Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 21, issue 1, 2011, pp. 87-93

THE PURPOSE OF THE MULTIPLE ELUTIONS FOR THE DETERMINATION OF ORGANOCHLORINE PESTICIDES IN COMPLEX FODDER

Adriana CHIŞ[1]*, Cornelia PURCĂREA[2] Cristina HORGA[3]
[1] University of Oradea, Faculty of Environment Protection, Oradea, Romania
[2] Institute for Public Health Cluj-Napoca, Cluj-Napoca, Romania

ABSTRACT. This paper presents the results of the determination (qualitative and quantitative) of organochlorine pesticides in complex fodder for nursing cows by gas chromatography. The applied method requires the use of three subsequent elutions with 200 ml ethylic ether/light petroleum each, in variable proportions. The paper analyses the appearance of the compounds in relation with the used eluents in order to identify if this massive consumption of solvent is really necessary. The practical determinations proved that the use of one particular eluent is enough for a specific level of certainty.

Keywords: fodder, organochlorine pesticides, variable elution

INTRODUCTION

The determination of the contamination degree of the fodders with organochlorinated pesticides is extremely important for the counterbalance of the contamination of the animal foods. In case of complex fodders in whose composition there are various materials, the potential contamination sources are multiple. In the European Union, the use of this type of pesticide was gradually decreased up to the total interdiction of the last product admitted – Endosulfane, starting with 2008. The residues are found at smaller and smaller concentration levels but it continues to be supervised at European level for reasons of food safety. Therefore, they must be detected (EFSA, a, b, 2005, EFSA, a, b, 2006, EU 2006). These studies are conducted even in neighboring countries of Romania which are not subject to the UE norms, such as Serbia. (Škrbič and Predojevič, 2008). Due to liposolubility and high persistence in the environment they are currently found both in environment factors and in food. Moreover, there are areas on the Globe, such as Asia and South America, where they still use certain organochlorinated pesticides, this is why in this area there were many studies regarding the impact of the pesticide contamination of fodder on the humans' health state (Gill et al., 2001, Singh et al., 1997 Kanaan et al., 1992). The effect can spread on other areas, as well, such as Romania, for various reasons. First of all, this way the pollution of the environment factors is maintained, a phenomenon which does not have boundaries (Wang et al., 2007). Moreover, there is the possibility that the complex fodders become impurified through a component coming from such an area, such as cereals where the presence of OCP was highlighted (Bai et al., 2006). Since we know that the decrease of the concentration makes the analytic determination more difficult, we chose the application of a multiple-elution method, which has high chances to highlight all the compounds present, even at ppb concentrations (SR EN 12393-2/2004). However, at the same time, we wished to highlight the use of the three elutions practices according to the purpose followed.

MATERIALS AND METHODS

For the study that makes the object of the present paper, three series of complex fodder samples have been subject to testing. The fodder, made of unique fodder mixes (TMR) come from a cow factory in the area of the Sânandrei village, nearby the city of Oradea. The samples 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, beer marc, grounded corn, grounded triticale, PMV premixed (protein-vitamin-mineral concentrate and soy grist), calcium carbonate, sodium chloride. The samples were taken in plastic water-tight containers. The recipients used for the determination of OCP were rinsed with petroleum ether; after cleaning, they were dried in the drying oven and kept with the lid on in order to prevent contamination. The preparation for the analysis consisted of the determination of their humidity because the OCP extraction technique from non-fat vegetal products depends on the water content of the samples (SR EN 12393-2/2004). The method used was the reference method, that is the drying in thermo-adjustable oven at 105 °C, for 4 hours (Bradley, 2003); the determination was performed immediately after the collection, in the same day, in the Food Toxicology Laboratory of the Faculty of Environment Protection from Oradea. The technique used is the solvent extraction, followed by the liquid/liquid partition – LLE (Schenck and Hobbs, 2004). The extract is purified on column with activated Florisil ®. The organohalogenated pesticides are eluted with ethylic ether/ light petroleum in variable proportions. The extraction and the determination of

***Correspondence:** Chiş A. M., University Of Oradea, Department Of Food Control, Environment Protection Faculty, No. 26 Gen, Magheru Bd., Oradea, 410048, Tel +40-744-696943, E-Mail : andichis @yahoo.com*
Article Received: November 2010; Published: February 2011

the contaminants were performed on fresh products in the following 48 hours after the collection, at the Institute of Public Health from Cluj Napoca. All the reagents used were Merck-type with chromatographic purity and there were no additional purifications performed, except for witness samples of the reagents on each work phase.

The first phase undertaken is the extraction of the toxic with an extraction mixture of acetonitryl and water (65:35 V/V), because the humidity was under 75%) followed by light-petroleum ether extraction. There follows the purification of Florisil column with three subsequent elutions of 200 ml mixture ethylic ether / light petroleum for each, in the following V/V proportions: 6/94 (eluent A), 15:85 (eluent B) and 50:50 (eluent C). Further on this paper, letters A, B and C refers at the specific eluent above nominated. We have used the method most recommended by specializated literature (Tadeo, Ed., 2008), as set forth by current legislation for the calculation of pesticide residues in vegetal origin 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 autosampler injection system with 6+2 spaces for vials, type AOC-210. Chromatography conditions :

Injection temperature (splitting)	250°C
Splitting temperature	163,5 kPa
Splitting gas	He with a 124 ml/min flow at scavenging 30 ml/min
Carrying gas	N_2 ultrapure 99,99%
Detector	320^0C, detector current of 2 nA, make-up flow = 30 ml/min

The device was connected to a computer and used a specific program for the interpretation of the results. Chromatograms are displayed on a singular monitor for the tested sample or together with the chromatogram of the used sample. The program supplies the retention times, the height of the picks and their surface, through automatic integration. 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" containing 20 POC at the concentration of 200 ppb. The standard was used at the 50 ppb dilution. Complex fodder tests submitted to the verification have undergone the procedures explained in "Test preparation for chromatography" part of the paper. The extract purified, was concentrated in a spinning evaporator, retaken in petroleum ether and submitted 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. For the qualitative determination, we compared the retention times for the significant picks that appear on the chromatogram of the unknown tests with the ones of the compounds from the standard sample. This way, we can determine which of the compounds that are present in the standard can also be found in the tested sample (Gocan, 1998).For the quantitative determinations, we have used the value of the surface of the picks (compounds that have been previously identified as being present in the tested sample). In order to prevent possible calculation errors, we introduced the data we obtained through the automatic integration of the unknown tests and the used standard in EXCELL calculation sheets, selecting only the surface of the picks we need, that is the compounds that were found from the qualitative point of view. In the case of the non-fat products (fodders) the calculation of the organochlorinated pesticide residues concentration refers to the amount of sample that passes through the Florisil purification column (S). S value is calculated according to the moisture of the products and using parameters that result from the development of all the work procedure for non-fat products as it can be seen in (1) formula (SR EN 12393-2/2004).

In the case of non-fat products, the values obtained are not corrected with the recovery degree (SR EN 12393,3-2004).

$$S = (m \times F \times P)/ T \times 100, g, \quad (1)$$

S	the amount of sample, g, passed through the purification column
M	the mass of the sample, g
F	the measured volume of the filtrate after the acetonitryl extraction, ml
T	total volume, in ml (ml of water in the sample plus ml of acetonytril added, minus the empyric contraction volume;
P	the measured extract of light petroleum, ml
100	the volume of the light petroleum used in the extraction, ml

RESULTS AND DISCUSSIONS

The results are written in table 1 for the qualitative determination. The compounds identified positively were marked with red.

The results of the quantitative determinations are present in concise form in table 3 for the three series of tested samples. The concentration inscribed in the table represent the sum of the values of the three applied

elutions, where necessary. The expression of the concentration as isomers for α and β HCH, endosulfan and DDT complies with the European (EFSA a,b 2005 and EFSA a,b, 2006) and national norms (Legis: Ordin 12/2006,118/2007, 160/2007) referring to the contamination of the fodders with organochlorinated pesticide.

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	Endosulfan sulfan	24.693	24.816			24.801			24.744	24.811	
20	Endrin cetona	26.030									

Legend:

A	*Elution order*
B	*Organochlorine compounds;*
C	*Retention time in the standard;*
D	*(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

The calculation of the amount of sample, complex fodder, TRM 1 type

Nr. sample	U, %	m,g	F, ml	P, ml	water,	T, ml	S, g
1-series 1	64.3	25.34	330	87	16.29	361.29	20.14
2- series 1	64.3	24.73	323	84	15.90	360.90	18.59
1-series 2	63.3	29.7	328	86	18.80	363.80	23.03
2- series 2	62.5	26.12	325	84	16.33	361.33	19.74
1-series 3	65.3	27.33	331	86	17.85	362.85	21.44
2- series 3	65.1	25.61	330	85.5	16.67	361.67	19.98

Legend : The significance of U, m, F, P, water T, S is the same as in (1) formula

In order to analyse the role and the contribution of the three elutions, in tables 4, 5 and 6 are presented the intake of residues divided on the three employed eluents in each series of the tested samples. In the calculation we took into account the values of the surfaces of the significant peaks appeared in the witness samples, separated in the three elutions practiced.

The analysis of the results obtained leads to two discussion pathways: Regarding the types of organochlorinated pesticides identified (table 1): Towards the 20 compounds identifiable according to the standard put at disposal, 16 compounds were identified in series (1), 13 compounds in series (2) and 15 compounds at series (3); In all the series of samples, the great majority of compounds are found in A: 93,8% in series (1) 92,3% series (2) and 86,6% series (3); One can observe that some of the compounds are to be found in more eluents, not necessarily identical, but very similarly, in all the series of samples; between 13% and 16% of the compounds identified in eluent A are to be found in eluents B and C; The compounds which are repeatedly eluted in the B eluent are γ Chlordan and Endrin and in the C eluent there is β HCH and α endosulfan.One compound, that is the β endosulfan isomer was found only in the B eluent in

series (1) and (3). Regarding the concentration of the identified organochlorinates pesticide residues, as well as their repartition in the used eluents, we can make the following considerations (tables 3, 4 and 5).

Table 3

Quantitative calculation of the OCP residues, in complex fodder TRM 1

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

Legend: (*) – it refers to the elution order of the organochlorinated compounds in the samples

Table 4

Quantitative calculation of the OCP residues, in complex fodder, TRM (1) series

Nr.	Organochlorine compounds	Concentration of OCP in the sample						
		eluent A		eluent B		eluent C		Total
		ppm	%	ppm	%	ppm	%	ppm
1	α HCH	0.0031	100					0.0031
2	γ HCH	0.0108	99	- 0.0001	- 1			0.0107
3	β HCH	0.0012	66	- 0.0001	- 6	0.0007	40	0.0018
4	δ HCH							
5	Heptachlor	0.0009	100					0.0009
6	Aldrin	0.0012	100					0.0012
7	Heptachlor epoxide	0.0009	100					0.0009
8	γ Chlordan	0.0010	18	0.0009	16	0.0036	66	0.0055
9	α Chlordan	0.0008	100					0.0007
10	4,4' DDE	0.0007	100					0.0007
11	α Endosulfan	0.0061	79	- 0.0002	-3	0.0020	25	0.0079
12	Dieldrin	0.0011	100					0.0011
13	Endrin	0.0029	53	0.0026	47			0.0055
14	4,4' DDD	0.0008	100					0.0008
15	β Endosulfan			0.0016	100			0.0016
16	4,4' DDT	0.0059	107	- 0.0003	- 5	- 0.0001	- 2	0.0055
17	Endrin aldehiyde							
18	Metoxiclor							
19	Endosulfan Sulfate	0.0040	100					0.0040
20	Endrin ketone							

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 21, issue 1, 2011, pp. 87-93

Table 5

Quantitative calculation of the OCP residues, in complex fodder, TRM (2) serie

Nr.	Organochlorine compounds	Concentration of OCP in the sample						
		eluent A		eluent B		eluent C		Total
		ppm	%	ppm	%	ppm	%	ppm
1	α HCH	0.0018	106	-0,0001	-6			0.0017
2	γ HCH	0.0102	78	0.0029	22			0.0131
3	β HCH			-0.0001	-20	0.0006	120	0.0005
4	δ HCH							
5	Heptachlor	0.0006	100					0.0006
6	Aldrin	0.0021	100					0.0021
7	Heptachlor epoxide	0.0005	100					0.0005
8	γ Chlordan							
9	α Chlordan	0.0021	100					0.0021
10	4,4' DDE	0.0006	100					0.0006
11	*α* Endosulfan	0.0080	87	-0,0002	-1	0.0014	15	0.0092
12	Dieldrin							
13	Endrin	0.0009	69	0.0004	31			0.0013
14	4,4' DDD	0.0031	100					0.0031
15	β Endosulfan							
16	4,4' DDT	0.0095	104	-0.0003	-3	-0,0001	-1	0.0091
17	Endrin aldehiyde							
18	Metoxiclor							
19	Endosulfan Sulfate	0.0023	100					0.0023
20	Endrin ketone							

Table 6

Quantitative calculation of the OCP residues, in complex fodder, TRM (3) serie

Nr.	Organochlorine compounds	Concentration of OCP in the sample						
		eluent A		eluent B		eluent C		Total
		ppm	%	ppm	%	ppm	%	ppm
1	α HCH	0.0008	100					0.0008
2	γ HCH	0.0189	100,5	-0,0001	-0.5			0.0188
3	β HCH	0.0008	74	-0,0001	-10	0.0004	36	0.0011
4	δ HCH							
5	Heptachlor	0.0012	100					0.0012
6	Aldrin	0.0024	100					0.0024
7	Heptachlor epoxide	0.0009	100					0.0009
8	γ Chlordan			0.0021	100			0.0021
9	α Chlordan	0.0011	100					0.0011
10	4,4' DDE	0.0012	100					0.0012
11	*α* Endosulfan	0.0093	92	-0,0002	-2	0.0010	10	0.0101
12	Dieldrin							
13	Endrin	0.0028	65	0.0015	35			0.0043
14	4,4' DDD	0.0011	100					0.0011
15	β Endosulfan			0.0023	100			0.0023
16	4,4' DDT	0.0018	120	-0,0003	-20			0.0015
17	Endrin aldehiyde							
18	Metoxiclor							
19	Endosulfan Sulfate	0.0039	100					0.0039
20	Endrin ketone							

Note: the „-„ values in tables 3, 4 and 5 refer to signals coming from the witness tests.

Most residues are fully eluted in the the A eluent; it is about the following compounds: αHCH, Heptaclor, Heptaclor epoxid, Aldrin (Dieldrin), αChlordan, 4,4’ DDE, 4,4’ DDD, 4,4’ DDT, Endosulfan sulphane.

γHCH is not constantly eluted, but in the A eluent; even when eluted in B as well, the amount in A is the most significant (78% in series 2); βHCH is identically eluted in series 1 and 3 in A (60%, 74%) and C (40%, 36%) but differently in series 2 when the residues appear entirely in C. t is observed that the value of the witness signal is significant in this compound, so that it might influence the result. The concentration of the residues of Endrin is divided between the A and B eluents but not identically in the three series ranging between 53 – 69% in A and 31-47% in B; For γChlordan, in the two series where it was identified (1 and 3), the residues are divided differently: in series 1 it is divided in all the eluents (A-18%, B-16%, C-66%) and in series 2 they appear entirely in the B eluent. For αEndosulfan the residues are divided mainly in the A eluent [(1)-79%, (2)-87%, (3)-92%) and the difference in the C eluent [(1)-11%, (2)-13%, (3)-8%).

CONCLUSIONS

For quick routine quantum analysis, the type A solvent use may be considered sufficient especially since β Endosulfan, which is found constantly only in the B eluent, is an isomer which is dosed only together with the Endosulfan isomers. The same aspect is highlighted within routine quantum analysis in which compounds are doses as isomer amount (α + β HCH, Chlordan isomers, Heptachlor isomers, Endosulfan isomers, DDT isomers). The only exception is Endrin, but in its case, the amount that appears in the B eluent is clearly bellow the one in eluent A. In case of precision analysis which aim all the components possible to be identified it is necessary to use all the three eluents. Also, in the case of the analysis with control purpose when the maximum admitted limits are ppm-ppb level, the three elutions are necessary for an efficient comparison with the MRL values for each compound separately and on groups of isomers. Since in these determinations, the solvent consumption are significant, we propose the investigation of other possibilities to decrease them, without affecting the precision of the results, respectively the testing of the way in which a proportional reduction of the method at the 1:2 and 1:5 report influences the qualitative determination of OCP in complex fodders taking into account the results of these tests on green fodders, results already reported. (Chiş, 2010).

REFERENCES

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

Bradley, R.L. Jr, 2003, Moisture and total solid analysis, Chap. 6, in Part II, Food Analysis, Third Edition, Springer Science (Suzanne Nielsen Ed.), USA Kluwer Academic New York, 81-103

Chiş Adriana - Studies regarding the optimization of the solvent consumption in the determination of organochlorine pesticides from green foder Vol.20/2010 a Studia Universitatis "Vasile Goldis", Seria Stiintele Vietii. ,ISSN 1584-3463, pag 81-87

EFSA, a, 2005, Opinion of the Scientfic 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

EFSA, a, 2006, Opinion of the Scientfic Panel on Contaminants in the Food Chain on a reques tfrom the Commission related to Endosulfan as undesirable substances in animal feed, The EFSA Journal, 234, 1-31

EFSA, b, 2005, Opinion of the Scientfic Panel on Contaminants in the Food Chain on a reques from the Commission related to Endrin as undesirable substances in animal feed, 2005, The EFSA Journal, 286, 1-31

EFSA, b, 2006, Opinion of the Scientfic Panel on Contaminants in the Food Chain on a reques tfrom the Commission related to DDT as undesirable substances in animal feed, The EFSA Journal, 433, 1-69

EU, 2006, Quality Control Procedures for pesticides Residues Analysis, Document nr. SANCO/10232/2006, Brussels, 2006 http://ec.europa.eu/food//fvo/ specialreport/pesticides_index_en.htm,

Gill, JPS, Aulakh, RS, Mundi, JPS and Sharma, JK, 2001, Pesticides residues in animal feed: a potential source of public health hazard. Paper in Post Conference proceeding of International Conference on Pesticides, Environment, Food Security from 19-23, November 2001 held at New Delhi. pp. 160-163.

Gocan S., 1998, High-performance cromatography, part I, gas chromatography, Dacia Publishing House, Cluj Napoca

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.

LEGIS – program informatic referitor la legislatia din Romania

Schenck, F.J. and J. Hobbs, 2004, Evaluation of a quick, easy, cheap, effective, rugged and safe (QuEChERS) approach to pesticide residue analysis, Bull. Environ. Contam. Toxicol.73(1), 24-30

Singh, R, Kumari, B, Madan, VK, Kumar, R and Kathpal, TS, 1997, Monitoring of HCH residues in animal feed. Indian J. Anim. Sci., 676: 250-52.

Škrbič, B. and Z. Predojevič, 2008, Levels of Organochlorine Pesticides in Crops and Related

Products From Vojvodina, Serbia :Estimated Dietary Intake, Environ. Contam. Toxicology, 54(4), 628-623

SR EN 12393-2, 2003, Produse alimentare negrase. Metode multireziduu pentru derminarea gaz-cromatografică a reziduurilor de pesticide, Partea 2 : metode de extracţie şi purificare

SR EN 12393-3, 2003, Produse alimentare negrase. Metode multireziduu pentru derminarea gaz-cromatografică a reziduurilor de pesticide, Partea 3 : Determinare şi teste de confirmare

Tadeo, J. L., (ed.), 2008, Analysis of Pesticides in Food and Environmental Samples, CRC Press, Taylor & Frances Group Boca Raton , London, New York

Wang G, X.-P., Yao T.-D., Cong Z-Y., Yan X.-L., Kang S.-C., Zhang Y., 2007, Distribution of persistent Organic Pollutants in Soil and Grasses Around Mt. Qomolangma, China, Arch. Environ. Contam. Toxicol., 52(2), 153-162.