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Concentrations of organochlorine pesticide residue in game meat from the gomoa east district of Ghana

Sarah.Blankson-Arthur, Anita Osei Tutu, Harriet Kuranchie Mensah, Dzifa Denutsui, Linda Maud Palm, Sampson Manukure Atiemo and Archibold Buah Kwofie

Nuclear Chemistry and Environmental Research Centre, Ghana Atomic Energy Commission, P.O. Box LG 80 Legon, Ghana.

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ABSTRACT

The study was conducted to determine the level of contamination of grasscutter (*Thryonomys swinderianus*) meat with p,p- DDT, p,p-DDE, hexachlorocyclohexane isomers, lindane (γ-HCH) and δ- HCH, dieldrin, aldrin, endrin, endrin aldehyde, endrin ketone, alpha-endosulfan, endosulfan sulfate, chlordane, heptachlor and methoxychlor.

The paper reports the levels of organochlorine pesticide residues in the meat of 15 grasscutter (*Thryonomys swinderianus*) samples obtained from the Gomoa district, a vegetable farming area in the Central Region of Ghana. The results indicated that all the analyzed samples were contaminated with the studied organochlorine pesticides. Aldrin and heptachlor were the principal contaminants in all the samples.

The levels of organochlorine pesticide residues detected in all the meat samples were below the accepted maximum residue limits (MRL), as adopted by the WHO/FAO Codex Alimentarius Commission.

Introduction

In Ghana, the meat of wildlife, popularly referred to as bush meat, continues to be a popular form of animal protein and over 80% of Ghanaians, both rural and urban, would eat bush meat if available (Tutu et al.,1996). Wild animals including the grasscutter (*Thryonomys swinderianus*) provide a significant proportion of protein consumed by most people in the African sub regions. Bush meat also constitutes an essential ingredient without which certain cultural and ceremonial events among African communities cannot be complete. However, evidence available indicates that hunters in their quest to generate higher incomes are now resorting to baiting bush meat with highly potent chemical pesticides probably including organochlorines. Highly potent pesticides used as baits, are smeared on the leaves of various crops including vegetables which serve as food sources for browsing animals. Bush meat killed in such manner has the tendency to retain the active ingredients in their tissues and pass them on to innocent consumers. No one is safe from this unconventional method of hunting bush meat. It is a risk not only to food security but to the very survival of the human race (Entsie, 2002). Available information indicate that some chemicals used for hunting have resulted in the death of consumers of bush meat which suggest that the chemicals are harmful not only to the animals but to humans as well (Mensah, 2003).

In Ghana, analytical investigations of a number of organochlorine pesticides in human organs, body fluids and other reported incidents suggest that some of these chemicals are still in use illegally (Ghana NIP, 2007). Although the use of almost all the POP-pesticides listed under the Stockholm and Rotterdam Conventions have been banned in Ghana since 1985, the intensive past applications of these chemicals for agricultural and public health purposes also suggest that they may still be present in the environment (Ghana NIP, 2007).

To protect the health of the consumer, there is the need to monitor pesticide residues in food in order to assess the extent of any pesticide use or misuse and in assuring the consumer about the quality or safety of the bush meat he consumes. The present study was undertaken to investigate the extent of contamination of grasscutter meat with p,p- DDT, p,p-DDE, hexachlorocyclohexane isomers, lindane (γ-HCH) and δ- HCH, dieldrin, aldrin, endrin, endrin aldehyde, endrin ketone, alpha-endosulfan, endosulfan sulfate, chlordane, heptachlor and methoxychlor in grasscutter meat slaughtered in the Gomoa District of the Central Region of Ghana in order to ensure its safety for human consumption.

Study Area

The Gomoa District of the Central Region is presented in Figure 1. The Gomoa District is one of the districts within the Central Region of Ghana. The district has two main vegetation zones, the coastal savannah and the moist semi-deciduous forest. The former consists mainly of grassland and trees of patches of scrub, while the latter is characterized by tall trees interspersed with grass cover, shrubs and soft woody species. These vegetation zones teem up with different kinds of game which are hunted and usually sold by the roadside.

Crop production, animal production and fishing, constitute the main economic activity of the district. The ecology of the district encourages the cultivation of crops such as cassava, maize, sugar cane, pineapple, rice, pawpaw, vegetable, citrus, yam and plantain. The farmers in the area use pesticides in their farming activities.

Materials and Methods

Sample Collection

A total of 15freshly killed grasscutters were purchased from hunters in the Gomoa District of the Central Region of Ghana. Samples were wrapped in aluminium foil and put in polythene bags, placed in icebox containing ice and transported to the

Tele:
E-mail addresses: plansis1960@yahoo.com

laboratory. They were then stored at -20°Cuntil analysis was conducted.

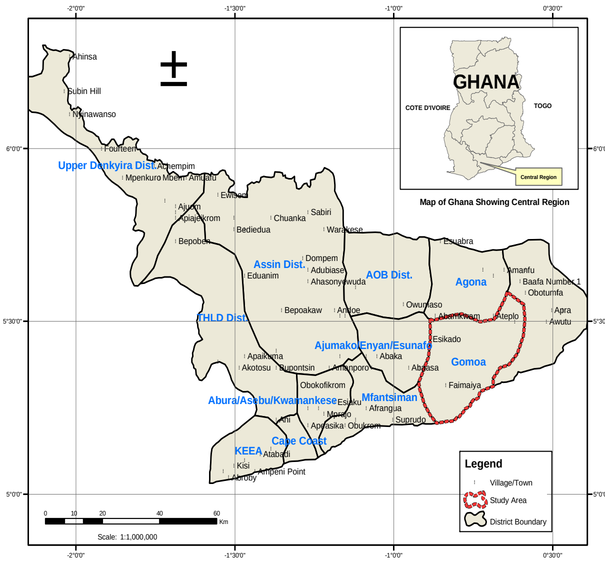

Figure 1: Map showing the study area

Chemical Analysis

The edible portion of grasscutter meat was homogenized using a meat blender. A 10g amount of the homogenized meat was weighed into a beaker containing 30g anhydrous sodium sulfate and thoroughly mixed. The sample mixture was transferred into an extraction thimble and placed into a soxhlet extractor. The mixture was extracted for 8h with 150ml of acetone-hexane mixture (30: 120ml (v/v)). The extracts were filtered and concentrated by rotary evaporation at 40°C till dryness.

Clean-up of the sample to remove residual fat and co-extractives was performed by transferring the extracts into a chromatographic column containing 1g activated florisil (60~100mesh) topped with 1g layer of anhydrous sodium sulfate to absorb any moisture present in the extract. The prepared column was first conditioned with 10ml of hexane. 1ml of each sample blank or matrix spike or sample extract was transferred onto the column using Pasteur pipettes. Prior to clean-up the sample extract was reduced to 10ml and 1ml of the 10ml extract subjected to clean up. The column was eluted with 10ml of hexane at a rate of 1-2ml/min. The collected eluate was concentrated on a rotary evaporator at a temperature of 40°C to dryness. The residue was dissolved in 1ml ethyl acetate and transferred quantitatively into a 2ml injection vials for analysis with electron capture gas chromatography.

Instrumental analysis

Organochlorine residues were determined by analysis of samples using electron capture gas chromatography. The gas chromatograph analysis was carried out using a Varian CP-3800 (Varian Associates Inc. USA) autosystem chromatograph equipped with ^{3}Ni electron capture detector (ECD). Carrier and make up gas was nitrogen at a flow rate of 1.0 and 29ml/min respectively. The temperature of injector operating in splitless mode was held at 225°C, oven temperature was 225°C and Electron Capture Detector was set at 300°C respectively. The column oven temperature was programmed as follows 60°C for 2min 180° C/min up to 300°C held for 31.80 min. The injection volume of the gas chromatograph was 1.0 µL. The residues detected by the GC analysis were confirmed by the analysis of the extract on 2 other columns of different polarities. The second column was coated with ZB-1 (methyl polysiloxane) connected to ECD and the second column was coated with ZB-17 (50% phenyl, methyl polysiloxane) and ECD was also used as a detector. The conditions used for these columns were the same. CTC Analytical combi PAL autosampler, split- splitless injector,PPC (programmed pneumatic control and a computer running Star Workstation data processor. For separation a 5% diphenyl 95% dimethyl siloxane capillary column (30m× 0.25mm× 0.25µm) ZB-5 (USA) was used. The identification of analytes was based on their retention times to the internal standard used for quatification. Multi- level calibration curves were created for quantification and good linearity (r^2>0.999) was achieved for tested intervals that included the whole concentration range found in samples. Peak area ratios (analyte response/internal standard response) were plotted against the concentration ratios (analyte concentration/internal standard concentration).

Determination of lipid content

Lipid content of muscle, liver and kidney samples were determined by soxhlet extracting 5g of sample using acetone-hexane (30:120ml v/v). The extracts were evaporated to complete dryness using a rotary evaporator. Lipid content of samples was determined gravimetrically using the formula;

$$\% \text{ lipid Weight} = \frac{\text{initial vial weight} - \text{final vial weight}}{\text{tissue weight}} \times 100$$

Quality control and Quality assurance

Procedural blanks were determined simultaneously for each set of the sample analysis by going through the same extraction and cleanup procedures.

Recovery Test

A recovery test was carried out in triplicate and fortified samples with 1.0ml of organochlorine mix standard. The fortified samples as well as the blank were subjected to the same analytical procedures of extraction, cleanup and analysis. The percentage of recoveries of organochlorines tested ranged from 85% to 98%. Residue levels for each pesticide were subsequently corrected for the recovery values.

Results and Discussion

The mean concentrations as well as the minimum and maximum concentrations of organochlorine pesticide residues in the grasscutter meat samples are shown in Table 1.

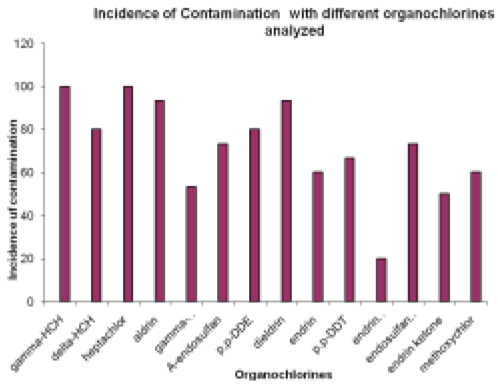

Figure 2: Incidence of contamination of the examined meat (n=15) with the studied organochlorine.

The incidence of contamination of the grasscutter meat samples with the studied organochlorines are shown in Figure 2. The mean concentration of aldrin was 4.682 µg/kg wet weight. Aldrin was detected in 11 (73.3%) of the examined meat samples. Although aldrin was detected in 66% of bovine meat and organ samples from Nigeria (Osibanjo and Adeyeye, 1997), it was detected in only 2% of the tested food samples from Taiwan (Doong et al., 1999) and it could not be detected in ruminant fat samples in Poland (Falandysz and Kannan, 1992)

In the present study heptachlor was detected in all 15 (100%) of the examined grasscutter meat samples. The mean concentration of heptachlor in the meat samples was 1.391 µg/kg wet weight.

γ- HCH and δ-HCH were detected in 15 (100%) and 12 (80%) of the grasscutter meat samples with mean concentrations of 0.686 and 0.404 µg/kg wet weight respectively. Studies in Spain indicated 100% of analyzed lamb and pork meat and meat products showed Lindane. (Herrera et al., 1994). Khalid et al (2007) also had an overall detection of 47% among the analyzed carcasses of camel, cattle and sheep in Egypt. It was also detected in 90% of analyzed meat and organs of cattle in Nigeria (Osibanjo & Adeyeye); however it was only detected in <10% of analyzed animal fat samples in Canada (Frank et al., 1990)

Dieldrin was detected in 14 (93.3%) at a mean concentration 0.337 µg/kg wet weight of the analyzed meat samples. A lower detection incidence of 44.4% was reported by Khalid et al (2007). Osibanjo and Adeyeye (1997) had an overall detection incidence of 100% of dieldrin in analyzed muscle and organs of goat and cattle slaughtered in Nigeria. However, a much lower detection incidence (<10%) for dieldrin was detected in fat samples of different animals in Canada (Frank et al., 1990) and also in meat and meat products in Spain (Herrera et al., 1996).

γ-chlordane was determined in 11 (73.3%) of the analyzed grasscutter meat samples In a study by Khalid et al., (2007) in Egypt, only 3.3% of the meat samples analyzed were contaminated with chlordane.

Out of 15 meat samples of grasscutter analyzed, 11 (73.3%) were positive for DDT and 12 (80%) were positive for DDE. The mean concentrations (µg/kg wet weight) of DDT and DDE in the grasscutter meat samples are 0.0.223 and 0.174 respectively (Table 1). The proportion of DDT/DDE has long been used as a rough indicator of the age of DDT residues in the environment. Ratios greater than one suggest relatively recent DDT application (Biddleman et al., 2005). Thus the ratio of 1.28 obtained in this study may be the result of recent input into the animals. The grasscutters might have been exposed through consumption of plants that had taken up DDT from contaminated. The frequency of detection of DDT in meat is higher in developing countries than in developed ones (Khalid et al., 2007). DDT was detected in 100% and 90% of bovine and lamb meat, respectively, in Iraq (Al-Omar et al., 1985). It was detected in a higher incidence of 96% of bovine meat and meat organs in Nigeria (Osibanjo and Adeyeye, 1997), and in 88% of meat and meat products in Spain (Herrera et al., 1996). An overall detection of 54.4% was found in meat from camel, cattle and sheep in Egypt (Khalid et al., 2007). In Canada however, DDT was only detected in 21% of analyzed fat samples of different slaughtered animals (Frank et al., 1990). The higher detection frequencies in DDT in some developing countries may have resulted from the illegal use of it in agriculture.

In the present study α-endosulfan and endosulfan sulfate were detected in 8(53.3%) and 11(73.3%) of the examined samples at mean concentrations of 0.915 and 1.092 µg/kg wet weight respectively. Ntow (2001) found endosulfan sulfate as the most frequently occurring (78%) organochlorine in water.

From the Table 2, the levels of organochlorine pesticide residues in all the grasscutter meat samples analyzed were below the F.A.O/W.H.O. Codex Alimentarius maximum residue limits (2005).

Conclusion

Residues of all the 14 organochlorines were detected at very low levels in the examined meat samples of the grasscutter tissues obtained from the Gomoa East District of the Central Region. The grasscutters from the Gomoa East District of the Central Region are therefore safe for consumption since the levels of the detected organochlorine residues were very low. The pesticide residues detected in the grasscutter meat may be the result of past usage of organochlorine pesticides in agriculture and in disease vector control. The grasscutters may have taken them into their body from ingesting plants grown on contaminated soil. From the Table 2, the levels of organochlorine pesticide residues in all the grasscutter meat samples analyzed were below the F.A.O/W.H.O. Codex Alimentarius maximum residue limits.

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Table 1: Mean concentrations (µg/kg wet weight) of organochlorine pesticide residues in grasscutter (*Thryonomys swinderianus*) meat. Lipid: 4.61%

Name of Organochlorine Pesticide	Min.	Max.	Mean (µg/kg wet weight)	Standard Deviation
	n = 15			
gamma-HCH	0.15	1.53	0.686	0.055
delta-HCH	0.21	1.41	0.408	0.065
Heptachlor	0.15	3.54	1.391	0.101
Aldrin	0.78	6.87	4.682	0.098
Dieldrin	0.13	0.95	0.337	0.025
gamma-chlordane	0.15	1.44	0.209	0.112
alpha-endosulfan	0.14	2.35	1.153	0.404
endosulfan sulfate	0.23	1.43	0.915	0.042
p,p'-DDT	0.23	2.02	0.223	0.211
p,p'-DDE	0.23	2.14	0.174	0.099
Endrin	0.11	0.87	0.211	0.007
endrin aldehyde	0.24	0.98	1.092	0.020
endrin ketone	0.22	1.87	1.440	0.043
Methoxychlor	0.20	1.34	0.901	0.031

n = Number of samples
Limit of detection = 0.01µg/g
Min=Minimum
Max=Maximum

Table: 2 Mean concentrations (µg/kg wet weight) of organochlorine pesticide residues in grasscutter (*Thryonomys swinderianus*) meat compared with the accepted Maximum Residue Limit as adopted by W.H.O./F.A.O./Codex Alimentarius Commission (2005)

Name of Organochlorine Pesticide	Mean conc. (µg/kg)	Maximum Residue Limit (µg/kg)
gamma-HCH	0.686	100
delta-HCH	0.408	100
heptachlor	1.391	200
aldrin	4.682	200
dieldrin	0337	200
gamma-chlordane	0.209	50
A-endosulfan	1.153	100
endosulfan sulfate	0.915	100
p.p-DDT	0.223	500
p,p-DDE	0.174	500
endrin	0.211	200
endrin aldehyde	1.092	200
endrin ketone	1.440	200
methoxychlor	0.901	-