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Analysis of pesticide residues in liquid milk using GC-MS

Abstract

Pesticides are essential for lowering disease rates and raising crop yields across the globe. It is imperative to talk about the history, types, and particular applications of pesticides, as well as pesticide behavior, contamination, and harmful impacts on the environment. Five Dhampur liquid milk samples were analyzed in order to determine the pesticide residues and contamination in the samples. GC-MS is used to extract pesticide residues from milk samples. Out of five samples, one was determined to have more residue than the maximum allowed. Through the drinking of tainted liquid milk, the estimated daily dosage of Bifenthrin was higher than what was considered tolerable for youngsters. Remarkably, at their detection level, none of the liquid milk samples showed signs of synthetic pesticides or routinely used organophosphorus.

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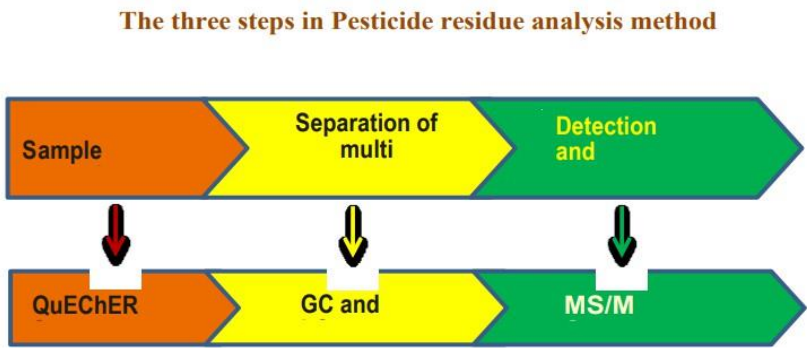
Introduction

The Gas Chromatography Technique is particularly helpful in this situation since the results presented in court must be extremely exact and samples frequently contain very complicated matrices. Pesticide residue testing is a process that identifies the pesticide residue levels in food products through an in-depth chemical and microbiological analysis that gives food manufacturers and producers confidence in their product ingredients. (Battu 2004)



Pesticides

Pest control agents are compounds known as pesticides. This comprises pesticides for insects, rodents, and animals as well as bactericides, microbicides, fungicides, and lampricides. Pesticide is defined by the Food and Agriculture Organization (FAO) as any substance or combination of substances meant to prevent, destroy, or control any pest, including those that spread disease to humans or animals, undesired plant or animal species, and substances that harm or otherwise interfere with food production, processing, storage, transportation, or marketing. It also includes wood and wood products, animal feedstuffs, agricultural commodities, and substances that may be given to animals to control insects, arachnids, or other pests in or on their bodies (Bedi 2013). Pesticides connected to chemicals:

Organophosphate: Most organophosphates are insecticides that work by interfering with the enzyme that controls a neurotransmitter, which has an impact on the nervous system. Carbamate: By interfering with an enzyme that controls neurotransmitter function, carbamate pesticides act on the neurological system similarly to organophosphorus pesticides. The effects of enzymes can, however, frequently be reversed.

Organochlorine insecticides: Previously widely used, these pesticides—such as DDT, chlordane, and toxaphene—have been banned from sale in many nations due to their persistent negative effects on human health and the environment. These are synthetic substitutes for the naturally occurring insecticide pyrethrin, which is present in chrysanthemum flowers. To maximize their stability in the environment, they were designed.

Herbicides with sulfonylureas: Sulfometuron-methyl, rimsulfuron, pyrazosulfuron-ethyl, imazosulfuron, nicosulfuron, oxasulfuron, nicosulfuron, flazasulfuron, primisulfuron-methyl, halosulfuron-methyl, flupyrsulfuron-methyl-sodium, ethoxysulfuron, chlorimuron-ethyl, bensulfuron-methyl, azimsulfuron, and amidosulfuron are among the sulfonylureas herbicides that have been commercialized for the purpose of controlling weeds.

Biopesticides: Certain pesticides known as "biopesticides" are made from naturally occurring substances like bacteria, plants, animals, and minerals.

These are made with harmful chemicals that are intended to be discharged into the environment on purpose. Even though the purpose of each pesticide is to kill a specific bug, a very high proportion of pesticides end up somewhere other than their intended target. Rather, they find their way into the water, air, sediments, and even our food. Pesticides have been connected to a number of health risks for people, ranging from immediate effects like headaches and nausea to long-term effects including cancer and reproductive damage. Additionally, using these reduces the soil's overall biodiversity. Higher soil quality results from the absence of pesticides, which also increases soil retention—a requirement for plant growth (Bedi 2015).

Toxic pesticides in food have caused great worry among the public and lawmakers due to their detrimental effects on the environment and human health. Pesticide behavior in the environment, such as volatilization from the treated area to the air, soil, and non-target plants, as well as residual pesticides transferred from soil and water to crops, vegetables, and fruits, are additional causes of food contamination in addition to the consequences of pesticide spraying for non-target plants. Pesticides' negative effects on the environment, including residues left behind, contaminate food and harm vegetation. For instance, the yields of potato plants may be decreased by exposure to the herbicide clopyralid. It just takes 1% of the applied clopyralid to volatilize and harm non-target plants. Applications of pesticides indirectly affect plants because they damage beneficial insects and soil microbes (Devi 2011).

Phorate

Phorate (chemical name O,O-diethyl-S-ethylthiomethylphosphorodithioate) is a phosphorodithioate pesticide. Phorate is marketed under several names, the most popular being Thimet, and is used in agriculture as a systemic and soil insecticide. Phorate works by first causing CYP to activate an oxon, which is then followed by the oxon inhibiting acetylcholinesterase. Although cytochrome metabolizes both P=S and thioether sulfur, flavin-containing monooxygenase also targets the thioether sulfur.

Atrazine

In agricultural and roadside uses, atrazine, also known by its chemical name, 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine, is widely used to control broadleaf weeds. The U.S. Environmental Protection Agency determined that although it was frequently found to be a well-water contaminant, it was not likely to cause cancer in humans. Nonetheless, it is acknowledged to be an endocrine disruptor. Research has been done on atrazine metabolism in humans and surrogate animals. The common term for a herbicide that is frequently used to eradicate weeds is atrazine. Most people use it on farms. The white, odorless powder known as pure atrazine is not extremely combustible, reactive, or volatile. In water, it will dissolve. Atrazine is not found naturally; it is created in a lab.

Metribuzin

Metribuzin belongs to the class of herbicides known as substituted as-triazinone. Interference with photosystem II electron transport in plant chloroplasts is the cause of activity. Numerous short- and long-term investigations have examined the metabolism of metribuzin in plants since the early 1970s. At first, side-chain deamination to generate deaminometribuzin (DA-metribuzin) and sulfoxidation and dealkylation of the 3-methylthio side chain to form diketometribuzin were the hallmarks of the photolysis (254 nm) of an aqueous solution of metribuzin. Deaminodiketometribuzin is the product of these two reactions.

These first photoproducts were degraded by further photolysis, with the 1,1-dimethylethyl side chain playing a major role. This eventually produced the very stable photochemical compounds 6-azauracile and 6-azathymine. It took long irradiation durations to find evidence of heterocyclic ring cleavage. Metribuzin's rapid photodegradation required the presence of oxygen (or hydrogen peroxide), and in the absence of oxygen, the rate of photodegradation was more than ten times slower.

Fipronil

5-amino-1-[2,6-dichloro-4-phenyl trifluoromethylsulfinyl] -4-[-1H-pyrazole-3-carbonitrile. Midway through the 1990s, the phenylpyrazole chemical fipronil was created as a practical pesticide. It works well against some insects that have developed resistance to the current insecticides, like the Colorado potato beetle. and some pests that affect cotton. Fipronil has an additional benefit as an insecticide in that it is far more poisonous to insects than to mammals.

Bifenthrin

Bifenthrin is a member of the pyrethroid group. It was created in the first half of the 1980s. Primarily, it is classified as a type I pyrethroid. Bifenthrin can occasionally induce oxidative damage, immunotoxicity, neurobehavioral toxicity, endocrine disruption, and developmental problems in nontarget organisms. Furthermore, bifenthrin is extremely harmful to aquatic organisms and is a major cause of insecticide-induced water toxicity.

Object and Study Area

The investigation's goal was to determine whether pesticide residues were present in the liquid milk from the Dhampur region. The tehsil of Uttar Pradesh's Bijnor district is called Dhampur. The primary source of Dhampur's renown is the Dhampur Sugar Mill (DSM). There are more than 60,000 residents in Dhampur.

Sampling

Five 500 mL samples of liquid milk, each wrapped in a polyethylene bag, were gathered from Dhampur dairy farms. All samples were stored in plastic bags at -20 °C before processing and were sent in Styrofoam boxes with recyclable ice. Raw milk samples were obtained directly from the cans or cooling tanks.

Materials and Method

Gas chromatography and liquid chromatography are the two primary analytical methods used in food analysis, and both enable the detection and measurement of pesticides.

Selecting the class of pesticide to be quantified, multiple types of quantification techniques are paired with GC (Fajgeli 2000). Different types of methods have been used to measure the presence of pesticide residues in grain samples, including mass-selective detection (MSD), flame-ionization detection (FID), nitrogen-phosphorus detection (NPD), and electron capture detection (ECD).

The Gas Chromatography/Mass Spectrometry (GC/MS) apparatus is used to identify the components at the molecular level (the MS component) and to separate chemical mixtures (the GC component). It is among the most precise instruments available for examining environmental sample data. The idea behind how the GC operates is that heat causes a mixture to split into its constituent substances. The hot gases are sent through an inert gas column (like helium). The separated materials flow into the MS when they exit the column aperture. Compounds are identified using mass spectrometry based on the mass of the analyte molecule. On a computer is kept a "library" of known mass spectra that covers thousands of substances (FAO 2016).

GC MS or GC MS/MS

The Gas Chromatography/Mass Spectrometry (GC/MS) apparatus is used to identify the components at the molecular level (the MS component) and to separate chemical mixtures (the GC component). It is among the most precise instruments available for examining environmental sample data. The idea behind how the GC operates is that heat causes a mixture to split into its constituent substances. After being injected into the GC inlet, the sample is evaporated and carried onto a chromatographic column by the helium carrier gas. As the sample passes through the column, the chemicals that make up the mixture of interest are divided according to how they interact with the carrier gas (mobile phase) and the column coating (stationary phase). The heated transfer line that the latter portion of the column travels through is where it ends up at the ion source entrance, where molecules that elute from the column are transformed into ions (Feo 2012). The sample molecules are ionized by an electron beam, forming smaller ions and molecular ions with distinct relative abundances that serve as a "fingerprint" for that chemical structure. After the ions are separated by the mass analyzer, they are detected. (Figure 1)

A device called GC-MS combines the capabilities of mass spectrometry and gas chromatography to identify various chemical substances found in organic matter, such as fatty acids, alkenones, steroids, and alkanes. The method of choice for monitoring organic compounds originating from a wide range of plants as well as in their ancient counterparts is GC-MS (Singh 2014). This technique may help reconstruct palaeoclimates and provide insight into the processes of evolution across time (Srimurali 2015 Tsiplakou 2010).

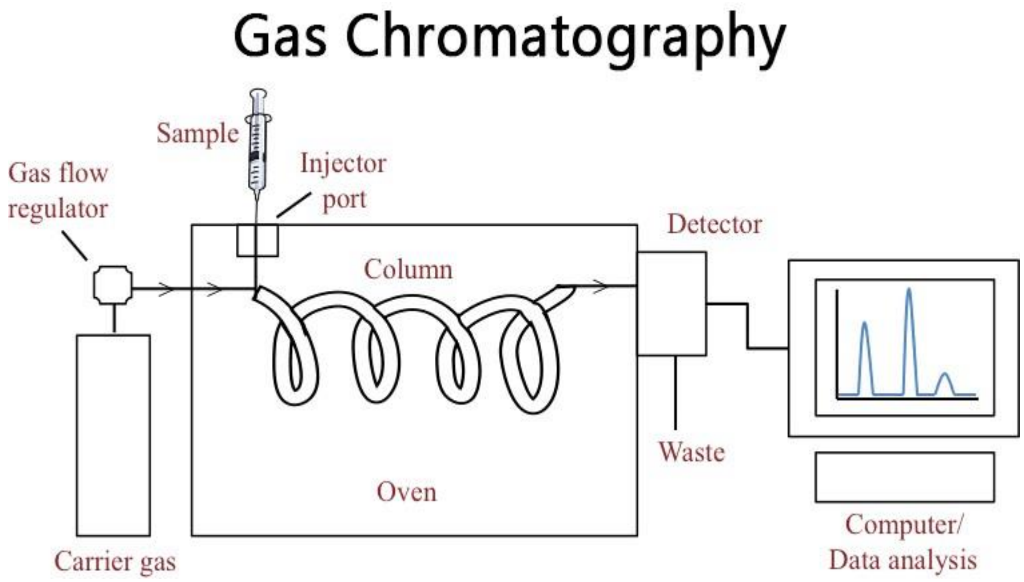


Figure 1: Representation of Gas Chromatography (source: Anthias Consulting).

Sample Preparation:

Took 5-10 grams of sample in a 50 ml polypropylene tube (centrifuge tube). Added 10ml of Mili Q water, vortex and add 10ml of Acetonitrile with 0.1% formic acid and shake well. Added 2.0 grams of Anhydrous Magnesium Sulphate + 0.5 grams of Sodium Acetate or QUECHERS Pouch. Handshake vigorously and vortex for 2 min. Centrifuge the tube at 5000rpm for 5 min. Pipetted out 4-8 ml of the supernatant into 15ml tube already consist of 200-500mg of anhydrous magnesium sulphate and 100-200mg of PSA or add 2ml supernatant in dispersive SPE 2ml. Vortex for one min, centrifuged at 5000rpm for 5 min. Pipetted out 2ml and dry under nitrogen gas, makeup the volume 1.0ml with Ethyle Acetate for GCMS/MS. Filter with 0.22µ nylon syringe filter into 2ml vial. Run in GCMS OR GCMS/MS Instrument (FSSAI 2011, MAFW 2015).

Results and Discussion

Using a standardized multi residue approach, the samples were determined for the presence of organochlorines, organophosphates, and synthetic pyrethroid residues. Table 1 displays the findings of the investigation, which revealed residues of phenol, atrazine, metribuzin, fipronil, and benthrin. GC–MS was used to confirm the structure of pesticide residues detected on GC by comparing the retention periods. The advantages of GC-MS include increased selectivity, sensitivity, and a wider range of applications. These characteristics, along with the bulk of determinations without derivatization, make GC-MS/MS the recommended technology currently available for assessing pesticide residues. At the detection limit of 0.01 mg per kg (whole milk basis), common organophosphorus chemicals such as monocrotophos, methyl parathion, chlorpyriphos, quinalphos, and triazophos were not detected. Likewise, at 0.01 mg per kg (whole milk basis), synthetic pyrethroids such as delta-methrin, fenvalerate, and cypermethrin were likewise undetected. Subsequent examination of five liquid milk samples from Dhampur dairy farms showed that two of the samples had levels of benthrin over the permissible limit of 0.05 mg per kg (whole milk basis). Low concentrations of phosphine, atrazine, metribuzin, and fipronil were discovered (IDF 1997).

Pesticides	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Unit
Phorate	10.38	19.85	49.89	99.68	200.17	ng/ml
Atrazine	11.81	21.50	45.61	100.41	200.64	ng/ml
Metribuzin	14.01	22.07	44.02	97.60	202.28	ng/ml
Fipronil	13.72	22.51	44.34	96.88	202.53	ng/ml
Bifenthrin	12.15	21.16	45.88	99.98	200.81	ng/ml

Conclusion

The examination of remaining pesticides in liquid milk is improving our knowledge of food safety and quality. Using a standardized multi residue approach, the samples were examined for the presence of organochlorines, organophosphates, and synthetic pyrethroid residues. The results of the current investigations have unequivocally demonstrated that the samples taken from Dhampur, dairy farms contain no detectable levels of phosate, atrazine, metribuzin, or fipronil. Even Nevertheless, milk samples had greater than average frequencies of Benthrin residues, surpassing the permissible limit. Out of five samples, one was determined to have more residue than the maximum allowed. The main causes of the rising use of pesticides are population growth and climate change, and future projections indicate a higher level of pesticide output worldwide. Pesticide use is on the rise, and while it can help produce more inexpensive, high-quality food and increase crop yields, there are several drawbacks for both the environment and public health. Because of the chemicals in pesticides, which are designed to kill pests and manage weeds, they can also be poisonous to other creatures, such as fish, birds, beneficial insects, and non-target plants, as well as various environmental media, such as soil, water, and crops. These chemical residues affect human health by contaminating food and the environment. Additionally, pesticide contamination spreads apart from the intended plant targets, causing pollution in the surrounding area. Pesticides can travel in several ways, such as through the air, wind, water, runoff, leaching, plants, animals, and people.

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