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FINDING OUT NON DEGRADING ORGANOCHLORINES IN A FRESH WATER FISH BY GLC AND STUDY THEIR ACCUMULATION DYNAMICS

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Different quantity of various organochlorine chemicals found in a fresh water cat fish of commercial utility *Heteropneustes fossilis.* This study is an alarming signal for possible toxicological exposure to human beings by way of consuming such exposed fish. GLC analysis of inhabiting water, specimen blood extract and specimen tissue extract has been done. It shows that major organochlorines in blood sample were Endsulfan, α-HCH, β-HCH and γ-HCH. The organochlorines detected in edible portion of fish were Endosulfan, δ-HCH, Aldrine pp-DDE etc. The quantity of these organochlorines were at or above the theoretical threshold limit and may be effectively toxic to fish consuming population

Keywords: Organochlorines, Heteropneustes fossilis, endosulfan, HCH (hexachlorocyclohexane), GLC (gas liquid chromatography).

INTRODUCTION

The whole life of any living being is a continuous flux of chemicals, that gives apprehension that it is better to apply chemistry both theoretically and technically to evaluate the effects of chemical discharge on the life of living being so as to reach the comparatively important and comprehensive result and to be more precise to evaluate pollution more accurately.

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].

Many techniques of isolation, cleaning of pesticides residue extracts from lipid containing tissues and blood have been carried out. Soxhlet extraction with a variety of organic solvents, such as hexane, ether, acetone, alcohol and their combinations, was among the most common method for extracting organochlorines from lipid containing tissues. 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 positive 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.

MATERIALS AND METHODS :

The specimen were collected from different ponds in Bhagwanpur near Roorkee, adjacent to agricultural land and industrial area. Medium sized fishes (12-16 cm, weighing about 250-350 grams) were collected for experiment. For collecting blood, they were cut slightly above the caudal fins and the blood was squeezed out. Flesh was excised from whole body and stored in deep freezer. Some samples of inhabiting water were also collected from same location.

INSTRUMENTATION:

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° C and injector port temperature 250°C. Detector ^{63}Ni ECD with temperature 25°C. GC was used in this analysis, and that was equipped with Fused Silica Open Tubular (FSOT) 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 Blood

Pesticidal residue from blood was extracted by the method described by Therdteppitk and Yammeng[9]. Final volume was made up with hexane upto 2ml. 5 micro-litre of this solution was injected into GLC attached with an Electron Capture Detector.

EXPERIMENTAL PLANNING

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 extraction. The temperature of heater was maintained at 60° C. The mixture wrapped 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° 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 transferred to milliliter 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; named as (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; named as (F_2).

RESULTS AND DISCUSSION:

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

$$\text{Concentration of analytes} = \frac{\text{area of analytes} \times \text{Concentration of standard}}{\text{area of standard}}$$

Table : 1 Concentration of analytes in microgram per millilitre of blood (µgm/ml).

Alpha HCH	Gamma HCH	Beta HCH	Endo- sulfan	o,p- DDT	p,p- DDT
0.118	0.1328	0.1066	2.448	0.1424	0.0826

(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-2 and Figure-3. Quantity of OCs was measured in microgm per gm (µg/gm) of fish tissue, as shown in Table.

Concentration of analytes in microgram per gram (µg/gm) of fish tissue.

OC's	F 1	F 2	Total conc.
Alpha HCH	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

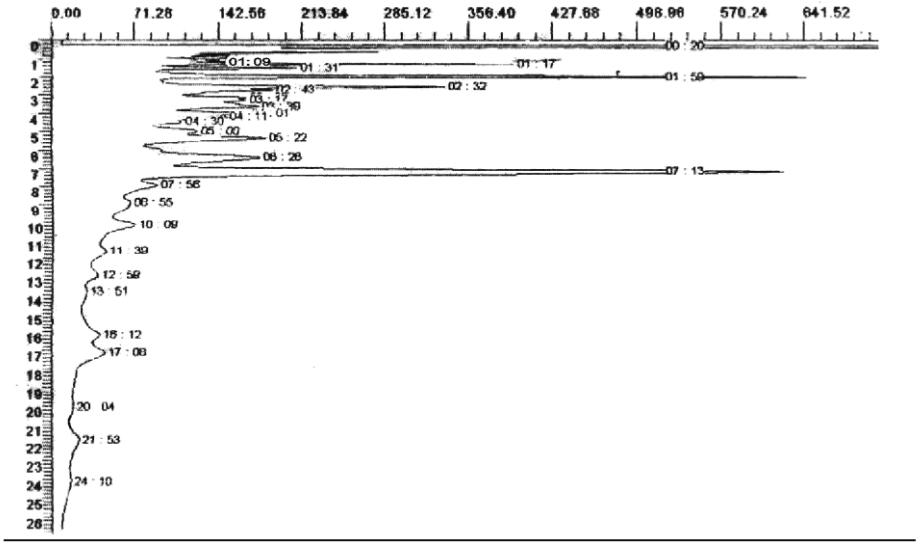

Figure-1: Chromatogram showing concentration of different organochlorines in the blood of fish.

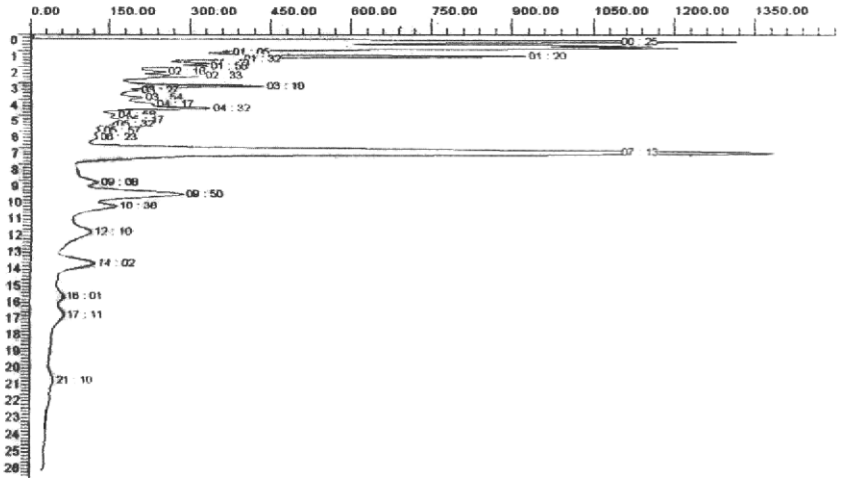

Figure-2: chromatogram showing concentration of different organochlorines, obtained through solvents mixture F_1.

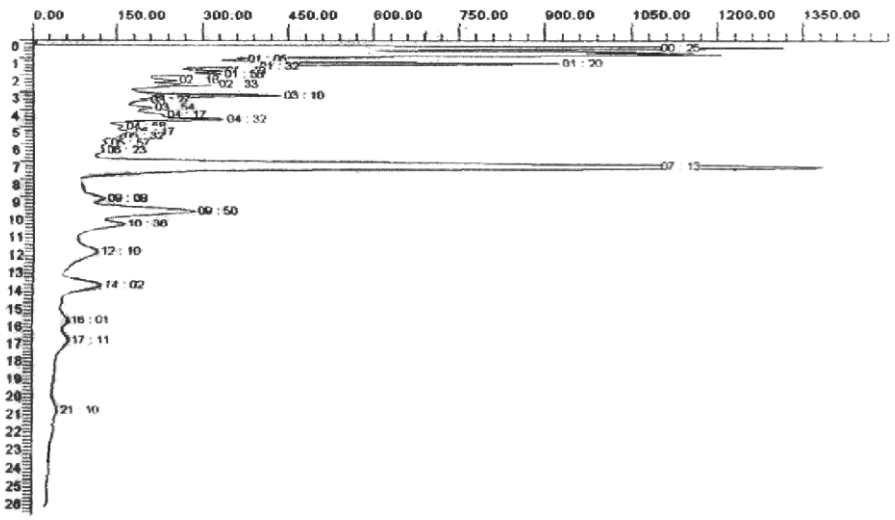

Figure-3: Chromatogram showing concentration of different organochlorines, obtained through solvents mixture F_2.

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, (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"[10].

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. 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. 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.

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, 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 consume 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.

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