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Determination of organochlorine pesticide residues in cow's milk marketed in Ahwaz city of Iran

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Abstract: Organochlorine insecticides are among the most important organotoxins and make a large group of pesticides. Physicochemical properties of these toxins, especially their high lipophilicity, facilitate the absorption and storage of these toxins in human and animal bodies. The existence of the residues of these toxins in milk which is one of the most widely used foodstuff containing lipids can be a quantitative and qualitative index for the presence of these toxins in animal bodies. In this research the residues of seven important organochlorine insecticides in 35 milk samples marketed in the city of Ahwaz, Iran, have been identified and measured. First the lipid phase was separated from the aqueous phase, then extraction of the toxin residues from the lipid phase and subsequent purification of each was achieved through a column of activated Florusil. Detection and measurement of the toxins were achieved by using HPLC instrument with a UV-Visible detector. The results obtained had shown that Lindane (0.042 mg/Kg) and DDT [(1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane)] (0.28 mg/Kg) are exceeded the standard limits recommended by FAO/WHO.

Keywords: Pesticides, DDT, lindane, aldrine, dieldrine, toxin.

Introduction:

A pesticide is a substance or mixture of substances used to kill a pest.[1] A pesticide may be a chemical substance, biological agent (such as a virus or bacteria), antimicrobial, disinfectant or device used against any pest. Pesticides can also be classed as synthetic pesticides or biological pesticides (biopesticides), although the distinction can sometimes blur. Broad-spectrum pesticides are those that kill an array of species, while narrow-spectrum, or selective pesticides only kill a small group of species.[2] A systemic pesticide moves inside a plant following absorption by the plant. Most pesticides work by poisoning pests.[3]In the 17th century, nicotine sulphate was extracted from tobacco leaves for use as an insecticide. In 1939, Paul Müller discovered that DDT (I) [4,4'-(2,2,2-trichloroethane-1,1-diyl)bis(chlorobenzene) ; **D**ichloro-**D**iphenyl-**T**richloroethane)] was a very effective insecticide. It quickly became the most widely-used pesticide in the world. In the 1940s manufacturers began to produce large amounts of synthetic pesticides and their use became widespread.[4] Some sources consider the 1940s and 1950s to have been the start of the "pesticide era".[5] Pesticide use has increased 50-fold since 1950 and 2.3 million tons of industrial pesticides are now used each year.[6] Seventy-five percent of all pesticides in the world are used in developed countries, but use in developing countries is increasing.[2] The agricultural use of DDT is now banned under the Stockholm Convention on Persistent Organic Pollutants, but it is still used in some developing nations to prevent malaria and other tropical diseases by spraying on interior walls to kill or repel mosquitoes.[7] Some types of organochlorides have significant toxicity to plants or animals including humans. Some insecticides such as DDT are persistent organic pollutants which pose dangers when they are released into the environment. For example, DDT accumulates in aquatic food chains. Because the body is not able to break down or dispose of it, and it interferes with calcium metabolism in birds, there were severe declines in some bird predator populations. Rachel Carson brought the issue of DDT pesticide toxicity to public awareness with her 1962 book Silent Spring. While many countries have phased out the use of some types of organochlorides such as the US ban on DDT, persistent DDT, PCBs, and other organochloride residues continue to be found in humans

and mammals across the planet many years after production and use have been limited. In Arctic areas, particularly high levels are found in marine mammals. These chemicals concentrate in mammals, and are even found in human breast milk. Males typically have far higher levels, as females reduce their concentration by transfer to their offspring through breast feeding.[8a,b] Pesticides are used to control organisms which are considered harmful.[9] Pesticides can save farmers money by preventing crop losses to insects and other pests. DDT, sprayed on the walls of houses, is an organochloride that has been used to fight malaria since the 1950s. Recent policy statements by the World Health Organization have given stronger support to this approach.[10] Dr. Arata Kochi, WHO's malaria chief, said, "One of the best tools we have against malaria is indoor residual house spraying. Of the dozen insecticides WHO has approved as safe for house spraying, the most effective is DDT".[10] However, since then, an October 2007 study has linked breast cancer from exposure to DDT prior to puberty.[8] Scientists estimate that DDT and other chemicals in the organophosphate class of pesticides have saved 7 million human lives since 1945 by preventing the transmission of diseases such as malaria, bubonic plague, sleeping sickness, and typhus.[2] However, DDT use is not always effective, as resistance to DDT was identified in Africa as early as 1955, and by 1972 nineteen species of mosquito worldwide were resistant to DDT.

Materials and methods

All chemicals were purchased from Merck, Germany. Standard samples of insecticides (Lindane, aldrine, dieldrine, o,p'-DDT, p,p'-DDT, p,p'-DDE, p,p'-DDD were supplied by WHO in Tehran Iran). Petroleum ether : acetonitrile (V/V 10:1), acetonitrile : petroleum ether (V/V 10:1), potassium oxalate aqueous solution (w/v 5g/100 mL), anhydrous sodium sulphate (commercial sodium sulphate was heated in an oven with temperature at 500 °C for 5 hours) then kept in a capped container, commercial Fluorisil was activated by heating first at 650 °C for 3 hours in an electrical furnace, then kept at 130°C for 5 hours in a desiccator. HPLC (Cecil, series 1000) instrument equipped with a UV detector was used.

Preparation of the milk samples: 35 cow's milk samples were purchased randomly from different parts in Ahwaz city of Iran, then, the following procedures were carried out on each sample prior to the analysis:

Extraction: to a 50 g of cow milk in a 500 mL separatory funnel, 5 mL of aqueous potassium oxalate solution (5% w/v) was added. 50 mL of methanol was added to the solution and shook for 1 minute. Then, 100 mL diethyl ether was added and shook slowly for 2 minutes. After that 50 mL n-hexane was added to the mixture and shook for 1 minute and left aside the mixture for 10 minutes. The lower aqueous layer was separated and discarded. The mixture was put aside for further 2 minutes and the lower aqueous layer was separated and discarded. Some glass wool was placed into an ordinary glass funnel and covered quite well with dried anhydrous sodium sulphate. Then the organic layer in the separatory funnel was passed through the glass funnel and collected into a weighed Erlenmeyer flask. The separatory funnel was washed twice and each time with 10 mL n-hexane and then passed through the glass funnel and collected into the Erlenmeyer. The solution was evaporated on a rotary evaporator until the clear oily colour was appeared. Finally, it was weighed and the amount of oil was measured.

Purification of oil: the extracted oil was transferred into a separatory funnel and the container was washed with 10 mL petroleum:acetonitrile solution (v/v 10:1) and added to the separatory funnel. Then, 20 mL acetonitrile : petroleum solution (v/v 10:1) was added and shook the separatory funnel vigorously for 2 minutes. The lower acetonitrile layer was separated and to it 500 mL of 2% aqueous sodium chloride solution was added. The upper ethereal layer was washed twice and each time with 20 mL of acetonitrile : petroleum solution (v/v 10:1). The acetonitrile layers were added to the sodium chloride solution. The whole sodium chloride solution was transferred to a separatory funnel and 100 mL of petroleum ether was added to it and shook vigorously for 2 minutes. The lower aqueous layer was separated and discarded. The ethereal layer was washed with 100 mL distilled water, then, the aqueous layer was discarded. The ethereal layer was passed through a 20 g anhydrous sodium sulphate column. The separatory funnel was washed twice and each time with 10 mL with petroleum ether, then passed through the anhydrous sodium sulphate column. Evaporation of the ether on a rotary evaporator resulted about 1 mL of an extract.

HPLC analysis of the extract:

The optimum chromatography analysis was achieved with the following conditions: reversed phase HPLC, acetonitrile:water (60:40 V/V) mobile phase, UV detector with λ_{max} 212 nm, flow rate programming (for the first 12 minutes1.5 mL/min; then for the next 10 minutes 2.5 mL/min, and finally for the last 8 minutes 3.5 mL/min). 5 mg of each of the standard insecticide was injected separately; a sharp and symmetrical peak was obtained for each of the standard. Then, a mixture of the standards was injected at the same conditions. The components of the mixture were separated quite cleanly. The extracted material was concentrated to the utmost, then n-hexane was added until the volume was reached 0.1 mL. Finally, 10 µL of the sample (extract) was injected to the column

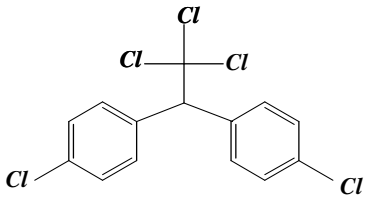
under the above conditions. A comparison of the HPLC chromatograms of the standards and the sample resutled in the identification of the components of the sample as Lindane; o, p'-DDT; p, p'-DDT; p, p'-DDE; p, p'-DDD; Aldrine; and Dieldrine.

Table 1. Statistical results of various toxins obtained from cow's milk marketed in Ahwaz city of Iran.

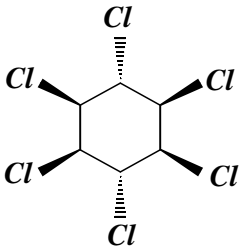
Toxin's name	**Lindane (II)**	**o, p'-DDT (III)**	**p, p'-DDT (I)**	**p, p'-DDE (IV)**	**p, p'-DDD (V)**	**Aldrine (VI)**	**Dieldrine (VII)**	**Total DDT**	**Total DDT +DDE+DDD**
Total No. of Samples	35	35	35	35	35	35	35	35	35
No. of samples contained the toxin	28	17	19	21	16	18	22	20	22
% of the samples contained the toxin	80	48.75	54	60	60	51	63	57	63
Max. amount, X_{max} (mg/kg)	0.042	0.135	0.23	0.056	0.092	0.406	0.095	0.28	0.406
Min. amount, X_{min} , (mg/kg)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total amount (mg/kg)	0.755	1.043	1.811	0.622	0.98	0.97	1.155	2.757	4.4
Average amount ,$\bar{X}$, (mg/kg)	0.021	0.0298	0.051	0.017	0.028	0.027	0.033	0.0787	0.125
Standard deviation (SD)	0.012	0.0378	0.058	0.016	0.034	0.033	0.032	0.089	0.128
Standard limit of the toxin (μ),(mg/kg)	0.004	-	-	-	-	0.005	0.005	-	0.05

Results and Discussion

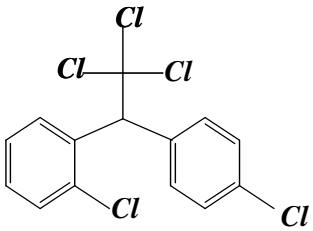
Many pesticides widely used in agriculture are organochlorides. These include DDT, dicofol, heptachlor, endosulfan, chlordane, mirex, and pentachlorophenol. These can be either hydrophilic or hydrophobic depending on their molecular structure. Organochlorine insecticides are among the most important organotoxins and make a large group of pesticides. Physicochemical properties of these toxins, especially their high lipophylicity, facilitate the absorption and storage of these toxins in human and animal bodies. The existence of the residues of these toxins in milk which is one of the most widely used foodstuff containing lipids can be a quantitative and qualitative index for the presence of these toxins in animal bodies. In this research the residues of seven important organochlorine insecticides in 35 milk samples marketed in the city of Ahwaz, Iran, have been identified and measured. First the lipid phase was separated from the aqueous phase, then extraction of the toxin residues from the lipid phase and subsequent purification of each was achieved through a column of actived Florusil. Detection and measurement of the toxins were achieved by using HPLC instrument with a UV-Visible detector. The total number of samples analysed was 35. The results had shown the presence of: Lindane [(1,2,3,4,5,6-hexachlorocyclohexane, also known as gamma-hexachlorocyclohexane, (γ-HCH), benzene hexachloride (BHC), gammaxene and Gammallin] **(II)** in 28 of the samples (80% of the samples) within the range of X_{min} = 0 to X_{max} = 0.042 mg/KG; o, p'-DDT **(III)** in 17 of the samples (48.57 of the samples) within the range of X_{min} = 0 to X_{max} = 0.135 mg/KG; p, p'-DDT **(I)** in 19 of the samples (54% of the samples) within the range of X_{min} = 0 to X_{max} = 0.23 mg/KG; p, p'-DDE (Dichlorodiphenyldichloroethylene; 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene) **(IV)** in 21 of the samples (60% of the samples) within the range of X_{min} = 0 to X_{max} = 0.056 mg/KG; p, p'-DDD (dichlorodiphenyldichloroethane; 1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene) **(V)** in 16 of the samples (60% of the samples) within the range of X_{min} = 0 to X_{max} = 0.092 mg/KG; Aldrine (1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene)**(VI)** in 18 of the samples (51% of the samples) within the range of X_{min} = 0 to X_{max} = 0.406 mg/KG; and Dieldrine (1,2,3,4,10,10-hexachloro-6,7-époxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-diméthanonaphthalène) (**VII**) in 22 of the samples (63% of the samples) within the range of X_{min} = 0 to X_{max} = 0.095 mg/KG. Meanwhile the total amount of DDT [The term "**total DDT**" is often used to refer to the sum of all DDT related compounds (p, p-DDT, o, p-DDT, DDE, and DDD) in a sample] and its metabolites were found in 22 of the samples in 22 of the samples (63% of the samples) within the range of X_{min} = 0 to X_{max} = 0.406 mg/KG; and total amount of DDT were found in 20 of the samples (57% of the samples) within the range of X_{min} = 0 to X_{max} = 0.28 mg/KG. The complete results are given in Table 1.



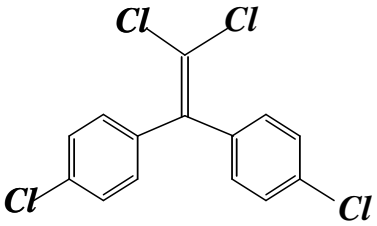
DDT ; (p,p'-DDT) (I)



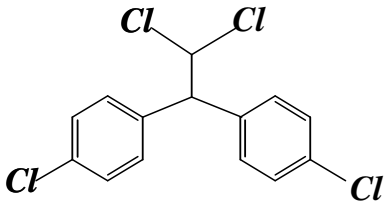
Lindane (II)



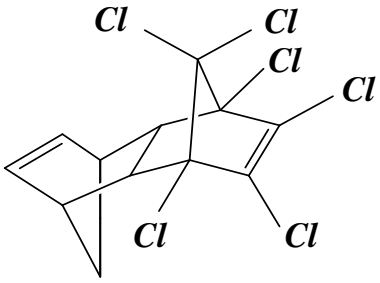
o,p' –DDT (III)



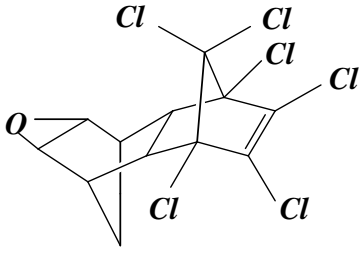
P,p'-DDE (IV)



P,p'-DDD (V)



Aldrine (VI)



Dieldrine (VII)

The results obtained had shown that Lindane (1,2,3,4,5,6-hexachlorocyclohexane) (0.042 mg/Kg) and DDT [(1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane)] (0.28 mg/Kg) are exceeded the standard limits recommended by FAO/WHO. Separation and identification of the components of the extract were also attempted by using column chromatography, thin layer chormatography (TLC) and gas chromatography – mass spectrometry (GC-MS) equipped with FID detector, but no satisfactory results were achieved. Unfortunately ECD detector was not available and we had to abandon the gas chromatography – mass spectrometry technique. It is worth mentioning that in this research, as it is well known, DDE and DDD are the major metabolites and breakdown products of DDT in the environment and their formation may be due to the extensive use of DDT in the past, as DDT is metabolized in plants by microorganisms to DDE and DDD.

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