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LEVELS OF DDT IN STERILIZED MILK

Aleksandra Tasić[1*], Ivan Pavlović[1], Slobodan Stanojević[1], Dušan Nikolić[2]

[1]Scientific Institute of Veterinary Medicine of Serbia, Belgrade, Serbia
[2]University of Belgrade, Institute for Multidisciplinary Research, Belgrade, Serbia
***e-mail: alekstasic79@gmail.com**

ABSTRACT

Milk is an important source of nutrients for newborns, babies, and children. Sterilized milk is a nutritional food of modern times and a fast lifestyle. On the other hand, concerns about the safety of milk date back to the end of the last century. Various sources of contamination can threaten the safety of milk and the health of children, but also adults. The concern is primarily related to the presence of pesticides, the main source of which is the feed and the environment. The aim of this work is to systematize the results obtained from milk control in recent years. Research was carried out by monitoring the presence of the sum of DDT metabolites in sterilized milk in the period from 2018 to 2022.
Key words: *DDT, sterilized milk, safety*

INTRODUCTION

Milk is a very important food for humans, and at the same time a suitable environment for microorganisms (which can enter the milk from the moment of milking and during further manipulation). Therefore, in order to preserve milk in the correct condition for a long time, modern thermal processing methods are applied, of which pasteurization and sterilization have found the most widespread use to date (Sedas et al., 2021). As the main difference in the characteristics of the milk obtained from the aforementioned thermal processing procedures highlight the different shelf life of pasteurized and sterilized milk from receipt to the time of their use.

Sterilized milk has a significantly longer shelf life, which with the current methods of sterilization is at least 30 days, and practically several months and up to a year. For longer storage and preservation of sterilized milk, refrigeration devices are not required and this gives it a greater possibility for different applications. Sterilized milk has a longer shelf life because it is obtained by heat treatment at temperatures above 100°C. In the sterilization process, vegetative forms and spores of microorganisms are destroyed. Due to the application of homogenization, sterilized milk has better organoleptic properties than pasteurized milk. Shredded fat droplets prevent cream from separating, which gives a uniform appearance in terms of color and composition of the entire mass of milk in the bottle. Homogenization also has an effect on the increase in viscosity. Due to homogenization, sterilized milk is more digestible and suitable for dietary nutrition and for feeding children.

The presence of DDT and its metabolites is widely studied in many parts of the world in environmental samples, primarily sediment, rivers, but also breast milk and food of animal origin (Sakan et al., 2017; Juvancz et al. 2015; Rêgo et al., 2019). DDT (dichlorodiphenyltrichloroethane) was the most widely produced pesticide in the world between 1950 and 1993, producing more than 2.6 million tons (Juvancz et al., 2015). The interest in the DDT levels in milk dates back to the end of the last century and is still a current topic in science primarily due to human health and safety reasons. Milk can be contaminated physically, while the source of chemical contamination comes from the use of pesticides and veterinary drugs, hormones, and it can also be contaminated by the presence of heavy metals and aflatoxins (Raheem et al.,2021). Almost every day people are exposed to a significant number of anthropogenic pollutants. In the human body, some substances are broken down quickly, while others are retained and digested slowly. Organochlorine pesticides (OCPs) were originally introduced in the 1940s and were widely used to control agricultural pests until they were banned by the government. OCP chemicals are lipophilic in nature and have a slow metabolism (Saxena et al., 1982). Due to their chemical composition, they can survive in the environment. These substances accumulate and persist in adipose tissue after exposure. The accumulation of organic pollutants, primarily pesticide residues in fatty foods causes concern due to the potential carcinogenic effect, as well as the possible negative impact on the work of the harmful glands, nervous and immune systems. Persistent organic pollutants (POPs) can enter the food chain and accumulate in the fatty tissue of animals and humans. The definition of persistent organic pollutants (POPs) is that they are organic compounds that have a long half-life in the environment and are capable of slow physical, chemical and biological degradation. These pollutants can pass through ecosystems and can travel long distances, both locally and globally (Ramezani et al., 2022). Examples of POPs pesticides are organochlorine compounds namely: aldrin, chlordane, DDT, dieldrin, endrin, heptachlor and many others (Lopez-Carrillo et al., 1996). POPs usually have a high level of solubility in lipids and therefore bioaccumulate in adipose tissues of living organisms. They also have a long half-life in the body that can be measured from several months to several years after exposure. Therefore, these characteristics represent a special danger and risk for the environment and human health. All POPs, including DDT due to its semi-volatility and persistence, continue to worry the public. For many reasons, they are still on the list of pesticides whose presence is mandatory during the monitoring and control of food of animal origin by the European Parliament and the Commission. At high concentrations, POPs cause serious environmental effects, such as reproductive and developmental effects in wild and laboratory animals (WHO, 2000; Snedeker, 2001). All organochlorine compounds are mostly used as insecticides. POPs persist for a very long time in the environment. Chronically hazardous pesticides can affect body weight, hormone status, and can disrupt the endocrine and reproductive systems. It is known that organochlorine pesticides interfere not only with immunity, but also with the metabolism of thyroid hormones, which later affects the mother's offspring due to the level of dioxin in breast milk (Hasan et al., 2022). Human exposure to persistent pollutants occurs through diet, occupation, accidents and environment, especially in countries where POPs are used or used in significant quantities in tropical agriculture.

The aim of this review is to present knowledge about the presence and determination of pesticides in milk, with emphasis on the determination of DDT content. The results obtained during the first controls for the presence of DDT are discussed, as well as the most recent ones obtained in recent years using new methods of extraction and control using modern techniques. Also, the results obtained by monitoring and controlling the presence of DDT metabolites in sterilized milk over a period of 5 years, from 2018 to 2022, are presented.

MATERIALS AND METHODS

The certified standards of DDT metabolite studied were purchased from Dr. Ehrenstorfer. All the standards were of high purity (over 98%). The stock solutions were prepared in ethyl acetate, at a concentration of 10 µg/mL. This stock solution was used to prepare the calibration standards from 0.01 to 0.10 µg/mL. They were used to obtain the calibration curves in the ethyl acetate and matrix. The 150 samples were extracted by a modified QuEChERS method described by Anastassiades et al. (2003). For the extract, 10.0 g of sterilized milk sample was weighed into a 50 mL polypropylene centrifuge tube, internal standard (TPP) and 10 mL of acetonitrile were added and the solution was mixed for 2 min using a vortex mixer. Then, 4.0 g of anhydrous magnesium sulfate, 1.0 g of sodium chloride, 1.0 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate were added to the tube and the mixture was mixed again first on a vortex, and then followed by centrifugation for 5 min (4000 rpm) to separate the layers. An aliquot of the upper layer of the extract was transferred to a 15 mL polypropylene centrifuge tube containing 900 mg of anhydrous magnesium sulfate and 150 mg of C18. The extract was vigorously shaken for 3 min and after that it was centrifuged, it was evaporated to dryness in a stream of nitrogen, hexane was added, after that it was filtered through a PTFE 0.45 µm filter and injected into the GC-MS system. The chromatographic analysis was performed using a gas chromatography (Clarus 680 PerkinElmer) with tandem mass spectrometry (Clarus SQ8T, US) (GC/MS). The instrument uses Turbo Mass software version 6.1.0.

The sterilized milk samples analyzed were samples from supermarkets sold in the territory of the Republic of Serbia. Most of the samples (80%) were imported into Serbia from neighboring countries, while a smaller percentage (20%) of those samples of sterilized milk were produced on the territory of Serbia.

RESULTS AND DISCUSSION

The used validated GC/MS method according to document SANTE/11312/2021 obtained a good response correlation coefficient in terms of linearity in the range of 10 to 100 µg/kg for DDT metabolites, with R^2 >0.99. Validation and investigation was conducted for all metabolites of DDT (p, p´-DDT; o, p´-DDT; p, p´-DDD; o, p´-DDD; o, p´-DDE; p, p´-DDE). The matrix effects (ME) was compensated with matrix match calibration. The chromatogram of real milk samples is given in Figure 1.

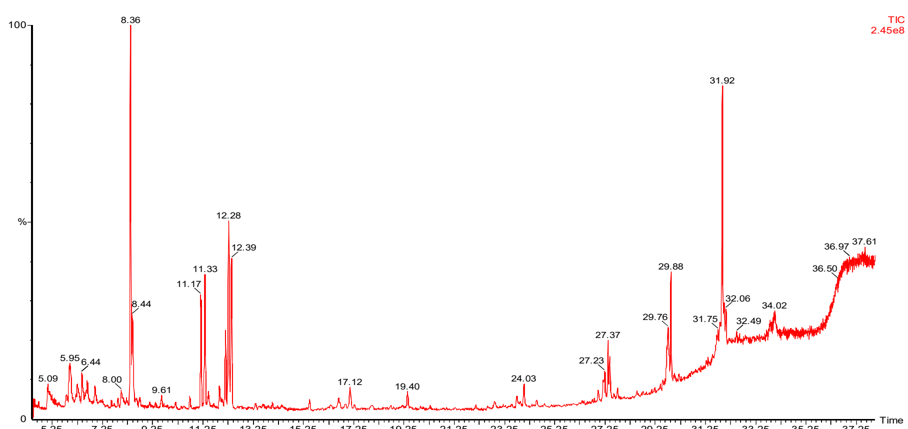

Figure 1. Chromatogram of real milk sample detected in full scan.

Figure 2 shows the detected p, p´ DDT in a spiked milk sample in determined recovery at a concentration level of 10 µg/kg (the level of the limit of quantification, LOQ). For quantitative quantification in selected ion monitoring (SIM) mode. Quantification was achieved by the detection of four quantification target ions: 165, 235, 236 and 237 according to the quantification of the internal standard.

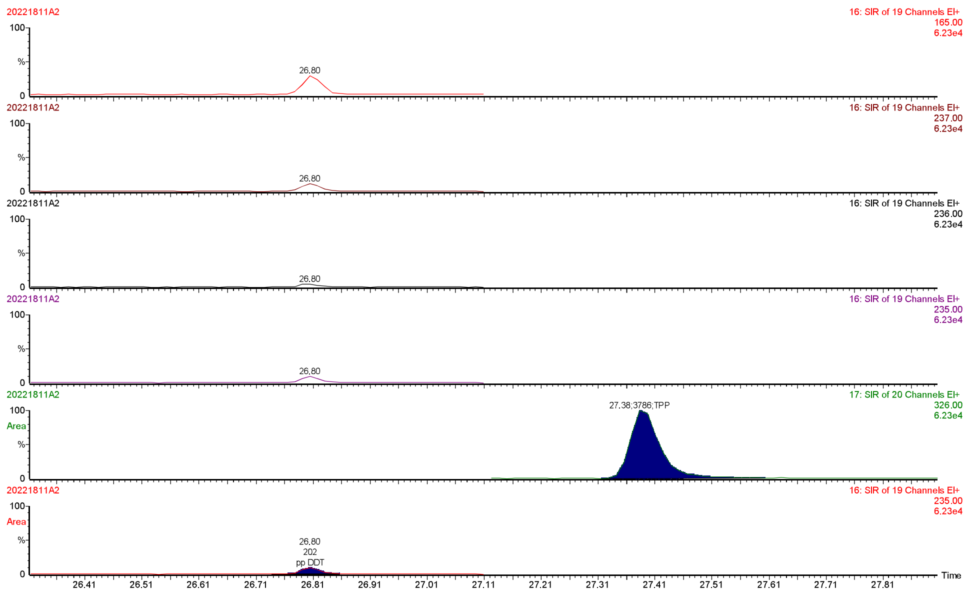

Figure 2. Ion chromatograms in SIM mode for p, p´- DDT and the internal standard peak

The LOQ as the lowest quantified value was set for each DDT metabolites on 0.01 mg/kg and it was confirmed experimentally. Recovery studies were satisfactory at two levels (20 and 100 µg/kg), added to blank milk samples.

RESULTS OF REAL SAMPLES

The analyses of 150 milk samples were done with this highly sensitive and selective of using GC/MS method. The presence of DDT metabolites was not detected in all examined milk samples. Considering this examination, it could be concluded that average milk consumption should not represent a threat and will not have a negative exposure assessment for human health when it comes to the presence of DDT. These results confirm the safety of milk when referring to the presence of DDT in the territory of the Republic of Serbia, but on the other hand, they do not exclude control and monitoring. These persistent pollutants are still present in the environment, they are present in samples of animal origin, and the directive of the European Commission requires their monitoring and testing in fatty foods.

STOCKHOLM CONVENTION

The Republic of Serbia is a signatory to the Stockholm Convention on Persistent Organic Pollutants (POPs). In 2009, the National Assembly of the Republic of Serbia adopted the Law on the Ratification of the Stockholm Convention ("Official Gazette of the RS - International Treaties", number 42/09), and in the same year the Government of the

Republic of Serbia adopted the National Implementation Plan for the Implementation of the Stockholm Convention on Persistent Organic Pollutants substances. By adopting the Law on the Ratification of the Stockholm Convention, Serbia undertook to fulfill all the provisions of the Stockholm Convention, including the harmonization of national legislation in all segments related to POP pollutants. These activities include the necessity to identify and establish norms for the emission and levels of polluting substances from the POP group. The plan included the control of implemented measures by creating a register of POPs, action plans for management and destruction, as well as systematic monitoring of POPs in various environmental media.

The Stockholm Convention on Persistent Organic Pollutants was adopted in 2001 in Stockholm under the coordination of the United Nations Environment Program. The Convention entered into force on May 17, 2004. and is the first global and legally binding instrument based on scientific criteria (Stockholm Convention, 2004). The first 12 POPs included in the Convention are aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, HCB, mirex, toxaphene, PCB, PCDD and PCDF. The main goal of the Stockholm Convention is to prohibit or limit the production, use, emission, import and export of toxic hazardous substances, which belong to the group of long-term organic pollutants, in order to protect human health and the environment. The criteria for prioritizing pollutants were concentration levels as well as specific physico-chemical characteristics, persistence, bioaccumulative potential, toxicity and the ability to find them even at long distances.

Integrated pest management strategies include: 1) biological control (eg, use of beneficial insects and pathogens, host plant resistance), 2) mechanical/physical control (eg, trapping), and 3) chemical control (eg, use of pesticides, attractants/repellents). In general, chemical pest control requires pesticides from different groups, usually insecticides, herbicides and fungicides. Their monitoring is especially important with fatty products of plant or animal origin, because most pesticides are highly lipophilic (well soluble in fat). Thus, fatty foods can contain relatively high amounts of pesticide residues and for this reason represent a risk of exposure to toxic substances for animals and humans (Madej et al., 2018).

POPs SOURCES

The sources of organic pollutants are very different, such as industrial processes and combustion sources, including traffic and agricultural uses. OCPs were used on a large scale in agriculture, forestry and public health. In agriculture, they act as insecticides, acaricides and fumigants to control pest. Examination of DDTs levels in milk may give basic information on the status, sources and human risk of OCPs in the environment. The research, which provides information on the degree of contamination of milk and milk products in the territory of Serbia are insufficient, especially in the field of organic and persistent substances. After the end of the war in the 50s of the last century, there was a sudden development of the chemical industry, and the increase in the population indicated the need for a larger amount of food, which led to the invention, adoption and use of pesticides. On the other hand, these same pesticides were not tested enough and nothing was known about their harmfulness. After the release of the harmful effects of pesticides, their production and use were banned, but they still remained present on our planet. In a separate study to identify sources of contamination, it was found that agricultural areas for rice field and vegetable cultivation are the main sources of environmental contamination by most organochlorine insecticides in Malaysia. Due to the need to meet local demand, Bangladesh started production of DDT in 1966 in Chittagong. The factory for the production of DDT was closed in 1995, because the declaration from the Stockholm Convention on the Limitation of the Production and Use of Organochlorine Pesticides prohibited the import, production and use of DDT in Bangladesh. In America, the production of DDT was in the factory, that is, the town of Monsanto, which is still uninhabited after half a century due to DDT contamination. At the end of the fifties, factories for the production of DDT were opened in all major countries of the world.

DDT

DDT was first isolated in Germany in 1874, but it was not until 1939 that Professor Paul Müller recognized the potential of this chemical as a nerve toxin for insects, which proved its effectiveness over the following decades. In 1948, Swiss professor Paul Muller received the Nobel Prize for the discovery of dichlorodiphenyl-trichloroethane (DDT). During World War II, soldiers and civilians were given the necessary doses of DDT to prevent insect-borne diseases, yellow fever, typhus, elephantiasis, and other serious diseases. In endemic areas in South Africa, p,p '-DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane) was used to interrupt malaria transmission. Field farmers and ranchers across the US used it without any restrictions during the fifties and sixties to combat harmful insects. At the beginning of its use, DDT was the best-known and most praised chemical preparation for fighting disease vectors such as malaria, even to the extent that its shortages were considered a public health hazard by the World Health Organization. Until the moment when the World Health Organization recognized the concern about the potential risks due to the presence of DDT in human milk as well as numerous negative confirmed cases. Of course, all these investigations should not mislead the public. The presence of DDT in breast milk should not lead the public, especially mothers not to use it, because milk has a beneficial effect because it is the optimal source of food for newborns. In particular, when information is shared with the public, it should be made clear that the presence of dioxins and PCBs in breast milk is not an indication to avoid breastfeeding as emphasized by the World Health Organization (WHO, 2009). In Mexico, two companies started producing DDT in 1959, and tests in 1995 confirmed the presence of organochlorine pesticides in 146 out of 439 food samples. When it comes to milk samples out of 202 samples, the presence of pp-DDT in the amount of 0.01 to 0.082 mg/kg was confirmed in as many as 43.5% (Lopez-Carrillo et al., 1996). In India, with the use of DDT preparations, the number of malaria cases decreased from 75 million to only 5 million in just one decade. Crop yields after using DDT in that period were doubled and livestock were healthier and gained weight faster. Prior to its manufacture and agricultural use of pesticides being outlawed in 1983, China, one of the countries with the biggest agricultural production, was a significant producer and consumer of pesticides. Since the 1950s, China has been a big producer and consumer of DDT. 0.4 million tonnes of DDT were generated over its 30 years of operation, making up 20% of the total global production (Wong et al. 2005).

The first public doubts were sown by the publication of the famous American biologist Rachel Carson, published in 1962 under the title "Silent Source". Some insect pests have gradually developed resistance to DDT pesticides (especially mosquitoes from parts of India and South America), causing their populations to explode, while at the same time their natural predators, such as hornets, are nearly eradicated by spraying the chemical. This led to DDT being banned in the US in 1973 except in cases of extreme danger to public health. Many other countries have also banned its use or drastically restricted it with strict control.

DDT LEVELS IN MILK AND TESTS

There are still challenges in the very way of determining and developing simple techniques and procedures for control. But the results obtained in recent years in all countries are uniform and they agree that the presence of DDT in milk is decreasing since the first contaminations in the seventies of the last century. The transmission of p, p'-DDT and its metabolites to infants via breast-feeding was studied in the Kwazulu area, South Africa, where DDT is used to prevent the transmission of malaria. Blood samples were taken from 23 babies, as well as breast milk samples from their mothers. The mean value of total DDT in blood was 127.03 μg/L, and in breast milk 15.06 mg/kg (calculated on milk fat) (Bouwman et al., 1992). The samples were taken at Mseleni Hospital, northern Kwazulu, during 1987 because the mothers lived in an area where DDT is used to control malaria. Mseleni is located in North Kwazulu is a malaria endemic area where DDT has been applied only to control malaria. In that area, DDT was not used in agriculture, because DDT has been banned for this purpose in South Africa since 1976. Multiplicative regression analysis showed that the mean DDT value in the blood of infants increased significantly with the age of the infants. Organochlorides were therefore largely transferred from the mother to the infant. The use of DDT in the Amazon (Brazil) against malaria was until 1992, and the control of 69 samples of breast milk in each sample confirmed the presence of DDT (Azeredo et al., 2008). According to some data, DDT in breast milk was higher in developing and former Soviet countries, including Malaysia, than in developed countries. Long-term analyzes of human milk tests in Norway and Sweden indicate a 90% reduction in DDT and its metabolite p,p'-DDE and smaller reductions in total PCBs, hexachlorobenzene (HCB) and polychlorinated naphthalenes (PCN). Also, in recent scientific literature, there is an increasing number of published papers in which organochlorine pesticides were not detected, but neither were pesticides of the newer generation in milk and milk products. Examination of different types of milk in Iran confirmed that no pesticides were detected in 91% of the tested samples. Apart from dimethoate, the presence of p,p′-DDT and p,p′-DDD was confirmed in three samples, where in one sample of human milk p,p′-DDE was higher than MDK values (Ramezani et al., 2022). The most common source of p,p′ DDT intake in humans is food in industrialized countries. In 2003, the compound p, p′ -DDT was detected in various foods in many countries with concentrations of over 1.0 mg/kg in fatty foods (Binelli and Provini, 2003). On the other hand, endosulfan present in animal feed is the primary source of endosulfan residues in milk. From the processed data of 12142 samples of animal origin tested in 2020, the European Food Safety Agency (EFSA, 2022) found no measurable pesticide residues in as many as 92% of the tested samples. On the other hand, 830 samples (6.8%) contained one or more pesticides in measurable concentrations, but below or equal to the MRL. MRL exceedances were determined in 145 samples, which is 1.2% of the total number of tested samples, of which 94 samples (0.8%) were non-compliant with legal regulations when measurement uncertainty was taken into account. The most frequently quantified substances were copper compounds (488 samples), DDT (131 samples), hexachlorobenzene (118 samples), thiacloprid (88 samples), mercury (52 samples). The persistence and confirmation of the results processed by the European Commission for the presence of DDT and hexachlorobenzene is in the fat of foods and milk and is confirmed here explained due to the ability of bioaccumulation.

Of course, there were generally great difficulties in obtaining data that included the quantities and types of unregistered, banned and smuggled pesticides used. On the other hand, there is a large amount of data in the scientific literature about the presence of DDT in milk, the amount of which is controlled in all countries at different locations, times and periods (Rai et al., 2017). No significant amounts of organochlorine pesticides were found in the Republic of Serbia, which is certainly the result of a good and timely policy of control and prohibition of these substances. Preparations of aldrin, dieldrin, chlordane and heptachlor were banned in Serbia even in the earlier seventies. As for DDT, it is the last POP insecticide from the list of the Stockholm Convention, which was withdrawn in Serbia in 1989.

DAMAGE CAUSED BY POPs

Serbia currently lacks any official information or particular study on which to build a cause classification of registered human disease cases (Milic et al., 2019). There are many examples of far-reaching consequences of POPs, such as endocrine disruption, reproductive and developmental damage, neurotoxicity, increased morbidity rate, etc. (Gill et al., 2020; Snedeker et al., 2001; Witt et al. 1966). The lack of information can be attributed to two factors. First, the general population was not aware of the existence of POPs. Second, due to the multiple exposures and multiple characteristics of pesticides, the range of diseases caused by pesticides is difficult to diagnose.

RATIFICATION OF THE STOCKHOLM CONVENTION

The aim of this project is to help prepare for the management of POPs and to identify actions at the national level for the control, minimization and eventual elimination of POPs. Since the entry into force of the Stockholm Convention, attention has been focused on the development of the national profile and assessment of POPs management. The plan includes an assessment of the production and use of POPs pesticides, an assessment of polychlorinated biphenyls, an assessment of unintentionally produced chemicals, a socio-economic analysis of the use of POPs, as well as raising awareness about POPs. Also, controls on the import and application of pesticides are carried out through the strengthening of legal regulations and normative bases such as: review of registration status, market surveillance, environmental monitoring and monitoring of poisoning cases. In this way, effective control and an effective system of protection of citizens and the environment is achieved. As a result of contamination, the quality of food can be compromised and thus lead to short-term or long-term hazards to human health, such as genetic disorders, birth defects and nervous system disorders.

The impact of chemical compounds such as DDT on the environment is serious and raises concerns about their persistence (Fång et al., 2015). DDT as a non-polar organic pollutant and many other residues such as paclobutrazol can accumulate in fatty tissues and enter the food chain due to its hydrophobic chemical structure and for this reason can end up in breast milk and milk products (Guo et al., 2020). In this way, milk is an important biomarker for the assessment of DDT and all POPs residues in the human body. Given that milk and milk products are consumed in large quantities by children and adults alike, milk safety has received serious attention (Gill et al., 2020).

CONCLUSION

Alternatives to POPs include the promotion and support of ecological (organic) agriculture. Although this tendency is in line with the so-called "green chemistry", i.e. "green science", it is still difficult to imagine plant and animal production without synthetic chemicals, given the current state of technology. Therefore, the application of good agricultural practices on the farm, improving the knowledge and awareness of pesticide users, as well as continuous monitoring of pesticides in crops, are the paths and guidelines that lead to safe and secure food. This study indicated that even if the pesticides applied many years ago have not permanently disappeared, there are still traces of DDT present in some places that can be detected.

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