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ANALYTICAL DATA

BHC RESIDUES IN MILK: A GAS CHROMATOGRAPHIC INVESTIGATION

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BHC residues were determined in milk samples of cows and buffaloes (Indore, M.P., India). α -, β - and γ -BHC were identified in samples from four different localities using gas chromatography. All samples were contaminated with BHC, with the toxic gamma isomer appearing in the largest concentration. Buffalo milk was more contaminated than cow milk, with maximum values of 17 and 12 $\mu\text{g/l}$, respectively.

INTRODUCTION

BHC (benzenehexachloride, (1,2,3,4,5,6-hexa-chlorocyclohexane or $\text{C}_6\text{H}_6\text{Cl}_6$) is one of the most commonly used organochlorine insecticides. It is very effective against many pest species, due to its long persistence, low water solubility and lipophilic characteristics. The very same properties of BHC pose serious environmental pollution problems: BHC residues enter into the food chain and accumulate in various tissues of target and non-target organisms. Because of phenomena like bioconcentration and biomagnification, the BHC residues accumulate in organisms in increasing concentrations at each trophic level in the environment. The toxic constituent of BHC is γ -BHC.¹

Milk-producing animals such as cows, buffaloes and goats (common Indian milk-producing breeds) accumulate residues of insecticides, while grazing in insecticide-applied areas or through contaminated food. Residues of organochlorine insecticides are conventionally determined using gas chromatography (GC). The present study summarizes data on BHC residues of milk of two common milk-producing animals, buffaloes and cows, using GC.

MATERIALS AND METHODS

Samples of milk of buffaloes (80 samples) and cows (80 samples) were collected from four different sources (Indore, Madhya Pradesh, India).

Table 1 BHC residues in cow milk ($\mu\text{g/l}$)^a

Locality	α -BHC	β -BHC	γ -BHC	Total BHC
1	0.2-1.1	ND-0.4	7.2-9.2	7.6-10.7
	0.7	0.2	8.0	8.9
2	ND-1.6	ND-0.2	6.8-8.4	6.8-10.2
	0.5	0.1	7.5	8.1
3	0.6-2.1	0.4-1.0	7.6-9.1	8.6-12.2
	1.1	0.7	8.1	9.9
4	0.1-0.5	0.1-0.7	6.1-9.7	6.3-10.9
	0.3	0.4	8.4	9.1

^aData are given for four localities, and give ranges ($t > 0.01$) and mean ($t > 0.01$) values.

For the extraction process 100 ml of sample were thoroughly mixed with 10 ml of saturated sodium chloride solution in a separatory funnel. 75 ml of pure and distilled hexane were added to the separator, which was shaken vigorously. The mixture was allowed to stand till the two phases were clearly separated. The aqueous phase was transferred to a second separator and the process was repeated. The hexane extracts were collected and subjected to clean-up procedure. 1 g of charcoal (analytical-grade; BDH, Glaxo Lab., Bombay) was added per 100 ml of extract in a conical flask which was shaken for 2 min. The adsorbent was allowed to settle down. The supernatant was transferred to a funnel fitted with Whatman No. 1 filter paper. The filtrate was collected and concentrated in a flash evaporator.

The residues were analysed by GC on a Packard 437-A model gas chromatograph equipped with an electron capture detector having a Ni^{63} radioactive foil and a Shimadzu Chromatopac integrator CR-3A. The $6' \times \frac{1}{4}''$ glass columns used had a 2 mm inner diameter and contained 15% OV-17 + 1.95% OV-202 on Chromosorb WHP, 80/100 mesh as stationary phase. Pure and dry nitrogen was used as the carrier gas at a flow rate of 25 ml/min. The injector, oven and detector temperature were all set at 220°C.

0.5 μl of the concentrated extract was injected into the gas chromatograph with a Hamilton microlitre syringe. The peaks of the BHC isomers were identified by comparison with the injection of standards. The BHC recovery from fortified

samples was above 80%. RESULTS AND DISCUSSION

The BHC residue levels in the milk samples of buffaloes and cows are reported in Tables 1 and 2. All samples were found to be contaminated with BHC. Buffalo milk was more contaminated than cow milk, which may well be due to the higher fat level of the milk (6% versus 4.5%). γ -BHC, which is the most toxic component of BHC, was present in much larger concentrations than α -BHC and β -BHC in the milk samples of both animals.

The maximum concentration of total BHC residues was 17.3 $\mu\text{g/l}$ and 12.2 $\mu\text{g/l}$ in buffalo and cow milk, respectively. The mean values per locality of the three

Table 2 BHC residues in buffalo milk ($\mu\text{g/l}$)^a

Locality	α -BHC	β -BHC	γ -BHC	Total BHC
1	1.2–2.1	0.6–0.9	9.8–13.6	11.6–16.6
	1.7	0.7	11.6	13.9
2	0.8–1.7	0.6–0.7	11.1–14.1	12.5–16.5
	1.2	0.6	12.9	14.7
3	0.3–1.5	0.1–1.2	8.6–10.8	9.0–13.5
	0.8	0.5	9.2	10.5
4	2.4–4.3	ND-1.2	9.1–11.8	11.5–17.3
	3.8	0.6	10.4	14.8

^aData are given for four localities, and give ranges ($t > 0.01$) and mean ($t > 0.01$) values.

isomers showed maxima of 8.1, 1.1 and 0.7 $\mu\text{g/l}$ for the γ -, α - and β -isomers, respectively, in cow milk, and of 12.9, 3.8 and 0.7 $\mu\text{g/l}$ in buffalo milk.

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Reference

1. Matsumara, F., *Toxicity of Insecticides* (Plenum Press, New York, 1980).