ORGANOCHLORINE PESTICIDES AND POLYCHLORINATED BIPHENYLS IN GAME ANIMALS FROM POLAND

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Abstract

The occurrence and concentrations of organochlorine pesticides and polychlorinated biphenyls (PCBs) were determined in 141 adipose tissue samples of wild boars, roe deer, and red deer from different regions of the country, collected in 2010/2011. The determinations of HCH isomers (α -, β -, and γ -HCH), HCB, DDTs (p,p'-DDT, o,p'-DDT, p,p'-DDE, and p,p'-DDD), and PCBs (six indicator PCB congeners 28, 52, 101, 138, 153, and 180) were carried out using the capillary gas chromatography. The mean concentrations of DDTs expressed on the fat basis ranged from 0.241 mg/kg (wild boar) to 0.032 mg/kg (red deer), and for PCBs from 0.015 mg/kg (wild boar) to 0.010 mg/kg (roe deer). The mean contribution of p,p'-DDE to DDTs and PCB 153, PCB 138, and PCB 180 to PCBs exceeded 80% and 90% respectively, in all game animal samples studied. Higher contents of chlorinated hydrocarbons were detected in game animals originating from industrial regions.

Key words: game animals, organochlorine pesticides, PCBs, residues.

Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are representatives of a large group of chlorinated hydrocarbons, listed as persistent organic pollutants (POPs) in the Stockholm convention. Environmental pollution caused by these compounds has constituted a serious global problem for over half a century. They are widespread in air, water, soil, and live organisms including wildlife and humans. Many of these compounds, due to their environmental persistence, possess the ability to accumulate in the food chain, mainly in adipose tissues. It is widely known from the literature that exposure to certain POPs may result in serious environmental and health effects including adverse effects on the nervous, immune, endocrine, and reproductive systems, and may induce certain types of cancer (1, 14, 15). The evaluation of food and environmental contaminations by chlorinated hydrocarbons began in the 1960s or early 1970s in many industrialised countries. Results of these studies led to introduction of restrictions and then prohibition of production and use of the organochlorine pesticides in agriculture and PCBs in industry. Studies on the OCP and PCB residues have become the basic element of each monitoring programme for food contamination.

Nowadays, none of pesticides containing the substances listed in the Stockholm convention are produced, used, imported, or exported in Poland. For over 30 years, the production of pesticides has been

based on substances, which do not contain POPs. In 1972, DDT-containing pesticides were gradually withdrawn, and in 1975 the use of these chemicals was completely terminated. The use of pesticides containing HCB was permitted for the last time in 1978. Lindane was completely banned in Poland from 1988 to 1990. PCBs have never been produced in Poland on a technical scale. Only small quantities of Tarnol and Chlorofen were produced (below 1,000 ton).

The levels of POPs in wildlife animals reflect environmental contamination by these compounds (2, 5, 7, 17). Game animals are good indicators of environmental contamination by chlorinated hydrocarbons. According to the Central Statistic Office, during the hunting season 2010/2011, 233,000 wild boars, 161,000 roe deer, and 54,000 red deer were shot (Concise Statistical Yearbook of Poland 2012).

The aim of the study was to monitor the occurrence and concentrations of organochlorine pesticides and indicator PCB congeners in adipose tissue of wild boars, roe deer, and red deer from different regions of Poland and to evaluate the possible toxicological hazard to the game meat consumer.

Material and Methods

Samples of adipose tissue from 84 wild boar (*Sus scrofa*), 32 red deer (*Cervus elaphus*), and 25 roe deer

(*Capreolus capreolus*) were collected during the hunting season from August 2010 to January 2011. Sex, age, and weight were recorded for each animal. Wild boar (42 females and 42 males) aged between 1 and 5 years and weighed from 20 to 120 kg. Red deer (17 females and 15 males) aged between 1 and 8 years and weighed from 24 to 156 kg. Roe deer (22 females and three males) aged between 1 and 5 years and weighed from 11 to 45 kg.

The studies were conducted in four industrial regions recognised as areas of significant environmental contamination, *i.e.* the Upper Silesian Industrial District (GOP, area around Miasteczko Śląskie and Tarnowskie Góry, mining industry, chemical industry), the Legnica-Głogów Copper District (LGOM, around Rudna, one of the biggest centres of copper mining and industry in Poland), the Bełchatów Brown Coal Basin (vicinity of Bełchatów and Piotrków Trybunalski, coal mining industry), and the Turoszów Brown Coal Basin (area of Bogatynia, coal mining industry). As a reference area no industrialised region of Warmia and Mazury Lake District was selected.

The determination of organochlorine pesticides (HCB, α -HCH, β -HCH, γ -HCH, p,p'-DDE, p,p'-DDD, o,p'-DDT, p,p'-DDT) and six indicator PCB congeners (PCB IUPAC No. 28, 52, 101, 138, 153, 180) was carried out using a capillary gas chromatography with electron capture detection. Chlorinated hydrocarbons were extracted from the adipose tissue samples using mixture of hexane and acetone. The extracts were cleaned up on the basis of sulphuric acid treatment ($\text{H}_2\text{SO}_4 + \text{H}_2\text{SO}_4$ 25% SO_3), while separation of PCB congeners from OCPs was achieved with the use of alkaline hydrolysis (10% KOH in ethanol). The purified extracts were analysed by GLC-ECD.

Gas chromatographic analyses were performed on an Agilent Technologies model 6890 Plus equipped with 7683B series autosampler, split-splitless injector in pulsed splitless mode, and ^{63}Ni - μEC detector. Chromatographic separation was achieved on HP-5MS capillary column (60 m $\times$ 0.25 mm ID $\times$ 0.25 μm film thickness, J&W Scientific, USA). The following oven temperature programme was used: initial temperature of 60°C for 2 min, increase to 180°C at 40°C/min and hold for 2 min, increase to 245°C at 2°C/min and hold 2 min, increase to 265°C at 4°C/min and hold for 15 min, and then increase to 290°C at 8°C/min and hold for 20 min. For confirmatory purposes a second capillary column DB 1701 (60 m $\times$ 0.25 mm ID $\times$ 0.25 μm film thickness, J&W Scientific, USA) was used. The injector and detector were operated at 280°C and 325°C, respectively. The GC was controlled by ChemStation software.

The study was carried out with the use of validated analytical methods in accredited laboratory. The validation was performed with the recommendations of the document SANCO/12495/2011 (Method validation and quality control procedures for pesticide residues analysis in food and feed). Validation parameters obtained for animal fat matrix demonstrate that the analytical method fulfil the method performance

acceptability criteria. Recoveries from fortified samples were in the range 86%-102%, coefficients of variation (CV), indicating the precision of the methods, were <12% for repeatability and <15% for reproducibility, uncertainty 25%, limit of determination was 0.001 mg/kg. The laboratory regularly and successfully participated in international proficiency testing organised by FAPAS (Food Analysis Performance Assessment Schemes, UK) and the European Union Reference Laboratory (CVUA Freiburg). In addition, the certified reference materials were analysed (CRM 430 - Organochlorine pesticides in pork fat, CRM 445 and 446 - Chlorobiphenyls in pork fat).

Results

The presence of DDT and its metabolites has been observed in all 141 samples of adipose tissue of game animals. Residues of p, p'-DDE occurred in all samples: p,p'-DDT in 53%, and p,p'-DDD in 44% of the samples. Among other examined pesticides, HCB and HCH isomers were detected. The low concentrations of HCB were found in 74% of the animals, in the range from traces to 0.025 mg/kg. HCB was detected in 67% of samples from wild boar, in 75% of samples from red deer, and in all samples from the roe deer. Concentrations were low; mean and median content did not exceed 0.010 mg/kg. Additionally, low contents of the isomers α -, β -, and γ -HCH were detected in a few percentages of the samples with the mean concentration of about 0.002 mg/kg fat.

The occurrence of PCBs was detected in 98.6% samples. Only in two wild boars from the region of Warmia and Mazury PCB congeners were not identified. The most frequently detected PCB congeners were as follows: PCB 153 in 98.6% of the samples; PCB 138 in 97.9% of the samples, and PCB 180 in 94.3% of the samples. Other PCB congeners were detected only in some samples.

The results of determinations of DDTs and PCBs in fat of wild boars, roe deer, and red deer from different regions of Poland, are reported in Tables 1 and 2. The mean concentration of DDTs (sum of p,p'-DDE, p,p'-DDD, o,p'-DDT, and p,p'-DDT) in game animal fat was 0.157 mg/kg, in that p,p'-DDE 0.129 mg/kg, p,p'-DDT 0.022 mg/kg, p,p'-DDD 0.007 mg/kg, and o,p'-DDT <0.001 mg/kg. The results of individual samples ranged between 0.001 mg/kg and 2.490 mg/kg. Values of median and 90th percentile and 95th percentile were 0.057 mg/kg, 0.391 mg/kg, and 0.638 mg/kg, respectively. The mean concentration of PCBs in game fat was 0.014 mg/kg; the results ranged from 0.001 mg/kg to 0.164 mg/kg.

The highest concentrations of pesticides were detected in tissues of wild boars. Mean concentration of DDTs in the fat was 0.241 mg/kg (median 0.140 mg/kg). Concentrations in tissues of other game animals were several times lower and were on average 0.037 mg/kg (roe deer) and 0.032 mg/kg (red deer). Number of tested wild boars allowed to compare environmental

contamination by chlorinated compounds between regions of animals' origin. In tissues of wild boars collected in four industrial regions, the concentrations of DDTs were several times higher (mean 0.309 mg/kg) than in wild boar from the region of Warmia and Mazury (mean 0.071 mg/kg). The highest levels of DDTs were found in wild boars from the Legnica-Głogów Copper District.

Mean concentration of PCBs in the tissues of wild boar was 0.015 mg/kg fat, in red deer 0.013 mg/kg fat, and in roe deer 0.010 mg/kg fat. In tissues of wild

boars from industrial regions, few times higher concentrations of PCBs (mean 0.020 mg/kg) were detected comparing to wild boar from the Mazury and Warmia region (mean 0.004 mg/kg). The highest content of PCBs was found in the fat of wild boars from the Turoszów Brown Coal Basin (mean 0.032 mg/kg).

The percentage of p,p'-DDE in the total DDTs and the sum of PCB 153, 138, and 180 in the total PCBs was about 82% and 98%, respectively (Fig. 1).

Table 1
Concentrations of DDTs and PCBs in adipose tissue of game animals (mg/kg fat)

	Species	Number of samples	Mean	Min – Max	Median	90 th percentile	95 th percentile
DDTs	Wild boar	84	0.241	0.004 – 2.490	0.140	0.572	0.795
	Red deer	32	0.032	0.002 – 0.083	0.026	0.064	0.076
	Roe deer	25	0.037	0.001 – 0.141	0.018	0.089	0.129
PCBs	Wild boar	84	0.015	0 – 0.164	0.007	0.034	0.053
	Red deer	32	0.013	0.002 – 0.053	0.007	0.025	0.036
	Roe deer	25	0.010	0.001 – 0.036	0.005	0.025	0.027

Table 2
Concentrations of DDTs and PCBs in adipose tissue of wild boars from different regions (mg/kg fat)

	Regions	Number of samples	Mean	Min – Max	Median	90 th percentile	95 th percentile
DDTs	LGOM	27	0.439	0.107 – 2.490	0.240	0.829	1.397
	Bełchatów	6	0.353	0.082 – 1.010	0.264	0.714	0.862
	Bogatynia	23	0.189	0.014 – 0.764	0.142	0.310	0.574
	GOP	4	0.049	0.014 – 0.140	0.021	0.106	0.123
	Warmia and Mazury	24	0.071	0.004 – 0.391	0.037	0.193	0.206
PCBs	LGOM	27	0.011	0.001 – 0.046	0.008	0.028	0.037
	Bełchatów	6	0.009	0.003 – 0.028	0.004	0.019	0.023
	Bogatynia	23	0.032	0.002 – 0.164	0.018	0.106	0.116
	GOP	4	0.017	0.001 – 0.054	0.007	0.041	0.048
	Warmia and Mazury	24	0.004	0.001 – 0.017	0.003	0.009	0.011

Fig. 1. Percentage contribution of DDT and its metabolites and PCB congeners in adipose tissue of game animals

Discussion

In the previous monitoring studies conducted between 1995 and 2003 under the Programme of Polish Ministry of Agriculture and Rural Development, focusing on the quality of soils, plants, food, and agricultural products, 1,145 game animals were tested. Mean concentration of DDTs in the fat of wild boars was 0.501 mg/kg (1995-2003, 511 animals, median 0.272 mg/kg), in roe deer 0.042 mg/kg (1995-2003, 447 animals, median 0.023 mg/kg), and in red deer 0.029 mg/kg (1996-2000, 187 animals, median 0.024 mg/kg). Contents of DDTs exceeding MRL were identified in samples from 51 wild boars and one roe deer. Mean concentration of total PCBs (as Aroclor 1260) in the tissues of wild boars was 0.038 mg/kg fat, in roe deer 0.024 mg/kg fat, and in red deer 0.041 mg/kg fat. The concentration of organochlorine pesticides and PCBs did not differ significantly between 1995 and 2003. Results of monitoring from 1995 to 2003 were published in annual reports (8).

In Poland, the results of the long-term systematic studies have shown a significant decrease in the concentration of organochlorine pesticides and the trend of slow decline in the content of PCBs in farm animal tissues, milk, and eggs (11). In comparison with the results for game animals, obtained during these and other authors' investigations, the declines of the mean levels of chlorinated hydrocarbons have been also observed (12, 16, 17). It can be assumed that contamination of roe deer and red deer by DDTs are now at the levels of 0.02-0.04 mg/kg. It is obvious that tissues of wild boars contain higher levels of DDTs than those for other species of game animals. The levels of DDTs were probably related with the species feeding habits. Roe deer and red deer are herbivorous species. Wild boar is a typical omnivorous animal and gets feed mainly by digging the ground in forests. Soils are still contaminated with persistent chlorinated compounds (3, 4).

Comparison of the PCB results with the results of other authors appears to be complicated by the fact that the authors used a variety of analytical methods, different methods of sample preparation, and different ways to present the results (*e.g.*, based on fat or tissue). In addition, the results of occurrence and contents of non dioxin-like PCBs in animals are presented in a variety of ways: as a sum of seven indicator PCB congeners IUPAC numbered 28, 52, 101, 118, 138, 153, and 180 (earlier EU recommendation), a sum of six congeners (PCB 28, 52, 101, 138, 153, and 180 - EFSA and EU recommendation), a sum of three congeners (PCB 138, 153, and 180) and even the only PCB 153, a sum of higher than seven selected congeners or as total PCBs (as *e.g.* Aroclors) (5, 6, 7, 9). The six PCB indicators were found to represent around 50% of the sum of the total PCBs, which were measured in the food samples. Results of the study show that contamination of game animals (wild boar, red deer, and roe deer) by PCBs (sum of six PCB indicators) in Poland is on the low level of 0.01–0.02 mg/kg.

In order to protect consumers and to promote trade, maximum residue limits (MRLs) of pesticide residues in food of plant and animal origin are laid down in Regulation (EC) No 396/2005. MRLs in meat, expressed on fat bases of different species of farm animals for following pesticides have been established: DDTs (1 mg/kg fat), HCB (0.2 mg/kg), α -HCH (0.2 mg/kg), β -HCH (0.1 mg/kg), and γ -HCH (0.02 mg/kg). Among tested game animals, exceedance of MRLs was identified only in tissues of wild boars from industrial regions. Concentrations of DDTs exceeded the limit value in three wild boar samples and γ -HCH, and β -HCH in one sample. Maximum levels (ML) for the sum of six non-dioxin like PCB indicators in food are laid down in the Commission Regulation (EU) No 1259/2011. PCB concentrations above 0.04 mg/kg (ML for cattle, pigs, and poultry) were found in fat of two red deer and six wild boars from industrial regions.

According to the available literature, the presence of residues of PCBs and some OCPs in tissues of game animals are still found in many countries (2, 5, 6, 7, 10). Naccari *et al.* (9) studied the chlorinated hydrocarbon contamination of 154 samples from wild boars (heart, liver, lung, kidneys, muscle tissue, and spleen) from Calabria (south of Italy). The results have shown the presence of low residue concentration of DDTs only in eight livers, three kidneys, and one heart sample and lack of PCB (Aroclor 1232) residues. In another study from Italy, *p,p'*-DDE was detected in 95% and PCBs (seven PCB indicator congeners) in 97% of the liver, and perirenal fat samples from roe deer resident in a protected area of the Emilia-Romagna region (10). Mean organochlorine concentrations from this study were comparable with the results obtained from the terrestrial herbivorous mammals from other parts of the world. Still, the problems with PCB contamination of food of animal origin in the areas of their former production occur. In Brescia (Italy) a PCB production plant, operated from 1930 to 1984, polluted soil and forage of surrounding fields, and caused a significant contamination of meat and milk of the cattle fed local forage (13). The indicator PCB levels were about 100 times higher than the regional background. Another contamination case occurred in eastern Slovakia (7). In the Michalovce district, 25-years of the manufacture of PCB (Delors, from 1959 to 1984) resulted in increased environmental pollution of the surrounding area. Fish living in contaminated Michalovce waters contained about hundred times higher PCB levels than those captured in a control area. Similarly, game animals (deer, roe deer, wild boar, and duck) hunted in contaminated forests contained several times higher levels than those hunted in control area. The project's results have showed that the food and human population of the Michalovce district are much more contaminated with PCBs comparing to the rest of Slovakia.

In conclusion, the results of the study indicate that game animals from different parts of Poland have low contamination with the analysed chlorinated hydrocarbons. While assessing the results obtained for DDTs and PCBs in tissues of wild boars, red deer, and

roe deer it should be noted that the mean concentrations of these compounds are similar and sometimes even lower to the results obtained by other authors investigating the samples of the same species. Due to environmental contamination, wild living animals can contain higher levels of PCBs, organochlorine pesticides, and other chemical contaminants than farm animals. The contents of DDTs and PCBs in game meat are not a hazard and should be considered safe for consumers. Further investigations on free-living animals as indicators of environmental pollution by chlorinated hydrocarbons should be continued.

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