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ORGANOCHLORINE PESTICIDE RESIDUES IN CATTLE FEED SAMPLES IN KARACHI

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Chlorinated hydrocarbon insecticides were monitored in animal feed samples collected from the Karachi Cattle Colony. Seventy nine random samples were screened for lindane, α-BHC, β-BHC, δ-BHC, endrin, aldrin, dieldrin, pp′-DDT, pp′DDE, p′p′, DDD, dicofol, heptachlor and heptachlor epoxide. Gas chromatographic analyses revealed that approximately 46 % of the samples were contaminated with different pesticides and their metabolites. α-BHC and γ-BHC were found to be present in most of the compounds.

Key words: Pesticides; Control of vector borne diseases; Animal feeds.

INTRODUCTION

Pesticides are toxic chemicals extensively used for the control of vector borne diseases, increase in food production and improvement in the quality of agricultural crops. They have, infact, become an integral part of modern farming but their continued use has also posed many serious problems. Most of the chlorinated pesticides are lipophilic and are highly persistent. Even if present in minute quantities, their variety, toxicity and persistence have an adverse effect on ecological systems such as animals, birds, fishes and trees with which human welfare is inseparably bound.

Livestock are mostly exposed to pesticides through feed which may be contaminated either through earlier treatment of soil with some pesticides or through direct application of sprays or dusts during crop protection measures. Pearson *et al.* [1] reported on the distribution of chlorinated pesticides in animal feed components and finished feeds. Frequency of occurrence and levels of selected organohalogens were monitored in animal feed components and animal feed over a seven year period. Numerous examples of contamination of animals through animal feeds are reported elsewhere in the literature [2-6].

This study presents the protocol and results of a monitoring programme carried out to assess the level of contamination of animal feed with organohalogen compounds. The entire programme was accomplished in three phases, namely, preliminary laboratory investigations using spiked feed samples to evolve a suitable analytical methodology, random sampling of animal feed from the Karachi Cattle Colony and finally the pesticide residue analyses/interpretation of results. Thirteen organochlorine pesticides or their metabolites have been studied. The compounds selected for screening were αBHC, β-BHC, γBHC, δ-BHC, pp′-DDT, pp′-DDE, DDD (TDE), dicofol, heptachlor, heptachlor epoxide, aldrin, dieldrin and endrin.

EXPERIMENTAL

Sampling. Karachi Cattle Colony comprises a total of four hundred and seventy four dairy farms. Due to financial constraints and the capacity of the laboratory to store a large number of samples for residue analyses, it was decided to draw random samples of prepared cattle feed from every sixth dairy farm. As far as possible, recommendations of the Codex Committee on pesticide residues were followed [7]. About 1 kg feed was considered sufficient for sub-sampling. The samples were properly labelled, packed in polythene bags and brought to the laboratory where each sample was subdivided into three sub-samples of equal size and frozen at -20° until analysed.

Extraction. For our work with animal feeds, a mixture of moisture free toluene and *n*-hexane (re-distilled in glass) was employed in the ratio of 1:3 whenever needed. 150 ml of this solvent mixture was added to a sample of 25 g animal feed in a 500 ml Erlenmeyer flask and shaken for three hours on an electric shaker. Subsequently, the extract was filtered through Whatman No. 542 filter paper containing a little anhydrous sodium sulphate. The contents of the flask were washed with 3x25 ml of the solvent mixture and filtered. The filtered extracts were combined and its volume noted. The extract was evaporated to almost dryness in a rotary vacuum evaporator at about 60° on a water bath and taken up in 25 ml n-hexane for acetonitrile partitioning.

Acetonitrile partition. The extract in *n*-hexane was transferred to a 100 ml separatory funnel and 3x15 ml portions of acetonitrile were added to it. The separatory funnel was shaken vigorously for 2 min., allowed the layers to separate and drained the lower acetonitrile layer into a receiving flask. Different acetonitrile layers were combined and *n*-hexane phase was discarded. Acetonirile retains pesticides while fats and other interferring components,mostly remain in *n*-hexane. Acetonitrile was evaporated completely in a rotary vacuum evaporator. The residue was dissolved in a small volume of *n*-hexane and transferred to Florisil column for further purification of the desired pesticides.

Cleanup. The procedure of Mills *et al.* [8] was employed for cleanup. The sample extract in *n*-hexane was quantitatively transferred to a clean and dry glass column (11 mm i.d. x 50 cm) previously packed with 10 g activated Florisil (Sigma Chemical Co., USA) and eluted with 15 % diethyl ether in petroleum ether. Flow rate through the column was adjusted @ 1 ml/min. 250 ml eluate was collected. In the recovery studies performed prior to monitoring work, this quantity of eluant was found to elute all the thirteen compounds studied. It was then concentrated down to 1 ml in a rotary vacuum evaporator for GLC determination.

Instrumentation. Screening of chlorinated pesticides was performed by gas liquid chromatography. Gas chromatograph (model: Pye-Panchromatograph) equipped with a tritium based electron capture detector was employed. For confirmation of the identity of unknown compounds in feed samples, two GLC columns, each packed with a different liquid stationary phase, were used.

GLC operating conditions. Two glass columns each 30 cm long x 4 mm i.d., packed separately with (i) a mixture of 7.5 % QF_1 + 5 % DC-200 and (ii) 1 % NGS, both supported on 80/100 mesh chromosorb W, Temp. column oven 150°, detector oven 175°, detector voltage, 1 Volt for column (i) and 5 Volts for column (ii) pulsed; electrometer setting, 10^{-10} amp. full scale; nitrogen (carrier gas) flow rate, 65 ml min^{-1}; Honeywell recorder, 10 millivolts, chart speed, 8 $mm.min^{-1}$. The two columns were conditioned at 175° for 24 hours before use. These operating parameters were employed for both the columns and found suitable for all the investigated compounds.

The instrument behaved in a linear manner throughout. 1-5 μl quantities of cleanedup sample extracts were injected into the gas chromatograph alongwith relevant pesticide standards for identification and quantitation. Each cleanedup sample extract was gas chromartographed three times to confirm reproducibility of results. For ease in calculation, the unit of parts per billion or μg/kg has been adopted throughout the study. A contorl sample processed in a similar manner did not give any interferring GLC response.

RESULTS AND DISCUSSION

Seventy nine samples of animal feed were drawn from the Karachi Cattle Colony, each of which was sub-divided into three sub-samples of equal size and analyzed by the described methodology. All quantitations are reported in Table e. Results of each sub-sample of animal feed are presented as a mean of pesticide residue alongwith standard error. 36 Feed samples were found to be contaiminated with residues of different chlorinated pesticides while 43 samples did not contain any thing, and hence not included in the table. According to the data obtained, 16 samples contained α-BHC residues at levels ranging from 1.3 to 17.7 μg/kg, 25 samples were found to contain γBHC at levels ranging from 2.3 to 19.6 μg/kg while β-BHC and δ-BHC were present in one sample each in quantities of 74.4 and 55.4 μg/kg respectively. pp -DDT was present in traces in two samples while pp′-DDE was reported in trace amounts to 53.4 μg/kg in two samples. Heptachlor epoxide was found in two samples in quantities of 20.9 and 21.2 μg/kg;

Table 1. Recovery of studied pesticides from spiked feed on two different GLC column materials

Sl.	Pesticide	Added μg/kg	% Recovery* 7.5 % QF_1 + 5 % DC-200	1 % NGS
1.	α-BHC	1.0	101.0 ± 3.39	102.0 ± 3.73
2.	β-BHC	30.0	104.5 ± 0.44	104.0 ± 0.39
3.	γBHC	2.0	97.6 ± 1.17	99.1 ± 1.01
4.	δ-BHC	30.0	87.8 ± 1.11	86.5 ± 1.61
5.	pp′.-DDT	50.0	98.8 ± 2.39	100.0 ± 2.35
6.	pp′-DDE	50.0	103.4 ± 2.05	104.9 ± 1.62
7.	DDD (TDE)	50.0	99.0 ± 1.81	99.0 ± 1.19
8.	Dicofol	75.0	101.3 ± 2.18	102.2 ± 2.48
9.	Heptachlor	20.0	102.0 ± 1.0	102.2 ± 1.88
10.	Heptachlor-epoxide	20.0	93.0 ± 0.58	94.4 ± 0.26
11.	Aldrin	5.0	96.5 ± 0.75	97.1 ± 0.61
12.	Dieldrin	20.0	104.0 ± 1.33	102.0 ± 0.92
13.	Endrin	10.0	105.4 ± 0.43	106.0 ± 1.3

*Mean and standard error of three analyses.

Table 2. Organochlorine pesticide residues (μg/kg) found in feed samples on two different GLC columns.

Sl. No.	Plot No.	Pesticide detected	Residue (μg/kg) with standard error*	
			7.5 % QF_1 + 5 % DC-200	1 % NGS
1.	7	Heptachlor epoxide	23.6 ± 0.29	21.2 ± 0.25
2.	25	γ-BHC	5.3 ± 0.16	4.9 ± 0.047
3.	31	α-BHC	3.5 ± 0.82	3.2 ± 0.047
		γ-BHC	3.4 ± 0.25	3.4 ± 0.047
4.	37	α-BHC	16.2 ± 0.12	16.1 ± 0.082
		γ-BHC	19.6 ± 0.28	18.0 ± 0.17
5.	49	α-BHC	8.1 ± 0.094	7.9 ± 0.047
		γ-BHC	15.0 ± 0.094	14.4 ± 0.047
6.	55	α-BHC	4.7 ± 0.047	4.6 ± 0.047
		γ-BHC	5.6 ± 0	5.5 ± 0.047
7.	61	γ-BHC	5.5 ± 0	5.3 ± 0.082
8.	79	β-BHC	74.4 ± 0.37	74.53±0.17
		γ-BHC	2.9 ± 0	3.27±0.17
9.	97	γ-BHC	17.1 ± 0.094	16.67±0.09
10.	163	Heptachlor epoxide	20.9 ± 0.12	20.93b0.29
11.	193	α-BHC	8.7 ± 0.82	7.9 ± 0.047
		γ-BHC	7.8 ± 0.047	8.3 ± 0.047
12.	199	α-BHC	7.8 ± 0.082	6.9 ± 0.47
		γ-BHC	3.3 ± 0	3.4 ± 0
		δ-BHC	55.4 ± 0.17	58.0 ± 0.17
13.	230	α-BHC	1.5 ± 0	1.4 ± 0
		γ-BHC	4.7 ± 0.047	4.8 ± 0.47
		pp′-DDE	231.0 ± 1.25	263.3 ± 1.2
14.	236	Aldrin	7.7 ± 0.047	7.6 ± 0.047
		Dieldrin	43.36±4.69	43.96±0.25
15.	254	γ-BHC	2.3 ± 0	2.4 ± 0.047
16.	266	Heptachlor epoxide	9.2 ± 0.047	9.4 ± 0.082
17.	296	α-BHC	1.3 ± 0.047	1.3 ± 0.047
		γ-BHC	6.7 ± 0.047	6.8 ± 0.047
18.	302	α-BHC	1.7 ± 0.047	1.7 ± 0.047
		γ-BHC	6.1 ± 0	6.0 ± 0.094
19.	326	α-BHC	7.7 ± 0.047	8.0 ± 0.094
		γ-BHC	9.1 ± 0.047	9.2 ± 0.047
20.	338	α-BHC	17.7 ± 0.017	16.8 ± 0.082
		γ-BHC	8.7 ± 0.047	8.7 ± 0.094
21.	344	pp′-DDT	Traces	Traces
		pp′-DDE	Traces	Traces
22.	356	Heptachlor epoxide	20.9 ± 0.047	21.0 ± 0.094
23.	368	α-BHC	2.1 ± 0	2.1 ± 0
		γ-BHC	8.2 ± 0.17	7.4 ± 0.047
24.	374	α-BHC	4.7 ± 0	4.8 ± 0
		γ-BHC	4.1 ± 0.047	4.3 ± 0.047
25.	386	Aldrin	6.6 ± 0.16	6.7 ± 0
		Diedlrin	46.4 ± 0.15	47.33 ± 0.15
26.	392	γ-BHC	5.1 ± 0.094	5.1 ± 0.047
27.	410	Dieldrin	32.4 ± 0.15	33.4 ± 0.15
28.	416	α-BHC	9.3 ± 0.047	9.3 ± 0
		γ-BHC	8.2 ± 0.047	8.3 ± 0.047
29.	428	α-BHC	1.4 ± 0	1.3 ± 0.047
		γ-BHC	6.3 ± 0.047	6.3 ± 0
		Aldrin	7.2 ± 0	7.5 ± 0.12
		Dieldrin	35.4 ± 0.235	35.9 ± 0.15
30.	434	pp′-DDT	Traces	Traces
31.	440	α-BHC	1.3 ± 0	1.4 ± 0
		γ-BHC	7.0 ± 0	7.1 ± 0
32.	446	pp′-DDT	Traces	Traces
		pp′-DDE	53.43 ± 0.2	54.46 ± 0.2
33.	452	γ-BHC	5.0 ± 0.047	5.1 ± 0.047
34.	458	γ-BHC	9.0 ± 0.082	9.3 ± 0.047
35.	464	Dieldrin	28.56±0.1	29.06±0.1
36.	469	γ-BHC	2.9 ±0.082	2.6 ±0.22

(Continued on column 2)

Traces = Numerical values can not be calculated.

*Each figure represents the mean value of three sub-samples with standard error.

aldrin in two samples in quantities of 6.6 and 7.6 μg/kg; and dieldrin has been reported in four samples in quantities ranging from 28.56 to 47.33 μg/kg.

It is evident from Table 2 that results obtained by both the GLC columns were consistent and comparable. Residues were qualitatively identified by comparison with standard pesticides while quantitative analysis was performed by the external standardization method. It has the advantage that calculation is based on a comparison of the size of the peak of a particular compound in the standard solution and the unknown and that no response factor or area correction factor is required.

Prior to monitoring work, the efficiency of analytical methodology was evaluated in model experiments in which different feed components procured from the market were mixed in a ratio stimulating the animal feed in the cattle colony. Weighed amounts of prepared feed samples were thoroughly ground and then spiked with known quantities of each studied pesticide separately and intimately mixed. It was then carried through the described

procedures and finally analysed by gas chromatography. Recoveries of different compounds ranged between 86.5 % and 106 %. Recovery data are presented in Table 1.

CONCLUSION

The presence of a large number of residues of different chlorinated pesticides or their metabolities in cattle feed indicates that its consumption may result in the deposition of these chemical compounds in fats and mammary glands of animals, its secretion in milk and ultimately its entry into the human body.

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