

REVIEW ON PRESENCE OF ORGANIC CONTAMINANTS RESIDUE IN FOOD BY DIFFERENT ANALYTICAL TECHNIQUES

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Abstract: Organic contaminant and their residue present in foods have been a major concern for food Safety due to their persistence and toxic effects. To ensure food safety and protect human health from contaminant residue, it is critical to achieve a better understanding of pathways into food and develop strategies to reduce human exposure. Organic Contaminant residue could present in food in the raw stages, transferred from the environment or artificially introduced during food preparation steps. Pesticides are widely used in agriculture mainly to increase crop yields to cater huge supply of food products for increasing world population as well as to protect crops from pests and control insect-borne diseases. Increased use of pesticides results in contamination of the environment and the excess accumulation of pesticide residues in food products, which has always been a matter of serious concern. Methods for determination of organic contaminants such as pharmaceuticals and pesticides in various food samples, using SPE as the extraction technique and LC-MS/MS with electro spray ionization, were developed and validated.

This review addresses recent advances in the analysis of organic contaminants, such as antibiotics, Pesticides, biological toxins, and food-borne pathogens, in foods by Liquid Chromatography, Mass spectroscopy. Organic Contaminant exposures have been linked to many human diseases such as Alzheimer, Parkinson, asthma, bronchitis, infertility, birth defects attention deficit hyperactivity disorder.

Key words: Organic Contaminants Residue, Pesticide Residue, Residue limit, Liquid chromatography, Mass spectrometry, Impact on Human health.

I. INTRODUCTION TO ORGANIC CONTAMINANT RESIDUE ^[1, 2]

Organic contaminants may be defined as **chemicals that are carbon based**, for example, organic solvents, pesticides, petroleum-based wastage, and gas or liquid phase volatile compounds. The presence of organic contaminants in the environment has recently become a great concern of Environmental Protection Agencies around the world, with special attention for pharmaceuticals and pesticides that are included in the so called emerging contaminants ^[1].

These compounds are continuously introduced into the environment and are found at trace or ultra-trace concentrations ($\mu\text{g L}^{-1}$), but, even so, can affect human health. Major concerns about the presence of organic contaminants in the environment involve development of bacterial resistance to antibiotics, carcinogenic activity and the still uncertain human risks of long term chronic exposure at trace levels ^[1].

There are many contaminants of an organic nature that can occur in food, including mycotoxins, pesticides, pathogenic organisms, and impurities associated with environmental pollution or with contact migration from food packaging. Food safety is ensured through legislation that relies heavily on appropriate analytical methods to detect contamination of food, both rapidly and reliably ^[2].

The analysis of organic contaminants in food is an important issue that encompasses many disciplines including chemistry, biochemistry, and microbiology. It is extremely intricate because these pollutants, which can be numbered in the thousands, are present at extremely low concentrations in a very complex mixture of natural food components. Thus, any instrumental technique that is to be used successfully in food analysis should achieve the separation of the matrix compounds and the identification of the contaminants at trace levels ^[2].

PESTICIDE RESIDUE ^[3, 4]

The term pesticide covers a broad variety of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematocides, plant growth regulators and others ^[3].

Depending on the chemical structure pesticides can be classified as:

1. Organochlorines (endosulfan, hexachlorobenzene),
2. Organophosphates (diazinon, omethoate, glyphosate),
3. Carboxylic acids and their derivatives,
4. Urea derivatives,
5. Heterocyclic compounds (benzimidazole and triazole derivatives),
6. Phenol and nitro phenol derivatives,

7. Hydrocarbons,
8. Ketones,
9. Aldehydes and their derivatives,
10. Metal organic and inorganic compounds,
11. Natural and synthetic pyrethroids.

Currently, pesticides are very valuable in developing nations, particularly those in tropical are as looking for an entry in the global economy by providing off-season fresh vegetables and fruits to nations in more temperate weathers. However, these goals cannot be achieved without the increased use of pesticides, mainly insecticides, herbicides and fungicides ^[2].

Increased use of pesticides results in contamination of the environment and the excess accumulation of pesticide residues in food products. Food products contaminated with toxic pesticides are associated with severe effects on the human health. More than 95% of sprayed insecticides and herbicides reach a destination other than their target species, including non-target species, air, water and soil ^[4].

Pesticide contamination of both surface and ground waters can also affect aquatic animals and plants, as well as human health when water is used for public consumption. For, Human a pesticide exposure means getting pesticide in the body. The toxic effect depend upon the exposure time and the concentration of pesticide ^[4].

II ORGANIC CONTAMINANT RESIDUE IN FOOD ^[5]

Persistent organic pollutants (POPs) present in foods have been a major concern for food safety due to their persistence and toxic effects. To ensure food safety and protect human health from POPs, it is critical to achieve a better understanding of POP pathways into food and develop strategies to reduce human exposure. POPs could present in food in the raw stages, transferred from the environment or artificially introduced during food preparation steps ^[5].

Exposure to these pollutants may cause various health problems such as endocrine disruption, cardiovascular diseases, cancers, diabetes, birth defects, and dysfunctional immune and reproductive systems. Contaminants have become a concern in terms of food safety due to pesticide residue and environmental contaminants detected in the food supply. A large amount of pollutants generated from rapidly developing industrial sectors have been released to the environment and found their way into the food supply ^[5].

In recent decades, there has been a focus on a subset of harmful organic chemicals, mostly of anthropogenic origin, that are commonly classified as **Persistent organic pollutants (POPs)**. POPs are a class of carbon-based organic chemicals that are persistent, bio- accumulative and have long-range transport potential ^[5].

There are three types of POPs present in the environment:

- 1) Especially organochlorine pesticides (OCPs) such as dichloro diphenyl trichloroethane (DDT) and its metabolites;
- 2) Industrial and technical chemicals including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and Perfluorooctanesulfonate (PFOS); and
- 3) By-products of industrial processes including polychlorinated dibenzofurans (PCDFs), and Polyaromatic hydrocarbons (PAHs).

POP include a range of substances that include:

- 1) Intentionally produced chemicals currently or once used in agriculture, disease control, manufacturing, or industrial processes. Examples include PCBs, which have been useful in a variety of industrial applications (e.g., in electrical transformers and large capacitors, as hydraulic and heat exchange fluids, and as additives to paints and lubricants) and DDT, which is still used to control mosquitoes that carry malaria in some parts of the world ^[6].
- 2) Unintentionally produced chemicals, such as dioxins, that result from some industrial processes and from combustion (for example, municipal and medical waste incineration and backyard burning of trash) ^[6].

Commonly Found Persistent Organic Pollutant [POP] in Food ^[5]

1. Chlorodibenzo Furan (CDF)
2. Polychlorinated Biphenyl (PCB)
3. Polybrominated Diphenyl Ether
4. Hexabromo Biphenyl
5. Hexachlorobutadiene (HCB)
6. Organochlorine Pesticide (OCPs)
7. Polyaromatic Hydrocarbons (PAHs)

POPs are resistant to environmental degradation including chemical, biological, and photolytic reaction. Therefore, once released POPs Can stay in the environment for long time. Some POPs could have half-lives of years or decades and they can stay in the environment until they are taken up by plants and animals.

POPs can bioaccumulate in the fatty tissue of living organisms and, therefore, become concentrated as they move through the food chain. Over 90% of human exposure to POPs is through the consumption of contaminated food, particularly food of animal origin. Fishes are among the major sources of exposure to POPs ^[5].

To protect consumers from POP-contaminated food, many national and international agencies such as the European Food Safety Authority (EFSA), World Health Organization (WHO), US Environmental Protection Agency (EPA), and US Food and Drug Administration (FDA) have developed regulations and guidelines to reduce the exposure to POPs ^[5].

Reason for Food Contamination^[7]

Food is a crucial contributor to human health well-being and a major source of worry, pleasures, and stress with one of the reasons behind the stress and worry, are the diseases caused as a result of contaminated food. There are multiple reasons for the contamination of food^[7].

Food preparation undergoes through a long chain of processing, where each stage is a potential source of chemical contaminants invasion of the food. Transportation of food can also lay the foundation for contamination of food, specifically under poor sanitary conditions. Likewise, some chemicals are mixed deliberately during the food preparation process to improve the shelf life of a food product^[7].

The contaminants may include impurity food when cooked in the kitchen; nevertheless, the transmission is mainly dependent on the effectiveness of the kitchen hygiene though. Chemical contaminants enter the food chain naturally as well with pathogens that are present in the environment and show high bacterial numbers on some key raw foods such as poultry meat.

Sources of POP Contamination in Food^[5, 8, 9]

Food preparation usually involves multiple steps including processing, packaging, transportation and storage. Each step could be a potential source for POP invasion. Food could be contaminated by POPs through different paths. For example, raw materials may contain POPs that are transferred from the environment. Since POPs are resistant to degradation, they can stay in the environment for an extended period time. Previously released POPs in the environment are a major source of the POP contamination of food and feed supplies^[5].

Plant foliage uptake of POPs can effectively transfer POPs from air to plant and subsequently to food. Other sources of POPs are food preparation steps, during which POPs may artificially be introduced by humans^[5]. The source of contaminants may be the environment. This is the case for metals such as lead and mercury, dioxins, and polychlorinated biphenyls (PCBs)^[8]. Agricultural may lead to food contamination. Potential sources of organic contaminants include: Industrial leaks and spills, improper application of pesticides, Careless disposal of cleaners, oil and antifreeze, Improper disposal of household wastes, Landfills and garbage dumps, accidents, spills during transportation (i.e. from rail or tank cars)^[9].

Residue in Food Chain^[10]

Table 1: Residue in Food Chain

SR.NO.	ITEMS	RESIDUE
1	Cattle Feed	DHT,BHC
2	Oils, Rice, Wheat flour	DDT,BHC
3	Dairy Product, Baby Milk Powder, Butter, Ghee	BHC,DDT, HCB, PCB, Heptachlor
4	Meat , Eggs	PCB, Carbaryl
5	Vegetables	Endosulfan
6	Human Breast Milk	BHC, DDT, Aldrin, HCH

III ORGANIC CONTAMINANT RESIDUE FOUND IN VARIOUS FOOD

A. Organic contaminant residue in Milk and Dairy product^[11]

Milk and dairy products are important components of the human diet. Some residue and contaminants in milk and dairy products can be classified as antibiotics, mycotoxins, anthelmintics, pesticides, dioxins, hormones and heavy metals^[11]. Presence of the residual concentrations of these compounds in milk and dairy products can be an important direct indicator for the quality status of the milk and their products, because these agents can easily transported to milk and dairy products which are evaluated the most important contamination sources for the human health^[11].

1. Mycotoxins:

Mycotoxins are toxic metabolites produced by various fungi growing in a wide range of food and animal food stuffs. The main mycotoxins that occur frequently are aflatoxins, ochratoxin A. The most common mycotoxins that are stable in milk and dairy products such as cheese, are citrinin, cyclopiazonic acid, and aflatoxin6. Aflatoxin B1 (AFB1) is produced by certain *Aspergillus spp.* during their growth on animal feeds. Mammals which consume feed contaminated with Aflatoxin B1, produce milk contaminated with Aflatoxin M1 (AFM1)^[11]. Aflatoxin M1 is a hepatic carcinogenic metabolite found in milk of lactating animals which consume food with Aflatoxin B1.

In relation to cheese, the presence of aflatoxins may be due to three causes:

- a) Presence of AFM1 in milk,
- b) Synthesis of AFB1, B2, G1, G2 by fungi,
- c) Use of powdered milk with AFM1, to enrich milk employed in cheese production.

2. Anthelmintics:

Anthelmintic drugs are widely used in the veterinary medicine for protecting or treating the animals mainly against gastrointestinal nematodes and lungworms. The anthelmintic drugs are generally of very low toxicity to mammalian hosts, but some benzimidazole compounds such as albendazole are known to be teratogenic. Benzimidazole anthelmintics are effective against nematode, cestode and trematode parasites, and they are used as antiparasitic drugs in veterinary and human medicines ^[11].

3. Dioxins:

These compounds are lipophilic unwanted by-products. Dioxins cause a wide array of adverse health effects in both animals and humans. They are potent cancer-causing agents and are considered to be a known human carcinogen by the World Health Organisation's. Exposure of humans to dioxins occurs mainly (> 95 %) through contamination of food. Milk and dairy products, bovine adipose tissue, eggs and fish are the main contributors to human dioxin exposure ^[11].

Dioxins in air settle onto soil, water and plant surfaces. They do not readily break down in the environment and over time they accumulate in the grazing animals. People ingest the dioxins with the consumption of the contaminated milk, dairy products, meat and eggs.

4. Hormones:

Milk contains many natural hormones like corticoids, oestrogens, Androgens etc. which are produced by mammals. The presence of these natural hormones in milk shows difference due to animal's physiological characteristics, different periods of lactation and pregnancy. In recent years, hormones and hormone like compounds have been used frequently in vegetables and livestock production to obtain a high performance in a short time. These anabolic compounds are used for increasing the rate of weight gain, improving the food efficiency, storing protein and decreasing the rate of fat storing 28-30 °C. But depending on the use of anabolics in animal food, residues which may occur in milk and dairy products present concerns human health risks and because of this many countries restrict or prohibit the use of anabolic compounds in livestock production ^[11].

Zeranol is a nonsteroidal, oestrogenic mycotoxin which is produced by several *Fusarium* species. It is used in livestock for increasing the rate of weight gain, improving the feed efficiency and for a good quality carcass with less fat. Zeranol residues should not exceed 0.05 ppb in daily human food ^[11].

5. Heavy metals:

Technological progress, various industrial activities and increased roadways traffic caused a considerable increase in environmental contamination. Heavy metals enter the human body through inhalation and ingestion. It is well established that lead and cadmium are toxic and children are more sensitive to these metals than adults. While copper and zinc are essential they can be toxic when taken in high doses. Determination of the residual concentrations of metals in milk can be an important direct indicator of the hygienic status of the milk and/or of its derived products, as well as an indirect indicator of the degree of pollution of the environment in which the milk is produced. Milk and dairy products constitute a major food, especially for infants and children, and relatively low levels of toxic elements can contribute significantly to dietary intakes and be hazardous for public health ^[11].

Heavy metals content of milk products are variable due to factors such as differences between species, geographical area, characteristics of the manufacturing practices and possible contamination from the equipment during the process ^[11].

B. Organic Contaminant residue in Honey ^[12]

Honey is a natural sweetener used widely across the world from ancient times. It is widely used for various applications including clinical and contains 200 distinct chemical compounds. Honey is recognised world-wide as a medicine, energy source and well known for biological, physiological and pharmacological activities. Due to agricultural intensifications and human interference, honey and various bee products are polluted by different source of contamination. The honey contamination especially with antibiotics and adulterations are very active which completely vanish the apiculture field and create poor reputation of beekeepers. Therefore it is the need to improve the quality of honey by excluding the different contamination sources and possibilities ^[12].

One of the major organic contaminants present in the environment are PCB's, (polychlorinated biphenyls) which originate from motor oil, coolants and lubricants, produced before. These substances are still persistent in the environment and can contaminate plants and thus, the bees and their products. The quantities, found in honey are low and safe, while those found in wax are higher. Small residues of polyaromatic compounds originating from oil were found in honeys ^[12].

C. Organic Contaminant Residue in Fish ^[13]

Although air and water are also major pathways of exposure to the organic contaminants, their concentrations in these media are lower than in fish. Moreover, pollutants in air and water can be removed by current purification technologies before being inhaled and drunk, whereas it is very hard to remove those pollutants in fishes.

Those polluted fishes would be ingested by human beings and other animals. Therefore, from both ecological and human health points of view, it is important to determine and evaluate the risks of pollutants in fish ^[13]. The organic contaminants, including polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), and polychlorinated biphenyls (PCBs), are Present in Fish tissue. They are of priority concern because of their persistence, toxicity, and long-distance transportation in global environment. Their residues in a daily consumed fish pose potential threat to human health and aquatic ecosystems.

D. Organic Contaminant Residue in Meat ^[14]

Meat and meat products are important for nutrition and the human diet. Meat are also one of the major routes of human intake of contaminants. The potential health risk associated with exposure to these residues from meat consumption. Contaminating substances may enter the food chain at many different stages. Through various constituents like fertilizer ingredients and contaminants, irrigation water, contaminants and pesticides can enter food crops through plant roots. Contaminants in forages and other feeds can be transmitted to animal products ^[14].

Environmental chemicals such as heavy metals (e.g., lead and mercury) from many sources have sometimes been found as food contaminants. Thus range of contaminants/toxicants found in food is varied but can be broadly subdivided into:

1. Food processing by products;
2. Agricultural and veterinary chemicals used in primary production;
3. Natural chemicals found in plants, fungi or bacteria associated with plants
4. Environmental contaminants, including heavy metals and organic contaminants.

A great number of chemical compounds are used either directly or indirectly during the processing and storage of meat and meat products. Concentration levels of some toxic chemical residues in meat sold in to assess the potential health risk associated with exposure to these residues from meat consumption ^[14]. During the processing of foods, products may be produced that, if present in large amounts, could potentially adversely affect health. For example, cooking certain meats at high temperatures creates chemicals that are not present in uncooked meats. A few of these chemicals may increase cancer risk, such as polycyclic aromatic hydrocarbons and heterocyclic amines. Some different HCAs (HeteroCyclic Amines) have also been found, resulting from the cooking of muscle meats that may pose human cancer risk ^[14].

IV PESTICIDE RESIDUE FOUND IN VARIOUS FOOD ^[3]

Pesticides are widely used in agriculture mainly to increase crop yields to huge supply of food products for increasing world population as well as to protect crops from pests and control insect-borne diseases. Increased use of pesticides results in contamination of the environment and the excess accumulation of pesticide residues in food products, which has always been a matter of serious concern ^[3].

Pesticide residues in food and crops are directly related to the irrational application of pesticides to the growing crops. Accumulated pesticide residues in food products have been associated with a broad variety of human health hazards, ranging from short-term effects to long term toxic effects. The impact of pesticide residues can be minimized by taking certain measures such as the rational use of pesticides, promoting organic farming, exploit natural and bio pesticides, and proper implementation and amendment of pesticide-related laws ^[3].

A. Pesticide residues in Grains:

Pesticide residue refers to the pesticides or metabolic products of the pesticides that may remain in food grains, vegetables and fruits after they are applied to crops. Many of these chemical residues, especially derivatives of chlorinated pesticides, exhibit bioaccumulation which could build up to harmful levels in the body as well as in the environment ^[3].

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 fruits and vegetables. Insects and pests can cause a loss in the quality and quantity of grains and their products. The application of pesticide is widely used for grains before harvest and after harvest to protect the grains from damage or loss. Cultivation and storage of grains often require an intensive use of pesticides, which may then be found in grains and in foods prepared from them. Cereal grains are treated with degradable pesticides, including organophosphates, carbamate, synthetic pyrethroids and insect growth regulators, both in storages and prior to shipment in order to prevent insect infestation. The most consumed pesticides for vegetables, fruits and food grains in India include sulphur, endosulfan, mancozeb, phorate, methyl parathion, monocrotophos, cypermethrin, isoproturon, chlorpyrifos, Malathion, carbendazim, quinalphos, copper oxychloride, and dichlorvos. Maximum residue level is the highest level of a pesticide residue that is legally tolerated in or on food or feed when pesticides are applied correctly ^[3].

In India, Food Safety and Standards Authority of India sets the maximum residue limits for pesticides in crops, foods, vegetables and fruits. The Food and Agriculture Organization and World Health Organization have recommended residue limits for bioresmethrin, bromophos, Carbaryl, chlorpyrifos-methyl, deltamethrin, dichlorvos, etrimfos, fenitrothion, fenvalerate, permethrin, phenothrin, pirimiphosmethyl and pyrethins used for the protection of grains ^[3].

B. Pesticide residue in Fruits:

The accumulation of pesticide residues depends on the physiochemical properties of the pesticide molecules as well as food. The use of pesticides during production often leads to the presence of pesticide residues in fruits after harvest. Most farmers and commercial producer fruit with pesticides on several occasions during growing because pesticides decrease toxins produced by food infecting organisms, increase productivity and are less labor intensive.

Many commodities contained more than one residue per product, up to 9 residues in grapes and tea were determined, up to 5 - 9 residues in citrus fruits like orange, mandarins, lemons, peaches, pears and up 3 - 5 residues in pomegranates, plums, cucumbers, strawberries ^[3].

C. Pesticide residue in Vegetables:

The accumulation of pesticide residues depends on the physiochemical properties of the pesticide molecules as well as food. The use of pesticides during production often leads to the presence of pesticide residues in vegetables after harvest. Most farmers and commercial producer treat vegetables with pesticides on several occasions during growing because pesticides decrease toxins produced by food infecting organisms, increase productivity and are less labor intensive.

According to a study, the cucumber was the crop with the highest number of pesticide residues with the predominant presence of methomyl, metalaxyl, and imidacloprid. Methomyl is a carbamate insecticide with restricted use because of its high toxicity to humans. The other pesticide residues found in cucumber samples were chlorpyrifos, cyprodinil, imidacloprid, metalaxyl and tebuconazole^[3]. A study on analysis of pesticide residue concentration in vegetables revealed that the risk to consumers varies with the season and it was reported that the winter season has the highest pesticide concentration in vegetables.

V ORGANIC CONTAMINANTS RESIDUE LIMIT IN FOOD^[15]

Table 2: List of Residues and their Limits^[15]

SR.NO.	NAME OF CONTAMINANTS	FOOD	MAXIMUM RESIDUE LIMIT (MRL in mg/Kg)
1	Carbaryl	Fish	0.2
		Food Grains	Wheat – 2.0 Maize –0.0.2
		Leafy Vegetables	10
		Other vegetables	5
		Milk and milk products	0.05
2	Carbendazim	Food grains	Wheat-0.05,and other food grain 0.1
		Vegetables	0.5
		fruit	5
		Eggs	0.1
		Milk and milk products	0.1
3	Carbofuran	Food grain	0.10
		Fruits and vegetables	0.10
		Milk and milk products	0.05
		meat	0.10
4	Cypermethrin	Wheat grains	2
		meat	2
		Milk and Milk products	0.05
5	Dichlorvos	Food Grains	Wheat-7.0,other food grains-1
		Vegetables	0.15
		Milk and milk products	0.01
		fruits	0.1
		Meat	0.1
		Fruits	0.2 -1
6	Malathion	Food Grains	Wheat – 10.0, and other food grains – 4
		Fruits	4
		Vegetables	3
7	Methyl Parathion	Grains	0.01
8	Permethrin	vegetables	0.05
9	Phorate	Food grains	0.05
		Fruits	0.05
		Eggs	0.05
		Meat	0.02
		Milk and milk products	0.05
10	Pirimiphosmethyl	Food grains	7
		Eggs	0.05
		Meat	0.05
		Milk and milk products	0.05
11	DDT (Dichlorodiphenyltrichloroe thane)	Milk	1.25
		Fruits and vegetables	3.5
		Meat	7.0

VI ANALYTICAL TECHNIQUE^[5, 16]

Methods for the analysis of organic contaminant residue and pesticide residue in various food matrices have been developed in recent decades. The commonly used analytical techniques for the detection of organic contaminant residue and pesticide residue in different food matrices are summarized in chart^[16].

Technique such as supercritical fluid extraction, solid-phase extraction, solid-phase microextraction, microwave-assisted extraction, liquid–liquid extraction, liquid-phase microextraction, pressurized liquid extraction and stir bar sorptive extraction ^[16].

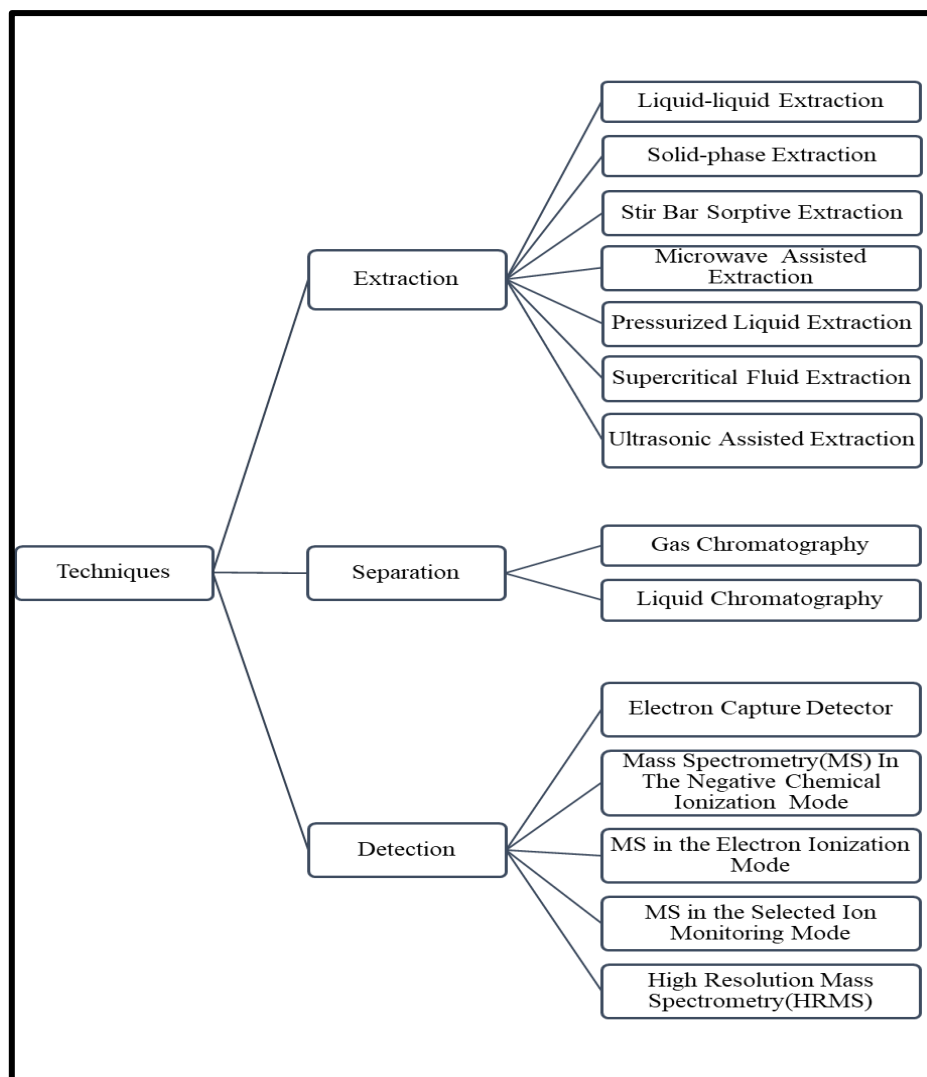


Fig.1: Analytical Techniques ^[5]

1 EXTRACTION ^[5, 16]

a) Liquid –Liquid Extraction

The selection of sample preparation techniques is dependent on the characteristics of the sample matrix. For liquid/aqueous samples, **liquid–liquid Extraction (LLE)** is the most conventional extraction method. The application of LLE has been widely accepted in standard methods for the analysis of contaminant in milk ^[16].

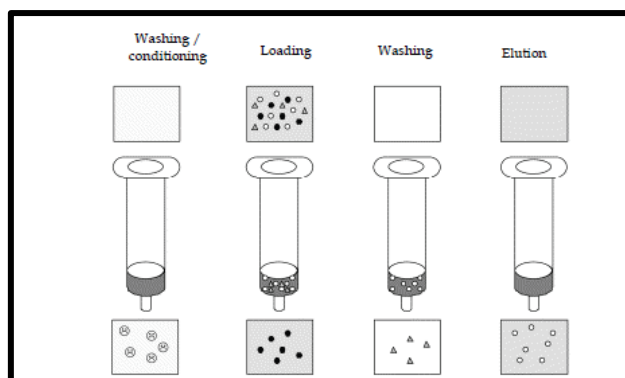
LLE separates compounds based on their relative solubilities in two immiscible liquids, usually water and organic solvent. Therefore, it requires large amounts of organic solvent. To reduce solvent consumption, a modified LLE method, dispersive liquid–liquid microextraction, was developed. Dispersive **liquid–liquid microextraction** is mainly used to extract organic analytes (PCB, OCPs, and PAHs) from samples ^[16].

b) Solid Phase Extraction

Solid phase extraction (SPE) was developed an alternative approach to LLE for separation, purification, pre-concentration and solvent exchange of solutes for solution. An SPE method always consists of three to four successive steps, as illustrated in Figure. **First**, the solid sorbent should be conditioned using an appropriate solvent ^[16].

This step is crucial, as it enables the wetting of the packing material and the solvation of the functional groups. The **second** step is the percolation of the sample through the solid sorbent. Depending on the system used. The **third** step (which is optional) may be the washing of the solid sorbent with an appropriate solvent, having low elution strength, to eliminate matrix components which have been retained by the solid sorbent, without displacing the analytes. The final step is the elution of the analytes of interest by an appropriate solvent, without removing the retained matrix components ^[16].

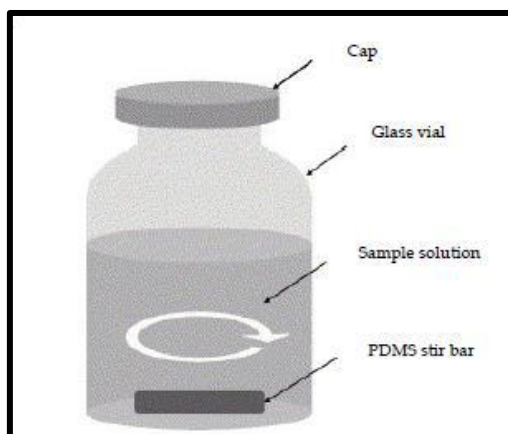
Solid-Phase Extraction is another alternative solvent reduction method for liquid samples. It has been used by the US EPA as an alternative to LLE when analyzing organic compounds in waste water. Solid-phase extraction separates compounds in a liquid mixture based on the different affinities for a solid phase (sorbent) between an analyte and interferences. It has been widely used to extract OCPs from food ^[16].

Fig.2: Solid – Phase Extraction ^[16]

c) Stir Bar Sorptive Extraction

Stir bar sorptive extraction (SBSE) was developed to overcome the limited extraction capacity of SPME fibers. A glass stirrer bar is coated with a potentially thick bonded absorbent layer (polydimethylsiloxane – PDMS) to give a large surface area of stationary phase, leading to a higher phase ratio and hence a better recovery and sample capacity. Normally, SBSE is applied to the extraction of aqueous samples containing low concentrations of organic compounds. For samples containing high concentrations of solvents, the solutions should be diluted before extraction ^[16].

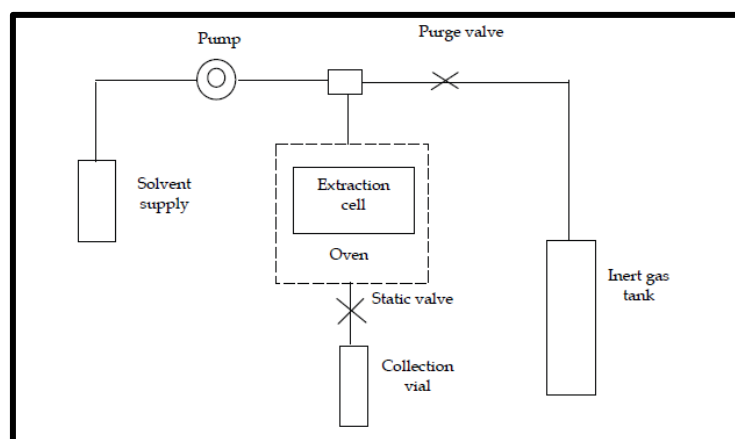
Both methods have been used to extract OCPs, PCBs and PBDEs in water samples. Common extraction techniques for solid samples include Soxhlet extraction, pressurized liquid extraction, supercritical fluid extraction, microwave- assisted extraction, ultrasonic-assisted extraction.

Fig.3: Stir Bar Sorptive Extraction ^[16]

The conventional Soxhlet extraction is still among the most common methods used for various matrices and analytes, especially for dioxins/furans and dioxin-like PCBs (Polychlorinated biphenyl) in food ^[16].

d) Pressurized Fluid Extraction

This technique, also named Accelerated solvent extraction (ASE) or pressurized liquid extraction (PLE), is a solid-liquid extraction process performed in closed vessels at relatively elevated temperature, usually 80 to 200 °C, and elevated pressures, between 10 and 20 MPa conditions for short time periods (5-10min) ^[16]. The time required for extraction is independent of the sample mass and the efficiency of extraction is mainly dependent on the temperature. Below shows a schematic diagram of a PFE system.

Fig.4: Pressurized Fluid Extraction ^[16]

In PFE, the pressure is applied to maintain the solvent in its liquid state. Elevated temperature and pressure are used to achieve high extraction of the components from sample matrices. Pressurized liquid extraction has been widely used for the extraction of PAHs, PCBs, PCDFs, in fatty foods including egg, fish and meat samples ^[16].

e) Microwave-Assisted Extraction

In this technique uses microwaves to heat the solvent and increase the solvent penetration into the sample matrix. It has been widely used to extract OCPs from food. Microwave-assisted extraction is very attractive because it requires less extraction time, reduces solvent usage, and improves extraction yield. However, it has some drawbacks such as expensive equipment, a polar solvent and a clean-up process afterwards ^[16].

f) Ultrasonic - Assisted Extraction

It is a simple and inexpensive alternative that can reduce operating temperature with the help of ultrasound waves. Ultrasonic-assisted extraction has been applied for the detection of OCPs and PAHs in food ^[16].

g) Supercritical Fluid Extraction

It uses supercritical fluid as the extracting solvent to separate analytes from the mixture. Carbon dioxide has been the mostly used supercritical fluid. This method has been used to extract OCPs from egg, butter, oil and meat products ^[16].

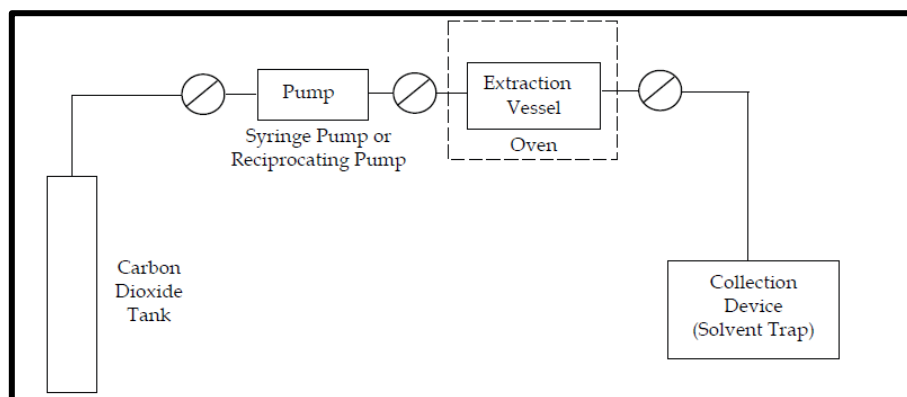


Fig.5: Supercritical Fluid Extraction ^[16]

2 SEPARATION & DETECTION ^[17]

Mass spectrometry (MS) coupled with chromatography is the mostly widely applied technique for POP quantification in food. Since food-based matrices are complex, selectivity is the primary concern. An overview on different separation and detection techniques are described. GC-MS is a method of choice for less polar pesticides; for more polar compounds, LC-MS is more suitable.

The need to deal with more polar pesticides as well as with pesticide metabolites, which are often more polar and less volatile than pesticide itself, is one of the main reasons for choosing LCMS/ MS over GC-MS ^[17]. Compounds which are thermolabile, not volatile, and have not been derivatized can be separated by LC-MS. LC-MS can analyze a much wider range of chemicals than GC-MS.

1) Liquid Chromatography:

Most of the procedures used to determine a considerable number of pesticides in food, employ the **HPLC technique** in a reversed-phase system, using gradient elution with a linear increase of the percentage of the organic solvent. The advantage of gradient elution is the possibility of achieving the optimum conditions of separation by decreasing the polarity of the mobile phase. The hydrophilic molecules leave the column first, followed by molecules with a lower polarity and, finally, hydrophobic molecules. Mixtures of acetonitrile, methanol, and water in various volume ratios are used as eluents ^[17]. The additives to LC mobile phases are restricted to volatile compounds; nonvolatile compounds such as phosphorous salts have to be avoided. In this group of sorbents, the abovementioned column BEH C1 8 and Zorbax C18, with the particle diameter of 1.8 μm , from Agilent Technology, are the most popular in the analysis of pesticide residues in food. The much greater separation efficiency of the chromatographic system in comparison with conventional HPLC, the UHPLC system is currently an optimum solution for the chromatographic separation of multicomponent mixtures ^[17].

2) Mass Spectrometry:

Mass spectrometry is a key analytical technique enabling the identification of various groups of chemical compounds, including pesticides. Regardless of its construction and designated use, a mass spectrometer measures the mass-to-charge ratio of charged molecules. Every mass spectrometer consists of three basic components: an ion source, an analyzer where ions are separated according to their mass-to-charge ratio, and a detector where ions are counted. A series connection of several analyzers is the most frequent modification of the system ^[17].

A) Ionization Method:

The possibility of ionizing a substance is a basic condition for analyzing it in a mass spectrometer. Ionization methods vary depending on the structure and principle of operation of an ionizer. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are the most frequent ionization methods used to determine pesticide residues in food by means of an LC-MS system ^[17].

(i) Electrospray Ionization (ESI):

Electrospray ionization is a basic method of molecule ionization used in the analysis of multicomponent mixtures in an LC-MS system. It belongs to a group of methods whereby “soft” ionization is carried out under atmospheric pressure. Under this method, the eluate stream leaving the chromatographic column is introduced into the ionization source through the capillary. At the outlet of the capillary, the sample dissolved in the solvent is exposed to a strong nebulizing gas (typically, nitrogen) and a very strong electric field, which results in the atomization of the sample into charged microdroplets. The ionization process is shown in Figure. ESI is the most frequently used ion source in the analysis of pesticides in food by means of LC-MS methods. The analysis of pesticide residues in food with LC-ESI-MS methods is conducted both in the positive ion mode and negative ion mode ^[17].

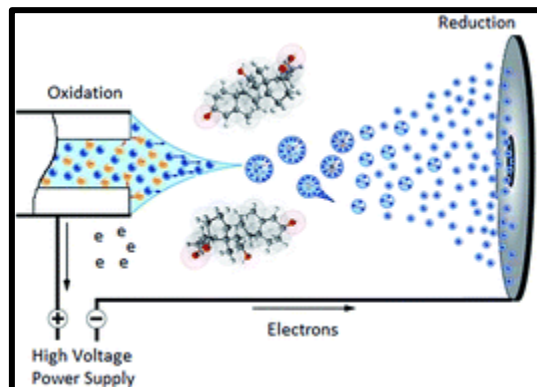


Fig.6: Electro – Spray Ionization

(ii) Kinds of Analyzers:

The analyzer is the most important component determining the performance of a spectrometer. Its task is to separate ions in terms of their mass-to-charge ratio and to direct the beam of focused ions to the detector ^[17].

The choice of a particular analyzer is determined by the kind of analysis conducted. In the multiresidue analysis of pesticides in food, the following analyzers are used most frequently: quadrupole (Q), time-of-flight (TOF), and ion trap mass analyzer (IT).

a) Quadrupole Analyzer

The quadrupole analyzer (Q) consists of four metal electrodes in the form of symmetrically arranged rods with a hyperbolic cross-section. It works like a mass filter that, with the specific parameters of the electromagnetic field, allows the passage of ions with the selected mass-to-charge ratio values, while other ions are dispersed and do not reach the detector ^[17].

Its separation efficiency is a function of ion mass and increases slightly as the mass increases. In the analysis of pesticide residue in food, it was used mainly in the first years of the period, later it was gradually replaced by the most popular triple quadrupole mass analyzer used in tandem mass spectrometry. This method has been used to extract OCPs from egg, butter, oil and meat products.

b) Time-of-Flight Analyzer

The time-of-flight mass analyzer (TOF) consists of an ion accelerating grid and a flight tube (about 1 m long), through which the ions travel to the detector. The analyzer separates ions accelerated by an electric field according to their velocity which depends on their mass and charge. The time-of-flight analyzer measures the time it takes an ion having a specific mass-to-charge ratio to reach the detector; the time is counted from the moment the ion is accelerated ^[17].

The greater the mass-to-charge ratio of an ion, the longer it takes to reach the detector. In the analysis of pesticide residues in food, the time-of-flight analyzer is mainly used to confirm the presence of the compounds of interest in matrices such as fruit and vegetables, honey and meat, fruit beverages, and wine.

c) Ion Trap Analyzer

One of the most popular ion trap analyzers (IT) is the quadrupole ion trap consisting of a ring-shaped electrode and two electrodes with a spherical cross-section, with the space between them forming a trap. The ion trap analyzer traps ions with a specific mass-to-charge ratio by means of an electric field. From the trap, the ions are sent to the detector, in the order of increasing m/z values ^[17].

The range of analyzed masses and separation efficiency of ion traps are similar to those offered by quadrupole analyzers. Alongside the quadrupole analyzer, the ion trap analyzer is one of the most popular single MS analyzers in the analysis of pesticide residues in food.

3 Gas chromatography (GC):

GC is the most commonly used technique for separation. The GC separation is dependent on the boiling points of the compounds and their interactions with the stationary phase of the column. Most POPs are semi-volatile, and their polarities are between moderate and non-polar. These physicochemical properties make most POPs well suited to being measured by GC-MS, except for PFAS-related chemicals, which are always measured using LC-MS/MS method ^[17].

However, there is no single column that can separate all congeners of PCBs and dioxins/furans. To overcome this difficulty, comprehensive two-dimensional GC was introduced. When passing two columns, there are two degrees of separation based on different physicochemical properties. Compared to single columns, the two-dimensional GC can significantly improve selectivity (peak capacity) and sensitivity.

VII TOXICITY RELETED ORGANIC CONTAMINANT RESIDUE AND PESTICIDE RESIDUE ^[18]

One of the recent issues that have globally attracted the concern of consumers is the presence of pesticide residues and Organic contaminant residue in food. These residual chemicals are persistent organic contaminants that have serious lethal effect on human health when exposed beyond certain levels ^[18].

Various illnesses have been reported to be associated with the consumption of plants food substances that have Organic Contaminant Residue beyond the Level with respect to the daily intake and acute reference dose (ARD). Based on this, a consumer is not considered to be at risk, when the dietary intake that is estimated is not beyond the ARD. The process of monitoring and establishment of permissible limits for Organic contaminant residue pesticide residues in various commodities and foods aid the efficient mechanism of control for human safety. Some of the adverse health effects associated with exposure to pesticides include vomiting, headache, irritation of skin, dizziness, difficulty in breathing, neurological diseases as well as other diseases associated with intense poisoning by pesticides such as cancer and eventually death. Some Persistent Organic residue and pesticides cannot be acted upon by microorganisms in the environment hence they remain for years in the environment and capable of bioaccumulating with a long half-life in the environment and human tissues ^[18]. Typical examples of pesticides that tend to remain for long in the environment include aldrin, Chlordane and DDT. Other kinds of pesticides include insecticides such as, permethrin, kelthane, and cypermethrin; fungicides such as mancozeb, viclozolin. They are reported to have different levels of toxicity ^[18].

Infertility plague has recently been identified by WHO as a social challenge that is connected to organic Contaminant residue and pesticides residue present in agricultural products. On this note, Emphasised that infertility is associated with pesticides build-up in the body and is responsible for reproductive challenges in about 15-20% cases of infertility in women. The various chemical contaminants present in human bodies go in mostly through ingestion or inhalation route. Pesticides such as and DDT (dichlorodiphenyltrichloroethane) tend to remain in the environment for a very long period of time ^[18].

A) Impact of Organic Contaminant residue and Pesticide on Human health ^[19]

1 Acute health effects of residue exposure:

The short-term acute adverse effects pesticide exposure on human health are stinging eyes, rashes, blisters, skin irritations, blindness, nausea, dizziness, diarrhea and death.

Exposure to pesticides in agricultural work can cause serious risks to the respiratory system causing chronic cough, dyspnea, wheezing and expectoration, decreased lung capacity, asthma, and bronchitis ^[19]. These respiratory problems were found in workers in flower crops, coffee plantations and banana plantations. In banana farming the use of various pesticides was related to the symptoms of burning in the throat and lungs, airway congestion, cramps, skin peeling, diarrhea, headache, chest pain, weakness, cough and skin irritation. Many studies have found positive associations with residue exposure and children's respiratory and allergic effects such as asthma, wheezing, coughs, acute respiratory infections, hay fever, rhinitis, eczema, chronic phlegm, and lung function impairments ^[19].

2 Chronic effects of pesticide exposure:

The long-term chronic adverse effects of pesticides exposure are cancers, birth defects, reproductive harm, neurological and developmental toxicity, immunotoxicity, and disruption of the endocrine system. The chronic effects of pesticides on human can be categorized into three major groups; neurotoxic effects, genotoxic and carcinogenic effects, and reproductive effects ^[19].

3 Neurotoxic effects:

Neurotoxicity can be defined as any adverse effect on the central or peripheral nervous system caused by chemical, biological or physical agents. A developing nervous system in children (During replication, migration, differentiation) is more susceptible to neurotoxic chemicals including pesticides and organic contaminant residue ^[19].

Pesticide molecules are small and lipophilic in nature, and can enter from blood to brain and then in neurons, glial cells and brain micro vessels. Pesticides can disrupt blood-brain barrier receptors in the central nervous system which enhance chronic toxicity and affect the receptor-mediated transcytosis ^[19].

The dysfunction of mitochondria and oxidative stress is responsible for several neurological diseases, including Parkinson's disease, seizure, cognitive dysfunction, attention and memory deficits, dementia, depression, and Alzheimer's disease.

4 Genotoxic and carcinogenic effects:

A genotoxic agent can be a physical, chemical or biological agent that can interact with the genetic material (DNA) causing alterations, damage or ruptures, and those that interfere with enzymatic processes of repair, genesis or polymerization of proteins involved in chromosome segregation. These alterations could lead to impaired embryonic development or be the initial steps in the development of cancer. Various contaminant residue exposure can cause genomic damage.

Genetic damage caused by pesticides is broadly classified into following classes;

1. Pre-mutagenic damage like DNA strand breaks and DNA adducts,
2. Gene mutations like insertion, deletion, inversion and translocation.

Genetic damage has been reported from exposure to Malathion, carbofuran (carbamate), triflururon (Insect growth regulator), imidacloprid, acetamiprid and thiamethoxam (neonicotinoid Insecticides), pentachlorophenol (OC), Emamectin benzoate (used in household, and veterinary medicine). **Cancer** is characterized by an uncontrolled cell growth with limitless replication, resistance to apoptosis, alteration of growth factors (GFs), and resistance to chemotherapy, metastasis and angiogenesis. Cancer develops as a result of multi-factorial complex interactions of genetic and lifestyle factors including, diet, stress, physical and biological agents, infections, and exposure to the hazardous chemical substances ^[19].

5 Leukemia:

Leukemia has been associated with occupational exposure with a higher risk in livestock farmers. The risk of chronic myelocytic leukemia (CML) and acute myeloblastic leukemia (AML) was found to be higher in women.

Children whose parents used garden and indoor insecticides, or whose mothers had been exposed while pregnant had increased rates of all types of leukemia. Children living on farms and those exposed to household pesticides have increased risk of leukemia ^[19].

VIII PREVENTIVE MEASURES TO REDUCE ORGANIC CONTAMINANT RESIDUE IN FOOD ^[20]

The risks associated with these substances, several steps have been taken to reduce or eliminate emissions of dioxins, dioxin-like PCBs and other related persistent organic pollutants. Many countries have now implemented the Stockholm Convention on POPs, which suggests ending commercial use of 12 POPs and reduce or eliminate their emission into the environment.

To monitor reduction in human exposure WHO and UNEP (United Nations Environment Programme) run a monitoring program for POPs in breastmilk. In regard to food contamination, the FAO/WHO Codex Commission, which is a risk management body comprised of 169 member countries, is developing a draft code of practice for dioxins and dioxin-like PCBs in food, which identifies source-directed measures to reduce their presence in food, including fish, as which provides an evaluation of the need for possible regulatory measures, such as limits in food and feed ^[20].

The results of this studies should be used to maintain the focus on reducing the exposure of humans to dioxins and dioxin-like PCBs. Specifically, efforts to reduce the level of such substances in animals used for human consumption should be actively supported, in particular source-directed measures such as reduction of contamination levels in animal feed.

FAO and WHO consider fish to be an important component of a nutritious diet, and that the risk of consuming contaminated fish must be weighted in view of the beneficial nutritive effects of fish ^[20].

A) Preventive measures to reduce pesticide residues in food ^[3]

Different types of pesticides are used in different countries for different types of crops to prevent pests, insects, and weeds. Because of the expansion of worldwide trade, more foods which are treated by pesticide are being imported into different countries.

These worldwide trades increase the expansion of pesticide residues in different areas of the world and it's the issue of public health concern. There are a number of strategies which can be used to minimize pest and disease problems and reduce pesticide residues in food grains, vegetables and fruits ^[3].

1 Organic farming:

One of the methods used to reduce the effect of pesticide residue in food is to eat organic foods than non-organic ones. According to standard meta-analyses the frequency of occurrence of detectable pesticide residues was four times higher in non-organic crops than organic crops. There is evidence that indicated organic food consumption can reduce exposure to pesticide residues in food. Organic crops, on average, have higher concentrations of antioxidants and a lower incidence of pesticide residues than the non-organic foods across regions and production seasons.

It is advisable to consume foods rich in antioxidant to reduce effects of the chronic disease by suppressing oxidative activity. An increased dietary intake of antioxidant rich foods protect Against chronic diseases, including cardio vascular diseases, certain cancers (e.g. prostate cancer) and neurodegenerative diseases ^[3].

2 Washing food products:

The second methods reported to reduce pesticide residue in food is washing. Washing may reduce pesticide residue in food commodities. Washing with water and various chemical solution for domestic and commercial applications is necessary to decrease the pesticide residues. The efficiency of the washing treatments on pesticide removal depends on the washing solution, the chemical properties of the pesticide, the surface area, the nature of the food, the length of time the pesticide is in contact with the food, and the formulation and application method of the pesticide. Usually, the pesticide is lodged in the outer wax-like layers and then moves to the inside, making washing and removal of the pesticides less effective ^[3].

CONCLUSION

The present work provides facts on organic contaminations and pesticide residue to understand its present in the Food. Many potential contaminant residue that may pose a high risk to human health and that need to be monitored. To better protect human health, more studies should focus on understanding the toxic effects of emerging POPs. As the technology advance, the detection of such contaminants become easier. However, there are several contaminants that are still unknown and research continue in this regard. Pesticides have become increasingly popular for agricultural, industrial, livestock husbandry and home use and represent a significant potential health risk for human and livestock. Some of the adverse health effects associated with exposure to organic contaminants include vomiting, headache, irritation of skin, dizziness, difficulty in breathing, neurological diseases as well as other diseases associated with intense poisoning by pesticides such as cancer and eventually death. The impact of pesticide residues can be minimized by preventive measures such as rational use of pesticides, washing and proper processing of food products, practising organic farming, use of natural pesticides and bio-pesticides, and strict implementation and amendment of pesticide-related laws. Establishing regulatory standard and management practices of using the organic contaminants and pesticides are the alternative ways to prevent the adverse effect of their residue on the environment.

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