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Screening of organochlorine insecticides (DDT and heptachlor) in dry fish available in Bangladesh

Md. Nurul Huda Bhuiyan[1], Habibur Rahman Bhuiyan[1], Matiur Rahim[2], Kabir Ahmed[1], K.M. Formuzul Haque[2], Md. Tariqul Hassan[2] and Md. Nazrul Islam Bhuiyan[1]

[1]BCSIR Laboratories Chittagong, Chittagong 4220, Bangladesh; [2]Institute of Food Science Technology, BCSIR, Dhaka, Bangladesh.

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Abstract

The concentrations of organochlorine insecticides DDT and heptachlor were investigated to estimate the current status of insecticides used in dry fish. The most popular dry fish– ribbon fish (chhuri), shrimp (chingri) and bombay duck (loittya) were selected for this study and these dry fishes were collected from different markets of Dhaka and Chittagong. The range of DDT used in all the samples was 3.04 to 875.0 ppb. The range of DDT in ribbon fish 131.6 to 149.4 ppb, in shrimp 3.04 to 318.2 ppb and in bombay duck 61.9 to 875.0 ppb was found. The range of heptachlor used in all the samples was 0.7 to 5.5 ppb. The range of heptachlor in ribbon fish 1.7 to 2.3 ppb, in shrimp 0.7 to 3.8 ppb and in bombay duck 1.8 to 5.5 ppb was found. The heptachlor was not found in ribbon fish (CTG-2), shrimp (DHK-2 and CTG-1) and bombay duck (DHK-1). The concentration of heptachlor in dry fish compare to DDT was found too much less.

Introduction

The natural gifts of aquatic resources, fish and other fishery items have played an important role on the culture and life style of the people of Bangladesh. Fisheries and aquatic resources are economically, ecologically, culturally and aesthetically important to the nation. In Bangladesh, at present there are 260 freshwater fish species, 12 species of exotic fish, 475 species of marine fish and 60 species of prawn and shrimp available (Chandra, 2006). Fisheries sector contributes to GDP 5.2%, animal protein supply 63% and foreign exchange earning 4.8% for the nation (Chandra, 2006). Huge amount of fish catches from fresh and marine water during winter season when the natural depression attain shallow water and even dry up, naturally the fish catch attains peak.

During this time the Bay of Bengal, the coastal crisscross channels and other depressions remain calm and quiet and as a result fishing activities are strengthened and huge fishes are harvested during this period than the other seasons. The fishes included shrimp, chhuri, loittya, faissya, popa, bailla, laukkya, puti, elgona, fewa, etc. The necessity to cultivate dry fish looms up when huge quantity of fresh fish caught everyday remains unsold because of shortage of customers. Such huge catch of fish cannot be sent to the towns or the metropolis on a daily basis either for

shortage of transport or fish traders not willing to pay the right amount of money. As a result winter is considered to be the peak season for processing dry fish and continues till the onset of the rainy season. The dry fish is properly known as "SHUTKI" (in Bangla).

For long conservation of fish by drying is common practice in Bangladesh. This practice is usually made in the remote coastal isolated islands and in inland depressions where chilling and freezing facilities are lacking. Most of the marine fishes which are caught in remote areas and islands viz., Afatiar Chor, Dublar Chor, Kutubdia, Khuruskul, Moheskhali, Rangabali, Sonadia and St. Martin's are simply sun dried. The finally dried fish products are generally stored in a dump warehouse either at the site or nearby coastal towns. In addition to this, the weather is humid particularly during the monsoon period. Due to high moisture content in the weather and dump condition of warehouse, the dry fishes absorbed moisture so rapidly that the fish becomes suitable for infestation by beetles and mites. Most unexpected cause of infestation is that the fishermen do not dry fishes properly due to loss of weight i.e., the fishermen want more profit selling the dry fishes in weight. For protection of dry fish from infestation they use insecticides whatever they are getting within their reach. These insecticides are health hazard both for users and consumers.

In many countries of the world like Bangladesh, a great deal of indiscriminate and dangerous usage of insecticides because of the absence of clear recommendations for control of insects and lacks of training in the nature and correct uses of insecticides. There are many insecticides sold in the markets without names and insecticides are not true to the label. Unknown diluted varieties rarely contain any instructions on heath hazards are sold to unaware users. Nobody can draw a statistic on insecticide causalities, although newspapers reports on deaths and sufferings or chronic effects are common in Bangladesh.

DDT (dichlorodiphenyltrichloroethane) and heptachlor are banned in Bangladesh but there is no statistical figure about these organochlorine insecticides (UNEP, 2002). Bangladesh is party to the Stockholm Convention. A national implementation plan is already underway from September 2002 for the POPs (Persistent organic pollutant) through the assistance of UNEP. The Department of Environment, a member of Pesticide Technical Advisory Committee (PTAC) control the pesticide registration scheme in Bangladesh. No pesticides and insecticides are registered without reviewing the available Toxicological and Eco-toxicological information of the candidate pesticide. However, there is no specific legislation for controlling the production and use of hazardous industrial chemicals (UNEP, 2002).

The most significant properties of the organochlorine insecticides are their extreme lipophilic nature and resistance to biodegradation, which results in their accumulation and concentration in fatty tissues and their extreme persistence in environment (Taqnnenbaum, 1979). Among the insecticides DDT is a commercial organochlorine insecticide that has been widely used on agricultural crops as well as for vector control (ATSDR, 1995). DDT and its by-products can persist in soil and sediments for more than 15 years and are known to bio accumulate in animal tissues. DDT had been banned for all uses in 49 countries and restricted to vector control in 23 (PANNA, 1995). The half-life of DDT in humans is approximately 4 years (Noren and Meironyte, 2000). Heptachlor is another organochlorine cyclodiene pesticide that has been used to control termites and as an insecticide on seed grains and food crops. Heptachlor epoxide, the main metabolite of heptachlor, is extremely persistent in soil. In some cases, trace amounts of heptachlor epoxide have been found in soil 14–16 years after application (Extoxnet, 1996). Plants can draw heptachlor epoxide directly from the soil, and the chemical bioaccumulates in animals. It has been banned or restricted in more than 60 countries (WHO, 1988). However, some of these countries still permit its use for termite and other pest control, and many developing nations still use for agricultural purposes (Noronha, 1998). Despite the imposition of a ban on use in the United States in 1988, U.S. customs data showed that it was exported in large quantities through 1994 (Panna, 1997). A study showed that countries have restricted and banned heptachlor; levels detected in breast milk have dropped, often by more than 10-fold (Jensen and Slorach, 1991). But another studies showed that in

the United States, levels in the Southeast were nearly double the levels in the rest of the country during the period when it was still used (Savage, 1981).

The objectives of this study were to detection and determination of the concentration level of insecticides (DDT and Heptachlor) in dry fish used for conservation of dry fish and to elucidate the contamination status of organochlorine insecticides. By physical inspection of the sites (Sonadia Island, Moheskhali, Cox's Bazaar, Kuakata and Dublar Chor) where the fishes are drying and processing by the fishermen, we become confirmed that some fishermen do not dry properly or they do not have proper knowledge about preservation and they used some poisons (insecticides) without label or label with improper instructions.

Materials and Methods

Sampling

Samples from New Market (DHK-1), Karwan Bazaar (DHK-2), of Dhaka and Asadgonj (CTG-1), Reajuddin Bazaar (CTG-2), of Chittagong was collected. Three most popular species of dry fishes namely ribbon fish (Chhuri), shrimp (Chingri) and bombay duck (Loittya) were collected from each market. Total number of samples were 12.

The control samples of three different fishes were collected from drying yards of Sonadia island that are known sample treated with no insecticides and taken into account as blank.

Apparatus

Mincer fish chopper (Weisser No. 81 K), Soxhlet extractor, separatory funnels (500 and 200 mL), chromatographic tube (20 mm i.d. 50 cm long), sample concentrator (Techne dry block DB.3), round bottomed flask (500 and 100 mL), volumetric flask (50 and 10 mL), gas chromatograph (GC-14B, Shimadzu), syringe (10 µL, Hamilton Co.).

Reagents

Acetone, diethyl ether, dimethyl formamide saturated with petroleum ether, *n*-hexane, petroleum ether (30-60°C), petroleum ether (30-60°C) saturated with dimethyl formamide, eluting mixture I (petroleum ether + diethyl ether 94:6 v/v), standard solutions, eosin solution (2 mg in 100 mL), sodium sulfate solution (2 g/100 mL $NaSO_4$ 10 H_2O), sodium sulfate anhydrous (heated for at least 2 hours at 550°C), florisil 60-100 mesh (heated for at least 2 hours at 550°C, cool and stored in tightly stoppered container, prior to use heated for at least 5 hours at 130°C, cool and add 5% w/w water, shake this mixture for at least 20 min and stored in a container for at least 10 hours), cotton wool.

All the solvents used for the analysis purchased from MERCK, Germany. DDT and heptachlor standards were obtained from Sigma Chemicals.

Sample preparation

All the samples are finely comminuted in a mincer; heating of the samples during comminuting is avoided by briefly chopping several times (Peter and Zeumer, 1987).

Extraction

Triturate a sample of 25 g, with sodium sulfate to dry, powdery mixture, with the aid of an extraction thimble; extract the mixture exhaustively with Petroleum Ether in Soxhlet apparatus. Concentrate just to dryness the extract solution by a concentrator and dilute to 25 mL with petroleum ether saturated with dimethyl formamide (Peter and Zeumer, 1987).

Clean up

Clean up was done in two steps- (Peter and Zeumer, 1987).

a) *Dimethylformamide-petrolium ether partition:* Transfer the solution (dissolved in 25 mL petroleum ether saturated with dimethyl formamide) to 250 mL separatory funnel. Rinse the flask with small portion of a previously measured amount of 75 mL dimethyl formamide. Then add the remainder of the dimethyl formamide to the separatory funnel, and shake vigorously for 1 min. Drain the dimethyl formamide phase, and again extract the petroleum ether phase with 10 mL dimethyl formamide. Transfer the combined dimethyl formamide phases to a 500 mL separatory funnel, and add 200 mL sodium sulfate solution. Add a few drops of eosin solution to achieve better recognition of phase separation in the subsequent partition. Then extract successively with a 40 mL

portion and three 25 mL potions of petroleum ether for 1 min each time. Wash the combined petroleum ether phases with 10 mL water, dry on sodium sulfate, filter through a cotton wool plug, add 5 mL *n*-hexane, and concentrate to approximately 5 mL.

b) *Florisil column chromatography:* About half filled a chromatographic tube with petroleum ether, and sprinkle with 30 g florisil in small portions through a funnel with stopcock open, tapping the column in the process. Cover the florisil with an approx. 2 cm layer of sodium sulfate. Drain the supernatant solvent to the top of the column packing. Pipette the sample solution on to the column. Let the solution percolate to a level of 1-2 mm above the top of the column. Then rinse the flask with small portions of eluting mixture I, add the rinsings to the column, and also let them percolate to a level of 1-2 mm above the top of the column. Next eluate the column with the remainder of the total 200 mL amount of eluting mixture I, at a flow rate of about 5 mL/min. Add 5 mL *n*-hexane to the eluate, concentrate the eluate to 5 mL and dilute with *n*-hexane to 10 mL.

Sample analyses

The DDT and heptachlor residues were analyzed by GC-14B, Shimadzu with an electron capture detector (ECD), a manual sampler and GC solution software. A column of 3.1 m x 3.2 mm; I.D glass spiral; stationary phase silicon OV-17, 5%, aging 300°C, support chromosorb-W-AW-DMCS, mesh 80/100, 1 µm film thickness was used for the chromatographic separation of insecticides. The temperature was fixed for the injector at 250°C, column at 280°C, detector at 280°C. The carrier gas was nitrogen with a 60 mL/min-flow rate. 1.0 uL sample was injected for each run and the running time was 25 min. Standards' peak were identified by injecting high concentration of the standard (0.5 ppm and 0.25 ppm) and the retention time for DDT and heptachlor were determined. Then calibration was done at 3 points (25, 50 and 100 ppb) by composite stock standard solution. GC system was calibrated using external standard technique. Individual standard stock solution (100 mg/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 hexane. Stock standard solution was used to prepare primary dilution standards. Appropriate volume of each individual stock solution was taken in a volumetric flask and mixed the solutions to obtain composite stock standard solution.

Analytical quality control

Gas chromatograph equipped with ECD was checked for linearity. Instrumental limit of detection for GC-ECD was 1.0 ug/L for organochlorine pesticides. An aliquot of dry fish samples which 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 to which known quantities of the pesticides were added in the laboratory in ppb range. This laboratory fortified matrix was analyzed exactly like the sample. Extraction and clean up were done as mentioned and the recoveries from untreated control samples of dry fish fortified with the analyzed compounds at level of 25 ppb were 96-100% for heptachlor and 98-100% 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 and heptachlor. 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.

Results and Discussion

No studies of OCPs (DDT and heptachlor) in the dry fish of Bangladesh have been carried out. The present study have been undertaken in order to provide the preliminary information on the concentration of DDT and heptachlor in dry fish for the first time and to investigate their contamination level. The detected compounds were DDT and heptachlor. The results obtained are alarming for Bangladesh. All of the samples contained organochlorine insecticides are shown in Table I. The concentrations of DDT in the samples of ribbon fish collected from DHK-1, DHK-2, CTG-1 and CTG-2 were 149.4, 131.6, 140.4

Table I

Summary of DDT and heptachlor concentrations in the dry fish samples

Dry fish	Insecticides	Name of locations			
		DHK-1	DHK-2	CTG-1	CTG-2
Ribbon fish Chhuri)	DDT	149.4	131.6	140.4	137.5
Ribbon fish Chhuri)	Heptachlor	1.7	1.9	2.3	ND
Shrimp (Chingri)	DDT	3.04	69.4	3.8	318.2
Shrimp (Chingri)	Heptachlor	0.7	ND	ND	3.8
Bombay duck (Loittya)	DDT	156.6	182.3	875.0	61.9
Bombay duck (Loittya)	Heptachlor	ND	1.8	5.5	2.3

The concentrations are in ppb unit; ND = Not detected

and 137.5 ppb respectively; in the samples of shrimp collected from DHK-1, DHK-2, CTG-1 and CTG-2 were 3.04, 69.4, 3.8 and 318.2 ppb respectively and in the samples of bombay duck collected from DHK-1, DHK-2, CTG-1 and CTG-2 were 156.6, 182.3, 875.0 and 61.9 ppb respectively. The range of DDT use in all the samples was 3.04 to 875.0 ppb. Among the samples the DDT (except CTG-2) was found comparatively lowest in shrimp and highest in bombay duck. The ribbon fish samples of all places contained almost about same concentration of DDT. The level of concentration of DDT in dry fish is a great concern but more concern is such a dangerous poison is still using in our some popular food items such as dry fish though it is banned in our country.

DDT is a slow poisoning substance. It can transfer from generation to generation through breast milk (Solomon and Weiss, 2001). It is classified as "moderately toxic" by the US National Toxicological Program and "moderately hazardous" by WHO, based on the rat oral LD_{50} of 113 mg/kg (WHO, 2005). Farmers exposed to DDT occupationally have an increased incidence of non-allergic asthma (Brow, 2007). Organochlorine compounds in general have been linked to diabetes (Jones et al., 2008). EPA classified it as a class B2 probable human carcinogen (US EPA, 1987). A study of malaria workers who handled it occupationally found an elevated risk of cancers of the liver and biliary tract (Rogan and Chen, 2005). Exposure to it before puberty increases the risk of breast cancer later in life (Clapp et al., 2008). In areas where it is used for malaria control, infants can be exposed via breast milk in levels that exceed the W.H.O's acceptable daily intake value for DDT (Bouwman et al., 2006; Ntow et al., 2008).

From the Table I, we found that the concentrations of another organochlorine, heptachlor in the samples of ribbon fish collected from DHK-1, DHK-2, and CTG-1 were 1.2, 1.9 and 2.3 ppb respectively but in CTG-2 was not detected; in the samples of shrimp collected from DHK-1 and CTG-2 were 0.6 and 3.8 ppb respectively but in DHK-2 and CTG-1 were not detected; in the samples of bombay duck collected from DHK-2, CTG-1 and CTG-2 were 1.7, 5.5 and 2.3 ppb respectively but in DHK-1 was not detected. The ranges of heptachlor use in all the samples were 0.7 to 5.5 ppb. Among the samples the heptachlor was found comparatively lowest in shrimp and highest in bombay duck. The ribbon fish samples of all places contained almost about same concentration of heptachlor. The concentration of heptachlor in dry fish compare to DDT was found too much less. In some samples (ribbon fish sample of CTG-2, shrimp sample of DHK-2, shrimp sample of CTG-1, bombay duck sample of DHK-1) heptachlor were not present. But in the chromatogram of ribbon fish sample of CTG-2 and shrimp sample of DHK-2 showed a big saturated peak which indicates a high concentration of organochlorine rather than DDT and heptachlor. That peak was not our targeted peak. But it can indicate that there is another organochlorine which is used in high concentration like DDT. It is also alarming to Bangladesh that a banned item like heptachlor is still using in dry fish as well as DDT and it can assume from the study that a composite mixture

of insecticides is used for the conservation of dry fish. Heptachlor has been shown to bioaccumulate in fish and cattle. Most of the heptachlor that is swallowed is absorbed into blood. Heptachlor can pass directly from a mother's blood to an unborn baby through the placenta (LDWG, 2007). Animals fed heptachlor throughout their lifetime had more liver tumors than animals that ate food without heptachlor. EPA has classified heptachlor as a probable human carcinogen (B2) and established an oral cancer slope factor of 4.5 per mg/kg-day (LDWG, 2007).

Almost all industrialized countries and many developing countries around the world have recognized the hazards of persistent pesticides and have banned them. But in some developing countries, POPs insecticides are readily available in spite of official bans or severe restrictions. In those countries a government may lack the resources and infrastructure to implement and enforce the legislation fully. People selling these chemicals in isolated villages may have no idea that sale or use of the pesticides is restricted.

The government of Bangladesh should take all the necessary steps to combat the situation, it can be the steps to implement the legislation and improving the awareness of the related people through some program, public education campaigns and announcement of harmful rule of those insecticides and make difficult the availability of those insecticides in market either its true pack or false pack. The stocker should dry correctly and should pack carefully so that the fish can not absorb moisture in monsoon. The other way of preservation can follow such as freezing, canning and curing. From the study, we can say that, the stocker used insecticides abundantly. They use DDT as a compulsory preserver of dry fish. Other insecticides are also used as a composite mixture of insecticides to get good preservation.

Due to our limitation of facilities, we could not study about other organochlorine and organophosphorus but we suspect that there are other organochlorine insecticides contaminations in the dry fish, as our chromatograms showed some unexpected peaks in every sample.

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Conflict of Interest

Authors declare no conflict of interest

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Author Info
Md. Nurul Huda Bhuiyan (Principal contact)
e-mail: nhbmb@yahoo.com