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Pesticide Residue in Marketable Meat and Fish of Nadia district, West Bengal, India

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Abstract

Pesticides are the group of chemicals that used for protection of agricultural and food products by controlling plant and animal pests. The pesticides have significant contribution to increase the yields of agricultural products and regulating vector borne diseases. The unscientific use of pesticide may accumulate in substantial quantity in crops, vegetable, fruits, fishes and meat etc. Pesticide pollution of local environmental may have severe impact on human as well as other animals, birds, fishes and livestock by different ways of exposure. India as populated and developing country is the significant producer of pesticides in and ranks 12th in the world for the use of pesticides. In the present study some locally used Organo-chlorine and Organo-phosphorus pesticide residue of marketable fishes and meats of Nadia district of West Bengal, India were analyzed through Gas chromatography. The result found that the mean values of Organo-chlorine pesticide residue in meat samples such as Heptachlor (0.174mg/kg), β-HCH (0.200 mg/kg), δ-HCH (0.070 mg/kg), Aldrin (0.187 mg/kg) and in fish samples were Heptachlor (0.272 mg/kg), Aldrin (0.187mg/kg). The Organophosphorus pesticide residue in meat samples are Chlorpyrifos (11.450 mg/kg), Fenchlorphos (8.071 mg/kg), Disulfoton (10.948 mg/kg), Methyl Parathion (5.340 mg/kg) and in fish samples were Chlorpyrifos (8.537 mg/kg), Fenchlorphos (15.598 mg/kg), Disulfoton (17.435 mg/kg), Methyl Parathion (6.0975 mg/kg), Famphur (10.57 mg/kg). It was found that some meat and fish samples were within and some are exceeded the permissible limits of Food Safety and standard for pesticides.

Key words: Pesticide, Pesticide residue, Organochlorine, Organophosphorus, Nadia

Introduction

Pesticides are the biocide substances meant for attracting and destroying or mitigating any pests (USEPA, 2007). The pesticide is mainly used for protection of crops from the damaging agents such as insects, fungi or weeds. The term pesticide includes the following categories, *i.e.*, herbicide, insecticide, insect growth regulator, nematocide, termiticide, molluscicide, piscicide, avicide, rodenticide, predacide, bactericide, insect repellent, animal repellent, antimicrobial, fungicide, disinfectant and sanitizer (Randall et al., 2008). The residual pesticides refers to the pesticides that may remain on or in food materials after they are applied to food crops. Many of these chemical residues, especially organochlorine and

organophosphate pesticides, exhibit bioaccumulation which could harmful to the human, animals as well as the environment. Persistent chemicals can be magnified through the food chain and have been detected in products ranging from meat, poultry, and fish, to vegetable oils, nuts, and various crops, fruits and vegetables (Crinnion, 2009).

Pesticides are always considered according to the type of pest they control. The pesticides are grouped into according to their persistence in nature, *i.e*., either biodegradable pesticides, which will be broken down by microbial action and other living beings into harmless compounds, or persistent pesticides, which may take long time (months or years) in nature before they are broken down. The suitable example of the persistence pesticide is DDT, which led to its accumulation in the food chain and its killing of birds of prey at the top of the food chain. Traditionally the pesticides are divided into two groups, *i.e*., organic and inorganic. Organic pesticides are based on chemicals having carbon as the basis of their molecular structure and are more complex than those of inorganic pesticides, and usually do not easily dissolve in water. The inorganic pesticides are simpler chemical compounds have a crystalline, salt-like appearance, are environmentally stable, and usually dissolve in water. The earliest chemical pesticides were inorganic, and included substance such as sulfur and lime. The majority of modern pesticides contain an organic chemical.

Organochlorine pesticides (OCPs) are the non-polar toxic chemical compounds classified as dichlorodiphenylethane cyclodienes and chlorinated benzenes (Ademoroti, 1996). The OCPs are common global environmental contaminants found in food stuffs, fish, meat and also drinking water (Ize-Iyamu et al., 2007). The toxicity of OCPs on human health, livestock and laboratory animals has been widely studies by several researchers (Bouman et al., 1990; winter, 1992 , Olea et al., 1998). Pesticides can be enters into the body through various ways like, oral exposure, inhalation, skin contact etc. (Zacharia, 2011). The toxicity effects of pesticides on human and animal health depend on the toxicity of the chemical and the length and magnitude of exposure (Lorenz, 2009). A number of pesticides *i.e.,* DDT, HCH compounds are hyper accumulate in biological system through food chain and causing serious threat to biological system (Gupta, 2004; Lallas, 2001). The exposure effects of pesticides can range from mild skin irritation to birth defects, tumors, genetic changes, blood and nerve disorders, endocrine disruption, coma or death. Children are more susceptible and sensitive to pesticides than adults. Pesticide can affect the reproductive system and early stages of fetus development (Hodgson and Levi, 1996).

In the present investigation a study was carried out to evaluate the residual pesticides like, Organochlorine pesticides (OCPs) and Organophosphorus pesticides(OPPs) in market available fishes and chicken meats in Nadia district of West Bengal, India.

Methods and Materials

Sampling location

The samples were collected from the markets of different blocks of Nadia district of West Bengal. The selected locations are Krishnanagar (23.4009°N, 88.5014°E), Kalyani (22.9751°N, 88.4345°E), Hanshkhali (23.3613°N, 88.6024°E), Majdia (23.4189°N, 88.7253°E), Chapra (23.5155°N, 88.5493°E), Nakashipara (23.5856°N, 88.3534°E), Chakdaha (23.0479°N, 88.5130°E), Nabadwip (23.4036°N, 88.3676°E) and Tehatta (23.7296°N, 88.5299°E). These areas are basically the lower Gangetic plane land.

Sample collection and analysis

The chicken meat and fish samples from the market of study areas were collected in replicates and analysed for residual Organochlorine pesticides (OCPs) and Organophosphorus pesticides(OPPs) following the method Aktar et al., 2009 and Anonymous, 2003.

Collection of meat samples

The chicken meat samples were collected from markets of the study area and thoroughly washed with 0.85% normal saline to maintain the isotonicity of the tissue and wrapped in sterile aluminum foil. The samples thus, collected were kept in ice boxes and transported to laboratory. The same were kept in laboratory at 4°C in freezer till the time of analysis, normally within 48 hours. Each sample was segregated into triplicate number and allowed suitable time to maintain room temperature before being processed for extraction and clean-up procedure.

Extraction methodology for multi residues of pesticides in chicken meat samples

The tissue was finely chopped with the help of sharp edged knife and was spread gently over Whatman filter paper over which another filter paper of the same dimension was kept and pressed with light pressure to remove the blood by soaking. An aliquot (1gm) of the tissue was accurately weighed and taken to a 50 ml conical flask with adding 5 ml of formic acid and 3ml of hexane. The mouth of the flask was tightly covered with the help of aluminum foil. The contents of the flask were shaken at 37^{o} C for half an hour in a shaker to allow the tissue to be dissolved in formic acid and facilitate the solvent to come in contact with the pesticides covalently bonded with the fatty acid of the tissue. The contents were then transferred to graduated centrifuge tubes with the help of additional amount hexane (2 ml) to ensure quantitative transfer to the solvent. The contents were subjected to centrifugation for 10 minutes at 6000 RPM at 20^{0} C. The upper layer of hexane was collected and the remainder was again extracted with 2 ml of hexane. The procedure was repeated and the hexane fractions were pooled.

Clean-up methodology for meat samples

While extracting the pesticides, fat is also co-extracted. As such the extracted samples are subjected to exhaustive clean-up method prior to their analysis is necessary not only to ensure better results but also to prevent the damage to the column. Therefore, clean-up of the extracted samples, in the qualitative and quantitative estimation of pesticides, is an important consideration and should be followed very precisely. The pooled hexane fraction was transferred to a test tube containing 1 ml of sulphuric acid (AR grade, 65% v/v). The contents were gently shaken for 2 minutes and then allowed to rest. The sulphuric acid would turn yellow (since it reacts with fat and forms yellow colour complex). Collect the upper hexane layer with the help of disposable suction pipette and transfer it to another test tube containing 1 ml of sulphuric acid and repeat the same treatment till the sulphuric acid does not turn yellow, which is an indication that the co-extracted fat has been completely removed from the pesticide extracted hexane. Finally the hexane fraction is transferred to the test tube containing 0.5 ml distilled water (Milli-Q) for the removal of sulphuric acid from the hexane fractions. After this the hexane fraction is kept in glass stopper GLC specimen bottle with few crystals of anhydrous sodium sulphate to absorb the moisture. The cleaned up samples were analyzed for the qualitative and quantitative determination of pesticides employing the Gas Chromatographic technique.

Collection of fish samples

Different composite fish samples larger than (1 kg) were collected from market and wrapped with an aluminum foil. The samples thus, collected were kept in ice boxes and transport to laboratory. After the samples were taken into laboratory, kept in deep freezer at 4°C till the time of analysis, normally within 48 hours.

Extraction methodology for multi residues of pesticides in fish samples

About 2gms of representative sample was churned with 2 x 20 ml acetonitrile and tetrahydrofuran mixture (1:1, v/v) in a warring blender for 5 min. The extract was filtered through Buchner funnel under low suction using Whatman No. 1 filter paper and the filtrate was transferred to a 500 ml separating funnel. It was then diluted with 250 ml of saturated aqueous NaCl solution and partitioned with n- hexane twice (2 x 50 ml).

Clean up Methodology for Fish Samples

The n-hexane portions were combined in clean dry flask through a funnel having cotton plugged with 10-15g anhydrous Na_2SO_4 (Kathpal and Dewan, 1975). The hexane phase containing the pesticide residues was concentrated to 30 ml in a rotary evaporator at 4^o C. The n-hexane extract was cleaned after addition of 2g of activated charcoal and shaking for 1-2 min and the flask was left undisturbed for 15 min. The mixture was filtered through Whatman No.1 filter paper and residue was washed sufficiently with hexane, acetone mixture (9:1. v/v). The filtrate was concentrated and the volume was made up to 10 ml with distilled hexane for GC analysis.

Analysis of residual pesticides

The extracted clean samples of meat and fish were analysed for residual Organochlorine pesticides (OCPs) and Organophosphorus pesticides(OPPs) through Gas chromatograph (Thermo Trace 1310) using FID and ECD detector and standard reference pesticides.

Results and discussion

The group of Organochlorine and Organophosphorus pesticide residues of the chicken meat and fish samples from the study areas were analysed through GC. The levels of Organochlorine (Heptachlor, β-HCH, δ-HCH, Aldrin) and Organophosphorus (Chlorpyrifos, Fenchlorphos, Disulfoton, Methyl parathion, Famphur) pesticide residues in chicken meat samples were illustrated in table 1 and the levels of Organochlorine and Organophosphorus pesticide residues in fish samples were recorded in table 2.

The present study it was found the Organochlorine and Organophosphorus pesticide in significant amount in the chicken meat sample (table 1). The amount of residual Organochlorine pesticide such as Aldrin was 0.1872 mg/kg, dHCH (Lindane) was 0.070 mg/kg and bHCH was 0.1997mg/kg. Whereas the residual Organophosphorus pesticide such as Chlorpyrifos was 11.45mg/kg and Methyl parathion was 5.34mg/kg. The residual concentrations of aldrin in fat of broiler chicken were in the range of 0.01 to 0.08 ppm with mean residual concentration of 0.04 ppm was studied by Sunil Kumar, (2013) from the retail markets of Hyderabad. The overall concentration of dieldrin in tissues of chicken was 0.043 ppm (0.01 to 0.077 ppm) found by Aboul-Enein et al*.,* (2010)**.** In Spain Castillo et al., (2011) found that Organochlorine pesticide such as Aldrin (0.2 mg/kg), dHCH (Lindane) (0.02mg/kg), bHCH (0.1mg/kg) and the Organophosphorus pesticide such as Chlorpyrifos (0.05mg/kg) and Methyl parathion (0.02 mg/kg) in chicken samples. The pesticide contaminated chicken feed might be the reason for the detection of aldrin and dieldrin chicken tissue. Comparing

Table 1. Organochlorine and Organophosphorus pesticide residues in chicken meat samples (mg/kg), (n=27).

Organochlorine Pesticides	Minimum	Maximum	Mean ± S.E
β-HCH	0.040	0.861	0.200 ± 0.031
δ-HCH (Lindane)	0.007	0.160	0.070 ± 0.027
Aldrin	0.131	0.265	0.187 ± 0.060
Heptachlor	0.012	0.198	0.174 ± 0.023
Organophosphorus Pesticides			
Chlorpyrifos	8.560	14.976	11.450 ± 0.781
Disulfoton	5.020	16.876	10.948 ± 2.501
Fenchlorphos	1.876	14.266	8.071 ± 1.939
Methyl Parathion	0.919	11.145	5.340 ± 0.430

Table 2. Organochlorine and Organophosphorus pesticide residues in fish samples (mg/kg), (n=27).

Organochlorine Pesticides	Minimum	Maximum	Mean ± S.E
Aldrin	0.070	0.299	0.187 ± 0.005
Heptachlor	0.078	0.465	0.272 ± 0.054
Organophosphorus Pesticides			
Chlorpyrifos	3.970	15.306	8.537 ± 1.875
Disulfoton	3.970	30.900	17.435 ± 5.299
Famphur	5.513	15.620	10.566 ± 1.491
Fenchlorphos	6.493	24.773	15.598 ± 1.352
Methyl Parathion	2.000	10.195	6.097 ± 1.401

the other findings a considerable amount of residual pesticides found in the present analysis, where the residual OPPs are found in higher concentration than OCPs.

Considering the residual pesticides in fish samples the Organochlorine (Aldrine, Heptachlor) and Organophosphorous (Chloropyryfos, disulfoton, famphur, fenchlorphos, methyl parathion) are found in considerable amount (table 2). Like meat samples the Organophosphorous are found in higher concentration than the Organochlorine compounds. It showed that 70-80% of sample having high level of contamination. Studies done by Kole et al*.,* (2001) reported the presence of endosulfan and HCH residues in fishes sold at Calcutta market. There are many studies on the presence of organochlorine residues in aquatic system; water and fishes (Sarkar et al*.,* 2003), freshwater fish (Kaur et al*.,* 2008). The HCH levels reported in freshwater fishes of Andhra Pradesh (2 mg/kg) and Punjab (7 mg/kg) (Amaraneni and Pillala 2001**;** Kaur et al., 2008). In a study from Nigeria the Organochlorine pesticide such as Aldrin (6.76 mg/kg) and the Organophosphorus pesticide such as Chlorpyrifos (1.97 mg/kg) found in fish samples (Akan, et al., 2014).

As per Food Safety and standard, Notification / Regulation 2011, among the all pesticide residues, such as Disulfoton, Fenchlorphos, Chlorpyrifos, Methyl Parathion, Famphur, Heptachlor, Aldrin, Lindane and Endosulfan are highly toxic and hazardous.

The tolerance limit of Disulfoton, Fenchlorphos, Chlorpyrifos is 0.1mg/kg and permissible limit of some OCP pesticides, *i.e.,* Heptachlor (0.1 mg/kg) (according to USEPA), Aldrin (0.2mg/kg) (Food Safety and standard, Notification / Regulation 2011) and dHCH, bHCH (0.003mg/kg).

The overall findings indicate that the OCPs and OPPs pesticide residue in marketable

sample of meat and fish of Nadia district, West Bengal is considerable high which is a major threat to the people of these areas. The use of agricultural chemicals such as fertilizer and pesticides magnify those impacts through bioaccumulation and biomagnifications process. While advances in agro chemistry have reduced those impacts, for example by the replacement of long-lived chemicals with those that rapidly degraded and biocontrol process.

The residual pesticides in animal food products has received worldwide attention from local and international regulatory bodies from the food safety point of view. Despite ban of some pesticides by WHO, still some of those are used in developing countries including India. As a results humans are exposed to higher dietary level of pesticide residues significantly through animal food such as meat and fishes. The proper management and handling of pesticide use is urgently needed otherwise the disaster like *Minamata* may happen in future. All concerned should aware about the toxic effect of the pesticide and Govt. should frame guideline for use of pesticide.

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