International Journal of scientific research and management (IJSRM)
||Volume||1||Issue||7 ||Pages|| 352-353||2013||
Website: www.ijsrm.in ISSN (e): 2321-3418

Screening and quantification of dichlorodiphenyltrichloroethane (DDT) and Dichlorovos in selected dry fish species of Bangladesh by GC-ECD detector

M. Nahid Hasan[1], H.M. Rakibul Islam[1], K.K.U. Ahmed[1], Y. Mahmud [2], [1]and S. Siddiquee*[3]

[1]Bangladesh Fisheries Research Institute,
Shrimp Research Station, Bagerhat, Bangladesh
nahid.bfri@gmail.com

[2]Bangladesh Fisheries Research Institute,
Freshwater Station, Mymensingh, Bangladesh
yahiamahmud@yahoo.com

[3]Biotechnology Research Institute,
University Malaysia Sabah, Jln UMS,
88400 Kota Kinabalu, Sabah, Malaysia
shafiqpab@ums.edu.my

Corresponding author: Siddiquee*, S Biotechnology Research Institute, University Malaysia Sabah, Jln UMS,
88400 Kota Kinabalu, Sabah, Malaysia
Email: shafiqpab@ums.edu.my

Abstract
An attempt was made to know the residual status of insecticides in dry fish during their long term preservation by Electron Capture Detector (ECD) of Gas Chromatography. Eleven popular fish species were selected and samples were collected from different markets of Sayedpur and Cox's bazar of Bangladesh. All samples were extracted and prepared for injection using standard protocols for residue analysis during November to February 2012. The DDT concentration ranges were found of 14.454 to 1249.686 ppb according to 209.185~755.032 ppb for ribbon fish, 262.185~485.809 ppb for bombay duck, 242.166~298.165 ppb for giant perch, 161.739~515.185 ppb for prawn, 146.886~487.102 ppb for needlefish, 34.279~204.403 ppb for puntio barb, 111.495~420.191 ppb for snakehead (taki & shol), 587.146~1249.686 ppb for freshwater shark (Shol) and 14.454~97.124 ppb for freshwater shark (Boal), respectively. Samples collected from Sayedpur, DDT was found of 33.498 ppb for Chinese pomfret and 370.461 ppb for striped dwarf catfish, not at all found in cox's bazar samples. No Dichlorovos residues were detected in all the samples. However, the residual DDT ranges within the safe limit, efforts should be given on limiting the uses of all classes of hazardous chemicals for avoiding any health hazard situation and ensuring food security.

Keywords: Dry fish; Organochlorine; insecticides; DDT; Dichlorovos; GC-ECD detector

1. Introduction

In Bangladesh, dry fish belong to one of the most popular food item as well as protein source in many areas including Chittagong, Sayedpur, Chandpur, Kuakata, Barisal etc. Blessed with geographical speciation, the coastal areas along with the coastal rivers and several haors have been specialized zone for producing dry fish. Mid October to mid April stands for the pick season for fish drying while the fish are mainly sun dried. During this time the Bay of Bengal, the coastal fringes and other depressions remain calm and quiet and as a result fishing activities are strengthened and more fishes are harvested during this period than the other seasons comparatively. Drying is common practice for long preservation of fish in

Bangladesh to the remote coastal isolated islands and in inland depressions where chilling and freezing facilities are hardly available. The end products are then generally stored in a dump warehouse nearby coastal towns. In addition to this, the humid weather particularly during the monsoon period pose rapid absorption of moisture into the dry fish products that makes them highly susceptible to beetles and mites. Most unexpected causes of infestation are 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 a mixture of organochlorine (DDT and heptachlor) insecticides [1]. At present import and production of all sorts of Organic Pollutants (POPs) like DDT and heptachlor have been banned in Bangladesh but at least five POPs pesticides including DDT are still in use under a different name or label [2]. However, the country lacks in central register or list of available chemicals using by the fish drying industry, some intergovernmental organizations like the Food and Agricultural Organization (FAO) and the World Bank have started working on developing a database for frequently used chemicals in the industry concerned. Subsequently, country-specific data was not found, this did not tell enough about usage to know specifically where and how much of these compounds are being used [2]. Among the dirty dozen the chlorinated hydrocarbons (organochlorine compounds) are the first generation of pesticides called wonder drug introduced following the Second World War which comprises DDT, Dieldrin, Heptachlor and others. They are designed to kill insects and which as broad-spectrum poisons. DDT can transfer from generation to generation through breast milk [3]. In areas where it is used for malaria control, infants can be exposed via breast milk in levels that exceed the WHO's acceptable daily intake value for DDT [4]. 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 [5]. Farmers exposed to DDT occupationally have an increased incidence of no allergic asthma [6]. Organochlorine compounds in general have linked to diabetes [7]. EPA classified it as a class B2 probable human carcinoge [8]. Exposure to it before puberty increases the risk of breast cancer later in life [9]. Thus, the present study was to design for screening and quantification of the concentration level of insecticides (DDT and Dichlorovos) in dry fish used for conservation of dry fish and to elucidate the contamination status of Organochlorine insecticides.

2. Materials and Methods

2.1 Sample collection

Eleven most popular dry fish samples namely Bombay duck (Loittya), Ribbon fish (Chhuri), Shrimp (Chingri), Seabass (vekti), Kakila, Tengra, Punti, Taki, Boal, Shol and Chinese pomfret (Rupchanda) were used and collected from Sayedpur (whole sell market for dry fish) of Dinajpur district and Cox's bazar, Bangladesh at winter (December-March) season of 2012. 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.

2.2 Apparatus

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

2.3 Reagents

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

2.4 Extraction and Cleanup Procedure

The extractions were carried out according to Abolagba (2011) [10], with some necessaries modifications were adopted such as extraction, separation and clean-up samples. A 25 mL 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 30 min. 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 25 mL 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 10 mL of (CH_2Cl_2) was added to filtrate to make sure that no trace element of pesticides was 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 (FDA, 1982) [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 30.0 m x ID 0.25 mm x film thickness 0.25µm. The GC was run under the following conditions: injector temperature: 200^0C; detector temperature 270^0C; oven temperature programme: 250^0C starting from 100^0C for 1 minute and continued at 5^0C/minute to 250^0C held for 3 minute; injected sample volume: 1µL; mode of injection: Split; The carrier gas was N_2 with a 172.0 kPa flow rate. Run time; 35 min. Standards' peak were identified by injecting high concentration of the standard (0.5ppm and 0.25 ppm) and the retention time for DDT was determined. Then calibration was done at 3 points (50, 100 and 500 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 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

Gas chromatograph equipped with ECD was checked for linearity. Instrumental limit of detection for GC-ECD was 1.0 µg/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 50 ppb were 96 to 100% for heptachlor and 98 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 and dichlorovos. 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 was identified and quantified by the chromatogram of standards. Sample results were quantitated 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

Eleven fish species were used, namely, Bombay duck (Loittya), Ribbon fish (Chhuri), Shrimp (Chingri), Chinese pomfret (Rupchanda) Gaint Perch (vekti), Needlefish (Kakil) Striped Dwarf Catfish (Tengra) Puntio Barb (Punti), Snake head (Taki), Freshwater shark (Boal) and Shol were analyzed to detect DDT and Dichlorovos. These results were indicated an alarming for Bangladesh. All of the samples contained DDT insecticides (except Chinese pomfret and Tengra from Cox's bazar samples). As shown in Table 1, different concentrations of DDT and Dichlorovos were found.

Table 1: Summary of DDT and Dichlorovos concentrations in dry fish samples

Dry fish	Insecticides	Name of location	
		Sayedpur	Cox'sbazar
Bombyduck(Loittya)	DDT	485.809	262.185
	Dichlorovos	ND	ND
Ribbonfish(Churi)	DDT	755.032	209.185
	Dichlorovos	ND	ND
Chinese pomfret (Rupchanda)	DDT	33.498	ND
	Dichlorovos	ND	ND
Gaint Perch (vekti)	DDT	298.165	242.166
	Dichlorovos	ND	ND
Prawn(Chingri)	DDT	515.185	161.739
	Dichlorovos	ND	ND
Needlefish	DDT	487.102	146.886

	Dichlorovos	ND	ND
Striped Dwarf Catfish (Tengra)	DDT	370.461	ND
	Dichlorovos	ND	ND
Puntio Barb(Punti)	DDT	204.403	34.279
	Dichlorovos	ND	ND
Snake head (Taki)	DDT	420.191	111.495
	Dichlorovos	ND	ND
Freshwater shark (Boal)	DDT	97.124	14.454
	Dichlorovos	ND	ND
Shol	DDT	1249.686	587.146
	Dichlorovos	ND	ND

ND*=Not Detected, The concentrations are in ppb

Samples collected from cox'sbazar, the different concentrations of DDT were found between range of 14.454 ppb to 587.146 ppb, in orderly; 262.185 ppb for Bomby duck (Loittya), 209.185 ppb for Ribbonfish (Churi), 242.166 ppb for Gaint Perch (vekti), 161.739 ppb for Prawn (Chingri), 146.886 ppb for Needlefish, 34.247 ppb for Puntio Barb (Punti), 111.495 ppb for Snake head (Taki), 14.154 ppb for Freshwater shark (Boal) and 587.146 ppb for Freshwater Shark Shol, respectively, except Chines pomfret and Tengra samples. The lowest concentration of DDT found in Chinese pomfret (Rupchanda) at 33.498 ppb and the highest concentration was found in Shol at 1249.686 ppb. In Sayedpur samples, the concentrations of DDT were of 485.809 ppb for Bombyduck (Loittya), 755.032 ppb for Ribbonfish (Churi), 33.498 ppb for Chinese pomfret, 298.165 ppb for Gaint Perch (vekti), 515.185 ppb for Prawn (Chingri), 487.102 ppb for Needlefish, 370.461 ppb for Striped Dwarf Catfish, 204.403 ppb for Puntio Barb (Punti) 420.191 ppb for Snake head (Taki), 97.124 ppb for Freshwater shark (Boal) and 1249.686 ppb for Freshwater shark (Shol), respectively. Both sampling area of Freshwater shark Shol, the highest DDT concentration was 1249.686 ppb and 587.146 ppb. Only DDT was found of Chinese pomfret and tengra samples values of 33.494 ppb and 307.461 ppb in Sayedpur areas but no DDT any residues found in Cox's bazar samples. No dichlorovos detection was found both location samples.

4. Discussion and Conclusion

Until today no researchers have been done on DDT and Dichlorovos in the dry fish of Bangladesh. To my best knowledge, this is the first time reported the preliminary information on the concentration of DDT and Dichlorovos in dry fish and their contamination level. These obtained results are alarming for Bangladesh. The level of concentration of DDT in dry fish is a great concern and also more concern is such a dangerous poison is still using in our some popular food items such as dry fish though its banned in our country. From this study, we found that fishes which are dried for selling at rainy season while generally stored in a dump warehouse either at the site or nearby coastal towns. The weather is particularly humid during the rainy season. Due to high moisture content in the weather and dump condition of warehouse, the dry fishes so rapidly absorbed moisture after that the fish became infestation by beetles and mites. Most unexpected cause of infestation, the fishermen did not properly dry fishes due to loss of weight and getting less profit when selling the dry fishes. Especially when high cost fishes are selling such as Chinese pomfret, Gaint Perch and Ribbon fish. DDT is a slow poisoning substance. It can transfer from generation to generation through breast milk (Solomon and Weiss 2001) [3]. 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) [5]. Farmers exposed to DDT occupationally have increased incidence of non-allergic asthma (Brow 2007) [12]. Organochlorine compounds in general have linked to diabetes (Jones et al. 2008) [7]. 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) [13]. Exposure to it before puberty increases the risk of breast cancer later in life (Clapp et al. 2008) [9]. In areas where it is used for malaria control, infants can be exposed via breast milk in levels that exceed the WHO's acceptable daily intake value for DDT (Bouwman et al., 2006) [14]. Almost all industrialized countries and many developing countries around the whole world have recognized the hazards of persistent pesticides banned. But still now some developing countries, POPs insecticides are willingly 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 are 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 implementation 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 difficulty the availability of those insecticides in market. The stocker should dry correctly and should pack very carefully so that the fish cannot absorb moisture in rainy monsoon. The other way of preservation can follow such as freezing, canning and curing. 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 of dry fish, as our chromatograms showed some unexpected peaks in every sample. Further work on DDT residues in blood serum of consumers of the study area is recommended.

ACKNOWLEDGEMENTS

This study was done under the research project "Assessment of Hazardous Pesticides and antibiotics in fish and shrimp of Bangladesh" Quality Control Laboratory, Shrimp Research Station, Bagerhat. The authors are indebted to the Director General, Bangladesh Fisheries Research Institute, Mymensingh for allocation of funds doing such research. The authors also wish to thanks Mrs. Rubia Akter, Scientific Officer for her cordial assistance in doing such research

References

[1] Bhuiyan, M.N.H., Bhuiyan, H.R., Rahim, M., Ahmed, K., Haque, K.M.F., M.T. Hassan and M.N.I. Bhuiyan. Screening of Organochlorine insecticides (DDT and heptachlor) in dry fish available in Bangladesh. Bangladesh Journal of Pharmacology 3: pp.114-120, 2008..

[2] ESDO,Environment and Social Development Organization. Country Situation Report on Persistent Organic Pollutants in Bangladesh pp. 5-33. 2005.

[3] Solomon, G. and P. Weiss. Healthy milk, healthy baby. New York, Natural Resources Defense Council. 2001.

[4] Ntow, W.J., Tagoe, L.M., Drechsel, P., Kelderman, P., H.J. Gijzen and E. Nyarko. Accumulation of persistent organochlorine contaminants in milk and serum of farmers from Ghana. Journal of Environmental Research 106 (1), pp 17–26, 2008.

[5] WHO, World Health Organization. The WHO Recommended Classification of Pesticides by Hazard. British Journal of Psychiatry 187: pp 583- 584, 2005.

[6] Anthony, J.B. Pesticide Exposure Linked to Asthma, Sci. Am. Journal of the American Medical Association 162 (9), pp. 890-897. 2007.

[7] Jones, O.A.H., M.L. Maguire and J.L. Griffin. Environmental pollution and diabetes: a neglected association. Lancet 371, pp. 287–288. 2008.

[8] US EPA, 1987. Integrated Risk Information System p, p'-Dichlorodiphenyltrichloroethane (DDT) (CASRN 50-29-3) II.A.1.Weight Evidence Characterization 52(130): 25720.

[9] Clapp, R.W., M.M. Jacobs and E.L. Loechler. Environmental and occupational causes of cancer: new evidence 2005-2007. Reviews environmental health *23*(1), pp. 1–37. 2008.

[10] Abolagba, O.J; Igene, J.O. and Usifoh, C.O. Studies of Pesticide Residues in Smoked Catfish (Clarias gariepinus) in Nigeria: some Health Implication. Australian Journal of Basic and Applied Sciences, 5(5),pp 496-502. (2011).

[11] FDA, Food and Drug administration: Pesticides Analytical Manual, 1. (1982).

[12] Brow, A.J. Pesticide exposure linked to asthma. Scientific America 162, pp. 890-897, 2007.

[13] Rogan, W.J. and A. Chen. Health risks and benefits of bis (4-chlorophenyl)-1,1,1-trichloroethane (DDT). Lancet 366 (9487), pp. 763–773. 2005.

[14] Bouwman, H., B. Sereda and H.M. Meinhardt. Simultaneous presence of DDT and pyrethroid residues in human breast milk from a malaria endemic area in South Africa. Environmental Pollution 144(3), pp. 902–917. 2006.