Pakistan J. Sci. Ind. Res., Vol. 31, No. 1, January 1988

MONITORING OF FRESH MILK FOR ORGANOCHLORINE PESTICIDE RESIDUES IN KARACHI

Zahida Parveen and S. Zafar Masud

Pest Management Research Institute, PARC, Old Block 9 and 10, Karachi University Campus, Karachi-32

(Received September 2, 1986; revised December 29, 1987)

Milk supplies in the Karachi Cattle Colony were monitored for organochlorine pesticide residues in the year 1984. A total of 79 samples of milk were analyzed out of which approximately 40% of the samples were found to be contaminated with either BHC isomers, pp'-DDT, pp'DDE, heptachlor epoxide, aldrin or dieldrin. The most frequently occurring pesticide was γ-BHC. The presence of aldrin residues in milk indicates that this product is still being used in Pakistan although its sale was banned in the 1970's. Recovery studies of pesticides added to milk are also included.

Key words: Chlorinated hydrocarbon residues, Fresh milk, Karachi.

INTRODUCTION

The use of pesticides in public health and agriculture has made a considerable impact on human health, production and preservation of food, fibre and other crops for increasing world population. High persistence of organochlorine pesticides in the environment, accumulation in living organisms, eggs and tissues of domestic animals has caused a reassessment of their beneficial effects.

Milk occupies an important place particularly in the diet of infants and children and plays a pivotal role in their growth and development. The presence of significant amounts of pesticide residues in milk is, therefore, undesirable. Organochlorine pesticides can persist in fat and mammary glands of animals and pass out in milk. It is, therefore, essential to obtain information on residue levels of these pesticides in milk in order to assess their health significance. Organochlorine pesticides have been detected in milk samples in various monitoring programmes conducted in several countries [1-7].

Studies presented herein have been undertaken with a view to assessing the levels of contamination of milk with persistent organochlorine pesticides in the Karachi Cattle Colony. The entire work has been completed in three phases. In the first phase, recovery studies were carried out to evolve a suitable analytical methodology for extraction, cleanup and quantitative determination of pesticides spiked with known amounts in milk. A total of thirteen pesticides or their metabolites were studied, namely, α-BHC, β-BHC, γ-BHC, δ-BHC pp'-DDT, pp'DDE, DDD (TDE), dicofol, heptachlor, heptachlor epoxide, aldrin, dieldrin, an endrin. In the second phase, random samples of milk were drawn from the cattle colony and frozen at -20° until analysis. In the third and final phase, the samples were systematically analysed for residues and the data were interpreted. A monitoring programme of such a magnitude has been undertaken for the first time in Pakistan.

METHODS

1. *Sampling.* The main objective was to obtain a sample representative of the lot in order to determine its average pesticide residue content. Sampling of milk was done from Karachi Cattle Colony which is spread over a vast area and comprises a total of four hundred and seventy four plots. These plots contain variable numbers of cows/buffaloes (mostly buffaloes), ranging between 30-400 animals per plot. Since sampling from each plot was not possible due to financial constraints and the capacity of the laboratory to store a large number of samples for residue analysis, it was decided to draw samples from every sixth plot. A total of seventynine samples were collected during the period from March to May 1984.

It became apparent from a discourse with the owners of various plots that they frequently use gammexane or other available insecticide formulations for pest control in their stores and surrounding area. They further informed the authors that they do not separate milk from cows and buffaloes and sell a mixture. Samples were drawn from their mixed lots and considered as final samples. Samples (0.5l) were kept in clean and dry glass bottles, sealed, properly labelled and brought to the laboratory. In the laboratory, each sample was subdivided into three subsamples of equal size and stored at −20° until analysis.

2. *Extraction and cleanup.* The method of Veierov and Aharonson [6] was followed with slight modification. The only modification was the addition of a saturated solution of sodium chloride at an appropriate stage in the process. It aids in breaking any emulsion that is formed.

The efficiency of this procedure was evaluated in model experiments with milk procured from the market. Milk (25 g) was taken in triplicate for each experiment and transferred to a 250 ml separatory funnel. It was then spiked with known amounts of each of the thirteen pesticide standards separately and shaken for 5 min. The spiked milk samples were allowed to stand for 30 min. at room temperature before proceeding for extraction and cleanup. Recovery was evaluated by means of gas chromatography and ranged from 60.0 to 114.3% except for dieldrin and endrin, in which case poor recoveries of only 1.30% and 2.4% respectively were obtained. Recovery data are presented in Table 1.

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

S. No.	Pesticide	Added	% Recovery*	
			7.5% QF-1/ 5% DC-200	1% NGS
		$\mu g\ Kg^{-1}$ (whole milk basis)		
1.	α-BHC	5.0	114.0 ± 1.41	114.3 ± 1.90
2.	β -BHC	10.0	71.0 ± 2.16	72.22 ± 1.34
3.	γ-BHC	5.0	83.67 ± 1.00	84.75 ± 1.07
4.	δ-BHC	10.0	70.82 ± 2.50	70.98 ± 1.76
5.	pp′DDT	50.0	70.80 ± 0.65	70.86 ± 0.61
6.	pp′-DDE	50.0	72.56 ± 1.25	72.18 ± 0.99
7.	DDD (TDE)	75.0	62.29 ± 1.35	62.67 ± 1.62
8.	Dicofol	100.0	60.00 ± 0.87	60.55 ± 1.42
9.	Heptachlor	9.0	70.46 ± 1.71	71.96 ± 1.33
10.	Heptachlor epoxide	9.0	71.21 ± 0.29	71.51 ± 0.29
11.	Aldrin	8.0	71.0 ± 1.41	72.45 ± 1.04
12.	Dieldrin	500	1.34 ± 0.02	1.30 ± 0.01
13.	Endrin	500	2.34 ± 0.008	2.4 ± 0.009

*Mean and standard error of three analysis.

3. *Gas-liquid chromatographic determination.* For identification and quantitation of studied compounds, a Pye-Pan Chromatograph equipped with an electron capture detector was employed. Confirmation of the identity of unknown compounds in milk samples was done on two GLC columns each packed with a different liquid stationary phase.

3.1 *Operating parameters.* 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 on 80/100 mesh chromosorb-W (Phase Separations Limited Cheshire, England), and (ii) 1% NGS supported on 80/100 mesh chromosorb W. Temperatures, column oven 150° detector oven 175°; detector voltage, 1V for column (i) and 5 V for column (ii) pulsed; electrometer setting, 10^{-10} amp. full scale; nitrogen (carrier gas) flow rate, 65 ml min^{-1}; Honeywell Recorder, 10 mv; Chart Speed, 8 mm min^{-1}.

The two columns were conditioned at 175° for 24 hr before use. The above operating parameters were employed for both columns and found suitable for all the investigated pesticides. Two different column materials have been used with a view to varying the retention times of studied pesticides to confirm their identity.

The linearity of response was checked by injecting different concentrations of analytical grade insecticides into the GLC column and noting their peak heights. The instrument behaved in a linear manner throughout. Retention times for thirteen studied compounds on two different column materials are presented in Table 2.

Table 2. Retention time values of studied pesticides on two different column materials

S. No.	Pesticide	Retention time (min)	
		A mixture of 7.5% QF_1 + 5% DC-200 on 80/100 mesh chrom. w.	1% NGS on 80/100 mesh chrom. w.
1.	α-BHC	1.06	0.55
2.	β-BHC	2.0	0.87
3.	γ-BHC	1.44	0.62
4.	δ-BHC	2.25	1.06
5.	pp′-DDT	15.0	7.60
6.	pp′-DDE	7.37	3.80
7.	DDD (TDE)	12.75	6.00
8.	Dicofol	16.25	2.70
9.	Heptachlor	2.19	1.10
10.	Heptachlor epoxide	5.25	2.45
11.	Aldrin	2.87	1.45
12.	Dieldrin	7.63	4.5
13.	Endrin	10.25	5.00

Each cleaned-up sample extract was analysed by gas chromatography along with its insecticide standard in *n*-hexane using 2-5μl injections. The amount of insecticide in each sample extract was calculated by comparing its peak height with that of the standard. The peak height was calculated by measuring the vertical distance from the peak apex to a line forming the base line of the peak. For ease

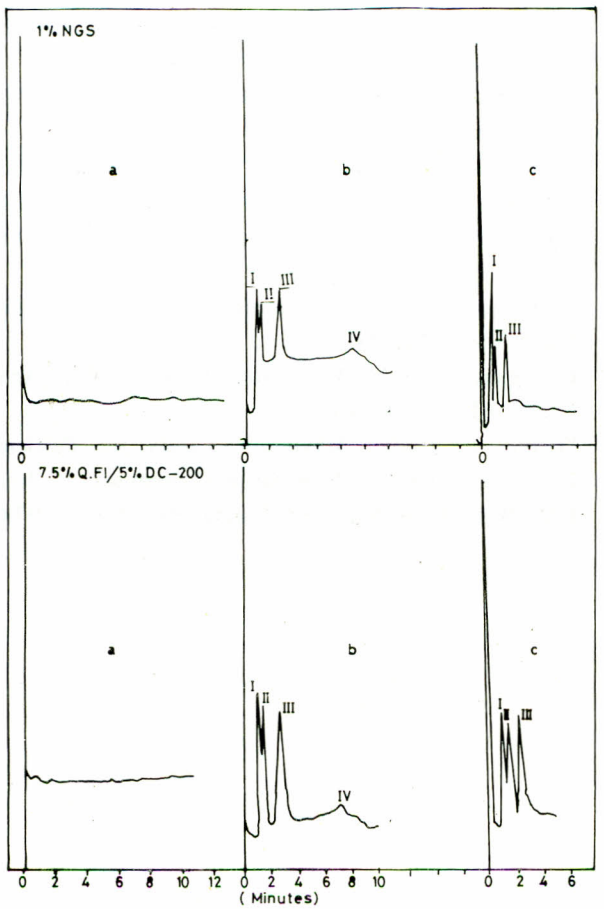

Fig: 1 Dual-column analysis of organochlorine pesticides in representative milk samples. (a) Control, (b) Sample from plot No. 236. peak identification (I) α-BHC (II) γ-BHC, (III) Aldrin and (IV) Deldrin, (c) sample from plot No. 266, peak identification (I) α-BHC, (II) γ-BHC & (III) δ-BHC.

in calculation, the unit of parts per billion or $\mu g/kg^{-1}$ has been adopted throughout the study. Whenever the sensitivity of the column went down resulting in poor GLC response for the compounds of interest, the column material was replaced and conditioned for optimum sensitivity as already described.

RESULTS AND DISCUSSION

Pesticide residue methods are the basic tools for the analyst in determining residues in samples of unknown history. In a residue determination, the aim is to get rapid, quantitative screening, identification and quantification of as many residues as possible within the shortest possible time.

The procedures are reliable, rapid, easy to standardize and suitable for monitoring eleven out of thirteen organochlorine compounds investigated (Table 1). Extremely poor recoveries were obtained for dieldrin and endrin by this method. This is due to the fact that sulphuric acid reacts with these two compounds resulting in low recoveries. The procedure is, therefore, not recommended for dieldrin and endrin.

In monitoring studies, out of 79 milk samples screened, 29 were found to be contaminated with different organochlorine pesticides or their metabolites. Range of different pesticide or their metabolites detected in contaminated milk samples is presented in Table 3. It is evident from the Table that α-BHC was detected in 19 samples in concentrations that ranged from 4.8 to 946.0 μgkg^{-1} γ-BHC was reported in 27 samples in concentrations from 5.1 to 850 μgkg^{-1} out of which 7 samples were found to contain residue levels higher than the recommended MRL (maximum residue limits); δ-BHC was monitored in four samples in concentrations ranging from 10.7 to 64.1 μgkg^{-1}; aldrin was present in quantities of 24.2 to 29.5 μgkg^{-1} in two samples only and both the values exceeded recommended MRL [8] fixed by Codex Alimentarius Commission; heptachlor epoxide was found (23.3 μgkg^{-1}) only in one sample and its value also exceeded MRL while pp'-DDE and dieldrin were found in traces.

Aldrin is no longer in use in Pakistan, but since the results were confirmed by two different GLC column materials, it was concluded that probably some old stock of aldrin had been used for pest control in some cultivated area or locality.

Table 3. Organochlorine pesticide residues (μgkg^{-1}) found in milk samples on two different GLC columns.

S. No.	Pesticides studied	No. of contaminated milk samples	Range of residues (μgkg^{-1})	
			7.5% QF_1 + 5% DC-200	1% NGS
1.	α-BHC	19	5.0–946	4.8–946
2.	β-BHC	N.D*	–	–
3.	γ-BHC	27	5.2–850.77	5.1–850
4.	δ-BHC	4	10.7–64.1	11.0–63.4
5.	pp′–DDT	N.D	–	–
6.	pp′-DDE	1	Traces**	Traces
7.	DDD (TDE)	N.D	–	–
8.	Dicofol	N.D.	–	–
9.	Heptachlor	N.D	–	–
10.	Heptachlor epoxide	1	23.6	23.3
11.	Aldrin	2	24.2–30.3	25.4–29.5
12.	Dieldrin	2	Traces	Traces
13.	Endrin	N.D.	–	–

* Not detected.

** Numerical value cannot be calculated

CONCLUSION

The above described monitoring studies demonstrate the presence of organochlorine pesticides or their metabolites in a large number of milk samples drawn from the Karachi Cattle Colony. On the basis of present investigations, it is suggested that similar studies should be undertaken in other major milk supply centres in the country in order to draw a national picture for overall assessment of the situation.

REFERENCES

1. D. Samuel Faust and H. Irwin, Suffet; Residue Reviews, **15**, 44, (1976).
2. S. Moore, W.N. Bruce, D.E. Kuhlman and R.E. Randell, Pestic. Monit. J., **11**, 94 (1973).
3. J.S. Wedberg, Moore, F.J. Amore and H. McAvoy, Pestic. Monit. J., **11**, 161 (1978).
4. K.L. Steffey, J. Mack, C.W. MacMonegle and H.B. Petty., J. Environ. Sci. Health, **B19**, (1), 49 (1984).
5. B.J. Devidow, Assoc. Off. Agric. Chem., **33**, 130 (1950).
6. D. Veierov and N. Aharonson, J. Assoc. Off. Anal. Chem. **63**, 532 (1980).
7. D. Veierov and N. Aharonson, J. Assoc. Off. Anal. Chem., **61**, 253 (1978).
8. "Guide to Codex Maximum Limits for Pesticide Residues: Codex Alimentarius Commision", Joint FAO/WHO Food Standards Programme, Rome (1978).