

Original Research

# Polychlorinated Biphenyl (PCBs) Residues in Suburban Red Foxes (*Vulpes vulpes*) – Preliminary Study

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## Abstract

The objective of our study was to determine residues of PCB indicator congeners in select organs of suburban foxes from the West Pomeranian area of Poland. Quantitative analysis was conducted by capillary gas chromatography using the mass spectrometry method. Mean concentrations of  $\Sigma$ PCBs (sum of PCBs: 28, 52, 101, 118, 138, 153, 180) in the liver and lung was 389.99 ng/g and 110.57 ng/g of lipid weight. The proportion of dominant congeners (#180, 153 and 138) in total PCB was 90.9% in the liver and 77.1% in lungs. The PCB concentrations obtained in the liver of foxes are lower than the literature data, especially with regard to highly chlorinated congeners.

**Keywords:** PCBs, biomonitoring, bioindicators, foxes, Poland

## Introduction

Polychlorinated biphenyls (PCBs) are a group of synthetic hydrocarbons, the production of which started on a commercial scale in 1929 in the United States. Due to their physical and chemical properties, they were used in the industry mainly as electrical insulating oils, cutting fluids, liquid heat exchangers, components of sealants and plasticizers, as well as performance chemicals for paints and lacquers, impregnated fabrics and fireproof boards, and pesticide carriers [1, 2]. Technical PCB preparations were produced globally in large amounts. In Poland, Chlorofen (1966-70) and Tarnol (1971-75) were manufactured for

several years [3]. By 1980, the global production of technical PCB preparations in countries of the Organization for Economic Co-operation and Development (OECD) was estimated to be 1.2-2.0 million tons [4, 5].

Long-term mass production and broad application of PCBs have contributed to environmental contamination by these compounds [6-10]. In the 1970s many countries abandoned the production of PCB preparations and/or restricted their use to closed systems when chlorobiphenyls were found to be considerably toxic, environmentally persistent, able to spread and contaminate ecosystems far away from emission sources, and capable of bioaccumulation. Despite the measures taken at the time, these compounds are still present in all environmental components [11-15].

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Polychlorinated biphenyls are a major group of anthropogenic contaminants that are harmful to both humans and animals. Health threats posed by exposure to these compounds result from their potential as endocrine and reproductive disruptors, teratogens, and carcinogens [16-19].

Wild terrestrial mammals such as roe deer, red deer, wild boars, wolves, and foxes are used as indicator organisms for biomonitoring industrial pollutants, including PCBs. Given the large territorial range of these animals, their role in identifying local pollution sources is small, but they provide important information about the degree of environmental pollution and potential threats. Scientific literature contains many reports of free-living ruminants [8, 20, 21], wolves [22, 23], mink [6], and foxes [11, 24-26]. These animals were used to evaluate current environmental contamination with polychlorinated biphenyls and to determine changing trends in PCB concentration and composition.

Not many such studies have been carried out in Poland. Research on PCB residues was conducted by Falandysz and Kannan in wild boars and roe deer [27], by Przybycin and Juszkiwicz in roe deer, red deer, and wild boars [28], by Szymczyk-Kobrzyńska and Zalewski in red deer [29], and by Zasadowski et al. in roe deer [7]. The available Polish literature provides no information about the use of foxes in PCB biomonitoring. Red foxes increasingly colonize suburban and urban habitats [30] and they are exposed to the same pollutants as the local human population. They are opportunistic predators, which means that they eat pretty much anything they can find. The stomachs of foxes from urban and suburban areas were found to contain human food scraps and other organic wastes, as well as garbage (empty food packages, paper, textiles) [31]. Contesse et al. [32] report that food scraps and garbage constituted more than half of the average stomach content of foxes from around Zürich. Dip et al. [26] indicate that urban and suburban foxes live in the same environment as humans and also share, in part, their diet. Therefore, we believe that as animals living in close vicinity to humans, they can be valuable bioindicators of contamination of the human environment, especially since, as reported by Corsolini et al. [25], the concentration of organochlorine compounds in fox tissues is similar to the level of these xenobiotics in humans.

The objective of our study was to determine indicator residues of PCB congeners in selected organs of suburban foxes from the West Pomeranian area of Poland.

## Materials and Methods

### Materials

The content of polychlorinated biphenyls was analyzed in the liver (n=19) and lungs (n=16) of 20 foxes (*Vulpes vulpes*), including 11 males and 9 females. The experimental foxes were shot by hunters in 2008 and 2009 or killed in road accidents near Szczecin. The collected organs were

homogenized and stored at -20°C until chemical analyses. The organs with gunshot injuries (bullet wounds) and impurities (shot pellets, soil, etc.) were not examined. The age of animals was not determined.

## Methods

### Chemical Analysis

Weighed amounts ( $\approx 15$  g) were triturated in a mortar with anhydrous sodium sulphate (Chempur). The extraction of analyzed congeners (#28, 52, 101, 118, 138, 153, and 180) with lipids was performed in a Soxhlet apparatus (6 h) using a 150 cm<sup>3</sup> mixture of n-hexane (Merck KGaA) and acetone (POCH) (v/v 1:2.5), and again a 50 cm<sup>3</sup> mixture of n-hexane and ethyl ether (POCH) (v/v 9:1). The extracts obtained were concentrated and the weight of lipids was determined gravimetrically. The content of test-tubes was then dissolved in 2 cm<sup>3</sup> n-hexane and purified by adding 6 cm<sup>3</sup> concentrated sulphuric acid (Merck KGaA). After layers' separation, the upper n-hexane layer was transferred to 8-cm<sup>3</sup> LiChrolut® glass columns filled with 1 g activated florisil (Sigma Aldrich) (120°C). The extracts were concentrated to a volume of 0.5 cm<sup>3</sup> and submitted to gas-liquid chromatographic separation using the method of capillary gas chromatography with mass spectrometry in Clarus 600 GC/MS Perkin Elmer, using an ELITE-5MS column (30 m×0.25 mm×0.5 µm). Chromatographic analysis was performed under the following conditions: column oven: 140°C (1 min.), increase rate 10°C/min. to 200°C (5 min.), increase rate 10°C/min. to 280°C (5 min.) and increase rate 10°C/min. to 300°C (15 min.). One sample was analyzed for 42 min. The flow rate of the carrier gas (helium) was 1 cm<sup>3</sup>/min. – splitless. Injection volume – 5 µl.

The analyzed PCB congeners were identified based on retention times of individual congeners and comparison of mass spectra with spectra of PCB congeners from the standard solution used (LGC Standards GmbH, Wesel, Germany). The accuracy of the analytical procedure was tested by determining the analyzed compounds in the reference material (ERM-BB445 LGC Standards GmbH, Germany), for which the concentrations were 71-92% of the reference value. For the calculation of means, samples with levels below the detection limit were assigned a value of one-half the detection limit. The limit of detection (LOD) for the analyzed compounds ranged from 0.03 to 0.05 ng/g.

### Statistical Analysis

Statistical analysis of the results was carried out using Statistica software (Statsoft Inc., ver. 7.1). Data distribution was evaluated using Shapiro and Wilk's W test. Differences in PCB concentrations in the analyzed organs between males and females were determined based on the Mann-Whitney U-test. Differences were considered significant at  $P < 0.01$ .

Table 1. Concentrations (ng/g lipids) of indicator PCBs in the liver and lungs of foxes.

PCBs	Liver (n=19)				Lungs (n=16)			
	Mean	Sd	Range	Median	Mean	Sd	Range	Median
All specimens								
#28	12.96	13.16	1.13-50.01	11.20	11.28	8.79	1.69-33.01	10.96
#52	2.17	1.83	0.30-7.43	1.85	2.82	2.07	0.28-6.54	2.71
#101	6.80	11.96	0.39-42.98	2.38	7.92	15.76	0.35-54.94	3.46
#118	4.37	7.01	0.13-26.42	1.50	3.26	2.89	0.24-8.95	2.96
#138	64.76	85.05	9.65-286.98	22.77	28.52	26.42	nd-83.26	21.95
#153	17.36	16.19	nd-53.32	7.40	13.61	13.03	1.76-47.80	9.52
#180	185.60	299.42	7.74-926.53	37.49	43.15	35.25	nd-117.41	30.63
∑PCB	289.99	393.18	38.65-1,199.46	110.50	110.57	51.42	29.98-187.65	106.90
Lipids [%]	2.98	1.77	1.02-7.11	2.71	3.01	1.96	1.01-6.12	2.93
Female								
#28	10.08	8.19	3.61-23.55	7.03	7.21	4.10	1.69-10.96	7.40
#52	1.29	0.76	0.30-2.22	1.16	2.32	1.69	0.46-4.14	1.79
#101	3.45	2.74	0.50-7.88	2.82	2.88	2.01	0.64-5.56	3.46
#118	1.63	1.97	0.41-5.07	0.61	2.08	2.25	0.30-5.64	0.88
#138	45.86	40.15	13.65-99.26	21.40	20.09	13.9	nd-38.03	24.52
#153	4.93	2.65	nd-7.40	6.27	8.04	5.31	1.76-11.69	7.79
#180	121.95	132.28	nd-395.07	40.24	27.03	21.67	nd-62.24	17.58
∑PCB	189.19	161.46	53.10-443.63	117.89	69.66	33.72	29.98-106.90	62.58
Male								
#28	14.76	12.78	1.13-50.01	13.39	14.68	10.52	4.23-33.01	14.78
#52	2.67	2.14	0.53-7.43	2.05	3.25	2.41	0.28-6.54	2.74
#101	8.89	15.10	0.39-42.98	1.92	12.13	16.15	0.35-54.94	3.55
#118	6.08	8.57	0.13-26.42	3.84	4.24	3.18	0.24-8.95	3.83
#138	76.58	95.18	9.65-286.98	25.50	35.54	30.13	11.12-83.26	21.99
#153	18.63	16.04	1.55-53.32	10.79	18.25	16.14	3.80-47.80	13.06
#180	225.39	266.27	7.74-926.53	33.20	56.57	33.20	24.19-117.41	50.27
∑PCB	352.99	488.17	38.65-999.46	103.03	144.66	36.19	102.45-187.65	146.91

n.d. – not detected

## Results and Discussion

The high capacity of PCBs for bioaccumulation and their ability to produce harmful effects made it necessary to monitor the content of these compounds in biotic and abiotic environments. In this study, we determined the content of indicator PCBs, i.e. congeners most widespread in the environment and in human and animal tissues [33]. The determinations were made on target organs, namely the liver (mainly for higher chlorinated PCBs; [34]) and lungs

(for low chlorinated PCBs; [21]) of the foxes. These organs were chosen because of their role in PCB metabolism and their capacity to accumulate these compounds. In general, the liver represents a depot organ for all PCBs, whereas lungs are a target organ for bioaccumulation of low chlorinated PCB congeners. These compounds reach lungs through both the blood stream and the respiratory passages, and are distributed throughout the lung parenchyma [35, 36].

The results of these determinations are shown in Table 1.

Table 2. Concentration (ng/g lipids) of PCB in muscle and adipose tissue of foxes.

	PCB, ng/g lipids								References
	#28	#52	#101	#118	#138	#153	#180	ΣPCB	
Muscle tissue	–	–	–	0.4-2.1	0.9-2.6	0.3-1.8	0.8-5.7	20.2	Corsolini et al. [25] Italy*
Adipose tissue	–	–	–	0.07-0.8	0.1-1.0	1.2-5.6	0.5-1.3	7.2-38.0	
Adipose tissue	–	–	–	–	3.9	10	18	50	Hoshi et al. [11] Japan**
Adipose tissue	5-26	6-47	15-328	–	66-711	163-1544	216-596	–	Georgii et al. [24] Germany
Adipose tissue	6	–	7	–	–	–	–	199 <sup>A</sup>	Dip et al. [26] Switzerland

\*data expressed in µg/g lipids

\*\*data expressed in ng/g wet weight

<sup>A</sup>-ΣPCB (138, 153, 180)

In the majority of studies that have used foxes as bioindicators, chemical analysis was made of the adipose tissue of these animals [11, 24-26, 37] (Table 2), which makes it difficult to compare them with our findings. Only Bachour et al. [21] determined the concentration of indicator PCBs in the liver of red foxes and obtained several to several dozen-fold higher concentrations of these compounds compared to our study. The only exception was PCB 28, the concentration of which was comparable. The largest differences concerned mainly PCB 153 as well as 180 and 138. In our study, the concentration of these congeners in the livers of foxes from the Szczecin area was 59-, 9-, and 8-fold lower, respectively.

Wang-Andersen et al. [38] determined levels and congener patterns of PCBs in arctic foxes from Svalbard (Norway). They reported that mean hepatic PCB concentration in foxes was 0.4 µg/g.

The concentration of PCBs in the adipose tissue of foxes from different countries of Europe varies within wide limits. The highest concentration of total PCBs was found in foxes from Italy (7.2-38 µg/g) [25], compared to 0.199 µg/g in suburban foxes from Switzerland [26].

When comparing the content of different organochlorine compounds in the adipose tissue of foxes from the urban centre, suburban and rural surroundings, Dip et al. [26] found the highest concentrations of PCB 138 and 153 in urban foxes, followed by suburban animals. The lowest concentration of these compounds was observed in foxes living in rural surroundings. PCB 180 occurred in the highest concentration in suburban foxes. These authors also observed that out of 192 animals, PCB 28 was found only in two foxes that came from suburban areas and PCB 52 was not detected. In our study, congeners 28 and 52 were detected in all animals.

The degree of bioaccumulation of individual PCB congeners in animal tissues varies due to differences in their physicochemical properties, bioavailability and environmental half-life. On the other hand, of primary importance is also the efficiency of physiological and biochemical processes determining the intake, distribution and elimination of these xenobiotics from the body. PCB 138, PCB 153, and PCB 180 are thought to be the dominant congeners in biological samples. In most animals they are

poorly metabolized and thus extensively accumulated in tissues [18]. In our study we indeed found PCB 138, 153, and 180 were the dominant congeners in the liver of foxes, but the highest concentrations in lungs were found for PCB 138 and 180 (Fig. 1). The proportion of dominant congeners (#180, 153, and 138) in total PCB mass (defined as the sum of seven indicator PCB) was 90.9% in the liver (64%, 4.6%, and 22.3%, respectively) and 77.1% in lungs (39%, 12.3%, and 25.8%, respectively). A similar relationship was observed by Corsolini et al. [37] and Hoshi et al. [11] in the adipose tissue of foxes, although the percentage of these congeners was much lower than in our study. Wang-Andersen et al. [38] reported that PCB 138 and 153 accounted for 65% of total PCBs in arctic foxes.

It is worth noting that in the lungs of foxes, the proportion of PCB 28 (trichlorobiphenyl) in ΣPCB was only slightly lower than that of PCB 153 (hexachlorobiphenyl) (10.2% vs. 12.3%). In general, the proportion of PCB 28 in ΣPCB is at least several times lower compared to PCB 153. As reported by Bachour et al. [21], the comparatively high proportion of low-chlorinated biphenyls in lungs may be explained by increased environmental concentration of low chlorinated mixtures and increased inhalation of these compounds from the atmosphere. In the recent years when tech-

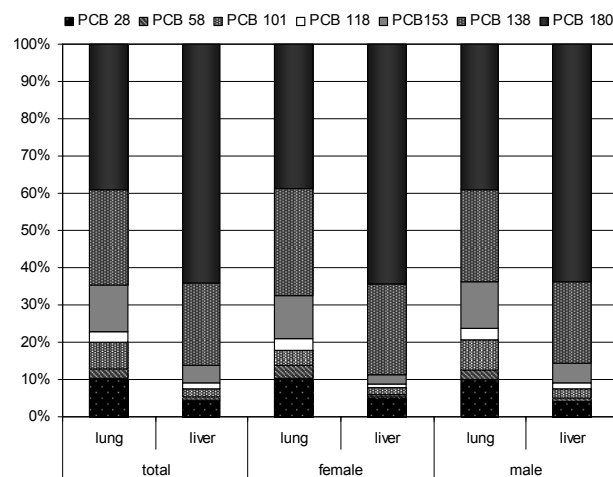


Fig. 1. Percentage of analyzed congeners in ΣPCB (sum of seven indicator PCB) in the analyzed organs of foxes.



nical preparations of polychlorinated biphenyls were used, only the mixtures of low chlorinated congeners were allowed, which could be reflected in the PCB profile of environmental matrices. A higher proportion of low chlorinated biphenyls in lungs Bachour et al. [21] observed also in humans (Germany). Furthermore, Georgii et al. [24] found that over a period of eight years (until 1991) there was a significant increase in the concentration of low chlorinated PCBs, including PCB 28, PCB 52, and PCB 101, in the adipose tissue of foxes from the area of Germany paralleled by a reduction in the concentration of highly chlorinated congeners. The same authors attributed this state of affairs to the fact that the use of Clophen A30, a preparation containing low chlorinated congeners, increased considerably after 1980, whereas the industrial application of Clophen A60 containing highly chlorinated chlorobiphenyls was banned. Lower chlorinated PCBs (mainly tri-, tetra-, and pentachlorobiphenyls) may also originate from the processing and recycling of paper (e.g. magazine covers) [2]. Paper mills are located both in Szczecin and in its surroundings (Schwedt in Germany). While production in the Polish plant was stopped in 2007, the Schwedt mill, situated 50 km away from Szczecin, has 4 independent paper manufacturing and processing factories, with high-quality paper obtained exclusively from waster paper and used for printing newspapers.

Our study also showed that the proportion of highly chlorinated PCBs in the lungs of foxes decreased in relation to the liver in favor of the congeners with a smaller number of chlorine atoms in the biphenyl molecule, in particular PCB 28 and PCB 101 (Fig. 1).

Despite the fact that the liver is a target (and storage) organ for polychlorinated biphenyls, especially those with a higher degree of chlorination [34], our study demonstrated that the liver had only a slightly higher concentration of PCBs than lungs (except PCB 101) (Table 1), and the differences between the organs were not significant ( $P < 0.01$ ). Although the mean concentration of  $\Sigma$ PCB in the liver was twice that in lungs (289.99 vs. 110.57 ng/g lipids), the median was similar at 110.50 and 106.90 ng/g lipids, respectively. However, when comparing the concentration of individual congeners according to sex, we observed that the concentration of PCB 52, 118, and 153 in the lungs of females was higher than in the liver. Many authors suggested that sex plays a considerable role in the bioaccumulation of polychlorinated biphenyls [8, 26, 37]. Most researchers attributed these differences between males and females to the possibility of eliminating part of the PCB pool accumulated in tissues during gestation and lactation. This is supported by studies in which PCB concentrations were found to be higher in young than adult animals [26, 37]. However, Corsolini et al. [37] suggest that there must be an additional mechanism associated with the intake and metabolism of these compounds in female foxes. This results from the observation of differences between males and females, although most of the analyzed females still had no offspring. It therefore seems that male foxes are better bioindicators. In our study, male foxes had a higher concentration of individual PCB congeners in relation to

females, but statistically significant differences were only found for PCB 153 and  $\Sigma$ PCB ( $P=0.0083$  and  $P=0.0057$ , respectively). Wang-Andersen et al. [38] found no significant differences in PCB levels between different sexes of arctic foxes.

Similar to Georgii et al. [24], we observed the concentration of PCB 180 (2,2',3,4,4',5,5'-heptachlorobiphenyl) in fox organs to be several-fold higher than that of PCB 153 (2,2',4,4',5,5'-hexachlorobiphenyl). The same authors believe this may result from the capacity for selective transport and/or metabolism of some polychlorinated biphenyls, including PCB 153. Foxes are thought to have cytochrome P-450 isoenzymes that allow for metabolism of chlorobiphenyls with chlorine atoms occupying positions 2, 4 and 5. Because PCB 180 has an additional chlorine atom at position 3 (meta), it shows a higher degree of bioaccumulation compared to PCB 153.

## Conclusions

Based on the results obtained, it is concluded that the area from which the foxes originated is contaminated with polychlorinated biphenyls to a slight extent. The concentrations of the analyzed compounds obtained in the liver of foxes are considerably lower than the literature data, especially with regard to highly chlorinated congeners. The relatively small differences between the content of hexa- and heptachlorobiphenyls and low chlorinated PCBs may indicate that the surroundings of Szczecin were or still are affected mainly by low-chlorinated PCBs. Changes involving a reduction of environmental contamination with highly-chlorinated congeners and a simultaneous increase in the concentration of low chlorinated mixtures were observed in Germany by Georgii et al. [24]. Further studies involving a larger group of animals investigated on a long-term basis are needed to conclusively determine the changing trends.

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