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# Presence of DDT in Animal Fat Aimed for Human Consumption in Albania

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**Abstract:**

*Under the frame of the National Monitoring Plan of residues in food products, Institute of Food Safety and Veterinary in Tirana, has carried out since years already analysis of pesticides residues in animal tissues. This paper gives the summary of data obtained from the analysis of organochlorine residues in fat tissues of sheep and goats, slaughtered for human consumption, during 2014-2015 in Albania. Because of their strong lipophilic nature and their tendency of passing up onto the food chain, DDT and its isomers are still encountered in the environment, though at a low level but at any case detected and identified as such. The results confirm the persistent presence of DDT, in animal fat tissue, even after a long time since their last agricultural use. The analysis was based on a modified QuEChERS method consisting in ethyl acetate extraction followed by freezing out as a first cleaning up step followed by a second cleaning up using PSA/C-18. The extract was injected in GC system coupled*

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*with a MS/MS detector. The results confirmed that major part of samples tested (74.5 %) showed a detectable contamination by p-p' DDE (< 20 ng/kg), although none of the results exceeded the maximum permitted level of 1 mg/kg established from EU Regulations.*

**Key words:** organochlorine, pesticides, *DDT,* persistence, animal fat

## AIMS AND BACKGROUND

*DDT* was one of the first chemicals in widespread use as a pesticide in our country as all over the world. Studies show a range of human health effects linked to *DDT* and its breakdown product, *DDE.DDT* was banned from use because it is highly persistent in the environment, gets accumulated in fatty tissues and can be bio concentrated on the food chain. *DDT* tends to accumulate in the fatty tissues of insects, wildlife, and people, but produces no known toxic effects while it is stored in the fat. *DDT* is metabolized into various breakdown products in the body including *DDE* etc. *DDT* is highly persistent in the environment as well. The half-life for *DDT* in soil is from 2 to 15 years. When fat stores are used during periods of starvation the breakdown products of *DDT* are released into the blood where they may be toxic to the liver and the nervous system. The organochlorine pesticides have been widely used for agricultural purposes even in Albania. They have been used intensively in the south-western regions of the country because accept for the agriculture purposes they were used against insects borne diseases- such as malaria.[1] The organochlorine pesticides more used in Albania have been *DDT*, *Lindane,* etc. *DDT* for example was greatly used even in Albania for years started from '45 until '70. Their use was banned after the years '90 although the organochlorine pesticides and their residues have constantly been reported in some environmental studies carried out in our country.[1,2,3] Non systematic information and data do exist relating to the imported quantity and usage of

*DDT* in public health sectors. What's more some non confirmed data say that *DDT* has been until recently used by the Ministry of Public Health for areas spraying against mosquitoes. According to the information gathered in some regions it results that *DDT* has been used until 15 years ago though not for agricultural purposes but for land and water areas spraying against mosquitoes. The quantities of *Lindane* and *DDT* (3 tons) which have been in storage from the Ministry of Public Health until 2005 have been destroyed under the frame of the project financed from Dutch Government. Under the frame of PHARE program in the year 2002 have been made the destruction in Germany of some other quantity of *DDT* remained at storage in the agricultural sector.[4] Though a number of studies, monitoring and surveys have been undertaken from different institutions and interested parts in Albania, a number of them under the frame of monitoring projects or environmental scientific research, there is still a need to correlate all the data obtained up to now, especially relating to evaluate the presence of *DDT* in different matrixes and geographic regions in order to fully estimate the risk Albanian customers and habitats run of the presence of POP in the all environmental spheres. The aim of this study is the evaluation of *DDT* [*1,1,1- trichloro-2,2-di (4-chlorophenyl) ethane*] presence in animals fat such as sheep and goats destined for human consumption. The number of samples for the two years of the monitoring is predetermined in the National Monitoring Plan according to the number of the animal (sheep and goat) registered in Albania.

## EXPERIMENTAL

*Sampling*

All the samples have been taken by Food Safety Inspectors from slaughterhouses in some of the main districts of Albania according to the respective methods of sampling for official

control described in detail in literature.[5] The samples have been transported to the lab in thermo box within the same day. The number of samples analyzed during these two monitoring years (2014-2015) was 72. The samples that could not be handled and analyzed during the arrival day have been processed and put to deep frozen storage conditions. The laboratory sample was prepared in accordance with the respective recommendations described in detail in literature.[6]

*Chemicals*

All chemicals used were of pesticide grades. The standard was *DDT* mix at concentration 10 ng /µl. Standard working solution and calibration curve solution at level 5, 10, 25, 50 and 100 ng /ml were prepared by diluting the right amount of solution in matrix blank extract. **Figure 1** shows the 5 points *p-p'DDE* calibration plot which shows a very good correlation at $R^2$=0.99 for the respective working zone.

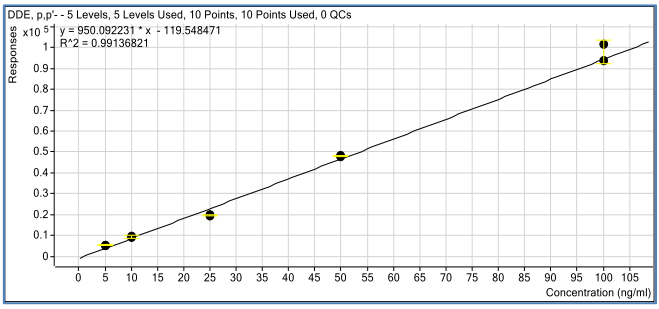


**Fig. 1. Calibration curve of p.p'- DDE (5, 10, 25, 50 100 ng/ml)**

*Extraction and clean-up*

The method used for DDT and its isomers determination is based on the ethyl acetate extraction using gas and liquid chromatography with tandem mass spectrometric determination.[7] Putting in into few words, 0.5 g of fat sample was accurately weighed and 10 ml of ethyl acetate were used for extraction. The samples were vortex vigorously and the extraction was followed by addition of some magnesium sulfate, vortex again, followed by centrifugation. The samples were

placed in -20° over night, re centrifuged and 1 ml of ethyl acetate layer was passed in tube containing PSA/C-18 for further cleaning up. After centrifugation the ethyl acetate layer was transferred into glass vial and injected into GC/MS/MS.

*Instrumental analysis*

The identification and quantification of DDT isomers in the extracts was determined by using gas chromatography couple to tandem mass spectrometry (GC/MS/MS Agilent) in multiple reaction monitoring (MRM) in EI MS/MS mode. The injection volume was 1 µl. The column used for separation was HP-5 MS (30 m x 250 µm x 0.2 µm). Carrier gas was Helium. Mass spectrometer was operating Electron impact Ionization (EI) mode, the electron energy was 70 eV and the temperature source at 290°C. The initial oven condition started at 50°C for 0,6 minute following by a temperature increase up to 180°C at a rate of 15°C/min held for 1 minute. Then the temperature was increased at 230°C by 7°C/min and the last step was up to 280 °C by 3°C/min. All the analysis have been performed in MS/MS mode. The transition used for the compounds identification and the quantification was taken from SweET method. **Figure 2** shows the signal acquitted from a positive resulting fat sample.

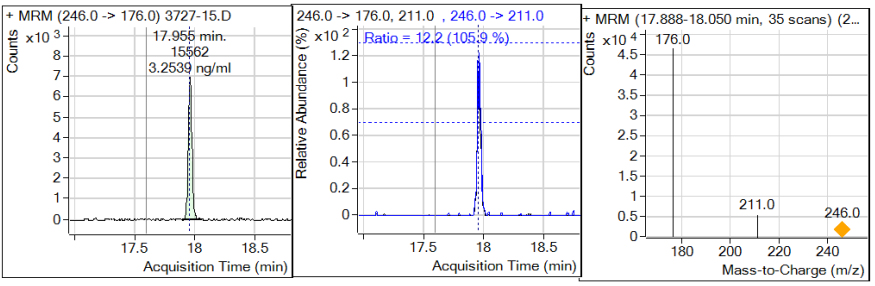


**Fig.2. Chromatogram of a fat sample where *p, p'- DDE* is detected RT=17.955'**

*Statistical analysis*

EXCEL software was used for statistical analysis of the data. Mean, standard deviation, minimum and maximum values were taken by using descriptive statistics. The significant

differences between *DDT* residues in goat fat and ship fat were calculated at $P < 0.05$. All analytic data of measurements were entered into a data matrix. Descriptive statistics were applied to interpret the results and to explain the variations in the data, between two categories of samples.

## RESULTS AND DISCUSSION

Statistical variables of the *DDT* residue values in fat for both types of animals are reported in the Table 1.

**Table 1. Descriptive statistics of *DDT* residue values in sheep and goats fat**

| Varaibles | Mean | Median | Standard Deviation | CV% | Kurtois | Skewness | Min | Max | Count |
|---|---|---|---|---|---|---|---|---|---|
| Sheep fat | 71.1 | 65.1 | 78.3 | 110 | 9 | 2.7 | 3 | 399.1 | 40 |
| Goats fat | 12.7 | 8.7 | 11.6 | 91 | 1.6 | 1.4 | 0.5 | 44.1 | 26 |

As we see in the table 1, the variance's coefficient of data for both types of fat is a little more than the limit values of 75 %, showing a good variance. We must emphasize that *DDT* residues values of sheep fat (max 399.1 ng /kg) are much more higher than goat fat (max 44.1 ng/kg).

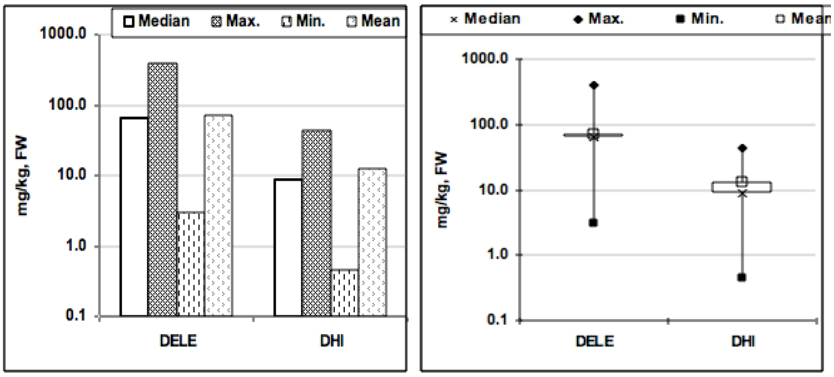


**Fig. 3. Trend distribution of the *DDT* residue values of sheep and goats**

In the **Figure 3** we have presented the minimum and maximum values of *DDT* residues with the most significant difference between two groups. This has been proven by Fischer test, where $F_{crit} < F_{stat}$ (4.16 < 12.93) and t-test where $t_{crit} < t_{-stat}$ (2.035 < 3.51). From these tests, which respective values are given in **Tables 2 and 3** we have found that there are significant differences between the goats and sheep from all districts.

**Table 2. F- test for *DDT* values between sheep and goats fat**

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Columns | 45890 | 1 | 45890 | 12.93 | 0.00 | 4.16 |
| Error | 110012 | 31 | 3549 | | | |
| Total | 276667 | 63 | | | | |

**Table 3. t-Test: Two-Sample Assuming Unequal Variances**

| | *Variable 1* | *Variable 2* |
|---|---|---|
| Mean | 66 | 12 |
| Variance | 7259 | 186 |
| Observations | 32 | 32 |
| Hypothesized Mean Difference | 0.000 | |
| df | 33.000 | |
| t Stat | 3.511 | |
| P(T<=t) one-tail | 0.0007 | |
| t Critical one-tail | 1.692 | |
| P(T<=t) two-tail | 0.0013 | |
| t Critical two-tail | 2.035 | |

Another important point of this study was the evaluation of the *DDT* presence and distribution in each district of the country by sampling sheep and goat fat from 5 distinct regions. The statistical variables for both groups of animals shown respectively in the **Tables 1-3** certify that the *DDT* residue

values are much higher in sheep fat than in goats fat and there are significant differences between animals of diverse districts.

In the **Tables 1 and 4** we have presented the mean, median and maximum for each district for the goats and sheep. Also, in the fig.4 below we have shown the difference of data between two types of animal according 5 districts in Albania. From **Figure 4** we see that max value of *DDT* is greater for the sheep fat in Dibra district (399.1 ng/kg) and in Korca district (130.8 ng/kg). But the mean of both districts are at the same order and we take the conclusion that the most contaminated animals are both districts Dibra and Korca. The statistical variables for goats fat reveal that the most contaminated fat with *DDT* residue are the animals from Korca district.

**Table 4. Descriptive statistical variables of *DDT* values for each group of animal in different districts**

| Sheep fat Variables | Mean | Median | Standard Deviation | Sample Variance | CV% | Kurtois | Skewness | Min | Max | Count |
|---|---|---|---|---|---|---|---|---|---|---|
| **Diber** | **127.2** | **44.1** | 160.7 | 25823 | 126 | -0.3 | 1.2 | 7.5 | **399.1** | 7.5 |
| **Elbasan** | 34.1 | 18.4 | 31 | 963 | 91 | 1.5 | 1.6 | 13 | 96.8 | 8 |
| **Fier** | 69.8 | 70.5 | 14.7 | 217 | 21 | 1.6 | -0.3 | 46.2 | 91.4 | 6 |
| **Vlore** | 15 | 13.4 | 7.2 | 52 | 48 | -0.8 | 0 | 4.4 | 24.7 | 7 |
| **Korca** | **95.4** | **102.6** | 29.6 | 877 | 31 | -1 | -0.5 | 39.8 | **130.8** | 13 |

| Goat Fat Variables | Mean | Median | Standard Deviation | Sample Variance | CV% | Kurtois | Skewness | Min | Max | Count |
|---|---|---|---|---|---|---|---|---|---|---|
| **Diber** | 9.59 | 10.6 | 8.33 | 69.45 | 87 | 0.69 | 0.9 | 1.25 | 22.4 | 5 |
| **Durres** | 13.99 | 13.9 | 8.13 | 66.15 | 58 | 1.85 | 1.29 | 6.5 | 27.17 | 5 |
| **Elbasan** | 11.97 | 8.6 | 10.19 | 103.91 | 85 | 4.57 | 2.1 | 4.87 | 29.99 | 5 |
| **Vlore** | 7.16 | 8.4 | 3.23 | 10.41 | 45 | -1.88 | -0.61 | 2.8 | 10.4 | 5 |
| **Korca** | 20.61 | 21.06 | 12.52 | 156.66 | 61 | 1.76 | 0.7 | 5.23 | 39.78 | 5 |

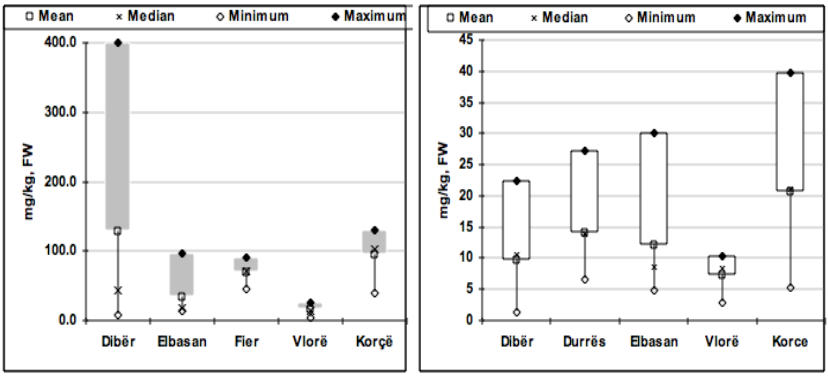


**Fig.4. Trend distribution of the *DDT* residue values of sheep and goats in each 5 district**

As conclusion we can say that higher values of *DDT* in the sheep fat comes from the fact of foraging behavior and diet selection of goats. Different conditions of feeding of sheep versus goats is reflected in *DDT* residue level much higher in sheep (mean level 71.1ng/kg) than goats (mean 12.2ng/kg). However, the *DDT* level in both types of animal fat are of ppb order, much lower of the permitted level by 1 ppm *DDT* in fat.

## CONCLUSIONS

Though *DDT* have been banned in Albania since more than 35 years ago, its presence proved by numerous but though sporadic attempts, is still evident in all the environmental spheres posing a threatening for the food chain and consequently to the consumers health. The samples of sheep and goat fat taken from different regions of Albania confirm the presence of *DDT* though at very low level but still clearly detected at most of the samples analyzed. The statistical data processing shows a significant difference between two groups of animals tested (sheep and goat) which relates to the specific grazing and feeding way of these two kinds of animal. This leads to *DDT* residue level much higher in sheep (mean level 71.1ng/kg) than goats (mean 12.2ng/kg). However, the *DDT* level in both types of animal fat are of ppb order, much lower of the permitted level of 1 ppm *DDT* in fat. Comparing the data between two types of animal for 5 districts monitored in Albania we draw the conclusion that the most contaminated animals are those of Dibra and Korca districts. The statistical variables for goats fat reveal that the most contaminated fat with *DDT* residue are the animals from Korca district which is the one of the most cultivated region of Albania.

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