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Residues of pesticides and metabolites in chicken kidney, liver and muscle samples from poultry farms in Dar es Salaam and Pwani, Tanzania

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HIGHLIGHTS

- Pesticide residues were determined in chicken organs in Tanzania.
- The highest concentrations of the contaminants were found in kidney and liver.
- The concentrations of the pesticide residues exceeded the MRLs in most samples.
- The contamination levels indicate the use of highly contaminated feed at the farms.

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ABSTRACT

The concentrations of organochlorine and organophosphorus pesticides and metabolites were investigated in chicken kidney, liver and muscle samples obtained from chickens collected from four poultry farms in Dar es Salaam and Pwani regions in Tanzania. The samples were extracted by solid dispersion using cyclohexane:ethyl acetate. The extracts were cleaned by adsorption column chromatography. The analytes were determined using gas chromatography-mass spectrometry (GC–MS). The concentrations of total DDT, total endosulfan and total HCHs in the samples ranged from 0.71 to 26, 0.3 to 7.9 and 0.02 –10.4 mg/kg lipid weight (lw), respectively. The highest concentrations of aldrin, dieldrin, chlorpyrifos, fenitrothion and pirimiphos methyl were 5.5, 4.8, 9.7, 5.6 and 7.8 mg/kg lw, respectively. The highest concentrations of the contaminants were found in the kidney and liver samples. The sites in Dar es Salaam showed the highest concentrations of the compounds. Most of the concentrations were above the maximum residue limits (MRLs) indicating risks and concerns for livestock and public health.

1. Introduction

Pesticides play important roles in agriculture and public health. Despite the many benefits of pesticides, the exposure of animals and the general population to the pesticides, especially those originating from pesticide residues in food, air and drinking water, even though may be in low doses, may cause many effects (Wang et al., 2013). Pesticides have been associated with a wide variety of human health effects, ranging from short-term impacts such as headache and nausea to chronic impacts like cancer, reproductive harm, immunosuppression and endocrine disruption (WHO, 2010).

Pesticides are used in poultry farms for fumigation and preserving poultry feedstuff (Van Barneveld, 1999; Windal et al., 2009). Contamination of chicken organs and products by pesticides can result from feed containing pesticide residues (Aulakh et al., 2006; Ahmad et al., 2010). Some of the pesticides are resistant to degradation; they persist in the environment and undergo bioaccumulation and biomagnification in organisms (Chen et al., 2005; Wu et al., 2005). Chicken products are important sources of nutrients to the human body. The presence of pesticides in chicken is of great concerns to the local consumers and international trade (Tao et al., 2009). Previously, no study had been undertaken to assess pesticide residues in chicken in Tanzania. Therefore, this study was conducted to determine the concentrations and status of organochlorine and organophosphorus pesticide residues in chicken kidney, liver and muscle.

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2. Materials and methods

2.1. Sampling

Chicken kidney, liver and muscle samples were obtained from chickens collected between January and April 2015 from four poultry farms which produce commercial poultry at a large scale in the Dar es Salaam and Pwani Regions. Two sampling sites were located in Dar es Salaam at Banana (located at latitude 6.8713°S and longitude 39.1898°E) and Kitunda (located at latitude 6.8898°S and longitude 39.2116°E) and the other two sites were located in Pwani at Misugusugu (located at latitude 6.7813°S and longitude 38.9929°E) and Nyumbu (located at latitude 6.7302°S and longitude 38.9385°E). The sites were designated as Banana = Site 1, Kitunda = Site 2, Misugusugu = Site 3 and Nyumbu = Site 4. Sixteen chickens that included four chickens from each farm were purchased from the poultry farms and slaughtered using clean stainless steel knives. The kidney, liver and muscle were removed and separately wrapped in aluminium foil. The samples were transported to the Chemistry Department, University of Dar es Salaam and kept frozen at −18 °C until extraction.

2.2. Sample preparation, extraction and clean-up

Sample preparation and processing were performed according to the procedures described by Åkerblom (1995). Each sample was homogenized by grinding using clean motor and pestle. Extraction was performed by solid dispersion. The homogenized sample (20 g) was mixed with anhydrous sodium sulphate (15 g) and shaken in a flask with cyclohexane:ethyl acetate mixture (1:1 v/v, 70 mL). The extraction was repeated by shaking the aqueous phase with 50 mL, then 40 mL of the solvent mixture. The extracts were dried with anhydrous sodium sulphate (10–20 g) and concentrated to 2 mL using a rotary evaporator. Clean-up was conducted employing adsorption column chromatography. A glass column (10 mm i.d. x 32 cm) was plugged with clean glass wool, and then packed with 10 g of florisil (previously activated at 200 °C for 24 h and cooled) and sodium sulphate (5 g). The column was rinsed with cyclohexane and ethyl acetate mixture (10 mL). The concentrated extract (2 mL) was eluted with cyclohexane:ethyl acetate (40 mL). The eluates were concentrated by a rotary evaporator and made up to 2 mL in cyclohexane:acetone mixture (9:1 v/v) ready for GC-MS analysis.

The fat content was determined applying the method described by the United States Department of Agriculture (USDA, 2009). Briefly, a weighed sample (3–4 g) was Soxhlet extracted using petroleum ether (85 mL) for 4 h and the extract was collected in a weighed flask. The solvent was evaporated on a steam bath, then, the flask and its contents were dried in an oven at 100 °C until a constant weight was obtained. The fat content was calculated based on the difference between the weight of the flask after extraction and weight of the flask prior to extraction.

2.3. Analytical quality assurance

The tools and glassware used were cleaned using detergents and tap water and rinsed using distilled water and then with acetone. The glassware was dried in an oven at 110 °C overnight. The samples, extracts and standards were kept frozen prior to analysis or transportation. The reagents and solvents (sodium sulphate, acetone, cyclohexane, ethyl acetate and petroleum ether) were from Thermo Fisher Scientific, UK and were of analytical grade with above 99% purity. Certified reference standards of high purity of above 95% (Dr. Ehrenstorfer, Germany) were used. The following 17 standards were used for the determination of the analytes in the samples: aldrin, dieldrin, dichlorodiphenyltrichloroethane (DDT) isomers (*p,p'*-DDT and *o,p'*-DDT), dichlorodiphenyldichloroethane (DDD) isomers (*p,p'*-DDD and *o,p'*-DDD), dichlorodiphenyldichloroethene (DDE) isomers (*p,p'*-DDE and *o,p'*-DDE), α-endosulfan, β-endosulfan, hexachlorocyclohexane (HCH) isomers (α-HCH, β-HCH, γ-HCH and δ-HCH), chlorpyrifos, fenitrothion and pirimiphos methyl. Procedural blanks were prepared using the solvents and the reagents. Recovery tests were carried out using selected samples which were spiked with different concentrations of all the reference standards. The blanks and recovery samples were processed and analysed like the test samples. Detection limits were based on the lowest concentrations of analytes that gave signals which were three times higher than the noise level.

2.4. GC-MS analysis, identification and quantification

The analyses were performed at the Department of Chemistry, Makerere University. A gas chromatograph–mass spectrometer (GC-MS 6890-5975, Agilent), equipped with HP-5MS capillary column (30 m × 0.25 mm x 0.25 μm), XL mass selective detector and injector, was used for analysis. The oven temperature programme was: 90 °C, held for 1 min, then raised to 180 °C at a rate of 30 °C/min then raised at a rate of 4 °C/min to the final temperature of 260 °C. The carrier gas was helium at the flow rate of 2.2 mL/min. Splitless injection of 1 μL volume was carried out at 250 °C injection port temperature with purge flow of 3 mL/min. The internal pressure was set at 150 kPa and the interface temperature was maintained at 300 °C. The mass spectrometer was operated in electron impact (EI) ionization with ion source temperature of 230 °C and full scan mode with the range of 45–500 *m/z*. Standards were analysed at the beginning on each day of analysis. The analytes in the samples were identified by comparing their retention times and mass spectra to those of reference standards. The NIST (National Institute of Standards and Technology) mass spectral library and AMDIS (Automated Mass spectral Deconvolution and Identification System) also aided the identification of the analytes. Quantification of the analytes was performed using the peak heights of the analytes and the concentrations of the standards.

2.5. Statistical data analysis

Statistical analysis of the data was performed using GraphPad Instat software (Motulsky, 1998). The variations were tested by analysis of variance (ANOVA) followed by post hoc tests. The differences between samples were compared by unpaired *t*-test.

3. Results and discussion

3.1. Blanks, recoveries and detection limits

No significant peaks appeared in the chromatograms of the procedural blanks. The percentage mean recoveries of the analytes ranged from 75 to 117% (n = 3) and were deemed acceptable (European Commission, 2015). The detection limits were 0.001 mg/kg for dieldrin, α-endosulfan, β-endosulfan, *o,p'*-DDT, α-HCH, β-HCH, δ-HCH, chlorpyrifos and pirimiphos methyl and 0.003 mg/kg for aldrin, *p,p'*-DDD, *o,p'*-DDD, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDT, γ-HCH and fenitrothion.

3.2. Organochlorine pesticides and metabolites

3.2.1. Aldrin, dieldrin and endosulfan

The concentrations of aldrin in chicken kidney, liver and muscle samples ranged from <0.003 to 5.5, 1.0 to 3, and <0.003–0.7 mg/kg lipid weight (lw), respectively. The concentrations of dieldrin ranged from <0.001 to 4.8 mg/kg lw in kidney, 0.2–2.1 mg/kg lw in