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DDT, HCH and PCB Residues in Fat of Red Deer (*Cervus Elaphus*) from the Region of Warmia and Mazury, 2000-2001

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Abstract

The authors presented the results of a study on the content of polychlorinated biphenyls (PCBs), hexachlorohexane (γ-HCH) and dichlorodiphenyltrichloroethanes (DDTs) in perirenal fat from red deer obtained from the region of Warmia and Mazury in the years 2000-2001. Separation of the analysed compounds was conducted by gas chromatography. The analyses showed the presence of DDT and DDE in all samples, whereas DDD was found only in 13 samples (24% of all the analyzed samples), predominantly in trace amounts. Concentrations of DDTs and their metabolites averaged: 16.5 μg/kg of DDTs, 8.2 μg/kg of DDT and trace amounts of DDD. The lowest concentrations of these compounds were found in hinds and the highest in calves. Of all the analyzed substances, γ-HCH residues appeared in the smallest amounts – average 0,6 μg/kg and whereas PCBs concentrations were the highest – average 23,7 μg/kg. The results show that the region of Warmia and Mazury is an area characterised by low levels of pollution caused by the analyzed xenobiotics.

Keywords: DDT, HCH, PCB, *Cerrus elaphus*, Warmia and Mazury

Introduction

For many years the natural environment has been polluted by chlorinated hydrocarbons (DDT, HCH) and polychlorinated biphenyls, which belong to persistent contaminants. The main source of the residues of these pesticides is their application in agronomic treatments to protect cultivated crops as well as in the protection of forests against pests infesting trees [1, 10]. These compounds, apart from being highly toxic, are easily accumulated in plant and animal tissues, creating a threat of poisoning not only at the moment of their application but also for a long period of time afterwards [2]. Chloorganic pesticides have either been completely withdrawn since the 1970s or else they are now being withdrawn from the list of substances permitted to be used in many European countries, in North America and in Japan. At the same time, the countries of the tropical and subtropical zone, and especially the countries in the southern hemisphere, still use such insecticides as DDT, lindane or chlordane [3]. Many persistent chloroorganic compounds are transported by air over distances as long as thousands of kilometres from the sites where they are applied to where they finally deposit [4,7,8,9]. Like DDT, polychlorinated biphenyls (PCBs) are highly persistent in the enironment. The presence of PCBs in food and cases of high concentration of these xenobiotics in animals' bodies, which form the final link in the food chain, together with scientifically proven high toxicity of PCBs, has forced many countries to introduce restrictions on or total ban of the production and application of these

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Table 1.Organochlorine pesticides and PCB in perirenal fat of red deer from Warmia and Mazury (μg/kg).

Group	γ-HCH			DDE			DDD	DDT			DDTs			PCB		
	x x_{min}-x_{max}	s	cv%	x x_{min}-x_{max}	s	cv%	x x_{min}-x_{max}	x x_{min}-x_{max}	s	cv%	x x_{min}-x_{max}	s	cv%	x x_{min}-x_{max}	s	cv%
Calves n=13	0.7 trace-2.0	0.6	86	9.8 3.2-20.5	5.4	55	n.d.-trace	9.6 3.2-31.5	7.1	74	19.4 9.5-37.4	8.1	42	22.6 12.8-44.9	10.2	45
Hinds n=21	0.6 trace-3.0	0.7	116	7.2 2.7-18.3	3.7	51	n.d-2.5	6.9 2.2-17.4	4.1	71	14.3 6.1-31.7	6.9	48	22.7 13.2-34.5	5.6	25
Bulls n=20	0.4 trace-2.0	0.5	111	8.3 2.1-16.5	4.2	51	n.d.-0.7	8.6 n.d.-28.1	8.6	100	17.0 2.4-31.6	10.0	59	25.6 10.0-63.1	12.0	47
All n=54	0.6 trace-3.0	0.6	100	8.2 2.1-20.5	4.4	53	n.d.-2.5	8.2 n.d.-31.5	6.8	83	16.5 2.4-37.4	8.5	51	23.7 10.0-63.1	9.4	40

n - number of samples; x - arithmetic mean; s - standard deviation; x_{min} - minimum; x_{max} - maximum; cv - coefficient of variation (%), n.d. - not detected

chemicals [16] in the 1970s. A potential source of PCB environmental contamination is transformer oil and masses which fill condensers. Trace amounts can also appear spontaneously, from proper precursors, when rubbish and waste are being burned or when potable water and sewage are being chlorinated [16].

The level of chlorinated hydrocarbons and polychlorinated biphenyls in tissue of wild game is a good indicator of environmental contamination by these substances. Wild game which stays in the polluted environment is under a greater threat created by these chemicals than livestock [5,6,2].

Materials and Methods

The analyses were made on samples of perirenal fat from 54 red deer obtained randomly from October 2000 to January 2001 from the region of Warmia and Mazury. The animals were obtained by individual game shooting or from wild game purchasing centres located in the area studied. Due to regulations in each hunting ground, it was impossible to obtain the same number of red deer in each sex or age group. All carcasses of animals were examined by a veterinarian.

In order to determine chlorinated hydrocarbons (γ-HCH, DDTs and metabolites: DDT, DDE and DDD; determined as DDT was - p,p'-DDT isomer) in the analyzed samples, fat was separated by grinding it with anhydrous sodium sulphate and next washed twice with n-hexane. The residual amounts of chloroorganic pesticides in the fat obtained were determined by the method described by the State Institute of Hygiene [11]. The method consists of the destruction of fat with concentrated sulphuric acid and extraction of chlorinated hydrocarbons into n-hexane. Following the determination of DDT and HCH, the solutions were thickened using a dehydrochlorinating agent, composed of potassium hydroxide and ethanol. The next step was extraction with the help of n-hexane and a solution of potassium bichromate and sulphuric acid as an oxidising agent (in order to eliminate other interfering organohalogenated compunds). The extract was then used to determine PCBs. Separation of the analyzed compounds was conducted by gas chromatography using a PYE 4600 Unicam with an electron capture detector. The conditions of the separation were: a glass column 2.1m in length and 4 mm in inner diameter; carrier – supelcoport 100/120 mesh; liquid phase – 1.5% SP-2250 and 1.95% SP-2401. Argon flowing at 60 cm^3/min was used as a carrier gas. The temperatures of separation were 465 K in the column, 523 K in the detector and 498 K in the evaporator. The compounds were identified by comparing times of retention of peaks on chromatographs of samples and standards. The results were analyzed statistically.

Results and Discussion

The main characteristic of DDT, γ-HCH and PCBs is their persistence, which is their ability to remain in different components of the environment for very long periods of time (for DDT the time of half-persistence in the environment is over 60 years) [12]. Among the major effects of its negative influence is that DDT is capable of damaging intercellular links. Any disturbance in the communication between cells caused by DDT may lead to loss of control over the growth and differentiation of cells. Some authors claim that this can be a cause of cancer. A DDT dose sufficient to promote cancer formation in animals ranges from 25 to 30 mg/kg b.w./day [13]. DDT can cause leukaemia and lung cancer. Degeneration changes in the central nervous system as well as inferior imperviousness of the walls of blood vessels are the two negative consequences of exposure to hexachlorohexane [15], which can also be responsible for the occurrence of leukaemia, lymphoma and sarcoma of soft tissues [14]. In addition, PCB poisoning causes symptoms similar to those observed in vitamin A deficiency. It also leads to changes in the immune system, digestive system and thyroid disorders and can cause hepatocellular carcinoma or stomach cancer [16].

The residues of the analyzed substances determined in the perirenal fat of red deer can be found in Table 1. The

Table 2. Organochlorine pesticide and polichlorinated biphenyl residues in fat of red deer from the northern parts of Poland (μg/kg).

PCB	HCH	DDE	DDD	DDT	DDTs	Regions	Years	Authors
< 50	9.7[B]	38	n.d.	2.0	40	-Olsztyn province	1984	Falandysz, Falandysz, 1986
-	20[A]	70	20	90	180	-Olsztyn province	1984/85	Zasadowski et.al.,1988
< 20	4.0[B]	28	<2.0	< 2.0	28	-north Poland	1985	Falandysz, 1993
-	19[A]	-	-	-	65	-Suwałki and Białystok provinces	1986/87	Rodziewicz, Hajduk, 1989
23	15[A]	38	19	4.0	61	-north Poland	1987/88	Falandysz, Kannan, 1992
-	6.0[A]	-	-	-	30	- Suwałki and Białystok provinces	1988/89	Rodziewicz, Hajduk, 1991
-	4.0[A]	-	-	-	27	- Suwałki and Białystok provinces	1990/93	Rodziewicz, Hajduk, 1995
24	0.6[B]	8.2	ślady	8.2	16.5	-province of Warmia and Mazury	2000/01	Szymczyk, Zalewski, (publ.)

n.d. - not detected; A - HCHs; B - γ-HCH

analyses showed the presence of DDT and DDE in all the samples, whereas DDD was found only in 13 samples (24% of all the analyzed samples), predominantly in trace amounts. DDTs therefore consisted of DDT and DDE metabolites, which amounted to 49% of the total amounts. The relevant literature [6,7,17,18,19,20] suggests that the percent ratio of DDT and DDE in fat of wild game can vary, but DDT usually predominates, constituting 39-95%. Most of the authors cited above emphasize that DDD is the metabolite reaching the smallest percentage of total DDTs, except for the red deer examined by Falandysz and Kannan, where it equalled 31%. In summer, when the amount of reserve fat in animals is reduced, chlorinated hydrocarbons can be transferred with blood to muscles and the liver, where they are biotransformed. Under environmental stress, this process in some animals can be so intensive that only DDE and DDD, but no DDT can be determined in their tissues [17, 21,22]. It should be noticed that the metabolite DDE is resistant to degradation by microorganisms present in stomachs of ruminants [20].

The concentrations of DDTs and their metabolites in the three analysed groups of red deer (calves, hinds and bulls) were on similar levels and equalled (on average): 16.5 μg/kg of DDTs, 8.2 μg /kg of DDT and trace amounts of DDD. The lowest concentrations of these compounds were found in hinds, which can be explained by the fact that lactation and permeation of chloroorganic pesticides through the placenta to the fetus are two main ways of removing these compounds by an organism. The affinity of DDT with fats leads to its elevated levels in milk, and the intake of DDT present in milk from the digestive tract of young mammals is very high (nearly 100%). In all lifetime, the risk of exposition to chloroorganic compounds is the highest when young animals are fed on mother's milk [13]. This could explain why the highest concentrations of DDTs and their metabolites were found in the calves we tested. Another fact worth remembering is that young organisms do not have fully developed mechanisms of detoxification, which may also contribute to higher concentrations of xenobiotics in their bodies [13, 23].

Of all the analyzed substances, γ-HCH residues (lindane) appeared in the smallest amounts. Detectable amounts of this compound were found in all the samples, but only in 13 (24%) was the substance found in trace amounts. The mean concentration of γ-HCH was 0,6 μg/kg fat. Compared to DDT, hexachlorohexane is more easily absorbed from the digestive tract, especially when in the presence of fats [23]. As for DDT, the highest amounts of γ-HCH were found in calves (0.7 μg/kg fat).

The data presented in Table 1 show that concentrations of polychlorinated biphenyls in perirenal fat of the tested red deer cannot be classified as high. PCB content in the samples belonging to the three groups was uniform and equalled on average 23.7 μg/kg. In the case of red deer obtained in the west of Poland differences in PCB concentrations between young and old animals were quite large (17.0 and 26.0 μg/kg, respectively) [24]. The authors' own research showed no distinct variation between mean concentrations of this compund between the calves and older animals. However, some differences were detected in the frequency of higher concentrations of polychlorinated biphenyls, for example PCBs in amounts exceeding 20.0 μg/kg of fat in hinds and bulls constituted, respectively, 66 and 65% of the samples, in contrast to calves, where such levels appeared in 46% of the samples.

The whole area from which the animals were obtained was divided into four sectors: north-east, south-east, north-west and south-west. The levels of the analyzed substances in those parts of the region was uniform, even though slightly higher concentrations of DDTs and DDT metabolites were detected in eastern parts of Warmia and Mazury, while the levels of γ-HCH in the north-eastern part of the region was half as low as in the other sectors (Tab. 3). These variations may be due to some differences in the amounts of plant protection chemicals used in different parts of the region in the earlier years.

The amounts of polychlorinated pesticides in perirenal fat of red deer determined in our study are lower than the values reported by other authors (Tab. 2) [6, 7, 17, 18, 19,

Table 3.Organochlorine pesticides and PCB in perirenal fat of red deer from different parts of Warmia and Mazury (μg/kg).

Group	γ-HCH			DDE			DDD	DDT			DDTs			PCB		
	x x_{min}-x_{max}	s	cv%	x x_{min}-x_{max}	s	cv%	x x_{min}-x_{max}	x x_{min}-x_{max}	s	cv%	x x_{min}-x_{max}	s	cv%	x x_{min}-x_{max}	s	cv%
North –East n=17	0.6 trace-1.9	0.5	84	8.3 4.8-16.5	3.1	37	n.d.-2.5	9.1 4.0-31.5	7.5	82	17.6 9.5-37.1	8.9	51	22.9 12.8-44.9	9.4	41
South-East n=17	0.6 trace-2.7	0.7	117	7.3 2.7-14.8	3.9	53	n.d.-trace	10.1 3.4-28.1	8.4	84	17.1 8.1-31.6	9.4	54	25.0 14.2-63.1	10.4	42
North -West n=6	0.3 trace-0.6	0.2	83	7.9 5.8-13.0	2.6	33	n.d.	6.6 4.1-9.4	2.2	34	14.7 11.2-22.4	4.2	29	24.3 15.4-43.8	10.9	45
Sout-West n=14	0.6 trace-1.6	0.7	119	9.5 2.1-2.0	6.4	68	n.d	5.4 n.d.-11.7	3.2	58	14.9 2.4-30.8	8.9	60	22.8 10.0-37.9	8.3	36

n - number of samples; x - arithmetic mean; s - standard deviation; x_{min} - minimum; x_{max} - maximum; cv - coefficient of variation (%), n.d. - not detected

20, 25]. The decrease in residues of DDTs since the 1980s reached 59%, while the levels of γ-HCH in the same period fell by up to 94%. The amount of PCBs in fat of red deer has not changed for the past twenty years, and equals 20 µg/kg of fat. The maximum levels of concentrations of polychlorinated pesticides in fat of red deer determined in this study were below the highest permissible levels in foodstuffs, which equal for both DDTs and γ-HCH 1 mg/kg recalculated into fat according to the Polish [26] and European norms [27]. The limits on PCBs binding in some European countries for animal produce are from 0.2 to 3 mg/kg of fat. The decision of the Commission of 9th July 1999 (99/449/EU) was to impose temporary obligation to test the content of selected PCB congeners as a preliminary evaluation to food contamination with dioxins. According to this regulation, the results of determinations of polychlorinated biphenyls in meat and meat products must not exceed the level of 0.2 mg/kg of fat. Should this level be higher, it is necessary to conduct analyses towards the presence of dioxins [28]. Similar regulations are binding in Poland [29], and this law can to some extent specify the permissible limits of PCBs in food.

The results presented in this paper indicate that red deer inhabiting the area of Warmia and Mazury are only slightly polluted by the analyzed xenobiotics. They can also indicate that there is a decreasing tendency in the concentrations of these chemicals. The levels of DDT, HCH and PCBs determined in the fat of red deer are certainly lower than the values tolerated in food products, both in Poland as well as in the countries of the European Union. As a result, 90% of red deer meat and meat products are exported abroad [30]. It can be added that the region of Warmia and Mazury is an area characterised by low levels of pollution caused by the analyzed xenobiotics. Similar observations were previously made by the authors regarding the presence of heavy metals [31].

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