

Full Length Research Paper

Perfluorooctanoic acid and perfluorooctane sulfonate in Nile Perch and tilapia from gulf of Lake Victoria

Francis Orata^{1,2*}, Quinete Natalia¹, Maes Anke¹, Werres Friedrich³, Wilken Rolf-Dieter^{1,2}

¹IWW Rheinisch-Westfälisches Institut für Wasserforschung gemeinnützige GmbH

²Institute of Geowissenschaften, Johannes Gutenberg-Universität, Joachim-Becher-Weg 21; DE-55099 Mainz.

³IWW Rheinisch-westfälisches Institut für Wasser, Moritzstr. 26 D-45476 Mülheim an der Ruhr.

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A report of the levels of Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA) in the fish from Lake Victoria is presented. Two fish species namely *Lates niloticus* (Nile perch) and *Oreochromis niloticus* (Nile tilapia) were obtained from Winam gulf of Lake Victoria, Kenya and analysed for PFOS and PFOA in muscles and liver using LC/MS/MS. Concentrations value of PFOS in Nile perch muscles of up to 10.50 and 35.70 ng/g for liver samples were obtained. Nile tilapia concentration values were of up to 12.40 and 23.70 ng/g for muscles and liver samples respectively. The accuracy and precision of the method were validated, and the effectiveness of the method in determining the contents of these two perfluorinated compounds in fish matrice was also demonstrated. The lowest limit of quantification (LOQ) was 0.5 ng/g and limit of detection was 0.05 ng/g. Typical values for precision obtained were 0.15 – 3.8% for HPLC/MS/MS, with concentrations ranging from 0.5 to 1000 ng/ml.

Key words: PFOS, PFOA, LC/MS/MS, fish, Lake Victoria.

INTRODUCTION

In recent years perfluorinated alkylated substances (PFAS) have appeared as a new class of global pollutant. Besides being an industrially important group of compounds, PFAS are regarded as highly toxic and extraordinarily persistent chemicals that pervasively contaminate human blood (Olsen et al., 2003; Hansen et al., 2001) and wildlife throughout the world (González-Barreiro et al., 2006; Lau et al., 2006; Abbott et al., 2007). They are therefore regarded as PBT (persistent, bioaccumulative and toxic) chemicals (González-Barreiro et al., 2006) and cause diverse toxic effects in laboratory animals including primates (Biegel et al., 2001; Butebhoff et al., 2002). Human toxicology for PFOA and PFOS has been reviewed recently (Kennedy et al., 2004; Kudo and Kawashima, 2003; Lau et al., 2007; OECD, 2002). Dietary intake seems to be the main source of exposure of the general population to PFOS and PFOA (Fromme et al., 2007b). In Germany, recently 12.2 g/L of PFOS and 5.3 g/L (median values) of PFOA were found in non-

occupationally exposed volunteers (14 - 16 years of age) living in the southern part of Bavaria, Germany (Fromme et al., 2007a). Previous reports on monitoring PFAS in fish have mostly concentrated on liver, plasma and whole body. Few studies report levels of PFOS in fish fillets. PFAS concentration limits in fish fillets ranging from non detectable to approximately 300 ng/g has been reported (Geisy and Kannan, 2001; Hoff et al., 2003).

The management of Lake Victoria has for the last half century been largely focused on fish production. Very little attention has been paid to the ecological effects of pollutants on biodiversity. Judging by the very limited number of publications on persistent organic Compounds studies done in Winam Gulf of Lake Victoria and its Wetlands in recent years, the trend suggests the need for further research. Assessment of persistent organic pollutants (POP's) in Lake Victoria aquatic resources is critical, considering the ban almost a decade ago (in April 1999) following a report that pesticides had been determined in fish samples from Lake Victoria (Abila, 2003). This ban resulted in a 68% decline in fish exports (World Trade Organisation 2006; Abila 2003). Nile perch is the major source of income in riparian states of East Africa that share Lake Victoria water. The two fish species under this study

*Corresponding author. E-mail: fraora@yahoo.com. Tel: +4969254908015. Fax: +4969254908009.

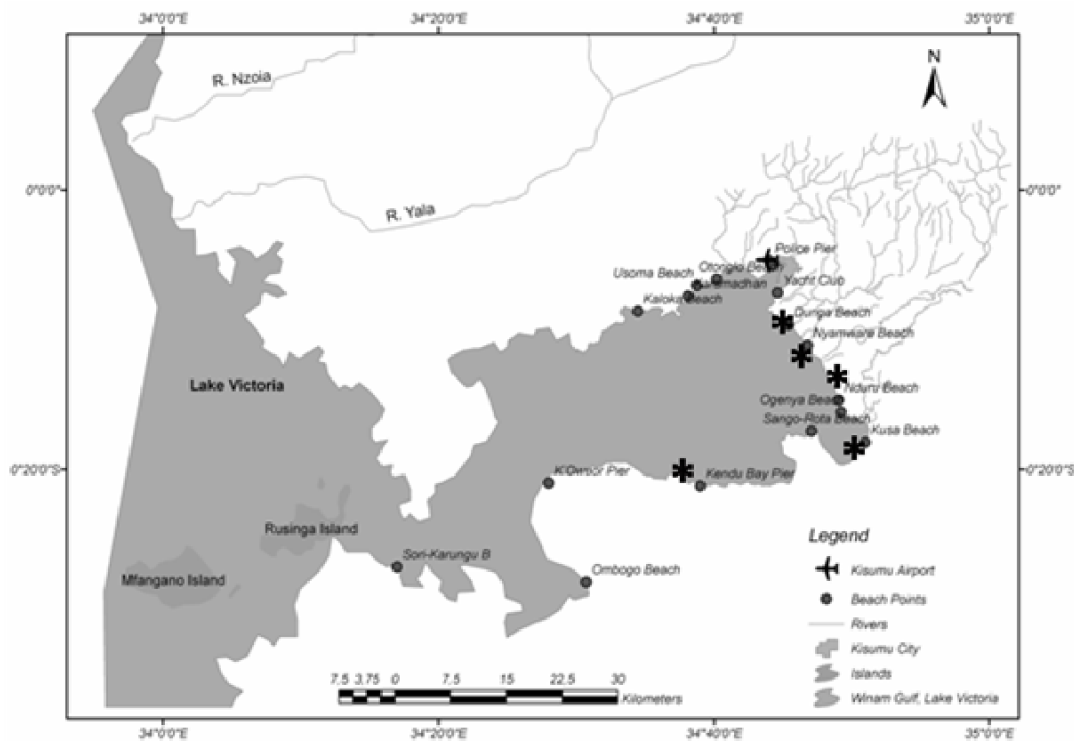


Figure 1. A map showing fish sampling beach locations (indicated by ·) within Winam gulf of Lake Victoria

are commonly found and are consumed both locally and internationally. Therefore, it is important to monitor perfluorinated alkylated substances as a new class of global pollutant in Lake Victoria biota and abiota matrices.

MATERIALS AND METHODS

Perfluorooctanoic acid, perfluorooctane sulfonate standards and an internal standard perfluorononanoic acid (PFNA) were obtained from Wellington Laboratories Canada. The counter-ion solution, 0.5 M tetrabutylammonium (TBA) hydroxide, was prepared by dissolving TBA hydrogen sulphate (Merck, Darmstadt, GER) in water; the pH value of the solution was adjusted to 10 with 2 M NaOH-solution. The stock solutions were prepared weekly. Methanol, (all suprasolve), analytical grade methyltert butyl ether (MTBE), Ammonium acetate (p.a.) and all other chemicals were obtained from Merck (Darmstadt, Germany). Ultra pure Milli-Q water was processed by a Millipore-Q-system (Millipore, S.A. Molsheim France).

Two fish species namely *Lates niloticus* (Nile perch) and *Oreochromis niloticus* (Nile Tilapia) were obtained from the coastal beaches of Winam gulf of Lake Victoria. Fish were restricted to only those of more than 25 and 40 cm full body length for Nile tilapia and Nile perch respectively.

Sampling was done during the period of November 2006 to January 2007. Samples were packed in coolers containing wet ice upon collection and shipped to the testing laboratory and deep frozen at -70°C until chemical analysis. In order to minimize the possibility of introducing PFOS contamination into samples, fluoro-polymer materials were avoided. Sampling in the beaches of Winam gulf catchments for this work were divided into locations namely; Dunga Beach, Nyamware Beach, Nduru beach, Kusa Beach and Kendu bay Pier Beach (Figure 1). Liver samples were

obtained after dissection of the fish.

More than 5 g fish muscles and about 1 g of fish liver were homogenised with 5 times its weight of water. 1 g of the homogenate to which an internal standard (perfluorononanoic acid) was added, was thoroughly mixed with 1 ml of 0.5 M tetrabutylammonium hydrogen sulphate solution (adjusted to pH 10) and 2 ml of 0.25 M sodium carbonate buffer. Target analytes were then extracted by adding 5 ml of methyl-tert butyl ether (MTBE) to the aqueous sample mixture and shaking for 20 min. The MTBE supernatant was then recovered and the extraction repeated a further two times. The MTBE fractions were all combined, concentrated to 1 ml and methylene chloride (1 ml) was added before proceeding to the extract clean-up. Sample extracts were cleaned up by low pressure chromatography using silica. The sample extract was then added to the column and eluted using 15 ml of dichloromethane to remove fat, followed by 30 ml of acetone which contained all target analytes. The acetone fraction was then evaporated just to dryness and reconstituted in 1 ml aliquot of methanol for HPLC-MS/MS analysis. HPLC-MS/MS analysis were carried out with HPLC (HP 1090) interfaced with an Ion Trap MS (Thermo LCQ-Duo). Solvent: A = 2 mmol/l Ammonium acetate in methanol and B: 1 mmol/l Ammonium acetate in water. Gradient program used: A 10% then increase to 30% at 0.1 min, increase to 75% at 7 min and from 7 to 10 increased to 100% where is kept at the level until 15 min. before reversion to original conditions. Flow rate 0.3 ml/min. Separation was done by a Betasil C18 column. MS conditions: Type: quadruple, Ionization: ESI negative. The method was calibrated with standards concentration range 0.5 - 1 ng/L and then 1 - 200 ng/L. Eight calibration curve points from enriched standards gave a value of $r^2 = 0.997$ was used for quantifications and were prepared routinely, to check for linearity. The LOQ of target chemicals was evaluated for each sample based on the average blank concentrations plus five times its standard deviation of ten blanks. Linearity of Matrix matched those of standard calibration curves. With 10 μl of the final sample volume of 1 ml injected in the splitless mode, the

Table 1. Concentrations range and mean with standard error (in brackets) in ng/g of PFOS and PFOA in muscles and liver of *Lates niloticus* and *Oreochromis niloticus* obtained from various sampling locations. *Only one to two samples are above the limit of quantification. Values below LOQ are denoted by '<'. Values below the LOQ were not included in the estimation of the mean.

Sampling location	Fish species	PFOS (ng/g)		PFOA (ng/g)	
		Muscles	Liver	Muscles	Liver
Dunga Beach	<i>Lates niloticus</i> (n = 5)	1.00 -10.50 (4.15 ±2.09)	6.20 -11.40 (8.55 ±0.85)	<0.50- 2.20* 2.20	<0.50- 1.80* (1.18 ±0.60)
	<i>Oreochromis niloticus</i> (n = 5)	0.90 -12.40 (4.89 ±2.11)	1.50- 19.70 (10.01 ±3.21)	<0.50- 0.90* (0.90)	<0.50- 1.90* (1.90)
Nyamware beach	<i>Lates niloticus</i> (n = 5)	1.00 – 9.41 (3.11 ±1.59)	15.03-35.70 (24.35 ±3.60)	<0.50	<0.50- 3.80* (2.06 ±0.28)
	<i>Oreochromis niloticus</i> (n = 5)	1.20 – 8.00 (3.70 ±1.17)	24.00 – 23.70 (11.75 ±3.70)	<0.50	<0.50- 1.00* (1.00)
Ndura Beach	<i>Lates niloticus</i> (n = 5)	0.90 – 5.00 (2.70 ±0.66)	1.40 – 13.20 (6.90 ±1.90)	<0.50	<0.50-1.20* (1.20)
	<i>Oreochromis niloticus</i> (n = 5)	0.90 – 3.00 (1.86 ±0.38)	2.60 – 14.20 (6.95 ±0.97)	<0.50	<0.50-1.00* (1.00)
Kusa Beach	<i>Lates niloticus</i> (n = 5)	1.00 – 2.20 (1.83 ±0.21)	2.20 – 5.60 (4.20 ±0.58)	<0.50	<0.50
	<i>Oreochromis niloticus</i> (n = 5)	1.00 – 2.00 (1.23 ±0.19)	4.20 – 7.30 (5.64 ±0.52)	<0.50	<0.50
Kendu bay pier Beach	<i>Lates niloticus</i> (n = 4)	1.80 – 3.20 (2.20 ±0.25)	3.20 – 6.70 (4.95 ±0.57)	<0.50	<0.50- 1.10* (1.10)
	<i>Oreochromis niloticus</i> (n = 4)	1.20 – 2.40 (1.80 ±0.21)	2.30 – 5.60 (4.15 ±0.55)	<0.50	<0.50

LOQ was 5 pg (absolute amount) corresponding to concentration of 0.5 ng/g for HPLC/MS/MS method. The monitored ion was 499 for PFOS and 413 for PFOA. Fish samples were spiked and analyzed to test the precision of the method. Recovery range was 87.7 to 104.6%. Results of accuracy and precision of analyses for the present investigation were found to be good. Reproducibility of the results done after every third day for (n = 4) number of times gave average absolute amounts (ng) 3.91 and percentage standard deviation 8.7%. Precision results as part of interlaboratory comparison analysis of PFOS and PFOA concentrations, for both fish analysis done by three different laboratories were between 6.38 and 10.97%.

RESULTS

Interesting observations regarding the concentration distribution of PFOS in fish liver and muscles for the two fish species were observed. The mean concentrations of PFOS obtained from all sampling locations in both fish analysed was between 1.23 to 11.75 ng/g. Higher concentrations of PFOS than PFOA in muscles and liver, with liver samples containing several orders of magnitude higher than in muscles in samples analysed was observed. Concentrations range of PFOS in Nile perch muscles was 0.90 to 10.50 ng/g and 1.40 to 35.70 ng/g for liver samples (Table 1). Table 1 shows the variation in range, mean and standard error PFOS and PFOA concentration (ng/g) in Muscles and liver of Perch and Nile Tilapia among the beach locations.

In Nile Tilapia samples, the concentration ranges for muscle and liver were 0.90 to 12.40 ng/g and 1.50 to 23.70 ng/g respectively. PFOA concentration was less than the limit of quantification for most samples analysed, however trace concentrations of PFOA were obtained in the liver samples. Pearson correlation test (2-tailed) in liver and muscles for Nile tilapia and perch gave values of $r = 0.852$ and 0.387 ($P < 0.001$) respectively, as shown in Figure 2. Pearson correlation test (2-tailed) obtained in Tilapia muscles and liver gave values more than 0.97 significant correlation at 0.01 level for three of the five sampling beach locations namely Dunga Beach, Nyamware Beach and Kendu bay pier.

DISCUSSION

Fish from Lake Victoria had relatively low levels (Nile perch up to 35.70 ng/g and Nile tilapia up to 23.70 ng/g) of the perfluorinated compounds studied in comparison to reports from other studies done. Giesy and Kannan (2001) obtained a concentration range of 7.5 to 46 ppb of PFOS in various fish species muscles from Scheldtz estuary in Belgium. Martin et al. (2004) working on archived lake trout samples obtained concentrations of up to 180 ng/g mean whole body PFOS concentrations. Hoff et al. (2005) conducted PFOS assessment on gibel carp (*Carassius auratus gibelio*), carp (*Cyprinus carpio*),

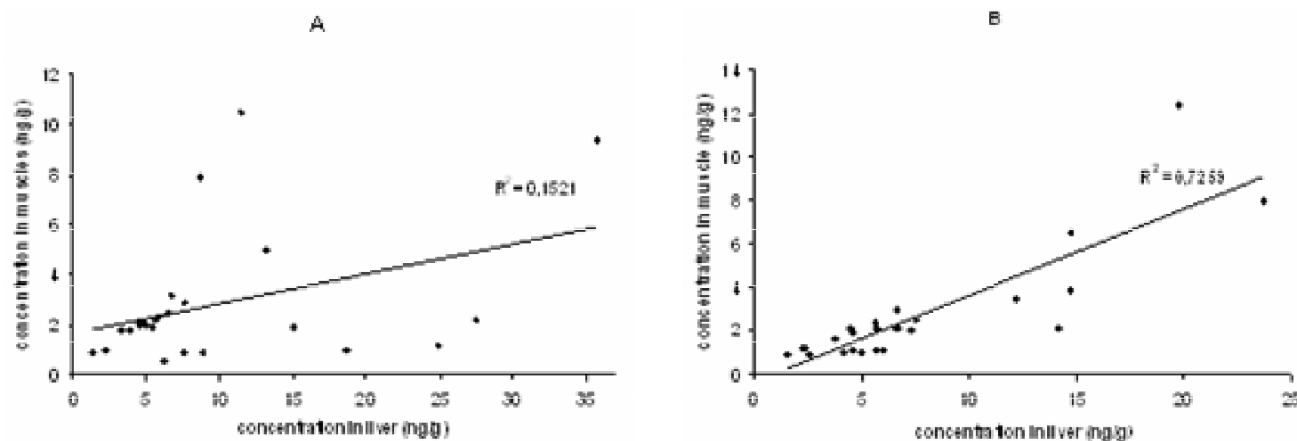


Figure 2. A scatter diagram showing correlation between Nile perch muscles and liver (designated A) and Nile tilapia muscles and liver (designated B).

and eel (*Anguilla anguilla*) in Flanders (Belgium). The liver PFOS concentrations in fish from the leperlee canal (Boezinge, 250-9031 ng/g wet weight, respectively) and the Blokkersdijk pond (Antwerp, 633-1822 ng/g wet weight) were higher than at the Zuun basin (Sint- Pieters-Leeuw, 11.2-162 ng/g wet weight) and among the highest in feral fish worldwide. In this study, only mature Tilapia fish (of length more than 25 cm for Nile tilapia) were analyzed for PFOA and PFOS concentrations. According to Balirwa (1998), the length at which 50% of the Nile tilapia first exhibited sexual maturity (Lp 50 values) in the littoral habitats of Lake Victoria as 18 cm and 24 cm total length (TL) for male and female fish, respectively. For Nile perch, only fish of more than 40 cm TL in length were obtained for further analysis. Assumption was made that at 40 cm TL, Nile perch is near maturity. Study report by Mkumbo et al. (2007) revealed that the size of Nile perch at first maturity was at 54.3 cm TL (1.6 yr) and 76.7 cm TL (2.5 yr), for males and females, respectively. Cunha et al. (2005) observed that PFOS burden was higher in mature than in non-mature mussel (*Mytilus galloprovincialis*) individuals, suggesting that at least part of the chemical is released during spawning. PFOS has been found in bird and fish eggs, supporting this hypothesis (Giesy and Kannan, 2002). In this study slightly higher concentration of PFOS than PFOA were noted in both fish species analysed, thus indicating that PFOS accumulates more in fish muscles and liver than PFOA. It can also be observed in this study that the per-fluorinated compounds concentration does not correlate significantly between the two fish species in this study, for both muscles and liver samples. This indicates that concentration does not depend on the trophic position for Nile perch and Nile tilapia which implies diet as the only source of PFOS and PFOA. It is also possible that atmospheric deposition can be another source given the near similarities in quantities observed. Although the environmental fate of PFOS is not completely under-

stood, the accepted mechanism by which it occurs in remote areas is by other more volatile PFCs, such as perfluoroalkyl sulfonamides, acting as precursors which carry a PFOS moiety, being transported for long distances and then being degraded or metabolised to PFOS (So et al, 2004 and Stock et al, 2004). There are data which show that PFCs produced by electrochemical fluorination can be broken down by microorganisms to PFOS and PFOA (Hekster et al., 2003).

Nile perch (a predator fish) is higher than Nile tilapia in the trophic position in the Lake Victoria ecosystem. Studies by Njiru et al (2004) of the food of introduced Nile tilapia showed that Nile tilapia originally known to be herbivorous; feeding mostly on algae has diversified its diet to include insects, fish, algae and plant materials. The shift in diet could be due to ecological and environmental changes in Lake Victoria, which have been associated with changes in composition and diversity of fish and invertebrate fauna, emergence and dominance of different flora (Njiru et al., 2004). Unclear correlation of PFOS concentration in Nile perch and Nile tilapia indicate a shift in food web relationship in Lake Victoria ecosystem. According to Njiru et al. (2000) feeding patterns observed in *O. niloticus* may be as a result of the species plasticity and response to changed ecological conditions prevailing in the lake following water hyacinth infestation. Due to this changed feeding behaviour in *O. niloticus* a new food web is emerging in the lake (Njiru et al., 2000). A test of correlation between the concentrations obtained in Tilapia muscles and liver using Pearson correlation test (2-tailed) for three of the five sampling beach locations namely Dunga Beach, Nyamware Beach and Kendu bay pier gave values more than 0.97 significant correlation at 0.01 level (N = 5). Although both three beaches are within urban centres, the correlation obtained between the concentrations of PFOS in Tilapia muscles and liver samples may be as a result of atmospheric deposition. This is because these urban centres either do not have or

have inefficient sewer treatment plants and major Industrial activities. PFOS in the Tilapia liver and muscle for all samples analysed were significantly correlated [$r = 0.852$, $P < 0.001$] as shown in Figure 2 (B). Probable reason for this is that Nile tilapia has a localized feeding habit and therefore the PFOS concentration observed in the liver and muscles reflect those in the environmental matrices at the sampling locations. There was no significant correlation (Pearson correlation value 0.387) for the same test between Nile perch muscles and liver as shown in Figure 2 (A).

Conclusion

Preliminary results show that PFOA and PFOS residues are present in two fish species studied namely Nile Tilapia and Perch from five beaches of Winam gulf of Lake Victoria ecosystem. The results of this study can be used as indicator information for further studies on the sources, behaviour and fate of PFOA and PFOS and residues in both abiotic and biota compartments of Lake Victoria and other lakes.

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