CALCULATION OF PERSISTENT ORGANO-CHLORINE RESIDUES IN A FRESH WATER FISH BY GLC AND FIND OUT THEIR ACCUMULATION PATTERN

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Abstract— Residues of various pesticides in a commercial fresh water catfish, *Heteropneustes fossilis* were measured. This study is relevant because of possible toxicological exposure to fish eaters by way of consuming such fish containing pesticides. Tissue extracts obtained by Soxhlet apparatus were used for analysis and the concentrations of analytes were determined by gas chromatography with electron capture detection.The organochlorines found in edible portion of fish (*i.e.*flesh) were mainly- Endosulfan, Delta-HCH, Aldrine , pp-DDE etc. The concentration of these organochlorines were at or above the theoretical threshold limit and hence are supposed to be effectively toxic to fish eaters.

Keywords— *Heteropneustes fossilis*, organochlorines, endosulfan, hexachlorocyclo hexane (HCH), gas chromatography

I. INTRODUCTION

Many persistent, bioaccumulative organochlorine pesticides (OCs) have been extensively used in many countries. These compounds such as DDT, dieldrin etc. persist in the environment for a long time and continue to contaminate aquatic food webs, often at a levels thought to be hazardous to both human health and ecosystem[1,2].

OCs enter in aquatic organisms through body surface, respiratory organs and food, these are transferred from lower to higher tropic levels through the food chain and are biomagnified in the process[3,4]. Man being omnivorous, accumulates the residues throughout life[5]. A relationship between plasma levels of poly chloro biphenyls (PCB's), dieldrin and alpha hexa chloro cyclohexane (α-HCH) with the consumption of salt water fish was found in elderly Germans[6]. Thus, determination of OCs contamination in fish is useful to understand the extent of aquatic pollution and the potential risk of human exposure.

Study area

The study area Agra is located at 27.10^0 N 78.05^0 E, in U.P. state of India. It's elevation is 164.46 meter from MSL. It is located on the bank of river Yamuna and one of the biggest and densely populated cities of India. Agra is famous for leather products and textile industries. Because of population and industrialization, it is also one of the most polluted cities of India.

Materials and Methods

The fish along with the sample of the water, that was inhabited by the fish was obtained from a local pond in the village of Agra district of UP in India, which is about 3 kilometers away from river Yamuna and appox. 14 km. from district head quarter. Only medium size fish were selected (10-15 cm long and weighing upto 250-350 grams). Muscles were excised from the whole body, especially post cephalic part and stored at -20^0 C.

Instrumentation and quantification

All the chemicals and reagents used in extraction and cleanup of organochlorines were HPLC grade and the glassware used were free from residue contamination. The gas chromatograph used in this experiment was NUCON (model 5765) equipped with electron capture detector (ECD). Carrier gas is nitrogen (IOL – AR grade), with flow rate 60ml/min. Column temperature was 190^0 C and injector port temperature 250^0 C. Detector ^{63}Ni ECD with temperature 25^0 C. GC was used in this analysis, and that was equipped with Fused Silica Open Tubular (FSOT) capillary column, which is most efficient and technologically latest type of capillary column. We quantified the samples by comparing the peak area of each with those of their respective standards. Extraction was done by techniques described by Dale et al.[7] and U.S. EPA[8] with some modifications.

Extraction of Pesticides from Inhabited Water

To the 500 ml of water sample taken in a 1000 ml capacity distillation flask was added with 60 ml dichloromethane (DCM). It was shaken well and the nozzle of distillation flask was opened after few seconds to eject out gases. The DCM was allowed to settle down in the distillation flask. This DCM was taken out by opening the nozzle carefully, in a 250 ml stoppered conical flask. The process was repeated thrice and a total of 180 ml DCM collected in a 250 ml conical flask with all the OCs content of water. The DCM was dried in Rota vapour pump (Bucchi). The wall of conical flask was washed well with hexane and the solution *i.e.* washings was made 1ml. It was kept in a borosil test tube, sealed by parafilm and used for GLC.

Extraction of Pesticides from Muscles

Five grams of homogenized muscles and 25 gm of anhydrous sodium sulphate were mixed and the mixture was put into extraction thimble for soxhlet apparatus. The temperature of heater was maintained at 60^0 C. The mixture wraped in double layer of

Whatman no.1 filter paper and was extracted for 4 hours with a mixture of acetone and n- hexane in the ratio of 1:4, using 40 ml acetone and 160 ml of n-hexane.

Extract was filtered through Whatman no.1 paper and the filterate was dried in vaccum rotatory evaporator, at 40^0 C. After drying, the flask was washed thrice, with little amount of hexane each time. The washings were collected in conical flask. Now, the extract was transfrrred to millilitre graduated Borosil test tube, and evaporated under Nitrogen stream, till it remains 1ml. Now sealed the tube with parafilm.

Preparation of Filtration Column

This extract was cleaned up by passing it through ion exchange column setup of florisil. In the column, consisted of a layer of glasswool, then a thick layer of 5 gm florisil and 1gm of anhydrous sodium sulphate at top, to absorb moisture.

Now, two solvent mixtures F_1(3.6 ml diethyl ether + 56.4 ml n-hexane = total volume 60ml.) and F_2(40 ml diethyl ether + 40 ml n- hexane = total volume 80 ml.) are prepared in two different conical flask.

Filtration

Filteration column was prewashed with 20 ml of n-hexane. Now, 1ml pesticide laden hexane from graduated borosil tube, which was collected by washing the conical flask, was poured into the filteration column. Then F_1 solvent was poured in the filteration column. Filterate was collected in a conical flask and dried in rota vapour pump. Solution was made upto 1 ml by washing the wall of flask with hexane. It was kept in clean Borosil tube for GLC; (F_1). Similarly, the solvent mixture F_2 was poured in the filteration column. Filterate was collected in another conical flask, dried in rota vapour pump and the solution was made upto 1 ml by washing the wall of the flask with hexane. It was kept in clean Borosil tube for GLC; (F_2).

II. RESULTS AND DISCUSSION

The concentration of analytes through the chromatograph was determined by the formulae,

Concentration of analytes =

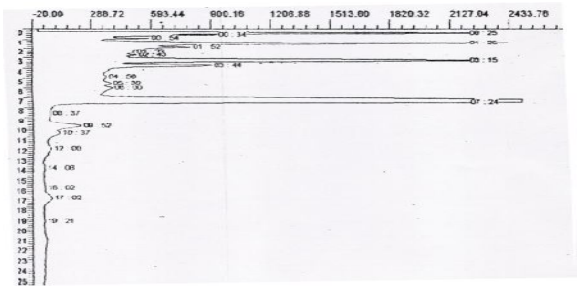
$$\frac{\text{area of analytes x concentration of standard}}{\text{Area of standard}}$$

(a) Estimation of OCs in the inhabited water

5 microlitres of extracted OCs, which were collected in 1ml graduated borosil test tube, was injected in to the GLC column. Quantity of OCs measured was in microgram per millilitre of water, as shown here –

Concentration of analytes in microgram per millilitre of water (µgm/ml).

Delta HCH	Endosulfan	Aldrin	ppDDE	Alpha HCH
8.58	17.9	2.32	0.918	0.412



Chromatogram showing various OC's concentration in the water inhabited by fish.

(b) Estimation of OCs in the muscle tissues of fish

2 microlitres of sample extract was injected in GLC column. Total concentration of analytes was obtained by adding the concentration of OCs, obtained through solvent mixtures F_1 and F_2, separately in Figure-A and Figure-B. Quantity of OCs was measured in microgm per gm (µg/gm) of fish tissue, as shown here-

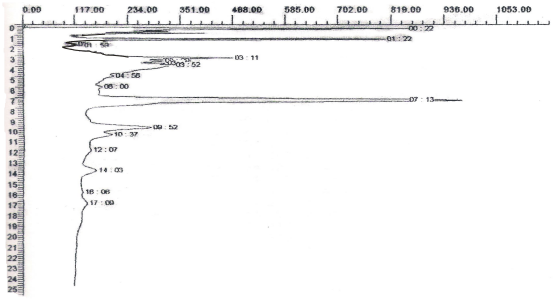


Figure A: chromatogram showing concentration of different organochlorines, obtained through solvents mixture F_1.

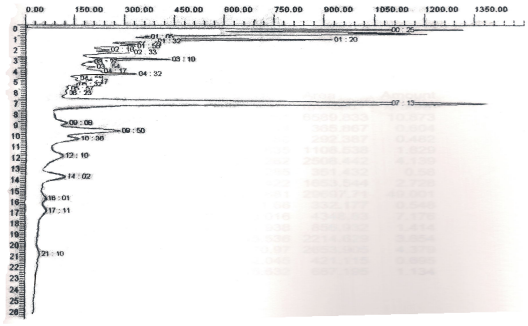


Figure B: Chromatogram showing concentration of different organochlorines, obtained through solvents mixture F_2.

These findings are very suggestive, that use of endosulfan as a major organochlorine pesticide has increased. This is also proved by production data. After ban on DDT, the world-wide production of endosulfan has increased rapidly. At present, it is estimated to be 10,000 tonnes per year. DDT is classified as a Group 2B carcinogen[10], (possible carcinogen to humans) by the international agency for research on cancer. It is therefore classified as a

persistent organic pollutant, and it's manufacture, distribution and use are prohibited by the "Stockholm Convention on Persistent Organic Pollutants"[11].

Various factors such as size, age, species, feeding habits are related to the bioaccumulation of chemicals. In the case of organic contaminants, lipid content is a major factor because most OCs are lipophilic. A positive correlation was observed between pesticide residues and lipid contents of fish[12,13]. Among the OCs analyzed, HCH compounds showed a significant correlation with lipid content in the muscular tissues of fish. However, DDT, chlordane, dieldrin and especially PCB compounds showed no significant correlation. Given sufficient time, lipophilic chemicals in the environment attain equilibrium with lipid compartment of the organism through equilibrium partitioning.

The data of pesticidal content in inhabited water can be correlated with the data of pesticidal content in blood as well as in flesh. However, if chemical input is in dynamic state and organism are migratory in nature, it is hard to establish the correlation. It has been found that due to bio-accumulation and bio-transformation, concentration of pesticides in blood increases and in the medium, concentration decreases[14]. Concentration of pesticide in blood exceeds even the LC_{50} concentration, but it is not lethal for the specimen, because it combines with blood protein and not available for metabolic breakdown again. So, pesticide's toxicity is not harmful for specimen any more, however it may be lethal for the person consuming such exposed fish[17].

	F 1	F 2	Total conc.
Alpha IICII	0.000718	0.001738	0.002456
Delta HCH	0.0084	0.01031	0.01871
Aldrin	0.006	0.0019	0.0079
Endosulfan	0.0901	0.1438	0.2339
p,p-DDE	0.0119	0.01864	0.03054

Human exposure to the environmental contaminants through fish consumption depends on the amount of fish consumed. OCs are known to accumulate in the subcutaneous adipose tissues[15], but here, only skin-off muscle tissues of fish were analyzed, as it is a source of exposure besides the known fat deposits. This is especially significant when small fish or sliced fish are consumed with skin. This means that if the people digest the skin along with flesh, the risk for exposure to the contaminants through fish consumption will increase.

Therefore, the present findings are interpreted in terms of fish living in general environment available to it, with usual contamination with pesticides and the fish is deliberately exposed to the said pollutant, and it also confirms that natural fish is not safe fish.

CONCLUSIONS

Bulk of OCs are used for agricultural crop protection, forest protection and public health programmes. From the study of the data of OCs content in inhabiting water, fish blood and fish flesh, we can see that concentration of endosulfan is higher in all samples. ppDDE and δ-HCH are also present in good concentration in inhabiting water as well as in flesh. α-HCH is also present in all samples, however in lower concentrations. Earlier studies have shown that endosulfan undergoes metabolic changes in animal as endosulfan diol and other hydrophilic metabolites. That is why the peaks of endosulfan occour slightly at different positions**.**

The proposed study for estimation of total OC's content in water and aquatic life is a promising and easy way to improve the management of these chemicals for agricultural and public health programmes.

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