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Organochlorine pesticide residues in poultry meats of Bangladesh

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Summary

Forty poultry meat samples from four large companies were analyzed for the presence of organochlorine pesticides. Homogenized samples were mixed with silica sand and anhydrous sodium sulfate, and extracted with ethyl acetate followed by *n*-hexane: MTBE. Clean up of samples were done by silica sulphuric acid and analyzed by GC-ECD, and confirmed by GC-MS. The limit of detection (LOD) and limit of quantification (LOQ) were 0.5, 0.5 and 0.7, and 2.5, 2.5 and 3.5 ng/g, respectively. The calibration curves were linear and the correlation coefficients (r^2) were 0.991, 0.992 and 0.997 for DDE, DDD and DDT, respectively and the recoveries for them were 76%, 78% and 80%. DDT and its metabolites, DDE and DDD were detected in 28 (70%), out of 40 samples and ranged 0.039-0.769 mg/kg.

Keywords: Bangladesh, DDT, organochlorine pesticides, persistent organic pollutants, poultry meats

Introduction

Organochlorine pesticides (OCPs) including DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane), aldrin, dieldrin, endrin, lindane, hexachlorobenzene (HCB), chlordane, methoxychlor, toxaphene, heptachlor, heptachlor epoxide and hexachlorocyclohexanes (HCHs) were used in Bangladesh to increase crop production and to eradicate vector diseases from early fifties (Rahman, 2000). In order to meet up the local supply, the DDT production factory in the country was started within Chittagong Chemical Complex area (CCC) in 1966. However, due to long persistent in the environment, bioaccumulation, and biomagnifications and accumulation to the fatty tissues of human through food chain, the use and production of OCPs became restricted worldwide from nineties (Jaga and Dharmani, 2003).

The Stockholm Convention identified twelve persistent organic pollutants (POPs) including DDT which are harmful for wildlife and human health and formulated a treaty in 2001 in order to stop production, use and elimination of OCPs (UNEP, 2001). Being a signatory country of the Convention, Bangladesh closed down the DDT factory in 1995. However, information about the disposal of unused DDT stored in the CCC factory is still lacking. Moreover, surveys and research conducted by our group in collaboration with the Department of Environment, Bangladesh reported the presence of huge amount of DDTs in the soil of the factory premises (Nahar, 2006; Al Mahmud et al., 2015). DDTs could be transported away from the CCC area via air and water, and can spread contamination to the surrounding areas. Although the use of DDT is banned in the country there were reports that it is continuously being used illegally (Takada et al., 2003). Residue of DDT and its metabolites, DDE and DDD were identified and quantified in fresh fish, dry fish and poultry feeds (Nahar 2008; Shoeb et al., 2009). Our previous studies also addressed human exposure to POPs indicating high exposure to *p*,*p*'-DDT and 2,2-bis(4-chlorophenyl)-1,1-dichloroethene (*p*,*p*'-DDE) and the exposure might be due to the contaminated food which originated from fishes and poultry meats (Zamir et al., 2008, Mamun et al., 2007, Linderholm et al., 2011). The presence of OCPs in poultry meats is already known worldwide (Ahmad et al., 2010). However, there are no studies regarding OCPs in poultry meats in Bangladesh.

Bangladesh is recently classified as a lower-medium income country with a population of 160 million, the seventh most populous country of the world (World Bank, 2015). The inhabitants are dependent on the agricultural products and industrial output is very low. Rice is the staple food for Bangladeshi and it is consumed every day with either fishes or poultry meats. Both fishes and chicken provide sources of protein. Chicken, ducks and pigeons are the main poultry flocks in Bangladesh (Frands Dolberg, FAO, 2008). Poultry industry is increasing in the country to meet the local supply and to export abroad. It is estimated that the production of chicken birds was 195 million in 2006 (Bangladesh Bureau of Statistics, 2006). Recently, there was some news in the daily newspaper that OCPs acquired from neighboring countries and chicken meats were contaminated due to the presence of heavy metals and the OCPs. The

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present study was aimed to investigate the level of residual organochlorine pesticides in chicken meats for human consumption in Bangladesh.

Materials and methods

Sample Collection

Forty poultry meat samples (average sizes of whole meat 1.5-2.0 kg) were bought from four large poultry meat companies named Aftab, Babu, Tareq and Sarwar within Dhaka city. All the samples were wrapped with aluminum foil and kept in a chilled box and transferred immediately to the laboratory and stored in a freezer at a temperature below -20 ºC until analysis.

Reagents, chemicals and solvents

All the chemicals, reagents and solvents used for analysis in the present work were analytical grade (purity 99.99%). Ethyl acetate, *n*-hexane and acetone were purchased from Merck (Darmstadt, Germany). Sulfuric acid (98%, w/w) and silica sand were bought from BDH (Poole, UK) and Kanto Chemical Co. Int. (Tokyo, Japan), respectively. Anhydrous sodium sulphate was heated at 300 ºC for at least 4 hours in an oven and cooled before use. Certified standard of organochlorinated pesticides, *i.e.*, *p,p´*-DDT and its metabolites (*p,p´*-DDE & *p,p´*-DDD) were purchased from Dr. Ehrenstrofer, GmbH, Augsberg-Germany.

Equipment

Gas chromatographic (GC) analyses were performed using a Shimadzu-2010 machine equipped with an electron capture detector (ECD, Shimadzu Corp. Kyoto, Japan). Injector and detector temperatures were set at 280 ºC and 320 ºC, respectively. Separations were performed on DV-5 (30 m x 0.25 mm i.d., film thickness 0.25 µm) where helium was used as carrier and nitrogen was used make-up gases and flow rate was 2 mL/min). Split ratio was 1:77 and injection volume of the sample was 1 µL. Oven temperature was programmed: 80 ºC (1 min) then, 20 ºC rise per min to 295 ºC and finally hold for 5 min. Agilent 6890 N gas chromatograph with an Agilent 7683B auto-sampler and Agilent 5975 N mass selective detector was used to confirm DDTs in the samples. An HP-5MS capillary column (30 m × 250 µm i.d., 0.25 µm film thickness, Agilent Technologies, CA, USA) was used to separate the pesticides. Helium was used as a carrier gas at a flow rate of 1.0 mL.min^{-1}. The following oven temperature program was employed: initial temperature of 70 ºC held for 1 min; increased at 10 ºC min^{-1} to 280 ºC; held for 3 min, post run 2 min. The detector and injector temperatures were 290 ºC and 280 ºC, respectively. The injection volume was 1 µL in split less mode. The ionization was carried out by electron impact (EI mode, 70 eV), and analysis was carried out in selected ion monitoring (SIM) mode, in which three characteristic ions were monitored: (*m/z* = 176, 246, 318) for DDE, (*m/z* =165, 199, 235) for DDD, and (m/z= 165, 199, 235) for DDT.

Extraction and clean-up

The chicken meat (edible part without bone) was separated after defrosting and chopped into small pieces and homogenized by a kitchen blender. The homogenized sample (10 g) was ground with silica sand (10 g) and anhydrous sodium sulfate (30 g) was added and mixed with a pestle until the mixture of the sample floated freely. The powdery sample was extracted successively with 50, 20, 20 and 20 mL of ethyl acetate by shaking 3 minutes each time. All the extracts were combined and filtered using filter paper with small of anhydrous sodium sulfate (~15 g). The filtrate was concentrated by evaporation and was reconstituted in hexane (~ 1.0 mL). Proteins in samples were denaturated by addition of hydrochloric acid (6 M, 1 mL) and 2-propanol (6 mL). Each sample was re-extracted with a mixture of *n*-hexane: MTBE (1:1, 6 mL). Organic and water phases were separated by centrifugation for 5 min at 3000 rpm. The organic phase was transferred and washed with aqueous potassium chloride (1%, 4 mL) prior to solvent evaporation. The lipid content of each chicken meat sample was determined gravimetrically. Solvent blank was also run simultaneously with samples. The lipids in 4 mL hexane were eliminated by a conc. sulfuric acid (2 mL). An additional clean up step was performed to get lipid free samples prior to instrumental analysis. The samples were purified in columns containing silica gel impregnated with conc. sulfuric acid (2:1 w/w, 1 g) and with *n*-hexane (8 mL) as mobile phase.

Recovery

Known amount of DDE, DDD and DDT (0.08 µg/mL in each case) were added to homogenized local chicken meat (10 g) and allowed to stand for 30 min. The samples were extracted and cleaned-up by following the same procedure as described above. Reagent blank samples were also analyzed.

Calibration curve, limit of detection (LOD) and limit of quantification (LOQ)

The standard calibration curves of DDE, DDD and DDT were made by injecting solutions at concentration 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, and 2.50 μg/mL of the certified standard DDE, DDD and DDT into the GC-ECD. The curve gave straight line with correlation coefficient (r^2) equal to 0.991, 0.992 and 0.997 for DDE, DDD and DDT, respectively. The limit of detection (LOD) and quantification (LOQ) were calculated by taking peak height (concentration of the standard) three times of noise level (S/N; 3:1) and S/N ratio, 9:1, respectively. The LOD of DDE, DDD and DDT were found to be 0.5, 0.5 and 0.7 ng/g, respectively while LOQ were 2.5, 2.5 and 3.5 ng/g for them.

Results and discussion

Chickens are the main protein sources of Bangladeshi people who consume meat almost every day due to their nutrient values and easy purchase. However, meats can be contaminated due to the presence of organochlorine pesticides, such as DDT from different sources. Out of 40 samples, more than 70% samples contained organochlorine pesticides DDT and its metabolites, DDE or DDD (Table 1). Nine samples from Aftab poultry farm contained DDT and the range were 0.031-0.669 mg/kg. The range of total DDTs (DDT, DDD and DDE) ranged from 0.039-0.769 mg/kg. DDT was found in five samples from Babu poultry farm in the range of 0.027-0.065 mg/kg and the ΣDDT were trace-0.083 mg/kg. Eight samples of Tareq poultry farm contained DDT (ranged 0.041-0.269 mg/kg) and the ΣDDT ranged from 0.056-0.269 mg/kg. Four samples from Sarwar poultry farm contained DDT in the range of 0.045-0.141 mg/kg and the range of ΣDDT was 0.008-0.159 mg/kg. DDT and its metabolites in meat samples were confirmed by GC-MS in selected ion monitoring (SIM) mode in which three characteristic ions (*m/z* = 176, 246, 318) for *p,p'*-DDE, (*m/z* =165, 199, 235) for *p,p'*-DDD, and (*m/z*= 165, 199, 235) for DDT were monitored. The percentage ratio of DDT/DDTs was around 1.0 for all poultry farms and indicated that their uses are recent and ongoing. Due to toxic and harmful effects of DDT and its metabolites the government and concerned authority should monitor the illegal use of DDT in food stuff. Most of the values are lower than the maximum residue limits (MRL) for DDTs in chicken meats of 0.1 mg/kg (FAO/WHO 2006). However, continuous consumption of contaminated meats can accumulate the pollutant in human body and produce toxic effects. There are no rules about the daily intake of contaminated chicken meats in Bangladesh as data are not available. The Government has established Bangladesh Food Safety Authority (BFSA) to implement rules to have safe food for the protection of human health and life. These data can help setting guidelines and monitor OCPs and other pesticides residues in foodstuffs to save human health.

Conclusions

Chicken meats are one of the main menus in every day meal in Bangladesh. DDT and its metabolites, DDE and DDD were detected in 28 (70%), out of 40 samples within range 0.039-0.769 mg/kg. The regulating authority should monitor the illegal use of contaminants in food stuffs.

Table 1. Residual organochlorine pesticides in fresh poultry meat of four farms

Organochlorine pesticide residues		Aftab (n=10)	Babu (n=10)	Tareq (n=10)	Sarwar (n=10)
		x 10^{-3} mg/kg			
DDE	Range	trace-100	trace-13	trace-49	trace -34
	Mean	30	5.0	10	12
	Median	18	5.0	trace	10
DDD	Range	trace-69	trace-18	trace-41	trace-6.0
	Mean	37	9.0	10	1.0
	Median	30	9.0	3.0	trace
DDT	Range	trace-669	trace-65	trace-269	trace-141
	Mean	219	20	97	29
	Median	296	trace	92	trace
DDT/DDTs	Range	trace-1.0	trace-1.0	trace-1.0	trace-1.0

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