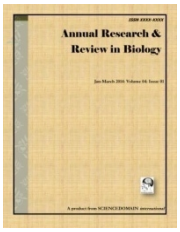

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# Determination of Dichlorodiphenyltrichloroethane Residues Levels in Commercial Marine Dry Fish from Different Regions of Bangladesh

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author MNH managed the literature searches, wrote the protocol and prepared the first draft of the manuscript. Authors RA, HMRI and SS managed the analyses of the study. Author YM managed the literature Searches and corrected the first draft. Author KKUA designed the overall study. All authors read and approved the final manuscript.*

***Original Research Article***

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## ABSTRACT

Dichlorodiphenyltrichloroethane (DDT) residual levels are available several commercially dry fishes. So these fishes were detected and determinate the contamination status of dichlorodiphenyltrichloroethane (DDT). The most popular dry fish-Ribbon fish (Lepturacanthus savala), Chinese pomfret (Pampus chinensis), Bombay duck (Harpodon nehereus) and *Shrimp sp*. (crustaceans) were selected for this study and these dry fishes were collected from nine different markets (three from each of Khulna, Chittagong and Cox's bazar district) of Bangladesh during December-March in 2013. A total number of 36 samples were selected for analyzing in the laboratory by using gas chromatography-mass spectrometry electron captured (GC-ECD) detector. The ranges of DDT in all samples were 2.81 to 877.82ppb. The ranges of DDT in the samples of Bombay duck, Chinese pomfret, Ribbon fish and Shrimp were 13.7-874.35ppb, 2.81-

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877.82ppb, 3.73-253.68ppb and 4.27-585.97ppb respectively. The mean concentrations of DDT were found highest amount from retailer markets while compared to that from the producers markets. Results indicated that the concentration of DDT in dry fish from Bangladesh are higher and may cause chronic disease and potential long-term risk for human health.

*Keywords: Dichlorodiphenyltrichloroethane (DDT); Dry fish; organochlorine insecticide.*

## 1. INTRODUCTION

Dry fish commonly known as SUTKI (in Bengali) is low cost dietary protein source and is used as a substitute of fish at the scarcity of fresh fish. About 15% of fishes are cured for mass people consumption at the scarcity of fresh fishes in Bangladesh [1]. It is a very favorite food item among Bangladeshi peoples and it has high demandable marketing besides fish and seafood products. Dry fish consumption frequency is very satisfactory in the Southeast Asian countries where people in Bangladesh consume it at least once a week in their daily meal [2]. Sundry is a common practice by the dry fish producing industries in the remote coastal isolated islands and in inland where chilling and freezing facilities are lacking. Most of the marine dry fishes are produced in remote areas and islands viz., Afatiar Chor, Dublar Chor, Kutubdia, Khuruskul, Moheskhali, Rangabali, Sonadia and St. Martin Island [3]. Dried fish products are generally stored in a dump warehouse either at the site or nearby coastal towns. During the storage period, sometimes fishers use insecticides to prevent dry fishes from insect infestation whatever they are getting within their reach. For protection of dry fish from infestation, fishermen and drier use mixture of insecticides [3]. In Kuakata (a fish processing zone of Bangladesh), high level of DDT powder (locally known as white powder) is used though Bangladesh banned the 'dirty dozen" in [4] and there is no statistical figure about these organochlorine insecticides in Bangladesh [5]. These insecticides are health hazard both for users and for consumers and have long- term potential health risk [3]. In the developed world, peoples are more concern about the risk and health issues [6]. In Bangladeshi peoples are more aware about health issues [7] and higher income peoples are more concern about harmful and health hazardous food intake [2]. Like other developing countries, a great number of indiscriminate and dangerous insecticides are sold in the markets without names and proper labeling.

In the present study, we have considered only marine fishes and shrimp. While collecting the samples by physical inspection in Chittagong, Najirattek, Khoruskhul, Khulna and Cox's Bazar etc. we are observed some processors and fishermen applying some poisons (insecticides) by spray and dipping method without label or label with improper instruction [8]. The present study work was undertaken for the detection and determination of the concentration level of DDT in some available marine dry fish and shrimp sp. as well as to elucidate to contamination status of using organochlorine insecticides (eg., DDT) in Bangladesh.

## 2. MATERIALS AND METHODS

### 2.1 Sampling

Samples were collected from Khulna New market (Station 1), Moilapota (Station 2) and Khalispur (Station 3), Najirartek (Station 4), Khuruskhul (Station 5), Borobazar (Station 6)

Chittagong Asadgonj (Station 7), Reajuddin Bazar (Station 8), Newmarket (Station 9). Four commercially available species of dry fish species namely Ribbon fish (*Lepturacanthus savala*), Bobay duck (*Harpodon nehereus*), Chinese pomfret (*Pampus chinensis*) and *Shrimp sp*. (crustaceans) were collected from each market during December-March season in 2013. Total 36 samples were collected. The control fishes samples (not used any insecticides for drying fishes) were collected from Marine Fisheries and Technology Station, Cox's bazar, Bangladesh Fisheries research Institute, Bangladesh [9].

## 2.2 Apparatus

Mincer fish chopper (Weisser No. 81K), round bottomed flask (500 and 100mL), volumetric flask (50 and 10mL), Homogeniger IKAR T25 digital ULTRA-Turrax, Nitrogen evaporator(N-EVAPTM111), SPE Cartidge ($C_{18}$-REC 300mg/3mL) Magnetic Starrier, Gas Chromatograph (GC-2010, Shimadzu), syringe (10µl, Hamilton Co).

## 2.3 Reagents

Dichloromethane, methanol (both, high purity 99.99%, HPLC grade) and anhydrous sodium sulphate were purchased from Merck Company (Germany). DDT Standard was obtained from Sigma Alorich Chemicals (USA).

## 2.4 Extraction and Cleanup Procedure

The extractions were carried out according to Abolagba [10] procedures, with some necessaries modifications were adopted such as extraction, separation and clean-up samples. A 25mL of methanol and water of ratio 1:1 solution was added to each sample in the beaker. The content in the beaker was gradually stirred for 30min. After stirring, the solution was filtered into a conical flask and the filtrate was used to estimate the pesticide residue in each fish sample. A 25mL of dichloromethane ($CH_2Cl_2$) was added to the filtrate and then stirred. The stirring was helped to partition the filtrate into aqueous and non-aqueous residues. The non-aqueous phase was pipetted into a beaker. Another 10mL of ($CH_2Cl_2$) was added to filtrate to make sure that no trace element of pesticides were left in the aqueous residues. The solution was then filtered and dried using anhydrous sodium sulphate which was helped to trap the water present in the filtrate and also to disintegrate the sample [11]. The solution was left to $N_2$ evaporator which was allowed it evaporate to dryness; leaving the pesticides to settle at the bottom of the tube. This was later reconstituted with $CH_2Cl_2$ and was cleanup using SPE cartridge. Then, it was pipetted into sample vials for GC-ECD analysis.

## 2.5 Sample Analyses

The DDT residues were analyzed by GC-2010, Shimadzu with an Electron Capture Detector (ECD), an auto injector (Shimadzu, AOC 20i) and GC solution software. The capillary column used was Rtx-5MS, length 30.0m x ID 0.25mm x film thickness 0.25µm. The GC was run under the following conditions: injector temperature: 260ºC; detector temperature 280ºC; oven temperature programme: 250ºC starting from 0 to 160ºC for 1 min and continued at 10ºC/min to 190ºC held for 1 min and continued at 5ºC/min to 250ºC; injected sample volume: 1µL; mode of injection: Split; The carrier gas was $N_2$ with a 172.0 kPa flow rate. Run time; 16 min. The methodology for calibration prescribed by Nahid et al. [12] with some necessary modification was applied. Standards' peaks were identified by injecting high

concentration of the standard (0.5 and 0.25ppm) and the retention time for DDT was determined. Then calibration was done at 3 points (50, 100 and 500ppb) by composite stock standard solution. GC system was calibrated using external standard technique. Individual standard stock solution (100mg/L) was prepared by weighing appropriate amounts of active ingredients in a brown bottle with a Teflon-lined screw cap and dissolving the weighed standard in HPLC grade methanol. Stock standard solution was used to prepare primary dilution standards. An appropriate volume of each individual stock solution was taken in a volumetric flask and mixed the solutions to obtain stock standard solution.

## 2.6 Analytical Quality Control

Analytical quality control was done followed by Nahid et al. [12]. Gas chromatograph equipped with ECD was checked for linearity. Instrumental limit of detection for GC-ECD was 1.0µg/l for Organochlorine pesticides. An aliquot of dry fish samples were collected as blank and treated exactly as a sample including exposure to all glassware, equipments, solvents and reagents used with the sample matrix. No analyte peak was detected in laboratory reagent blank. An aliquot of fortified samples matrix were prepared for known quantities of the pesticides which were added in the laboratory in ppb range. This laboratory fortified matrix was analyzed. Extraction and clean up were done as mentioned and the recoveries from untreated control samples of dry fish fortified with the analyzed compounds at the level of 50ppb was 96 to 100% for DDT. Prior to injection of the first sample solution, a standard solution was injected at least three times to check the operating conditions and the constancy of the detector signals. Further linearity of the ECD signal was checked by injecting serial dilutions of DDT. A standard solution injected after at least every other sample solution so that any alterations of the gas chromatographic system recognized due to column contamination.

Any insecticide detected from the tested samples were identified and quantified by the chromatogram standards. Sample results were quantities in ppb automatically by the GC software, which represented the concentration of the final volume injected and from the value, the actual amount of insecticide residues present in the sample was determined by using the following formula:

$$= \frac{\text{Concentration of obtained in injected volume (ppb)} \times \text{Quantity of final volume (L)}}{\text{Amount of sample taken (kg)}}$$

## 3. RESULTS AND DISCUSSION

The present study have been undertaken in order to provide the preliminary information on the concentration of DDT in dry fish and to investigate their contamination level. The results obtained from the samples from nine different sampling sites that are alarming for the consumers of Bangladesh. Most of the samples contained DDT is shown in Table 1. The mean concentrations of DDT in the samples of Bombay duck collected from 9 Stations were ranged of13.7-874.35ppb (except for Station 1, 2 and 4). All samples of Chinese Pomfret collected from 9 different stations contained DDT residues were ranged from 2.81-877.82 ppb. The mean concentration of Ribbon fish from the sampling stations were ranged from 3.73-253.68 ppb (except for station-1). The range of DDT use in shrimp sp. was ranged from 4.27-585.97ppb. The range of DDT use in all samples was 2.81-874.35ppb. Among the samples (except station 1, 2, 4 for Bombay duck and station - 1 for both Ribbon fish and

Shrimp sp.) the DDT was found comparatively lowest in chinese pomfret from station-1 and highest in bombay duck from station - 8. The comparisons of DDT concentration of different station among the various species are presented in the Fig. 1.

A few studies have been carried out so far to be revealed the current status of insecticides used, in particular, DDT in dry fish of Bangladesh. For last few years, application of a number of health hazard pesticides (e.g., dichloro-diphenyl-trichloroethane) have been exposed nakedly in the dry-fishe industries, therefore, the present study carried out to investigate the concentration of DDT in some commercially available marine species. The most concerning issue with DDT contamination is the risk of chronic health hazards as DDT is a slow poising substance and show vertical transmission from generation to generation through breast milk [13]. However, the contamination level is classified as “moderately toxic” by the US National Toxicological Program and “moderately hazardous” by WHO, based on the rat oral LD 50 of 113mg/kg [14]. Farmers exposed to DDT occupationally have an increased incidence of non-allergic asthma [15]. The mean concentration of DDT in ribbon fish collected from all stations was ranged from 3.73 to 253.68ppb. For ribbon fish, no DDT residues were detected from station 1, however, the highest concentration of 253.68ppb was detected from station-2 followed by 3.73ppb, the lowest concentration for the species from station-3. The mean concentration of DDT in shrimp sp. was recorded from 4.27 to 585.97 ppb. No detection of DDT was found for shrimp sp collected from station-1. The range of DDT use in the samples was 2.81 to 877.82ppb. Among the samples of the DDT were found comparatively both lowest and highest in chienes pomfret. The chinese pomfret samples of all station contained DDT residues. No DDT residues concentrations of all samples were collected from the station-1 except for chienese pomfret was 2.81ppb. Through dryfish is a popular food for the coastal belt people of Bangladesh, it is not so popular like Coxs-bazar and Chittagong in Khulna. The commercial market of dryfish in Khulna is not so developed like Chittagong and Cox's-bazar of Bangladesh. Most of the consumers produced dry fish for their own consumption. This may be one of the causes which protect the retailer to spray of DDT in their products. Almost all the samples, DDT was found comparatively in highest concentration from the station-8. The mean concentration of DDT in all samples collected from station-8 was recorded ranged between 141.43 to 877.82ppb. The mean concentration of DDT collected from Reajuddin Bazar showed a big saturated peak which indicates a high concentration of organochlorine rather than DDT and heptachlor [3]. However, it is found that the residues concentration varies from producer to retailer markets. Among our sampling sites Stations 6, 8 and 9 are known as retailer market are found more concentration of DDT compared to the stations 4, 5 and 7 (known as whole sell market) known as producer market. A number of studies showed that DDT is responsible for non-allergic asthma [14] and have direct link with diabetes [16]. A study found elevated risk of cancers of the liver and biliary tract for workers that handled DDT to control the malaria vector [17]. A number of studies have argued that the accumulation of DDT in human body before puberty increases the risk of breast cancer for the women [18].

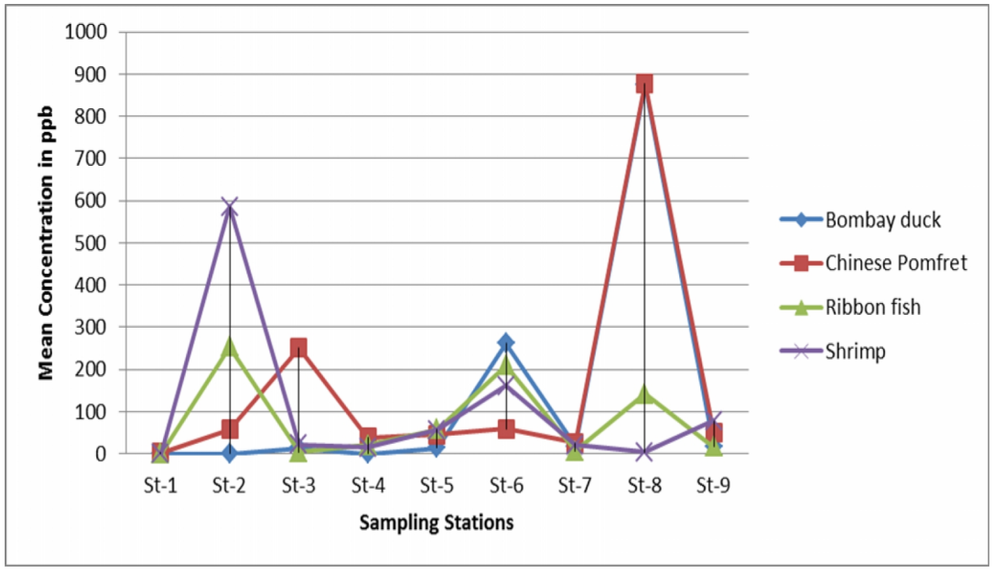


**Fig. 1. Comparisons of DDT concentration among different types of dry fishes collected from nine sampling sites**

**Table 1. Concentration of DDT (in ppb) in dry fish samples collected from nine different sampling sites (Mean concentration ± Standard deviation)**

| Dry fish | Station-1 (Khulna New market) | Station-2 (Moilapota) | Station-3 (Khalispur) | Station-4 (Nagirartek) | Station-5 (Khuruskhul) | Station-6 (Borobazar) | Station-7 (Asadgonj) | Station-8 (Reajuddin Bazar) | Station-9 (Chittagong New Market) |
|---|---|---|---|---|---|---|---|---|---|
| Bombay duck | ND | ND | 13.7±0.58 | ND | 14.09±0.12 | 261.89±0.52 | 17.42±0.61 | 874.35±0.97 | 17.99±0.67 |
| Chinese Pomfret | 2.81±0.31 | 58.01±0.74 | 250.47±1.0 | 38.27±0.92 | 45.17±0.21 | 58.32±0.19 | 26.27±0.72 | 877.82±1.5 | 50.95±0.34 |
| Ribbon fish | ND | 253.68±2.45 | 3.73±0.31 | 20.62±0.77 | 58.24±0.08 | 209.13±0.15 | 7.19±1.49 | 141.43±1.1 | 16.28±0.43 |
| Shrimp | ND | 585.97±0.62 | 21.39±0.35 | 15.42±0.26 | 56.16±0.07 | 161.67±0.13 | 20.84±2.67 | 4.27±0.49 | 78.78±0.54 |

**ND=Not detected Value= mean of triplicate analysis ± sd*

Almost all industrialized countries and many developing countries around the world have recognized the hazards of persistent pesticides and have banned them. But some developing countries, Persistent Organic Pollutants' are readily available in spite of official bans or severe restrictions. In Bangladesh application of DDT for protection of crops and dried fish from insects still available although its production and application is banned [8]. Government should strict to combat the situation by implementing the legislation. Awareness build up program is necessary among related people about the residuals impact of DDT in human body. The stocker should dry fishes appropriately and should pack carefully so that the fish cannot absorb moisture in monsoon.

## 4. CONCLUSION

Our analysis has showed that the fishers and the dried fish stocker in Bangladesh have using DDT as a compulsory preserver of dried fish without concerning health hazard issues. The concentration levels of DDT in the collected samples are higher which may causes health disease to the consumers for long time. In general, the mean concentration of DDT in samples is higher, in most cases, than the recommended WHO Maximum Residue Level (MRLs) in food items so it should give cause for concern. Other insecticides are also using as a composite mixture of insecticides to get good preservation. Due to our limitation of facilities, we could not perform analysis about other organochlorine and organophosphorus but we can suspect that there are other organochlorine insecticides contaminations of dried fish as our chromatograms showing some unexpected peaks in every sample**.** Further work on DDT residues in blood serum of consumers of the study area is recommended.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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