Evaluation of contamination level of mare's milk with selected chlorinated hydrocarbons

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Investigations into the contamination of mare's milk with chlorinated hydrocarbons were conducted in two horse studs - Racot and Rzeczna, in the years 2004-2005. Samples of milk were collected from 15 mares in the year 2004 and from 20 mares in the year 2005. The material collected was determined for contents of residues of γ-HCH and DDT and its metabolites - DDD, DDE. The results obtained in the study confirmed the continuous presence of those contaminants in the milk of mares as well as a dependency of the level of contamination on the place of horse breeding. Milk samples collected from mares originating from the Horse Stud Rzeczna contained lower quantities of γ-HCH and higher quantities of DDT than the milk samples of mares originating from the Horse Stud Racot. The milk of mares from the Horse Stud Racot was additionally characterized by a higher percentage of DDE in tDDT, which may indicate a greater intensity of DDT transformation on that area as compared to the area of the Horse Stud Rzeczna. The study demonstrated also a slight decrease in the level of DDT and γ-HCH in the milk of mares collected in the year 2005 in respect of the year 2004. In addition, no DDD residues were found in milk collected in the years 2005 from mares bred in either Horse Studs.

KEY WORDS: mare's milk / chlorinated hydrocarbons / pollution of environment

A variety of biological and chemical methods of plant protection against pests has been applied since ancient times. However, a rapid increase in the demand for chemical agents occurred in the XIXth century as a result of intensive changes proceeding in agriculture. In 1939, insecticidal properties of DDT and two years later of γ-HCH (lindane) were discovered. Ever since, those substances have been commonly applied for the eradication of detrimental insects. For several decades, those toxins have been introduced into the environment on a mass scale. Their harmfulness became the focus of interest in the 1950s, yet the ban on their use was only implemented 20 years later. The withdrawal of DDT and γ-HCH from use is connected with their detrimental effect on the health of humans and animals. Those substances may lead, among other things,

to: damage of the central nervous system, haematopoietic system, hormonal system and parenchymatous organs; they may also induce necroses. Such an action is, however, observed at very high doses of those substances. Yet, the current problem is posed by their capability to accumulate in the food chain. Organochlorine insecticides, being lipophilic substances, are accumulated mainly in fatty tissue. It usually does not pose any problem to an organism, for such toxins are blocked in this way. However, moving along the food chain, the level of detrimental substances becomes substantially higher in the successive links. Those compounds are the most dangerous to organisms at the end of the food chain [2, 5, 7, 11, 19, 25, 26].

Due to their capability to penetrate through the placenta to a foetus and through the mammary gland to mother's milk, chlorinated carbohydrates pose a severe threat to newborns and sucklings whose defense mechanisms are not fully developed in the first period of life and who have been exposed to those toxins practically since the foetal life [2, 5, 19]. Investigations concerning the contamination of human and animal milk with those compounds have been carried out in Poland and abroad for several decades. Such analyses have also been conducted for the milk of mares originating mainly from areas of the north-eastern Poland. They demonstrated, among others, the impact of environmental conditions on the level of milk contamination as well as considerable changes in the level of contamination over time [4, 9, 10, 15, 16, 18, 24, 27]. For this reason, a study was undertaken which was aimed at comparing the contamination of mare's milk with chlorinated hydrocarbons in two horse studs from different regions of Poland over two successive years.

Material and methods

The experimental material was milk collected from mares originating from two Horse Studs: SK Rzeczna - located on the area of Żuławy Wiślane (7 mares in the year 2004 and 10 mares in the year 2005) and SK Racot - located in the Wielkopolska region (8 mares in the year 2004 and 10 mares in the year 2005). Samples of milk were collected in each horse stud twice, in a one-year interval. Milk was collected from mares of different breeds (Wielkopolski horse, Polish half-bred horse, Thoroughbred), with clinically-healthy udders. Due to the fact that the concentration of chlorinated hydrocarbons in milk increases up to the third month of lactation and then begins to decrease [23], mares in the first four months of lactation were selected for analyses (Tab. 1). In addition, owing to the considerable variation of foaling terms, in some studs it was difficult to gather a sufficient group of mares that were in a shorter lactation interval than between l and 4 months on the day of milk sample collection. The mares were fed feeds originating from own crops.

Ca. 2 hours before milk sampling, the foals had muzzles put on or were fixed in boxes neighboring with boxes of their mothers, so that a greater quantity of milk could be accumulated in the mother's udder. Next, the milk was hand-milked (ca. 250 ml), preserved with a hydrogen peroxide solution (ca. l ml/250 ml milk) and cooled for

Table l - Tabela l
Number of mares in particular months of lactation
Liczba klaczy w poszczególnych miesiącach laktacji

Mo ıth of lactation	SK R ẓeczna		SK R ıcot	
M esiąc laktacji	2004	2005	2004	2005
I	_	4	4	5
II	4	3	2	4
III	3	3	3	1
IV	2	-	-	-

transportation. Until subjected to chemical analyses, the milk was kept at a temperature of –21°C.

In order to determine the contents of organochlorine insecticides – γ-HCH (γ-hexachlorocyclohexane) and DDT (l,l,l-trichloro-2,2-bis(4'-chlorophenyl)ethane), and their metabolites - DDD (l,l-dichloro-2,2-bis(4'-chlorophenyl)-ethane) and DDE (1,1-dichloro-2,2-bis(4'-chlorophenyl)ethylene), the samples of milk were subjected to fat extraction. Fat was extracted from milk twice with acetone and petroleum benzine (50 ml of each per 50 ml of milk). The collected mixture was evaporated onto an evaporator at a temperature of 72°C, and the fat content was then determined with the gravimetric method. Chlorinated hydrocarbons contained in fat were assayed with a simplified method described by Amarowicz et al. [2], according to which slightly heated up fat was transferred by means of n-hexane (5 cm^3) to a Hehner's cylinder and then sulfuric acid (ca. 20 cm^3) was instilled. The hexane layer containing chlorinated hydrocarbons was collected to a measuring cylinder and 80 cm^3 of distilled water were instilled. The hexane layer re-collected to test tubes was left in a warm place for evaporation. The obtained chlorinated hydrocarbons were dissolved in l cm^3 of n-hexane and injected onto a column of a gas chromatograph. A PYE 4600 Unicam apparatus with an electron capture detector and a glass column (1.5 m x 4 m) filled with chromosorb W/A/W DMSC 80/100 mesh and a liquid phase 5% DC-11 was used to this end. Argon delivered at a flow rate of 60 cm^3/min was used as a carrier gas. The separation was run at the following temperatures: column - 411 K, detector - 523 K and evaporator - 498 K. Determinations were recorded using a Philips 10 mV recorder. The compounds were identified by comparing retention times of their peaks on chromatographs with those of the standards. The results obtained were subjected to a statistical analysis by means of STATISTICA software.

Results and discussion

The chemical analyses of the collected material demonstrated the presence of contaminants in all samples of mare's milk collected in the year 2004 and in most of the samples collected in the year 2005. Noteworthy is the fact of the permanent presence

of γ-HCH and DDT in the environment despite not having been introduced into it for over 30 years, which has been confirmed by results of investigations conducted on various biological material [3, 8, 14, 17].

In the case of γ-HCH, mean contamination of mare's milk fat, analyzed over the period of 2 years, reached 0.17 μg/kg in the Horse Stud Rzeczna and 1.73 (μg/kg in the Horse Stud Racot, and in the case of DDT and tDDT (total DDT, i.e. the sum of DDT and its metabolites – DDD and DDE) accounted for, respectively: 13.77 μg/kg and 21.6 μg/kg in the SK Rzeczna and for 2.11 μg/kg and 10.93 μg/kg in the SK Racot (Tab. 2). The statistical analysis of results demonstrated highly significant differences in the mean content of lindane and DDT as well as tDDT in milk depending on the place of horse breeding. The samples of milk originating from mares from the SK Rzeczna contained considerably smaller quantities of γ-HCH residues and more DDT, than those collected from mares of the SK Racot. That dependency was noticeable both in mean values from the two years examined and in the values determined in each year separa-tely.

Table 2 - Tabela 2
Mare milk fat contamination by chlorinated hydrocarbons dependent on breeding place of the horses
Zanieczyszczenie tłuszczu mleka klaczy węglowodorami chlorowanymi w zależności od miejsca hodowli koni

Specification Wyszczególnienie		Chlorinated hydrocarbons - Węglowodory chlorowane					
		γ-HCH (μg/kg)	DDT (μg/kg)	DDD (μg/kg)	DDE (μg/kg)	tDDT (μg/kg)	% DDE in tDDT % DDE w tDDT
		2004 and 2005 - Lata 2004 i 2005					
S K Rzeczna	$\overline{X}$	0.17^{A}	13.77^{A}	0.37	7.31	21.46^{B}	38.3^{A}
n=17	Sd	0.22	8.68	0.47	3.07	11.28	11.9
S K Racot	$\overline{X}$	1.11^{A}	2.11^{A}	0.25	8.58	10.93^{A}	80.2^{A}
n=18	Sd	1.02	2.31	0.31	6.1	6.3	20.8
		2004 - Rok 2004					
S K Rzeczna	$\overline{X}$	0.10^{A}	17.88^{A}	0.91^{A}	8.89	27.67^{A}	32.2^{A}
n=7	Sd	0.16	7.09	0.19	3.59	9.67	7.7
S K Racot	$\overline{X}$	2.22^{A}	3.84^{A}	0.57^{A}	6.44	10.84^{A}	60.1^{A}
n=8	Sd	0.83	1.43	0.18	0.91	1.69	8.8
		2005 - Rok 2005					
S K Rzeczna	$\overline{X}$	0.22^{A}	10.9^{A}	0	6.22	17.12	42.5^{A}
n=10	Sd	0.25	8.83	0	2.21	10.63	12.8
S K Racot	$\overline{X}$	1.34^{A}	0.72^{A}	0	10.29	11.01	96.2^{A}
n=10	Sd	1.03	1.93	0	7.9	8.53	10.7

Means in columns in particular years marked with the same letters differ significantly at P≤0.01
Średnie w kolumnach w poszczególnych latach oznaczone tymi samymi literami różnią się wysoko istotnie (P≤0,01)

According to Dubniak [6], over the period of the intensive application of organochlorine pesticides, the utilization of DDT in the area of the Wielkopolska region, the SK Racot is located on, was substantially higher than on Żuławy Wiślane, the SK Rzeczna is situated on. Taking into account that the current contamination of milk with DDT is, for all that, higher in Rzeczna than in Racot, it should be stated that the contemporary level of contamination is affected, to a greater extent, by transformations of the analyzed xenobiotics in the environment than by the level of their utilization on the selected area in the future [21]. The rate of those changes is determined by a variety of environmental factors, including: climatic conditions, humidity and structure of soil. chemical composition of water or the structure of flora and fauna [13]. The major metabolite of DDT in organisms of mammals as well as a product of its photolytic degradation is DDE [12, 19]. Hence, the percentage of DDE in tDDT (% DDE in tDDT) indicates the intensity of DDT transformations proceeding in the environment. Highly significant differences in the percentage of DDE in tDDT (Tab. 2) between the milk originating from the SK Racot (80.2% DDE in tDDT) and the milk collected in the SK Rzeczna (38.3% DDE in tDDT), point to a greater intensity of transformations proceeding on the area of the SK Racot. In addition, the increase in percentage contribution of DDE in tDDT in 2005 as compared to 2004 indicates „the ageing" of the product introduced into the environment, i.e. DDT, and together with the observed decrease in its concentration in the milk of mares - confirms that this compound was not re-introduced to the environment (Tab. 3).

Owing to the ban on the use of organochlorine pesticides since the beginning of the 1970s, a constant decline has been observed in the level of environment contamination with those xenobiotics [3, 9]. This, in turn, results in the purification of the milk of mares. It has been confirmed by results of analyses conducted in the SK Plękity in the years 1989, 1999 and 2002. The content of lindane in milk fat of mares bred in that stud decreased from 27 μg/kg in 1989 to 17 μg/kg m 1999, and then to 9 μg/kg in 2002 [20, 22, 26]. In turn, the content of tDDT in the respective years reached: 86 μg/kg, 38 μg/kg and 11 μg/kg fat. Also the comparison of mean levels of milk contamination, together in horse studs Racot and Rzeczna between the year 2004 and the year 2005, demonstrated a slight, though statistically significant, decrease in γ-HCH and DDT (Tab. 3). Noteworthy is the fact that no DDD residues were detected in the milk sampled in the year 2005 from mares originating from either studs. In respect of the values determined in milk from 2004, it yielded highly significant differences, which - undoubtedly - confirms the successive purification of the environment. Also in each horse stud analyzed separately, the contamination of mare's milk displayed similar tendencies over the analyzed period. They were more tangible in the SK Racot, where the decrease in the level of DDT was highly significant, likewise the increase in the percentage contribution of DDE in tDDT. It should additionally be emphasized that DDT was no longer detected in 8 out of 10 milk samples collected in the SK Racot in the year 2005.

The results obtained indicate that despite the permanent presence of residues of chlorinated hydrocarbons in the milk of mares, a successive decrease has been observed in the level of those contaminants. In addition, the reported study confirms the signifi-

Table 3-Tabela 3
Mare milk fat contamination by chlorinated hydrocarbons dependent on year of milk sampling
Zanieczyszczenie tłuszczu mleka klaczy węglowodorami chlorowanymi w zależności od roku, w którym pobrano próby mleka

Specification		Chlorinaled hydrocarbons – Węglowodory chlorowane					
		Y-HCH (µg/kg)	DDT (µg/kg)	DDD (µg/kg)	DDE (µg/kg)	tDDT (µg/kg	% DDE in tDDT % DDE w tDDT
SK Racot + SK Rzeczna							
2004	$\overline{X}$	1.23	10.39	0.73^{A}	7.58	18.70	47.1^{a}
n=15	Sd	1.25	8.67	0.25	2.75	10.82	16.5
2005	$\overline{X}$	0.79	5.81	0^{A}	8.25	14.06	69.4^{a}
n=20	Sd	0.93	8.12	0	6.02	9.89	29.9
SK Racot							
2004	$\overline{X}$	2.22	3.84^{A}	0.57^{A}	6.44	10.84	60. 1^{A}
n=8	Sd	0.83	1.43	0.18	0.91	1.69	8.8
2005	$\overline{X}$	1.34	0.72^{A}	0^{A}	10.29	11.01	96.2^{A}
n=10	Sd	1.03	1.93	0	7.9	9.27	10.7
SK Rzeczna							
2004	$\overline{X}$	0.10	17.88	0.91^{A}	8.89	27.67	32.2
n=7	Sd	0.16	7.09	0.19	3.59	9.67	7.7
2005	$\overline{X}$	0.22	10.9	0^{A}	6.22	17.11	42.5
n=10	Sd	0.25	8.83	0	2.21	10.63	12.8

Means in columns in particular studs marked with the same letters differ significantly: small letters - at P≤0.05; capital letters - at P≤0.01
Średnie w kolumnach, w poszczególnych stadninach, oznaczone tymi samymi literami różnią się istotnie: małe litery - przy P≤0,05; duże litery - przy P≤0,01

cant effect of the site of horse breeding on the level of contaminants in milk and on the rate and tendencies of transformations the chlorinated hydrocarbons are subject to.

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Ocena poziomu skażenia mleka klaczy wybranymi węglowodorami chlorowanymi

S t r e s z c z e n i e

Badania dotyczące skażenia mleka klaczy węglowodorami chlorowanymi przeprowadzono w dwóch stadninach koni - Racot i Rzeczna, w latach 2004-2005. Próby mleka pobrane zostały od 15 klaczy w 2004 roku i 20 klaczy w 2005 roku. W zebranym materiale oznaczono zawartość pozostałości γ-HCH i DDT oraz jego metabolitów – DDD, DDE. Uzyskane wyniki potwierdziły ciągłą obecność tych zanieczyszczeń w mleku klaczy, a także zależność poziomu skażenia od miejsca hodowli koni. Próby mleka pochodzące od klaczy z SK Rzeczna zawierały mniejsze ilości γ-HCH, a więcej DDT. niż próby mleka od klaczy z SK Racot. Zaobserwowano również wyższy procentowy udział DDE w tDDT w mleku klaczy z SK Racot, co może świadczyć o większej intensywności przemian DDT na tym terenie, niż na terenie SK Rzeczna. Przeprowadzone badania wykazały niewielki spadek poziomu DDT i γ-HCH w mleku klaczy pobranym w 2005 roku, w odniesieniu do roku 2004. Ponadto w mleku pobranym w 2005 roku od klaczy z obydwu stadnin nie wykryto już pozostałości DDD.