

INCIDENCE OF DDT AND ITS METABOLITES IN DRY FISHES AND EVALUATION OF WASHING AND BOILING TREATMENT FOR DIETARY INTAKE

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ABSTRACT

A qualitative and quantitative analysis was carried out to determine the level of o,p'-DDT, p,p'-DDT, DDD and DDE residues in ten species of dried fishes collected from Dhaka, Gazipur, Chittagong and Coxbazzar metropolitan market in Bangladesh, and to evaluate washing and boiling treatment to the fishes for decontamination. The results indicated that all the fishes were found to be contaminated with DDT's residues. The residues of DDT in the fish samples ranged from 0.05 to 6.73 ppm. The concentration of p,p'-DDT was found greater than all other metabolites. The concentration of DDT's in the fish samples, except in promfret collected from coxbazzar exceeded the permissible maximum residues limit (MRL) recommended by FAO/ WHO. The maximum concentration of DDT was found in spider prawn followed by white shrimp. Washing and boiling treatment did not significantly reduce the residues. Normal washing removed 8-25% and washing plus ten minutes boiling treatment removed 3-30% residues. Therefore, it is better to avoid DDT contaminated dry fishes for consumption.

Keywords: DDT, DDD, DDE, dry fish, residues.

INTRODUCTION

Dichloro-diphenyl-trichloro-ethane (DDT) and its metabolites such as Dichloro-diphenyl-dichloroethane (DDD) and Dichloro-diphenyl-dichloro-ethylene (DDE) residues are organochlorine insecticide which cause genotoxicity and endocrine disruption in humans (Mostafalou and Abdollahi 2013). Altogether, these are

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called DDT's and have been linked to diabetes (Bhuiyan *et al.* 2008). DDT's also causes breast, liver and pancreatic cancer in human being. As a persistent environmental contaminant and widespread use, DDT's has resulted in worldwide contamination. DDT's can be stored in all tissues but is found most prominently in fats. With its long half-life and its global use, no living organism may be considered DDT free (Turosov *et al.* 2002). It is estimated that it would take 10 to 20 years for an individual to become DDT free, even if all DDT exposures could be avoided during that time. In Bangladesh, DDT was banned in 1997 (Rahman *et al.* 2012). Despite a prohibit imposed by World Health Organization (WHO) on use, but it is still used in large quantity in dried fishes.

During the monsoon period, the dry fishes absorbed moisture rapidly which act as predisposing factor to be infestation by flies, beetles and mites (Hill 2002). Most unexpected causes of infestation are that the fishermen do not dry fishes properly to avoid loss of weight. For protection of dry fish from infestation they use a mixture of organochlorine (DDT and heptachlor) insecticides (Cohn 2007). Some reports in Bangladesh show alarming pollutants in fish like DDT and heptachlor in fish (BCAS 1990). In Kuakata (a fish processing zone in Bangladesh), high level of DDT powder (locally known as white powder) is used frequently (Dureja and Rathore 2012).

Residue studies on organo-chlorine compound were confined mostly in fresh water fishes for quality control. Only DDT and some polychlorinated biphenyls (PCBs) was assessed in India (Kaur *et al.* 2008) and Iran (Kafilzadeh *et al.* 2012). Many authors studied on quality assurance (Quevauviller 2008, Bhuiyan *et al.* 2008), seasonal trend (Bellassoued *et al.* 2013), pesticide screening (Bhuiyan *et al.* 2008) and toxicological effects (Niimi, 2011) of dry fishes. But very little investigations have been made on the research efforts on the quantification of DDT and its metabolites in dry fishes and evaluation of cooking effect for its dietary intake. Therefore, the present work was undertaken to find out the status of DDT's in ten species of dry fishes procured from four metropolitan markets namely Dhaka, Gazipur, Chittagong and Cox's bazar in Bangladesh and to evaluate the washing and boiling effect on the reduction of DDT's for dietary purpose.

MATERIALS AND METHODS

The study was conducted during January to December, 2013 by collecting dry fishes from the metropolitan market of Bangladesh during January to December, 2013. The selected markets are Gazipur (Joydevpur, Bazar), Dhaka (Kawran bazar), Chittagong (Reazuddin bazar) and Cox bazaar (Teknaf bazaar) in Bangladesh. Both wholesale and retail shops were chosen in the market to purchase dry fish.

Instruments: The samples were finely comminuted in a fish mincer (a grinding machine). Fat was extracted by Soxhlet apparatus (Soxtherm, Gerhardt Bonn, Germany). The elute, extract or filtrate was concentrated by rotary vacuum evaporator (Yamato, Japan). The pesticide detection and analyses were performed in GC-17A (Gas Chromatography, origin Shimadzu Co. Ltd, Japan) with Electron Capture Detector (ECD) and auto sampler (AOC 20i).

Chemicals and reagents: In this study, the analytical grade organic solvents (*n*-hexane, acetone, ethyl alcohol and di ethyl ether and petroleum ether) were obtained from Merck (Darmstadt, Germany). Anhydrous sodium sulfate (Na_2SO_4), Conc. H_2SO_4 , Florisil (60-100 mesh size) and Silica gel was also purchased from the market.

Pesticide standards: The Organochlorine pesticide standard Dichloro-diphenyl-triichloroethane (o,p'-DDT and p,p'-DDT), Dichloro-diphenyl-dichloroethane (DDD) and Dichlorodiphenyldichloroethylene (DDE) standard (from Dr. Ehrenstorfer GmbH) was bought from registered chemical importer of Bangladesh.

Standards and working solutions: The Concentration of stock standard solution was prepared at concentration of 10 ppm. For calibration, standard solutions of DDT were prepared at concentrations of 1.0, 2.5, 5.0, 10.0 ppm in *n*-hexane.

Sample collection and storage: Ten species of fish (Table 1.) were selected and depending upon their availability each species was collected from four markets (weighing 400–500 g) which constitute a total sampling of 40 fish. Samples were collected in airtight polythene bag with proper tagging and labeling. The samples were transported to laboratory of pesticide and environmental toxicology of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) under chilled conditions and were stored at - 20 C till processing as described by Uddin *et al.* 2007.

Table 1. List of selected fish for residue analysis

Sl. No.	Local name	Common / English name	Scientific name
1.	Chapila	Indian gizzard	<i>Gonialosa manmina</i>
2.	Kaski	River sprat	<i>Corica soborna</i>
3.	Loittyia	Bombay duck	<i>Harpadon nehereus</i>
4.	Nona illish	Hilsha	<i>Hilsa ilisha</i>
5.	Rupchanda	Promfret	<i>Pampus argenteus</i>
6.	Deshi chanda	Glassy fish	<i>Parambassis ranga</i>
7.	Koral	Barramundi	<i>Lates calcarife</i>
8.	Puti	Pool barb	<i>Puntius sophore</i>
9.	Gura chingri	Spider prawn	<i>Macrobrachium tenuipes</i>
10.	Chaka chingri	White shrimp	<i>Penaeus indicus</i>

The first halves (SS1) were used for residue analysis individually in respect of four metropolitan markets and second halves (SS2) for examining washing and boiling effect on dietary intake. Here same fish species from different market are mixed to form a composite sample. The composite samples (SS2) were divided into three parts. 1st part was prepared as it was remained (without washing); 2nd part was prepared after vigorous washing with normal water (Normal washing); 3rd part was prepared after washing followed by 10 min boiling (boiling wash).

Extraction: Fifty grams dried fish sample was taken from SS1 and each part of SS2. The samples were sliced and oven dried for overnight at 75°C. Then it was grounded by grinding machine. The powdery mass was sieved and prepared for extraction and clean up. Twenty gram (20 g) of each sample were then taken into mortar and pestle with 2-5 g of anhydrous sodium sulfate (Na_2SO_4). Then it took few minutes for equilibration and the samples became moisture free. The powdery mass was extracted exhaustively with 100 ml *n*-hexane in soxhlet apparatus for 8 hrs. The anhydrates material was then poured into 250 ml conical flask with 100 ml *n*-hexane and placed into orbital shaker for 12 hrs continuous shaking. After shaking, the slurry was filtered through a funnel with suction. The flask and filter cakes were rinsed with 25ml of *n*-hexane each. The extract was concentrated to 50 ml using a rotary vacuum evaporator. The filtrate was collected in a centrifuge tube which was then centrifuged at 14000 rpm for 10 minutes. After centrifuge, supernatant was collected in 250 ml conical flask for cleanup.

Clean up: The concentrated extract was primarily cleaned up by adding 40 ml conc. H_2SO_4 drop wisely as developed by Gupta (2012). The cleaned up n-hexane was then passed through anhydrous Na_2SO_4 and concentrated in rotary evaporator to 20 ml. Finally, it was cleaned up by florisil column chromatography. Briefly, a glass column (2 cm wide x 50 cm long) with Teflon stop cock was taken and clumped it vertically. A cotton plug was inserted at the the bottom of the column with the help of glass rod. About 1 cm layer of anhydrous Na_2SO_4 was placed above the cotton plug. The column was packed with activated florisil 10-15 g and tapped gently to get uniform packing. Again 1 cm layer anhydrous Na_2SO_4 was placed at the top of the florisil. The 20 ml concentrated n hexane extract was transferred to the column. The flask was rinsed with 5-10 ml eluting solvent (n-hexane) and transfer carefully to column. The residue was concentrated in a rotary vacuum evaporator and finally made up the volume to 20 ml for estimation.

Analysis: After extraction and clean up, the sample was injected (1 μ l) into the column of Gas Chromatograph (GC-17A, Shimadzu Co. Ltd, Japan). The analysis was made in the following GC Conditions (Table 2.): The chromatogram of standards and a sample of dry fish are presented in Appendix III.

Table 2. Details of gas chromatographic conditions using in this study

Parameter	: Condition			
Column	: Column name: Rtx-CL pesticide (Sl# 726625) Column Length: 30 mtr Inner Diameter: 0.32 mm Film thickness: 0.50 μ m Column Max Temp: 340 $^{\circ}$ C			
Column temperature program	: Rate $^{\circ}$ C/min	Temperature ($^{\circ}$ C)	Hold time (min)	Total program
	---	160	1.0	14 min
	15	220	4.0	
	10	240	3.0	
Oven temperature	: 300 $^{\circ}$ C			
Injector temperature	: 280 $^{\circ}$ C			
Detector temperature	: 240 $^{\circ}$ C			
Gas flow rate	: Nitrogen as carrier, 30 mL min ⁻¹			
Injection volume	: 1 μ L			

Estimation of DDT residues: The quantity of residue present in the sample was calculated by using both formula (noted below) and lab solution software for GC-17 using calibration curve.

$$\text{Residue (ppm)} = \frac{\text{Area produced in unknown sample} \times \text{quantity of final volume}}{\text{Area produced standard sample}}$$

An aliquot was injected into the GC with auto injector. Tentative identification of the suspected insecticide was carried out in relation to retention times of the pure analytical standard.

RESULTS AND DISCUSSION

Forty samples of ten different dry fishes were analyzed to detect and quantify the residues of DDT and its metabolites to elucidate their contamination level. Table 3 represents the concentration of o,p'-DDT, p,p'-DDT, DDD and DDE residues in the dried fish samples available in four metropolitan markets. All the fish species were found contaminated with DDT's residue. Food and Agricultural Organization (2005) has fixed recommended maximum residue limits (MRLs) for fishes which is usually called codex MRLs. The general value codex MRL for fish is 0.2 to 0.3 ppm (Kaur *et al.* 2008). The concentration of DDT's in all fish samples ranged from 0.05 to 6.73 ppm.

DDT and its metabolites contamination in dry fishes: The highest level of DDT residue (6.73 ppm) was found in spider prawn sample collected from Chittagong metropolitan city market (Reazuddin Bazar) which was followed by Indian white shrimp (5.49 ppm) (Table 3). The DDT's concentration in shrimp was approximately 22 times than the recommended MRLs set by Codex alimentarius commission. The levels of DDT's were more than FAO/WHO recommended Maximum Residue Limits (MRL), except the samples extracted from promfret (*Pampus argenteus*) collected from Coxbazzar. There is only 0.05 ppm which is below the Codex MRL.

DDD and DDE were not detected in the fish sample of promfret of Cox's bazar and Indian glassy fish of Gazipur market. The absence of DDT's in promfret might be due to the high value of this fish and fishermen and fish trader do not need to preserve the fish for longer period. Besides, the promfret is usually sold in super market and shopping mall where the quality is strictly maintained. On the other hand, glassy fish could preserve many days without any chemical treatment

and it might be the reason for no appearance of DDT's in this dry fish species. Without a few exceptions a general trend of sequential variation was observed in DDT's level in four metropolitan markets. The trend is: Chattagong > Coxbazzar > Dhaka > Gazipur. The reason for greater concentration in Chittagong region could be the high availability of DDT powder in Chittagong medical depot, where a large amount of unused DDT remains stockpiled since 1983 (IPEP 2005). Among the selected pesticides p,p'-DDT concentration was found higher in most of the samples which was followed by o,p'-DDT, DDE and DDD.

Table 3. Quantity of DDT and its metabolite residues in dried fishes in Bangladesh.

Name of the fish species	Area of collection	Quantity of DDT, DDD and DDE residues (ppm)				Σ DDT
		p,p'-DDT	o,p'-DDT	DDD	DDE	
1. Indian gizzard	Gazipur	0.58	0.51	0.32	0.35	1.75
	Dhaka	0.60	0.52	0.36	0.33	1.81
	Chittagong	0.63	0.56	0.35	0.38	1.92
	Coxbazzar	0.62	0.55	0.34	0.38	1.88
2. River sprat	Gazipur	1.18	1.04	0.64	0.72	3.58
	Dhaka	1.16	1.02	0.63	0.70	3.52
	Chittagong	0.84	0.74	0.46	0.51	2.56
	Coxbazzar	0.52	0.46	0.29	0.32	1.59
3. Bombay duck	Gazipur	1.03	0.90	0.56	0.62	3.12
	Dhaka	0.98	0.86	0.54	0.60	2.98
	Chittagong	1.06	0.93	0.58	0.64	3.22
	Coxbazzar	0.95	0.84	0.52	0.58	2.88
4. Hilsha	Gazipur	0.71	0.63	0.39	0.43	2.16
	Dhaka	0.84	0.74	0.46	0.51	2.56
	Chittagong	1.09	0.95	0.59	0.66	3.29
	Coxbazzar	0.70	0.62	0.38	0.43	2.13

5. Promfret	Gazipur	0.12	0.11	0.07	0.07	0.37
	Dhaka	0.14	0.12	0.08	0.08	0.42
	Chittagong	0.15	0.13	0.08	0.09	0.45
	Coxbazzar	0.02	0.01	nd	0.01	0.05
6. Glassy fish	Gazipur	0.30	0.27	0.17	nd	0.74
	Dhaka	0.38	0.33	0.21	0.23	1.15
	Chittagong	0.45	0.39	0.24	0.27	1.35
	Coxbazzar	0.25	0.22	0.14	0.15	0.75
7. Barramundi	Gazipur	1.03	0.90	0.56	0.62	3.12
	Dhaka	1.02	0.90	0.56	0.62	3.10
	Chittagong	1.10	0.97	0.60	0.67	3.33
	Coxbazzar	0.94	0.83	0.51	0.57	2.86
8. Pool barb	Gazipur	0.46	0.40	0.25	0.28	1.38
	Dhaka	0.41	0.36	0.23	0.25	1.25
	Chittagong	0.37	0.32	0.20	0.22	1.12
	Coxbazzar	0.32	0.28	0.17	0.19	0.96
9. Spider prawn	Gazipur	2.15	1.89	1.17	1.30	6.25
	Dhaka	2.05	1.80	1.12	1.24	6.20
	Chittagong	2.22	1.95	1.21	1.35	6.73
	Coxbazzar	2.04	1.79	1.11	1.24	6.18
10. White shrimp	Gazipur	1.68	1.47	0.91	1.02	5.08
	Dhaka	1.73	1.52	0.94	1.05	5.23
	Chittagong	1.81	1.59	0.99	1.10	5.49
	Coxbazzar	1.45	1.27	0.79	0.88	4.39

Notes: nd = Not detected

Average residues of DDT : The average residues also calculated from individual metropolitan market (Fig. 1.). The highest concentration was observed in spider prawn (6.41 ppm) followed by white shrimp (5.05 ppm). The lowest concentration was observed in Promfret (0.33 ppm).

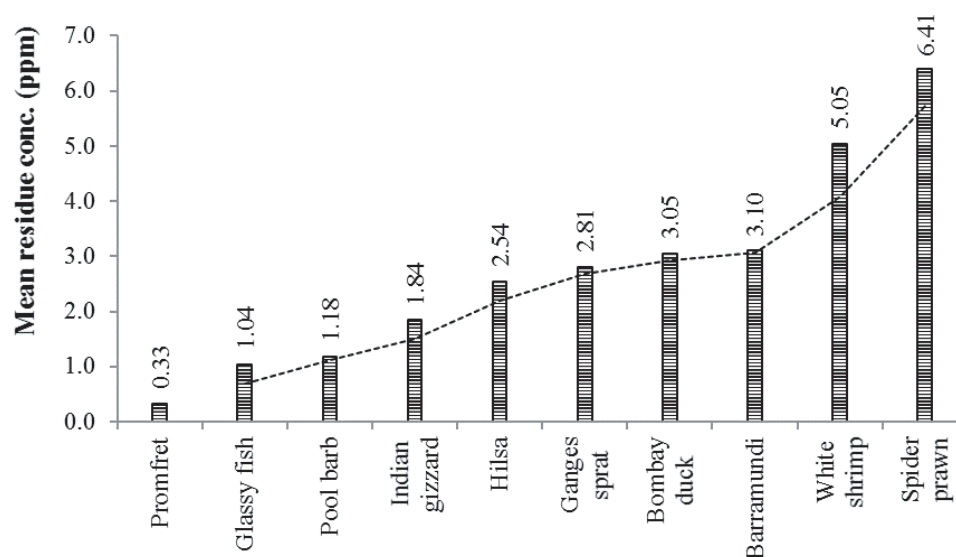


Fig. 1. Sequential order of DDT concentration (low to high) observed in dry fishes

Considering the average residue in four metropolitan market, the ten dry fish species follows the following order: Spider prawn > White shrimp > Barramundi > Bombay duck > Ganges sprat > Hilsha > Indian gizzard > Pool barb > Glassy fish > Promfret (Fig. 2).

Effect of washing and boiling treatment in reducing DDT's residues

The composite sample of each individual fish species collected from four metropolitan markets were studied to know the effect of washing and boiling treatment. The investigation was primarily aimed to estimate the effect of various washing and boiling/cooking to elucidate the concentration level for dietary purposes. The results are presented in Table 4 & 5.

From this investigation it was found that all the samples of dry fishes contained DDT and its metabolites (DDD and DDE) and these were not removed 100% by any type of washing, even after washing followed by boiling for 10 min.

Table 4. Residues of DDT's dry fishes after washing and boiling treatment.

Name of the fish species	Type of cooking treatment	Quantity of DDT, DDD and DDE residues (ppm)				
		p,p'-DDT	p'-DDT	DDD	DDE	Σ DDT
1. Indian gizzard	Without washing	0.67	0.43	0.25	0.55	1.9
	Normal Washing	0.59	0.39	0.21	0.45	1.72
	Washing & Boiling	0.5	0.35	0.18	0.35	1.35
2. River sprat	Without washing	1.64	1.32	0.35	0.65	3.96
	Normal Washing	1.48	0.98	0.28	0.53	2.81
	Washing & Boiling	1.36	0.76	0.23	0.42	2.18
3. Bombay duck	Without washing	1.13	0.81	0.62	0.56	3.12
	Normal Washing	0.9	0.64	0.54	0.49	2.57
	Washing & Boiling	0.78	0.48	0.45	0.37	2.2
4. Hilsha	Without washing	0.95	0.64	0.7	0.77	3.06
	Normal Washing	0.72	0.55	0.57	0.65	2.85
	Washing & Boiling	0.58	0.47	0.45	0.52	2.3
5. Promfret	Without washing	0.28	0.19	0.16	0.35	0.98
	Normal Washing	0.23	0.16	0.14	0.28	0.77
	Washing & Boiling	0.18	0.13	0.12	0.22	0.68
6. Glassy fish	Without washing	0.52	0.36	0.41	0.29	1.58
	Normal Washing	0.44	0.31	0.35	0.24	1.25
	Washing & Boiling	0.36	0.26	0.31	0.19	1.02
7. Barramundi	Without washing	1.21	0.9	0.59	0.6	3.3
	Normal Washing	0.95	0.8	0.47	0.52	2.8
	Washing & Boiling	0.85	0.65	0.38	0.45	2.1
8. Pool barb	Without washing	0.5	0.38	0.27	0.23	1.38
	Normal Washing	0.38	0.34	0.23	0.19	1.25
	Washing & Boiling	0.31	0.28	0.2	0.16	1.05
9. Spider prawn	Without washing	2.39	1.78	0.12	1.18	6.25
	Normal Washing	1.92	1.62	0.09	0.95	5.31
	Washing & Boiling	1.62	1.45	0.07	0.77	4.36
10. White shrimp	Without washing	1.85	1.38	0.81	1.12	5.16
	Normal Washing	1.58	1.34	0.67	0.98	4.1
	Washing & Boiling	1.39	1.05	0.55	0.78	3.57

Our people think that these insecticides remain only on the surface of the dry fish and can be washed out with vigorous washing. They also believe that if can be removed washing followed by boiling the poison will be destroyed and washed out with water. But DDT, DDD and DDE are nearly insoluble in water and has a good solubility in most organic solvents, fats and oils. Moreover, these are highly stable molecule. In this experiment a big amount of DDT's was washed out at various washing but a significant amount was remained due to their water insolubility, fats solubility and high stability. The concentration of DDT's was decreased by washing due to the removal of the organochlorine insecticides present in the outer surface of the dry fish.

Table 5. Percent reduction of DDTs by washing and washing & boiling

Name of the fish species	Type of cooking treatment	% reduction of DDTs residues				
		p,p'-DDT	o,p'-DDT	DDD	DDE	Σ DDT
1. Indian gizzard	Without washing	-	-	-	-	-
	Normal Washing	11.94	9.30	16.00	18.18	9.47
	Washing & Boiling	15.25	10.26	14.29	22.22	21.51
2. River sprat	Without washing	-	-	-	-	-
	Normal Washing	9.76	25.76	20.00	18.46	29.04
	Washing & Boiling	8.11	22.45	17.86	20.75	22.42
3. Bombay duck	Without washing	-	-	-	-	-
	Normal Washing	20.35	20.99	12.90	12.50	17.63
	Washing & Boiling	13.33	25.00	16.67	24.49	14.40
4. Hilsha	Without washing	-	-	-	-	-
	Normal Washing	24.21	14.06	18.57	15.58	6.86
	Washing & Boiling	19.44	14.55	21.05	20.00	19.30
5. Promfret	Without washing	-	-	-	-	-
	Normal Washing	17.86	15.79	12.50	20.00	21.43
	Washing & Boiling	21.74	18.75	14.29	21.43	11.69
6. Glassy fish	Without washing	-	-	-	-	-
	Normal Washing	15.38	13.89	14.63	17.24	20.89
	Washing & Boiling	18.18	16.13	11.43	20.83	18.40

7. Barramundi	Without washing	-	-	-	-	-
	Normal Washing	21.49	11.11	20.34	13.33	15.15
	Washing & Boiling	10.53	18.75	19.15	13.46	25.00
8. Pool barb	Without washing	-	-	-	-	-
	Normal Washing	24.00	10.53	14.81	17.39	9.42
	Washing & Boiling	18.42	17.65	13.04	15.79	16.00
9. Spider prawn	Without washing	-	-	-	-	-
	Normal Washing	19.67	8.99	25.00	19.49	15.04
	Washing & Boiling	15.63	10.49	22.22	18.95	17.89
10. White shrimp	Without washing	-	-	-	-	-
	Normal Washing	14.59	2.90	17.28	12.50	20.54
	Washing & Boiling	12.03	21.64	17.91	20.41	12.93

Further decrease of organochlorine insecticide concentration in washing plus boiling treatment than normal washing treatment was due to more loss of fats and oils with hot water and organochlorine insecticides were possibly drained out with this lost fats and oils. Evaluating the results obtained from this study it may revealed that normal washing removes 8-25% and washing plus 10-minute boiling reduce 3-30% of DDT and its metabolites. This removal is not significant because the retained amount is much more than Accepted Daily Intake (ADI). Therefore, it may be recommended that, we intake a significant amount of DDT and metabolites with diet when palatable dry fish is our one of the food item in the menu.

CONCLUSION

Dried fish samples from all four metropolitan markets were found to be contaminated with different concentrations of DDT's residues indicating unrestricted and unplanned use of DDT for controlling infestation during processing and storage. The national MRLs should be generated for dried fish to make a massive awareness among people. The concerned authorities should take necessary steps to combat the situation through public awareness, about the harmful role of DDTs and restricting the unlawful distribution of these insecticides in the local market.

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