Bull. Environ. Contam. Toxicol. (2001) 67:554–559

DOI: 10.1007/s00128-001-0159-y

Bulletin of Environmental Contamination and Toxicology

Monitoring of Market Fish Samples for Endosulfan and Hexachlorocyclohexane Residues in and Around Calcutta

R. K. Kole, H. Banerjee, A. Bhattacharyya

AICRP on Pesticide Residues, Pesticide Residue Laboratory, Department of Agricultural Chemicals, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur 741252, West Bengal, India

Received: 12 January 2001/Accepted: 1 June 2001

The widespread use of organochlorine (OC) pesticides has created significant impact in our environment. Although the use of most of the chlorinated pesticides has been curtailed, the chemical and physical stability of these compounds has resulted in their continuing presence in the environment and in the food chain (Sannino *et al.* 1996). The residue burden in fish have been observed to decline since the banning of many OC insecticides in Western countries (Murty 1986), but their presence is continued in fish from different regions of developing countries (Kole and Bagchi 1995). Fish, particularly those living in paddy fields, may be an important source of residues in the diet. In Africa, residues of OC pesticides in edible freshwater fish are often so high that only a small quantity consumed could exceed the acceptable daily intake (Conway and Pretty 1991). Therefore thorough monitoring of the OC pesticide residues is crucial for proper assessment of human exposure to these toxic contaminants through food. As a part of our research programme under All India Co-ordinated Research Project on Pesticide Residues, we reported the occurrence of endosulfan residues in fish from the markets in and around Calcutta, West Bengal during the period 1988–1993 (Chakravarty *et al.* 1996). West Bengal is the largest producer of inland fish and consumes 1.0 million tonnes of it annually, the highest among all the states in India (Hunter 1995–96). With a view to generate residue data and to determine the trend of OC pesticide residues occurring in market fish samples in and around Calcutta, the earlier study was continued in respect of endosulfan as well as HCH residues for yet another five year period (1993–94 to 1997–98) for safety evaluation to consumers. The results are reported in the present paper.

MATERIALS AND METHODS

Fish samples belonging to the species *Labeo rohita* and *Catla catla* were collected in two different groups of size viz. large (0.5 – 1.0 kg) and small (50 – 200 g) from different markets of Calcutta and suburbs during the period from 1993–94 to 1997–98. Large fish samples (~ 500 g) were collected by combining different pieces from 4 – 5 large fish after removing scales, fins and other non–edible portions. Small fish samples (~ 500 g) were constituted of 5 – 6 numbers of fish after removing scales, fins and other non-edible portions. All the samples were cut into small pieces, taken in ice-box and carried to the laboratory for analysis.

Correspondence to: A. Bhattacharyya

Samples were thoroughly blended in a mixer to form a pulp. For endosulfan, the sample pulp (5.0 g) was extracted with acetonitrile : tetrahydrofuran (1 : 1 v/v) mixture (Chakravarty *et al.* 1996) and was further partitioned to hexane after adding saturated aqueous Sodium Chloride solution. The hexane extract containing the endosulfan residues was cleaned up by activated charcoal (Darco G – 60) and filtered using hexane : acetone (9 : 1 v/v) mixture as washing solvent. The filtrate was concentrated in a rotary vacuum evaporator (RVE) and the volume was made upto 10 ml with hexane for analysis of endosulfan isomers by gas chromatography (GLC).

For analysis of HCH residues, 5.0 g of fish pulp was ground in a mortar with anhydrous Sodium Sulfate to form a free flowing powder. Dry powder was extracted with 200 ml of hexane in a Soxhlet apparatus for 8 hr. The hexane extract was concentrated in a RVE and cleaned up with concentrated Sulphuric acid. After thorough washing with distilled water, the hexane layer was further cleaned up using a florisil column (AOAC 1990). The elution with 6% diethyl ether in petroleum ether (200 ml) was concentrated and the volume was made upto 10 ml with hexane for estimation of HCH isomers by GLC.

The residues of endosulfan and HCH in the cleaned up extracts were estimated by GLC (Hewlett Packard, Model 5890 A) coupled with an integrator (HP 3392 A) using electron capture detector (ECD). The glass columns (1.8 m × 2 mm id) were packed with 3% OV – 101 (for endosulfan) and with mixture of 3% OV–17 + 1.95% OV – 210 (for HCH) on chromosorb WHP (80 – 100 mesh). The oven, detector and injector temperatures were maintained at 200, 300 and 210^{0}C respectively for endosulfan and 160, 300 and 210^{0}C respectively for HCH residues. Nitrogen was used as the carrier gas with a flow rate of 60 ml min^{-1}.

The methods described for analysis of endosulfan and HCH residues were standardised following fortification of α and β - endosulfan, α, β, γ and δ - HCH each at 0.25, 0.5 and 0.1 μg g^{-1} concentrations in both large and small fish samples. The average recovery was found in the range of 88 – 94%.

RESULTS AND DISCUSSION

The results regarding endosulfan residues occurring in fish samples are presented in Table 1. More than 50% of 235 samples analysed during the period 1993–94 to 1997–98 were found to be contaminated with endosulfan residues in the overall range of 0.01 – 1.41 μg g^{-1}. About 16% of the samples were above the maximum residue limit (MRL) of endosulfan (0.2 μg g^{-1}) prescribed for meat on fat basis (FAO / WHO 1978). The distribution of endosulfan residues in large and small fish samples has been presented in Figure 1 (a and b respectively). Majority of the contaminated samples were observed in the residue range of 0.01 – 0.20 μg g^{-1}.

The results of HCH residues detected in fish are shown in Table 2. The percent contamination by HCH residues was much higher than that observed by endosulfan residues. More than 80% of 157 samples were detected for HCH

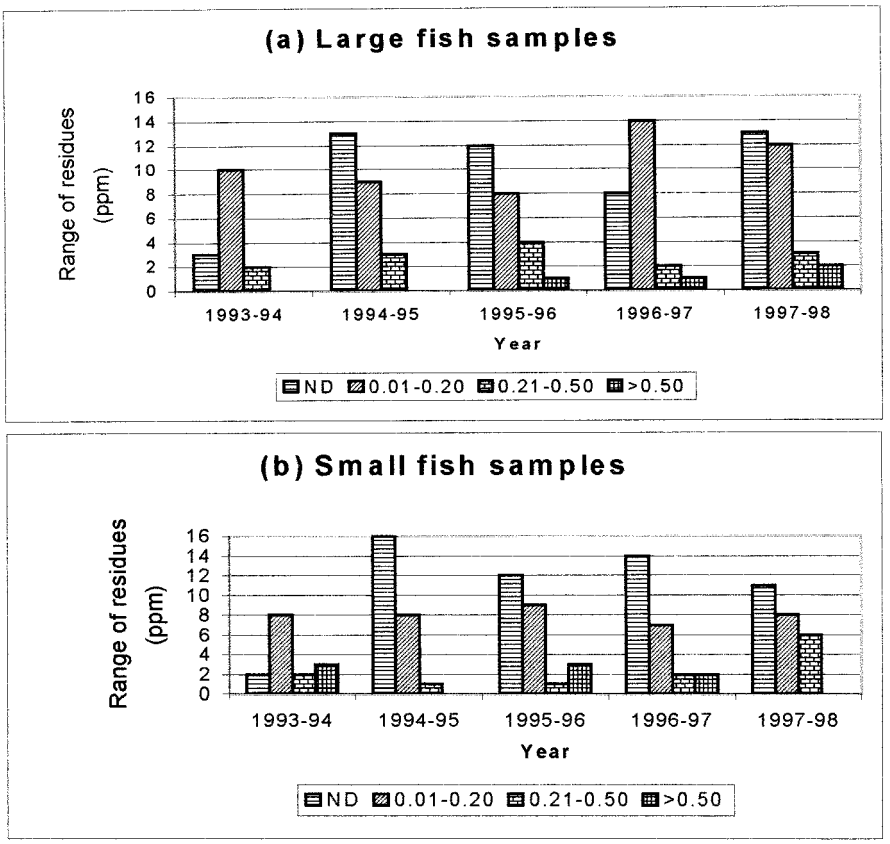

Figure 1. Distribution of endosulfan residues in fish.

Table 1. Extent of fish contamination with endosulfan residues.

Year	No. of fish samples analysed	No. of samples found contaminated	Range of residues of endosulfan (α+β) in $\mu g\ g^{-1}$	No. of samples above MRL
1993-94	30	25	0.01 – 1.24	7
1994-95	50	21	0.02 – 0.48	4
1995-96	50	26	0.01 – 0.87	9
1996-97	50	28	0.01 – 1.08	7
1997-98	55	31	0.01 – 1.41	11
Total :	235	131	0.01 – 1.41	38

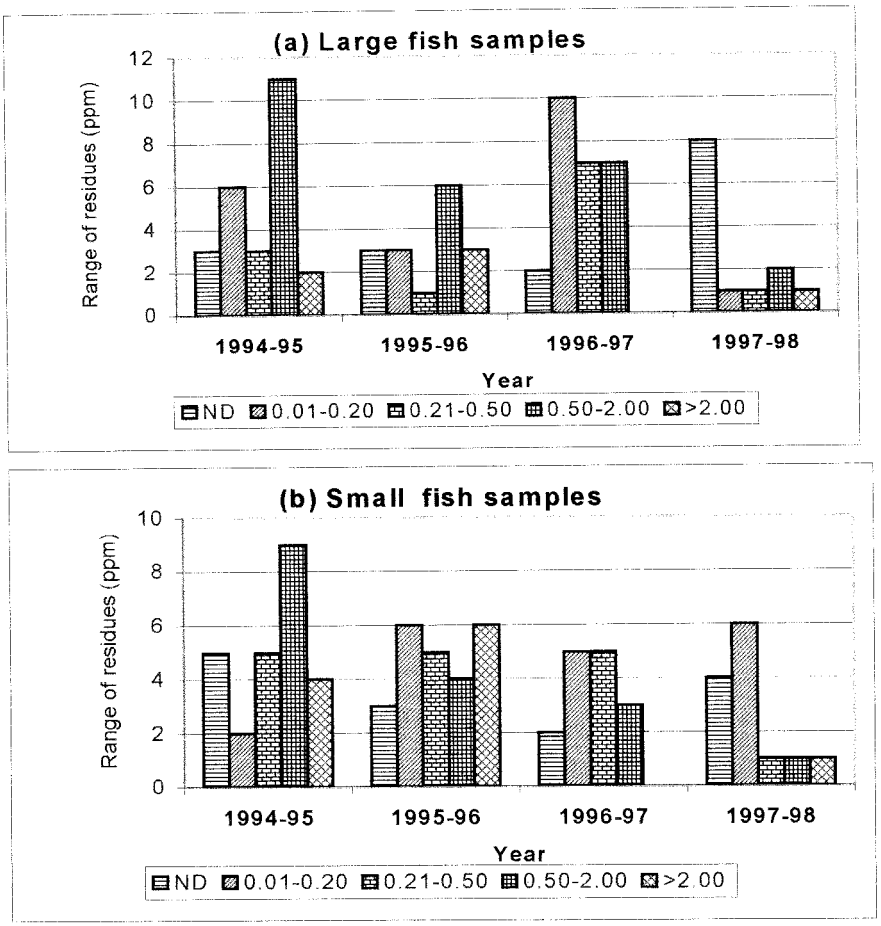

Figure 2. Distribution of HCH residues in fish.

Table 2. Extent of fish contamination with HCH residues

Year	No. of fish samples analysed	No. of samples found contaminated	Range of residues of HCH ($\alpha+\beta+\gamma+\delta$) in $\mu g\ g^{-1}$	No. of samples above MRL
1994-95	50	42	0.10 – 3.76	26
1995-96	40	34	0.10 – 8.69	19
1996-97	41	37	0.02 – 1.53	10
1997-98	26	14	0.01 – 9.72	5
Total :	157	127	0.01 – 9.72	60

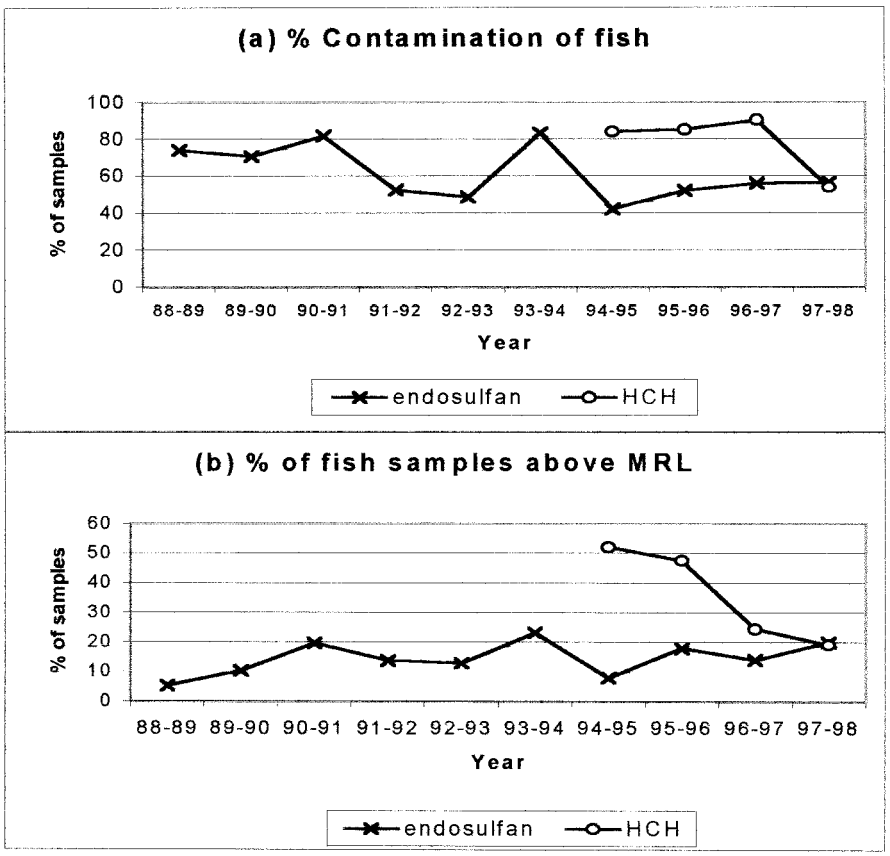

Figure 3. Trend in fish contamination with endosulfan and HCH residues.

residues in the overall range of 0.01 – 9.72 μg g^{-1}. The extent of contamination was, therefore, much higher by HCH than that by endosulfan residues. About 38% of the samples were above the legal limit (0.5 μg g^{-1}) of HCH (FAO 1983). The distribution of HCH residues in large and small fish samples is presented in Figure 2 (a and b respectively). The majority of the contaminated samples were in the range of 0.21 – 2.00 μg g^{-1} of HCH residues. However, the extent of HCH residues in small fish considerably decreased during 1996–98 over the previous period.

The trend regarding fish contamination with endosulfan and HCH residues is presented in Figure 3. The contamination with endosulfan residues was compared with the results of the previous study (Chakravarty *et al.* 1996) for the period of 10 years 1988–89 to 1997–98. A slow decreasing trend of contamination of fish with endosulfan residues was observed during the recent years (Figure 3a). However, the percentage of samples above MRL exhibited almost a steady state (Figure 3b). The percentage of samples above MRL value of HCH is much higher than that obtained for endosulfan. Interestingly, a declining trend in

percentage contamination as well as in percentage samples above MRL was observbed in case of HCH residues (Figure 3 a, b). Dogheim *et al* (1990) also reported fish samples from two Egyptian Governorates to exceed the residue limits of several organochlorine pesticides including HCH isomers.

Acknowledgments. We thank the Scientists of the Pesticide Residue Laboratory, BCKV for their cooperation. They also express their gratitude to AICRP on Pesticide Residue for financial support and to BCKV for infrastructural facilities.

REFERENCES

AOAC (1990) Pesticide and industrial chemical residues. In : Helrich K (ed) Official methods of analysis of the Association of Official Analytical Chemists, 15th edn., AOAC, Inc., Virginia, p 279

Chakravarty A, Pal S, Das AK, Sukul P, Bhattacharyya A, Adityachaudhury N (1996) Monitoring of endosulfan residues in market fish samples. Pestic Res J 8 : 164 – 167

Conway GR, Pretty JN (1991) Pesticides and human health. In : Unwelcome harvest – agriculture and Pollution, Earthscan Publications Ltd., London, p 137 – 139

Dogheim SM, Nasr EN, Almaz MM, El – Tohamy MM (1990) Pesticide residues in milk and fish samples collected from two Egyptian Governorates. J Assoc Off Anal Chem 73 : 19 – 21

FAO (1983) Compilation of legal limits for hazardous substances in fish and fishery products. Food and Agriculture Organization of the United Nations, Rome, Italy

FAO / WHO (1978) Acceptable daily intake, residue limits and guideline levels proposals at Joint Committee meeting. Food and Agriculture Organization of the United Nations, World Health Organization, p 23

Hunter B (1995 – 96) The Statesman's Year Book, 132 edn., Macmillan Press Ltd., London, p 753

Kole RK, Bagchi MM (1995) Pesticide residues in the aquatic environment and their possible ecological hazards. J Inland Fish Soc India 27 : 79 – 89

Murty AS (1986) Toxicity of pesticides to fish, Vol 1, CRC Press, Inc. Boca Raton, Florida

Sannino A, Mambriani P, Bandini M, Bolzoni L (1996) Multiresidue method for determination of organochlorine insecticides and polychlorinated biphenyl congeners in fatty processed foods. J Assoc Off Anal Chem 79 : 1434 - 1446