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SHORT COMMUNICATIONS

DETERMINATION OF γ-BHC IN BREAST MILK OF KENYAN WOMEN

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ABSTRACT. Residues of γ-benzene hexachloride (lindane) were determined from samples of human milk procured from a hospital in Nairobi, Kenya. The pesticide residues were extracted using n-hexane. Detection and quantitation of the pesticide residues was made by a gas chromatograph employing an electron capture detector. Most samples were found to contain detectable but low levels of lindane residues in the concentration range of 9.0×10^{-6} to 1.0 parts per million (ppm).

INTRODUCTION

Understandable anxiety is being expressed by increasing numbers of individuals and governmental agencies about possible risks to public health from modern pesticide chemicals in foodstuffs. The prolonged ingestion of even small amounts of some of these chemicals added as pest control agents to our food and to the feed of our livestock can unquestionably cause harm (1). On the other hand, farmers' demand for pesticides keeps on increasing in order for them to meet the needs of our pyramiding populations.

In Kenya lindane is used in the control of household pests, seed dressing, dusting of live plants and in the storage of beans. It is also applied directly to control soil pests. Lindane can enter the human food chain through absorption and translocation to the edible parts of plants growing on contaminated soils (2). The presence of lindane in human food chain is indicated by residues in human milk and blood (3,4). This pesticide has also been shown to get distributed in the tissues of swine (5) and get incorporated in beans treated during storage (6).

Accumulation of lindane in the human body is very likely since the compound is not readily metabolised (7). The major effect of lindane is the induction of porphyria (a liver disease) (8) and therefore the pesticide is considered to be a potent inducer of hepatic porphyria in humans. Lindane, aldrin, DDE and DDT have also been shown to act as antagonists to pregnancy in humans (9).

Toxicological studies with lindane have shown carcinogenic effects in rats (10,11), histopathological changes in the livers of pigs (12), wide-spread abdominal pathological lesions in dogs (13), convulsions in rabbits (14) and degenerative ovarian changes in female Rhesus monkeys (15). At high concentrations, lindane has been shown to cause a reduction in sex hormone levels in the female catfish (16) and induce considerable ultrastructural changes in the cellular components of the neurohaemal organs on the median nerves of the Stick insect (17). Contamination of human milk with residues of organochlorine insecticides represents a major environmental problem because this is a major source of infant exposure to these toxic chemicals during lactation. This paper reports the results of a study to determine the level of residues of lindane in breast milk of Kenyan women.

MATERIALS AND EXPERIMENTAL METHODS

Materials. Human milk samples were obtained from one hospital in Nairobi. Glass vials of size 4 x 2 cm were used. Teflon caps lined with aluminium foil were used to seal the vials. All vials were washed with soap and warm water and rinsed three times with deionized water followed by triple distilled acetone twice. The vials were dried in the oven, cooled and transported to the hospital. The head nurse would express the milk from the mothers into a 500 cm^3 glass beaker (washed as stated above) and pour the milk into the vials. The vials with milk were placed in the refrigerator at +2 to +4°C awaiting collection. Every day the vials would be picked up from the hospital and stored in a freezer at -12°C to -18°C in the Chemistry Department.

The solvents used, acetone and n-hexane, and the reagent propan-2-ol (all Analytical Reagents grade) were triple distilled. Anhydrous sodium sulphate was soxhlet extracted with n-hexane for two hours then dried at 130°C prior to use. The glass wool used was also soxhlet extracted with n-hexane.

Samples were analysed using a Pye-Unicam 104 Gas Chromatograph fitted with an electron capture detector and using a column (1.8 m x 0.4 mm) packed with 1.5 % OV-17/1.95% OV-20. Column temperature was maintained at 200°C using nitrogen (white spot) as carrier gas.

Preparation of lindane standards. Ten standards in the concentration range $1 - 10^{-5}$ ppm were prepared by diluting a standard solution of known concentration appropriately. For each of the ten standards, 5 µl samples were drawn and injected into the column. The retention time and peak heights for these standard solutions are given in Table 1.

Preparation of lindane derivative standards. Five standards of the disubstituted ether derivative (bis-isopropoxytetrachlorobenzene, BITB) in the concentration range $0.05 - 5 \times 10^{-4}$ ppm were prepared by diluting a standard solution of known concentration appropriately. 5 µl portions were analysed and their results are given in Table 2.

Extraction and analyses. An established analytical procedure (18) was followed. The milk sample (0.5 g each) was thoroughly mixed with 0.5 cm^3 of n-hexane in a test tube. The mixture was then transferred to a narrow column for cleanup. A column packed with 100 mm of Florisil adsorbent (PR grade) and topped with 12 mm anhydrous Na_2SO_4 had been held in an oven at 130°C for 16 hours and prewashed with 50 cm^3 hexane just before use. The test tube containing the sample was then rinsed with two 0.5 cm^3 portions of n-hexane and added to the column. The column was then eluted with 200 cm^3 of n-hexane at a flow rate of 5 cm^3/minute.

The eluent was collected in a Kuderna-Danish concentrator assembly fitted with a 25 cm^3 graduated evaporative concentrator tube. The concentrator tube was then immeresed in boiling water bath to about one third of its depth and the eluent (extract) concentrated to 10 cm^3. The concentrator tube was removed from the Kuderna-Danish assembly and cooled. The joint was rinsed with 3 cm^3 of n-hexane. The tube was placed under a nitrogen stream (white spot) and the extract reduced to approximatelyy 3 cm^3. The sides of the tube were rinsed with n-hexane and the volume adjusted to 5 cm^3.

A 5 µl portion of the extract was injected into the column for direct analysis of γ-BHC residues. At the start of each working day, several consecutive injections of standards were done to "prime" the column. Results of this analysis are given in Table 3.

The calculation of concentration of lindane was somewhat dependant on peak shape. Tall narrow peaks with no overlap were determined by comparing peak

height with that of the standard (most fell in this category). Where there was overlap or unsymmetrical peak or sloping baseline, triangulation or integration was used. The peak heights of standards and samples did not vary more than 25% and concentration had to fall in the linear range of the detector. The same attenuation setting was used for all samples and standards.

Derivatization. Those milk samples that snowed residues of γ-BHC (lindane) were derivatized to enable further gas chromatographic analysis so as to confirm the presence of the pesticide. The derivatization process was specific for lindane and was followed as outlined in the USA EPA manual (18). Five concentrations of γ-BHC standards were derivatized along with the samples. Concentrations were chosen that bracket the concentration of the γ-BHC present in the sample as determined by the intitial GC analysis.

To each sample in a 25 ml graduated evaporative concentrator tube, 5 drops of 1% paraffin oil in n-hexane were added. The mixture was then evaporated under a gentle stream of nitrogen in warm water until 0.1 - 0.2 cm^3 of n-hexane remained. 0.2 cm^3 pyridine and 0.5 cm^3 of 10% KOH in propan-2-o1 were then added. A modified Micro-Snyder column was then attached to the tube. The tube was then placed in a boiling water bath for exactly 45 minutes, removed and cooled under tap water. 10 cm^3 of 2% Na_2SO_4 aqueous solution and exactly 2.0 cm^3 of n-hexane were then added into the contents of the tube. The tube was then stoppered and shaken vigorously for one minute. The aqueous and the n-hexane layers were then allowed to separate completely. 5 µl of the n-hexane (upper) layer was then injected into the column. Results of this analysis are given in Table 4.

RESULTS AND DISCUSSION

The chromatograms obtained had peaks close and sometimes overlapping the γ-BHC peak. One can only assume that these are either heptachlor or the α- and β-isomers of γ-BHC. Further work with a variety of columns and use of specific standards would resolve the problem.

Results in Tables 1 and 2 show varying amounts of lindane detected in the milk samples. All standards were run until a constant peak height (3 times) was obtained and the average is reported in Table 1. All samples were run twice and the average reported. If the two were too dissimilar a third or fourth injections were made.

Table 1. Results from chromatograms of lindane standards.

Sample No.	Concentration (ppm)	Peak height (cm) arithmetic mean	Standard deviation (SD)
1	1.00	18.4	2.55
2	0.5	11.93	0.95
3	0.1	10.6	0.78
4	0.05	8.1	1.23
5	0.01	6.13	0.53
6	0.005	5.0	0.56
7	0.001	4.0	0.7
8	5×10^{-4}	3.0	0
9	1×10^{-4}	2.0	0.10
10	1×10^{-5}	0.95	0.13

off should be close to 0

Retention time (R_T) = 1.6 minutes

Table 2. Results from chormatograms of bis-isopropoxytetrachlorobenzene (lindane derivative) standards.

Sample No.	Concentration (ppm)	Peak height (cm) arithmetic mean	Standard deviation (SD)
1	0.05	19.95	0.51
2	0.025	19.30	0.15
3	0.001	18.0	0.28
4	0.005	15.20	1.88
5	5×10^{-4}	10.01	0.82

Retention time (R_T) = 2.2 minutes

Table 3. Results from chromatograms of sample analysis for lindane.

Sample No.	Weight of milk analysed (g)	Lindane peak height (cm) at R_T = 1.6 min.	Concentration of lindane in sample (ppm)
1	0.5791	no peak	-
2	0.5703	9.3	5.7×10^{-2}
3	0.5433	no peak	-
4	0.5373	no peak	-
5	0.6189	22.0	beyond detector range
6	0.5334	no peak	-
7	0.4871	no peak	-
8	0.5310	no peak	-
9	0.6249	no peak	-
10	0.7116	no peak	-
11	0.5172	3.5	8.75×10^{-4}
12	0.5351	1.0	1.05×10^{-5}
13	0.5449	no peak	-
14	0.5253	10.2	0.096
15	0.5610	1.6	8.0×10^{-5}
16	0.7141	3.5	8.75×10^{-4}
17	0.5228	12.5	0.524
18	0.6518	2.45	1.225×10^{-4}
19	0.4860	no peak	-
20	0.6066	9.2	0.057
21	0.5328	very short peak	-
22	0.6886	7.15	0.044
23	0.6326	1.45	7.25×10^{-5}
24	0.6841	no peak	-
25	0.6088	no peak	-
26	0.6592	no peak	-
27	0.5826	no peak	-
28	0.5941	no peak	-
29	0.6520	no peak	-
30	0.5408	8.4	0.052
31	0.5101	2.3	1.15×10^{-4}
32	0.5312	10.6	0.1
33	0.5110	6.2	0.010
34	0.5210	5.3	0.005
35	0.5551	no peak	-
36	0.5066	no peak	-
37	0.4834	no peak	-
38	0.5110	9.2	0.057
39	0.5502	no peak	-
40	0.4995	2.5	1.25×10^{-4}
41	0.5244	3.4	5.67×10^{-4}
42	0.5373	no peak	-
43	0.5113	no peak	-
44	0.4066	13.0	0.545
45	0.6134	0.9	beyond detector range
46	0.5663	11.1	0.465
47	0.5339	no peak	-
48	0.5312	no peak	-
49	0.5469	no peak	-

The amounts of lindane residues detected in the milk samples occur in concentrations varying from 9.0×10^{-6} to 1.0 ppm. Most of the milk samples however had the residue in concentrations lower than the average concentration of 0.0024 ppm obtained from a similar study with Amercian mothers (19).

The confirmation of γ-BHC was done by derivatization. These peak heights are separate and NOT comparable to the original γ-BHC peak height. Tables 2 and 4 show the results of the derivatization. In the EPA work (18) and again in this work, there are some unresolved problems with the formation of only the disubstituted bis-isopropoxytetrachlorobenzene derivative. Significant quantities of mono-substituted derivative were often formed that prevented accurate quantitations. Therefore, this derivative scheme is only considered a semi-quantitative confirmation of the γ-BHC.

As this study was only a pilot study to see if γ-BHC could be detected at all, recoveries were NOT calculated. Future work would include recoveries from spiked samples to determine the accuracy of the experimental method. In addition, another expermental technique would be employed as a comparison.

Table 4. Results from chromatograms of lindane derivative in milk samples after derivatization.

Sample No.	Weight of milk analysed (g)	Derivative (BITB) peak height (cm) at R_T = 2.2 min.	Concentration of BITB in derivatized sample (ppm)
2	0.5702	12.5	6.24×10^{-4}
5	0.6189	7.75	3.87×10^{-4}
11	poorly resolved	-	-
12	0.5351	17.5	9.72×10^{-4}
14	poorly resolved	-	-
15	0.5610	16.9	9.39×10^{-4}
16	0.7141	8.85	4.42×10^{-4}
17	0.5228	15.2	0.005
18	0.6518	9.2	4.60×10^{-4}
20	0.6066	14.3	4.70×10^{-3}
22	0.6886	very short peak	-
23	0.6326	very short peak	-
30	0.5408	13.2	4.34×10^{-3}
31	0.5101	10.9	5.0×10^{-4}
32	0.5312	13.5	4.44×10^{-3}
33	0.5110	8.2	4.1×10^{-4}
34	0.5210	very short peak	-
38	0.5110	15.1	0.005
40	0.4995	very small peak	-
41	0.5244	very small peak	-
44	0.6066	15.0	4.93×10^{-3}
45	poorly resolved	no peak	-
46	0.5663	14.3	4.70×10^{-3}

CONCLUSION

The results obtained in this study indicate that residues of lindane are present in breast milk of Kenyan women in varying concentrations. It is possible that residues of other halogenated pesticides are also present in the milk. More research on this subject is desirable considering the fact that breast milk is the major source of infant exposure to these toxic chemicals during lactation.

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