

Pesticides Residues: Endosulfan and DDT in Cow's milk in Gezira State, Sudan

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Abstract

This study was performed in the Gezira Area to investigate the organochlorine insecticide residues in raw cow's milk. Five of raw cow's milk samples have been collected from different places in Gezira Area, which suffered of previous health problems caused by unsafe use of pesticides. The samples were analyzed by applying different chromatographic methods so as to investigate Endosulfan and DDT residues. The findings showed that all investigated samples were found to contain endosulfan and DDT residues from 0.01 to 0.3 ppm for endosulfan and from 0.11 to 2.18 ppm for DDT respectively. It is important to mention that, the concentration of residues in some samples exceeded the standard levels were cited by FAO and WHO for acceptable daily intake as 0.006 ppm. for endosulfan and 0.002 ppm for DDT. This study recommended the need to prohibit the use of endosulfan in agricultural activities in Gezira Area, promote health education programs among the farmers and encourage the routine pesticides residues studies in food.

Keywords: Residues of pesticides, Cow's milk, Sudan

1. Introduction

The Era of chemical pesticides began during the World War II. The synthetic organic insecticides were produced, the first of which was DDT. Pesticides use is now necessary to increase the agricultural production. Sudan, being an agricultural country, its growing demand of pesticides has increased year after year. During the period (1974 – 1977) the quantities sprayed annually of P,P'-DDT, Gammexane (BHC) and Endosulfan in the Gezira, were 3×10^3 , 3×10^4 and 1.4×10^6 , pound a.i. respectively [1-8]. In a field visit to the Gezira scheme it has been found that the quantities, used annually of endosulfan,

were 150.000 liters, 100.000 liters are pure endosulfan and 50.000 liters were mixed with another pesticide such as Dimethoate . However the standard quantities applied in the Gezira scheme . were cited as 0.75 liter / feddan of endosulfan of which 375 g/feddan is an active ingredient (a.i) The above figures apply to ground spraying , where as in ULV technique the quantities used are 0.70 liter / feddan of endosulfan , but the active ingredient is 350 g/feddan (Personal communication Administration of plant protection – Gezira scheme, 2012). After harvest of fields the cattle are allowed to graze on crops and fodders land which were previously sprayed with pesticide in Gezira scheme . In addition cotton seed and ground nuts cakes are also given to cattle in concentrated rations. The endosulfan pesticide was reported to cause poisoning . It accounts in the Sudan for 55% of the total poisoning accounts caused by pesticides during the last 30 years .

The endosulfan classified in moderately hazardous pesticide (Class II), DDT and malathion was categorized as slightly hazardous pesticides [9]. These pesticide chemicals are toxic in high levels and carcinogenic in animal tests. Epidemiological studies indicate that some of the synthetic pesticides are acting as endocrine disturbing chemicals and causing several hormone-related cancers.

Recently several author reported Diamanti-Kandarakis et al. [7] and Fleseriu [8] reported that pesticides aldrin, chlorpyrifos, DDT, endosulfan, malathion and monocrotophos pesticides are acting as endocrine disturbing chemicals on endocrine system of animals and may cause cancer risks[12-16].

The objectives of the investigation was to observe the effective levels of endosulfan and DDT residues. Also to provide information regarding the knowledge attitudes and practice (KAPs) of handlers of pesticides . The findings are essential to establish public health measures to control the effect of endosulfan .

2. Experimental

The Gezira State is considered as an area of agricultural activities . The main schemes are the Gezira scheme being the biggest scheme , as well as the Rahad and Guneid schemes . The cultivated area amount to 766.000 feddans in the last year 2011 and the average yield of feddan was 1.7 sack . As far as the animal resources are concerned , the Gezira State possesses a great potential.

In 2001 the animal resources constituted 3.188.000 heads of cattle, 2.364.000 of sheep , 1.531.000 of goats and 81.000 of camels. Furthermore the state is estimated to have 3.529.000 pieces of poultry and approximately 71 Tons of fish [7].

In the Gezira state the application of pesticides beings normally in the last week of August and ends in December during the season . The applications are reach their peak in

September to October . It is important to mention that in the Gezira state there are 112.000 tenant farmers [7].

2.1. Samples of raw cow milk

Five raw cow's milk samples were collected randomly from different places in Gezira State after field spraying by endosulfan. Half liter of each sample was produced in a clean stoppered bottles and then taken for chemical analysis and each sample replicated three times (table 1).

Table 1. Code of raw cow's milk samples

Sample code	Place of collection
A	ElHassahiesa
B	ElMasallamia
C	ElNishieshiba
D	ElAbbassia
E	Barakat

Reagents and standards

All chemicals and solvents were of analytical and chromatographic grade. The organic solvents were obtained from Panreac (Barcelona, Spain). Each standard was dissolved in acetone to obtain stock solutions of approximately 200 mg/L and these were stored in the dark at 4°C until further use. The fresh working standard solutions were obtained by dilution with n-hexane. Florisil adsorbent (16–30 mesh) was obtained from Sigma–Aldrich (St. Louis, MO, USA). Endosulfan and DDT were more than 99% pure and were purchased from Dr. Ehrenstorfer, GmbH (AUGSBURG, GERMANY)

2.2. Instruments and chromatographic conditions

Analysis of all pesticides was carried out with a Hewlett–Packard 6890 gas chromatograph (Palo Alto, CA, USA) equipped with an μ ECD Detector, split/splitless injection port, and fused silica capillary HP-5 column (5% diphenyl and 95% dimethylpolysiloxane, 25 m \times 0.32 mm ID, 0.52 μ m film thickness); and temperature programming from 80 °C to 250 °C (15 °C/min); injector temperature 250 °C and detector temperature 300 °C. Carrier gas (helium) flow rate, 2.6 mL/min; makeup gas (nitrogen) flow rate, 60 mL/min; injection volume, 1 μ L; and splitless time, 0.1 min. Data were acquired and the equipment was controlled using HP (Palo Alto, CA, USA) Chem-Station software, which was run under Microsoft Windows NT on an HP compatible personal computer. The identification of the a.i. peaks and the absence of interfering substances were assessed by comparing the sample chromatograms with those of a pesticide standard mixture and of an untreated sample. The

internal standard method was used to quantify the residues by measuring the peak areas vs. concentrations.

2.3. Liquid-liquid and solid-phase extraction procedure

The method used to extract the pesticides from milk was adapted from CHARLES and RAYMOND [17]. Each 50 g of sample and 150 mL of acetone was added. The mixture was homogenised for 2 min and then mixed for 2 h. The acetone residues were partitioned using saturated aqueous sodium chloride (30 mL) and dichloromethane (70 mL) in a separating funnel.

The extraction was repeated with another 70 mL of dichloromethane and the combined extracts were dried over anhydrous sodium sulphate. The dichloromethane fraction was collected and evaporated on a rotatory vacuum evaporator at 40 °C and the residues were dissolved in an acetone/hexane (1:9) mixture (10 mL). In the clean-up step, 1 mL of the extract was passed through a florisil column previously conditioned with 5 mL of acetone/diethyl ether (6:4) and 5 mL of diethyl ether. The pesticide residues were eluted with acetone/diethyl ether (6:4) (4 mL). Samples were analysed by gas chromatography.

3. Results and Discussions

3.1. Quality assurance procedure

The Codex quality assurance criteria [18] were followed to assess the performance of the method. The recovery rates of GC analyses ranged from 90 to 100% at spiking level values equivalent to 0.003 ppm. For the analysis of pesticide residues at the µg/g levels, accuracy and recovery of 70–120% are considered acceptable [19-24] and fulfil the criteria for quantities methods [25].

The results of the analyses were not corrected for recoveries. Blank samples were fortified with the pesticide mixture and analyzed as normal samples with each set of samples. The results were recorded on control charts. Repeated analyses of old samples were regularly carried out to assess reproducibility.

The limit of detection (LOD) was taken to be the amount of analyte that gave a signal that was clearly distinguishable from the background noise of the instrument. The LODs calculated in this way ranged from 0.181 to 1.297 ng mL⁻¹. The limit of quantification (LOQ), defined as the concentration of analyte that gave a signal equivalent to the blank signal plus ten times its standard deviation, ranged from 0.708 to 3.425 ng mL⁻¹ for the pesticides studied.

The precision, expressed as relative standard deviation (RSD), was calculated for each pesticide using standard solutions of 100 ng mL⁻¹ concentration. For 5 replicate analyses, it was found that 2.9 < %RSD < 8.4%.

Good linearity was achieved in the 10-200 ng.ml⁻¹ concentration range, for both pesticides, with a correlation coefficient of 0.9998.

3.2. Analysis of milk samples

Five raw cow's milk samples were investigated by chemical quantitative and qualitative analysis for the presence of organo chlorine insecticides residues (Endosulfan and DDT) from different places in Gezira Area. All samples have shown the residues in different concentrations. Table (2) shows the concentration of endosulfan and DDT residues in raw cow's milk samples. For endosulfan the concentrations of residues in all samples are found 0.3, 0.03, 0.02, 0.01 and 0.1 ppm respectively, where as for DDT residues the concentrations are 2.18, 1.00, 0.11, 0.50 and 0.42 respectively.

Table 2. Concentration of endosulfan and DDT residues in raw cow's milk samples

Sample	Endosulfan (ppm)	DDT (ppm)
A	0.3	2.18
B	0.03	1.00
C	0.02	0.11
D	0.01	0.50
E	0.1	0.42

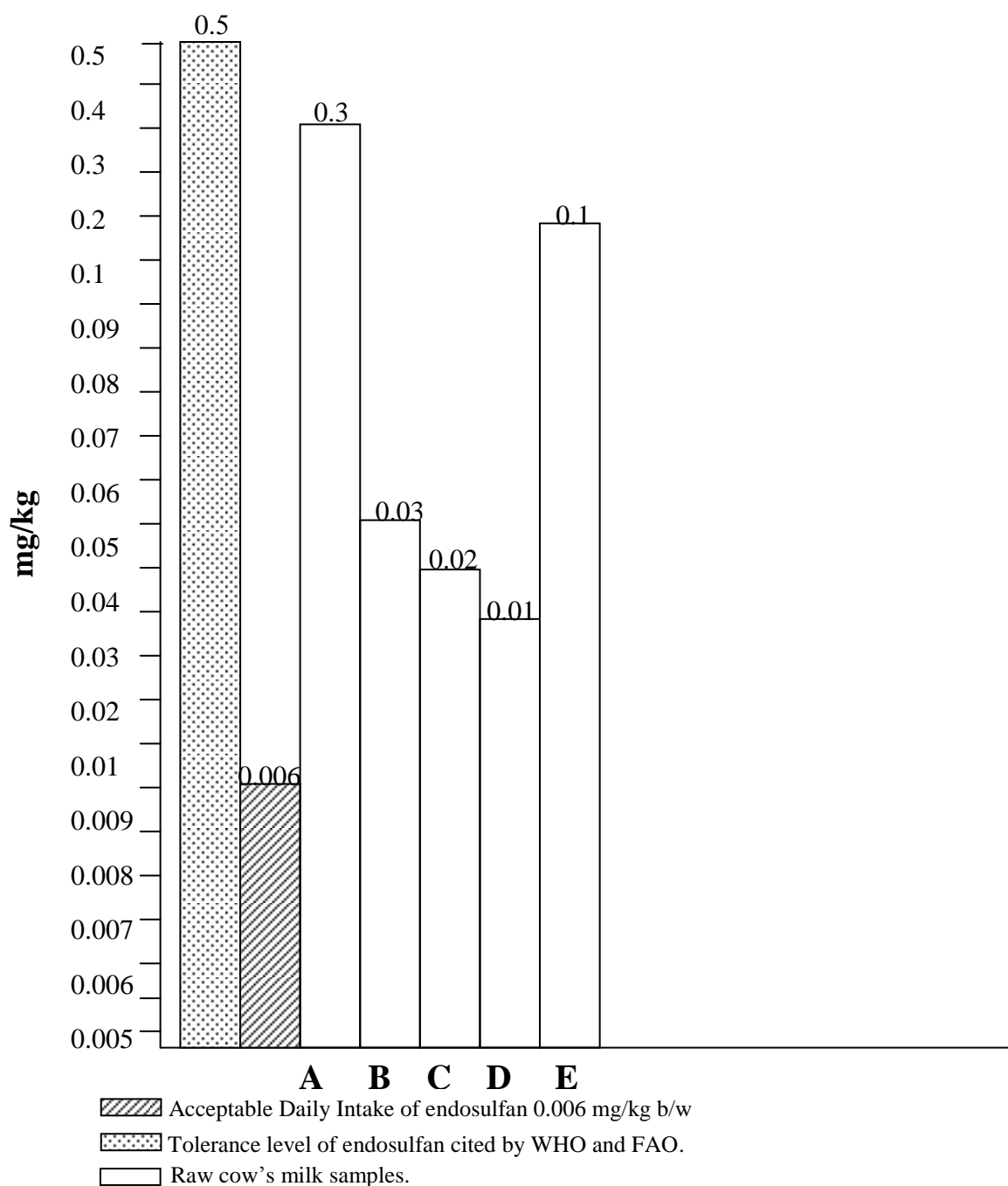
Of the total raw cow's milk samples, endosulfan and DDT residues were detected in all samples. Endosulfan residues in the raw cow's milk samples are below the tolerance level (0.5 mg/kg) established for milk and milk products by the FAO / WHO [2]

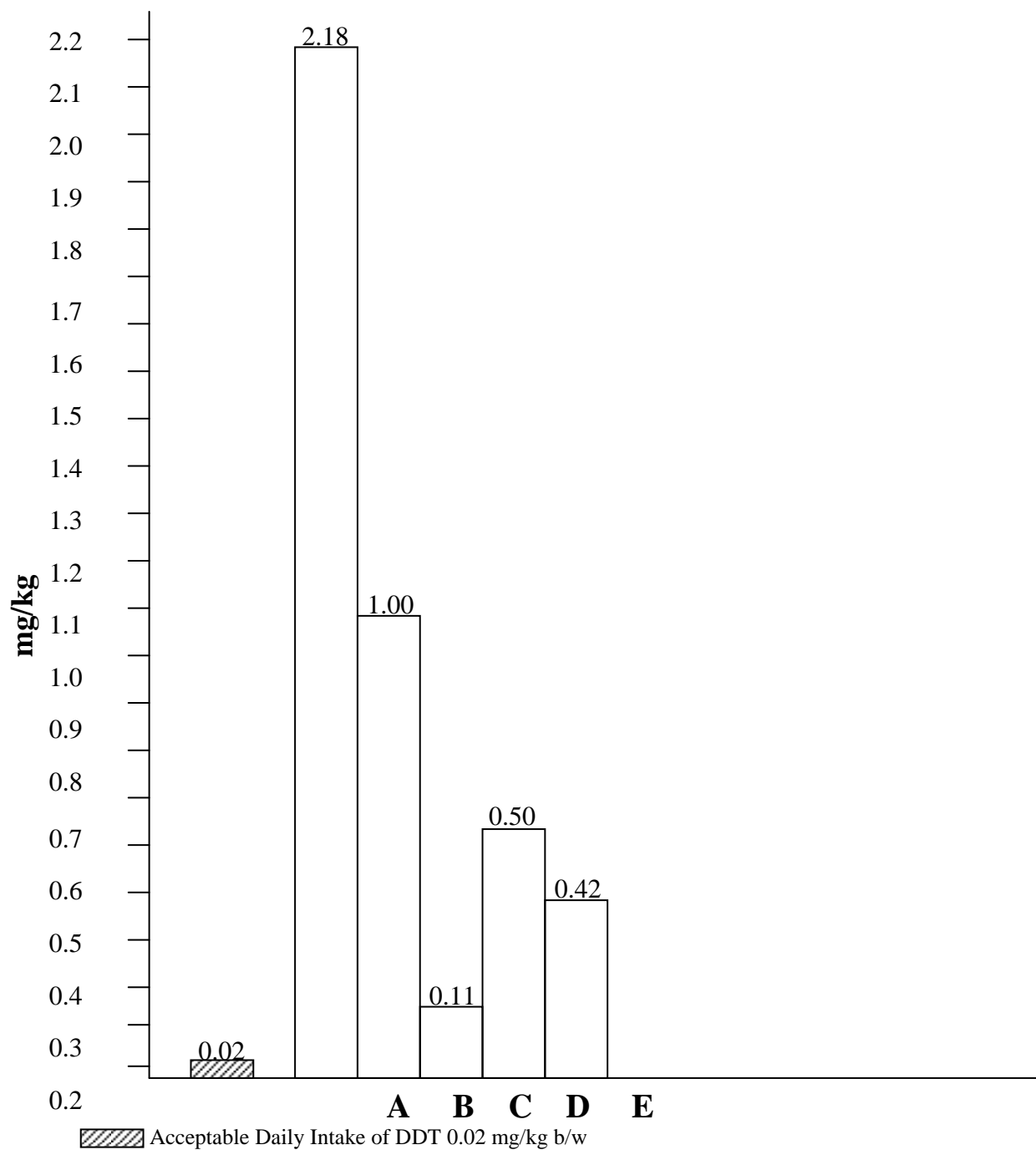
The concentration of endosulfan and DDT in the raw cow's milk samples ranged from 0.01 to 0.3 ppm for endosulfan and 0.11 to 2.18 ppm for DDT respectively. These concentrations are above acceptable daily intake (ADI), however the standard levels of Codex Alimentarius Commission (CAC) were cited as 0.006 mg/kg body weight for endosulfan and 0.02 mg/kg body weight for DDT respectively.

The pollution of food by endosulfan became an international problem because there are excessive amounts of it used in agricultural activities. In 1974 to 1977 the quantity which had been sprayed annually from endosulfan in Gezira was 1.4×10^6 pound active ingredient [26]. There are many similar studies which investigated endosulfan in the food as general and in the milk in particular. The findings of the present study showed that all investigated samples were found to contain unacceptable endosulfan daily intake particularly in Hasahiesa town. All investigated samples were found to be below the tolerance level cited by [4]

Despite the restriction of dichlor diphenyl trichloro ethane (DDT) by Environmental Practice Agency (EPA) in 1973, but it had still penetrated virtually every food chain that was studied. This was the result of its extreme stability in the environment.

This has been confirmed by the findings of [26] in their study conducted to determine the risk of organochlorine upon migratory birds in South Africa, however the results showed different concentrations of DDE, DDD and DDT residues in the blood samples of birds. They concluded that continued illegal DDT was in use.





Raw cow's milk samples.

Also a similar study, performed by Ijani et al [13] investigated the effects of organo chlorine pesticides in birds in Tanzania. This study revealed those large quantities of DDT, endosulfan, dieldrin; Lindane and toxaphene are still applied against insect pests and disease vectors.

In the Sudan particularly in Gezira Area DDT usage had begun in the season 1945 to 1946 in Gezira Scheme for controlling cotton pests. In 1974 to 1977 the quantity which has been sprayed annually from DDT was 3×10^6 pounds active ingredient [26]. The findings of the present study showed that all investigated raw cow's milk samples were found to contain unacceptable DDT residues levels particularly in Hasaheisa town. This complies with what has been mentioned by [16] who expressed that DDT accumulates in animals that eat plant tissue, bearing even traces of DDT, for example the dairy cows excretes a large share of the ingested DDT in its milk fat. According to [16] DDT and its chemical relations are all nerve poisonous. They increase the nervous excitability, and cause tremors and convulsions. The medium lethal dose of DDT for humans is about 250mg per of body weight.

Residues of both endosulfan and DDT were confirmed in samples by applying Thin Layer Chromatography technique (TLC). β -endosulfan was not detected in the standard concentration of (30 ml * 2mg/ml) but it was detected by using gas liquid chromatography technique in standard concentration of 5ng because the latter is more sensitive. This finding was in agreement with who mentioned that thin layer chromatography was an excellent method for confirming the identity of chloro organics already detected by gas liquid chromatography technique.

Farmers in Gezira Area possessed a good educational level, 16.67%, 18.18%, 15.66%, 32.32% and 17.17% of them had primary, intermediate, secondary and university education respectively. So health education program could be applied so as to improve their knowledge about health hazards caused by pesticides because 6.06% of farmers think that pesticides do not cause health hazards and 1.01% of them have no idea about health hazards of pesticides.

For specific hazards of pesticides 26.63% of farmers believe that pesticides cause poisoning, 17.93% cancer, 3.81% congenital deformation, 15.76% allergy and 35.87% of them think that pesticides cause all above health problems. According to [4] pesticides may also kill other organisms and most of them are poisonous to human. The World Health Organization [5] estimated that one million people are affected by insecticides poisoning every year and that 2000 die as a result of being unaware of the risk involved in handling insecticides George, (1978) mentioned that under certain condition, pesticides may be toxic to humans. Some pesticides are much more toxic than others, and severe illnesses may result from ingestion of only a small amount of certain chemical, while with compounds no serious effects result even from ingestion of large quantities.

The public health hazards have a bearing with the use of endosulfan insecticides; this was expressed by [9]. They confirmed that endosulfan is highly toxic via the oral and dermal route, and alpha isomer is more toxic than beta isomers.

Stimulation of the central nervous system is the major characteristic of endosulfan, this was proved by [1, 2].

Teratogenic and mutagenic effect of endosulfan were also confirmed by the experiment of While [1,2]. Found that endosulfan is not carcinogenic.

The findings showed that 33.84% of farmers store the pesticides in cereal stores. This represents a bad practice because [5] stated that pesticides store should only contain pesticides, all other goods or objects should be removed.

From the questionnaires in the framework of this study it was found that 78.79% of farmers deal with endosulfan in Gezira area table (20) . 74.36% of farmers use it for spraying tomatoes and vegetables, whereas 78.21% of farmers sprayed more than 0.75 liter per feddan (this is the permitted dose of endosulfan per feddan which was cited by Gezira Scheme). While 50% of farmers mixed endosulfan with another pesticide, this practice may lead to complex health hazards. This was in line with what [27] mentioned, namely combinations of pesticides and various additives should not be used unless the specific combination has been tested and is recommended by the appropriate authorities, because of their potential toxicity.

All above findings represent risky practices affecting public health of humans in Gezira area, first and major reason of all these practices was the missuse handling of pesticides in local markets in Wad Medani town, the Capital of Gezira State.

Cow's milk consumption was found to be high in Gezira area, as 62.12% of farmers own cows from different herds.

Cows in Gezira area were fed by different methods such as silage (aalaf) and/or grazing fodder's in the field. The previous methods of feeding may lead to the appearance of the pesticides residues in the cow's milk, because after harvesting of field crops, the animals depend on their grazing on the residues of these crops and fodder's which were grown in Gezira area, this also in addition to the cake of ground nuts and cotton seeds. These crops and fodder's were exposed to pesticides application to control pests, which appeared on them, and so humans and animal, were exposed to pesticides directly or indirectly. In a similar study conducted by Ijani [13] in Pakistan the findings of their study revealed that laboratory investigation of cotton seed samples showed contamination in 73.6% of samples with 24 different pesticides. While [15] mentioned that direct application of pesticides may result in an accumulation of their residues in soil. Another source of pesticides in soil is the residues of these chemicals in the atmosphere.

From this study it was found that 56.35% of farmers questioned believed that endosulfan affect their cow's milk from feeding.

The findings of the present study showed that there is a significant correlation between the level of education and the knowledge of farmers about the health hazards caused by pesticides. (P value = 0.002).

The relation between the agricultural products sprayed by endosulfan and the source of obtaining it was represented by the findings of this study, a significant correlation has been found in the comparison. (p value = 0.003).

A significant correlation with P value of 0.008 was found in the relation between the practice of farmers for storing pesticides and the assessment of endosulfan effectiveness.

4. Conclusion

This study revealed the presence of (0.01 to 0.3 ppm) for endosulfan and (0.11 to 2.18 ppm) for DDT respectively . Endosulfan and DDT were detected in all samples analyzed. The concentration of endosulfan residues in all samples has exceeded the acceptable daily intake (ADI) 0.006mg/kg cited by Codex-1999, but it is below the tolerance level 0.5mg/kg established for milk and milk products by the [1].

For DDT all investigated samples were found to exceed the codex standards. Additional efforts are needed to minimize the undesirable environmental consequences of the use of pesticides.

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