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Summary of Ph.D. Thesis

INCIDENCE AND HYGIENIC SIGNIFICANCES OF ORGANOCHLORINE PESTICIDES IN SOME SAMPLES OF FOOD AND ANIMAL FEED IN BISTRITA-NASAUD COUNTY

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Keywords: organochlorine pesticides (OCP), the incidence, hygienic significance, maximum level admitted (MLA), liposolubility, retention time.

Chapter 1. PESTICIDES

A modern agriculture requires pest plants and agricultural products. Between the means used more intensively include pesticides, synthetic chemicals. Number of pesticides and other plant protection products is about 670 divided into 10 categories. Classification of pesticides is done according to several criteria: their origin, action against pests, physical form of presentation, etc. chemical structure. Depending on the degree of toxicity, pesticides are grouped into: Group I - highly toxic marked with red labels, group II - highly toxic, marked with green labels, group III-moderately toxic, marked with blue labels, Group IV - low toxicity, marked with black labels. Depending on chemical structure, are classified as pesticides: organochlorine, organophosphorus, organocarbamics respectively thiocarbamate and nitrophenol. Organochlorines in group III of toxic organic substances which are structurally alkyl, cyclic, or aromatic, substituted with one or more chlorine atoms. Their widespread use, followed by entering the biogeochemical cycle of ecosystems affect humans. In terms of health implications are large because of the complexity of using pesticide-plant interactions, pesticide, animal, pesticide-plant-animal-man. Food contamination can be direct food processing plant which is consumed directly by humans after indirect result of food pollution by residual pesticides in soil, air, water, transfer pesticides to animals concerned meat, milk, eggs. Selectively accumulate chlorinated insecticides in the animal organism, the decreasing order mesenteric fat tissue, fatty tissue deposit, perirenal fatty tissue and then the muscle. The overall direction is to reduce the use of organochlorine insecticides in agriculture, because pesticides entered the body can cause nervous system disease, coronary insufficiency, atherosclerosis, cirrhosis. In humans, DDT has been reported for the first time, in 1948 the population in different areas of the U.S. Subsequently, it
was established presence as part of the fat of entire population of the world. In our country research has been conducted by Aizicovici, Unterman and Sirghie. They have shown that pollution is the same order of magnitude of pollution recorded worldwide and the need to take appropriate measures to reduce pollution.

Chapter 2. RESEARCH OBJECTIVES

Research objectives proposed in this thesis aim subscribe thesis: "The incidence and hygienic significance of OCP in some food and feed samples Bistrița-Năsăud County." They are:

OBJECTIVE 1 - Determination of organochlorine pesticide residues in some fat dairy products;
OBJECTIVE 2 - Determination of organochlorine pesticide residues in some meat products;
OBJECTIVE 3 - Determination of organochlorine pesticide residues in feed.
OBJECTIVE 4 - Optimize consumption of solvents to determine organochlorine pesticide residues in animal feed using new methods for complex sample preparation.

All samples were collected from manufacturing establishments in Bistrița-Năsăud county area.

2.1. MATERIALS USED

For all test products were made in two periods: 06-08.2009 and 01-03.2010. For cream were tested:
-8 Samples fermented cream 20% fat (two types, concerned SA1 and SB1 two samples of each type);
-8 Sweet cream 32% fat samples (two types, concerned, and SA2 SC2, two samples of each type);

For cheeses were tested:
-Cheese Dalia 12 samples (three concerned CDA, CDB and CDC two samples of each type);
-Mozzarella, 8 samples (two MA and MB concerned two samples of each type);
-cheese skin 12 samples (three concerned BA, BB and BC two samples of each type);

Of processed meat products and comercializate in the county were tested:
-8 Bacon samples (two KA and KB concerned two samples of each type);
-8 Goiter with paprika samples (two GA and GB concerned);
-8 Smoked samples (concerned two SLA and SLB, two samples of each type);
-8 Lebărvuști samples (two concerned LA and LB, two samples of each type);
-8 Sausage samples (CA and CB concerned two, two samples of each type);
-8 Summer sausage samples (SA and SB concerned two, two samples of each type);
-8 Beef tallow samples (two SVEand SVC two samples of each type).

Materials tested suspected contamination by organochlorine pesticides have been tracing feed and feed Green Table complex type used on farms TRM1 (for lactating cows) and TRM2 (for cows resting breast) samples were collected in June and July 2010 and were tested every 4 samples of each type of feed.

2.2. DETERMINATION OF PESTICIDES

Steps for determining organochlorine pesticides in food and feed were: sample preparation and determination as such.

2.2.1. Preparation of samples

For extraction of fat from cream to the cold extraction method used and the hot extraction on meats. Extraction of fat from cheese I made it with the Soxhlet apparatus. Sample preparation consisted of separate feed extract with pesticides - method of extraction with acetonitrile water mixed with liquid-liquid partition with petroleum ether.

2.2.2. Extraction and purification residues

Purification of extracts fat dairy products, meat and feed products was achieved by liquid-liquid partition method (LLE) with acetonitrile and purification on florisil ® column.
2.2.3. Pesticides determination of fat and fat-free food

It was used capillary column gas chromatography using an electron capture detector (ECD). For qualitative and quantitative determination of residues of OCP to use a gas chromatograph type Perkin Elmer Auto System XL model. When determining the OCP qualitative identification of residues in the samples investigated was based on comparing the retention times for compounds existing in the standard record retention times in samples.

2.2.3.1. Qualitative determination

To have a degree of safety as the identification, we used statistical calculation based on the distribution of "t". Were used as reference values that retention times of standard organochlorine compounds in different concentrations and compared with the values of retention times for the same compound in the samples investigated. By interpreting the value of "t" results showed the classification to "insignificant differences" (ns) for the compounds considered to be present in the sample.

2.2.3.2. Quantitative determinations

A. Dairy products, meat and beef tallow

Were injected equal volumes of sample and standard (1μl) and final volume was 10 ml resume. Thus the general formula for the determination of residues of products OCP above becomes:

\[
P_{OC} = \frac{c_{et} \times A_{pr} \times 10}{A_{et} \times m \times \frac{100}{R}}, \text{ppm}
\]

- \(A_{et}\) - component peak area for investigation, the standard;
- \(A_{pr}\) - peak area for component investigated in evidence;
- \(c\) - the component in the standard concentration, ppm The value of \(c = 25, 50, 100, 150, 200\) depending on the mode specific to each type of product analyzed.
- \(m\) - mass of the sample taken into work g fat products was taken into account fat mass;
- \(R\) - recovery rate of waste.
B. Fat free products (feed)

If fat-free products (feed) is introduced in calculating the amount of sample passing through florisil ® column purification (S), calculated according to the moisture proof and using the resulting parameters covering specific operating mode for fat-free products, concerned values F and P:

\[ S = \frac{m \times F \times P}{T \times 100}, g \]

S - amount of sample in grams passed through the column;

m-sample weight in grams;

T - total volume in milliliters, the green fodder, T = ml water sample addition ml acetonitrile added, less volume shrinkage empirical, complex feed, T = ml water added plus ml extraction mixture minus correction for volume contraction; P - measured by petroleum ether extract, in milliliters; 100 - volume of petroleum ether used to extract, in milliliters; F-volume of the filtrate measured in milliliters.

Volume contraction is considered to 5 ml samples with humidity between 80 and 95% when using 200 ml acetonitrile extraction.

CHAPTER 3. RESULTS ON ASSORTMENT

3.1. OBJECTIVE 1

Concentration on organochlorine pesticide residues found in:

• Cream:
  - Isomers \( \alpha, \beta, \gamma \) HCH, DDT (alone or isomers), chlordane and HCB residue concentrations are lower than the value of AML;
  - Endrin concentration calculated for 62.5% of AML samples was equal, and the remaining samples of the same order of magnitude to MRL.

• Cheese:
  - Isomers \( \alpha, \beta, \gamma \) HCH, Heptachlor and HCB gives values of organochlorine pesticide residues below the MRL;
  - Endrin, presents concentration values comparable to the value of AML, but differences between varieties of cheese tested:
- cheese - concentration to 20% of MLA samples was equal;
- Mozzarella - concentration ranged between 5% and 5.75% of MLA;
- cheese skin - concentration for 16.66% of MLA samples had value;

If endosulfan, all samples have values close to the maximum allowed, but different cheese samples tested, as follows:

- Cheese: for 16.67% of the samples, the concentration was 45.1% in MLA, for 66.67% of the sample concentration was 64.3% in MLA and 16.67% the concentration was 99.9 % of MLA;
- Mozzarella cheese: for 25% of the sample value is equal to MLA;
- Cheese bellows: for 50% of MLA samples equal value.

For residues of 4,4 'DDT isomer alone or with 4,4' DDE situation is different between the cheeses tested in the same type, the types:

- Cheese: calculated concentrations ranged between 22.23% and 55.28% of MLA;
- Mozzarella cheese: the concentrations ranged between 4.51% and 45.45% of MLA;
- Cheese skin: the range BC (06.-08.2009), found evidence that the concentration calculated is equal to MLA in the second period value was calculated to be 67.78% of MLA.

- Butter:
  - Isomers (α, β, γ) HCH gives values of organochlorine pesticide residues below the MRL;
  - HCB organochlorine compounds present in oil samples tested of 50% with values in MLA, from 06.-08.2009, the evidence in the second period at 100% concentration values between 56% and 40.4% of MLA.

3.2. OBJECTIVE 2

Concentration on organochlorine pesticide residues in:

- meat:
  group-HCH pesticides (isomers α, β, γ) residue concentrations are much lower than in AML samples with bacon and paprika crop;
- The beef tallow αHCH isomer appears in 75% of samples taken in the work, and γHCH βHCH occur in 50% of the samples investigated;
- the sausage, liver sausage, summer sausage HCH isomers range from (0.0010 to 0.0014 ppm).
- HCB, Aldrin and Dieldrin concentrations are insignificant compared to MLA samples of bacon, crop with paprika, bacon, and beef tallow;
   Endrin is found in meats such:
- in 50% of samples tested bacon concentration value was equal to MLA;
- the crop with paprika endrin in 50% of the samples shows the concentration equal to the MLA and the remaining 50% of the samples have concentrations of the same order of magnitude with MLA, the endrin concentration Beef tallow was also calculated with MLA. The presence of endosulfan in samples of bacon and sweet paprika crop samples is highlighted in Figure 1 and 2:

![Concentrations of Endosulfan in samples of kaizer](image)

Figure 1 - Comparison of calculated endosulfan concentrations in samples of bacon
Figure 2 - Comparison of calculated endosulfan concentrations in the samples of Goiter with paprika. Legend: MLA - the maximum allowed, P1 - from 06.-08-2009 P2 - from 01.-03.2010.

- in smoked bacon and beef tallow endosulfan concentrations have values equal to MLA
- the sausage, liver sausage and summer sausage endosulfan concentrations presented values ranging from (0.0004 to 0.0007 ppm).
- DDT alone or with its isomers are found in samples of bacon, jowl with paprika, smoked bacon, beef fat, sausage, liver sausage and summer sausage concentration values lower than MLA.
- Heptachlor, alone or with its isomer, is found in all samples with elevated concentrations of bacon in MLA;
- 50% of the crop with paprika samples have concentrations representing 56% of MLA, and the remaining samples have concentration values representing 62.75% of MLA;
- The sausage, liver sausage and summer sausage concentrations ranged from (0.0001 to 0.0002 ppm).
- Chlordane is found in one sample of bacon in a concentration close to MLA;
- The smoked bacon, sausage, sweet paprika goiter, liver sausage, beef tallow and summer sausage Chlordane concentrations were lower than the MLA.
3.3. OBJECTIVE 3

In respect of the MLA in plant products is observed differences between green products and cereals, in that in both cases allowed the grain boundaries are higher. These are $\alpha$ HCH (0.02 ppm-feed complex) and the sum chlordane isomers. TRM type compound containing both types of crops that have been nominated as separate. The MLA for the compound was considered a range (0.01 to 0.02 ppm Chlordane) between the maximum allowed value allowed for grain and green plants, because the analyzed samples containing mixtures of alfalfa, grain, corn, triticale in different proportions.

Green feed positively identified organochlorine pesticides in green forage samples have values of residues in MLA, the differences being of the order of magnitude.

This is due to the short period of "life" of green mass and also the short period of contact with the ground and through which water may become contaminated.

3.3.1. Complex animal feed (TRM1 and TRM2)

Values for HCH isomers are comparable to European ones. For endosulfan, the calculated values are the same size as those in Germany. For endrin calculated values are comparable with the minimum values found in feed in European monitoring programs, except Denmark who reported higher values. For DDT, calculated values are comparable with those in Germany and Estonia. In compound complex compounds found almost all veterinary watch. The difference in composition leads to differences of contamination, and this difference is the type and quantity of grain used. Differences between the degree of contamination presented by the two types of feed compounds reflects on Lindane and endrin, as exemplified in Figures 3 and 4.
3.4. OBJECTIVE 4

Regarding types of OCP identified in the complex feed samples investigated shows that the working version differences reduced to 1:2 normal mode are small, in both types of feed being found almost the same compounds, eluted in the same way. The reduced version 1:5 exchange differences are visible to the normal version in the sense that no. eluted compounds is greatly diminished. Compounds
are no longer found: Heptachlor (isomers), Aldrin, Chlordane, Dieldrin and DDT (isomers). In the reduced version endosulfan concentration values are between 70% and 90% of the normal version.

Method reduced the ratio of 1:2 leads to the same results as in 1:1 mode for both types of feed tested complex.

Chapter 4. CONCLUSIONS

On the types and incidence of organochlorine pesticides that are found in all samples investigated: DDT alone or sum of isomers, isomers (α, β, γ) HCH, HCB, endosulfan and endrin. Pesticides that occur sporadically: Heptachlor, Aldrin, Dieldrin and Chlordane. Heptachlor is absent in cream (Gr = 20% and Gr = 32%) and feed. Aldrin and Dieldrin does not appear in the dairy and feed preparations. Chlordane was not found in cheese samples and feed samples investigated.

These data could be explained as follows:
Recent-enough period to have been in use legally in the EU and therefore in our country and γHCH endosulfan;
γHCH-isomer, lindane as single or as a component of the combined type of fungicides was allowed to use in the EU by 2002, and in our country to our accession to the European body in 2007;
Long-period with widespread use of organochlorine compounds technical HCH and DDT isomers;
Life-long period of pesticide that isomers of HCH, Endrin, Dieldrin and especially since some use in some parts of the world (e.g. DDT in Asia).

Regarding the evaluation of organochlorine pesticide residues concentration value:
-no evidence showed that the calculated values exceed the maximum allowed;
-pesticidal compounds which have equal values with MLA: endrin, endosulfan and DDT. These values may be due to different fat content (20% and 32% cream) which lead to different reservoirs for lipid-soluble compounds with strong
character, endrin, or use raw material from other species (sheep) because the amount of DDT s equal to MLA occurred in cheese skin.

Strong liposolubility character of DDT isomers resulted in registration of residue levels, compared with the MLA group, higher than for cream. This may have several explanations: -fat content is much higher than in cream; -DDT alone or sum of isomers is a pesticide that was used on a massive scale and despite the fact that no use is found in all environmental factors and food so because high bioaccumulation capacity. If the presence of endosulfan might be due to short of his removal from service, from 01.01.2008, it being found in the products investigated, especially in the first period 06-08.2009. The degree of contamination with OCP testing is influenced by the variety of meat dishes, such samples smoked had the lowest degree of contamination, beef tallow and the highest degree of contamination.

The fact that the complex feed, regardless of type, are found all compounds veterinary track could mean that one of the components is contaminated. It could be grain, because:

Green feed-meal showed a low contamination;
-acquisition and accumulation of OCP is louder than the green bean plant;
-containing compound different types of grains (corn, triticale) so the effect can be cumulative;

the use feed imported from parts of the world where permitted OCP use today.

The presence of pesticides in animal feed preparations proves the idea that these compounds accumulate in the food chain due to milk.

**Chapter 5. RECOMMENDATIONS**

- Determinations in parallel on components of the feed and the feed end product complex to detect the true source of contamination;
- OCP-tracking in the feed, milk and preparations;
- analysis of preparations especially with higher fat content;
- increase the number of samples in national programs.
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