correlation was used to investigate the correlation among the concentrations. An individual *t*-test was carried out to compare the mean value of the "farmed" and "wild" Ugandan samples. Factor analysis was done using "KMO and Bartlett's test of sphericity" as a correlation matrix and extracting principal components (PCA) based on eigenvalue > 1. Statistical analysis was performed using IBM SPSS 20.0 (Chicago, IL, USA), with a bilateral test ($P < 0.05$).

Human exposure

The average insect consumption for the adult population in Uganda was estimated to be 62.6 g/day during grasshopper seasons (lasting about 3 months, i.e., from March to May and from November to January)[17]. Since no insect consumption figures are currently available for Nigeria, the average insect consumption of grasshoppers in Uganda was thus used for the risk assessment estimation. In addition, to cover the most conservative scenario, the insect consumption rate was assumed to be constant throughout the year.

The estimated dietary intake (EDI) of OCPs and CUPs was calculated for each compound with DF > 30% by multiplying the median concentration of that pesticide (ng/g dw) by the average consumption rate of edible insects (62.6 g/day) by the adult population and dividing by the average body weight of the adult population for each country (i.e., 62 kg and 60 kg for the adult population of Uganda[18] and Nigeria[19], respectively). The hazard quotient (HQ or the potential risk of non-carcinogenic effects) per each compound was calculated by dividing the total EDI by the relative oral reference dose factor (RfD). HQ values ≥ 1 indicate a potential health risk for the population. The potential carcinogenic risk (CR) was calculated by multiplying the EDI by the relative oral cancer slope factor (SFO). A public screening criterion sets a threshold of CR < 1.0×10^{-6} as acceptable[20].

RESULTS AND DISCUSSION

Levels of pesticides in edible insects

Targeted OCPs were < LOQ in all analysed insect samples, with the sole exception of HCB, measured in NGR-02 (0.45 ng/g dw), UGD-01 (0.62 ng/g dw), and UDG-05 (0.87 ng/g dw) [Supplementary Table 7]. No pattern of contamination was identified, considering the species, wild/farmed source or processing method, suggesting low background contamination with OCPs in the environment. HCB levels in the analysed samples were also comparable with levels measured in farmed edible insects from Europe and Asia (0.1-69 ng/g lipid weight)[15,21].

Out of 44 CUPs, a total of 23 compounds were detected in edible insects, among which 13 chemicals have a detection frequency higher than 30% [Table 2]. Generally, there was a wide variation in the concentrations and patterns of the different CUP residues in the analysed edible insects [Figure 1]. The organophosphate insecticides chlorpyrifos and dichlorvos were detected in 100% and 89% of the samples, with total median concentrations of 2.6 and 5.0 ng/g dw, respectively. For both compounds, the highest concentrations were observed in the wild sample NGR-02 (156 and 26 ng/g dw, respectively) [Figure 1, Supplementary Table 8]. As of 2020, chlorpyrifos was banned in the EU[22], and in 2021 the US EPA announced a ban on the use of chlorpyrifos on food crops in the US[23]. Unauthorised application of chlorpyrifos in certain SSA countries has, however, been identified and highlighted as potentially posing a threat to human health[24]. Dichlorvos has been reported to be applied as an agricultural insecticide on crops, stored products and mosquitoes in SSA countries, including Nigeria[25]. In addition, these two compounds are reported to be affordable and readily available, supporting our outcomes. Concentrations of chlorpyrifos and dichlorvos were also highly correlated with each other ($P = 0.007$) [Supplementary Table 9], suggesting a similar source and fate in the environment. Another organophosphate insecticide, ethoprophos, was detected in only three insect samples, with a total limited median concentration of 0.1 ng/g dw.

The carbamate insecticide aldicarb was detected in 78% of the samples, with a total median concentration of 8.9 ng/g dw, while other carbamates, such as oxamyl and propoxur, were detected in 44% and 67% of the samples, with median concentrations of 0.6 and 1.2 ng/g dw, respectively. Aldicarb is a systemic pesticide applied to control certain insects and nematodes on both food and non-food crops. Due to its toxic effects, control actions to ban or severely restrict aldicarb use have been reported by European countries, while its use for agricultural purposes is still allowed in some countries, including SSA[26]. Aldicarb presence was highly correlated with both dichlorvos and cyfluthrin (both $P < 0.001$) [Supplementary Table 9], possibly indicating that these pesticides were applied together or in a similar pattern. Propoxur has less toxic effects on pests than aldicarb, and it has both contact and systemic activity[26]. It is used on a variety of pests in either agricultural or non-agricultural applications, including malaria control activities[27]. Interestingly, the highest individual concentrations of aldicarb and propoxur were measured in the wild sample NGR-02, reaching 118 and 327 ng/g dw, respectively. Propoxur was also the highest measured pesticide concentration in all analysed edible insects [Figure 1, Supplementary Table 8]. This can be attributed to the harvesting style of giant crickets, which are manually dug out from their soil burrows by insect pickers[28]. Aldicarb, usually applied as soil fumigant or as direct soil solution[29], can thus have been taken up by the crickets during their underground lifetime. Similarly, the long residual effect of propoxur after application on turfs, forestry, household, and agricultural environments[30] could have been a major reason for the high levels found in this soil insect species.

The pyrethroid insecticide cypermethrin, detected in 67% of the samples, showed the highest median concentration measured in edible insects, up to 17 ng/g dw, while the median levels of other pyrethroids, such as cyfluthrin and permethrin were 4.5 and 1.0 ng/g dw, respectively. Cypermethrin is a toxic broad-spectrum insecticide that has been reported to be widely used by farmers to control populations of variegated grasshopper (*Zonocerus variegatus*), which is among the main pests of crops grown in West and Central Africa (including Nigeria)[31]. This is confirmed by the generally higher concentrations of cypermethrin measured in insects from both Uganda and Nigeria compared to the other pyrethroids, but also generally to the other investigated insecticides [Supplementary Table 8]. Interestingly, this grasshopper is among the most consumed species of Orthoptera in Africa[32]. Therefore, even if this species was not analysed in the frame of this study, this highlights the threats potentially represented by the use of insecticides in relation to the consumption of edible insects.

Table 2. Descriptive statistics of CUPs (ng/g dw) in edible insects from Uganda and Nigeria. In bold, compounds with df > 30%. Individual concentrations of all CUPs are reported in Supplementary Table 8

	ALL (*n* = 9)						UGD (*n* = 5)					NGR (*n* = 4)				
Compound	**df (%)**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Mean**	**SD**	**Min**	**Median**	**Max**
Fenpyroximate	11	0.1	0.3	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.2	0.4	0.0	0.0	0.9
Metalaxyl	11	0.3	1.0	0.0	0.0	2.9	0.6	1.3	0.0	0.0	2.9	0.0	0.0	0.0	0.0	0.0
Boscalid	11	0.3	0.9	0.0	0.0	2.8	0.6	1.2	0.0	0.0	2.8	0.0	0.0	0.0	0.0	0.0
Myclobutanil	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tebuconazole	22	0.5	1.0	0.1	0.1	2.8	0.1	0.0	0.1	0.1	0.1	1.1	1.3	0.1	0.7	2.8
Diuron	11	0.1	0.2	0.0	0.0	0.7	0.1	0.3	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0
Isoproturon	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Atrazine	22	0.2	0.3	0.0	0.0	1.0	0.1	0.2	0.0	0.0	0.5	0.3	0.5	0.0	0.0	1.0
Prometryn	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Propazine	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sebuthylazine	22	0.3	0.8	0.0	0.0	2.4	0.1	0.2	0.0	0.0	0.4	0.6	1.2	0.0	0.0	2.4
Simazine	**67**	**6.6**	**8.8**	**0.1**	**0.8**	**22**	**12**	**9.0**	**0.6**	**15**	**22**	**0.3**	**0.4**	**0.1**	**0.1**	**0.8**
Terbuthylazine	11	0.2	0.5	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.4	0.8	0.0	0.0	1.7
Terbutryn	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Novaluron	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Spirotetramat	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aldicarb	**78**	**20**	**37**	**0.2**	**8.9**	**118**	**10**	**7.1**	**2.3**	**15**	**17**	**32**	**58**	**0.2**	**4.6**	**118**
Carbofuran	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methiocarb	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methomyl	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oxamyl	**44**	**2.9**	**5.0**	**0.6**	**0.6**	**16**	**1.3**	**1.0**	**0.6**	**0.6**	**2.9**	**4.9**	**7.4**	**0.6**	**1.6**	**16**
Propoxur	**67**	**37**	**109**	**0.2**	**1.2**	**327**	**0.9**	**0.7**	**0.2**	**1.2**	**1.7**	**83**	**163**	**0.2**	**1.4**	**327**
Acetamiprid	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dinotefuran	**44**	**0.6**	**0.6**	**0.1**	**0.1**	**1.5**	**0.1**	**0.0**	**0.1**	**0.1**	**0.1**	**1.3**	**0.2**	**1.0**	**1.3**	**1.5**
Imidacloprid	**33**	**0.2**	**0.1**	**0.1**	**0.1**	**0.4**	**0.1**	**0.1**	**0.1**	**0.1**	**0.3**	**0.2**	**0.2**	**0.1**	**0.21**	**0.4**
Thiacloprid	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Thiamethoxam	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chlorpyrifos	**100**	**20**	**51**	**1.1**	**2.6**	**156**	**3.9**	**2.8**	**1.6**	**2.9**	**8.8**	**40**	**77**	**1.1**	**2.0**	**156**
Diazinon	11	0.1	0.1	0.0	0.0	0.3	0.1	0.1	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0

Dichlorvos	**89**	**7.5**	**7.7**	**1.3**	**5.0**	**26**	**6.8**	**3.8**	**3.0**	**5.1**	**13**	**8.2**	**11.6**	**1.3**	**3.0**	**26**
Dimethoate	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ethoprophos	**33**	**0.7**	**0.8**	**0.1**	**0.1**	**1.9**	**0.8**	**1.0**	**0.1**	**0.1**	**1.9**	**0.5**	**0.7**	**0.1**	**0.1**	**1.5**
Malathion	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phosmet	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fipronil	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bifenthrin	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cyfluthrin	**67**	**7.1**	**8.6**	**1.0**	**4.5**	**27**	**5.6**	**4.9**	**1.0**	**4.5**	**13**	**9.0**	**12.5**	**1.0**	**3.9**	**27**
Cypermethrin	**67**	**27**	**31**	**0.4**	**17**	**72**	**32**	**36**	**0.4**	**17**	**72**	**22**	**27**	**0.4**	**15**	**57**
Deltamethrin	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Permethrin	**33**	**5.2**	**7.4**	**1.0**	**1.0**	**22**	**5.2**	**9.5**	**1.0**	**1.0**	**22**	**5.2**	**5.2**	**1.0**	**4.2**	**11**
Flonicamid	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chlorantraniliprole	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Piperonyl butoxide	22	0.4	0.7	0.1	0.1	1.9	0.1	0.0	0.1	0.1	0.1	0.8	0.9	0.1	0.6	1.9
Paclobutrazol	**67**	**4.5**	**4.9**	**0.2**	**3.0**	**14**	**4.9**	**3.4**	**0.2**	**5.2**	**8.6**	**4.0**	**6.9**	**0.2**	**0.8**	**14**

Imidacloprid and dinotefuran, two neonicotinoid insecticides, were both present in median concentrations of 0.1 ng/g dw. Dinotefuran, in particular, was detected only in wild samples from Nigeria and in similar concentrations among the four insect species [Supplementary Table 8]. Neonicotinoids are considered particularly toxic to insects and can kill both targeted pests and not-targeted insects, including bees and other pollinators[33]. For this reason, the use of imidacloprid was banned in the EU in 2018[34], while in SSA, both imidacloprid and dinotefuran are still in use[35]. In addition, these two insecticides can also be applied at the same time to reach a combined effect of quick action and longer protection[36]. Thus, the low concentrations of imidacloprid and dinotefuran measured in the edible insects from this study seem to suggest a past or diffuse background environmental contamination.

The herbicide simazine was detected in 67% of the samples, with a median concentration of 0.8 ng/g dw, indicating the use of this compound in the SSA environment, although it has been withdrawn from the EU with "essential use" derogations[37]. Finally, the plant growth regulator and fungicide, paclobutrazol, was detected in 67% of the samples, with a median concentration of 3 ng/g dw. Paclobutrazol is known to protect several crops from various environmental stressors, including drought and heat radiation and can thus be used to improve the growth and yield of crops[38]. In addition, in this case, the highest individual concentration was measured in sample NGR-02 (14 ng/g dw).

Cypermethrin was the highest contributor to the median CUPs in the insect samples, up to 38% in all insects, 25% and 39% in Ugandan and Nigerian insects, respectively [Supplementary Figure 2], followed by aldicarb (20%, 22% and 12%). Simazine and permethrin accounted then for 22% and 11% of the Ugandan

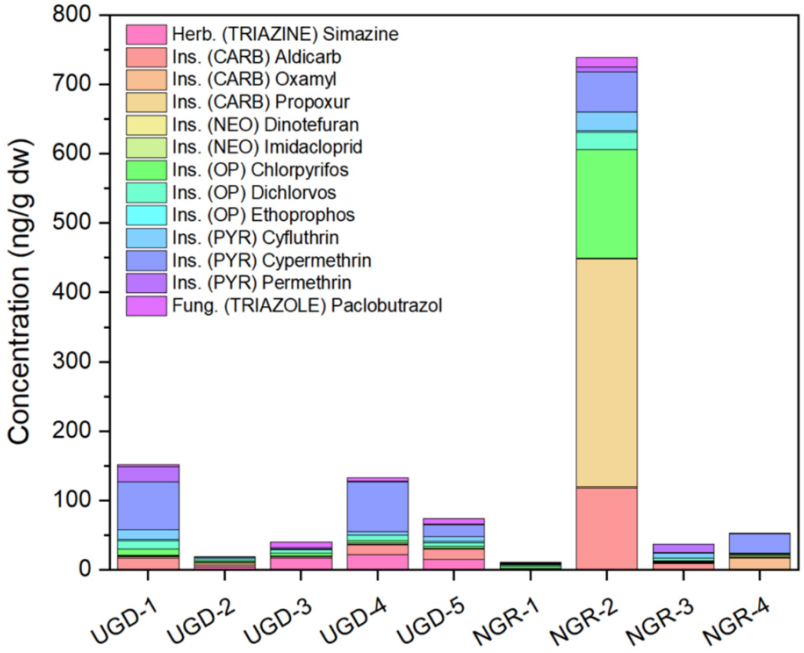

Figure 1. CUP concentrations (ng/g dw) in edible insects from Uganda (UGD) and Nigeria (NGR) and their individual contribution to the overall contamination.

and Nigerian median contamination with CUPs.

Differences between CUPs in Ugandan farmed and wild insects were compound-specific [Supplementary Table 8]. For simazine and paclobutrazol, the average concentration in farmed house crickets (2.2 ng/g dw and 1.6 ng/g dw, respectively) was significantly lower ($P < 0.05$) than the average concentration in wild grasshoppers (18 ng/g dw and 7.2 n/g dw). These results suggest that the contamination with these CUPs in a farming setting could be more easily controlled or that this difference could be species-specific. However, in the case of the aldicarb, measured in similar concentrations in the farmed UGD-01 (17 ng/g dw) and in the wild UGD-04 and -05 (15 ng/g dw), as opposed to the lower concentrations in farmed UGD-02 (3.1 ng/g dw) and wild UGD-03 (2.3 ng/dw). Oxamyl was measured only in farmed Ugandan house crickets (average 2.3 ng/g dw), similar to the concentration measured in wild NGR-02 (giant cricket, 2.5 ng/g dw), but lower than NGR-04 (African palm weevil, 16 ng/g dw), while it was < LOQ in the other Ugandan wild insects. In this case, it is possible that the insect order, habitat or life stage played a role in the accumulation of the contaminant. Permethrin was measured in UGD-01 (22 ng/g dw), while it was < LOQ in the other farmed or wild Ugandan samples, suggesting a site-specific application of this insecticide. No significant or notable differences were observed between farmed and wild insects for the other CUPs.

The principal component analysis (PCA) of pesticide residues in the current study suggests that the residue levels were distributed according to their application. The first and second principal components (PC) in the factors extracted based on compounds accounted for 48.4% and 20.4% of the total variance, respectively [Figure 2A]. PC1 reflected the residue levels of the pesticides. PC2 showed a difference attributable to the main pesticide use, clearly separated into insecticides, herbicides, and fungicides. In addition, the statistically significant correlations between chlorpyrifos and dichlorvos and between aldicarb, dichlorvos and cyfluthrin [Supplementary Table 9] were also confirmed by the PCA, supporting the hypothesis that

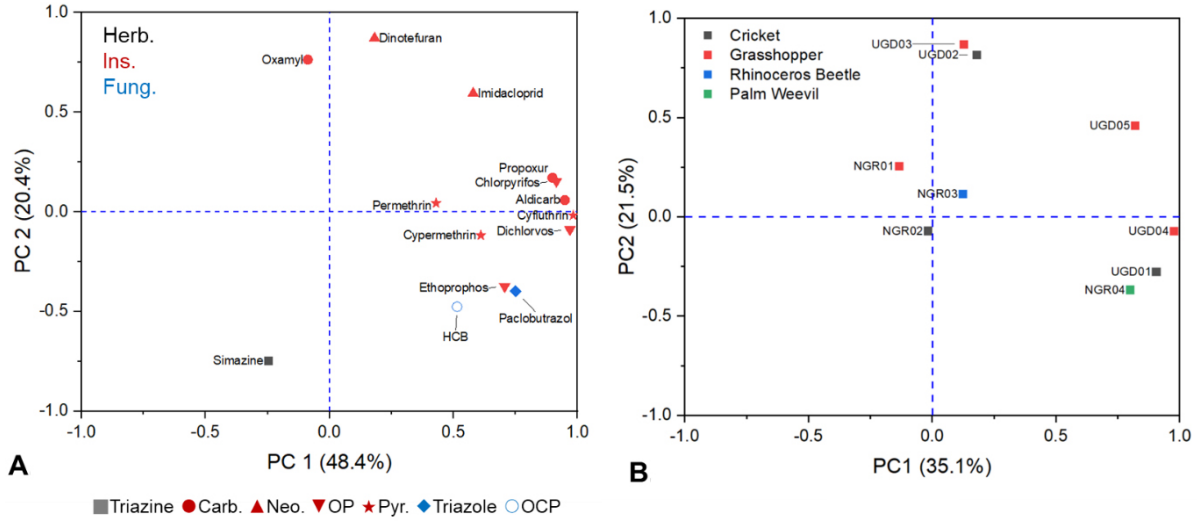

Figure 2. Principal component analysis (PCA) of the pesticide residue in edible insects. Factors extracted based on compounds (A) and edible insect samples (B). Herb: Herbicide in black; Ins: insecticide in red; Fung: fungicide in blue; Carb: carboxamide; OP: organophosphate; Neo: neonicotinoids; Pyr: pyrethroid, OCP: organochlorine pesticide.

these compounds are commonly used in mixtures depending on the scope of application. Factors extracted based on the residue level of the samples [Figure 2B] only reflected limited variations among the samples, with PC1 of 35.1% and PC2 of 21.5%, respectively. Samples could not be clustered in PC1 nor PC2, indicating that the residue levels were not significantly impacted by the species of the insects, region of origin, or habitat. In this preliminary study, only a limited number of edible insect samples were analysed, which strongly hampered the interpretation of the data. This is because obtaining edible insects from SSA countries presented several hurdles, mostly represented by their partial availability throughout the year and their site-specificity. In addition, most samples were purchased from vendors and traders in open markets, with no possibility of gaining specific information on the exact location and situation of the collection site of wild insects. This, together with the limited available information on the use and application of pesticides in these areas, made it difficult to draw further conclusions.

To the best of our knowledge, no previous study investigated the occurrence and concentrations of the same pesticide residues in edible insects commonly consumed in SSA countries, particularly in Uganda and Nigeria. The levels of CUPs were therefore compared with concentration levels reported for other food commodities from the same and neighbouring countries. The median concentrations of dinotefuran and imidacloprid in edible insects from this study were lower than those reported for honey from South Africa (0.89 and 0.49 ng/g, respectively)[33]. In addition, the mean concentration of chlorpyrifos in insects was significantly lower than the levels measured in smoked fish from Mali (i.e., 2591 ng/g), but generally higher than the average levels in a wide variety of other food commodities, including cereals, vegetables and fruit[39]. Concentrations of chlorpyrifos in insects were in the same range as fruit, vegetables, wheat, and coffee beans from Ethiopia[40] and Ghana[41]. Mean concentrations of dichlorvos, propoxur, chlorpyrifos, and ethoprophos in insects were generally higher than or comparable to those recorded in cereals, pulses, fruit and vegetables from Nigeria[19]. Finally, the mean concentration of cypermethrin in the insects was similar to the levels measured in vegetables and fish, but generally higher than those detected in legumes, fruit, eggs and dairy products from Benin, Cameroon, Mali and Nigeria[39].

Human exposure

For the average adult population, the dietary intake of pesticides via consumption of edible insects, based on the consumption figures of grasshoppers in Uganda[17], was comparable between the two countries, ranging from 0.1 to 17 ng/kg bw/day in Uganda and from 0.1 to 16 ng/kg bw/day in Nigeria. The highest EDI was estimated in both cases for cypermethrin. The estimated EDIs were then compared to the available compound-specific RfD and SFO values [Table 3]. The calculated HQ and CR values were orders of magnitude lower than the indicated thresholds, while the CR of simazine and dichlorvos were close to the acceptability threshold of 1.0×10^{-6}. This suggests that the exposure of the adult population to pesticides through the consumption of edible insects is low. However, it is relevant to point out that data on the per-capita consumption of edible insects are lacking in most SSA countries. Moreover, when available, they concern only a limited number of species among the several widely consumed, giving an incomplete representation of the insect consumption pattern.

Finally, no MRLs (i.e., the highest concentration of a pesticide residue legally tolerated in a food commodity when pesticides are correctly applied)[42] are currently available for edible insects; thus, the pesticide residues in insects were compared with the most conservative MRLs available for meat from mammals or poultry [Table 3]. Concentrations of individual pesticides in insects, calculated in ng/g wet weight - considering an average dry fraction of 0.5, were generally below their considered respective MRLs. However, the concentrations of aldicarb, chlorpyrifos, dichlorvos, and cyfluthrin in sample NGR-02 were above the selected MRLs. According to these findings, the adult population of Uganda and Nigeria is not expected to suffer adverse effects from the ingestion of pesticide residue through insect consumption. It is, however, necessary to highlight that insects represent only a part of the daily diet, and their consumption is dependent on availability and seasonality. To be fully representative, these data should thus be revisited in light of the availability of per-capita consumption data of most consumed edible insects and, eventually, the availability of MRLs set specifically for insects.

CONCLUSIONS

Information on pesticide residues in edible insects from sub-Saharan African countries is currently lacking. This preliminary study yielded the first insights into the concentrations of OCPs and CUPs in edible insects from Uganda and Nigeria.

Results showed no contamination of the insects with OCPs but highlighted the presence of several CUPs at notable levels, likely attributable to their extensive use in SSA countries accompanied by a lack of relative rigorous legislation, regulations, and proper training for handlers. In this study, the concentrations of pesticide residues were below the available MRLs (for meat), suggesting that the selected edible insects were safe for consumption. Finally, the dietary risk assessment did not indicate health threats for the adult population. However, due to the challenges encountered when conducting this study, we suggest that the monitoring of the chemical safety of edible insects in SSA should be further investigated and insects should be integrated into more extensive dietary studies.

Table 3. Dietary risk assessment for pesticides detected in more than 30% of the insect samples

Compound	MRLs (ng/g)[a]	RfD (mg/kg per day)	SFO (mg/kg per day)$^{-1}$	UGD		NGR	
				HQ	CR	HQ	CR
Simazine[b]	-	5.00E-03	1.20E-01	3.04E-03	1.82E-06	1.99E-05	1.19E-08
Aldicarb[b]	10	1.00E-03	-	1.46E-02	-	4.78E-03	-
Oxamyl[b]	10	2.50E-02	-	2.55E-05	-	6.59E-05	-
Propoxur[c]	-	5.00E-03	-	2.36E-04	-	2.97E-04	-
Dinotefuran[d]	20	2.00E-02	-	6.37E-06	-	6.59E-05	-
Imidacloprid[b]	20	5.70E-02	-	1.68E-06	-	3.88E-06	-
Chlorpyrifos[b]	10	1.00E-03	-	2.87E-03	-	2.11E-03	-
Dichlorvos[b]	10	5.00E-04	2.90E-01	1.03E-02	1.49E-06	6.28E-03	9.10E-07
Ethoprophos[e]	10	1.00E-04	2.81E-02	9.55E-04	2.68E-09	9.94E-04	2.79E-09
Cyfluthrin[b]	10	2.50E-02	-	1.79E-04	-	1.61E-04	-
Cypermethrin[b]	100	1.00E-02	-	1.71E-03	-	1.57E-03	-
Permethrin[b]	100	5.00E-02	-	1.91E-05	-	8.84E-05	-
Paclobutrazol[b]	-	1.30E-02	-	4.01E-04	-	6.35E-05	-
HCB[b]	-	8.00E-04	1.6E+00	1.63E-04	2.09E-07	1.70E-04	2.17E-07

[a]MRL values available for meat from mammals or poultry[42]

[b]RfD and SFO from[20]

[c]RfD and SFO from[27]

[d]RfD and SFO from[43]

[e]RfD and SFO from[44]

MRLs: Maximum residue levels (in ng/g ww); RfD: reference dose; SFO; HQ: hazard quotient; CR: carcinogenic risk.

DECLARATIONS

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Methodology, writing - review and editing: Bombeke J, Altamirano J

Conceptualization, methodology, resources, writing - review and editing, supervision, project administration, funding acquisition: Covaci A

Availability of data and materials

Additional data for this study are presented in the Supplementary Material.

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None.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

None.

Consent for publication

Not applicable.

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