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Short Communication

Analysis of organochlorine DDT residue along with its metabolites in dry fishes from some selected markets of Dhaka city

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Abstract: A study was investigated to estimate the current status of health hazardous organochlorine insecticide DDT and its metabolites DDE and DDD in different species of dry fish. To achieve the goal of this experiment, ten different sun-dried fish samples were collected from different markets of Dhaka city. The concentration of DDT, DDE and DDD was determined using the capillary column coupled to Gas Chromatograph with Electron Captured Detector (GC-ECD). Most of the dry fishes, seven out of ten samples, were found to be contaminated with DDT along with its metabolites DDE and DDD ranging from 0.029-1.22 mg/kg which is a serious concern because of the nature of long persistency and bioaccumulation of DDT in the environment. The highest concentration of DDT (1.22 mg/kg) was observed in *Pampus chinensis* dry fish whereas low concentration was detected in *Otolithoides pama* (0.029 mg/kg).

Keywords: dry fish; DDT; DDE; DDD; organochlorine; GC-ECD

1. Introduction

Fish drying is a very popular and common practice applied in Bangladesh for preserving fishes that are not instantly consumed or sold in the fresh markets. Dry fish is one of the most popular food items which fulfill the demand of protein of many people in different areas of Bangladesh. Recently, it has been observed that this sun-dried fishes are exported to overseas countries to meet the need of its consumers who are mostly the immigrants and workers of the third world country (Bhuiyan *et al*., 2009). The fish drying technique usually works by removing water from the fish and therefore helps inhibit the growth of microorganisms. It has been reported that fish drier and traders face great challenges during processing and storage of the dry fishes due to rapid moisture absorption which causes the dried fishes more suitable for infestation by different insect pests including beetles and mites. To overcome the damages caused by pest infestation and to reduce the microbial load, different types of unauthorized pesticides are used during processing. But it has been a big issue that these frequently used insecticides are health hazardous even in small quantity in the food and may pose serious health issues to users and consumers.

A slow poisonous organochlorine (OC) pesticide DDT was the first insecticide applied during World War II to control lice and mosquitoes combating typhus and malaria, is now applied in dry fishes to control pest infestation and it was reported that this hazardous chemical is transferred from generation to generation through breast milk (Solomon *et al*., 2001). The embryonic and fetal period are appeared as the most vulnerable time of

exposure to organochlorines (Hayes *et al*., 1991). Due to hydrophobic nature of this organochlorine substance, it has the ability to bio-accumulate in different tissues of living organisms. As a result, it may pose toxic threats to humans and animals. In a study, it was reported that dichlorodiphenyltrichloroethane (DDT) can cause pancreatic cancer in humans under circumstances of heavy and prolonged exposure (Garabrant *et al*., 1992). Exposure to DDT may increase the incidence of non-allergic asthma, link to diabetes, an elevated risk of cancers of the breast, liver and biliary tract and the risk of Alzheimer's disease (AD) (Rogan *et al*., 2005; Brown *et al*., 2007; Clapp *et al*., 2008; Jones *et al*., 2008). Additionally, it was identified that ATP-binding cassette transporter A1 (ABCA1) and insulin degrading enzyme (IDE) are the downstream target genes adversely affected by DDT (Hayes *et al*., 1991).

In technical grade DDT, both dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) can be found where DDT undergoes dechlorination to form DDE or DDD (Gold *et al*., 1982). The presence of chlorine atoms in DDT and its metabolites, in conjunction with their low solubility and tendency to partition preferentially into the lipophilic phase, makes them highly toxic, especially to higher organisms. The United States Environmental Protection Agency (EPA) has classified DDT and its metabolite products, DDD and DDE, as priority pollutants (Sayles *et al*., 1997; Foght *et al*., 2001). The metabolite DDD is more toxic than DDT. It has been reported that DDE is more persistent than DDT (Krieger *et al*., 1994). The half-lives for DDT and DDE in humans were reported to be 4.2–5.6 years and approximately 8.6 years, respectively. In human body, DDT is metabolized to DDE which persists for longer periods of time in the body's adipose tissue and the lipid fraction of other tissues than DDT (Kirman *et al*., 2011). A number of epidemiologic studies showed an association between high concentration of DDE blood levels and mammary cancer (Wolff *et al*., 1993; Krieger *et al*., 1994). It has been calculated that it would take between 10 and 20 years for DDT to disappear from an individual if exposure would totally cease, but that DDE would possibly persist throughout the life span (Hayes *et al*., 1991). In Alzheimer's disease (AD) patients, the presence of DDE was found higher in a significant amount when compared with control groups (Singh *et al*., 2013).

Considering the potential for negative effects, it is necessary to address the persistence of this insecticide in environment. In a survey, it was revealed that the existence of DDT was present in an increased concentration in blood plasma of fishermen and fishermen's wives which is an indication of the use of DDT in fish drying to protect the fish from infestation and remain fresh on the market (Zamir *et al*., 2009).

Again, it has been a great concern that DDT is a banned item in Bangladesh as a member of the United Nations. But because of lacking of effective monitoring and control system regulation, people involved in dry fish processing are randomly using this hazardous pesticide. Residue analysis confirmed contamination of commercial dried fish with DDT and detected substantial levels of residues (including degradation products of the parent compound) in a number of species of dried fish marketed across the country. A study in Kuakata (a fish processing zone in Bangladesh) reported that high level of DDT powder is used on dry fishes (Barua, 2007). Several studies revealed that dry fishes contained DDT and this DDT could not be removed 100% by any type of washing, even after washing followed by 10 minutes boiling (Bhuiyan *et al*., 2009). Therefore, this study is a great necessity to estimate the current status of DDT insecticide residue along with its metabolites DDE and DDD applied in different species of dry fishes collected from Dhaka City markets of Bangladesh.

2. Materials and Methods

2.1. Reagents

Hexane (Extra pure, Merk, Germany), Acetone (Merck, Germany), Anhydrous sodium sulfate ($2Na_2SO_4$), Diethyl ether (BDH, England), Florisil (Sigma, U.S.A., Mesh size: 60-100), Methanol (Merck, Germany).

Analytical Standards: Mixture of Lindane, Heptachlor, Aldrin, Dieldrin, DDT, DDE and DDD (Analytical standards PolyScience, Germany).

2.2. Equipment

Centrifuge machine (England), Rotary vacuum evaporator with temperature-controlled water bath (EYELA, N-N Series, Cifton), Fractional distillation system (Schott Duran, Germany), Digital analytical balance (Model Mettler, AE-200), EYELA Electronic Vibriofox VFl (Janke & Kunkel), Nitrogen blow down unit, Gas Chromatograph (GC) Hewlett Packard 5890 II with electron capture detector ($_{63}Ni$).

2.3. Sample collection and storage

From Dhaka city market areas, ten different dried fish samples were collected in airtight polythene bag with tagging each sample and were brought to the Agrochemical and Environmental Research Unit (AERU), Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Ganakbari, Savar,

Dhaka as early as possible and preserved in refrigerator at -20°C until extraction for avoiding loss of the residues.

2.4. Sample preparation, extraction and clean-up

Hundred grams dried fish sample was taken from storage and kept at the room temperature for thawing. Then, the samples were sliced, ground, sieved and prepared for extraction and clean up. A relatively simple method for surface extraction of organochlorine compounds, especially DDT, was used for this study in place of laborious soxhlet extraction procedures. Five grams of each dried fish samples was finely grinded and dissolved in 2% diethyl ether in hexane (30 ml) for 10 minutes, stirred with a glass rod and finally centrifuged. The residue was extracted twice using the same solvent. The combined extract was evaporated to dryness with a stream of nitrogen and dissolved in hexane (1 ml). To clean-up the extract, florisil column chromatography was applied (DFG manual, 1987). The top 1.5 cm of the florisil column was packed with anhydrous sodium sulphate. Elution was done by 2% diethyl ether in hexane (5 ml/min.). The elute was concentrated to a small volume on a rotary vacuum evaporator and transferred to a glass stoppered test tube. Solvents were completely evaporated under mild flow of pure N_2. The evaporated sample was dissolved in double distilled hexane and made up to volume (5 ml) in a volumetric flask.

2.5. Analysis

After cleaning up all the samples, sample volume was made as per required by adding double distilled hexane and was injected (1 µL) into the column of Gas Chromatography (Hewlett Packard 5890 II). The analysis was made in the GC Conditions (ECD Mode) showed in Table 1. Aliquot was injected by micro syringe into the GC. Tentative identification of the suspected insecticide was carried out in relation to retention times of the pure analytical standard(s). Quantification was made using a freshly drawn standard curve of the concerned insecticide. Confirmation of the GC identified pesticide was performed by TLC on silica gel coated glass plates using distilled hexane as developing solvent. The chromatograph was developed by spraying an ethanolic solution of silver nitrate and irradiating with germinal UV light (254nm) for 30 minutes (D.C Abbott.).

2.6. Efficiency of extraction and clean-up

5 gm sample was spiked with ^{14}C-p,p-DDT (0.2 ml) corresponding to a radioactivity of 1,00,000 dpm. The spiked sample was extracted and cleaned up in the same way as the unspiked samples. Radioactivity was determined at different stages of extraction and cleaned-up by means of Liquid Scintillation Counter (LSC) (Matin *et al*., 1995).

3. Results and Discussion

Ten different species of dry fishes have been collected from Dhaka City markets to analyze the presence of DDT along with its metabolites DDE and DDD. The results obtained from the study have been presented in Table 2. The results are very alarming which revealed that most of the dry fish species tested were found contaminated with organochlorine insecticide DDT residue and its metabolites DDE and DDD. The total DDT used in all the samples was found within a range of minimum 0.029 to maximum 1.22 mg/kg. Most of the species including *Pampus chinensis*, *Harpodon nehereus*, *Sarotherodon melanotheron*, *Macrobrachium dayanum*, *Corica soborna* and *Parambassis ranga* samples showed the existence of organochlorine DDT insecticide residue. In three samples of dry fishes including *Leander styliferus*, *Scomberomorus gutlatus* and *Pellona ditchela*, no DDT residue was identified. The highest concentration of DDT (1.22 mg/kg) was observed in *Pampus chinensis* dry fish whereas low concentration was detected in *Otolithoides pama* (0.029 mg/kg). The results indicated that DDT was used in controlling and limiting the pest infestation of dry fishes during processing and storage. Slowly biodegraded DDT and its metabolites may pose risk, if it enters once in the food chain and in the tissues of living organisms. Symptoms such as headache, nausea, vomiting, confusion, and tremors were reported after low direct exposure to DDT. Moreover, accumulation of DDT in the body can affect the neurobehavioral functions, increase tumor production, and is associated with pancreatic and liver cancer (Turusov *et al*., 2002; Gautam *et al*., 2007). In addition to a possible carcinogenic effect, DDT has been reported to be associated with premature births. Prenatal exposure to DDT and other organochlorine insecticides has been found to affect the immune status of the children and increase their susceptibility to infections (Dewailly *et al*., 2000).

Our results showed that *Macrobrachium dayanum* was found to contain higher amount of DDT whereas no DDT residue was detected in *Leander styliferus*. The reason is that the fishers pay much more attention during

conservation of bigger size shrimp and use higher amount of DDT than the smaller size shrimp (Siddique *et al*., 2012).

Though DDT is banned in Bangladesh but unfortunately, many of the banned pesticides are still marketed and in use illegally at lower price because of their illegal transboundary entry, which are preferred to the dried fish processors and traders for their lower price and longer persistent efficacy (ASIP, 2000). Dried fish processors and traders of our country have a great dependence on pesticides. Therefore, most fishermen use pesticides indiscriminately to protect their products from harmful pests. They are not aware of harmful effects of pesticide residues on human health. Also, they are not aware of the use of DDT as well as other organochlorines are restricted to use in Bangladesh. But it is true that these banned pesticides reach to our fishermen through some illegal traders. The government of Bangladesh should take all the necessary steps to combat the situation by implementing the legislation and improving the awareness. Awareness among people who are involved with fish drying industry may be increased arranging different training programs which will help to let them master skill on biological control of ordinary pest, strengthen the ability for economically and effectively controlling pests.

Table 1. Details of gas chromatographic conditions.

Parameters	Condition
Column	Fused Silica Capillary, 30m×0.32mm×0.25µm
Oven temperature	Initial 175ºC and final 255 ºC
Injector temperature	260 ºC
Detector temperature	275 ºC
Carrier gas	Nitrogen
Gas flow rate	75 ml/min
Column head Pressure	8 ml/min
Injection volume	1µL

Table 2. DDT and its metabolites in dried fish samples of Dhaka City markets.

Local name	Scientific name	Residues detected (mg/kg)			
		p,p′-DDE	p,p′-DDD	p,p′-DDT	Total
Rupchanda	*Pampus chinensis*	0.417	0.157	0.649	1.22
Churi-Loittya	*Harpodon nehereus*	0.109	0.062	0.020	0.192
Topshe	*Sarotherodon melanotheron*	0.073	0.042	0.084	0.20
Surma	*Scomberomorus gutlatus*	ND	ND	ND	Nil
Choto Chingri	*Macrobrachium dayanum*	0.070	0.023	0.068	0.162
Gura Chingri	*Leander styliferus*	ND	ND	ND	Nil
Choukka	*Pellona ditchela*	ND	ND	ND	Nil
Kaski	*Corica soborna*	0.073	0.062	0.153	0.289
Tek chanda	*Parambassis ranga*	0.115	0.111	0.199	0.425
Poa	*Otolithoides pama*	0.029	ND	ND	0.029

Detection limit: 0.01 µg/kg, ND: Not Detected

4. Conclusions

From the findings, it can be concluded that the organochlorine pesticide DDT is still used in Bangladesh as a compulsory preserver of dry fish without concerning health hazard issues. Our results of the current status of DDT and its metabolite products DDE and DDD on dry fishes could play an important role for the policy maker and regulatory authority for the control over the unrestricted use of these compounds in dry fish during processing and storage. Again, more research works should be performed to prepare a data base regarding pesticide residue levels, acceptable daily intake (ADI), maximum residue limits (MRLs) which will make a massive awareness among people about the health hazard concern. Besides, regular monitoring activities on drying and packaging fishes without applying toxic health hazardous pesticides for preservation should be run by the government.

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Conflict of interest
None to declare.

Authors' contribution
M.A. Uddin and M.A.Z. Chowdhury designed and supervised the experiment. M.A Rahman, M.H Rashid and Z. Fardous carried out the experiment. M.A. Uddin and M. Nesha wrote the manuscript and finalized in consultation with M.A.Z. Chowdhury, M.A Rahman, M.H Rashid and Z. Fardous. All authors have read and approved the final manuscript.

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