Chapter 13

Analysis of Pesticide Residue in Fish

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Pesticides are chemical compounds that are used to kill pests, including insects, rodents, fungi, and unwanted plants (weeds). Over 1000 different pesticides are used around the world. Pesticides are used in public health to kill vectors of disease, such as mosquitoes, and in agriculture to kill pests that damage crops.

Pesticide can be defined as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest such as insect, rodent, nematode, fungus, weed, other forms of terrestrial or aquatic plant or animal life or viruses, bacteria, or other microorganisms on or in living man or other animals, which declares to be a pest, and any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant.

Pesticides can be classified in many ways on the basis of use, toxicity, mode of entry, mode of action, chemistry, and formulations. The major chemical types of pesticides include

(i) **Organochlorine pesticides (OC)** – This group consists of the polychlorinated derivatives of cyclohexane (Lindane), polychlorinated biphenyls (DDT, dicofol), and polychlorinated cyclodiene (Endosulfan).

Properties of OCs

Physical property: OCs are solids that possess low volatility, low solubility in water, high solubility in oils, fats, lipids, *etc*., and they are not prone to environmental degradation.

Chemical property: Organochlorine pesticides shows isomerism

Toxicity: These compounds possess high acute toxicity as well as chronic toxicity

Biological stability: OCs are not rapidly degraded by enzymes, not rapidly exerted, but get stored in the fatty tissues.

A number of organochlorine pesticides have been banned globally and they are controlled via the Stockholm convention on persistent organic pollutants (POP's). These include: aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex, and toxaphene

(ii) **Carbamates**: Carbamates are esters of either carbamic acids or thiocarbamic acids. Carbamates may be further subdivided into three sub-groups,

Group	Example
Aryl N methyl carbamate	Carbaryl, Propoxur
Hetero cyclic mono or dimethyl Carbamates	carbofuran
carbamoylated oximes	methomyl
Thiocarbamates	cartap hydrochloride (neriestoxin group of insecticide)

Properties:

Physical property: The organo-carbamates are available as nonvolatile solids. Carbaryl, and carbofuran are having very low water solubility (40-6000ppm) whereas Cartap hydrochloride is hygroscopic in nature. And these compounds undergo degradation by environmental factors.

Chemical property: These compounds are unstable in an alkaline medium.

Toxicity: The organo-carbamate compounds exhibit moderate to extreme toxicity, and they do not display chronic toxicity

Biological stability: The organo-carbamate compounds undergo enzymatic degradation and are rapidly metabolized and excreted. Biomagnification is almost absent in this group of pesticides and chronic toxicity is insignificant.

(iii) **Organophosphates (OP):** These are the esters of derivatized phosphoric acid, thiophosphoric acid and dithio phosphoric acids which are called phosphates, thiophosphates and dithiophosphates respectively. Some of the examples of each class of pesticides are as follows:

Group	Example
Phosphates	monocrotophos,phosphamidon, 2,2-dichlorovinyl dimethyl phosphate (DDVP) or Dichlorvos
Thiophosphates	methyl parathion, fenitrothion, Phosphorothiates oxy demeton methyl
Dithiophosphates	phosporodithioates dimethoate, pphosphorothioates

Based on the organic moiety attached to the phosphoric acid these can also be classified into aliphatic, phenyl, and heterocyclic derivatives.

Properties

Physical property: These compounds are available as liquids or semi-solids and possess significant vapour pressure and are comparatively volatile in nature. Some of these compounds are slightly soluble in water (example: Phosphamidon).

Chemical property: These compounds which are esters of phosphoric acid are not stable in alkaline pH, but stable over a narrow range of pH. Thiophosphates and dithiophosphates

undergo molecular rearrangements, forms isomers with increased toxicity and undergo oxidation to give oxo compounds with increased toxicity. The organo phosphorous pesticides undergo the conversion of one pesticide into another pesticide. The following are some examples.

Trichlorfon → dichlorvos

Formothion → dimethoate

Acephate → methamidophos

Toxicity: These compounds exhibit acute extreme toxicity to slight toxicity. LD50 values may change with the purity of the compound. These compounds are having low chronic toxicity. They undergo rapid conversion into low fat-soluble metabolites which are excreted.

Biological stability: The OP compounds undergo enzymatic degradation and the metabolites are fat-soluble and easily get excreted. Biomagnification is almost absent and chronic toxicity is insignificant

(iv) **Pyrethroids** – Living organisms do contain naturally a large number of chemicals some of which give them protection from foreign invasive substances. Many such chemicals have been isolated, identified, and evaluated for their biological activity. The flowers of chrysanthemum contain compounds called pyrethrins which are found to have possessed very good pesticidal activity but are found to be less stable in the environment. The pyrethrins are chemically the esters of chrysanthemic acid and pyrethric acid (which contains dimethyl cyclopropane group) with alcohols, namely pyrethrolone, cinerolone and jasmolone.

Synthetic Pyrethroids: Allethrin was the first synthetic pyrethroid developed in 1949, followed by resemethrin. However, they have failed to contain the desired properties and proved to be highly photolabile. The first photo sable pyrethroid developed was permethrin. This was followed by cypermethrin, deltamethrin, and fenvalerate. The synthetic Pyrethroids contain a halogenated derivative of dimethyl cyclopropane carboxylic acid and cyano phenoxy benzyl alcohol. Fenvalerate is an exception with the acid portion being p-chlorophenyl isopropyl acetic acid instead of cyclopropane carboxylic acid. In the case of permethrin, the alcohol portion does not have cyano - group, but it is simply phenoxy benzyl alcohol.

Water bodies around the world are threatened by various anthropogenic activities, resulting in poor water quality. The pesticide contamination in fish mainly comes through agricultural runoff and municipal sewage effluent. Later, intensive aquaculture practices with insecticides such as trichlorfon and dichlorvos to kill unwanted organisms or as algaecides to control water quality in fish/shrimp farms of different regions of the world also lead to pesticide

contamination in fish. Several studies have reported the presence of organochlorine pesticides in fish harvested from Indian waters also.

Procedure for Pesticide residue analysis in fish by GC-MS/MS

- Take a 2 kg fish sample, and homogenize the muscle tissue. Weigh 5 g representative sample in 50 ml centrifuge tube
- *Extraction*: Add 5 ml distilled water and Vortex for 1 minute. Then add 10 mL of acetonitrile containing 100 μL of acetic acid and vortexed for 1 minute. Add 6 g of magnesium sulphate ($MgSO_4$) and 1.5 g of sodium acetate (CH_3COONa) and vortex for 2 minutes. Centrifuge the content at 4000 rpm for 5 minutes. Keep the supernatant at -20°C for 20 minutes n to avoid loss of thermo-labile analytes due to heat generated during dSPE clean-up
- *Clean up*: Transfer the extract to dispersive-SPE (dSPE) tubes containing 50 mg PSA sorbent + 150 mg MgSO4 per mL as per AOAC- QuEchERS 2007.01. Vortex the mixture for 1 minute. Centrifuge at 4000 rpm for 5 minutes
- Filter 2ml extract through 0.2µ PTFE membrane filter; Inject and analyse in GC-MS

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