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Original scientific paper

Residues of Lindane in Adipose Tissue of Lambs and Sheep from Islands Krk and Cres

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A B S T R A C T

Concentrations of lindane from adipose tissue i.e. omentum of sheep from homesteads of island Krk that were treated with lindane, were compared with the concentrations of lindane measured in adipose tissue of non treated sheep from husbandry cooperative on island Cres. Evaluation of food safety for this samples were estimated by comparing obtained results and maximum tolerance concentration (MTC) for lindane (2.0 and 0.2 mg/kg). In treated lambs from Krk measured concentrations of lindane were in range from 0.0038 to 1.8644 mg/kg, and in treated sheep from 0.0094 to 1.646 mg/kg. In control group of lambs and sheep concentration of lindane was ≤0.0051 mg/kg. Estimation of food safety based on the results from this research are discussable, regarding the fact that MTC prescribed before year 2007 was 2.0 mg/kg. When the new law Regulations of MTC for residues of pesticides in food and animal feed (NN 119/07) came into the force prescribed MTC was 0.02 mg/kg. If food safety of sheep meat from Krk would be estimated based on latter, then 73.4% of samples of lamb meat and 80% of sheep meat could be proclaimed unsafe for human consumption. It is encouraging that after completion of using lindane in year 2005, levels of lindane in adipose tissue of lamb from Krk in year 2006 were under the concentration set by new legislate (< 0.02 mg/kg).

Key words: *lindane, residues, adipose tissue, sheep, Cres, Krk*

Introduction

Organochlorine pesticides were extensively used in agriculture, forestry, and medicine in second half of 20th century. Lindane was used in agriculture to protect food crops, seed and soil against phytophagic insects. Thirty years ago the knowledge of harmful effects of organochlorine pesticides on nontarget organisms began to contribute to their gradually withdrawall from market. In 2009 the production and agricultural use of lindane was banned under the Stockholm Convention on persistent organic pollutants[1]. Some organochlorine pesticides are still in use in some »third world« countries.

In Croatia lindane was used as an ectoparasiticide formulated as a emulsifiable concentrate for cattle and sheep. The product, named Gamacid® T-50, was produced until 2005 by the pharmaceutical company Pliva Ltd. later Veterina Ltd. The preparation was registered for the treatment of sheep by dipping them in medicinal bath containing lindane in concentration 0.03 to 0.06%. This was effective against ectoparasites such as ticks, parasitic arthropods (*Sarcoptes, Psoroptes, and Chorioptes* spp.), sucking and chewing lice (e.g. *Bovicola ovis*), sheep ked (*Melophagus ovinus*) etc.

Lindane is well absorbed after peroral ingestion or inhalation. In the most cases it is absorbed transdermally after a longer retention on the skin and fleece.

Lindane acute oral LD_{50} for rats is roughly 90 mg/kg, therefore it is classified into group of dangerous insecticides, i.e. highly toxic compounds[2,3]. Due to the extremely good ectoparasiticide efficacy of lindane compared to the other compounds, i.e. organophosphates, avetmectins and milbemicins etc., the use of lindane in veteri-

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nary medicine was allowed in Croatia to August 17th 2005 (NN 114/00)[4].

Since products from sheep are respectable in human nutrition in Croatia, a human health risk assessment for lindane can be carried out by considering it's residual concentrations in adipose tissue of treated ewes and lambs.

Available lindane pollution data regarding products from sheep in Croatia are scarce and sporadic, especially in the period after 1999, unlike in other countries with developed sheep farming.

This paper attempts to contribute the knowledge about the content of lindane in adipose tissue of sheep from the Croatian island Krk and Cres, and evaluates food safety by taking into account prescribed MTC value.

Material and Methods

Samples

Sheep control samples were collected from husbandry cooperative on the island Cres from animals that were not treated with arthropocides. Sheep meat are certified as organic production (during the slaughtering at the Cres Agricultural Cooperative slaughterhouse) in the spring and autumn 2003.

Samples from treated sheep were collected from animals farming households on island Krk by the principle of random selection. The herds were treated with lindane (Gamacid T-50) once or several times during the lifetime by applying the preparation on the neck or dipping in medicinal bath[5]. Samples were taken during the slaughtering in the spring and autumn of 2003, 2004, 2005 and 2006. Lambs were 4–5 month old, and ewes 6–7 years.

Samples of abdominal adipose tissue e.g. omentum were stored at –20 °C prior to analysis in May 2009.

Methods

Residues of chlorinated hydrocarbons in adipose tissue of sheep and lambs were determined according to the method IS/N.GCECDBr.1[6–9] in the laboratory of Health and Environmental Department, the Department of Common Techniques of the Institute of Public Health of the Primorsko-Goranska County in Rijeka.

GCMS technique identified the gamma-HCH e.g. lindane. Comparing the peak of lindane from mass spectra of the sample with the peak of lindane in the mass spectra of standards NIST 27 and NIST 147 the similarity of 83% was determined.

Statistical analysis

Kolmogorov-Smirnov test was used to verify the normality of distribution of collected data in each group. Significance of differences between average lindane concentrations in tissues of control and treated lambs, and as well as ewes, were analysed with Mann-Whitney U-test. Kruskal Wallis analysis of variance was used for determining the significance of differences between lindane concentrations in all groups. All the above procedures were performed using the Statistica 8.0 software.

Results

In control group of lambs and sheep (island Cres) the maximal concentration of lindane was ≤0.0051 mg/kg. In treated sheep from Krk the concentrations of lindane were in range from 0.0094 to 1.646 mg/kg (Table 1), and in treated lambs from 0.0038 to 1.8644 mg/kg (Table 2). Samples of lamb and sheep fat tissues exceeded prescribed MTC for lindane (0.02 mg/kg) in 73.4% and 80% respectively (Table 1 and 2).

Median lindan concentrations of control and treated animals of same age categories were significantly different ($p<0.001$). Difference in values between control groups of lambs and sheep was not statistically significant, as well as difference between treated groups. Although, the average value of lindane in adipose tissue of sheep was higher than in lambs, but it was not statistically significant (Table 3).

Discussion and Conclusions

The results of this research on lindane residues in abdominal adipose tissue of lambs and sheep from islands Krk and Cres provide insight on their concentrations in relative to MTC, which is one of the basic for assessing

TABLE 1
LINDANE CONCENTRATION IN ABDOMINAL ADIPOSE TISSUE FROM CONTROL – (ISLAND CRES 2003) AND TREATED SHEEP (ISLAND KRK 2003–2005)

SHEEP				
Animal No.	Control – Cres		Treated – Krk	
	year	lindane mg/kg	year	lindane mg/kg
1.	2003	0.0003	2003	0.0397*
2.	2003	0.0007	2003	1.6460*
3.	2003	0.0016	2003	1.2820*
4.	2003	0.0041	2003	0.7870*
5.	2003	0.0033	2003	0.0614*
6.	2003	0.0009	2003	0.0753*
7.	2003	0.0015	2004	0.0117
8.	2003	0.0004	2004	0.0094
9.	2003	0.0008	2004	0.0682*
10.	2003	0.0020	2004	0.0104
11.	2003	0.0011	2004	0.0434*
12.	2003	0.0006	2004	0.0743*
13.	2003	0.0003	2005	0.4722*
14.	2003	0.0007	2005	1.0964*
15.	2003	0.0005	2005	0.8720*

*exceed prescribed MTC of lindane year 2007 (0.02 mg/kg)

TABLE 2
LINDANE CONCENTRATION IN ABDOMINAL ADIPOSE TISSUE FROM CONTROL – (ISLAND CRES 2003) AND TREATED LAMBS (ISLAND KRK 2003–2006)

LAMBS				
Animal No.	Control – Cres		Treated – Krk	
	year	lindane mg/kg	year	lindane mg/kg
1.	2003	0.0012	2003	0.5807*
2.	2003	0.0027	2003	0.0038
3.	2003	0.0020	2003	0.0305*
4.	2003	0.0051	2003	0.0958*
5.	2003	0.0008	2003	0.1596*
6.	2003	0.0012	2003	0.0999*
7.	2003	0.0008	2003	0.0549*
8.	2003	0.0015	2005	0.0452*
9.	2003.	0.0026	2005	1.0353*
10.	2003	0.0010	2005	0.0321*
11.	2003	0.0010	2005	1.8644*
12.	2003	0.0011	2005	1.1510*
13.	2003	0.0021	2006	0.0109
14.	2003	0.0009	2006	0.0060
15.	2003	0.0011	2006	0.0088

*exceed prescribed MTC of lindane year 2007 (0.02 mg/kg)

their safety. Taking into account that, at the time when the samples were collected (2003–2006), MDK prescribed for lindane in Croatia was 2.0 mg/kg, what indicates that concentration in analysed samples was less than MTC in all control and treated animals. Lindane residues concentrations in control animals ranged from 0.0003 to 0.0051 mg/kg, and in treated animals from 0.0038 to 1.8644 mg/kg. These values does not exceed the MTC for lindane. According to former regulations, analysed meat samples could be proclaimed safe for human consumption. On the contrary, if assessment of safety was made according to new Regulations of MTC for residues of pesticides in food and animal feed (NN 119/07)[10], than 73.4% samples of lamb meat and 80% samples of sheep meat would be proclaimed unsafe for human consumption.

TABLE 3
STATISTICAL VALUES OF LINDANE CONCENTRATION IN ADIPOSE TISSUE FROM CONTROL AND TREATED LAMBS AND SHEEP

Group	N	$\overline{X}$±SD	median	min	max
Control lambs	15	0.0017±0.0011	0.0012	0.0008	0.0051
Treated lambs	15	0.3453±0.5653	0.0549*	0.0038	1.864
Control sheep	15	0.0013±0.0011	0.0008	0.0003	0.004
Treated sheep	15	0.4366±0.5556	0.0743*	0.0094	1.646

*$p<0.001$ (Mann-Whitney U test)

Although the average measured value of lindane in adipose tissue of sheep was higher than in lambs, that difference was not statistically significant. The determined difference would probably be more significant if we had the opportunity to work on a larger sample. Lindane concentrations in control sheep and lambs from Cres ranged from 0.0003 to 0.0051 mg/kg which is considerably lower than the earlier and later valid MTC (2.0 and 0.02 mg/kg). Certainly, the lindane concentrations measured in control animals are a consequence of their widespread use during the last forty years, which reflects the basic biological contamination of this northadriatic area. This contamination can be the result of lindane input through the food chain because of its intensive use in the past decades – especially in agriculture. Additionaly, it's source can be parent animals who were once treated and the residues of this substance has a potential to be transferred from generation to generation[11,12]. It should be noted that HCH and lindane was the most extensively used pesticide in former Yugoslavia[13].

Maximum lindane concentration in treated lambs was 1.8644 mg/kg and it was higher than the maximum lindane concentration in treated ewes – 1.646 mg/kg (Table 3). Since we don't known details about applying Gamacid® T-50 (concentration of lindane in the bath, intervals of treatment, compliance of withdrawal period, etc.), we are not able to determine the cause of such findings. The high levels of lindane in treated lambs, especially when compared to a valid MTC (0.02 mg/kg) are considered to be a result of its use against ticks. The use of Gamacid® T-50 is contraindicated in lambs younger than three months and in high-pregnant and lactating sheep. However, the surveys show that the prescribed instruction were not always respected in practice by the farmers[5].

Results of our research show the higher lindane concentrations when compared with concentrations of lindane in meat, milk and related products measured during regular national monitoring programs in the period 1986 to 1999. In the period 1986 to 1989 measured values of lindane in meat and products were higher (0.025 mg/kg fat) in comparison with 1999 (0.006 mg/kg fat). Levels of lindane in milk products were as well higher in the period 1986 to 1989 (0.024 mg/kg fat) compared to 1999 year (0.006 mg/kg fat)[14]. In our research the data obtained from treated ewes (0.437 mg/kg fat) and lambs (0.345 mg/kg fat) were not in accordance with the above trend of lowering the concentration of lindane in food of animal origin.

High values of lindane in adipose tissue of lambs (n=358) were also measured by other researchers. The results on lindane residues monitoring in Yugoslavia (1991–1996) revealed that 2.5% of lamb samples containe lindane higher than MTC, with mean value of even 4.75 mg/kg[15]. Considering the dynamic of lindane depletion from edible sheep tissues, interesting results were reported in France[16]. The authors determined that seven days after treatment of sheep with 0.025% bath the concentration in adipose tissue was 30.46±14.6 mg/kg. The

levels of lindane decreased under the value of MTC 2.0 mg/kg after 59.7±9.7 days. If we compare the measured values of lindane residues in samples of lambs and sheep from Krk with those dynamics of depletion, it can be concluded that farmers from Krk did generally comply with the prescribed withdrawal period.

As mentioned before, the details about lindane treatments including withholding period compliance during the sheep farming process are often unavailable and unknown, so the data obtained in this research can only serve as an indicator of the safety of sheep and lamb meat.

Also, it is encouraging that after the ban of lindane use in Craotia the levels of its concentration in fat tissue of lamb from Krk in year 2006 were under the new MTC. It can be concluded that this is a positive indicator of cessation of sheep lindane contamination, which in addition ensures health safety of sheep and lamb meat, thus decreasing health risks in the population[17–19].

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OSTACI LINDANA U MASNOM TKIVU JANJADI I OVACA S PODRUČJA OTOKA CRESA I KRKA

SAŽETAK

U istraživanju su uspoređene koncentracije lindana iz masnoga tkiva Gamacid®-om tretiranih ovaca i janjadi sa seoskih gospodarstava otoka Krka, sa koncentracijama lindana izmjerenih u masnome tkivu netretiranih ovaca i janjadi iz Poljoprivredne zadruge na otoku Cresu. Usporedbom dobivenih rezultata s najvećim dopuštenim koncentracijama za lindan (2,0 i 0,02 mg/kg) procjenjena je zdravstvena ispravnost ovČjeg i janjećeg mesa. U tretirane janjadi s otoka Krka utvrđene vrijednosti lindana bile su u rasponu 0,0038–1,8644 mg/kg, a u tretiranih ovaca u rasponu 0,0094–1,646 mg/kg. U kontrolnim tj. netretiranim skupinama janjadi i ovaca te su koncentracije bile ≤0,0051 mg/kg. Procjena zdravstvene ispravnosti mesa na osnovu dobivenih rezultata, a s obzirom na Činjenicu da je NDK za lindan do stupanja na snagu Pravilnika o maksimalnim razinama ostataka pesticida u hrani i hrani za životinje (NN 119/07) bila 2,0 mg/kg, a nakon toga 0,02 mg/kg može biti dvojaka. Ako se zdravstvena ispravnost mesa sa seoskih gospodarstava otoka Krka procjeni prema novoj vrijednosti NDK (0,02 mg/kg) onda je 73,4% uzoraka janjećeg i 80% uzoraka ovČjeg mesa bilo zdravstveno neispravno. No, prema ranije važećem NDK (2,0 mg/kg) svi su uzorci bili zdravstveno ispravni. Ohrabrujući je nalaz da je razina lindana, nakon prestanka korištenja Gamacid®-a T-50 u 2005. godini, i u janjadi s otoka Krka u 2006. bila manja od novopropisane NDK.