

# Toxicological Screening of Forage Used in Cattle Feed in Order to Determine the Degree of Contamination by Pesticides

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## Abstract

Pesticides enter the body via the respiratory, cutaneous and oral. The oral route is predominant. Feeding animals with forage treated with pesticides that can be processed as a result of surface treatment during the vegetation, can be a potential cause of occurrence of severe toxicosis in animals. The purpose was to assess the quality of CFF feed type used in large and small ruminants raised on an intensive type farm, using toxicological exams in order to determine pesticide pollution degree. There were collected and examined eight samples from four different categories of feed (sunflower shrot, CF 0-2 T2-steers, corn, soybean grist). From the total organochlorine insecticides nine chemicals were selected for toxicological examination: Aldrin, Dieldrin, Endrin, Chlordane, Endosulfan. From laboratory analysis it was found that in none of the eight feed samples were detected organochlorine compounds.

**Keywords:** cattle, CFF, organochlorine, pesticides.

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## 1. Introduction

Organochlorine insecticides are the alkyl compounds, cyclic, and aromatic substituted with one or more chlorine atoms. Legally, they are currently considered to be decommissioned, but we can say that in fraudulent situations they are still being used. It has been dropped using products that contain  $\gamma$ -HCH from 01.01.2007 and from 01.01.2008 at Endosulfan.

The health risk can be both acute and chronic, POC value of the chronicity to being higher than two indicating a contaminant bioaccumulable [1].

Organochlorine pesticides accumulate selectively in the animal body, in the descending order: mesenteric fatty tissue, deposit fatty tissue, perirenal fatty tissue and muscle tissue. According to the literature, there are multiple ways of water contamination and feed with these compounds that

have cumulative properties in organs and tissues. They are persistent and have half-lives (the time taken for half of the quantity of pesticide to be degraded) ranging from months to years and in some cases decades.

Also due to their high persistence and their ability to accumulate in the environment, and later on contaminating the feed, make these insecticides to be further monitored. Although organochlorine insecticides are classified in group III toxicity, which means they have a moderate degree of toxicity [2] they are still a topical issue especially in the context of agriculture chemicalization in our century.

However, most of these insecticides have been withdrawn from use but remain as environmental contaminants as they are quite stable in soil [3]. So, *Stockholm Convention*, which is a global treaty to protect human health and the environment from persistent organic pollutants (POPs), are targeted to eliminate or heavily restrict the production and use of POP pesticides and PCBs: include Aldrin, Chlordane, DDT,

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Dieldrin, Dioxins, Furans, Hexachlorobenzene, Heptachlor, Mirex, PCB s and Toxaphene [4].

## 2. Materials and methods

The research was carried out over a period of six months, between January and June 2013, on a farm growing ruminant type of grass in southwestern Romania.

In order to determine the extent of contamination with pesticides residues in compound feed type CFF, selecting the category organochlorine insecticides, there were collected and examined eight samples from four different categories of feed:

- CF T2-0-2 steers;
- Sunflower grits;
- Soybean grits;
- Corn.

In order to evaluate the comparative level of accumulation of insecticides in feed, there were chosen in this research both combined fodders containing different plants (maize, triticale, etc.) with cumulative effects, but also soy grits, sunflower grits and corn.

As the feed is more complex and large variations in the chemical composition, the higher its possibility of chemical pollution. Also opted not to collect green fodder for control, because contact with possible pollutants is of short duration (period sitting on the ground) [5].

The determination of organochlorine insecticides was done in accordance with STAS 14181:2001 - *Animal fodder. Determination of organochlorine pesticide residues* [6]. As a work methodology it was chosen: the gas chromatographic method. Determination of organochlorine insecticides residues can not be achieved directly, but it is necessary to go through two steps:

- stage of sample preparation (isolation and purification of the extract);
- phase determination of itself (qualitative and quantitative).

Identification of the components from the sample was made according to the retention time, compared with the retention time of the compound in the yardstick.

By using an automatic integration system we performed automatic marking retention time, chromatographic peak integration, directly

displaying the concentration of pesticide from the sample after the introduction of the dilution factor. Residues were detected by the combination of their retention time with the standard and the quantity by comparing the area with the standard using the HP enhanced integrator algorithm.

MAC values POC for residues in food of plant origin are listed in order ANSVSA no. 12/286/173/1/124 of 23.01.2006 as amended and supplemented [7].

## 3. Results and discussion

Although organochlorine insecticides belong to Group III toxicity (moderately toxic), their widespread use (banned in many countries, including our country) followed by entering the ecosystem circuit can affect animal organisms and humans by default. The motivation realizing this toxicology screening of feed produced in a unit CFF type was determined by the complexity of interactions insecticide-plant insecticide-animal, insecticide-plant-animal-human. Also the possibility of residues of organochlorine insecticides in feed is justified because of the persistence and large capacity of accumulation into the environment where they can be taken over by forage, especially since some compounds such as isomers of HCH and Endosulfan's were used in our country until 2007-2008 [8]. Another reason why fodder monitoring from a toxicologically point of view is important resulted from the fact that feeding of livestock with contaminated feed, even in small doses, causes a significant accumulation of residues for 1-2 months and some of them are eliminated by milk, this being the way of detoxification for females [9]. To assess the quality of the feeding stuff type CFF toxicological exams were made in order to determine the degree of pollution with pesticides, organochlorine insecticides category were selected for our research 5 chemical compounds belonging: chlorinated dienes, indeno chlorinated and endosulfan group, as follows: Aldrin, Dieldrin, endrin, Chlordane, Endosulfan. For a better accuracy of the results it was chosen to harvest two samples from each type of feed. Sampling was done in stages, thereby in January-March 2013 and also in April-June 2013. Data processing was done differentiated so (Table 1).

**Table 1.** Determining the level of pesticides contamination in samples

No. sample	Type forage	Origin forage	Reference values (mg/Kg)	Results obtained (mg/Kg)
1.	Sunflower shrot	CFF	Aldrin and Dieldrin LOD=0.004mg/kg LOQ=0.007mg/kg Endrin LOD=0.005mg/kg LOQ=0.01mg/kg Endosulfan LOD=0.004 LOQ=0.008 Clordan LOD=0.005mg/kg LOQ=0.01mg/kg	undetectable
2.	Sunflower shrot	CFF	Aldrin and Dieldrin LOD=0.004mg/kg LOQ=0.007mg/kg Endrin LOD=0.005mg/kg LOQ=0.01mg/kg Endosulfan LOD=0.004 LOQ=0.008 Clordan LOD=0.005mg/kg LOQ=0.01mg/kg	undetectable
3.	Corn	CFF	Endrin LOD=.005mg/kg LOQ=0,01mg/kg Clordan LOD=0.005mg/kg LOQ=0,01mg/kg Endosulfan LOD=0.004 LOQ=0.008	undetectable
4.	Corn	CFF	Endrin LOD=0.005mg/kg LOQ=0.01mg/kg Clordan LOD=0,005mg/kg LOQ=0.01mg/kg Endosulfan LOD=0.004 LOQ=0.008	undetectable
5.	NC T2-0-2 steers	CFF	Clordan LOD=0.005mg/kg LOQ=0.01mg/kg	undetectable
6.	NC T2-0-2 steers	CFF	Clordan LOD=0.005mg/kg LOQ=0.01mg/kg	undetectable
7.	Soybean grist	CFF	Aldrin andDieldrin, Endrin, LOD=0.005mg/kg LOQ=0.01mg/kg Endosulfan LOD=0.004 LOQ=0.008	undetectable
8.	Soybean grist	CFFF	Aldrin and Dieldrin, Endrin, LOD=0.005mg/kg LOQ=0.01mg/kg Endosulfan LOD=0.004 LOQ=0.008	undetectable

a. samples of *corn* and *sunflower grits* were subjected to toxicological testing by the gas chromatographic method for 5 compounds: **Aldrin** (C12 H7 CL6 (hexachloro-hexahydro-endo-exo-dimetan-naphthalene), **Dieldrin** (C12 H8 CL6 O (hexachloro-epoxioctahidro -endo-exo-naphthalen-dimetan), **Endrin** (C12H8Cl6O (1,2,3,4,10,10-hexachloro-6,7-epoxy-1, 4,4 a, 5,6,7,8,8 a-octahydro-1 ,4-endo-endo-5, 8 dimethanonaphthalene), **Chlordane** (-C9 H4 CL9 (1,2,4,5,6,7,8,8, octacos-2, 3,3 a, 4,7 , 7a-hexahydro-indan methane 4.7) and **Endosulfan** (C9H6Cl6O3S (6,7,8,9,10,10-Hexachloro-1, 5,5 a, 6,9,9 a-hexahydro-6,9-methano-2,4,3 benzadioxatien 3-oxide);

b. *soy grits* was subjected to toxicological examination for: **Aldrin** (C12 H7 CL6 (hexachloro-hexahydro-endo-exo-dimetan naphthalene), **Dieldrin** (C12 H8 CL6 O (hexachloro-endo-exo-epoxioctahidro dimetan-naphthalene), **Endrin** (C12H8Cl6O (1,2,3,4,10,10-hexachloro-6,7-epoxy-1, 4,4 a, 5,6,7,8,8 a-octahydro-1,4-endo-endo-5 8 dimethanonaphthalene), **Endosulfan** (C9H6Cl6O3S (6,7,8,9,10,10-Hexachloro-1, 5,5 a, 6,9,9 a-hexahydro-6,9-methano-2,4,3-benzadioxatien 3-oxide);

c. *CF steers T2-0-2* (combined fodder) has undergone to toxicological analyzes for determining residues of **Chlordane** (C9 H4 CL9 (1,2,4,5,6,7,8,8, octacos-2, 3,3 a, 4,7,7 a-hexahydro-indan methane 4.7).

Following the investigations it was found that in all of the 8 samples collected from various forage products CFF type, the residues of organochlorine insecticides were at the detection limit, according to the reference values from STAS 14181:2001. *Animal fodder. Determination of organochlorine pesticide residues.*

Given the negative results obtained in the present study it can be appreciated that feed produced in the unit type CFF, used in cattle bred in the intensive system, are not considered a source of pollution for animal or human. However we shouldn't neglect certain aspects of waiving or limitation of the use of organochlorine insecticides recently for our country, especially considering the fact that DDT and chlordane isomers in the forms  $\alpha$  and  $\gamma$  are persistent in soil, organic pollutants that can be detected together with their metabolites in a proportion of 50% up to 15-20 years [10]. Thus we believe that the research

should be pursued with determination in parallel on multiple types and categories of feed and on milk samples and by-products with high fat, so being able to detect any source of pollution. Pesticides are a problem because they persist in the environment and, when consumed, are stored in animals' fat deposits with risk of contamination in meat and milk products of cattle.

Globally there is a constant concern for pesticide monitoring, and at the European level the specialists of the EFSA prepared synthetic studies on isomers of HCH, Endosulfan, Endrin and DDT, as undesirable substances in animal feed. Today for example, Dieldrin is therefore banned in most country of the world (it is one the list of Stockholm Convention), but due to very slow environmental degradation of the substance, Dieldrin is still a widespread of the soil contaminant in Europe and other places. Monitoring data from de Netherlands show that the courgettes grown in greenhouses from the western part of the country do not always comply with the existing of 0.02 mg/Kg. The Netherlands request an increase of MRL-value of 0.05 mg/Kg [11].

In our country pesticides are strictly monitored by the SV Norms specific in foodstuffs of animal origin and plant, domestic and import, in line with European standards in the field (LEGIS) [12]; Order no. 35, February 15, 2010 to establish the Monitoring Plan of pesticide residues in plant and vegetable feed in accordance with Regulation (EC) no. 396/2005; Common Order MAAP, MSF and MAPM no. 396/707/1944/2002 prohibiting use on the Romanian territory of plant protection products containing certain active substances, published in Official Gazette no. 829/18.11.2002 etc. [13].

## Conclusions

The toxicological screening was conducted on CFF type feeding stuffs consisting of soybean grits, sunflower grits, corn, and CF T2-0-2 steers over a period of 6 months.

To assess the degree of pollution with pesticide residues there have been selected 5 compounds belonging to organochlorine insecticides: Aldrin, Endrin, Dieldrin, Chlordane and Endosulfan. As a result of the research conducted using as toxicological analysis the gaschromatographic

method it has been established that the residues of organochlorine insecticides from the 8 samples, were all under the detection limit.

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