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OCCURRENCE OF SELECTED PERFLUOROACIDS IN MUSCLE AND LIVER FROM WILD BOAR: RELEVANCE FOR FOOD SAFETY/FOOD SECURITY ISSUES

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Introduction

Wild game and feral fish have been described as sentinel of the overall environmental quality, as matter of their exposure to persistent organic pollutants (POPs) present in environmental matrices. Due to the POPs bio-accumulative behavior, the regular consumption of muscle and offals from such food resource may determine exposure progressively approaching the tolerable daily intakes (TDI), as guidance values for chronic toxicity [1]. Previous papers demonstrated the presence of bioaccumulative perfluoroacids (PFAAs), such as perfluoroctanoic-carboxylic (PFOA) and- sulfonic (PFOS) acids in feral fish [2] and wild games [3]: owing to the above, anglers and shooters may be reasonably considered as sensitive group for their food habits. Specific environmental quality standards have been fixed for PFOS and its salts in freshwater (0.65 ng/L) and related biota (9.1 ng/g fresh weight) by the European Legislation (Directive 2013/39/EU) [4], to prevent unacceptable intakes via fish consumption. In this paper we wish to describe the occurrence of C4 – C13 perfluorcarboxylic acids (PFCAs) and C4 – C8 perfluorosulfonic acids (PFSAs), in Italian wild boar, along with the food safety and food security issues related to the intake of muscle and liver by Italian shooters, upon the availability of the results of a targeted Food Frequency Questionnaire delivered to a such sensitive group.

Materials and methods

Muscle (N = 79, as diaphram, tongue) and liver (N = 62) of wild boar were drawn from officially appointed centers in charge to manage the carcass of stalked animals, to allow the place on the market of such food items with respect to the presence of Trichina spp (muscle), and to monitor the presence of hog cholera virus among wildlife (liver). The animals were representative of the Northern, Central, and Southern Italy. Determination were performed according to the isotope dilution method with labelled mixture provided by Wellington Labs (Chemical Research 2000, Rome, Italy), starting from 1 g as test portion, minced in 20 mL MeOH, sonicated, centrifuged at 3600 x g, 20 min, + 4° C. An aliquot of 10 mL of the supernatant, diluted 1:1 (v/v) with water 0.1% formic acid was cleaned up on SPE cartridges 6 mL 150 mg Oasis WAX (Waters), according to Stahl et al.[4]. The extracts were reduced to a final volume of 100 µl and 10 µl were injected into the UPLC-MS/MS (Acquity UPLC I Class provided with the PFC kit, coupled with a Quattro Premier XE Triple Quadrupole instrument, Waters, Milford, MA, USA) using a Luna® 3 µm C18 100 Å, LC Column 100 x 2 mm (Phenomenex Italia. QA/QC was represented by solvent and procedural blanks to control the release of PFAS from solvents, tubing and disposable materials, and from food samples with a consensus value to monitor method performances. Acquisition was performed in ESI negative ion, with a Limit of Determination of 0.50 ng/g fixed on the basis of the lowest point of the calibration curve where the noise from procedural blanks as average of different analytical session (N = 10) accounted less than 10% of the peak area. Interferences due to the presence of biliary salts were overcome with the transitions m/z 499/99 as qualifier and m/z 499/80 as quantifier for PFOS. The resolution between linear and non-linear PFOS isomers was acquired, but not shown in this paper as well the muscle/liver pair correlation, when available from the same animals. The consumption of wild boar muscle and liver was derived from a food frequency questionnaire, delivered to 766 Italian shooters. Consumers of wild boar muscle (positive answers N = 354) indicated a mean and a maximum amount of 6 and of 100 g/person/day, while those of wild boar liver (positive answers N = 117), ranged from 3 (mean) to 33 (maximum) g/person day. PFOS and PFOA intakes have been computed on deterministic basis according to the average and worst case scenarios, and expressed as ng/kg bw/day assuming a default value of 64 kg bw for adults, for appropriate comparison with the proposed TDI of 150 and 1,500 ng/kg bw/day for PFOS and PFOA, respectively identified by EFSA [5] on dislipemia and liver enlargement end-points, and with the RfD of 20 and 30 ng/kg bw /day, respectively recently

proposed by US EPA accounting for developmental toxicity [6] within the frame of health advisories for PFOS an POA in drinking water.

Results and discussion

Figure 1 shows the percentage of left censored data on the total of muscle (N =79) and liver (N = 62) samples analyzed. In Table 1 we report the occurrence descriptors of selected PFAAs in muscle and liver samples from wild boar: statistics were performed on determined data, only. In Figure 2 the PFOS and PFOA intake estimates are exemplified under the average and worst case scenario.

The analysis of the occurrences reported in Table 1 indicates that PFOA only among PFCAs, and PFBS and PFOS, among PFSAs show a determination rate above the 90% of the muscle samples. The almost constant presence of a short chained PFBS, not reported as bio-accumulative, suggests a wide environmental presence of such compound, as matter of an extended environmental release (Figure 1). The median and maximum concentration of PFOA (2.75 – 15.9 ng/g) and of PFOS (2.47 – 12.8 ng/g) resulted in the same order of magnitude of those reported in wild boar in Germany by Stahl et al., (2012). In liver, PFOS has been determined in all samples, as matter of its strong bio-availability and bio-accumulative behavior, followed by PFOA, and PFNA (Figure 1). In terms of concentration, median and maximum values of PFOA (6.7- 39 ng/g and PFOS (95 – 397 ng/g) again are rather in line with the literature data from Stahl el al., [3] (3.5 – 45 ng/g for PFOA, 51 – 1,780 ng/g for PFOS). It is worth noting that liver is able to mark better than muscle the presence of C9 - C13 PFCAs, that despite a reduced bioavailability with respect to PFOA as matter of the longer aliphatic chain, are also reported to bio-accumulate. This may indicate the presence of environmental spots characterized by the presence of PFCAs precursors, intercepted by wild boars as degradation products, and/or the possibly boars could metabolize precursors into PFOA.

The ratio between muscle and liver concentration computed on mean and maximum concentrations (0.03 – 0.03 for PFOS, 0.51-0.40 for PFOA) clearly discriminate the bio-accumulative behavior of PFOS in such organ. The concentration found in wild boar are well above those recently reported by Zefeiraki et al.,[7] in farmed pig liver with the highest concentration set at 4.2 ng/g fresh weight.

In terms of intakes, on average scenarios, the muscle and liver from wild boar is no able itself to determine exposures of relevance for PFOA. In the case of PFOS, with respect to the baseline intake of the Italian general population – adults (average = 0.50; P95 = 1.48, expressed as ng/kg bw, upper-bound) [8], the daily intake of 3 g of boar liver may rise the external dose up to 4.45 ng/kg bw/day under the average scenario, while under the worst case, there is a trespass of the EFSA TDI fixed at 150 ng/kg/bw/day (Figure 2). Nevertheless, such intake estimation should be integrated with other contribution coming from other food items such as fish and eggs, nown to contribute to the alimentary exposure in a relevant way. To conclude wild boar represents a sentinel of the overall quality of the environment, and the monitoring of time trends with respect to PFAS occurrence in such species may be of interest both for the environment and for the food safety/food security issues linked to the sociology of hunting.

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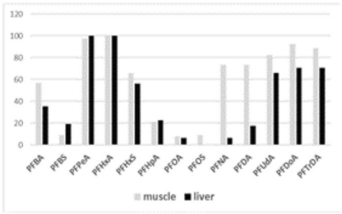

Figure 1. Percentage of left censored data (<0.50 ng/g) in wild boar muscle (N = 79) and liver (N = 62) for the considered C4 – C13 PFAAs.

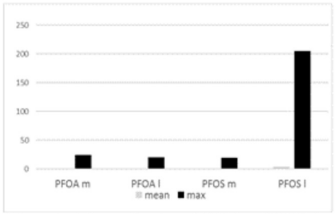

Figure 2. Estimated intakes of PFOA and PFOS from muscle (m) and liver (l) under the average and worst case scenario in Italian shooters. Values expressed as ng/kg bw per day, computed on an adults of 64 kg bw.

Table 1. Occurrence descriptors of quantified C4 – C13 PFCAs and of C4-C8 PFSAs in muscle (tongue, diaphram) and liver of Italian wild boars. NA = Not Applicable.

	Carbon lenght	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C4	C6	C8
matrix		PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUdA	PFDoA	PFTrDA	PFBS	PFHxS	PFOS
muscle	mean	1.78	1.02	NA	1.71	3.43	1.24	1.94	1.29	2.83	1.64	2.08	1.20	3.04
	P50	1.40	NA	NA	1.64	2.75	0.96	2.11	1.19	NA	1.59	1.38	1.16	2.47
	min	0.99	0.94	<0.50	1.09	1.56	0.40	1.11	0.66	1.20	1.40	0.89	1.06	0.92
	max	7.37	1.10	<0.50	2.68	15.6	3.89	3.70	2.11	7.16	2.05	18.4	1.54	12.8
liver	mean	3.22	1.49	NA	2.61	6.70	19.9	9.01	5.56	8.67	9.34	1.59	2.06	94.5
	P50	2.12	1.16	NA	1.91	4.96	10.1	8.32	4.83	5.70	4.89	1.34	1.67	83.1
	min	1.05	0.85	<0.50	1.10	6.04	13.5	0.79	1.50	1.70	1.70	0.90	1.14	9.10
	max	18.2	3.14	<0.50	10.1	1.10	1.20	93.2	38.6	14.9	27.3	5.50	4.11	397