INTERIM REPORT
ANNEX B

INVESTIGATION OF PESTICIDE RESIDUE LEVELS IN FLOODPLAIN FISH IN BANGLADESH

FAP 17
FISHERIES STUDIES AND PILOT PROJECT

JULY 1993

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Contents

1 SUMMARY

1.1 In May 1992 samples of four fish species were collected from four small perennial beels in the Tangail Compartmentalisation Pilot Project area. All beels were surrounded by HYV rice fields receiving organophosphate and carbamate pesticides in March and April 1992. Pesticide residue analyses carried out in Bangladesh detected no organophosphate residues in fish tissue but revealed low levels of organochlorine pesticides: DDT, dieldrin and endosulphan. Further analyses undertaken in the UK as a cross-check of the results revealed low levels of DDD and DDE, metabolites of DDT, and also dieldrin but no endosulphan. The results indicated slight organochlorine pesticide pollution in floodplain ecosystems. The implications of the results relating to fish survival and human health are discussed.

2 INTRODUCTION

2.1 One of the principal aims of flood control and drainage schemes in Bangladesh is not only to provide protection for people and infrastructure against annual monsoon flooding but also to convert seasonal wetlands to agricultural land for increased rice production. In recent years reliance has been placed on high yield varieties (HYV) of rice which generally require greater amounts of pesticides and fertilisers to maintain production targets.

2.2 Pesticide use is not effectively controlled in Bangladesh and toxic persistent pesticides such as DDT, although banned from agricultural use, are reported to be imported illegally from India and also obtained from sources within country for protection of rice crops. The amount of registered pesticides used in Bangladesh has increased considerably in recent years. These comprise mainly organophosphates such as diazinon, dimecron and nogos used to protect HYV boro rice (Anon, 1989). There is growing concern about their possible harmful effects on floodplain fish and people (Abul Bashar & Reazuddin, 1990; World Bank, 1991). The situation is unlikely to improve in the absence of adequate monitoring programmes.

2.3 A literature search revealed only two previous studies on pesticide residue levels in floodplain ecosystems in Bangladesh, both of which were supported by the British Overseas Development Administration (Roberts *et al.*, 1986 and 1989). These studies were carried out in connection with surveys of ulcerative disease in fish, but included very few samples from floodplain habitats receiving pesticides. It was therefore decided that as part of the FAP 17 fisheries programme a preliminary investigation of pesticide residue levels in floodplain fish should be undertaken in areas known to receive a range of pesticides used to protect HYV rice in the winter season. However, since FAP 17 did not have the capability to carry out pesticide residue analyses, a survey was first made of laboratory facilities and technical expertise in various scientific institutions to assess the feasibility of undertaking such analyses in Bangladesh.

2.4 Details of institutions visited during an earlier FAP 17 Design Study (July-September, 1991) and as part of FAP 17 (January-April, 1992) are given in Appendix I. Of these only the Institute of Food and Radiation Biology (IFRB) of the Atomic Energy Research Establishment at Savar had both laboratory facilities and technical expertise to immediately undertake pesticide residue analysis. The Institute had previously carried out DDT analyses in dried fish and had examined levels of a range of organochlorine and organophosphate pesticides in agricultural crops.

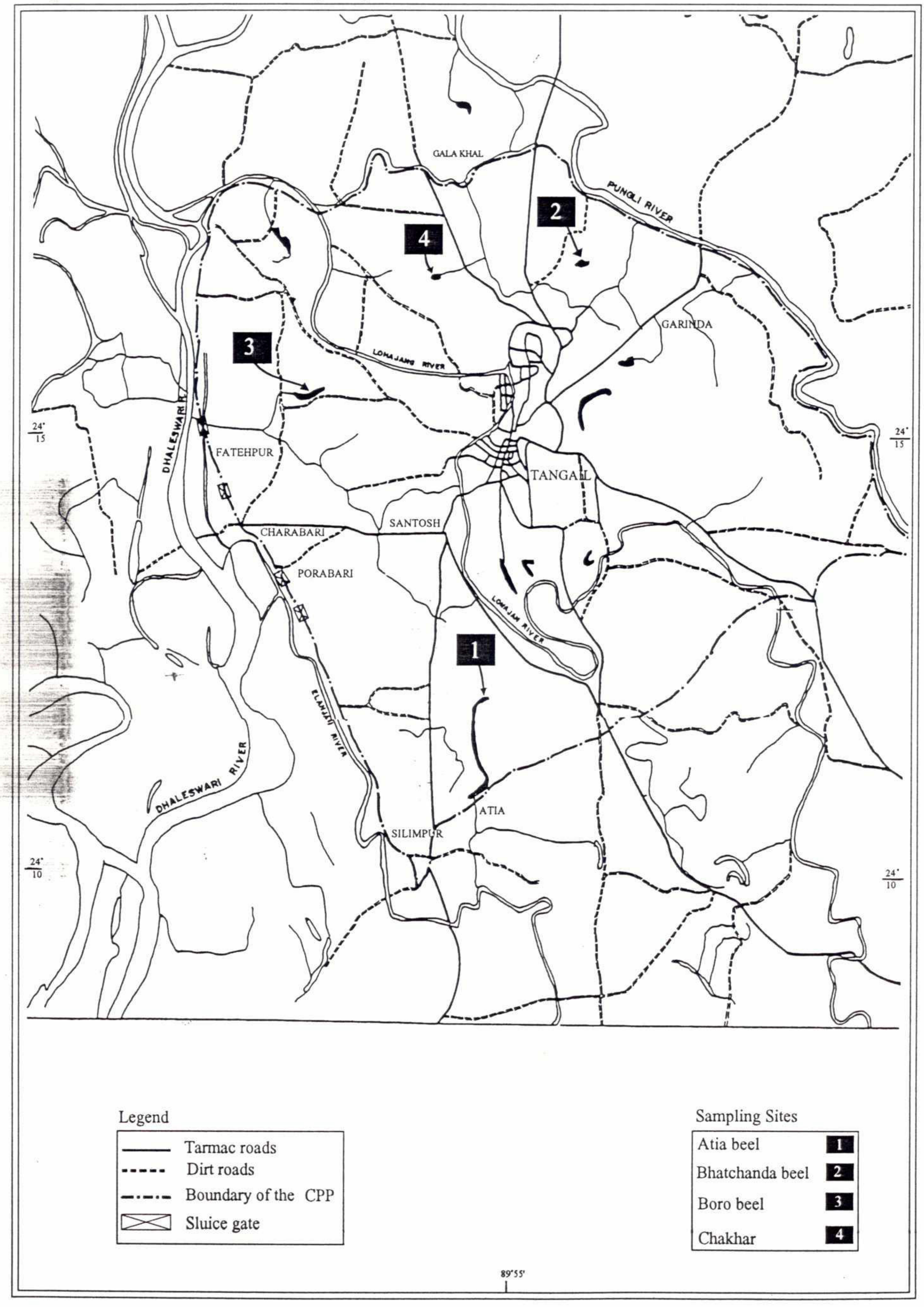
Study Area

2.5 The Tangail Compartmentalisation Pilot Project (CPP) was selected as the study area (Figure 2.1). The area surrounds the town of Tangail and lies within a flood control scheme designed to prevent annual floods from the Northern Dhaleswari and Pungli rivers. The area contains about 15 small perennial beels all of which are surrounded by HYV irrigated rice fields during the dry season. Based on information supplied by FAP 20, four beels were selected, ranging in size from 8 to 100 acres of water in the dry season. The areas immediately surrounding these beels planted with HYV rice ranged from 260 to 564 acres. Interviews with 10 farmers growing IRRI rice next to the beels revealed that a range of pesticides, mainly organophosphates, had been applied to protect the crop during March and April 1992 (Table 2.1). None of the farmers reported knowingly using DDT or any other organochlorine pesticide. No fishermen or farmers reported fish mortalities in the beels resulting from pesticide use in adjacent rice fields but fish from all beels suffered from ulcerative disease in 1991.

Table 2.1 Pesticides Used during 1992 to Protect IRRI Rice Crop at Selected Sampling Sites

Site	Pesticides Used	Pesticide Group	Months Applied
Atia beel	Dimecron Nogos (=Dichlorvos) Furadan	Organophosphate Carbamate	March/April
Bhatchanda beel	Basudin (=Diazinon)	Organophosphate	April
Boro beel	Dimecron Diazinon Basudin (=Diazinon)	Organophosphate " "	March/April
Chakhar beel	Basudin Furadan (=Carbofuran)	Organophosphate Carbamate	March/April

Figure 2.1 Location of Sampling Sites



3 METHODS

Field

3.1 Fresh fish samples were collected from catches of fishermen in four beels on 6 May 1992. The catches were wrapped in aluminium foil and stored in cool boxes containing ice made from deionised water. From the catches four species of fish were selected for pesticide residue analysis. The selected species included piscivores such as *Channa punctatus* and detritivores such the spiny eel, *Mastacembalus armatus* and the loach, *Lepidocephalus guntea* both of which also prey on invertebrates in the mud. Details of the samples are given in Table 3.1. All fish were weighed, coded and labelled then stored in freezers in Tangail and Dhaka prior to dispatch to IFRB in Savar.

Table 3.1 Fish Samples Collected for Pesticide Residue Analysis

Site	Sample Code	Species	Numbers of Fish	Total WT(g)	Length Range (cm)
Atia beel	ODA-1	*Mystus vittatus (tengra)*	20	130	77-98
Atia beel	ODA-3	*Mastacembelus armatus (baim)*	13	90	98-147
Atia beel	ODA-5	*Lepidocephalus guntea (gutum)*	26	110	64-90
Atia beel	ODA-4 + ODA-10	*Channa punctatus (taki)*	7	340	119-220
Bhatchanda beel	ODA-15	*Mastacembelus armatus (baim)*	20	160	110-160
Bhatchanda beel	ODA-16	*Mystus vittatus (tengra)*	20	160	80-101
Bhatchanda beel	ODA-17	*Channa punctatus (taki)*	6	350	120-200
Boro beel	ODA-7	*Mastacembelus armatus (baim)*	20	90	81-125
Boro beel	ODA-8 + ODA-11	*Lepidocephalus guntea (gutum)*	20	80	66-92
Chakhar beel	ODA-12	*Lepidocephalus guntea (gutum)*	20	110	70-96
Chakhar beel	ODA-14	*Mastacembelus armatus (baim)*	20	140	105-132

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Laboratory

3.2 Frozen fish samples were analysed at IFRB of the Atomic Energy Research Establishment in Savar near Dhaka. In all cases, samples were identified only by code numbers so as to avoid unintentional bias of the results. In all but one case, analyses were carried out on homogenised muscle tissue and not on organs such as the liver where residue levels would normally be expected to be higher. This approach was taken because fish of the size range analysed are usually gutted for human consumption and one of the aims of this investigation was to relate pesticide residue levels in fish to health risks for humans. Between 3 and 20 individual fish of each species, depending on the size range, were pooled to provide a single sample of 25 grammes for analysis. Thus, pesticide levels reported in this study represent averages for a number of individuals for each species.

3.3 Standard methods of extraction, clean-up and analysis using gas-liquid chromatography (GLC) were employed at IFRB to determine the concentration of a range of organochlorine and organophosphate pesticides. Various checks on the reliability of the methods were carried out at IFRB. These included "blind" replicate analyses on one sample (code ODA-16), duplicate analyses on other samples and the use of samples "spiked" with known concentrations of pesticide standards to determine recovery rates. As a further check on the reliability of these methods, three samples of muscle were sent to laboratories of the Natural Resources Institute (NRI), UK for analysis of organochlorines.

4 RESULTS

4.1 Results of analyses carried out at IFRB are presented in Table 4.1. A total of 10 samples comprising 126 individual fish of four species was screened for organochlorine and organophosphate pesticide residues. Low concentrations of three types of organochlorine pesticides were detected by GLC analysis, these comprised DDT, dieldrin and endosulphan. These pesticides were reported not to have been used by local farmers during 1992. In contrast, the organophosphate pesticides used by the farmers in March and April 1992 were not detected by IFRB but these normally degrade very rapidly in the environment in contrast to DDT and dieldrin which are highly persistent and can accumulate in certain tissues, especially those with a high lipid content (Muirhead-Thomson, 1987). IFRB lacked the analytical equipment needed for the detection of carbamates. Therefore information on the effect of Furadan applications by farmers is lacking.

4.2 There was generally close agreement between the results of duplicate analyses of DDT and dieldrin carried out at IFRB. Even when "blind" replicates were used, agreement was good in all but one sample (Table 4.1).

4.3 The results of analyses undertaken at NRI, on 3 fish muscle samples are shown in Table 4.2. These results are compared with those of IFRB for the same samples (Table 4.3). The analyses by NRI revealed no DDT but detected very low concentrations of DDT breakdown products, DDD and DDE. This difference may be expected if further metabolism, especially anaerobic metabolism, occurred during storage or transit of samples to the UK. In fact the flight to the UK was delayed for 9 hours but NRI reported that the samples still arrived fully frozen.

4.4 The NRI analyses revealed no trace of endosulphan I and II in contrast to the IFRB results which indicated low levels in two of the three test samples. In addition, dieldrin was found in only one of the three samples analysed by NRI at a concentration within the range reported by IFRB.

4.5 In all but one case analyses were carried out on muscle. In this one case, analyses were carried out by mistake on whole ungutted fish (*L. guntea*) from two combined sites (sample: ODA-5+8+11). Since organochlorines such as DDT and dieldrin accumulate more readily in fat deposits, higher concentrations are normally found in organs such as the liver and ovaries. Therefore, higher levels should be expected in whole fish samples. However, the results revealed the opposite: whole fish samples showed the lowest levels of DDT and the third lowest levels of dieldrin (Table 4.1).

4.6 Analysis of spatial variation in pesticide residue levels was difficult since only one species (spiny eel) was found in the catches at all sites. In addition, concentrations were generally very low and in the case of DDT and dieldrin many were near the lower limits of detection. Therefore small variations in concentrations between sites were probably meaningless.

4.7 Pesticide residue levels recorded by IFRB varied between different species, highest levels were found in the snakehead, *C. punctatus*, a predatory species at the top of the aquatic food chain where persistent pesticides would normally be expected to accumulate. However, the results from NRI relating to DDD and DDE levels showed the opposite trend with highest values found in the spiny eel and lowest in the snakehead.

Table 4.1 Pesticide Residue Levels in Fish (Analysis by IFRB, AEC Savar, Dhaka)

Species	Sample code N°	N° fish in one sample	DDT (mg/kg)	Dieldrin (mg/kg)	Endosulfan Sulfate II (mg/kg)	Endosulfan I (mg/kg)	Organophosphorus compounds (mg/kg)
Mastacembelus armatus	ODA - 3	13	0.0024 0.0018 0.0021	0.0012 0.0026 0.0019	ND	ND	ND
Mastacembelus armatus	ODA - 7	8	0.0039 0.0022 0.0031	0.0014 0.0016 0.0015	ND	ND	ND
Mastacembelus armatus	ODA - 14	7	0.0040 0.0046 0.0043	0.0062 0.0060 0.0061	ND	ND	ND
Mastacembelus armatus	ODA - 15	6	0.0027 0.0028 0.0028	0.0018 0.0020 0.0019	ND	ND	ND
*Lepidocephalus guntea**	ODA - (5+8+11)	20	0.0017 0.0005 0.0011	0.0018 0.0005 0.0012	0.0121	Trace	ND
Lepidocephalus guntea	ODA - 12	13	0.0013 0.0013 0.0013	0.0008 0.0008 0.0008	0.0103	Trace	ND
Channa punctatus	ODA - 17	3	0.0249	0.0054 0.0064 0.0059	0.2275	0.0544	ND
Channa punctatus	ODA - (4+10)	7	0.0171	0.0074 0.0060 0.0067	0.1495	0.0544	ND
Mystus vittatus	ODA - 1	20	0.0061	0.0032	0.0743	Trace	ND
Mystus vittatus	ODA - 16	19	0.0015	0.0010	0.0214	Trace	ND
*Mystus vittatus***	ODA-X=16	19	0.0012	0.0007	0.0170	Trace	ND
*Mystus vittatus***	ODA-Y=16	19	0.0048	0.0006	0.0186	Trace	ND

Notes: ND = Not detectable (limits of detection not given), * = Analyses carried out by mistake on whole ungutted fish, ** = "Blind" homogenised replicates provided by FAP 17

Table 4.2 Pesticide Residue Levels of Fish Analysis by NRI, UK.

Sample: ODA 10, Species: *Channa punctatus*	
mg kg^{-1} p'p-DDD :0.005 p'p-DDE :0.004 o'p-DDT : <0.003 p'p-DDT : <0.004	mg kg^{-1} Dieldrin : 0.003 Endosulphan I : <0.001 Endosulphan II : <0.001
Sample: ODA 12, Species: *Lepidocephalus guntea*	
mg kg^{-1} p'p-DDD :0.007 p'p-DDE :0.007 o'p-DDT : <0.004 p'p-DDT : <0.005	mg kg^{-1} Dieldrin : <0.001 Endosulphan I : <0.001 Endosulphan II : <0.001
Sample: ODA 15, Species: *Mastacembalus armatus*	
mg kg^{-1} p'p-DDD :0.012 p'p-DDE :0.012 o'p-DDT : <0.002 p'p-DDT : <0.004	mg kg^{-1} Dieldrin : <0.001 Endosulphan I : <0.001 Endosulphan II : <0.001

Notes:

1. The results are reported above as mg active ingredient per kg of fish ("wet weight"). No corrections have been made to the results to take moisture or fat content into account.

2. Where no residues were detected, a result of less than (<) the limits of detection of the analytical technique used are reported. These lower limits of detection also take into account the individual sample weights, hence the difference in some of the figures.

Table 4.3 Comparison of the Results of Pesticide Residue Analysis from IFRB (AEC), Bangladesh and NRI, UK.

Pesticide	Sample code no.	IFRB (mg/kg)	NRI (mg/kg)
DDT	ODA - 15	0.0028	ND(<0.007)
	ODA - 12	0.0013	ND(<0.009)
	ODA - 10	0.0171	ND(<0.006)
Dieldrin	ODA - 15	0.0019	ND(<0.001)
	ODA - 12	0.0008	ND(<0.001)
	ODA - 10	0.0067	0.003
Endosulphan I	ODA - 15	ND	ND(<0.001)
	ODA - 12	Trace	ND(<0.001)
	ODA - 10	0.0544	ND(<0.001)
Endosulphan II	ODA - 15	ND	ND(<0.001)
	ODA - 12	0.0103	ND(<0.001)
	ODA - 10	0.1495	ND(<0.001)

Note: Limits of detection of methods used at NRI are given is parentheses when pesticides could not be detected.

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5 DISCUSSION

5.1 The results of this study indicate low level pollution of floodplain habitats by organochlorine pesticides DDT and dieldrin. Some doubt remains concerning endosulphan pollution since its presence in fish tissues was not confirmed by analytical tests made in the UK. This compound is the least persistent in the environment of the organochlorine pesticides. The source of the pollution has not been confirmed in this study but it seems likely to originate from the use of unregistered, illegal pesticides by farmers to protect their rice crops. The detection of DDT and its metabolites DDD and DDE together with dieldrin in fish muscle tissue gives little indication of the time of application of these compounds onto the floodplain environment since they are capable of not only persisting and bioaccumulating in various organisms within the food chain but can also persist in soil, silt and mud for long periods of time (Muirhead-Thomson, 1971, 1987; WHO, 1989).

5.2 With regard to DDT pollution and its derivatives DDD and DDE, concentrations of 0.025 mg/kg found in the most contaminated fish, snakehead (sample ODA-17, Table 4.1) and the loach (sample ODA-15, Table 4.2) were well below safe values for tissue levels: 0-0.2 mg/kg (EPA, 1977). The same is true for dieldrin levels with safe concentrations of 0.3 mg/kg.

5.3 In terms of human health, levels of DDT were well below the Codex Alimentarius maximum residue limit for meat, 7 mg/kg (FAO/WHO, 1978) and the US Food and Drug Administration action level for DDT in fish fillets, 5 mg/kg. Even the most contaminated fish would have been safe to eat given the Codex Acceptable Daily Intake of DDT residue limit of 0.005 mg/kg body weight. Thus, a person weighing 50kg could safely consume 0.25 mg/kg of DDT per day which is equivalent to 10 kg of the most contaminated fish (0.025 mg/kg) per day. This is an enormous amount compared with the estimated daily per capita fish consumption of about 30g.

5.4 A literature search revealed only two previous studies of pesticide residues in freshwater fish in Bangladesh (Roberts *et al.*, 1986 and 1989). The studies were ancillary parts of broader studies of epizootic ulcerative fish disease. The first study found no detectable levels of pesticide residues in the two snakeheads analysed. The later study found no organophosphate or carbamate residues in fish tissues but detected low levels (0.01-0.03 mg/kg) of the organochlorines, dieldrin, p'p-TDE and αHCH. The studies reported no direct link between pesticide pollution and the incidence of ulcerative fish disease in Bangladesh and Southeast Asia.

5.5 The absence of organophosphate pesticide residues in fish tissues found in this study and reported in previous studies carried out in Bangladesh does not imply that there is no pollution problem threatening fish survival in rice fields and adjacent water bodies. Although these compounds are reported to degrade rapidly (within days) in the environment they are highly toxic to fish, even at low concentrations. The immense damage caused by pesticides to fish cultured in the rice fields of Southeast Asia and beyond has been well established (e.g. Arce and Cagauan, 1988). However, in Bangladesh rice-fish culture is still in an infancy stage (CARE, 1992) and very little is known about the size and species composition of wild fish populations in boro rice fields. In a survey of 610 farmers throughout Bangladesh, 71% reported seeing fish mortalities when they applied pesticides to their boro rice crops (Anon., 1989). Unfortunately, the study did not report the magnitude of fish mortalities observed by farmers.

5.6 In Bangladesh, efforts are currently being made to promote the reduction in pesticide use in rice fields by the adoption of rice-fish culture associated with integrated pest management techniques (CARE, 1992). Such efforts should be encouraged since they offer large-scale potential not only for decreased environmental pollution but also increased cultured fish production in the winter season.

6 CONCLUSIONS

6.1 The results revealed the presence of low concentrations of organochlorine pesticide residues in fish tissues which were not considered harmful to human consumers.

6.2 From a total of 14 scientific institutions visited during this study to assess the feasibility of undertaking pesticide residue analysis in Bangladesh, only one was able to do so but needed assistance from FAP 17 to provide certain consumable items.

6.3 In Bangladesh, there is a serious lack of quantitative information on the effects of pesticides on inland floodplain fisheries.

6.4 There is a large potential to increase rice-fish culture in Bangladesh but the widespread use of pesticides to protect rice crops poses a major constraint on development.

7 RECOMMENDATIONS

7.1 On the basis of the preliminary results obtained in this study, it is not considered appropriate for FAP 17 to continue more detailed and extensive investigations into pesticide residue levels in floodplain fish which would require considerable additional financial support to the project.

7.2 There is a need to strengthen the institutional capability to undertake pesticide residue analysis in Bangladesh. This would involve the provision of analytical equipment with the necessary maintenance services and training to improve technical expertise.

7.3 More information is needed on the effect of pesticides on the wild fish populations in Bangladesh. The Department of Fisheries and the Department of Agricultural Extension should use their existing field personnel to carry out systematic, quantitative surveys of fish mortalities caused by pesticides.

7.4 In view of the great potential for increased rice-fish culture in Bangladesh, it is recommended that the development of small-scale integrated pest management schemes such as that established by CARE be included as an integral part of future rice-fish culture projects. Such projects must carefully consider the role of rapidly degradable pesticides in their development programmes.

ACKNOWLEDGEMENTS

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Close cooperation was provided by the Department of Fisheries and special thanks go to Mr R Banik, Senior Scientific Officer who collected data on pesticide use at each sampling site. FAP 20 kindly provided background data on several beels in the CPP from which our site selection was made. Finally, thanks go to Mr C Price (BAFRU) who, despite a 9 hour flight delay managed to deliver frozen fish samples to the UK for further analysis at NRI, UK.

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Appendix I

Institutions and personnel visited during a survey of institutional capability for pesticide residue analysis in Bangladesh (July 1991 - March 1992).

1. Atomic Energy Research Establishment (AERE), Institute of Food & Radiation Biology (IFRB), Savar.
   Contacts: Dr. N. Choudhury, Director
   Dr. M. Matin, Chief Scientific Officer

2. Bangladesh Agricultural Research Institute (BARI), Gazipur.
   Contacts: Dr M K Chowdhury, Senior Scientific Officer

3. Bangladesh Agricultural University (BAU), Mymensingh
   Contacts: Professor Whab, Lecturer

4. Bangladesh Council of Scientific Industrial Research (BCSIR), Dhaka.
   Contacts: Dr. A. Rahman, Director

5. Fisheries Research Institute (FRI), Mymensingh
   Contacts: Professor M. Aminul Islam, Director

6. Fisheries Research Institute (FRI) Riverine Laboratory, Chandpur
   Contacts: Mr. U. Haroon, Senior Scientific Officer

7. Institute of Postgraduate Studies in Agriculture (IPSA), Salna, Gazipur
   Contacts: Dr. E. Ahsan, Director

8. International Centre of Diarrhoeal Disease Research, Bangladesh (ICDDR, B), Dhaka.
   Contacts: Professor D. Habte, Director

9. Ministry of Agriculture, Department of Agricultural Extension, Plant Protection Wing, Dhaka.
   Contacts: Mr. A K M Azad, Entomologist

10. Ministry of Environment and Forest, Department of Environment, Environment and Water Pollution Laboratory, Dhaka
    Contacts: Dr M A H Pramanik, Director General

11. River Research Institute, Faridpur
    Contacts: Mr A K M Shamsul Hoque, Director General

12. University of Dhaka, Dept of Biochemistry
    Contacts: Professor M. Rahman, Chairman
    Dr. A. Hossain, Assistant Professor

13. University of Dhaka, Institute of Nutrition and Food Science
    Contacts: Dr. S. Keramat Ali, Director

14. University of Dhaka, Dept. of Zoology
    Contacts: Professor Abu Tweb Abu Ahmed, Chairman