



Determination of perfluorinated substances (PFAS) in muscle tissue from wild boar from three districts of Serbia – Zlatibor, Pčinj and Raška

Ognjen Krnjaja¹ , Nikola Borjan¹ , Milenko Babić¹ , Damjan Gavrilović¹ , Aleksandar Bajčić¹ , Branka Borović¹  and Jasna Đinović-Stojanović¹ 

¹ Institute of Meat and Hygiene and Technology, Kačanskog 13, 11000 Beograd, Serbia

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are recognized as significant environmental pollutants due to their widespread industrial use and persistence. Wild boars are known bioindicators of environmental contamination with PFAS, as they accumulate these compounds through their omnivorous diet and foraging behaviour. In this study, muscle tissue samples (n = 15) from 15 respective wild boars were collected during the 2024–2025 hunting season as part of the Serbian National Residue Monitoring Program. Samples were obtained from three Serbian districts—Zlatibor, Pčinj, and Raška—and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). PFAS compounds were detected in 9 of the 15 wild boars, with total PFAS concentrations ranging from <0.170 to 3.179 µg/kg and an average concentration of 1.030 µg/kg. The results suggest regional differences in PFAS exposure and confirm the suitability of wild boar as a bioindicator species for PFAS contamination in Serbia.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are anthropogenic synthetic chemicals, that number over 4,000, and they are used extensively throughout the world in many industrial sectors. They are resistant to degradation at high temperatures, and are water, oil and dirt repellent (Buck *et al.*, 2011). Also, they resist biodegradation, photooxidation, and hydrolysis due to the strength of the carbon-fluorine bond (Sznajder-Katarzyńska *et al.*, 2019).

In the latest scientific opinion of the European Food Safety Agency (EFSA), four PFAS com-

pounds were assessed as toxic—perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexanesulfonic acid (PFHxS) and perfluorooctanesulfonic acid (PFOS). This requires countries to eliminate or reduce the release of these chemicals into the environment. (European Food Safety Authority, 2020).

Because wild boars are omnivores and search for food by burrowing into the ground, it is assumed that wild boar meat is most likely contaminated with PFAS compounds (Food and Agriculture Organization of the United Nations, 2018). Over the past twenty years, various studies have shown high lev-

*Corresponding author: Ognjen Krnjaja, ognjen.krnjaja@inmes.rs

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els of PFAS compounds in the tissues and organs of wild boars across Europe, especially in the regions of Central Europe (Kowalczyk *et al.*, 2018; Stahl, *et al.*, 2012; Felder, *et al.*, 2023; Schröder *et al.*, 2024; Arioli *et al.*, 2019; Death *et al.*, 2021). In Hesse, Germany, 506 muscle tissue samples from wild boars were tested for the presence of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). PFOA concentrations $\leq 7.4 \mu\text{g/kg}$ and PFOS concentrations $\leq 28.6 \mu\text{g/kg}$ were detected in muscle tissue. The concentration of PFOA was lower than the LOQ of $1 \mu\text{g/kg}$ in 456 samples (90.1%), a majority of all of the samples examined. PFOS concentrations lower than the LOQ were found in 329 of the 506 (65%) wild boar muscle samples. In 2019, PFOS was detected in 25% of muscle samples from wild boar collected in North West Italy at concentrations lower than those reported from Germany (Arioli *et al.*, 2019).

In Regulation of the European Union Commission from December 7 2022 (European Commission, 2022), the maximum permissible amounts for four PFAS compounds (PFOS, PFOA, PFNA, PFHxS) in meat of wild animals (except bear meat) were prescribed individually as well as the aggregate value, and they are an integral part of the regulations of the Republic of Serbia (Official Gazette of the Republic of Serbia, 2024). The maximum levels (applies to the sum of linear and branched stereoisomers, whether they are chromatographically separated or not) in meat of game animals are: PFOS – $5.0 \mu\text{g/kg}$, PFOA – $3.5 \mu\text{g/kg}$, PFNA – $1.5 \mu\text{g/kg}$, PFHxS – $0.6 \mu\text{g/kg}$ and sum of all four PFAS – $9.0 \mu\text{g/kg}$. For the sum of PFOS, PFOA, PFNA and PFHxS, lower bound concentrations are calculated on the assumption that all the values below the limit of quantification are zero.

The aim of this study was to determine the concentration of four PFAS compound (PFOS, PFOA, PFNA and PFHxS) in tissue (leg muscle) of wild boars in three districts of Serbia, Zlatibor, Pčinj and Raška, and compare the results. Zlatibor and Raška districts border each other, while the Pčinj district is about 300 km away from Zlatibor. This is the first study in Serbia to examine the concentration of PFAS in game meat.

2. Materials and methods

2.1. Sampling

PFAS levels were measured in tissue samples from wild boars in the period of one hunting sea-

son, within the framework of the Serbian National Residue Monitoring Program (autumn-winter 2024–2025). The total number of wild boars analysed was 15, with one tissue sample per animal. Wild boar tissue samples were stored at -18°C . Frozen samples were thawed at 4°C one day before the analysis and subsequently homogenized.

2.2. Reagents and standards

All solvents used were of HPLC grade—acetonitrile (Honeywell, Germany), methanol (Honeywell, Germany), water (Honeywell, Germany), ammonium acetate (Sigma Aldrich, Germany) and a QuEChERS kit (Phenomenex, USA). PFAS standards were of analytical purity manufactured by Wellington Laboratories Inc., Canada. Native Perfluorinated Compound Solution/Mixture was used as a calibration standard and Mass-Labelled PFAS Extraction Standards Solution was used as an internal standard. As a control, FAPAS QC Material /Fish/T0687QC and FAPAS QC Material /Dried Egg/T06142QC (FAPAS, UK), were used.

2.3. Sample preparation and measurement

During the development of the method and sample extraction plan, the guidelines stated in the works that dealt with the topic of PFAS compound analysis in samples of animal origin were taken into account (EURL for Halogenated POPs in Feed and Food, 2022; Kowalczyk *et al.*, 2018). Homogenized sample was weighed into a polypropylene centrifuge tube. Acetonitrile and internal working standard were added. The tube was centrifuged and the entire supernatant was transferred to a polypropylene tube. QuEChERS were added, and the tube was shaken vigorously. The tube was again centrifuged and the supernatant was transferred to a glass cuvette, and evaporated to dryness under a stream of nitrogen at 50°C . The dry residue was dissolved in acetonitrile and vortexed. The sample was transferred to a polypropylene cuvette and centrifuged. The supernatant was transferred to an HPLC vial.

2.4. Instrumental analysis

Boar tissue samples, which were previously determined to be free of PFAS compounds, were used as blank samples, calibration and spiking. Four blank samples of muscle tissue were spiked with PFAS calibration working standard, within the cali-

bration range of 0.1 to 5 µg/L. One blank sample was spiked with PFAS calibration working standard corresponding to concentrations of 2 µg/L (QC spike).

Analysis was performed by LC-MS/MS with a mass detector, using an HPLC device and a mass detector LCMS-8050 (Shimadzu, Japan). LC-MS/MS system equipped with RP Kinetex C18 column 100 mm × 2.1 mm, particle size 2.6 µm, and PFAS Delay Column Restek 50 × 2.1 mm, particle size 5 µm. The chromatographic separation was performed on a gradient with 2mM ammonium acetate in water/acetonitrile, 95%:5%, v/v, and 10% of acetonitrile/methanol, 60:40, v/v, as mobile phases.

2.5. Quality control

The results for QC materials were within the range of certified values, and the results for QC spike were within the range of 80-120%. The limit of quantification (LOQ) obtained in the validation process was 0.1 µg/kg.

3. Results and discussion

Tissue samples from Zlatibor District (numbers 1 to 6) and Pčinj District (numbers 7 to 12) came

from sows about 4-5 years old and that weighed between 70 and 80 kg. Tissue samples from Raška District (numbers 13 to 15) came from young wild boars, about 2 years old and that weighed around 40 kg. The results are presented in Table 1.

In samples 1, 2, 3 and 6 from Zlatibor District, only PFOS was detected out of the four PFAS compounds. Accordingly, concentrations of PFOS in these samples was equal to the concentrations of total PFAS, and they ranged from 0.170 µg/kg to 0.267 µg/kg. In samples 4 and 5, no PFAS compounds were detected.

In the samples from Pčinj District, PFOS was detected in samples 7, 8, 9, 11 and 12, PFOA in samples 7, 8, 9 and 11, and PFNA in samples 7, 9 and 11. PFHxS was not detected in any wild boar from Pčinj District. The concentrations of total PFAS in tissue samples from Pčinj District were higher than in those from Zlatibor District, due to the higher concentration of PFOS that ranged from 0.216 µg/kg to 0.675 µg/kg, but also because of presence of PFOA and PFNA that were not detected in the wild boar from Zlatibor District (Figure 1). In wild boar from Pčinj District, the concentration of PFOA was the highest of all the PFAS compounds, as it ranged from 0.355 µg/kg to 1.909 µg/kg, and it had the greatest impact

Table 1. PFAS concentrations in tissue of wild boars from three districts in Serbia. Perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexanesulfonic acid (PFHxS) and perfluorooctanesulfonic acid (PFOS). ND = not detected.

District	Wild boar tissue sample number	Concentration in µg/kg				
		PFOS	PFOA	PFNA	PFHxS	Total PFAS
Zlatibor	1	0.170	ND	ND	ND	0.170
	2	0.183	ND	ND	ND	0.183
	3	0.267	ND	ND	ND	0.267
	4	ND	ND	ND	ND	ND
	5	ND	ND	ND	ND	ND
	6	0.207	ND	ND	ND	0.207
Pčinj	7	0.586	1.706	0.662	ND	2.954
	8	0.216	0.355	ND	ND	0.571
	9	0.234	0.450	0.160	ND	1.415
	10	ND	ND	ND	ND	ND
	11	0.675	1.909	0.595	ND	3.179
	12	0.321	ND	ND	ND	0.321
Raška	13	ND	ND	ND	ND	ND
	14	ND	ND	ND	ND	ND
	15	ND	ND	ND	ND	ND

of the four compounds on the high concentration of total PFAS in these game animals. The concentration of PFNA ranged from 0.160 µg/kg to 0.662 µg/kg. In samples 7, 9 and 11 (from Pčinj District), all three PFAS compounds were detected. The concentration trend was PFOA > PFOS > PFNA, except for

sample 7, in which the concentration of PFNA was higher than that of PFOS and accordingly, for this wild boar tissue, the concentration trend was PFOA > PFNA > PFOS.

In wild boar tissue from Raška District, no PFAS compounds were detected.

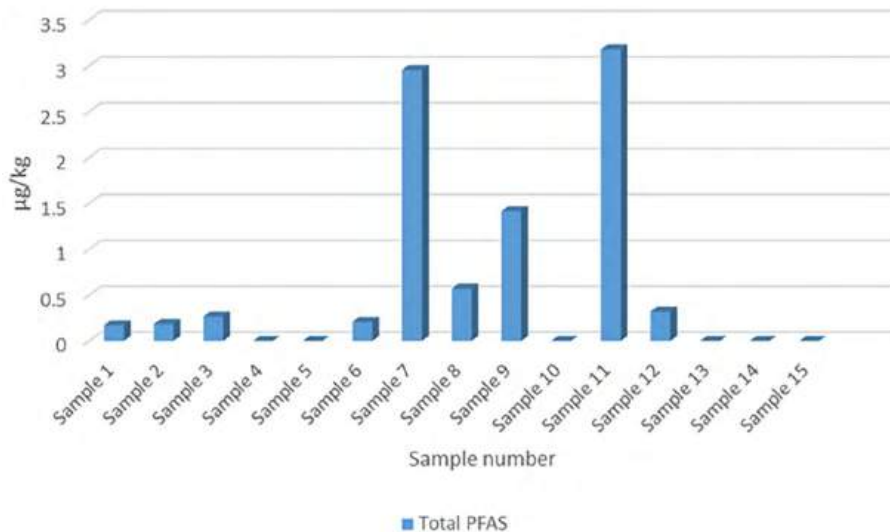


Figure 1. Total PFAS concentrations in wild boar from three districts in Serbia

4. Conclusion

The present study on wild boar leg tissue suggests that wild boars living in Serbia are exposed to PFAS. All obtained concentrations of PFAS compounds were within the limits prescribed by European and Serbian regulatory authorities. Comparing the districts, the highest concentration of PFAS compounds in the boar tissue was found in Pčinj District. Among these samples from Pčinj District, PFOA had the highest concentration of the four studied PFAS compounds. The higher concentration of PFAS compounds in Pčinj District compared to in Zlatibor District can be attributed to greater environmental pollution, due to a higher concentration

of industrial plants and larger settlements in the former location.

The absence of PFAS compounds in wild boar from Raška District can be explained by the fact that the wild boars from which the samples were taken were younger than those from Zlatibor and Pčinj Districts, rather than by the absence of environmental pollution.

This study shows that the wild boar can be used as bioindicators of environmental pollution with PFAS in Serbia in future research. The results so far indicate the need for continued monitoring of PFAS compound concentrations in the game meat.

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Authors info

Ognjen Krnjaja, <https://orcid.org/0009-0005-2242-0457>

Nikola Borjan, <https://orcid.org/0000-0003-4067-3755>

Milenko Babić, <https://orcid.org/0009-0006-6467-6757>

Damjan Gavrilović, <https://orcid.org/0009-0000-3786-496X>

Aleksandar Bajčić, <https://orcid.org/0000-0003-2923-4137>

Branka Borović, <https://orcid.org/0000-0003-0371-0991>

Jasna Đinović-Stojanović, <https://orcid.org/0000-0003-4602-0835>