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Organochlorine Pesticide Residues in Cow's Milk in Uganda

F. Ejobi,[1] L. W. Kanja,[2] M. N. Kyule,[2] P. Müller,[3] J. Krüger,[3] J. H. P. Nyeko,[4] A. A. R. Latigo[1]

[1]African Biodiversity Institute, P.O. Box 14126, Nairobi, Kenya
[2]Department of Public Health, Pharmacology and Toxicology, University of Nairobi, P.O. Box 29053, Nairobi, Kenya
[3]Centre for Environmental Research, Institute for Biogeography, University of Saarland, W-66041 Saarbrücken, Germany
[4]Department of Parasitology and Microbiology, Makerere University, P.O. Box 7062, Kampala, Uganda

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In the early 1970s organochlorine pesticides were identified as one of the major classes of environmental pollutants. Developed countries have, since then, reduced or banned their use (UNEP 1992). However, in Uganda as in many other developing countries, these chemicals are still used for crop protection and vector control. About 80 tonnes of DDT per year is used against cotton pests and for controlling mosquitoes in Uganda. Approximately 392 tonnes of dieldrin per year is used for controlling banana weevils and termites, and an additional 30 tonnes per year of dieldrin is used for ground spraying and selective treatment of tree trunks for tsetse control in the country (Aryamanya-Mugisha 1993). Other organochlorine pesticides used in the country include lindane, aldrin, hexachlorobenzene, camphechlor, chlordane, and heptachlor (Bazirake 1993). Organochlorine pesticides persist in the environment after they are used and are translocated and biomagnified along the natural food chains, with possible deleterious effects on organisms placed at higher trophic levels. For example, hyporeflexia in infants has been associated with DDE levels as low as 4 mg/kg milk fat in breast milk (Rogan 1986), DDT causes egg-shell thinning in some birds (Chang and Stockstad 1975), aldrin and dieldrin are potentially carcinogenic (IARC 1974), and lindane produces liver tumors in mice (IARC 1974).

In spite of the use organochlorine pesticides in Uganda, no studies had previously been conducted to monitor the levels of these chemicals in cow's milk. The main objective of the present study was to identify and quantify the levels of organochlorine residues in cow's milk so as to assess the extent of environmental contamination by these pesticides and also to evaluate their toxicological significance to the health of the consumers.

MATERIALS AND METHODS

Twenty one and 19 farms in Kampala and Iganga districts respectively were randomly selected and subsequently visited for collection of cow's milk. A total of 141 samples were collected, 69 from farms located in the pet-i-urban areas of Kampala District and 72 from farms in the rural areas of Iganga District.

Correspondence to: F. Ejobi

Kampala District embodies the capital city of Uganda and is located in central Uganda while Iganga District is located in eastern Uganda. All the samples were collected between October 1992 and January 1993. A questionnaire was completed in each farm visited to collect information on the system of animal rearing, water source(s) for the animals, animal breed (s), lactation length, crops grown in the farm, and pesticides used on crops and animals. About 20 ml of milk sample were obtained from each lactating cow randomly sampled. The samples were put in pre-cleaned universal bottles with aluminium foil lined caps and kept in a cool box containing ice packs and frozen at -20°C on the same day of collection. The samples were later transported in a cool box with dry ice to the Centre for Environmental Research, Institute for Biogeography, University of Saarland, Saarbrücken, Germany where the laboratory chemical analysis was done.

Extraction and clean-up was carried out according to the United States Environmental Protection Agency Method 608 (US-EPA 1980). Briefly, the method involved grinding 10 to 15 g of milk with anhydrous sodium sulphate to yield a dry free-flowing powder which was then transferred into a glass extraction column of length 30 cm and internal diameter 2 cm. The dry column was then eluted with 80 ml of dichloromethane with the first 40 ml allowed to stay in contact with the powder for 20 to 30 minutes. The eluate was collected in a pre-weighed round bottom flask. Dichloromethane in the eluate was removed using a rotary evaporator at about 35°C and under reduced pressure. The flask was weighed until a constant weight was obtained. The difference between this weight and the original weight of the flask constituted the fat content of the sample. The raw fat extract was clean-up in a glass column packed with florisil (3% water) using elution mixture of petroleum ether/dichloromethane at the ratio of 4:1. The flow rate of the elution mixture was controlled so as not to exceed 5 ml/min. The eluate was concentrated to a small volume (approximately 5 ml) using a rotary evaporator at about 35°C under reduced pressure. This was then transferred to a 10 ml flask and evaporated to almost dryness. One millilitre of iso-octane was added to the flask, mixed thoroughly with a whirl mixer and then transferred to autosampler vials ready for gas chromatography.

A gas chromatograph (GC) SIGMA 1 (Pet-kin Elmer) with integrator LCI 100 (Perkin Elmer) and autosampler AS-100 (Perkin Elmer) was used for the analysis. The GC parameters and operating conditions were: detector, ^{63}Ni electron capture; carrier gas, hydrogen; make-up gas, Ar/CH_4; column: length 25 m, internal diameter 0.25 mm, film thickness 0.25 µm, stationary phase S-54(5% phenyl-, 1% vinyl-, 94% methylsilicon); temperatures, detector 300°C, injector 250°C, oven 90-250°C; injection volume, 1 µl splitless 1 min, detection limit was 1 pg. Sum-DDT was calculated as p,p'DDT + o,p'DDT + 1.11(p,p'DDE + p,p'DDD), 1.11 being the conversion factor for the low molecular weight of the DDT metabolites.

Confirmation of the identity of the residues was done using coupled gas chromatography-mass spectrometry (GC-MS) system; Gas Chromatograph Series

8500 (Perkin Elmer) coupled with mass specific detector ITD-800 and Epson PC-AX with NBS/EPA mass spectra library with following parameters and working conditions: detector, Ion Trap (ITD-800); ionization, electron-ionization (70 eV); scan range, 105-400 amu; column, 30 m-FS-DB-5MS-CB-0.25µm (length 30 m, stationary phase 5% diphenyldimethylsiloxon, internal diameter 0.25 mm, film thickness 0.25 µm); carrier gas, helium (80 kPa); temperatures, detector 250°C, injector 290°C, oven 100-290°C; injection volume, 2 µl splitless for 2 min.

Recovery tests were done using market cow milk spiked with one millilitre of chlorinated pesticide mixture. The average percent recoveries for all the compounds varied from 83% for p,p'DDD to 94% for α-HCH. Analysis of variance (ANOVA) and Turkey's highest significance tests (Wayne 1983) was carried out to determine if there were significant differences in the means of residue levels among the variables. A 5% significance level was used.

RESULTS AND DISCUSSION

Out of all the samples analyzed, eight organochlorine pesticide residues were detected. These were: p,p'DDE, dieldrin, α-HCH, p,p'DDD, p,p'DDT, o,p'DDT, β-HCH and lindane. The frequency, mean and range of the residues detected are presented in Tables 1 and 2. The residue levels are expressed in mg/kg milk fat without correcting for percent recoveries. The mean percent extractable fat was 4.3% with a range of 0.7 to 9.0%.

Using one way analysis of variance (ANOVA), milk samples of cows in the peri-urban areas of Kampala District (n = 69) had significantly higher (p = 0.0018) mean level of sum DDT than those from the rural areas of Iganga District (n=72). Furthermore, milk samples from zero-grazed cows (n=46) showed significantly higher (p=0.0232) mean level of sum DDT than those from out-door grazed cows (n=95). No significant differences (p=0.7443) were also found in the mean levels of sum DDT between milk samples from local (n=75) and exotic (n=66) breeds of cows. The source of water for the cows did not have any significant effect on the levels of the residues in the milk.

This study is the first report on the occurrence and levels of organochlorine residues in cow's milk in Uganda. The major organochlorine contaminants found in the cow milk were pp'DDE (88.7%) and dieldrin (87.2%). The only comparable study on organochlorine residues in cow's milk in the East African region was done by Maitho (1978) who reported mean levels in mg/kg milk fat of the residues as follows: p,p'DDT 0.006, p,p'DDE 0.009, dieldrin 0.039, aldrin 0.002 and lindane 0.004. Generally, the mean levels of p,p'DDT of 0.028 mg/kg milk fat and p,p'DDE of 0.023 mg/kg milk fat observed in this study were higher than those reported by Maitho (1978). However, the mean level of dieldrin of 0.027 mg/kg milk fat in this study was lower than the one reported by Maitho (1978). In this study, no quantifiable levels of aldrin were detected.

The significantly higher mean level of sum DDT observed in cow milk samples

Table 1. Frequency, mean, standard error of the mean (S.E.M) and range of α-HCH, β-HCH, lindane and dieldrin detected in cow's milk from Kampala and Iganga districts, Uganda. The pesticide residue levels are expressed in mg/kg milk fat without correcting for percent recoveries.

Compound	Kampala District (n = 69)	Iganga District (n = 72)	Both Districts (n = 141)
α-HCH			
no. positive (%)	4 (5.8%)	50 (69.4%)	54 (38.3%)
mean ± S.E.M.	0.002 ± 0.0003	0.006 ± 0.0004	0.006 ± 0.0004
range	0.002 - 0.003	0.002 - 0.014	0.002 - 0.014
ß-HCH			
no. positive (%)	-	10 (13.9%	10 (7.1%)
mean ± S.E.M.	-	0.007 ± 0.001	0.007 ± 0.001
range	-	0.003 - 0.018	0.003 - 0.018
lindane			
no. positive (%)	3 (4.3%)	4 (5.6%)	7 (5.0%)
mean ± S.E.M.	0.018 ± 0.002	0.018 ± 0.006	0.014 ± 0.004
range	0.006 - 0.013	0.007 - 0.036	0.006 - 0.036
dieldrin			
no. positive (%)	55 (79.7%)	68 (94.4%)	123 (87.2%)
mean ± S.E.M.	0.036 ± 0.009	0.020 ± 0.002	0.027 ± 0.001
range	0.006 - 0.413	0.002 - 0.115	0.002 - 0.413

no. positive = number of samples with quantifiable residue levels
mean was calculated from positive quantifiable samples only
(-) = no quantifiable levels of residue detected

Table 2. Frequency, mean, standards error of the mean (S.E.M) and range of the DDT compounds in cow's milk from Kampala and Iganga districts, Uganda. The pesticide residue levels are expressed in mg/kg milk fat without correcting for percent recoveries.

Compound	Kampala District (n = 69)	Iganga District (n = 72)	Both Districts (n = 141)
p,p'DDE			
no. positive (%)	58 (84.1%)	67 (93.1%)	125 (88.7%)
mean ± S.E.M.	0.034 ± 0.0004	0.014 ± 0.001	0.023 ± 0.002
range	0.002 - 0.131	0.002 - 0.050	0.002 - 0.131
p,p'DDD			
no. positive (%)	15 (21.7%)	9 (12.5%)	24 (17.0%)
mean ± S.E.M.	0.013 ± 0.001	0.014 ± 0.002	0.014 ± 0.001
range	0.007 - 0.025	0.007 - 0.024	0.007 - 0.025
o,p'DDT			
no. positive (%)	3 (4.3%)	10 (13.9%)	13 (9.2%)
mean ± S.E.M.	0.036 ± 0.005	0.017 ± 0.003	0.021 ± 0.032
range	0.026 - 0.045	0.007 - 0.027	0.007 - 0.045
p,p'DDT			
no. positive (%)	8 (11.6%)	15 (20.8%)	23 (16.3%)
mean ± S.E.M.	0.016 ± 0.004	0.035 ± 0.001	0.028 ± 0.006
range	0.005 - 0.034	0.003 - 0.116	0.003 - 0.116
sum DDT			
mean ± S.E.M.	0.042 ± 0.005	0.025 ± 0.003	0.033 ± 0.003
range	0.002 - 0.168	0.002 - 0.130	0.002 - 0.168

means are calculated from positive quantifiable samples only
no. positive = number of samples with quantifiable residue levels

from the peri-urban areas than those from the rural areas could be attributed to different management systems. In the peri-urban areas, cows were usually zero-grazed while in the rural areas, out-door grazing was predominant. Zero-grazed cows were usually fed on crop residues like banana stems, maize stovers, potato vines or commercially prepared feeds which could be contaminated by pesticides. On the other hands, out-door grazed cows were pastured in the paddocks or communal grazing areas where pesticides are not usually applied. However, the present study did not analyze feeds of the zero-grazed cows for organochlorine residues as it was not the objective of the study.

The mean sum DDT level of 0.033 mg/kg milk fat in this study was below the Extraneous Residue Limit (ERL) of 1.25 mg/kg (fat basis) set by the FAO/WHO Codex Alimentarius Commission (1986). All the samples had sum DDT levels below the set ERL. The mean level of dieldrin of 0.027 mg/kg milk fat was also below the ERL of 0.15 mg/kg (fat basis) set by the FAO/WHO Codex Alimentarius Commission (1986). However, 2 (1.4 %) samples had dieldrin levels above the set ERL. The mean level of lindane in the study was 0.014 mg/kg milk fat which was slightly above the ERL of 0.01 mg/kg (fat basis) set by FAO/WHO Codex Alimentarius Commission(1986), and 4 (2.8 %) samples had lindane levels exceeding the set ERL.

From public health point of view, the observed levels of organochlorine residues in cow milk's in this study do not pose a serious health risk to the consumers. However, the presence of lindane and dieldrin in some samples above the ERLs suggests a possible risk to the health of the consumers. Anyhow, since cow's milk for human consumption is usually pooled, these individual high levels might not be very significant.

The results of this study have shown that organochlorine residues occur in cow's milk in Uganda. This indicates that the general environment is contaminated by these chemicals as a result of their use in the country. From the present findings, cow's milk is a good bioindicator of environmental contamination by organochlorine pesticides. Periodic biomonitoring of organochlorine pesticide residues in the environment should be carried out so as to assess the trends of environmental contamination by these chemicals. The government and other appropriate agencies should educate farmers on good agricultural practices (GAP) in the use of pesticides.

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