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Organophosphate and carbamate pesticide residues and accompanying risks in commonly consumed vegetables in Kenya

Isaac Omwenga[a,b,c], Laetitia Kanja[b], Paul Zomer[d], Jochem Louisse[d], Ivonne M.C.M. Rietjens[a], and Hans Mol[d]

[a]Division of Toxicology, Wageningen University and Research, Wageningen, The Netherlands; [b]Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Sciences, University of Nairobi, Nairobi, Kenya; [c]Department of Animal Science, Meru University of Science and Technology, Meru, Kenya; [d]Wageningen Food Safety Research, Part of Wageningen University and Research, Wageningen, The Netherlands

ABSTRACT
The current study was conducted to assess the levels of organophosphates and carbamates in vegetables in Kenya and to examine potential consumer health risks. A total of 90 samples were analysed by liquid chromatography/high-resolution tandem mass spectrometry. Residues of acephate, chlorpyrifos, methamidophos, omethoate and profenofos were found in 22% of the samples, ranging from 10 to 1343 µg/kg. The EU MRL was exceeded in 21%, 10%, 8% and 22% of the samples of French beans, kales, spinach and tomatoes, respectively. Chlorpyrifos in spinach had an acute HQ of 3.3 and 2.2 for children and adults, respectively, implying that potential health risks with respect to acute dietary exposure cannot be excluded. For chronic dietary exposure, all chronic HQs were below 1. The HI for the pesticides was 0.54 and 0.34 for children and adults. Routine monitoring of OPs and carbamates in vegetables is recommended to minimise consumer's health risks.

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Vegetables; Organophosphate; pesticides; food safety; dietary exposure assessment; human health Risk

Introduction

Pesticide use is essential for control of pests in horticultural crops and adequate production of food supplies for the ever-increasing world population as well as for control of vector-borne diseases. Most developing countries with a rapidly growing population require pesticides to improve food production (Akoto et al. 2015). However, the extensive use of pesticides has led to adverse effects to human health as well as other non-target species and the environment (Bravo et al. 2011; Stadlinger et al. 2013; Li et al. 2017; Bhandari et al. 2019).

Organophosphates (OPs) and carbamates were considered safer than their predecessors, the persistent organochlorine insecticides like aldrin, dieldrin and DDT (Fernńandez et al. 2000; Pogačnik and Franko 2003). However, over 40 OP pesticides, including the most commonly used ones such as chlorpyrifos have been classified by the WHO Food and Agriculture Organisation and US Environmental Protection Agency (EPA) to be moderately or highly hazardous to human health (WHO, 2009; Roberts and Reigart 2013). The intensive use of OP pesticides in various crops may lead to accumulation of levels higher than those permitted and subsequently affect quality of agricultural products and raise concerns with respect to human health and international trade (EC, 2005; Boobis et al. 2008). The OPs and carbamates produce their effects by inhibiting acetylcholinesterase (AChE), the enzyme that terminates the action of the neurotransmitter acetylcholine (ACh) within neuromuscular junctions and nerve tissue, leading to overstimulation of the post-synaptic membrane (Timchalk and Poet 2008). Reported effects related to exposure to OPs vary from mild short-term impacts such as nausea and headaches to chronic effects such as infertility, birth defects, blood disorders, nerve disorders, endocrine disruption and reproductive effects (Mansour 2004; Alavanja et al. 2013). Consumption of conventionally grown fruits and vegetables provide the major pesticide exposure route for the general population (Bradman et al. 2015). Consequently, biomonitoring studies have found pesticides and their metabolites in biospecimens in several countries including USA and Canada (Centers for Disease Control, 2017; Haines et al. 2017).

Despite regulation of pesticides through maximum residues limits (MRLs), there is lifelong combined exposure to low amounts of hundreds of various pesticides from one food item containing multiple compounds or combinations of food items, each containing different residues (Boobis et al. 2008). If these pesticides have a similar mechanism of action and end point, assessing the dietary risk of exposure differently may lead to underestimation of the health risk (Boon et al. 2008). Consequently, recent

CONTACT Isaac Omwenga isaac.omwenga@wur.nl.

approaches in pesticide risk assessment include cumulative and probabilistic approaches in order to include the amount and variety of pesticide residues likely to be present in the vegetable sample (Jensen et al. 2015; Boon et al. 2015; Elgueta et al. 2019). The initial step in assessing pesticide mixtures is to identify substances with a similar mechanism of toxicity (Gallagher et al. 2015). For example, acetylcholinesterase inhibitors such as OPs and carbamates have been assigned the same cumulative assessment group by the United States Environmental Protection Agency (USEPA, 2006, 2007, 2011) hence their choice in this study.

The use of chemical pesticides is still indispensable in Kenya due to the hot and humid tropical environmental conditions that are conducive to the development of pests, weeds and disease vectors (Omwenga et al. 2016). There is increased use of chemicals, especially fertilisers, veterinary chemicals and pesticides, since agriculture accounts for 60% of Kenya's foreign exchange earnings and provides raw materials for the industries (NIP, 2006). Many small-scale farmers are not aware of the hazards associated with these chemicals. For example, a previous study in Ghana revealed that up to 45% and 20% of farmers wore partial or no personal protective equipment at all when applying pesticides to cocoa plants hence increasing their risks of exposure to pesticides (Okoffo et al. 2016). In addition, the study reported other poor operational habits such as talking, eating, drinking, stirring pesticides with bare hands and smoking among others during the process of pesticides application (Okoffo et al. 2016). Other indiscreet practices, such as application of higher than recommended doses of pesticides on crops and failure to implement the pre-harvest time frame after application, may lead to consumers' exposure to pesticides at levels of concern (Akoto et al. 2015). Furthermore, OP pesticides of environmental and human health concern that have been banned in developed countries such as EU, USA and Canada are still used in developing countries (Picciotto et al. 2018). For example, pesticides such as chlorpyrifos, profenofos, acephate, metamidophos, dimethoate and omethoate which are banned in Europe and other countries are still in use in developing countries such as Costa Rica, Guatemala (Bravo et al. 2011), Mexico (Gonzales 2018) and Kenya (PCPB, 2019). The focus of occupational and public health institutions in developing countries have been on acute poisonings, as delayed and chronic effects remain undetermined (Wesseling et al. 2005; Toe 2010; Bravo et al. 2011).

Vegetables provide vitamins, minerals and active compounds known to reduce the effect of cardiovascular diseases (Ivey et al. 2015). According to the WHO, vegetables and fruits constitute on average 30% of food consumption (WHO 2003). However, in Kenya, it is estimated that fresh fruits and vegetables constitute 25% and >80% of the diets in the urban and the rural areas, respectively (Mungai et al. 2000; Kunyanga et al. 2018). Among these, kales (*Brassica oleraceae var acephala*), spinach (*Spinacia oleracea*), tomatoes (*Solanum lycopersicum*) and to a lesser extent French beans (*Phaseolus vulgaris*) are the most common vegetables used in various dishes (Karanja et al. 2012; Okello et al. 2012: Mutai et al. 2015: RSA, 2015; Njuguna et al. 2019). In general, it is reported that the amounts of pesticides used in vegetable crops are three times higher than what is used for cereal crops (Fan et al. 2015), which are staple foods in many other countries. Consequently, it is expected that vegetables contain higher pesticide residue levels as compared to cereal-based foodstuffs, not only because of the large amount of pesticide usage but also because some, like tomatoes, are eaten raw in salads (Baig et al. 2009; Knežević et al. 2012). Monitoring pesticide residues in vegetables is therefore key in determining and mitigating possible risks to human health from daily dietary pesticide exposure of consumers (Curl et al. 2003).

Developed countries such as USA, Canada and in the EU have implemented pesticide monitoring programmes. However, little effort has focused on long-term adverse health effects of pesticides on the agricultural workers and local consumers in developing countries (Ecobichon 2001). Many studies have reported on the levels of pesticide residues in various food products, including milk products (Golge et al. 2018), poultry and sheep fat (EFSA 2019), cereal (Lozowicka et al. 2014; EFSA 2019), olive oils (Razzaghi et al. 2018), fish (Sapozhnikova 2014) and fruit and vegetables from various countries (Lee and Lee 2012; Zhao et al. 2014; Quijano et al. 2016; Muñoz et al. 2017; EFSA 2019). However, few data are available on the presence and levels of pesticide residues in vegetables consumed in Kenya (Ngatia and Kabaara 1976; Karanja et al. 2012; Mutai et al. 2015; Kunyanga et al. 2018).

The objective of this study was, therefore, to determine OP pesticides and carbamates in tomatoes, kales, spinach and French beans and evaluate the associated dietary exposure and the associated potential health risks to children and adults upon acute and chronic exposure.

Materials and methods

Chemicals and reagents

Pesticide reference standards for organophosphate pesticides were obtained from Restek (Restek Corporation, Bellefonte, USA) while carbamate standards were from LGC standards (Teddington, UK) and Sigma Aldrich

(Zwijndrecht, the Netherlands) with certified purity ranging from 97% to 99%. Acetonitrile and acetic acid were obtained from Rankem (New Delhi, India) and anhydrous magnesium sulphate, primary and secondary amine from Phenomenex (Phenomenex Inc, Torrance, California, USA). All other organic solvents used were high-performance liquid chromatography (HPLC) grade.

Study site and study design

This study was conducted in urban and peri-urban Nairobi. Nairobi is the capital city of Kenya and is very likely to reflect what is anticipated in other counties in the country. With an altitude of 1,670 m (5,480 feet) above the sea level, urban and peri-urban Nairobi receive 1,050 millimetres of rainfall, twice a year with the long rains between March and May. The short rains occur between October and December. The mean annual temperature is 17°C, with mean daily maximum and minimum temperatures of 23°C and 12°C, respectively.

A total of 90 vegetable samples consisting of 19 French beans, 29 kales, 24 spinach and 18 tomatoes samples were collected from Mkulima, Kangemi, Kawangware, Limuru, N-market and Kiambu open-air markets in April, May and June 2018. The selection of these vegetables for this study was based on their high consumption and commercial significance in Kenya (Karanja et al. 2012; Okello et al. 2012; Mutai et al. 2015; RSA 2015; Njuguna et al. 2019).

Sampling was performed in accordance with the general principles and methods of the European Commission directive 2002/63/EC (EC, 2002) for compliance verification with MRLs in food commodities. Dust from the samples was removed with light brushing, without washing prior to placing them into the collection bags. Only fresh, high-quality vegetables that were free from blemishes or rot were sampled. Samples were double packed and transported on ice from the markets to the Department of Public health, Pharmacology and Toxicology, University of Nairobi, Kenya. In the laboratory, samples were placed in the refrigerator at 4°C and extracted within 5 days of collection.

Extraction procedure

The extraction and clean-up method used was based on QuEChERS (quick easy cheap effective rugged and safe) sample preparation method for pesticides (Anastassiades et al. 2003). About 1 kg of each vegetable sample was homogenised in a blender (SM1030 SANYO Electric Co. Ltd., Osaka, Japan) and 15 g of the homogenised sample was accurately weighed and transferred into a 50-mL polypropylene centrifuge tube with screw cap. Extraction was conducted according to the AOAC Official Method 2007.01. Briefly, a 15-mL volume of 1% acetic acid in acetonitrile was added as an extraction solvent and the tube was tightly capped and vigorously mixed for 5 min by hand shaking. The tube was opened and 6 g of anhydrous $MgSO_4$ and 1.5 g sodium acetate were added. The tube was closed immediately after addition and vigorously mixed by hand for 1 minute to avoid formation of salt lump. The mixture was centrifuged at 4000 rpm (Sorvall ST 16, Thermo scientific, Waltham, MA, USA). The acetonitrile phase was transferred into cryotubes and stored in the freezer (−80°C). Samples were transported at ambient temperature to the Netherlands for instrumental analysis within 15 hours. After centrifugation, an aliquot of the acetonitrile phase was diluted 1:1 with water and filtered. The final extract contained 0.5 g of matrix equivalent per ml.

LC-hrMS screening

Applying the method described by Zomer and Mol (2015), the extracts were first analysed using LC-hrMS. Following a non-targeted measurement, the extracts were screened in a targeted manner for a total of 118 cholinesterase-inhibiting compounds (organophosphate and carbamate pesticides) including some of their metabolites. Suspect screening was done based on expected retention time, a protonated molecule (NH_4- or Na-adduct in some cases) and a fragment ion. The compound specific parameters that were used for data processing during the pesticide screening can be made available upon request. As quality control, a mix solution of pesticides at 10 ng/ml was included in the sample batch to verify the detection capabilities of the method. These QC samples confirmed that similar detection limits (typically ≤10 μg/kg) were obtained as described by Zomer and Mol (2015). The sample extracts in which pesticides were detected during screening were re-analysed for confirmation and quantification, using an LC-MS/MS method.

LC-MS/MS confirmation and quantification

LC-MS/MS analysis was performed with a system consisting of an Acquity UPLC coupled to a TQ-S triple quad MS, equipped with an HSS-T3 C18 column (1.7 μm, 2.1 × 100 mm), using water (eluent A) and MeOH:H_2O 95:5 (eluent B), both containing 5 mM ammonium formate and 0.1% formic acid (Waters, Millford, MA, USA). The LC flow rate was 0.4 ml/min, the column temperature 45°C and the injection volume 5 μl. The LC gradient started with 100% A for 1 min, followed by a two-step linear increase to eluent B (45% B at 2.5 min, 100% B at 8.5 min), which was maintained for 3 min and then back to initial conditions in 30 s, allowing equilibration for 2 min before the next injection.

The MS was used in positive MRM mode with the following source parameters: capillary (3 kV), source offset (50 V), source temperature (150°C), desolvation temperature (590°C), cone gas flow (150 L/Hr), desolvation gas flow (1000 L/Hr) and nebuliser gas flow (7 bar). The transitions were grouped into two scan events to enable use of longer dwell times for each transition. Table 1 shows the two transitions measured for each compound. The second transition (Ql) was used to calculate an ion ratio between Qn and Ql. In positive samples, this value was compared to the average ratio in the standards and used for identification. The linear range was established by injecting a calibration curve in solvent. Positive samples were quantified by 1-point bracketed calibration using a standard in matrix prepared from a blank sample from the corresponding matrix. As QC samples the same spiked samples as used in the LC-hrMS screening were measured.

Data processing

For suspect screening by LC-hrMS, the software package Tracefinder (version 3.2 SP1, Thermo Scientific) was used to process the data. For confirmatory LC-MS/MS analysis, Masslynx was used. Calculations were further conducted using Microsoft Excel 2010.

Exposure analysis and risk characterisation

Dietary exposure (µg/kg bw/day) is calculated by dividing [residue concentration in food (µg/kg) x food consumption (kg/person/day)] with the body weight (kg bw/person). Consumption of vegetables contaminated with pesticides can have acute and chronic risks, which can be quantified using hazard quotient (HQ) and hazard index (HI) approaches.

Acute/short-term hazard quotient (aHQ)

The aHQ was calculated by aHQ = ESTI/ARfD, where ESTI is the estimated short-term intake, calculated by [the highest level of residue x the highest large portion of food consumption per day]/[body weight] and ARfD is the acute reference dose.

Chronic/long-term hazard quotient (cHQ)

The cHQ was calculated by cHQ = EDI/ADI, where EDI is the estimated daily intake, calculated by [mean level of residue x average portion of food consumption per day]/[body weight] and ADI the acceptable daily intake.

Information on ARfDs and ADIs were obtained from the EU pesticides database (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-db_en). When HQ values exceed 1, a health risk cannot be excluded.

Results and discussion

In Kenya, vegetables play an important role in the nutrition and health of both the urban and rural population and their intake is estimated to constitute up to 80% of the diets (Mungai et al. 2000; Kunyanga et al. 2018). Therefore, potential exposure to vegetable contaminants such as pesticide residues is likely to be high. The mean consumptions of kales, spinach, tomatoes and French beans per day are 200, 100, 200 and 10 g/day, respectively (Karanja et al. 2012; Kariathi et al. 2016; Kunyanga et al. 2018; Njuguna et al.

Table 1. Compound-specific parameters of the LC-MS/MS system.

Name	Precursor ion	Product ion	Cone voltage [V]	Collision Energy [V]	Dwell [s]
Methamidophos Qn	142.2	94	20	13	0.05
Methamidophos Ql	142.2	125	20	13	0.05
Acephate Ql	184	125	10	20	0.1
Acephate Qn	184	143	10	17	0.1
Omethoate Ql	214	155	22	17	0.05
Qmethoate Qn	214	183	22	10	0.05
Fenthion Qn	279	169	30	18	0.01
Fenthion Ql	279	247	30	12	0.01
Pirimiphos-methyl Ql	306	108	30	25	0.01
Pirimiphos-methyl Qn	306	164	30	25	0.01
Triazophos Qn	314	119	25	35	0.01
Triazophos Ql	314	162	25	20	0.01
Azinpohs-ethyl Qn	346	132	10	15	0.01
Azinpohs-ethyl Ql	346	160	10	10	0.01
Chlorpyrifos Qn	350	198	20	17	0.01
Chlorpyrifos Ql	352	200	20	17	0.01
Profenofos Ql	373	97	30	33	0.01
Profenofos Qn	373	303	30	18	0.01

2019). As data of Kenyan large portion consumption of the vegetables were not available, for aHQ, the maximum consumption value was assumed to be 560 (g/day) for tomatoes, kales and spinach based on a study conducted in Tanzania (Kariathi et al. 2016) and an estimate of 50 g/day for French beans was made. The dietary exposure was calculated for both the consumer groups of adults (aged above 18 years) and children (3–10 years old). For dietary exposure assessment, a body weight of 70 kg for adults (aged above 18 years) and 23 kg for children (aged 3–10 years) was applied as recommended by EFSA (2012). In children, the vegetables consumption (g/day) was assumed to be half that of an adults' daily vegetable consumption.

Major sources of uncertainties in the exposure assessment are food consumption rate, body weight, sampling, processing factors, left-censored data (non-detects), analytical bias and variation (EFSA 2006c). Processing factors were not considered during exposure assessment in this study. The substitution method was applied for non-detects as described in the EFSA scientific report (EFSA 2010b). The non-detect results were replaced with zero and the value of the LOQ (10 µg/kg), according to a Lower and Upper Bound (LB and UB) scenario, respectively. The risk assessment of the cumulative chronic exposure to residues was performed by summing the chronic Hazard Quotients (cHQ) for the individual pesticide residues as Hazard Index (HI) = ΣcHQ (Reffstrup et al. 2010). An HI exceeding 1 indicates that a health risk related to the combined exposure cannot be excluded.

OP pesticides and carbamates in Kenyan vegetables

In this survey, 118 organophosphate and carbamate pesticides were screened in vegetable samples comprising of French beans, kales, spinach and tomatoes to assess health risk. After the screening, acephate, azinphos-ethyl, chlorpyrifos, fenthion, methamidophos, omethoate, pirimiphos-methyl, profenofos, triazophos, aldicarb, carbaryl, carbetam, carbofuran, ethiofencarb, methiocarb and pirimicarb were tentatively detected. However, during confirmatory quantitative analysis, only chlorpyrifos, profenofos, acephate, metamidophos and omethoate had levels above the default limit of quantification of 10 µg/kg. Out of 90 vegetable samples analysed, 20 (22%) were found to contain residues of the targeted pesticide.

The most frequently detected pesticides in the positive samples were profenofos (40%; only in tomatoes), followed by chlorpyrifos (35%), acephate (30%), methamidophos (30%) and omethoate (15%). The findings are summarised in Table 2. The presence of multiple residues in French beans and tomatoes was 17%. Acephate and methamidophos were found in three French beans while the fourth had acephate, methamidophos and chlorpyrifos. Methamidophos can be used as an insecticide as such, but is also a metabolite of acephate. Since it was always found together with acephate (in a ratio of 0.3–0.5) it was concluded that its presence was the result of acephate applications. Omethoate is another OP pesticide that can be applied as such but also is a degradation product of dimethoate. Dimethoate was not detected, indicating that it might have been applied as such or it was a metabolite of dimethoate as observed in previous studies (Bhandari et al. 2018; Kunyanga et al. 2018). Most of the pesticide residues were found in tomatoes followed by French beans, kales and lastly spinach.

Chlorpyrifos is one of the most used pesticides worldwide due to its effectiveness against a wide range of pests and rapid action (Angioni et al. 2011) and also still registered for use in vegetable crops as well as soil treatment in Kenya (Mutai et al. 2015; PCPB 2019). It is registered for use in pineapples, roses, cotton, coffee, barley and wheat to control various pests. Its presence in French beans, kales, spinach and tomatoes could be due to plant uptake from the soil where it is applied to control soil-based pests (Zhang et al. 2015), spray drift, or direct application on vegetables. Poor compliance of the pre-harvest interval due to high market demand for the vegetables has also been reported to result in high residue levels as observed in Ghana (Ntow et al. 2006; Darko and Akoto 2008; Akoto et al. 2015). Ngigi et al. (2011) made a similar observation in Kenya among 85% of peri-urban farmers. Furthermore, other studies have

Table 2. Frequency and levels of organophosphate residues detected in/on various vegetables.

Vegetables	French beans (n = 19)		Kales (n = 29)		Spinach (n = 24)			Tomatoes (n = 18)		
Pesticides	n > LOD (%)	Min-max (µg/kg)	n > LOD (%)	Min-max (µg/kg)	n > LOD (%)	Min-max (µg/kg)	EU MRL (µg/kg)	n > LOD (%)	Min-max (µg/kg)	EU MRL (µg/kg)
Profenofos	0	-	0	-	0	-	10	8(44)	10–958	10000
Omethoate	0	-	0	-	0	-	10	3(17)	11–98	10
Chlorpyrifos	1(5.3)	117	2(6.9)	26–315	2(8.3)	24–1343	10	2(11)	78–107	100
Acephate	4(21)	41–66	1(3.4)	43	0	-	10	1(5.6)	409	10
Methamidophos	4(21)	20–25	1(3.4)	13	0	-	10	1(5.6)	182	10

indicated that farmers with low perception of harmful effects of pesticides may use them at higher than recommended doses on crops leading to higher pesticide residues (Horna et al. 2008).

In the current study, OP pesticides were detected in 22% of the screened vegetables. These prevalence is lower compared to published reports from other developing countries such as Chile, where pesticide screening in 118 leafy vegetable samples found 72% of spinach samples to contain pesticide residues at higher concentrations than the current study (Elgueta et al. 2017). In Tanzania, Kiwango et al. (2018) found 31% of sampled vegetables to contain detectable levels of OP residues including dimethoate, acephate, profenofos, dichlorvos and malathion at a mean of 8,560 µg/kg, 2,900 µg/kg, 8,440 µg/kg 20,800 and 5,470 µg/kg, respectively. In Algeria, Mebdoua et al. (2017) found OP pesticide residues in 58% of the 120 vegetables analysed. Another study by Elgueta et al. (2019) reported a higher frequency of chlorpyrifos detection at 21% and a mean of 10 µg/kg in lettuce, while the detection frequency in spinach and chard was 13% and a mean of 130 µg/kg for both leafy vegetables. Furthermore, methamidophos was found in spinach, chard and lettuce with a frequency of 8% and a mean of 60 µg/kg. The levels of pesticide residues in vegetables in this study were higher than those reported in India (Bhanti and Taneja 2007).

Profenofos and chlorpyrifos were the most frequently detected pesticide residues in vegetables followed by acephate and its metabolite methamidophos. These results are in agreement with previous studies involving pesticide screening in vegetables in peri-urban Nairobi that reported profenofos (Karanja et al. 2012) and chlorpyrifos (Kunyanga et al. 2018) as the most frequently detected pesticides among others. Although omethoate is restricted for use in fruits and vegetables, it was detected in 15% of the vegetable samples. This might be due to spray drifting from neighbouring farmers' fields, or cross-contamination by different vectors since it is registered for use as insecticide for coffee and ornamental flowers (PCPB 2019). Its presence could also be due to illegal use or its persistent nature (Xu et al. 2015). A previous study by Mutai et al. (2015) found kales and French beans in peri-urban Nairobi to contain up to 100 and 700 µg/kg of chlorpyrifos and dimethoate residues, respectively. Furthermore, the pesticide levels were reported to be higher during the dry period than during the wet period in that study, though there was no significant difference. Seasonal variability in pesticide residues has been observed, a phenomenon that leads to inadequate assessment of total exposure to pesticides (Mtashobya 2017). In the same study, significant pesticide residues were found in French beans as compared to kales, an observation that Mutai et al. (2015) related to the coarse nature and dense texture of French beans that slow down the rate of volatilisation, a process by which pesticides dissipate in contrast to smooth texture of kales (Bedos et al. 2002). Other factors that could affect the occurrence of pesticides and their residues in different matrices include lipid content, rainfall, temperature, soil properties and soil organisms (Bento et al. 2016).

Pesticide residues need to comply with maximum residue levels (MRLs) which are based on Good Agricultural Practice (GAP). Since GAP may differ in different countries, also MRLs can differ. Exceedance of local MRLs are an indication that local GAP is not well followed. There are no national MRLs in Kenya. Therefore, codex MRLs were used instead as benchmark. For the five OPs found in the four crops in this study, Codex MRLs were available for only five OP/crops combinations, thus most vegetables in this study had no established codex MRL for comparison with the measured OP levels. Chlorpyrifos exceeded the codex MRL of 10 µg/kg in French beans. Profenofos and acephate were below codex MRLs in tomatoes. Similarly, methamidophos was below the codex MRL in French beans. Due to lack of Codex MRLs, findings were also compared to EU-MRLs, which were exceeded in 8–33% of the vegetable samples. Acephate/methamidophos and omethoate have no registration in the EU and for their residues no import tolerances apply. Hence, an EU-MRL set at the default LOQ of 10 µg/kg applies and may obviously be exceeded when these pesticides are applied in the field. Several exceedances were observed for chlorpyrifos, banned in the EU since January 2020, but at the time of collecting and analysing the samples, it was still having MRLs above the 10 µg/kg default for certain crops (e.g. tomatoes). With a relatively high MRL for profenofos in tomatoes, no exceedances were observed for this OP pesticide although it was the most frequently detected pesticide in this study. In a previous study, Karanja et al. (2012) reported levels of profenofos, diazinon, cypermethrin and bitertanol above EU permissible limits in kales in peri-urban Nairobi.

Risk characterisation of the detected pesticides

The HQs related to exposure via dietary intake of the investigated vegetables contaminated with OPs for children and adults are presented in Table 3. It also shows acute reference dose and acceptable daily intake values used in this study. The consumption of spinach was found to imply an acute risk in children and adults with an aHQ of 3.3 and 2.2 for chlorpyrifos in children and adults, respectively. In all other cases, the aHQ and cHQ for the individual pesticides were below 1, implying no risk. Chlorpyrifos, methamidofos and omethoate contributed most to chronic HQs with 0.32, 0.13 and 0.04 in children and 0.21, 0.09 and 0.03 in adults, respectively. The aHQs for the individual pesticides for children and adults varied from 0.05 to 3.27 and from 0.03 to 2.15, while the cHQ varied from 0.00 to 0.25 for children and 0.00 to 0.14 for adults at the UB, respectively. The HQ for each residue is higher for children than for adults, due to the fact that exposure per kilogram body weight is highest for children.

Due to extensive use of OPs to control pests in vegetable farming, one expects consumer exposure to insecticide mixtures. Indeed, a number of biomonitoring studies have reported the presence of several organophosphate metabolites in urine and inhibition of acetylcholinesterase in plasma of agricultural workers (Timchalk and Poet 2008). Therefore, many studies begin to characterise the toxicological effects of exposures to concurrent or sequential OP pesticide mixtures (Timchalk and Poet 2008; Reffstrup et al. 2010). In this study, some vegetables showed more than one type of pesticide residue. The presence of multiple residues could be a result of plant uptake of persistent pesticides (Zhang et al. 2015), spray drift (Coronado et al. 2011) and poor agricultural practices that involve mixing of more than one kind of pesticides during application based on the conception that as a mixture they will be more potent, as has been observed among Nepalese farmers (Bhandari et al. 2018). Indeed, the practice of using pesticide cocktails that sometimes include unapproved or banned pesticides is worrying but in practice among some farmers in Africa.

Exposure to two or more pesticides may result in additive and/or interactive effects (Reffstrup et al. 2010;). We used the hazard index method proposed by the US EPA to assess risk posed by a group of pesticides that act by a common mechanism (i.e. AChE inhibition) or that are toxicologically similar, assuming additive effects (US EPA 2000; Reffstrup et al. 2010). Chronic HIs for OPs in all vegetables sampled were <1 in both children and adults, implying no health risks upon long term combined exposure.

Uncertainties related to dietary exposure assessment

Scientific uncertainties affect dietary exposure assessments and therefore should be considered during interpretation of the results. Due to paucity of data on Kenyan large portion consumption of the vegetables for aHQ determination, the maximum consumption value was assumed to be 560 (g/day) for tomatoes, kales and

Table 3. HQs for detected pesticides in French beans, kales, spinach and tomatoes.

			Short-term Risk								Long-term Risk			
			Children		Adult						Children		Adult	
	Pesticide	ARfD	ESTI	aHQ	ESTI	aHQ	HR	ADI		Mean	EDI	cHQ	EDI	cHQ
F. beans	Methamidophos	3	0.03	0.01	0	0	No	1	LB	2.47		0	0	0
									UB	11.4	0	0	0	0
	Acephate	100	0.07	0	0.05	0	No	30	LB	11.8	0	0	0	0
									UB	19.7	0	0	0	0
	Chlorpyrifos	5	0.13	0.03	0.08	0.02	No	1	LB	8.32	0	0	0	0
									UB	16.7	0	0	0	0
Kales	Acephate	100	0.12	0	0.08	0	No	30	LB	1.48	0.01	0	0	0
									UB	11.1	0.05	0	0.03	0
	Chlorpyrifos	5	3.83	0.77	2.52	0.5	No	1	LB	11.8	0.05	0.05	0.03	0.03
									UB	21.1	0.09	0.09	0.06	0.06
	Methamidophos	3	0.16	0.05	0.1	0.03	No	1	LB	0.45	0	0	0	0
									UB	10.1	0.04	0.04	0.03	0.03
Spinach	Chlorpyrifos	5	16.35	**3.27**	10.74	**2.15**	Yes	1	LB	57.0	0.12	0.12	0.08	0.08
									UB	66.1	0.14	0.14	0.09	0.09
Tomatoes	Acephate	100	4.98	0.05	3.27	0.03	No	30	LB	22.7	0.1	0	0.06	0
									UB	32.2	0.14	0	0.09	0
	Chlorpyrifos	5	1.3	0.26	0.86	0.17	No	1	LB	5.94	0.03	0.03	0.02	0.02
									UB	19.2	0.08	0.08	0.05	0.05
	Methamidophos	3	2.23	0.74	1.46	0.49	No	1	LB	10.2	0.09	0.09	0.03	0.03
									UB	20.1	0.09	0.09	0.06	0.06
	Omethoate	2	1.19	0.6	0.78	0.39	No	0.3	LB	9.67	0	0	0.03	0.09
									UB	18.6	0.08	0.27	0.05	0.17
	Profenofos	1000	11.66	0.01	7.66	0.01	No	30	LB	100	0.44	0.01	0.29	0.01
									UB	106	0.92	0.03	0.3	0.01

ESTI = Estimated short-term intake (μg/kg bw); aHQ = acute/short term Hazard Quotient; cHQ = chronic/long term Hazard Quotient; ARfD (μg/kg bw) and ADI (μg/kg bw/day) values were extracted from the EU pesticides database (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-db_en); HR = Health risk can no longer be excluded; EDI = Estimated daily intake (μg/kg bw/day); LB = lower bound (LOQ is set 0); UB = upper bound (LOQ-value = 10 μg/kg); aHQ values in bold represent a potential risk upon exposure to the pesticides via dietary exposure in adolescents and adults. No HR could be established in all cases.

spinach based on a study conducted in Tanzania (Kariathi et al. 2016). Though the dietary habits of the two countries are similar to a large extent, there is a possibility to under- or over-estimate the consumption and therefore in future a dietary recall questionnaire should be used to assess large portion consumption of vegetables in Kenya. In addition, processing factors such as washing, peeling and cooking were not considered during exposure assessment in this study. Generally, inclusion of processing factors could have led to lower estimated exposure. Finally, the high proportion of left-censored data may have introduced uncertainties in the overall estimate. The substitution method applied for non-detects as described in the EFSA scientific report (EFSA 2010b) could lead to an over-estimation of the exposure (substitution of non detects with LOQ (10 μg/kg)) or under-estimation (substitution of non detects with zero). Nevertheless, given that all individual cHQs and His, even with the worst-case approach, were below 1, indicates no risk resulting from long-term consumption of the vegetables.

Since the dietary pesticide intakes determined in the current study considered only exposures from the major vegetables, it did not cover the total dietary exposure to OP pesticides. Other food products such as fish, meats, dairy, grains and drinking water, and exposure due to occupational or residential sources may add to the overall exposure.

Conclusion

This study revealed the occurrence of several organophosphate pesticide residues in French beans, kales, spinach and tomatoes, sampled from peri-urban Nairobi. Chlorpyrifos, acephate, omethoate and methamidophos exceeded the Codex and/or EU MRLs. However, only the aHQ of chlorpyrifos in spinach revealed that a potential acute risk to both children and adult consumers could not be excluded. There were no indications for chronic risks, not for the individual OPs found and also not for the combined chronic exposure assessed through the HI. Routine monitoring of OPs and carbamates in vegetables is recommended to minimise consumer's health risks.

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Disclosure statement

The authors declare that there are no conflicts of interest.

ORCID

Ivonne M.C.M. Rietjens http://orcid.org/0000-0003-1894-3544

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