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THE DETERMINATION OF THE CONTAMINATION LEVEL BY ORGANOCHLORINE PESTICIDES OF SOME COMPLEX FODDER

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Abstract

The present paper presents the results of the qualitative and quantitative determination of organochlorine pesticides under sanitary veterinary surveillance in complex fodder. The purified extract was analyzed by gas chromatography. The results confirmed the presence of the majority of the compounds surveilled. For one of them, that is γHCH the concentration of the residues exceeded the MRL value.

Keywords: complex fodder, organochlorine pesticide contamination

INTRODUCTION

The main pathway for the contamination of animal food by organochlorine pesticides (OCP in the paper), is the ingestion of the contaminated food and/or water by the animals. (Gill and al., 2001, Singh and al, 1997). So the most accurate determination of those contaminants in fodder is very important in the purpose of monitoring a great source of OCP income having impact on human health (Kannan and al, 1992).

Speaking about, complex fodders has multiple contamination sources, depending on their components and their origin. So the cereals can proceed from globe zones where the legislation for pesticides is different compared to the European one. The ground and water quality from the cultivation areas have repercussions upon the contamination of fodders components, especially pollution is shown to be present all over and the incriminated substances are found in areas where they are not in use. (Wang et al, 2007).

While in EU the use of OCP for crops protection or silo treatments has been banned for more than 20 years, regarding the great majority of the compounds, in Asia, South America or South Africa some of them are still in use especially for the malaria control. Due to their distinct persistence in the environment maintained by the still use in other areas, the EFSA[1] specialists are drawing up and coordinating monitoring programs about the presence of OCPs in fodders (EFSA a, b, 2005, a, b, 2006).

[1] European Food Safety Authority

MATERIALS AND METHODS

Materials

For the study that makes the object of the present paper, three series of complex fodder samples was tested. The fodders, made of unique fodder mixes (TMR) come from a cow farm in the area of the Sântandrei village, near the city of Oradea. The samples were collected between 2008 and 2010 and were coded TRM1 (1), (2) and (3).

TMR1 are combined fodders for high productivity lactating cows – that is between 25-40 l/day. They consist of a mixture of several components: silo alfalfa, brewery mash, grounded corn, grounded triticale, PMV premixed (protein-vitamin-mineral concentrate and soy grist), calcium carbonate, sodium chloride

Methods

The preparation of the samples for the chromatographic analysis

The OCP are extracted from the matrix by an extraction mixture of acetonitril/water (65:35 V/V), followed by liquid/liquid partition with light petroleum. The extract is purified on an activated Florisil column. The OCP are eluted with mix of ethylic ether/light petroleum in variable proportions. (SR EN 12393/2/2004). The preliminary determination consisting of a humidity check was performed in the Research Laboratory for Food at the Environmental Protection Faculty from Oradea. The extraction and the determination of the contaminants were performed on fresh products in the following 48 hours after the collection, at the Cluj Napoca Institute of Public Health. All the reagents used were Merck-type with chromatographic grade and there were no additional purifications performed, except for witness samples of the reagents on each work phase. The final stage both at the extraction and the purification steps was the evaporation of the solvent under reduced pressure using a spinning evaporator.

The determinations of the residues of organochlorine pesticides in the studies products

We have used the method most recommended by specialized literature (Tadeo, Ed., 2008, Hura, 2006), as set forth by current legislation for the calculation of OCP residues in vegetal products. It is the gas chromatography (SR EN 12393-3/2004).

We have used a GC 2010 Shimadzu gas chromatograph with a capillary column type RTX -CL- pesticides 30 m length an 0,25 mm diameter, detector with electrons capture (ECD), nuclid ^{63}Ni – 370 MBq (10mCu) and an auto sampler injection system with 6+2 spaces for vials, type AOC-210

Qualitative and quantitative calculations

For the qualitative and quantitative calculation of the contaminants possibly present in the tested products, we used a standard produced by the RESTEK company No 32292, Lot nr A021837, type "Organochlorine pesticide Mix AB ≠2" having a concentration of 200 ppb. Notice the fact that the standard contains, with one exception (hexaclorbenzen) all the organochlorine compounds under sanitary-veterinary surveillance in vegetal and animal foods. The standard was used at 50 ppb dilution.

The purified extract, retaken in petroleum ether was subject to the chromatography under the same conditions as the standard test, as well as the blanks-test of the used reagents, according to the separation/purification method.

RESULTS AND DISCUSSIONS

The results are written in table 1 for the qualitative determination, separated on the three elutions performed. The compounds identified positively are marked with red.

The results of the quantum determinations are presented in table 2 for the three series of samples tested. The concentration written in the table represents the amount of the values from the three elutions applied, where it was necessary. The expression of the concentration as isomer amount for α and β HCH, endosulfan and DDT complies with the European norms (EFSA a, b 2005 and EFSA a, b, 2006) and the national ones, respectively Order 12/2006, 118/2007, 160/2007 referring to the contamination of fodders with OCPs (Legis).

Analysing the data from table 1 one can observe that in the three series of samples of tested complex fodders all the types of OPC compound surveilled from the sanitary-veterinary perspective and existing in the standard appear alone or as isomer sum. The differences refer to the isomers which are quantitavely dosed together: so in series (2) towards series (1), the β endosulfan, γ chlordan isomers and Dieldrin isomers are missing; in series (3) towards series (1), Dieldrin is missing and towards series (2) β endosulfan appears extra. In all series of samples the great majority of the compounds are found in eluent A: 93,8% in series (1), 92,3%, series (2) and 92,8%, series (3);

The values of MRL for the OCP residues in vegetal foods are currently written in the Orders of the President of ANSV-SA[2] mentioned above. These acts transpose the community legislation in this field without mentioning separately the category "Fodders". However, within the

[2] Sanitary veterinary and Food safety National Authority

Table 1

Qualitative calculation of organochlorine pesticides residues, TMR1 complex fodder

A	B	C	D – TRM1(1)			D-TRM1(2)			D-TRM1(3)		
			E_A	E_B	E_C	E_A	E_B	E_C	E_A	E_B	E_C
1	α HCH	7.194	7.181			7.179			7.186		
2	γ HCH	8.426	8.418			8.411	8.422		8.409		
3	β HCH	8.886	8.873		8.845			8.864	8.881		8.871
4	δ HCH	9.552									
5	Heptaclor	10.372	10.375			10.366			10.362		
6	Aldrin	11.657	11.656			11.662			11.667		
7	Heptaclor epoxid	14.652	14.64			14.640			16.644		
8	γ Chlordan	15.274	15.261	15.289						15.282	
9	α Chlordan	15.951	15.942			15.941			15.956		
10	4,4' DDE	16.518	16.538			16.525			16.530		
11	α endosulfan	16.753	16.748		16.766	16.745		16.811	16.755		16.761
12	Dieldrin	17.766	17.763								
13	Endrin	18.878	18.866	18.867		18.856	18.877		18.868	18.861	18.882
14	4,4' DDD	19.853	19.848			19.831			20.066		
15	β Endosulfan	20.076		20.068						20.081	
16	4,4' DDT	21.305	21.286			21.282			21.313		
17	Endrin aldehida	22.385									
18	Metoxiclor	24.346									
19	Sulfat endosulfan	24.693	24.816			24.801			24.744	24.811	
20	Endrin cetona	26.030									

Legend A –Elution order; B –Organochlorine compound from the sample; C –Retention time in the standard; D – TRM1(1), TRM1(2), TRM1(3)–retention time in complex fodder sample series 1, 2,3 ; E_A, E_B, E_C – Elution solvents used: A, B, C

Table 2

Quantitative calculation of the OCP residues, in complex fodder TRM

(*) Nr.	Organochlorine compound found in the sample	CMA MRL ppm	Concentration in the sample, ppm		
			TRM 1 (1)	TRM 1 (2)	TRM 1 (3)
1 3	HCH (Sum of isomers α and β)	0.01 -0.02	0,0049+ 0,0018= 0,0067	0.0017+ 0.0005= 0.0022	0.0008+ 0.0011= 00019
2	γ HCH	0.01	0,0107	0.0131	0.00188
57	Heptaclor (Sum of heptachlor and heptachlor-epoxide expressed in heptachlor)	0.01	0,0009+ 0,0009= 0,0018	0.0006+ 0,0005= 0,0011	0.0012+ 0,0009= 0,0021
6	Aldrine alone or combinated, expressed as dieldrin	0.01 -0.02	0,0012+ 0,0011= 0,0023	0,0021	0.0024
89	Chlordane (sum of cis and trans isomers and oxichlordane expressed in chlordan)	0.01 -0.02	0,0055+ 0,0007= 0,0062	0,0021	0,0021+ 0,0011= 0,0033
11 15 19	Endosulfan (Sum of α and β and endosulfan sulfat expressed in endosulfan)	0.05	0,0101+ 0,0014 0,0020= 0,0135	0,0092+ 0,0023= 0,0115	0,0101+ 0,0023 0,0039= 0,0163
13	Endrin	0.01	0,0055	0,0013	0,0043
10 14 16	Sum of DDT, DDE and DDT isomers expressed in DDT	0.05	0,0007+ 0,0008 0,0055= 0,0070	0,0006+ 0,0031 0,0091= 0,0128	0,0012+ 0,0011 0,0015= 0,0061

Legend: (*) – it refers to the elution order of the compounds in the samples;
MRL – MAXIMUM RESIDUE LEVEL

scientific opinions issued by the EFSA upon request of the European Commission, regarding the presence of the organochlorinated pesticides in fodders, the following problems have been identified (EFSA 2005 a, b, 2006, a, b):

- The complex fodders are made of a relatively big number of ingredients of which some are processed. It is not obvious, hence, which MRL value is applicable to which fodder compound and a lot of calculations are involved as well as uncertainties and "unknowns" (factors related to the processing);
- The pesticide related legislation does not yet cover the marine products which are regularly used in the animal fodders (indirect application);
- The pesticide related legislation does not yet cover the typical products for the animal fodder (which are not used in human foods) such as: green fodder, gross fodder, fish oil, fish meal.

However, considering the type of samples taken into consideration, the present paper considered the maximum allowed values for the OCP contaminants in vegetal foods like the ones found in the tested fodders which are written in table 2, column MRL. Since there are differences

between the green products and cereals, meaning that in three cases the allowed limits in cereals are higher (the α and β HCH, aldrin + dieldrin and chlordan isomer amounts) for these compounds value ranges were written.

By analyzing the concentration values found, the following situations appear:

In all the series of tested samples, the concentration of the γHCH compound is higher than the MRL values. This pesticide has been used until 2007 under the name of Lindan, as well as in mixture with other substances active as insect fungicide. Thus, its presence in cereals in higher concentration than the other HCH isomers is plausible and probable all the more that cereals are kept in silos for further use. A monitoring study performed between 1998 and 2000 in Denmark regarding HCH isomers (EFSA a, 2005) mentions the fact that the highest values were registered in the additives for the cattle and swine (female) meal and the fact that in some cases αHCH was predominant, means that there were treatments performed upon some components of the fodder with technical HCH, not with γHCH (Lindan). The source of contamination was declared unknown.

Endosulfan (isomer sum) and DDT (isomer amount) have values within the same range order as the MRL value. Endosulfan is the last organochlorinated forbidden product starting from January 2008, so its presence in the environment factors is high even if it tends to bio accumulate less than other organochlorinated products. The values of the concentrations calculated for DDT can be put on behalf of its persistence in the environment, next to the massive use in the past.

The sources can also be incidental as happened in 2002 in Germany. Some fodder samples tested (‘Eco” flour) proved a high DDT contamination, between 11 – 180ppb, values which were put on behalf of the storehouse where the fodders were stored and which had been previously used for pesticides (EFSA, a, 2006).

The rest of the compounds qualitatively identified, respectively isomer amount (α and β) HCH, Heptaclor (isomer sum), Aldrin, Chlordan (isomer sum) and Endrin has values lower than the MRL value, between 10^{-1} and 10^{-2} towards it. It is about pesticides which have not been used in a long while, some of them even 20 years, but which were used to a lower extent than γHCH or the DDT isomers DDT. Referring to Endrin, in what regards plats, it can be found rather in the oleaginous ones that in cereals because it is liposoluble.

CONCLUSIONS

The fact that all the sanitary-veterinary surveilled compounds can be practically found in complex fodder seem to indicate the fact that one of the

components is impurified. Most probably, it is about the cereals, based on the following considerations:

- The take-over and accumulation of the organochlorinated is more pregnant in the beans than in the green part of the plants;
- Complex fodders contain different types of cereals (corn, triticale) so the effect can be cumulative;
- The possibility of using in the complex fodders some imported components, from world areas where the organochlorinated pesticide contamination is still high. Thus, a study performed entirely in the Shaanxi farming land from China, showed in cereals the incidence of 53,3 % for ∑HCH and 5% for ∑DDT (Bai et al., 2006).

The brewery mash from fodders is also a potential source of pesticides through hop plant. The fact that the legislation admits MRL level of the OCP higher in the case of dry hop that other plants, indicates the susceptibility to contamination of this vegetal product according to the provisions of Order 12/2006 (Legis).

Hence, it is confirmed the supposition that the fodders are a major source of contamination for animal origin food such as milk and dairy. The aspect was verified through the testing of food products coming from the same geographical are in which the same OCP compounds were found, already reported. (Chiş a and b 2008). The same problem was reported by researchers in other countries like Jordan (Salem et al. 2009) or India (Nag and Raikwar, 2008 and 2010).

In what regards the calculated concentration for OCP residues, the distinct situations observed show the fact that even if some products have not been used in a long while (DDT, Aldrin, Endrin, α and β HCH), they can be found in non-fat agricultural products. So their wide persistence in the environment prevails upon the liposoluble feature.

In the limit of the fact that in the studies performed at European level by the EFSA specialists the types of fodders tested are not entirely mentioned, the comparison with the concentration values found in the tested samples, leads to the observation that the values found for the HCH, Endosulfan, Endrin isomers and for DDT (isomer amount) are comparable as range order with the European ones, registered after the year 2000 (EFSA a, b, 2005, a, b 2006).

The testing of other types of complex fodders used in the cattle meals can bring extra data to this matter, which is our goal for a future paper.

REFERENCES

1. Bai, Y., L. Zhou, J. Li, 2006, Organochlorine pesticide (HCH and DDT) residues in dietary products from Shaanxi province, People's Republic of China, *Bull. Environ. Contam. Toxicol., 76, 422-46*

2. Chiş Adriana and Cristina Horga, a, 2008, Determinations regarding the organochlorine pesticides contamination in milk from Bihor county, Studia Universitatis "Vasile Goldis", Seria Stiintele Vieţii, 18/2008, 297-305
3. Chiş Adriana, C Horga, V. Bara, 2008, Calculations regarding the organochlorine pesticides contamination in some sour-cream types from the Bihor county, Journal of USAMV-CN,65/2008 (2), 216-222
4. EFSA, a, 2005, Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Gamma – HCH and other hexachlorocyclohexanes as undesirable substances in animal feed, *The EFSA Journal, 250, 1-39*
5. EFSA, a, 2006, Opinion of the Scientfic Panel on Contaminants in the Food Chain on a request from the Commission related to Endosulfan as undesirable substances in animal feed, *The EFSA Journal, 234, 1-31*
6. EFSA, b, 2005, Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Endrin as undesirable substances in animal feed, 2005, *The EFSA Journal, 286,1-31*
7. EFSA, b, 2006, Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to DDT as undesirable substances in animal feed, *The EFSA Journal, 433, 1-69*
8. Gill, JPS, RS Aulakh, JPS Mundi and JK Sharma, 2001, Pesticides residues in animal feed: a potential source of public health hazard. P*aper in Post Conference proceeding of International Conference on Pesticides, Environment, Food Security from 19-23, November 2001 pp. 160-163.*
9. Hura Carmen, 2006, Laboratory guide – Analysis methods for food products, Editura Cermi, Iaşi
10. Kannan, K, Tanabe, S, Ramesh, A, Subramanian, A and Tatsukawa, R, 1992, Persistent Organochlorine residues in foodstuffs from India and their implication on human dietary exposure. *J. Agric. Food Chem., 40: 518-524.*
11. Legis – program informatic referitor la legislatia din Romania
12. Nag SK and MK Raikwar, 2008, Organochlorine pesticides in bovine milk, Bull Environ Contam Toxicol, 2008 Jan;80(1):5-9
13. Nag SK and MK Raikwar, 2010, Persistent organochlorine pesticides in animal feed, Environ Monit Assess, 5 May, . [Epub ahead of print]
14. Salem NM, R Ahmad, H Estaitieh, 2009, Organochlorine pesticide residues in dairy products in Jordan, Chemosphere. 2009 Oct;77(5):673-8
15. Singh R, B Kumari, VK Madan, R Kumar and TS Kathpal, 1997, Monitoring of HCH residues in animal feed. *Indian J. Anim. Sci.,* 676: 250-52.
16. SR EN 12393 -2, 3, 2003, Produse alimentare negrase. Metode multireziduu pentru derminarea gaz-cromatografică a reziduurilor de pesticide
17. Tadeo, J. L., (ed.), 2008, Analysis of Pesticides in Food and Environmental Samples, *CRC Press, Taylor & Frances Group Boca Raton , London, New York*
18. Wang G, X.-P., T.-D. Yao, Z-Y. Cong, X.-L. Yan, S.-C. Kang, Y. Zhang, 2007, Distribution of persistent Organic Pollutants in Soil and Grasses Around Mt. Qomolangma, China, *Arch. Environ. Contam. Toxicol., 52(2), 153-162*