Perfluorinated Compounds in Environmental Matrices of Lake Victoria Gulf

"Their Management Implications and Degradation Studies of Emerging Perfluorinated Surfactants Substitutes"

Dissertation zur Erlangung des Grades
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vorgelegt von **Francis Orata**
geboren in Kenya

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DEDICATION

For Christabell

"…….In the middle of my work, she gave me

the much needed courage and motivation

to keep on keeping on……"

DECLARATION AND COPYRIGHT

ABSTRACT

One of the major ecological challenges on Lake Victoria resources is the existence of "hot spots", caused by human waste, urban runoff, and industrial effluents. The lake is tending towards eutrophication which is attributed to the increasing human population in its watershed. The rate of population growth, estimated at seven per cent within 100 kilometres around the Lake Victoria catchment area, had outpaced the continental average, reflecting growing dependence and pressure on the lake's resources. The population growth around the continent's largest lake is significantly higher than the rest of Africa. If Lake Victoria is not managed properly, it will loss sustainability for the future. The lake is experiencing increasing anthropogenic loads of pollutants from expanding urban, agricultural and industrial development. So far, very little or no work has been done to determine the levels or impact of non-conventional trace organic pollutants in the Lake Victoria. The negative health impacts caused by some of the trace organic pollutants are well documented in other studies. It is in this view that the motivation of doing this study arose. The choice of pollutant for this study was perfluorinated compounds because it is an emerging non-conventional pollutant of concern worldwide.

A report of the levels of perfluorooctane sulfonate and perfluorooctanoic acid in environmental matrices of Lake Victoria is presented, and management implication of perfluorinated compounds and similar potential organic pollutants examined. Report on degradability of new emerging substitutes for perfluorinated alkyl surfactants and an alternative method for analysis of perfluorocarboxylic acids are presented.

Two widely consumed and economically important fish species namely *Lates niloticus* (Nile perch) and *Oreochromis niloticus* (Nile tilapia) were obtained from Winam gulf of Lake Victoria, Kenya, and analysed for perfluorooctane sulfonate and perfluorooctanoic acid in muscles and liver using liquid chromatography coupled with mass spectroscopy. Concentrations value of perfluorooctane sulfonate 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 matrices was also demonstrated. The lowest limit of quantification (LOQ) was 0.5 ng/g for fish samples.

Variability in the concentrations of perfluorooctanoic acid or perfluorooctane sulfonate in river waters (range perfluorooctanoic acid 0.4 – 96.4 ng/L and perfluorooctane sulfonate < 0.4 – 13.2 ng/L) was higher than for Lake waters (range perfluorooctanoic acid 0.4 – 11.7 ng/L and perfluorooctane sulfonate < 0.4 – 2.5 ng/L respectively). Both analytes were detected in Lake and River sediments with concentrations of perfluorooctanoic acid and perfluorooctane sulfonate ranged from 1.4 – 99.1 ng/g and < 1 – 57.5 ng/g respectively, which was higher than concentrations obtained from Lake sediments (range perfluorooctanoic acid < 1 – 24.1 ng/g and perfluorooctane sulfonate < 1 – 4.0 ng/g). The results obtained suggested generalized point sources such as domestic and industrial waste. The lowest limit of quantification (LOQ) in water samples was 0.4 ng/L for both analytes while for sediments it was 1 ng/g for both analytes. Typical values for precision obtained were 0.14 – 3.7 % for water samples. Recovery range obtained for sediments were 78 – 109 %, from spiked amounts ranging from 1 to 150 ng. Sampling sites within and near sewage and water treatment facilities gave the highest concentrations of both analytes. Analysis of water and fish samples from the Rhine river (Germany) for comparison, calibration and as part of analytical quality management was performed.

Significant correlations were tested between perfluorinated compounds levels in sediments, fish and water. Wastewater treatment plants and other anthropogenic sources have been identified as significant sources or pathways for the introduction of perfluoroalkyl compounds into Lake Victoria ecosystem. In this study, elevated concentrations of perfluorooctanoic acid and perfluorooctane sulfonate was found in two wastewater treatment plants (WWTPs) in Kisumu, City of Kenya.

An alternative analytical method to liquid chromatography/ mass spectroscopy for analysis of perfluorocarboxylic acids in abiotic and biotic matrices where high concentrations are expected is also presented. Derivatisation of the acid group to form a suitable alkyl ester provided a suitable compound for mass spectroscopy detection coupled to gas chromatography instrumental analysis. The acid is esterified by an alkyl halide i.e benzyl bromide as the alkylating agent for Perfluorocarboxylic acids quantification.

The study also involved degradability measurements of emerging perfluorinated surfactants substitutes. The stability of the substitutes of perfluorinated surfactants was tested by employing advanced oxidation processes (AOP), which was based on the degradation by ultra violet (UV)-Lamp, hydrogen peroxide (H_2O_2) or both, followed by conventional tests, among them an automated method based on the manometric respirometry test [OECD 301 F] – Oxi-Top-, Closed bottle test- CBT [OECD 301 D] and standardized fix bed bioreactor [FBBR] on perfluorobutane sulfonate (PFBS), a fluoroethylene polymer, fluorosurfactant (Zonyl), two fluoraliphaticesters (NOVEC ™ FC4430 and NOVEC ™ FC4432) and 10-(trifluoromethoxy) decane-sulfonate. Most of these new surfactants are well-established in the market and have been used in several applications as alternatives to PFOS and PFOA based surfactants.

The perfluorinated compounds concentrations obtained in water, sediments, and fish in this study are mostly lower than those obtained previously in various study areas around the globe for all matrices analysed. Specifically, the perfluorinated compounds levels in water observed in this study fall much below the known health based guide standards, for example the guide standards in Germany. The results of this study can be used as pioneer information for further studies on the sources, behaviour and fate of PFOA and PFOS and other related compounds in both abiotic and biota compartments of Lake Victoria and other lakes. The data obtained in this study can particularly be considered when formulating policies and management measures for preservation and sustainability of Lake Victoria resources.

This thesis has in parts been published and presented in the following papers and conferences.

Francis Orata, Natalia Quinete, Friedrich Werres, Rolf-Dieter Wilken. *Determination of Perfluorooctanoic Acid and Perfluorooctane Sulfonate in Lake Victoria Gulf Water*. Bull Environ Contam Toxicol. **2008.** Volume 82, Issue2 (2009), Page 218**.**

Orata Francis, Quinete Natalia, Maes Anke, Werres Friedrich, Wilken Rolf-Dieter. *Perfluorooctanoic acid and perfluorooctane sulfonate in Nile Perch and tilapia from gulf of Lake Victoria*. Pure and Applied Chemistry Vol. 2 (8), pp. 075-079, August, **2008**

Francis Orata, Natalia Quinete, Rolf-Dieter Wilken. *Long Chain Perfluorinated alkyl acids Derivatisation and Identification in Biota and Abiota Matrices using Gas Chromatography.* Bull Environ Contam Toxicol. DOI : 10.1007/s00128-009-9756-y

Francis Orata, Anke Maes, Friedrich Werres, Rolf Dieter Wilken. *Perfluorinated Compounds Mapping and Source Identification in Lake Victoria Gulf Basin by Sediments Analysis.* Soil and Sediment Contamination. (Accepted)

Natalia Quinete, Francis Orata, Friedrich Werres, Isabel Moreira, Rolf-Dieter Wilken. *Determination of Perfluorooctane Sulfonate and Perfluooctanoic Acid in the Rhine River, Germany*. FEB Vol.18 Reference-No: F-2009-043

Natalia Quinete, Francis Orata, Anke Maes, Markus Gehron, Karl - Heinz Bauer, Isabel Moreira, Rolf Dieter Wilken. *Degradation Studies of Emerging Perfluorinated Surfactants Substitutes.* Chemosphere. (In process).

2nd EuCheMS Chemistry Congress. www.euchems-torino2008.it. **2008** september 16 – 20 Torino, Italy. **V.3 / P-047 /** *Emerging Perfluorinated Chemicals Surfactants Substitutes Degradation Studies*

1st international Workshop / Fluorinated Surfactants: New Developments, Idstein, Germany. June **2008**. Title: (**PB 05**). *Emerging perfluorinated chemical surfactant substitutes degradation studies.* (**PB 06**). *Gas chromatography as an alternative analytical methodology for fluorinated alkyl surfactants in biotic and abiotic matrices*

Wasser 2008: a Conference organised by Water Chemical Society, Germany, at Trier, Germany. April 2008. Title: (**PAn05**). *Gas Chromatography as an Alternative Analytical Methodology for Fluorinated alkyl acid Surfactants in Biotic and Abiotic Matrices.* April **2008**

International Conference on Water and Developments in Kunming University of Agriculture, China. Sept. 2007. Title: *Analysis of PFOS and PFOA in Water and Fish from Lake Victoria and study of New Perfluorinated surfactants substitutes.* **Oral presentation**. September **2007**

Sustainable use of Natural recourses: organised by UNEP and Tongji University in Shanghai China. *Properties and fate of PFCs in the Environment.* **Oral presentation**. August **2007**

Deutsch-Russischer Workshop. „New Environmental Technologies". Kessel, Germany. *Determination of perfluorooctane sulfonate and perfluooctanoic acid in the Rhine River Germany*. **Oral presentation.** May 2009

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LIST OF ABBREVIATIONS

AFFF:	Aqueous Film-foaming Foams
AOP:	Advanced Oxidation Processes
APHA:	American public Health Association
ATSDR:	Agency for Toxic Substances and Disease Registry
BCFs:	Bioconcentration factors
BMF:	Biomagnification
BOD:	Biological Oxygen Demand
BRE :	Better Regulation Excecutive
CAS:	Chemical Abstract System
CBT:	Closed Bottle Test
C.C.F.A.C:	Committee on Food Additives and Contaminants.
CONTAM:	Scientific Panel on Contaminants in the Food Chain
COWI:	Consultancy within Engineering, Environmental Science and Economics
COT:	Committee on Toxicity
DHHS:	Department of Health and Human Services
DWI:	Drinking Water Inspectorate for England and Wales
ECF:	Electro-chemical Fluorination
EFSA:	European Food Safty Authority
ESR DIR:	Existing Substances Regulation Directorate
ETL:	Engineering Technical Letter
EU:	European Union
FAO:	Food Agricultural Organization
FBBR:	Fix-bed Bioreactor
FDA:	Food and Drug Administration
FDUEA:	2H-perfluoro-2-dodecenoic acid [$CF_3(CF_2)_8CFCHCOOH$]
FHUEA:	2H-perfluoro-2-octenoic acid [$CF_3(CF_2)_4CFCHCOOH$]
FNUEA:	2H-perfluoro-2-dodecenoic acid [$CF_3(CF_2)_7CFCHCOOH$]
FOUEA:	2H-perfluoro-2-decenoic acid [$CF_3(CF_2)_6CFCHCOOH$]
FSA:	Food Standards Agency
GC:	Gas Chromatography
GEF:	Global Environmental Facility
GIWA:	Global International Waters Assessment

GLNPO: Great Lakes National Program Offices

HPA: Health Protection Agency

HPLC: High-performance Liquid Chromatography

EC: International Electrotechnical Commission

ICRAF: Internal Centre for Research and Agroforestry

ISE : Ion Selective Electrode

KMFRI: Kenya Marine and Fisheries Institute

K_{OW}: Octanol-Water Partition Constant

LC: Liqiud Chromatograhy

LOD: Limit of Detection

LOQ: Limit of Quantification

LVEMP: Lake Victoria Environmental Management Program

LVFO: Lake Victoria Fisheries Organisation

MPCA: Minnesota Pollution Control Agency

MS: Mass Spectrometric

NAPAP: National Acid Precipitation Assessment Program

NEAP: National Environmental Action Plan

NEMA: National Environmental Management Authority

NESI : Negative Electro-spray Ionization

NEtFOSE: N-ethylperfluorooctane sulfonamidoethanol [$CF_3(CF_2)_7SO_2N(CH_2CH_3)(CH_2CH_2OH]$

NOAEL No Observed Adverse Effect Level

OECD: Organisation for Economic Co-operation and Development

OHCs: Organohalogen compounds

POPs: Persistent Organohalogenated Compounds

PASs: Perfluoroalkyl surfactants

PEEK: Polyetheretherketone [$–(C_6H_4COC_6H_4OC_6H_4O)_n$-]

PFBS: Perfluorobutane sulfonate [$C_4F_9SO_3$]

PFAS: Perfluoroalkyl sulfonates

PFAS: Perfluorinated Alkylated Substances

PFCs: Perfluorinated Compounds

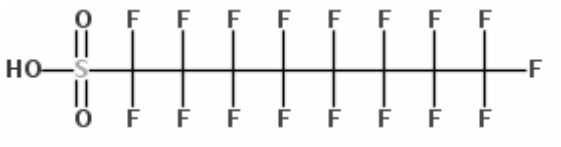
PFDA: Perfluorodecanoic acid [$C_{10}HF_{19}O_2$]

PFDoA: Perfluorododecanoate [$C_{11}F_{23}COO$-]

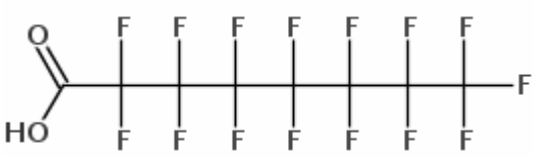
PFHS : Perfluorohexane sulphonate [$C_6F_{13}SO_3$]

PFOSA Perfluorooctanesulfonic acid [$CF_3(CF_2)_7SO_3H$]

PFOSF:	Perfluorooctanesulfonyl fluoride [$CF_3(CF_2)_7$-SO_2F]
PFPA:	Perfluoropentadecanoate [$CF_3(CF_2)_{13}COO-$]
PFTA:	Perfluorotetradecanoate [$CF_3(CF_2)_{12}COO-$]
PFTrA:	Perfluorotridecanoate [$CF_3(CF_2)_{11}COO-$]
PFUnA:	Perfluorou-n-decanoate [$C_{10}F_{21}COO$-]
POPs:	Persistent Organic Pollutants
PTFE:	Polytetrafluoroethylene [$(CF_2$-$CF_2)_n$]
RIKZ:	Rijksinstituut voor Kust en Zee
RSC:	Royal Society of Chemistry
RPA:	Policy Analysts Limited
SNURs:	New Use Rules
STPs:	Sewage Treatment Plants
TBA	Tetrabutylammonium
TDI:	Tolerable Daily Intake
TFE:	Tetrafluoroethylene)
TMF:	Trophic Magnification Factor
TSCA:	Toxic Substances Control Act
UF:	Uncertainty Factor
UK :	United Kingdom
U.S. EPA:	United States Enviromental Programs Agency.
WHO:	World Health Organisation
WTO:	World Trade Organisation
WWF:	World Wildlife Fund
WWTPs :	Wastewater Treatment Plants



Perfluorinated sulfonate (PFOS; $C_8F_{17}SO_3H$)



Perfluorooctanoic acid (PFOA; $C_7F_{15}COOH$)

CHAPTER ONE

1 INTRODUCTION

1.1 General Background

Perfluorooctanoic acid (PFOA; $C_7F_{15}COOH$) and perfluorinated sulfonate (PFOS; $C_8F_{17}SO_3H$) are used as water and oil repellants in the treatment of fabrics and leather, paper industry, personal care products and in many other industrial use (Kissa, 2001; Martin et al., 2003a; Berger et al., 2004). Perfluorinated compounds production volume of 2.1 x 10^7 kg (in 2004) was reported in the United States alone (Forman, 2004) and more than 4 x 10^7 kg world-wide (Merck, 2006) represents only a small fraction (0.4 %) of the total surfactants production.

The characteristics of Perfluoroalkylated compounds that cause them to persist in the environment are also the characteristics that made them attractive compounds for industrial usage for over 50 years. In recent years perfluorinated alkylated substances (PFAS) have been considered as a new class of global pollutant, and have attracted considerable attention among environmental scientists, toxicologists, epidemiologists, and policy makers. Perfluorinated alkyl substances are regarded as highly toxic and extraordinarily persistent chemicals that pervasively contaminate human blood (Butebhoff et al 2002; Olsen et al. 2003a; Hansen et al. 2001 and Chau, 2006) and wildlife throughout the world (González-Barreiro et al. 2006; Lau et al. 2006 and Abbott et al. 2007). They are therefore regarded as PBT (persistent, bioaccumulative, and toxic chemicals) (González-Barreiro et al. 2006).

Due to the importance that is still upheld for perfluorinated surfactants, efforts are being made to find new substitutes for perfluorinated surfactants. Recently, the following substitutes have been developed; Perfluorobutane sulfonate standard, two fluoroaliphatic esters NOVEC ™ FC4430 and fluoroaliphatic ester NOVEC ™ FC4432, fluoroethylene polymer, 50 % fluorosurfactant (Zonyl) produced by 3M and DuPont companies, and 10-(trifluoromethoxy) decane-sulfonate, a prototype from Merck company. The determination of the biodegradability should be checked before “new” chemicals are used for the first time, not only for environmental reasons but also for minimizing disposal charges. Thus, performing degradability test on emerging new surfactants is of great concern and interest.

Sinclair et al., (2004) found that ion-pairing, liquid liquid extraction method was suitable for measurements of concentrations in ng/L and μg/L levels for PFOA and PFOS analysis. Therefore we have applied this specific analytical method in this study. Effort has been made to use derivatisation of the acid group to determine whether GC/MS/MS as an alternative method to analyse long chain perfluorinated acids namely; perfluoro-n-octanoic acid (PFOA), 2H-perfluoro-2-octenoic acid (FHUEA; $CF_3(CF_2)_4CFCHCOOH$), 2H-perfluoro-2-decenoic acid (FOUEA; $CF_3(CF_2)_6CFCHCOOH$) and 2H-perfluoro-2-dodecenoic acid (FNUEA; $CF_3(CF_2)_7CFCHCOOH$) in water and fish. The method involves derivatisation of these acids to form a suitable ester for GC analysis. Derivatisation procedure for a GC instrumental analysis involved using benzyl bromide solution and acetone to form benzylperfluorooctanoate (benzyl ester) as presented by the equation below.

$$CF_3(CF_2)_6COOH + C_7H_7Br \rightarrow C_{15}H_7F_{15}O_2 + HBr$$

Due to the unique properties of the carbon-fluorine bond and the polarity of perfluoroalkyl groups, potential substitutes in most cases continue to be perfluoroalkyl based. Thus, issues of persistence in the environment remain (3M, 2002). With the prospects of completely banning the sulfonate based perfluorinated surfactants, acid based surfactants will continue to be market available. Therefore, analytical methods for acid based surfactants analysis advancement are required.

1.2 Ecological Challenges facing Lake Victoria Resources

Due to the high population densities along the shores of Lake Victoria, exploitation of its resources has subjected it to tremendous pressures. The population pressure has contributed to the existence of “hot spots”, caused by human waste, urban runoff, and industrial effluents. Over 70 % of the populations of the three countries that share Lake Victoria waters are strongly engaged in agricultural production mostly as small scale farmers with products like sugar, tea, coffee, maize, cotton, livestock keeping, and horticulture within the lake catchment. The use of agro-chemicals such as herbicides and pesticides has resulted in increase of pesticides residues level in Lake Victoria ecosystem. Nutrients such as phosphorus and nitrogen loaded into Lake Victoria have resulted towards eutrophication of the lake. It is reported that untreated sewage and industrial waste flow into Lake Victoria every day from

Kisumu City in Kenya, thus being the main potential source of perfluorinated compounds (PFCs) and other pollutants in Lake Victoria. This is basically due to inadequate sewage treatment facilities, most requiring repairs and upgrading to cope with the ever increasing population. Consequently, watershed degradation, industrial effluents and agricultural runoff have contributed to significant build-up of heavy metals, pesticides, nutrients and various surfactant chemicals. Nutrient loads into Lake Victoria have caused prolific growth of algae, and affliction with the Water Hyacinth (*Eichhornia crassipes*) infestation. Unsustainable utilization of the major Lake Victoria wetlands through agricultural activities and livestock keeping may affect the buffering capacity of the wetlands. Recent trends in Lake Victoria suggest dramatic change in limnological parameters and native fishery stocks from late 1960s to 2003. The reasons for the declining fishery and water quality has been blamed on over fishing, exotic species introductions (*Lates niloticus* and *Oreochromis niloticus*), disruption of traditional food-web relationships, deleterious land use practices, and pollution from diffuse sources. These factors have largely contributed to the oxygen depletion and mass extinction of indigenous fishes which is now taking place. These pressures have also resulted in massive loss of native fish species, which are the nutritional requirements for the local populations, this is partially caused by the introduction of exotic fish species such as *Lates niloticus* (Mbuta) and *Oreochromis niloticus* (Nyamami), and the increasing conversion of the fishery to an export commodity rather than being a local protein source. Evidently, Lake Victoria is facing major ecological challenges which require concerted efforts by all scientists, to monitor, in order to reverse the declining water quality and dwindling fishery resources.

The management of Lake Victoria has, for the last half century, been largely focused on fish production and management. Very little or no attention has been paid to the ecological effects of both conventional and emerging 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, suggests the need for further research. Assessment of persistent organic pollutants (POP's) and especially non conventional pollutants like perfluorinated compounds in Lake Victoria aquatic resources is critical, considering the ban of such products 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 (Abila, 2003 and World Trade Organisation, 2006). Surface water is the major source of drinking water in riparian states of East Africa that share Lake Victoria's water. The limited number of publications on perfluorinated compounds in sediment matrix

suggests the urgent need for more research in this area. In this study, a detailed report on levels of PFOA and PFOS in Lake Victoria aquatic ecosystem sediments is presented. Therefore, it is of major importance to monitor perfluorinated alkylated substances in Lake Victoria.

1.3 Geographical Setting and economic importance of Lake Victoria

Lake Victoria is the world's second largest freshwater lake by surface area, second to Lake Superior. It is bordered by Tanzania, Kenya and Uganda. It stretches 412 km from north to south between latitudes 0°30'N and 3°12'S, and 355 km from west to east between longitudes 31°37' and 34°53'E. The lake is situated at an altitude of 1,134 m above sea level. It has a volume of 2,760 km^3, an average depth of 40 m and a maximum depth of 80 m. Lake Victoria is the largest lake in Africa with a surface area of 68,800 km^2 and a catchment area of 193,000 km^2. The lake contains numerous islands and has a highly indented shoreline, which is estimated to be about 3460 km long. The flushing time (volume/average outflow) is 138 years and the residence time is 21 years. The lake is shared between three riparian states as follows; Kenya (6%), Tanzania (51%) and Uganda (43%). The overall lake catchment area encompasses 193,000 km^2 with Tanzania occupying approximately 44 %, Kenya 22 %, Uganda 16 %, Burundi 7 % and Rwanda 11 %.

In East Africa, Lake Victoria and its catchment supports some 30 million people, which constitutes about one third of the population of Kenya, Tanzania and Uganda. About 3 million people earn their source of livelihood directly or indirectly on the fish industry of Lake Victoria in the three countries. Lake Victoria is a source of livelihood for more than 30 million people whose living directly or indirectly from 800,000 tonnes of fish catches annually. Lake wide fish production is estimated at between 400 to 500 metric tons with Tanzania landing 40%, Kenya 35% and Uganda 25%. In approximation, fish from Lake Victoria earns US $590 million (Sh41.3 billion) of which US $340 million (Sh23.8 billion) is generated at the source. The remaining US $250 million (Sh17.5 billion) is earned in export from the Nile Perch fishery.

1.4 Description of study area

Kisumu, the third largest City in Kenya is located on the shores of Lake Victoria, at an altitude of 1160 m above sea level. The City covers approximately 417 km^2. Kisumu is situated approximately 00º06' south of the equator and 34º45' east of the Greenwich.

In 2005 the approximated population of Kisumu City is approximately 500,000 persons (Kisumu City Council, 2005).

Kisumu has a sub-humid and semi-humid tropical climate with high mean temperature of about 23° C and rainfall that varies with altitude (Kisumu City Council, 2005). The mean annual rainfall varies from 1100 mm in the south to 1500 mm to the north with potential evaporation of 2200 mm and 1900 mm respectively. In the Lake Victoria basin region, there are two wet seasons, long rains from April to June and short rains from October to November. The region experiences dry seasons from December to March. The precise timing and extent of each season varies annually.

In the study catchment area, the main rivers are Nzoia, Sio, Yala, Nyando, Kisat, Kibos, Sondu-miriu, Kuja, Migori, Riaria and Mawa. However, rivers entering the lake from Kenya, which contains the smallest portion of the lake, contribute over 37.6 % of the surface water inflows (LVEMP, 2003). About 86 % of total water input falls as rain and evaporative losses account for 80 % of the water leaving the lake (COWI, 2002). The mass balance of water in the lake based on the inflow and outflow is 33 m^3/ s. This accounted for the observed rise in the lake water level of about 1.0 m between January 1950 and December 2000 (COWI, 2002). However, the annual fluctuations in levels range between 0.4 - 1.5 m (Balirwa, 1998).

Since the region is basically an agricultural producing region, most of the industries that have been established are mainly agro-based such as sugar and allied industries. The Kisumu Railway Pier and fishing beaches (Kaloka, Usoma, Otonglo, Dunga, Tako, Ogal, Ogenya, Nyamware and Nduru) are within the Gulf area (see Figure 1). Other important sites within the Gulf are the Yacht Club and the Police Pier (see Figure 2). The Kikomi Textiles also resided along the river. There are other numerous cottage industries in the vicinity of Kisat River. The fish industry is of tremendous economic importance to the countries of East Africa since fish export earns these countries enormous foreign income. Figures 1 and 2 show us the maps of Winam Gulf of Lake Victoria and Winam bay respectively, within Kisumu

City, showing Nyalenda Sewage Ponds. Rivers Kisat, Saka, Auji and Kibos are illustrated in Figure 2. The sampling sites are highlighted in the respective Figures.

1.4.1 State of waste treatment and domestic water supply within the study area

The main water supply intake point serving Kisumu City is at the Gulf area near Hippo point, within the larger Dunga area. Another water abstraction facility is located upstream Kibos river. There also exists a water reservoir at Kibuye. According to Ombogo, 2003, by the year 2003, the existing water infrastructure in Kisumu City only served 40 % of the City. Water production was 15,000m^3/day against a water demand of 48,000m^3/day to 60,000m^3/day during the day. The water quality was unreliable (Ombogo, 2003).

Sanitation coverage within Kisumu city by the year 2003 was much less at 23%. Most of the town is either using septic tanks or pit latrines. Sanitary facilities and sewerage within Kisumu City are municipal wastewater treatment system along Kisat river which comprises of four trickling filter plants and oxidation ponds. By the time the field work of this study was being carried out, this wastewater treatment facility at Kisat was inefficient in its processes. Another waste water treatment facility is the Nyalenda waste water treatment plant that is located along Kibos-Nyalenda-Auji river system. The Nyalenda wastewater treatment ponds (Lagoons) are situated on the eastern side of Kisumu City on the eastern end of the Winam Gulf. They were constructed in 1976/77 to treat sewage from the south-eastern sector of the town. Initial design included three Lagoons arranged in three rows. One of the lagoons in each row is facultative and the rest are the maturation lagoons with a liquid depth of 1.75 m and volume of 60,334 m^3. The first three maturation lagoons have each a liquid depth of 1.20 m and a volume of about 15,000 m^3. The lagoons were designed to treat a sewage flow of 7,000 m^3 per day. With continued urbanization and industrial development of the Kibos area of the town, the ultimate flow was expected to reach 17,350 m^3 a day, catering for a population of about 100,000 people (Getabu, 1990).

Most factories (sugar, textile, dairy and paper factories) situated in Kisumu City have their own industrial wastewater treatment systems which are anaerobic ponds and aerobic oxidation ponds.

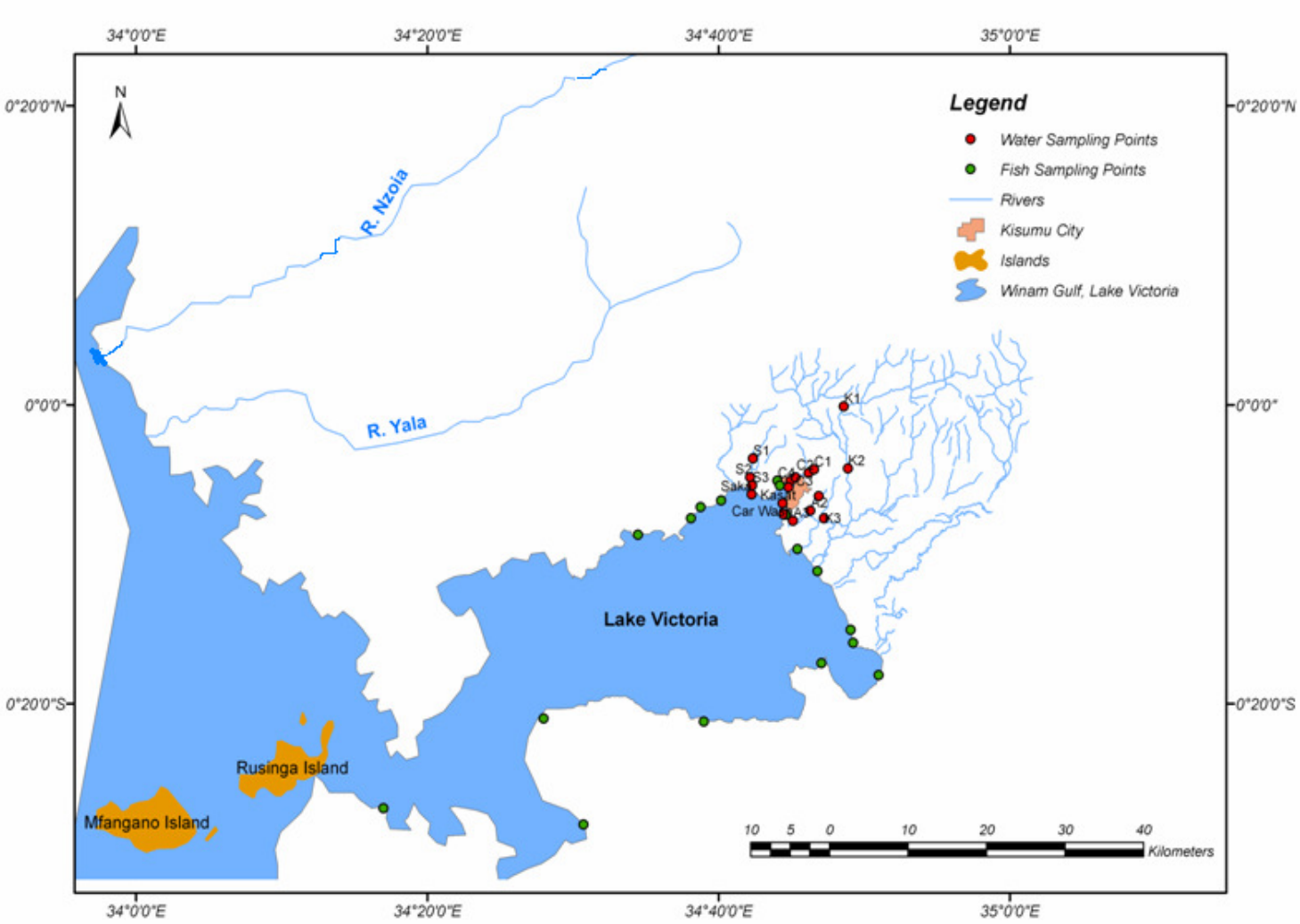
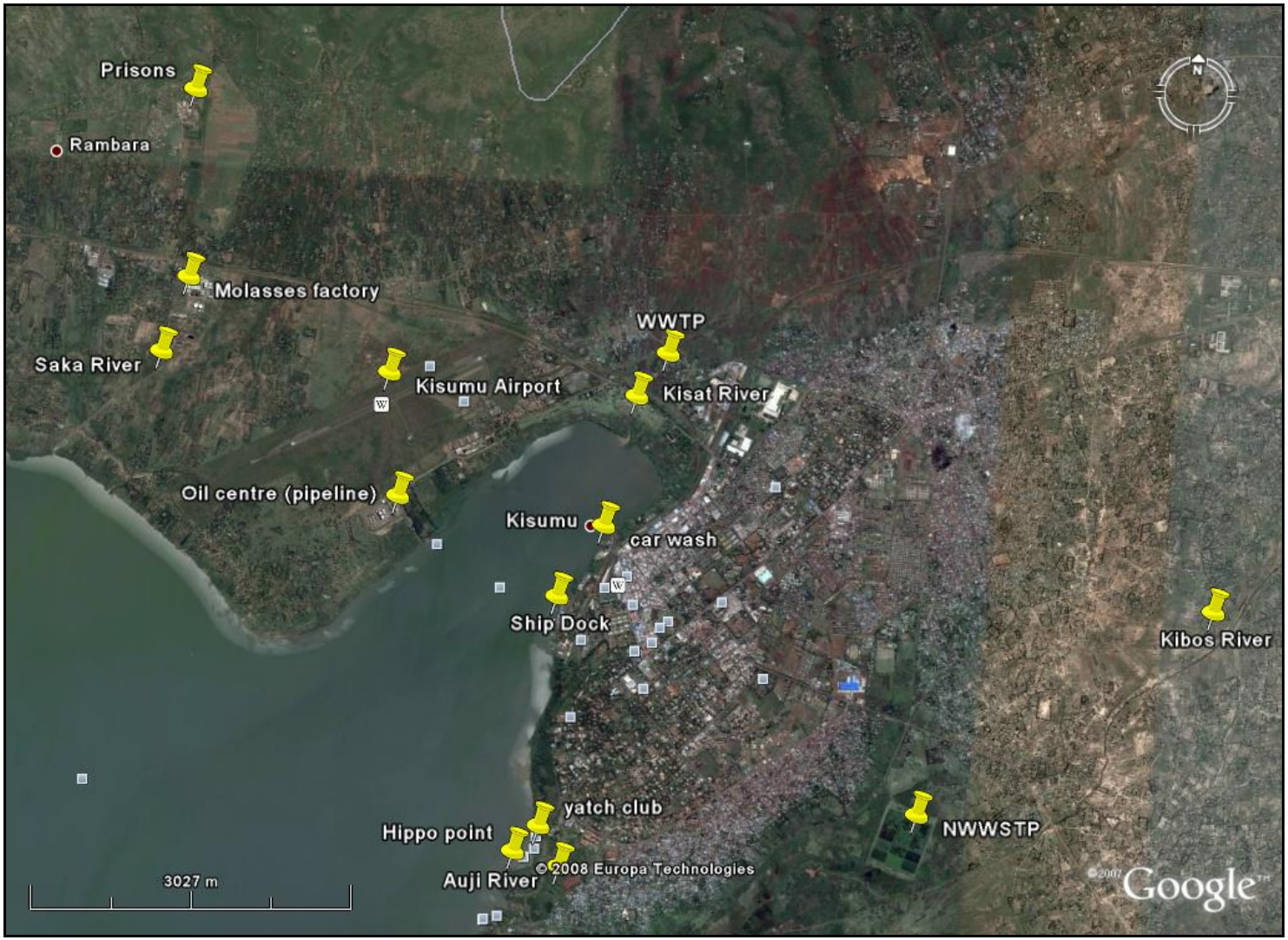


Figure 1. A map showing sampling locations along the rivers and within Winam gulf of Lake Victoria.



WWTP: Waste water treatment plant. NWWSTP: Nyalenda waste water treatment and stabilization ponds. Adapted from Google earth.

Figure 2. A Satelite image of the study area showing major sampling locations within Kisumu City.

1.5 STATEMENT OF THE PROBLEM

The sustainability of Lake Victoria's recourses involves management policies which include trace organic pollutants control. Detailed studies are necessary to thoroughly investigate occurrence and sources of trace organic contaminants of aquatic resources such as fish and water in the Lake Victoria Catchment. Generally, there was no available data on perfluorinated compounds (PFCs) levels in the Lake Victoria fresh water environment at the time of commence of this study. Hence, this research project aims to establish projected source and levels of PFOS and PFOA in water of Winam Gulf in Kenyan side of Lake Victoria, and to quantify levels in water, sediments and fish species namely *Lates niloticus* and *Oreochromis niloticus*.

Emerging perfluorinated surfactants substitutes continue to be fluorine based and are projected to be acid based. Thus, the issue of persistence of these compounds in the environment is still of concern. The need to test degradability of these new surfactants, find new or improve the already existing analytical methods is important.

1.6 OBJECTIVES

The main objectives of this study are to trace the source and levels of PFCs in sediments, water and fish from Winam Gulf of Lake Victoria, and its source rivers with an aim of providing control measures of PCF´s and of similar pollutants in Lake Victoria**.** Another objective in this tudy is to test new emerging perfluoroalkyl surfactants substitutes compounds for degradation, especially biodegradation. Improvement of analytical methodology in perfluorinated compounds analysis is also the focus of this study. Outlined below are the specific objectives that this study aims to achieve;

1.6.1 Specific Objectives

- To provide experimental data on and levels of PFCs in the fish, water and sediments along the Kenyan side of Lake Victoria namely Winam Gulf.
- To perform degradation tests on emerging surfactants.
- To develop alternative analytical method to LC/MS for Perfluorinated acids surfactants.

- To correlate the concentrations of PFOA and PFOS in Lake Victoria environmental matrices with an objective of identifying potential sources of pollution.
- To discuss and offer sustainable management solutions to PFCs and other similar organic pollutants which are present currently or may emerge in the future within the study area, with reference to the findings in this study.

1.7 HYPOTHESIS

Preliminary investigations through reconnaissance study on pollution status within Lake Victoria region and literature search on Physical-chemical properties of emerging perfluorinated surfactants substitutes and analytical methods used for perfluorinated compounds, the following hypothesis can be deduced for this study

I. There exist significant correlations between PFCs levels and other indicators of anthropogenic pollution in aqueous urban environments of Winam gulf of the Lake Victoria's basin.
II. New substitute compounds to PFCs as surfactants which are fluorine based are not readily degradable in the environment
III. Gas chromatography coupled with mass spectroscopy (GC-MS) can be used as an alternative method to analyse Perfluorinated compounds.

CHAPTER TWO

2 LITERATURE REVIEW

Environmental contaminants impacting the quality of food for man are a continuous challenge. Examples of such problematic chemicals include methylmercury and many persistent organohalogenated chemicals, notably the popular insecticides, DDT and its analogues, dieldrin, polychlorinated biphenyls (PCBs), -naphthalenes (PCNs), -dibenzo-*p*-dioxins (PCDDs), -furans (PCDFs), and more recently polybrominated diphenyl ethers (PBDEs). Perfluorinated chemicals (PFCs) have attracted considerable attention among environmental scientists, toxicologists, epidemiologists, and policy makers. Perfluorinated alkylated substances (PFAS) have been classified as a new class of global pollutant. Fluorinated surfactants are widely used (Kissa, 2001 and Martin et al., 2003a) and their production volume of 2.1 x 10^7 kg (in 2004) in the United States alone (Forman, 2004) and more than 4 x 10^7 kg world-wide (Merck, 2006) represents only a small fraction (0.4 %) of the total surfactants production.

2.1 Synthesis and Production of Perfluorinated Surfactants

The main production process of PFOA and PFOS related substances is Electro-Chemical Fluorination (ECF) and it is utilized by 3M, the major global producer of PFOA and PFOS-related substances prior to 2000. The process is given by the equation below:

- *Direct fluorination, electro-chemical fluorination (ECF)* :

  $C_8H_{17}SO_2Cl + 18\ HF \rightarrow C_8F_{17}SO_2F + HCl$ + by products ---------- (2)

The reaction product, perfluorooctanesulfonyl fluoride (PFOSF) is the primary intermediate for synthesis of PFOS-related substances. The ECF method results in a mixture of isomers and homologues with about 35 - 40 % of an 8-carbon straight chain PFOSF. However, the commercial PFOSF products are a mixture of approximately 70 % linear and 30 % branched PFOSF derivate impurities. Commercial synthesis of fluorinated alkyl substances originated in the late 1940s when 3M Company licensed and began developing an Electro-Chemical Fluorination (ECF) process invented by Joseph Simons, and DuPont Company began

developing a telomerisation fluorination process (Kissa, 2001). Electro-Chemical Fluorination, which refers to the fluorination of organic compounds in anhydrous hydrogen fluoride, primarily yields perfluorinated sulfonyl and carbonyl fluorides (Kissa, 2001). Perfluorooctane sulfonyl fluoride and perfluorooctanecarbonyl fluoride, the most abundant products, are the precursors respectively for the fluorosurfactants PFOS and PFOA. Telomerization is the other commercially important process used for synthesizing perfluoroalkyl substances, which begins with fluoroiodination of tetrafluoroethylene (TFE) to produce pentafluoroiodoethane, and then followed by a reaction with TFEs varying in number of "n" to yield a mixture of perfluoroalkyl iodides. The homologous fluoroalkyl chains generated during the telomerization process are all linear and contain only even numbers of fluorinated carbons in contrast to ECF, which produces a mix of linear and branched chains with both odd and even numbers of fluorinated carbons. The perfluoroalkyl iodides resulting from the reaction are commonly reacted with ethylene to produce intermediate perfluoroalkylethyl iodides. These iodides can be easily converted to yet other intermediates, such as olefins, alcohols, thiocyanates, sulfonyl chlorides, and thiols (Kissa, 2001).

Nearly all industrial output of such substances derives from the production either of perfluorinated sulfonyl and carbonyl fluoride intermediates by ECF or of perfluoroalkyl iodide intermediates by telomerization are known. Although these intermediates are not marketed as products themselves, they are nevertheless likely to be present as impurities in the finished products. It is largely intermediates or byproducts like PFOS that have been detected in humans (Hansen et al., 2001) and the environment (Giesy and Kannan, 2001). In as much as 3M Company is the sole manufacturer of fluorinated alkyl substances by ECF in the United States, the approximately three million kilograms of materials produced by 3M from perfluorinated sulfonyl fluoride intermediates in the year 2000 give some sense of the scale of sulfonyl fluoride production in the United States. Regrettably, it is impossible from this number for perfluorinated sulfonyl fluoride intermediates to estimate the quantity of these compounds that might eventually find their way into the environment (Melissa et al., 2003). The global production of PFOSF by 3M until the production ceased is estimated to have been 13,670 metric tones (1985 to 2002), with the largest yearly production volume, 3500 metric tones, in 2000 (UNEP, 2006). By the end of 2000 about 90 % of 3M's production of these substances had stopped and in the beginning of 2003 the production ceased completely. 3M´s voluntary phase-out of PFOS production has led to a significant reduction in the use of PFOS-related substances. This is due not only to the limited availability of these substances, but also

to the action within the relevant industry sectors to decrease companies´ dependence on these substances. Fluorinated alkyl substances based on telomerisation are produced by a number of companies including DuPont, Asahi Glass, Atofina, Clariant, and Daikin, but no production numbers are available for the telomere-based fluorinated alkyl substances. However, this list may not be exhaustive or current. According to the submission from Brazil and Japan three years ago, one manufacturer in Japan was still producing PFOS with a production amount of 1-10 tonnes by 2005 (UNEP, 2006). The submission from Brazil stated that lithium salt of PFOS is produced but that no quantitative data is available (UNEP, 2006).

2.2 Uses and application of perfluorinated surfactants

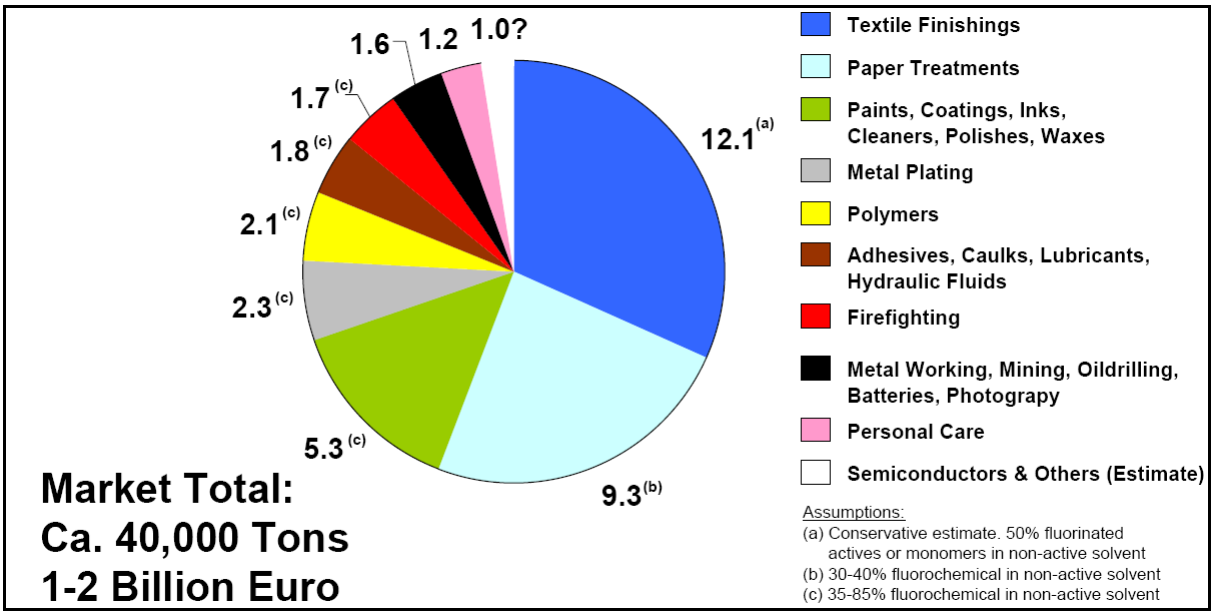
Perfluorinated surfactants with long carbon chains are both lipid-repellent and water-repellent. Therefore, they are commonly used as surface-active agents in different applications. The unique physical and chemical properties of fluorinated alkyl substances make them useful in a wide range of industrial and commercial applications. These properties are particularly manifested in fluorosurfactants. Surfactants are a class of chemicals that, at low concentrations, reduce the interfacial tension between the liquid in which they are dissolved and the gas, liquid, or solid phase which the surfactant solution is in contact with. Fluorosurfactants have alkyl tails that have both hydrophobic and oleophobic and they reduce interfacial tension to a greater degree than hydrocarbon surfactants (Kissa, 2001). Consequently, fluorocarbon surfactants are more versatile wetting agents than their hydrocarbon analogues (Kissa, 2001). This versatility character of fluorocarbon is exploited, for example, in aqueous film-foaming foams (AFFF), which use a mixture of fluorinated surfactants and hydrocarbon surfactants to extinguish hydrocarbon-fueled fires (Kissa, 2001). The dual hydrophobic/ oleophobic nature of the fluorinated surfactants in the AFFF formulation enables them to act both as the principal fire-extinguishing chemicals and as the vapor sealants that prevent reignition of fuel (Kissa, 2001). 3M reported in the year 2000 that 41% of its American production of perfluorooctane sulfonyl fluoridebased fluorinated alkyl substances was coated onto paper and packaging products; 37 % was impregnated into textile, leather, and carpet goods; 10 % was used as ingredients in industrial surfactants, additives, and coatings; and 3 % was incorporated into firefighting foams (U.S. EPA, 2000). 3M further reported that higher percentages of its perfluorooctane sulfonyl fluoride-based substances were used in Europe or textile, leather, and carpet goods (49 %) and for industrial surfactants,

additives, and coatings (15 %) than in the United States, whereas a lower percentage was used or paper and packaging products (33 %) (UNEP, 2006). The extreme persistence of these substances makes them suitable for high temperature applications and for applications in contact with strong acids or bases. It is the very strong carbon-fluorine binding property that causes the persistence of perfluorinated substances. The main uses and applications of perfluorooctane sulfonate are in fire fighting foams, carpets, leather/apparel, textiles/upholstery, paper/packaging, coatings/coating additives, and herbicides/pesticides, industrial and household cleaning products

In the UK study the following sectors were documentated by RPA and BRE (2004) to be using PFOS-related substances:

- Fire fighting foam stock
- Photographic industry
- Photolithography and semiconductor
- Hydraulic fluids
- Metal plating

The sectors presented above account for the UK. However, deviation in the current use pattern between EU countries can not be excluded (E.U. COM, 2005). PFOS and its precursors are not manufactured in most countries but rather are imported as chemicals or products for various uses. They may also be components in imported manufactured articles. In Canada, for example, it is estimated that the majority of PFOS have been used as water, oil, soil and grease repellents (e.g. on fabric, leather, paper, packaging, rugs and carpets) and as surfactants (e.g. in fire fighting foams, coating additives) (Environment Canada, 2004). Figure 3 shows a world market breakdown for fluorosurfactants and repellents use.



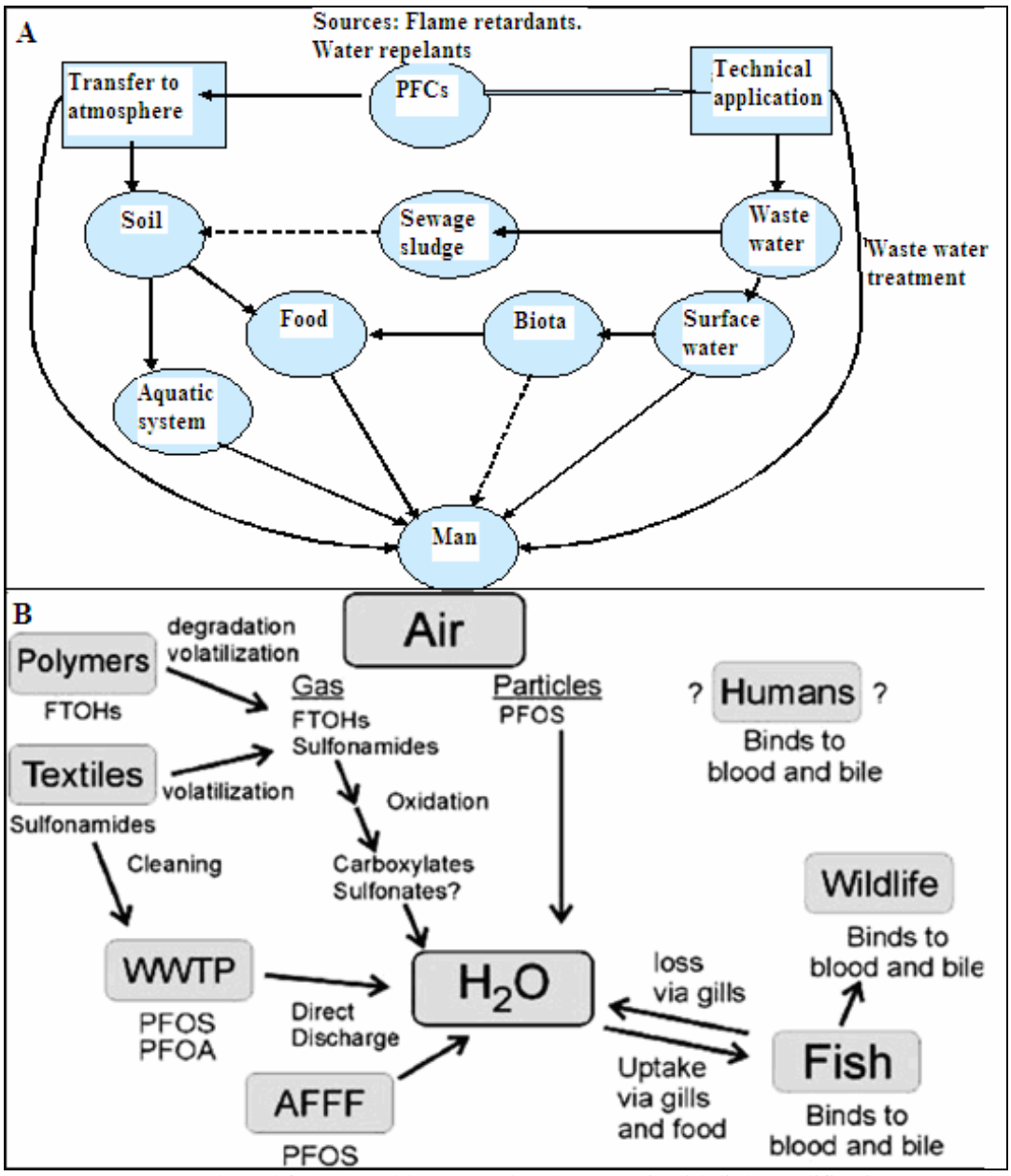
Sources: BCC, SRI, GIA, Freedonia, extrapolations to worldmarket in part by Merck

Figure 3. World Market Breakdown for Fluorosurfactants and Repellents

2.3 Sources, releases and environmental fate of PFCs

The existence of PFCs in the environment is a result of anthropogenic manufacturing and use, since PFCS are not a naturally occurring substance. Releases of PFCs and their related substances are likely to occur during their whole life cycle. They can be released at their production, at their assembly into a commercial product, during the distribution and industrial or consumer use as well as from landfills and sewage treatment plants after the use of the products (3M, 2000). One route for PFOA and PFOS-related substances to the environment may be through sewage treatment plants (STPs) and landfills, where elevated concentrations have been observed compared to concentrations that are often observed in remote areas. Once released from STPs, PFOS will partially adsorb to sediment and organic matter. A substantial amount of PFOS may also end up in agricultural soil, due to the application of sewage sludge to improve plant nutrients in the soil. The primary compartments for PFOS are therefore believed to be water, sediment and soil (RIKZ, 2002). Manufacturing processes constitute a major source of PFCs to the local environment. During these processes volatile perfluorinated substances may be released to the atmosphere. Perfluorinated substances could also be released via sewage effluents (3M, 2000). High local emissions are supported by one study that showed extremely high concentrations of PFOS in wood mice collected in the immediate vicinity to 3M's fluorochemical plant in Antwerpen, Belgium (Hoff et al., 2004). Figure 4 presents a summary of PFCs life cycle in different environmental matrices. However there are

significant gaps in knowledge of the environmental chemistry of PFCs as observed in the schematic diagram (part B).



Source: Adopted from Swackhamer, Deborah L (2005) with little modification.

Figure 4. The life cycle of perfluorinated compounds. Part A shows the general life cycle of perfluorinated compounds and part B shows the global transport and fate of perfluorochemicals.

Fire training areas have also been revealed to constitute a source of PFOS emissions due to the presence of PFOS in fire-fighting foams. High levels of PFOS have been detected in neighbouring wetlands of such an area in Sweden (Swedish EPA, 2004) as well as in groundwater in the US close to a fire-training area (Moody et al., 2003). The releases of

sulfonated perfluorochemicals from different product usages have been estimated. For example, garments treated with home-applied products, are expected to lose 73 % of the treatment during cleaning over a 2-year life span. A loss of 34 % to air is expected from spray can products during use, while up to 12.5 % of the original content may remain in the cans at the time of disposal. Boulanger et al., 2005, working on identifying the presence of PFOS and PFOA in samples from wastewater treatment plants, noted that cleaning and care of surface-treated products, such as carpets and clothing by consumers, and use in industrial processes causes the release of PFCs to municipal wastewater treatment systems. One possible route for perfluorinated substances to the environment may be through sewage treatment plants (STPs) and landfills, where elevated concentrations have been observed. Dispersion of PFOS in the environment is thought to occur through transport in surface water, or by oceanic currents (Yamashita et al., 2005; Caliebe et al., 2004), transport in air (volatile PFCs), adsorption to particles (in water, sediment or air) and through living organisms (3M, 2003).

The mechanisms and pathways leading to the presence of perfluorinated compounds in wildlife and humans are not well researched, but it is likely there are multiple sources (Kannan et al., 2002a). PFOS is stable, chemically inert and has the potential to bioaccumulate in living organisms (Corsolini and Kannan, 2004). It has a high water solubility, which makes it less likely to partition and be transported by air, (Giesy and Kannan, 2002), and it is fairly involatile. These properties mean that PFOS is unlikely to enter the atmosphere directly and undergo long-range transport on air currents to remote regions (Shoeib et al., 2004; Stock et al., 2004). Although the environmental fate of PFOS is not completely understood, 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 (Shoeib et al., 2004; So et al., 2004; 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). The widespread detection of environmentally persistent perfluorinated acids (PFCAs) such as perfluorooctanoic acid (PFOA) and its longer chained homologues (C9 > C15) in biota has instigated a need to identify potential sources. It has recently been suggested that fluorinated telomer alcohols (FTOHs) are the most probable precursor compounds that may undergo transformation reactions in the environment leading to the formation of these potentially toxic and bioaccumulative PFCAs (Dinglasan et al., 2004). Dispersion of PFOS in the environment is

thought to occur through transport in surface water, or by oceanic currents (Yamashita et al., 2005; Caliebe et al., 2004), transport in air (volatile PFOS-related substances), adsorption to particles (in water, sediment or air) and through living organisms (3M, 2003).

2.4 Developments in the analysis of fluorinated compounds

Issues and concerns regarding analytical methods for determination of persistent fluorinated acids (PFCA) and other perfluorinated substances in the environment were recently reviewed, noting contamination, calibration, recovery, and separation as recurrent difficulties to be overcome (Martin et al., 2004b). Determination of PFCs in environmental and biological matrixes is quite challenging, due to their lack of volatility for gas chromatographic (GC) analysis and the lack of a suitable chromophore for liquid chromatographic (LC) analysis using ultraviolet detection, (Martin et al., 2004b). Gas chromatography with electron capture detection (Belisle and Hagen, 1980) or mass spectrometric (MS) detection (Ylinen et al., 1985; Moody et al., 1999) can be used to sensitively and selectively measure derivatized fluorinated carboxylates.

Fluoroalkyl carboxylates have been derivatized by means of diazomethane (Belisle and Hagen, 1980), a liquid/ liquid extractive alkylation method that utilizes benzyl bromide (Ylinen et al., 1985), and by a strong anion exchange extraction method coupled with methyl iodide derivatization (Moody and Field, 1999). In contrast, perfluorinated sulfonates like PFOS, which do not form stable, volatile derivatives, can not be analyzed by GC/MS. A quantitative MS analysis of perfluoroalkyl sulfonates was reported in which samples with no preparation were injected directly into the mass spectrometer (Hebert et al., 2002). Although this technique is less time consuming, its developers do not recommend it for the analysis of blood serums, whole tissues, or wastewaters because of possible, interfering matrix effects. High-performance liquid chromatography (HPLC) followed by fluorescence detection (Ohya et al., 1998) was used to quantitatively determine perfluorinated carboxylates in biological samples. Fluorescence detection has good sensitivity, but unfortunately this advantage is offset by the limited specificity and excessive susceptibility to interference from sample matrices. High Perfomance Lliquid Chromatography (HPLC) coupled to a single quadrupole mass spectrometer (HPLC/MS) has been used to analyze for perfluorochemicals, including PFOS, in surface water and fish (Simcik and Dorweiler, 2002).

Regrettably, biological and environmental samples are so complex that many of the substances contained in such samples (analytes and nonanalytes alike) unavoidably coelute in the chromatographic stage of this technique, and as often as not, these coeluting species cannot be resolved in the single stage of mass spectrometric detection that follows. Consequently, the use of HPLC/MS for quantitative analysis still requires considerable sample cleanup to sufficiently reduce such interferences.

Analytical methods based on HPLC negative electrospray ionization (NESI) MS/MS were developed to analyze perfluoroalkyl carboxylates in human serum (Sottani and Minoia, 2002). A HPLC/NESI/MS/MS method was used to survey the global distribution of PFOS (Giesy and Kannan, 2001) and its accumulation in marine mammals (Kannan et al., 2002b), fish-eating water birds (Kannan et al., 2001), and oysters (Kannan et al., 2002a). Due to its sensitivity and selectivity, HPLC/NESI/MS/MS is, at present, the analytical method of choice for fluorinated alkyl surfactants in biological and environmental samples. With the use of high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), a method detection limit in the range of pg/L in water and at pg/g levels in biological matrices can be achieved (Taniyasu et al., 2005). Sinclair et al., (2004) found that ion-pairing, liquid liquid extraction method was suitable for measurements of concentrations in ng/L and µg/L levels for PFOA and PFOS analysis. Water analysis of PFOS and PFOA has been carried out with several methods. The most commonly used methods involve solid phase extraction (SPE) followed by HPLC-MS-MS. Scrupulous care must be taken in the laboratory to avoid sample contamination, because these compounds appear to be ubiquitous in analytical laboratory products.

In a recent study of PFOS and PFOA in river water, PFOS and PFOA were detected in the background blanks that included extracts from bottled drinking water and field blanks (Hansen et al., 2001). With only one exception, the analytes were at levels below the limit of quantitation (PFOS - LOQ range 10 - 25 ng/L; PFOA - LOQ range 25 - 50 ng/L). The levels of background contamination may vary from one instrument to another. When analyzing fluorinated alkyl substances, background interferences can be minimized by not storing aqueous samples in glass and PTFE containers; if glassware, including LC vials, must be used, it should be thoroughly rinsed with deionized water and methanol prior to use (Hansen et al., 2001). Aluminum foil should not be used to seal or cover containers because fluorinated alkyl substances are used to lubricate the mill-rollers used in foil manufacturing. Packaged, stored, and wrapped foods must be kept out of the laboratory, because paper food and

wrappings are treated with fluorinated alkyl substances. New clothes may be treated with fluorinated alkyl substances; thus, impurities, such as PFOS, may be present. Consequently, field or working clothes and laboratory coats must be washed thoroughly before personnel wear them.

2.5 Levels of perfluorinated compounds in abiotic compatment

2.5.1 Levels of perfluorinated compounds in Sediments and sludge

Quantifying PFCs in sediments may provide insight into their origins, distribution, and mobility in the environment. However, a major limitation to obtaining sediment data has been the lack of a sufficiently sensitive and accurate analytical method that accounts for the matrix effects typically observed in complex environmental media (Higgins et al., 2005). Furthermore, while sorption of PFC surfactants onto sediments has been suspected, it remains unclear whether the organic-carbon partitioning paradigm for hydrophobic organic contaminants is applicable to PFC surfactants. Sediments have been suggested as one of two final sinks of perfluorinated compounds, the other being the deep oceans (Prevendouros et al 2006). Studies to determine PFAS have been carried out in other Lake and river sediments. As example, data from a survey of San Francisco Bay Area sediments suggested widespread occurrence of PFCs in sediments at the low ng/g to sub-ng/g level (Higgins et al., 2005). Senthilkumar et al. (2007) obtained concentrations of PFOA of range 1.3 – 3.9 ng/g (wet weight) in Kyoto River, Japan. In another study, Nakata et al., (2006) reported PFOS, PFOA, PFHxS and PFOSA (perfluorooctanesulfonic acid) concentrations of ranges 0.09 – 0.14, 0.84 – 1.1, 0.33 – 0.55 and < 1.5 ng/g (wet weight) respectively in sediments from the Ariake sea in Japan.

Treated waste water released from local WWTPs is a main source of PFOA and PFOS in rivers. Becker et al. (2008) assessed the concentrations of PFOA and PFOS in sediments in relation to their levels in river water receiving effluent from a waste water treatment plant (WWTP) of the Roter Main River, Bayreuth, Germany. In the study, PFOS concentrations in sediment ranged from less than 50 ng /kg to 570 ng /kg dry weight, with the highest variations 500 m downstream the WWTP. PFOS was up to 17-fold higher than PFOA, due to its stronger adsorption potential. Levels of PFCs present in the effluents of industrial or municipal wastewater treatment plants only reveals the potential for direct releases to

receiving water bodies. According to the data collected by 3M (2001) the presence of PFOS in nearly all WWTPs was determined in biosolids samples.

The important role of sediments in the environmental fate of hydrophobic organic contaminants has long been recognized (Luthy et al., 1997). Hydrophobic contaminants such as polychlorinated biphenyls (PCBs) are expected to partition from the water column into organic-matter-rich sediments (Higgins et al., 2005) and sorption of linear alkyl benzene sulfonate surfactants, which exhibit both hydrophobic and hydrophilic functionalities, has been reported for sediments (Westall et al., 1999). Yet it is unknown whether the factors controlling the sorption of these surfactants also govern the sorption of anionic PFCs to environmental solids (Higgins et al., 2005). Losses of PFOS and PFOA in rivers is due to sorption to sediments (Hansen et al. 2002), while other data suggest sorption of PFOS to sediments and sludge (3M, 2000). Additional studies show elevated concentrations of PFCs in benthic dwelling organisms (Martin et al., 2004a) as opposed to offshore (pelagic) organisms which are limited to sediments exposure (Van de Vijver et al., 2003) in aquatic food webs. An accurate conceptual model of the movement of PFCs into aquatic food webs development is required. Therefore, it is important to evaluate sediments as a potential source of PFCs to the Food web (Higgins et al., 2005). A study of sediments from Nordic countries found differences between the countries. Samples from Sweden, Iceland and Faeroe Islands hardly contained any detectable PFCs whereas those from Norway were dominated by PFOS and PFOA and those from Finland were dominated by PFOS (Berger et al., 2004). In sewage sludge samples, PFOS and PFOA were the dominating PFCs. Total PFCs ranged from 150 pg/g wet weight to 3800 pg/g wet weight. For sewage effluent samples, the median concentration of PFOA was 20.5 ng/l and for PFOS was 12.7 ng/l (Berger et al., 2004).

2.5.2 Levels of perfluorinated compounds in freshwater

Studies have been carried out to establish concentrations of PFCs in some marine and fresh water ecosystems. Generally PFOS and PFOA concentrations in ambient waters, with no point source of pollution, are less than 5 ng/L (Yamatisha, 2004). Concentrations of PFOS and PFOA in the Great Lakes waters in the USA ranged from 21 - 70 and 27 - 50 ng/L, respectively (Boulanger et al., 2005). In another study, Simcik and Dorweiler (2005) obtained PFOS concentrations of upto 1.2 ng/L and from 2.4 to 47 ng/L in remote and urban surface

water respectively within the Lake Michigan environments. PFOA concentrations in that area ranged from 0.14 to 0.66 ng/L and from 0.45 to 19 ng/L. Sinclair et al. (2004) measured PFOS and PFOA in water samples collected from the Michigan waters of the Great Lakes and found concentrations upto 29 ng/l for PFOS and a concentration range of 2 to 5 ng/l in he background samples. For PFOA, the maximum concentration was 36 ng/l with background concentrations in the range of < 8 to 16 ng/l. PFOA was also detected in rainwater at a median concentration of 13.1 ng/l. A study was also conducted in Lake Ontario to determine the major sources of perfluorooctane surfactants of the Lake (Boulanger et al., 2005). The greatest source came from the inflow of Lake Erie. The second major source was from the wastewater discharge to the lake.

The concentrations of PFCs in freshwater samples from the USA in the study from Sinclair et al. (2004) were similar to those found from freshwater samples from Lake Biwa in Japan which ranged from < 4 to 7.4 ng/l for PFOS (Taniyasu et al., 2003). Another study in Japan revealed a wider range of contamination levels for PFOS (0.24 – 37.32 ng/l) and PFOA (0.1 – 456.41 ng/l) in rivers (Saito et al., 2004 In another study, it was reported that levels of PFOS in the middle stream of the Tama River in Japan were exceptionally high (upto 157 ng/l) (Harada et al., 2003; Taniyasu et al., 2003). A further study on waters from the middle stream of the Tama River was undertaken to confirm those findings (Harada et al., 2003). Harada et al managed to identify the origin of the high PFOS contamination in the Tama river came from sewage treatment effluents that have been discharged into the river. Concentrations in the discharge water were measured to 303 - 440 ng/l. The study also analysed PFOS in drinking water from different waterworks in Japan. In most drinking water, PFOS levels were detected to be less than 4 ng/l.

However, in drinking water originating from the middle stream of the Tama River there was heavy contamination (measurements of 43.7 and 50.9 ng/l) discovered. In another research on Japanese surface waters, comparatively high levels of PFOA (40 ng/l) were also found in drinking water in the Osaka area (Saito et al., 2004). Drinking water represents a possible source of human exposure to PFCs in these areas. In a study conducted on water samples from Nordic countries, PFOA concentrations were again slightly higher than PFOS concentrations (Berger et al., 2004). Median concentrations in lake waters were 7.8 ng/l and < 1ng/ml for PFOA and PFOS respectively.

2.5.3 Perfluorinated compounds in seawater

A study on concentrations of PFCs in the North Sea reported concentrations of PFOA of around 500 pg/l in the open sea whereas PFOS was below the limit of detection (Caliebe et al., 2004). However, another study conducetd in open ocean waters of the Pacific and Atlantic Oceans detected both PFOA and PFOS in pg/l concentrations, where PFOA was the more dominant chemical species (Taniyasu et al., 2004). For example, the concentrations in the central to eastern Pacific waters were 15 to 62 pg/l for PFOA and 1.1 to 20 pg/l for PFOS. The study noted that these values appeared to be background values for remote marine waters far from local sources. The study also showed that PFOS and PFOA were detectable in trace quantities in samples collected from the open ocean at depths of > 1000 m. A study of seawater from Nordic countries similarly found PFOA (median 5.2 ng/l) to be present in higher concentrations than PFOS (median < 1ng/l) (Berger et al., 2004). Coastal (offshore) waters had concentrations of PFOS and PFOA at an order of magnitude higher than the open ocean waters of the Pacific and Atlantic (Taniyasu et al., 2004). For instance, PFOA ranged from 1.8 – 192 ng/l and PFOS from 0.338 – 57.7 ng/l in the waters of Tokyo Bay. Again levels of PFOA were somewhat higher than PFOS.The high concentrations of PFOA and PFOS in Tokyo Bay compared to that of the open ocean waters suggest sources of PFCs that are associated with urban and industrial areas in Tokyo. A study on PFCs in coastal waters of Hong Kong and South China reported that PFOS and PFOA were detectable in all samples that were analysed (So et al., 2004). Concentrations ranged from 0.02 to 12 ng/L for PFOS and 0.24 to 16 ng/L for PFOA. Other PFCs were also detectable in about 90 % of the samples at concentrations less than those for PFOS and PFOA. These included perfluorooctanesulfonamide (PFOSA; $C_8F_{17}SO_2NH_2$) perfluorohexanesulfonate (PFHS; $C_6F_{13}O_3$), perfluorobutanesulfonate (PFBS; $C_4F_9O_3$) and perfluorononanoic acid (PFNA $C_8F_{17}COOH$). Taniyasu et al. (2004) made the observation that PFOA pollution is more ubiquitous than PFOS in oceanic waters.

2.6 Levels of perfluorinated compounds in biotic compartment

Widespread distribution of PFOS in wildlife tissues collected from several regions of the globe has been reported (Giesy and Kannan, 2002; Martin et al., 2004b). Additional perfluorinated organic contaminants, such as perfluorohexanesulfonate (PFHS), perfluorooctanoate (PFOA), and perfluorooctanesulfonamide (PFOSA) have been reported to

occur in the environment, however at lower concentrations and frequencies than PFOS. Earlier studies have suggested that concentrations of PFOS tend to be higher in predatoryorganisms than in lower trophic-level organisms of aquatic food chains (Kannan et al., 2002b). Human toxicology for PFOA and PFOS has also 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). Recently in Germany, 12.2 µg/L of PFOS and 5.3 µg/L (median values) of PFOA were found in non-occupationally exposed volunteers (14 - 67 years of age) living in the southern part of Bavaria, Germany (Fromme et al., 2007a). Other PFCs, including PFOA, perfluorooctanesulfonamide (PFOSA), perfluorohexanesulfonate (PFHS), PFBS and perfluorononanoic acid (PFNA) were also found in other living organisms although often to a lesser extent than PFOS (So et al., 2004).

2.6.1 Concentration of PFCs in invertebrates and fishes in freshwater

Perfluorinated compounds have been detected in freshwater fishes from different regions such as the Great Lakes in the USA, Japan and the Canadian Arctic (Taniyasu et al., 2003; Martin et al., 2004a respectively). A study of PFCs in a food web in Lake Ontario showed that some PFCs, including PFOS and PFOA, bioaccumulated in fish (Martin et al., 2004a). It also suggested that biomagnification, that is, a progressive increase in levels of PFCs in the bodies of organisms going up through the food chain, may be occurring at the top of the food web for PFOS, perfluorodecanoic acid (PFDA; $C_{10}HF_{19}O_2$) perfluoroundecanoate (PFUnA; $C_{10}F_{21}COO$-) and perfluorotridecanoate (PFTrA; $CF_3(CF_2)_{11}COO$-). The study on Lake Ontario`s food web analysed various PFCs in 3 species of fish. The mean PFOS concentrations in whole body homogenates of the fish were 450 ng/g (wet weight) for sculpin, 110 ng/g (wet weight) for smelt and 46 ng/g (wet weight) for alewife. Other PFCs that were detected in the fish included the homologous series of perfluoroalkyl carboxylates (PFCAs) – perfluorooctanoate (PFOA), perfluorononanoic acid (PFNA), perfluordecanoate (PFDA), perfluoroundecanoate (PFUnA), perfluorododecanoate (PFDoA; $C_{11}F_{23}COO$-), perfluorotetradecanoate (PFTA; $CF_3(CF_2)_{12}COO$-) and perfluoropentadecanoate (PFPA; $CF_3(CF_2)_{13}COO$-). PFCs were also detected in 2 aquatic invertebrates, Mysis and Diporeia. Mysis had a PFOS concentration of 13 ng/g (wet weight) and Diporeia, 280 ng/g (wet weight) respectively. The high concentrations found in Diporeia were unusual because, of the species

analysed, it occupied the lowest trophic level in the food web. It was hypothesised that the sediments of the lake were a major source of PFCs to the food web rather than the surrounding water and that the chemicals are consequently taken up by Diporeia, which is a benthic organism.

A study of fish from Michigan waters of the Great Lakes and inland water bodies of New York detected PFOS in the livers of a number of species that were tested (Sinclair et al., 2004). Concentrations ranged from < 7 to 381 ng/g wet weights, which were similar to those concentrations found in fish liver from Tokyo Bay measured in another study. A study of fish in Nordic countries found that PFOS was the predominant PFC species (Berger et al., 2004). The highest concentration of PFCs was detected in Finnish pike, a top predator in Nordic lakes (PFOS 551 ng/g wet weight, PFOSA 141 ng/g wet weight). Levels of PFOS in 3 fish species from Lake Biwa in Japan have also been investigated (Taniyasu et al., 2003). Concentrations in blood ranged from 33 to 834 ng/ml and in livers from 3 to 310 ng/g wet weight. A research group from the Canada examined organisms from the Canadian Arctic and collected fish from the mouth of the Great Whale River at Kuujuarapik and from Lake Minto, Quebec (Martin et al., 2004b). PFOS was found in all samples of fish liver at concentrations ranging from 5.7 to 50 ng/g. FOSA was detected in fish liver at similar concentrations to PFOS (2.0 to 18 ng/g). The study noted that these concentrations differ from birds and mammals in which levels of FOSA are usually lower than PFOS. PFOA level in fish was below the limit of detection.

2.6.2 Concentration of perfluorinated compounds in marine fish

An investigation on fish from the different coastal regions in Japan was conducted to measure concentrations of PFCs in samples of blood and liver (Taniyasu et al., 2003) PFOS was detected in all samples and the concentrations in blood ranged from 1 to 834 ng/ml and from 3 to 7900 ng/g (wet weight) in liver. The concentrations in fish varied depending on the species and their origins. A report from Taniyasu et al. (2004), who worked in the Pacific and Atlantic Oceans, noted that PFOS appeared to be the predominant compound in wildlife samples which were collected from several areas. It was noted that this discrepancy suggests that the bioaccumulation potential of PFOA is comparatively lower than PFOS. A study on marine fish from Nordic countries revealed a high variability in PFC levels reflecting

differences in trophic levels, feeding habits and location (Berger et al., 2004). A study on fish collected from the Mediterranean, specifically the Italian coast, investigated bluefin tuna (*Thunnus thynnus*) and swordfish (*Xiphias gladius*) (Kannan et al., 2002a). Harta et al. (2008) analysed concentrations of nine PFCs in the livers of 60 skipjack tuna (*Katsuwonus pelamis*) which were collected from offshore waters and the open ocean along the Pacific Rim, including the Sea of Japan, the East China Sea, the Indian Ocean, and the Western North Pacific Ocean, during 1997 – 1999. At least one of the nine PFCs was found in every analyzed tuna sample. Overall, perfluorooctanesulfonate and perfluoroundecanoic acid were the predominant compounds found in livers of tuna at concentrations of < 1 – 58.9 and < 1 – 31.6 ng/g, (wet weight), respectively. Long-chain perfluorocarboxylates such as perfluorodecanoic acid (PFDA; $C_{10}HF_{19}O_2$) and perfluorododecanoic acid (PFDoDA; $C_{11}F_{23}COOH$) were also common in the tuna livers.

2.6.3 Concentration of perfluorinated compounds in human blood

Perfluorooctane sulfonate and perfluorooctanoic acid have been detected in the blood of occupationally exposed workers at a few mg/L concentrations (mean 1.32 mg/L and 1.78 mg/L, respectively), and in the general population at µg/L concentrations (mean 28.4 µg/L PFOS) (Olsen et al., 2003b and Hensen et al., 2001). PFOS and PFOA were found in samples of umbilical cord blood which indicates possible exposure to the foetus in the womb. In individuals who have been exposed to PFCs through their occupation, concentrations of perfluorinated compounds in their blood are much higher than the general population (PFOS up to 12830 ng/ml, PFOA up to 81 300 ng/ml) (Allsopp et al., 2005). Kannan et al. (2004) assessed the level of various PFCs in human blood serum from a number of countries. The samples were collected in city locations and from a diverse age range of individuals. As such, it was suggested that the samples provided a reasonable representation of the populations in question. The mean concentration of PFOS in serum for the USA (34.9 ppb) was in the category of > 30 ng/ml (Olsen et al., 2003b). One exceptionally high value (1656 ng/ml) which is similar to concentrations found for persons with occupational exposure to PFCs, was detected. Results from an investigation in Sweden showed a mean equivalent concentration of PFOS in serum of 9.1 ng/ml (Kärrman et al., 2004). Such levels were within the same range as those found in Belgium (e.g. mean 13.9 ng/ml), Kannan et al. (2004). The production of certain PASs, particularly those that are PFOS-related, has been phased out due to the concern

of bioaccumulation, their detection in human serum and sparse knowledge of their toxicology (Taniyasu et al., 2003). In a study that involved the general population, blood samples from families including three generations living in 12 European countries were tested for a large number of chemicals including PFOS and perfluorooctane sulfonamide (FOSA) (WWF, 2005). PFOS was present in 37 out of 38 samples with out of 38 samples with concentrations from 0.15 to 2.04 ng/g blood (WWF, 2005).

2.7 Risk and hazard assessment of perfluorinated compounds

The hazard assessment of PFOS, prepared by the OECD in 2002, concluded that the presence and the persistence of PFOS in the environment, as well as its toxicity (González-Barreiro et al., 2006) and bioaccumulation potential, indicate a cause of concern for the environment and human health (OECD, 2002). Chronic health effects of PFCs in humans are still under debate, because of interspecies variation in the mode of action. Laboratory animal studies and in vitro tests have suggested that certain PFCs can act as potent peroxisome proliferators, inhibitors of gap junction intercellular communication, and tumor promoters (Andersen et al., 2008). An environmental risk assessment, prepared by the UK-Environment Agency, and discussed by the EU member states under the umbrella of the existing substances regulation (ESR DIR 793/93) shows that PFOS is of concern (Swedish Chemicals Inspectorate, 2006). The Environment Canada/Health Canada Draft Assessment of PFOS, its salts and its precursors were released for public comment in October 2004. The ecological risk assessment has concluded that PFOS and related substances are persistent, bioaccumulative, and inherently toxic (Environment Canada, 2004). The proposed Swedish regulation prohibits products which wholly or partly contain perfluorinated substances (Swedish EPA, 2004). These products are not allowed to be offered for sale or handed over to consumers for individual purposes or used commercially. Norway is now considering a proposal to prohibit the use of fire fighting foams containing PFCs, which is the major application of these compounds today.

The Environmental Protection Agency (EPA) in the USA finalized two Significant New Use Rules (SNURs) in 2002, requiring companies to inform the EPA before manufacturing or importing 88 listed PFOS-related substances (U.S. EPA, 2002). The EPA proposed an additional SNUR under section 5(a)(2) of the Toxic Substances Control Act (TSCA) in March

2006 to include within the scope of this regulation another 183 perfluoroalkyl sulfonates (PFAS) with carbon chain lengths of five carbons and higher. The EPA further proposed an amendment to the Polymer Exemption rule in March 2006 which would remove from exemption, polymers containing certain perfluoroalkyl moieties consisting of CF_3- or longer chains, and would require that new chemical notifications to be submitted on such polymers (U.S. EPA, 2002).

The Canadian government has also taken significant steps against fluorochemical release. In December 2006, regulations were proposed to prohibit manufacture, use, sale and import of perfluorooctane sulfonate (PFOS) and its salts and compounds (RSC, 2007).

European Union´s ministers and parliament agreed on a directive to severly restrict the marketing and use of PFOS in the EU, which member states will have to enact in national law. The Commission aims to ensure that PFOS is phased out as soon as the use of safer alternatives becomes technically and economically feasible. In the mean time it will continue to review the PFOA situation. The EU is now working on a proposal on the prohibition of PFCs in some products and chemical mixtures.

There is also a talk of adding perfluorinated chemicals to the Stockholm convention's 'dirty dozen' of persistent organic pollutants (RSC, 2007). The US firm, 3M, was ahead of the game in phasing out fluorinated products. In 2000, 3M voluntarily halted the production of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS; used in its Scotchguard product) and PFOS related products including *N*-ethylperfluorooctane sulfonamidoethanol (NEtFOSE; CF_3 $(CF_2)_7SO_2N$ (CH_2CH_3) (CH_2CH_2OH)). By 2002, it had completed its phase-out of PFOS production and no longer makes or sells it, with one exception. One of its subsidiaries still uses PFOA to produce fluoropolymers, but has developed a technology to recover and recycle the chemicals and monitors the health of its employees (RSC, 2007).

2.7.1 Persistence and degradation tests for perfluorinated alkyl based surfactants

Biodegradation studies of organic compounds in the aquatic environment gives important information of the final fate of chemicals in the environment. In the initial step of microbial degradation action, known as the primary degradation, minor alterations in the chemical

structure of the molecule occur, often resulting in the loss of the surface-active properties of the molecule. The total degradation of a compound, which can occur either by mineralization or assimilation, is measured as a substance-independent sum parameter such as evolution of CO_2, oxygen consumption or removal of the dissolved organic carbon (DOC) (Knepper et al., 2003).

There are a number of standardized and non-standardized methods for measuring biodegradability, all of which fall in two main categories: those involving direct measurement of bioconversion of a test compound and those involving indirect measurement such as cumulative oxygen uptake. The common principle of all these methods is to expose the chemical to microorganisms in a test medium (inoculum) in the presence of excess oxygen. Advanced Oxidation Process (AOP) refers to the use of UV light in combination with ozone or hydrogen peroxide or both to generate a very reactive free radical such as hydroxyl radicals (OH-) to destroy the organic contaminants. The hydroxyl radicals formed by AOP increases the rate of reactions over 100 to 1000 times higher than that observed with either oxidants or UV if applied separately (ETL 1110-1-161, 1996). As a result, many organic compounds which are normally resistant to powerful oxidants can be destroyed by the AOP in a short time, and most of the inherent shortcomings of chemical oxidation can be overcome (ETL 1110-1-161, 1996). Ready biodegradable substances are assumed to degrade rapidly in common environmental conditions, without the formation of toxic and persistent metabolites or by-products. The multilevel protocol is completed with simulation tests providing information on substance behaviour in specific environmental conditions, for example assesment tests which simulate biodegradation in the surface waters can be applied (Žgajnar Gotvajn and Zagorc-Končan, 2003).

The majority of OECD ready biodegradation test methods require a relatively high concentration of test substance. Such high dosage concentrations are unsuitable for screening of inhibitory or biocidal compounds. Unfortunately, the method with the lowest dosage concentration (Closed Bottle Test, 301D) also has the lowest biodegradation potential. Otherwise the Manometric respirometry (301F) test method has the ability to study biodegradation of test substances at concentrations of a few mg L^{-1} (O'Malley, 2006). The biological oxygen demand (BOD) is a measure for the quantity of oxygen required for the biodegradation of organic matter in water. The BOD tests have been used for over a century to determine the amount of biodegradable organic matter in wastewater (Roppola et al., 2006). Roppola showed in his study that the manometric respirometric test has many

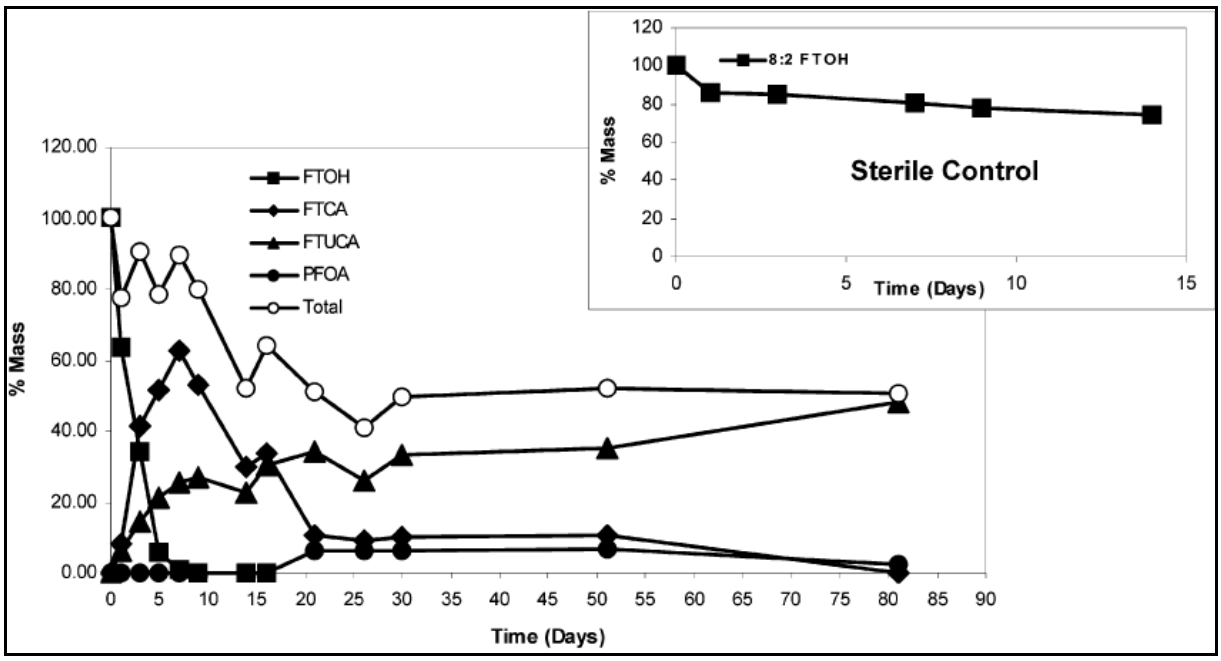
advantages compared to the classical BOD methods including reduced sample preparation time, use of non-diluted samples, easy and continuous reading of the measuring data, and faster measuring time (Roppola et al, 2006). This respirometric test is based on automatic pressure measurement in a closed bottle under constant temperature. Micro-organisms consume oxygen by degrading organic matter and the formed CO_2 gas is chemically bound by the sodium hydroxide pellets. The overall result is a pressure decrease in the bottle. The instrument calculates automatically the BOD value using the ideal gas law modified for conditions in a closed space. The BOD value can be read continuously during the test.

The biological fixed bed reactor (FBBR) is considered to be a good model system, due to the fact that with it one is able to simulate the biological degradation in different waters. It has been satisfactorily used for investigating both the primary degradation of various organic chemicals and their further breakdown pathway (Knepper et al., 2003). The FBBR system was completely described in Knepper et al. 2003. A standardized fixed bed bioreactor (FBBR) was utilized by Peschka et al. (2008a) running with surface water. The experimental set-up was based on a mixed microbial community from a natural setting other than isolated cultures, as this guaranteed a higher environmental relevance of the outcomes. (Peschka et al., 2008a).

Alternative compounds in use as surfactants are typically fluorinated compounds with shorter chain length such as fluorotelomer alcohols (mainly C_6 chain length), PFBS (perfluorobutane sulfonate), or perfluorinated polyethers based on a CF_3 or a C_2F_5 structure. Among the polyfluorinated alkyl compounds the bioaccumulation potential and hazard increase by increasing the length of the alkyl group (Poulsen et al., 2005). Polyfluorinated compounds with an alkyl chain length of C_5 or below do not seem to be significantly bioaccumulative and toxic. They are, however, still substances that will persist in the environment for decades, and the implications for human health and the environment are unclear.

A study on the hydrolysis of PFOS in water has been performed following US-EPA OPPTS protocol 835.2210 in 2003 by U.S. EPA (2003). The study was conducted at pH varying from 1.5 – 11.0 and at a temperature of 50 °C, to facilitate hydrolysis, but did not indicate any degradation of PFOS. The half-life of PFOS was set to be greater than 41 years. A study on the photolysis of PFOS in water following US-EPA OPPTS protocol 835.5270 has been conducted. No evidence of direct or indirect photolysis was observed under any of the conditions tested. The indirect photolytic half-life of PFOS at 25 °C was calculated to be more than 3.7 years. Biodegradation of PFOS has been evaluated in a variety of tests. Aerobic

biodegradation of PFOS has been tested in activated sewage sludge, sediment cultures and soil cultures in several studies (OECD, 2002). Neither of the studies demonstrated any signs of biodegradation. The only known condition whereby PFOS is degraded is through high temperature incineration under correct operating conditions (3M, 2003). Potential degradation at low temperature incineration is unknown.



Adopted from Dinglasan et al. (2004). Typical transformation kinetics and mass balance of metabolites observed in degradation experiments; degradation of 8:2 FTOH in active microcosm (vessel B). Loss of 8:2 FTOH, production of 8:2 FTCA, 8:2 FTUCA, PFOA, and overall mass balance. No observable loss of 8:2 FTOH in sterile control (inset). PFOA values were obtained using standard additions at day 81.

Figure 5. Typical transformation kinetics and mass balance of metabolites observed in degradation experiments.

Dinglasan et al. (2004), examined the aerobic biodegradation of the 8:2 telomer alcohol (8:2 FTOH, $CF_3(CF_2)7\text{-}CH_2CH_2OH$) using a mixed microbial system. Telomer acids (CF_3 $(CF_2)7CH_2COOH$; CF_3 $(CF_2)6CFCHCOOH$) and PFOA were identified as metabolites during the degradation, the unsaturated telomer acid being the predominant metabolite was measured. The overall mechanism involved the oxidation of the 8:2 FTOH to the telomer acid via the transient telomer aldehyde. The telomer acid via α-oxidation mechanism was further transformed, leading to the unsaturated acid and ultimately producing the highly stable PFOA. Telomer alcohols were demonstrated to be potential sources of PFCAs as a consequence of biotic degradation (Dinglasan et al., 2004). The study however provided evidence for one dominant mechanism that the degradation of the 8:2 FTOH was presumably due to microbial

activity since the sterile control showed little to no transformation during the experimental period as shown in Figure 5. Tolemer alchohols have been detected at tropospheric concentrations typically ranging from 17 to 135 pg m^{-3} (Martin et al., 2002 and Stock et al., 2004) with urban locations apparently having higher concentrations than rural areas. A study by Ellis et al. (2003) shows that the atmospheric lifetime of short chain FTOHs as determined by its reaction with OH radicals is approximately 20 days.

These results demonstrate that fluorotelomer alcohols are widely disseminated in the troposphere and are capable of long-range atmospheric transport. Ellis et al. (2001) have identified thermolysis of fluoropolymers as an abiotic mechanism that can potentially lead to the production of these persistent compounds, while Hagen et al. (1981) have been the first to observe the production of PFOA from the 8:2 telomer alcohol in a biotic system, and more recently, the production of perfluorocarboxylic acids were observed from a telomer alcohol biodegradation screening study by Lange (2002).

2.7.2 Bioaccumulation biomagnification and bioconcetration of perfluorinated compounds

To bioaccumulate literally means to accumulate in a biological system. However, it is commonly taken to measure the uptake over time of a substance, called a bioaccumulant, which can accumulate in a biological system. Bioaccumulation can be divided into bioconcentration and biomagnification. Bioconcentration considers uptake from the non-living environment while biomagnification describes uptake through the food chain. For many fat-soluble and persistent chemicals (POPs), biomagnification is the dominant factor. Everything in a biological system has a biological halflife, that is, a measure of how long it will stay in that system until it is lost, excreted, degrades, reacts into something different, or ends its presence in some other way. Most substances have a short half-life, as they are metabolized, or excreted as waste.

However, some compounds may stay in a system for a much longer period of time. It should be noted that PFOS does not follow the “classical” pattern of partitioning into fatty tissues followed by accumulation, which is typical of many persistent organic pollutants. This is because PFOS is both hydrophobic and lipophobic (Kerstner-Wood et al., 2003). Instead, PFOS binds preferentially to proteins in the plasma, such as albumin and β-lipoproteins (Kerstner-Wood et al., 2003), and in the liver, such as liver fatty acid binding protein

(Luebker et al., 2002). Due to the unusual physical-chemical characteristics of PFOS, it´s mechanism of bioaccumulation probably differs from other POPs. Studies on fish have shown that PFOS has bioconcentrating properties. In investigations on bluegill sunfish (*Lepomis macrochirus*) and rainbow trout (*Oncorhynchus mykiss*) bioconcentration factors (BCFs) have been estimated to be 2796 (whole fish) as well as 2900 (liver) and 3100 (plasma), respectively. The major route of uptake is believed to be through the gills (Martin et al., 2003).

Since PFOS is claimed to be released to the environment mainly through water from Sewage treatment Plants (STPs), one major route for PFOS into food chains could be through fish. PFOS has shown a high oral uptake (95 %) within 24 hours in the gastro-intestinal tract in studies on rats (OECD, 2002). Taken together, this could constitute the basis of the highly elevated levels that have been observed in top predators in food chains containing fish. In humans, the highest concentrations of PFOS have been detected in workers at 3M's manufacturing plant for perfluorochemicals in Decatur, US, where the levels in serum in the last year of measurement (2000) ranged between 0.06 – 10.06 ug/g (OECD, 2002). In a study following OECD protocol 305, the bioaccumulation of PFOS in bluegill sunfish (*Lepomis macrochirus*) has been tested. The whole-fish kinetic bioconcentration factor was determined to be 2796 (OECD, 2002). In another study on rainbow trout (*Oncorhynchus mykiss*), a bioconcentration factor (BCF) in liver and plasma was estimated to be 2900 and 3100, respectively (Martin et al., 2003c). Study by Rossana Bossi et al. (2005), showed that PFOS was the predominant fluorochemical in the biota analyzed, followed by perfluorooctane sulfonamide (PFOSA). PFOS was found at concentrations above LOQ (10 ng/g wet weight) in 13 out of 16 samples from Greenland and in all samples from the Faroe Islands.

When we strictly look at the BCF values, it is clear that these values are below the numeric BCF criteria in Stockholm Convention Annex D (the reported BCF values are below 5000) but, in this particular case, as noted above, the BCF numeric criteria may not adequately represent the bioaccumulation potential of the substance. Thus, monitoring data from top predators at various locations show highly elevated levels of PFOS which demonstrate the substantial bioaccumulation and biomagnification (BMF) properties of PFOS. It is also worth to mention that the concentrations of PFOS found in livers of Arctic polar bears exceed the concentrations of all other known individual organohalogens (Martin et al., 2004b). Based on

the concentration of PFOS in predators (e.g., the polar bear) in relation to the concentration in their principal food (e.g., seals), hypothetical BMF values can be calculated.

In a study by Kannan et al., (2005), the whole body BCF for round gobies (*Neogobius melanostomus*) were calculated to be approximately 2400, which is comparable with laboratory data. PFOS concentrations in fish (whole body of round gobies) compared to concentrations in liver of salmon results in BMFs of approximately 10 - 20. In bald eagles, the mean PFOS concentration in the livers, 400 ng/g (wet weight), gives a BMF of 4 to 5 when compared to fish at higher trophic levels in the study. For mink, BMFs from 145 to 4000 can be calculated when based on the mean liver concentration, 18 000 ng/g (wet weight), compared to their prey items such as crayfish (whole body), carp (muscles) and turtles (liver).

In general, it can be concluded that animals at higher trophic levels have higher concentrations of PFOS than animals at lower trophic levels, indicating that biomagnification is taking place. For instance, a trophic magnification factor (TMF) of 5.9 was calculated for PFOS based on a pelagic food web including: one invertebrate species, Mysis; two forage fish species, rainbow smelt and alewife; and a top predator fish species, lake trout. A diet-weighted bioaccumulation factor of approximately 3 was determined for the trout (Martin et al., (2004a). Morikawa et al. (2005) showed a high bioaccumulation in turtles. Results from a study performed by Tomy et al. (2004) indicated that PFOS biomagnified in an eastern Arctic marine food web (liver concentrations of PFOS were used for seabirds and marine mammals). Houde et al. (2006) showed PFOS biomagnification in the Atlantic Ocean bottlenose dolphin food web. Serum albumin is most likely the binding pool of PFOS (Jones et al., 2003) and several studies have been carried out with regard to bioconcentration in plasma. Ankley et al. (2005), presented the bioconcentration in fish was studied at concentrations of PFOS in water up to 1 mg /L; the concentration of PFOS in water and plasma followed an almost linear relationship in the doses tested up to 0.3 mg/l without any signs of saturation (1 mg/l was not tested due to mortality at that dose). This is far above environmentally relevant concentrations. In a study conducted by 3M (2003), the bioconcentration factor (BCF) in whole fish was determined to be approximately 2800 at a PFOS concentration of 86 µg/l, based on calculations of uptake and depuration of PFOS. Steady-state levels were attained after 49 days of exposure. Depuration occurred slowly and 50 % clearance for whole fish tissues was estimated to be 152 days. Due to mortality, a BCF could not be calculated for the

other concentration used 870 µg/l. Thus, it is not likely that saturation of serum protein binding sites will limit the bioconcentration of PFOS in fish.

Bioaccumulative substances are of great concern because of their potential to attain toxicologically significant tissue and organ residue concentrations in higher-trophic-level species such as predatory fish, birds, mammals and humans (Kelly et al., 2004). Unlike many other persistent and bioaccumulative environmental pollutants, PFOS and other PFCS do not accumulate in lipids of the body. Instead, these chemicals accumulate in the blood and in the liver and gallbladder (Renner, 2001). In an investigation on fish, the common shiner, the bioaccumulation factor for PFOS was reported to vary between 6 300 and 125 000 (Hekster et al., 2003). A study on wild fish from different coastal regions of Japan reported the existence of PFOS in all tissue samples of the fish (Taniyasu et al., 2003). Bioconcentration factors for PFOS in livers of fish were estimated for two species of marine fish and one freshwater species. Bioconcentration factors for PFOS ranged from 274 to 41 600. A study was carried out to investigate dietary accumulation of PFCs in fish (Martin et al., 2003c). Juvenile rainbow trout were fed for 34 days on food spiked with various perfluorosulfonates and perfluorocarboxylates. The fish were sacrificed at varying time intervals during and after the feeding study and the PFCs in their tissues were analysed. The results showed that PFCs had accumulated in the tissues of the fish with bioaccumulation factors ranging from 0.038 to 1.0. The bioaccumulation factors of less than 1 indicated that PFCs do not biomagnify in juvenile rainbow trout, however it cannot just be assumed that this will be the case in mature rainbow trout or in other fishes. The bioaccumulation factors increased with increasing length of the perfluorinated chain, as was also the case in the water exposure study discussed above (Martin et al., 2003b). Perfluorinated sulfonates bioaccumulated to a greater extent than perfluorinated carboxylates (Martin et al., 2003a).

The ability of a chemical to bioaccumulate in aquatic organisms has been assessed by using its partition coefficient, Kow. This has been adopted in some policies to assess the bioaccumulation potential of chemicals. However, its use is limited because it does not take into account other air-breathing organisms and therefore may be inaccurate. Presently, there is evidence that the Kow classification of chemicals is not an adequate model to identify substances with a bioaccumulative potential in food webs that include mammals, birds and humans (Kelly et al., 2004). For instance, PFOS does not meet the current Kow criterion for bioaccumulative substances and does not biomagnify in fish. However, studies show that

PFOS is efficiently absorbed via dietary exposures, biomagnifies and persists in the liver and blood of air-breathing animals and is inherently toxic (Kelly et al., 2004).

2.7.3 Long range environmental transport

The geographical distribution of perfluorinated compounds in Greenland was similar to that of persistent organohalogenated compounds (OHCs), with the highest concentrations in east Greenland, indicating a similar geographical distribution to that of OHCs, with higher concentrations in east Greenland than in west Greenland (Rossana Bossi et al., 2005). The potassium salt of PFOS has a measured vapour pressure of 3.31×10^{-4} Pa (OECD, 2002). Due to this vapour pressure and a low air-water partition coefficient ($< 2 \times 10^{-6}$), PFOS itself is not expected to volatilise significantly. It is therefore assumed to be transported in the atmosphere predominantly bound to particles, because of its surface-active properties, rather than in a gaseous state. It should be noted that some of the PFCs have a considerably higher vapour pressure than PFOS itself, and are as a result more likely to be volatile. This may allow a wider transport of PFCs through air than for PFOS itself. Once they reached the atmosphere, they can remain in gas phase, condense on particles present in the atmosphere and be carried or settle out with them, or be washed out with rain (3M, 2000). Martin et al. (2002) measured the air in Toronto and Long Point, Ontario for some precursors of PFOS. They found an average N-MeFOSE alcohol concentration of 101 pg/m^3 in Toronto and 35 pg/m^3 at Long Point. The average concentrations of N-EtFOSE alcohol were 205 pg/m^3 in Toronto and 76 pg/m^3 in Long Point. PFOS has been detected in rainwater from an urban center in Canada with a concentration of 0.59 ng/L. Whether or not PFOS originates from precursors either being transported and subsequently wet deposited and degraded to PFOS, or atmospherically degraded and then wet deposited, is unclear. Measurements of potential precursors for PFOS were not performed in this study (Loewen et al., 2005). The indirect photolytic half-life of PFOS at 25 °C has been estimated to be more than 3.7 years (OECD, 2002).

2.8 Previous research done in Lake Victoria on organic pollutants

Pollution resulting from increased human activities is threatening Lake Victoria, its effects being characterized by eutrophication and the occurrence of low dissolved oxygen levels in some parts of the lake. Mitema and Gitau (1990) worked on eighty-two samples of either fat or muscle of the Nile perch fish which were collected from the Kenyan region of Lake

Victoria for detection of organochlorine residues. Nine organochlorine residues were detected in the following percentages: α-BHCIHCB- 40 %; P-BHC/HCB- 40 %; y-BHC/HCB/lindane- 4 %; aldrin- 9 %; dieldrin-1 %; p, p'-DDE- 73 %; p, p'-DDD- 9 %; o, p'-DDT-170; and p, p'-DDT- 11 %. Previous assessment of pollution sources revealed that nutrient input originated mainly from atmospheric deposition and land runoff, together accounting for an estimated 90% of phosphorous and 94 % of nitrogen input into the lake (Scheren et al., 2000). Getenga et al., (2004) found α-BHC, β-BHC, lindane, endosulfan, heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin and methoxychlor residues in water samples collected from rivers draining the sugarcane fields, and in soils from sugarcane fields that constitute Lake Victoria basin. They found residues levels of lindane and α-BHC to be the highest, with α-BHC showing average concentration of 0.219 ± 0.091 mg/l for six points, except one point that showed concentration as high as 0.691 mg/l. The same study reported the highest concentration of lindane in water as high as 1.240 mg/l. Lake Victoria serves as the basic food and economic resource for millions of people in the region and recent studies points to the destruction of its catchment area.

Agricultural chemicals and their residues have been detected in Lake Victoria waters at varying concentrations but not high enough to be of threat to human health, export products, or ecosystem integrity. However, because of the ability of most organics to bioconcentrate and bioaccumulate in the food chain, their long-term impact to ecosystem integrity cannot be underestimated. Low concentrations (0.01 - 0.03 mg/L) of α-endosufan, β-endosurfan and endosulfan sulphate were detected in fish (Henry and Kishimba, 2002). Recent studies of pesticide residues in the Kenyan part of the drainage system of Lake Victoria have shown presence of p, p'-DDT, o, p'-DDE, p, p'-DDD, α-, β-, γ-HCH, aldrin, dieldrin, endrin, α-, β-endosulfan, endosulfan sulphate, heptachlor, heptachlor epoxide, ethyl parathion, malathion, fenithrothion, dimethoate, and diazinon (Madadi, 2004). The study reported residue levels ranging from < 0.002 - 0.439 µg/l in water, < 0.002 - 65.478 µg/g in soils, < 0.001 - 10.073 µg/g in weeds and < 0.001 - 481.178 µg/g in fish. Most of the residues in water were below WHO guidelines (IUPAC, 2003), whereas soil, weeds and fish had some residues above the recommended guidelines in water.

2.9 Quality assurance/quality control (QA/QC)

Analytical data must be of demonstrably high quality to ensure confidence in the results. Quality control (QC) comprises a system of planned activities in an analytical laboratory whereby analytical methods are monitored at every stage to verify compliance with validated procedures and to take steps to eliminate the causes of unsatisfactory performance. Results are considered to be of sufficiently high quality if they meet the specific requirements of the requested analytical work within the context of the defined problem; there is confidence in their validity and if the work is cost effective (Keales and Haines, 2002). Our institutional laboratory observes and applies the principals of **Analytical Quality Management (AQM)** in it´s research field. Quality control processes should include;

- Check of accuracy and precision of the data using statistical tests
- Detailed records of calibration, raw data, results and instrumental performance
- Observation on the nature and behaviour of the sample and unsatisfactory aspects of methodology
- Control charts to determine system control for instrumental and repeat analysis.
- Provision of full documentation and traceability of results to recognised reference material through recorded identification
- Maintenance and calibration of instruments to manufacturers specifications
- Management and control of laboratory chemicals and other materials including checks and quality
- Adequate training of laboratory personnel to ensure understanding and competence
- External verification of results wherever possible
- Accreditation of the laboratory by an independent organisation.

The overall management of an analytical laboratory should include the provision of evidence and assurances that appropriate QC procedures for laboratory activities are being correctly implemented. **Quality assurance** (**QC**) is a managerial responsibility that is designed to generate confidence in the analytical results. Part of the QA is to build confidence through the laboratory participating in **interlaboratory studies** where several laboratories analyse one or more identical homogenous materials under specified conditions. **Proficiency testing** is a particular type of study to access the performance of a laboratory or analyst relative to others, whilst **method performance studies** and **certification studies** are undertaken to check a

particular analytical method or reference material respectively. The result of such studies and their statistical assessment enable the performances of individual participating laboratories to be demonstrated and deficiencies in methodology and the training of personnel to be addressed.

2.9.1 Accreditation System

Defined **quality standards** have been introduced by a number of organisations. A number of organisations that offer accreditation suitable for analytical laboratories and their corresponding quality standards are given in Table 1 below

Table 1.Accreditation organisations ad their quality standards

Name of Accreditation organisation	**Quality standard**
Organisation of Economic Co/operation and Development	Good Laboratory Practices
The International Organisation for Standardisation (ISO)	ISO 9000 series of quality standards ISO Guide 25 general requirements for competence of calibration and testing laboratories
European Committee for Standardisation (CEN)	EN 29000 series EN 45000 series
British Standard Institution (BSI)	BS 5750 quality standards BS 7500 series
National Measurement Accreditation Service (NAMAS)	NAMAS

2.9.2 Assessment of Accuracy and Precision

The following definitions are important for analytical quality management and results interpretation and presentation:

Accuracy and precision: Accuracy is the closeness of an experimental measurement or result to the true or acceptable value. Precision is the closeness of agreement between replicated measurements or results obtained under the same prescribed conditions

Standard Deviation: The standard deviation of a set of values is a statistical based on the normal error (Gaussian) curve and used as a measure of precision

Relative Standard Deviation (Coefficient of variation): is the standard deviation expressed as percentage of measured value.

Pooled Standard Deviation: A standard deviation can be calculated for two or more sets of data by pooling the values to give a more reliable measure of precision.

Variance: This is the square of the standard deviation, which is based on some statistical tests

Overall precision: An estimate of the overall precision of an analytical procedure can be made by combining the precisions of individual measurements

Confidence interval: This is the range of the value around an experimental result within which the true or accepted value is expected to lie with a defined level of probability.

Quality Control Charts: These are graphical representations of quantitative data from on going series of mearsurements that can be used to monitor the stability of the system for quality control purposes. Control charts are used to routinely monitor quality. Depending on the number of process characteristics to be monitored, there are two basic types of control charts. The first, referred to as a univariate control chart, is a graphical display (chart) of one quality characteristic. The second, referred to as a multivariate control chart, is a graphical display of a statistic that summarizes or represents more than one quality characteristic.

Standard Operating Procedures (SOP)**:** The step-by-step procedures used in all the processes of the monitoring system, i.e. in the field, laboratory, and data management areas. **Objectives of SOPs** is to provide a method to ensure that all personnel perform the same procedure to avoid the variance of data quality between personnel in charge and that they conduct their works with good understanding of QA/QC.

Good laboratory Practice (**GLP):** It deals with the organization, process and conditions under which laboratory studies are planned, performed, monitored, recorded and reported. GLP practices are intended to promote the quality and validity of test data. GLP is a regulation and therefore a laboratory must have a specific organizational structure and procedures to perform and document laboratory work. The objective is not only quality of data but also traceability and integrity of data.

2.9.3 Method Validation and Standard Analytical Methods

Often, laboratories will develop their own in/house methods or adapt existing ones for specific purposes. Method development forms a significant part of the work of most analytical laboratories, and method validation and periodic revalidation is a necessity.
Selection of the most appropriate analytical method should take into account the following factors:

- The purpose of the analysis, the required time scale and any cost constraints.
- The levels of analytes expected and the detection limit required.
- The nature of the sample, the amount available and the necessary sample preparation procedure.
- The accuracy required for quantitative analysis.
- The availability or reference materials, standards, chemicals, solvents, instrumentation and any specific facilities.
- Possible interference with the detection or quantitative measurement of the analytes and the possible need for sample clean-up to avoid matrix interference.
- The degree of selectivity available. Methods may be selective for a small number of analytes or specific for only one.
- Quality control and safety factor.

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.
Analytical methods need to be validated or revalidated,

- before their introduction into routine use;
- whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); and
- whenever the method is changed and the change is outside the original scope of the method.

Method validation has received considerable attention in the literature and from industrial committees and regulatory agencies. *United States Pharmacopoeia* (*USP*) has published specific guidelines for method validation for compound evaluation (General Chapter 1225, 2007). USP defines eight steps for validation with the following **Parameters for Method Validation**:

- Accuracy
- Precision
- Specificity
- Limit of detection
- Limit of quantitation
- Linearity and range
- Reproducibility (also referred to as Ruggedness)
- Robustness

2.9.3.1 Precision and Reproducibility

The precision of a method is the extent to which the individual test results of multiple injections of a series of standards agree. The measured standard deviation can be subdivided into 3 categories: repeatability, intermediate precision and reproducibility (ISO/IEC 17025, 2005). Repeatability is obtained when the analysis is carried out in a laboratory by an operator using a piece of equipment over a relatively short time span. For environmental and food

samples, precision is largely dependent on the sample matrix, the concentration of the analyte, the performance of the equipment and the analysis technique. It can vary between 2 percent and more than 20 percent.

Intermediate precision is a term that has been defined as the long-term variability of the measurement process. It is determined by comparing the results of a method run within a single laboratory over a number of weeks. A method's intermediate precision may reflect discrepancies in results obtained

- from different Operators,
- from inconsistent working practice (thoroughness) of the same operator,
- from different instruments,
- with standards and reagents from different suppliers,
- with columns from different batches or a combination of these.

Reproducibility is defined by the USP (U.S. FDA, 2000 and ISO/IEC 17025, 2005) as the degree of reproducibility of results obtained under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials. It is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst. Reproducibility is determined by the analysis of aliquots from homogeneous lots in different laboratories.

2.9.3.2 Accuracy and Recovery

The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree. Accuracy can also be described as the closeness of agreement between the value that is adopted, either as a conventional, true or accepted reference value, and the value found.

The true value for accuracy assessment can be obtained in several ways. One alternative is to compare the results of the method with results from an established reference method. This approach assumes that the uncertainty of the reference method is known. Secondly, accuracy can be assessed by analyzing a sample with known concentrations (e.g., a control sample or

certified reference material) and by comparing the measured value with the true value as supplied with the material. If certified reference materials or control samples are not available, a blank sample matrix of interest can be spiked with a known concentration by weight or volume. After extraction of the analyte from the matrix and injection into the analytical instrument, its recovery can be determined by comparing the response of the extract with the response of the reference material dissolved in a pure solvent. Because this accuracy assessment measures the effectiveness of sample preparation, care should be taken to mimic the actual sample preparation as closely as possible. If validated correctly, the recovery factor determined for different concentrations can be used to correct the final results.

The range of an analytical method is the interval between the upper and lower levels (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. The range is normally expressed in the same units as the test results (e.g., percentage, parts per million) obtained by the analytical method.

For assay tests, the minimum specified range to be 80 to 120 percent of the test concentration, and for the determination of an impurity, the range to extend from the limit of quantitation, or from 50 percent of the specification of each impurity, whichever is greater, to 120 percent of the specification.

2.9.3.3 Limit of Detection and Limit of Quantitation

The limit of detection is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. The limit of detection is frequently confused with the sensitivity of the method. The sensitivity of an analytical method is the capability of the method to discriminate small differences in concentration or mass of the test analyte. In practical terms, sensitivity is the slope of the calibration curve that is obtained by plotting the response against the analyte concentration or mass. In chromatography, the detection limit is the injected amount that results in a peak with a height at least two or three times as high as the baseline noise level. Figure 6 illustrates the limit of quantitation (along with the limit of detection,

range and linearity). Figure 7 illustrates both the limit of detection and the limit of quantitation. Besides this signal/noise method there are three additional methods:

I. Visual inspection: The detection limit is determined by the analysis of samples with analyte of known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.
II. Standard deviation of the response based on the standard deviation of the blank: Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.
III. Standard deviation of the response based on the slope of the calibration curve: A specific calibration curve is studied using samples containing an analyte in the range of the limit of detection. The residual standard deviation of a regression line, or the standard deviation of y-intercepts of regression lines, may be used as the standard deviation.

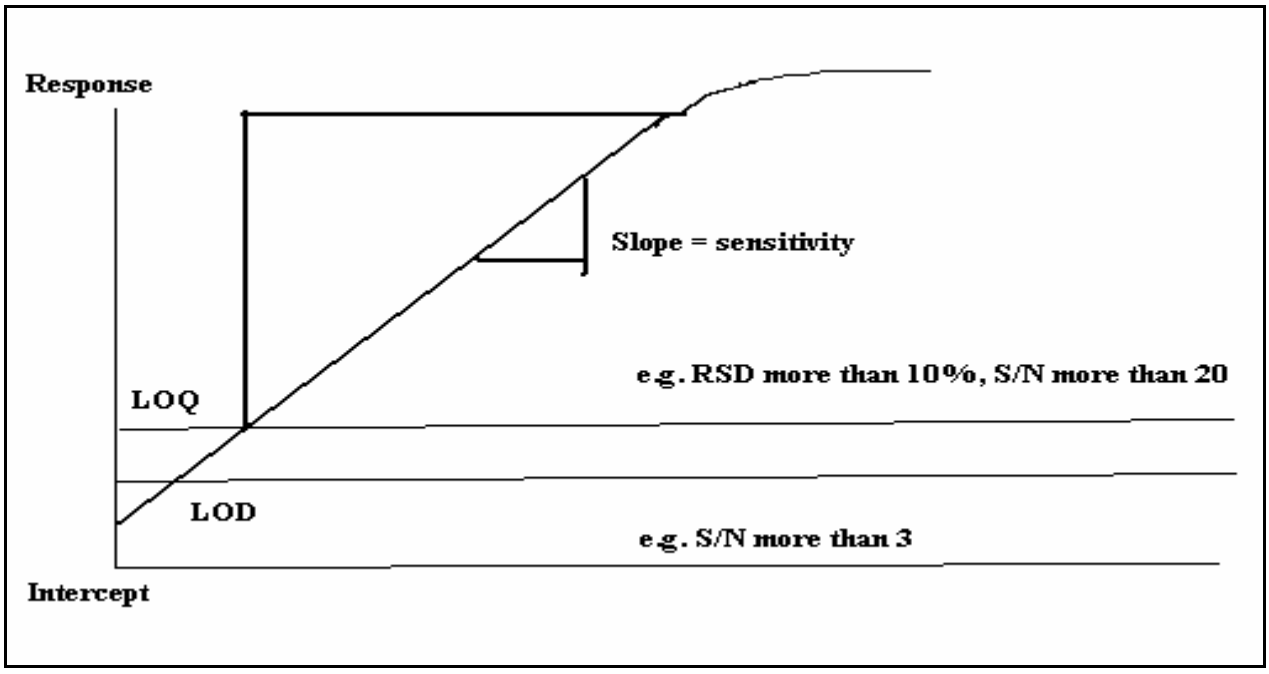


Figure 6. Definitions for linearity, range, LOQ, LOD

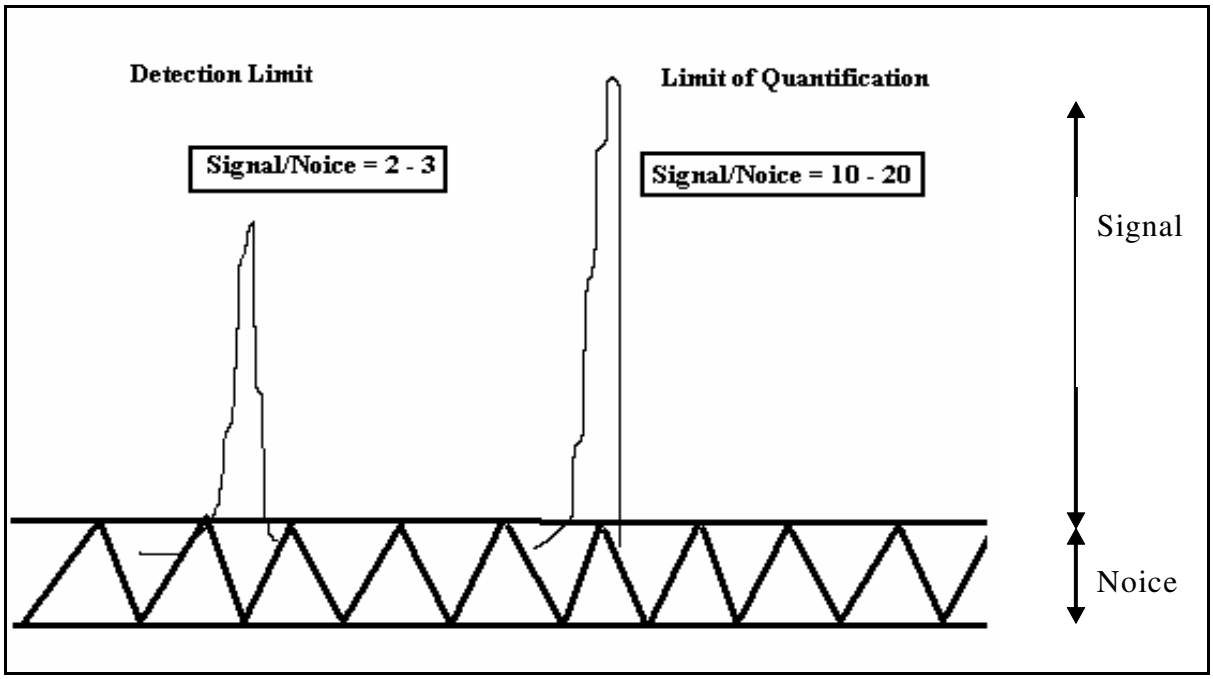


Figure 7. Limit of detection and limit of quantitation via signal to noise

The limit of quantitation is the minimum injected amount that produces quantitative measurements in the target matrix with acceptable precision in chromatography, typically requiring peak heights 10 to 20 times higher than the baseline noise. Detection limits are usually based on estimates of the standard deviation of replicate measurements of prepared blanks. A detection limit of two or three times the estimated standard deviation of the blanks above their mean is often quoted, where as many blanks as possible (at least 5 to 10) have been prepared and measured. This is somehow arbitrary, and it is perfectly acceptable to define the alternatives provided that the basis is clear and comparisons are made at the same probability level (Keales and Haines, 2002). An alternative method, reported by Eurachem (1993) based on the target value of the relative standard deviation (RSD) applicability is demonstrated in Figure 8. Figure 8 shows graphical interpretation of the EURACHEM (80) method. In this method, samples with decreasing amounts of the analyte are injected six times. The calculated **relative standard deviation** (RSD) percent of the precision is plotted against the analyte amount. The amount that corresponds to the previously defined required precision is equal to the limit of quantitation. It is important to not only use pure standards for this test but also spiked matrices that closely represent the unknown samples.

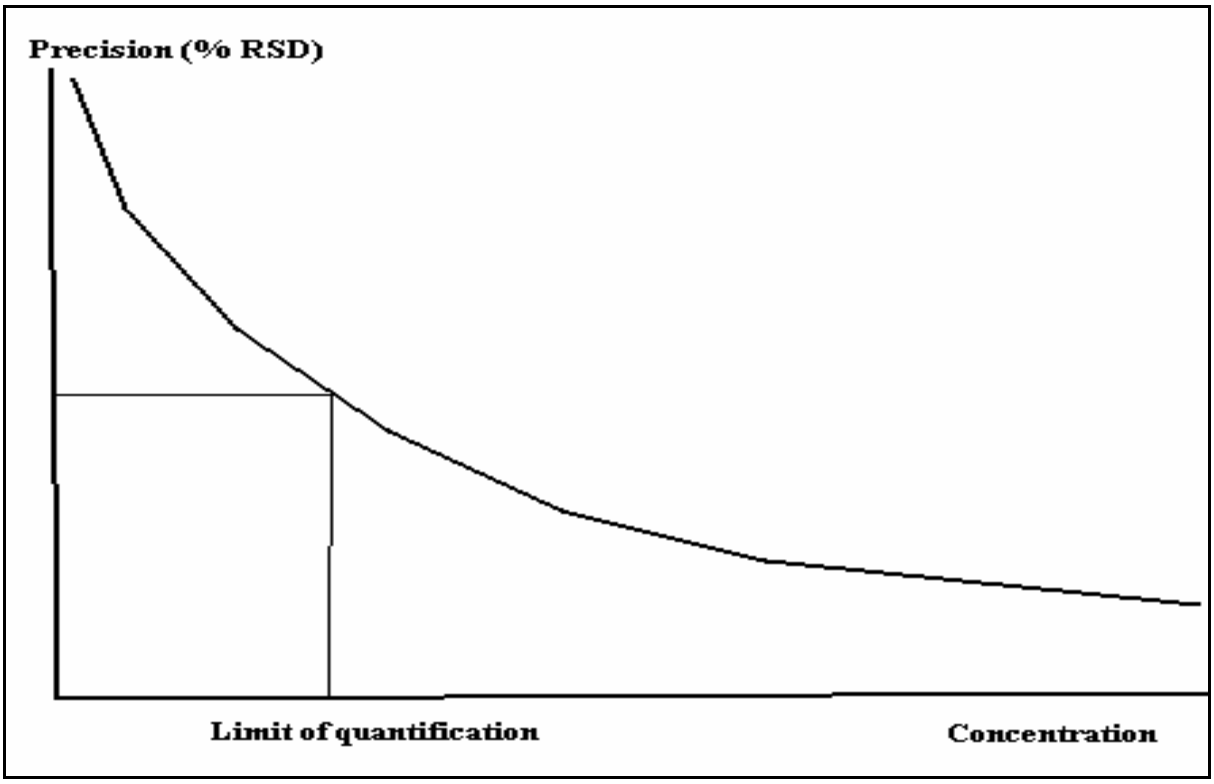


Figure 8. Limit of quantitation with the EURACHEM method.

Any results of limits of detection and quantitation measurements must be verified by experimental tests with samples containing the analytes at levels across the two regions. It is equally important to assess other method validation parameters, such as precision, reproducibility and accuracy, close to the limits of detection and quantitation.

2.9.3.4 Robustness

Robustness tests examine the effect that operational parameters have on the analysis results. For the determination of a method's robustness, a number of method parameters, for example, pH, flow rate, column temperature, injection volume, detection wavelength or mobile phase composition, are varied within a realistic range, and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range.

2.9.3.5 Strategy for the Validation of Methods

The validity of a specific method should be demonstrated in laboratory experiments using samples or standards that are similar to unknown samples analyzed routinely. The preparation and execution should follow a validation protocol, preferably written in a step-by-step instruction format (see example in Figure 9). This proposed procedure assumes that the instrument has been selected and the method has been developed. It meets criteria such as ease of use; ability to be automated and to be controlled by computer systems; costs per analysis; sample throughout; turnaround time; and environmental, health and safety requirements. Possible steps for a complete method validation are listed in below.

1. Develop a validation protocol, an operating procedure or a validation master plan for the validation
2. For a specific validation project define owners and responsibilities
3. Develop a validation project plan
4. Define the application, purpose and scope of the method
5. Define the performance parameters and acceptance criteria
6. Define validation experiments
7. Verify relevant performance characteristics of equipment
8. Qualify materials, e.g. standards and reagents for purity, accurate amounts and sufficient stability
9. Perform pre-validation experiments
10. Adjust method parameters or/and acceptance criteria if necessary
11. Perform full internal (and external) validation experiments
12. Develop SOPs for executing the method in the routine
13. Define criteria for revalidation
14. Define type and frequency of system suitability tests and/or analytical quality control (AQC) checks for the routine
15. Document validation experiments and results in the validation report

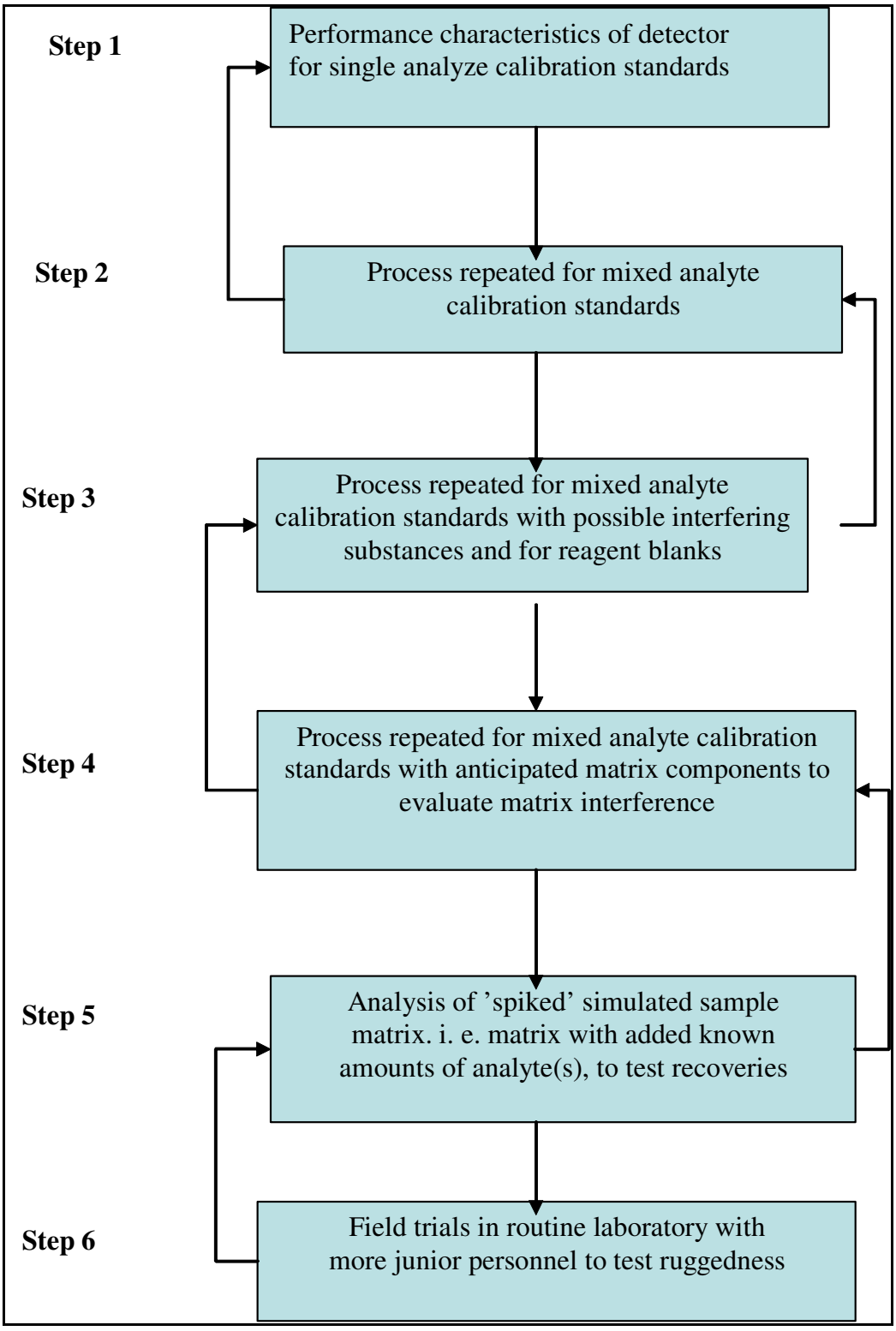


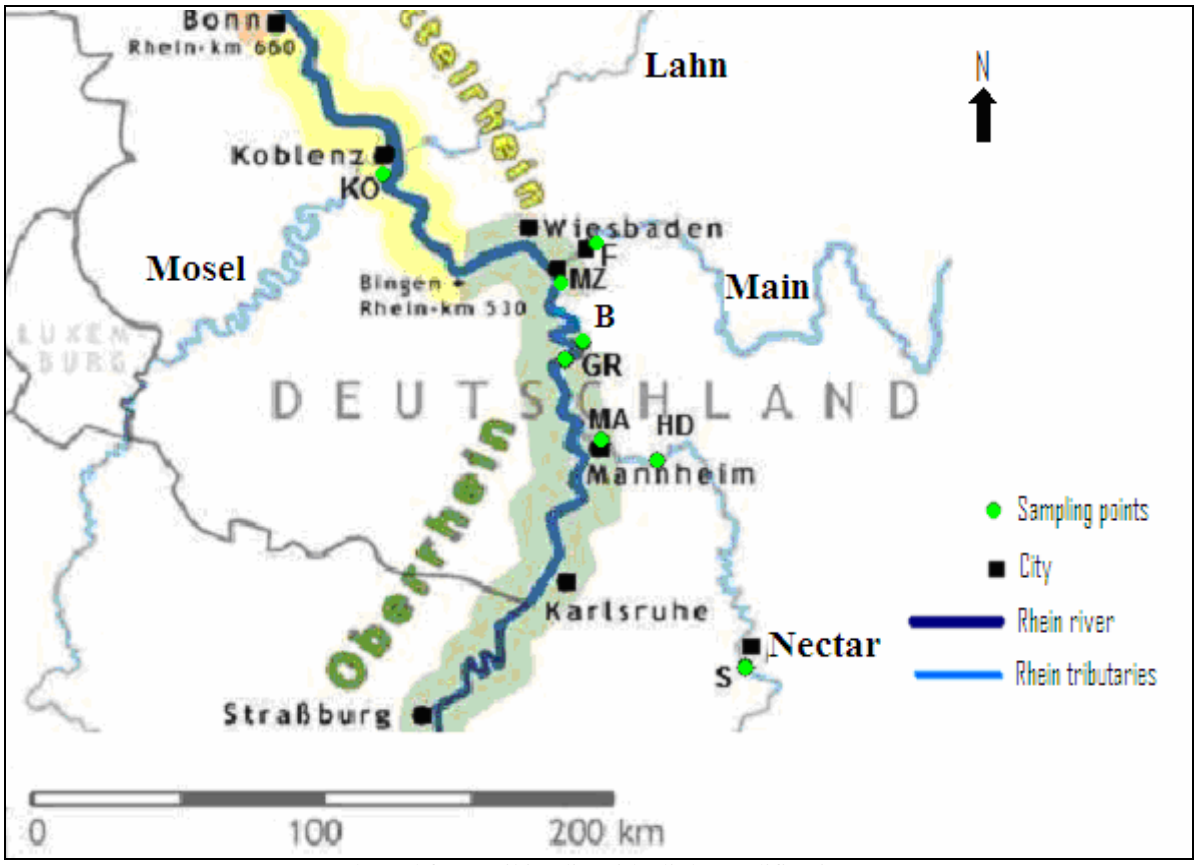
Figure 9. Flow chart for method validation

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Samples used in study

Sampling for water, fish and sediments was done during the period of November 2006 to January 2007 as well as in May and June 2008. Water and sediment samples were collected in areas where the detection of PFOA and PFOS was most expected due to anthropogenic inputs. Sites selected included along rivers that flow near industries, residential estates and waste treatment facilities. Also sites were selected near places where various commercial activities are carried out within Winam Gulf of Lake Victoria. Control sites were selected as sites far away from these commercially active sites. Five field blanks were prepared in selected locations. 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. Fishes were restricted to only those of more than 25 cm and 40 cm full body length for Nile tilapia and Nile perch respectively. Samples of fish and water were also obtained from the Rhine river in Germany. Water samples along the Rhine River and its tributaries were collected in August and December 2006. Two fish species, namely *Rutilus rutilus* (Roach, family Cyprinidae) and *Salmo trutta fario* (Trout/ Forelle, family Salmoninae), obtained from Rhine River (Lampertheim) and Main River (Taunus), respectively. Those samples from Germany were taken as representative samples for calibration, in QA and comparison of concentrations with those obtained from Lake Victoria. Among the sampling areas for water samples, four were taken from the Rhine river near Gernsheim (GR), Mainz (MZ), Mannheim (MA) and Koblenz (KO) and five from its tributaries: Main river at Frankfurt (F); Neckar river at Stuttgart (S), Heidelberg (HD) and Mannheim; and Mosel river at Koblenz. Figure 10 shows the sampling points at the Rhein river and its tributaries.



Adopted from wikipedia with slight modifications.

Figure 10. Sampling points for water at the Rhine river and its tributaries.

3.2 Sampling strategy

Fish samples were collected using a vessel of the Kenya Marine and Fisheries Research Institute. Trawling was done along the sampling sites or purchased from the local fishermen. Samples were collected from various beach locations in within Winam Gulf (Figure 11). Water and sediments samples were collected along stream/rivers that drain into Lake Victoria namely: Auji/Nyamasaria, Kisat and Saka and Kibos which are largely located in the Kisumu district in the Nyanza Province of Kenya and all draining into the eastern part of the Nyanza Gulf of Lake Victoria. River Kibos is located on the Northern part of Kisumu city. It originates from the western ridge of the Mau and Nyando escapment. The predominant type of land use in the river's basin is forestry and subsistence farming in the upstream. A few sugar cane farms are located in the middle reaches. Downstream, the river passes the eastern outskirts residential area of Kisumu town and enters the Nyakach bay through a papyrus swamp. Kisat is a small river and rises on the northern outskirts of Kisumu town. Subsistence farming is practiced on the upper reaches, which is also increasingly becoming urban.

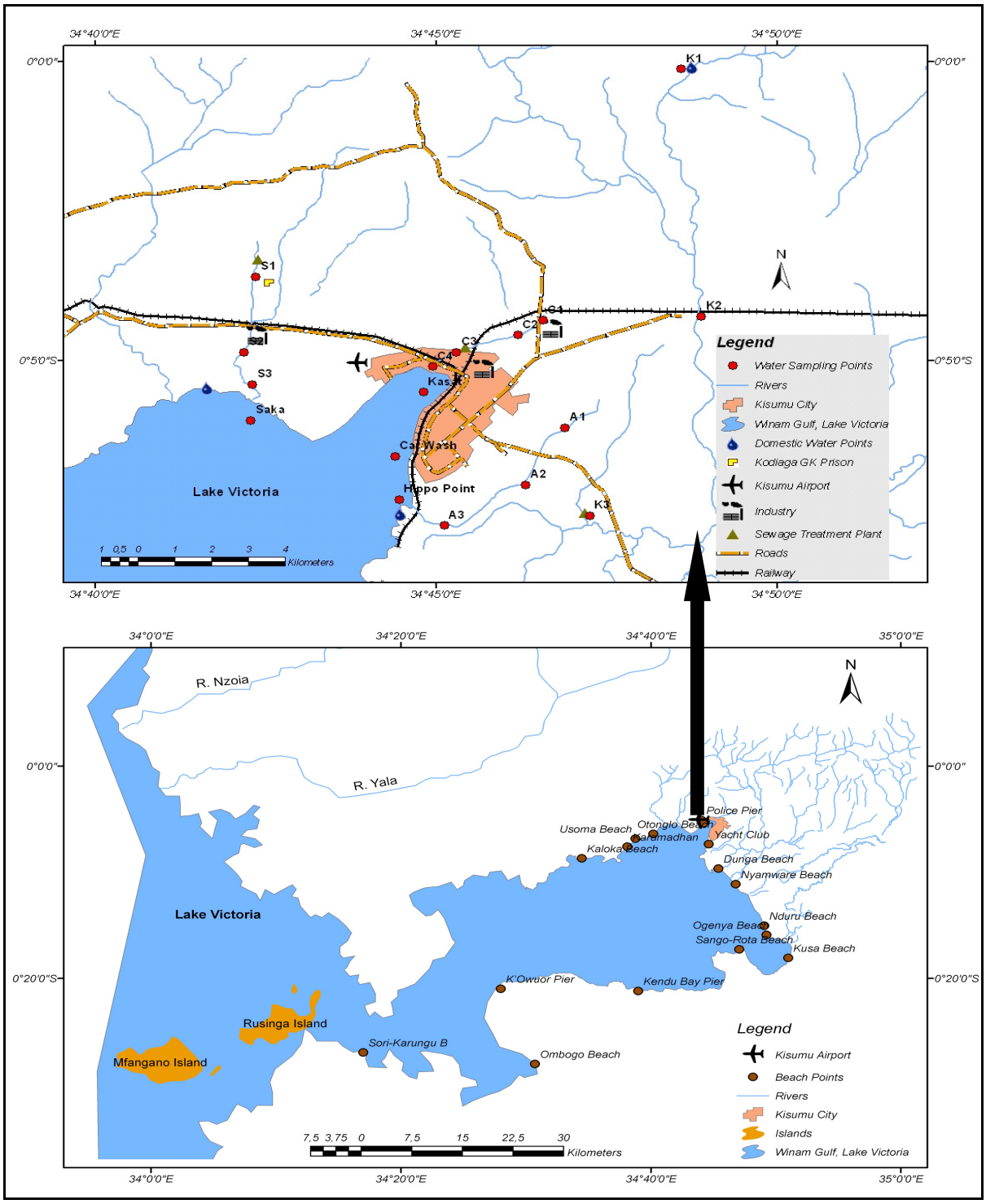


Figure 11. A map showing sampling locations within Winam gulf of lake Victoria and sampling points along rivers draining into the gulf.

Table 2. Description of the sampling stations on the rivers Kibos (K1-K3), Auji (A1-A4), Saka (S1-S3) and Kisat (C1-C4)

Code	Station name	Altitude (m a. s. l.)	Characteristics of catchments and possible pollution sourses
K1	Water station	1248	Limited agricultural activities (water is abstracted above this station for Kisumu town)
K2	River side	1213	Small agricultural holdings and urban settlements
K3	Nyamasaria/Nyalenda	1170	Domestic waste treatment and stabilization ponds, sand extraction and residential area
A1	Auji upstream	1165	Fed by streams from Nyalenda waste water ponds, Residential area, papyrus swamp
A2	Auji Middlestream	1165	Residential area
A3	Auji downstream first station	1160	Residential area
K4	Auji downstream near outlet	1170	Urban settlements, urban run-off
C1	Kisat upstream	1171	Industral area, urban run-off
C2	Obunga-mbuta	1165	Domestic sewage from slums,

			local breweries
C3	Kudho-Kotur	1164	Industrial area, urban run off
C4	Gulf Course	1159	Industrial area, sewage treatment plant
S1	Prisons	1200	Urban settlements and Government prison sewage treatment plant
S2	Kisumu molasses plant	1160	Chemicals and Mollases industry and urban settlement
S3	Saka Downstram	1158	Limited agricultural activities and urban settlement.

The river passes through densely populated slums at Obunga and through an industrial estate in the lower reaches. A municipal sewage treatment plant is located in the final part of the river before it discharges into Kisumu bay of the Winam gulf. River Auji catchment connects the Swamp at the large Nyalenda area, and draws its waters to Lake Victoria near where water for domestic use is drawn from the Lake, for use in Kisumu City. Saka River runs through Kisumu Kodiaga prison, kisumu molasses plant, and residential areas before finally reaching the Lake. Three to four stations were sampled on each river (see Figure 11). The ecological condition of each river especially up and downstream each sampling station was carefully considered. They include land characteristics, upstream basin (for example type of land use, river water use, urban and industrial effluents, etc.) and natural conditions influencing water quality. The code, station name, altitude (m a. s. l.) and characteristics of catchments and possible pollution sourses of some selected stations are tabled (see Table 2)

3.2.1 Water and sediments sampling

Water samples were collected in 1L polyethylene bottles, packed in coolers containing wet ice upon collection and shipped to the testing laboratory. In order to minimize the possibility of introducing PFOA and PFOS contamination into samples, fluoropolymer materials were avoided. Sampling in the open waters of Winam Gulf catchments were divided into locations (Figure 11) and each location with a number of sampling points. Water samples were collected along rivers that drain into Winam Gulf of lake Victoria namely: Auji, Saka, Kibos and Kisat designated A, S, K and C respectively (see map in Figure 11). Sampling was also done within Nyalenda Municipal Waste Treatment Ponds (MWTS) which is within the Kibos river catchment. Sediment samples were collected at the same spot in which water samples were collected. Surface sediments (1 – 5 cm) were taken. In areas were water depth was high, Ekman grab was utilized in sampling. Most rivers are largely located in the Kisumu and Nyando districts in the Nyanza Province of Kenya which all drain into the eastern part of the Nyanza Gulf of Lake Victoria. Nyalenda Municipal Waste Treatment Ponds location is shown in Figure 2 and 11. Sampling in the open waters of Winam Gulf catchments were divided into locations namely; Kaloka Beach, Karamadhan, Usoma Beach, Police Pier, Pipeline, Dock, Car wash, Yatch Club, Hippo Point, Dunga Beach, Nyamware Beach, Nduru beach, Ogenya Beach, Songa-Rota beach, Kusa Beach, Kendu bay Pier, K"Owuor Pier, Ombogo Beach, Sori-Karungu Beach, each with a number of sampling points. Samples were stored at 4 °C in the laboratory. Sediment samples were air dried, followed by oven drying overnight to achieve constant weights. Dried samples were then packed in labeled polythene bags, ready for futher analysis.

3.2.2 Fish sampling

More than seven samples of each species were collected at every site. Fish samples were transferred to ice boxes upon being caught. Samples of freshly caught *Lates niloticus* (Nile perch) and *Oreochromis niloticus* (Nile Tilapia), in the maturity life stage, were initially obtained from locations in the beaches of Winum gulf of lake Victoria namely; Dunga Beach, Nyamware Beach, Nduru beach, Kusa Beach and Kendu bay Pier Beach (See Figure 12). All sample collections trips were arranged with the help of staff and facilities from Kenya Marine and Fisheries Research Institute (KMFRI).

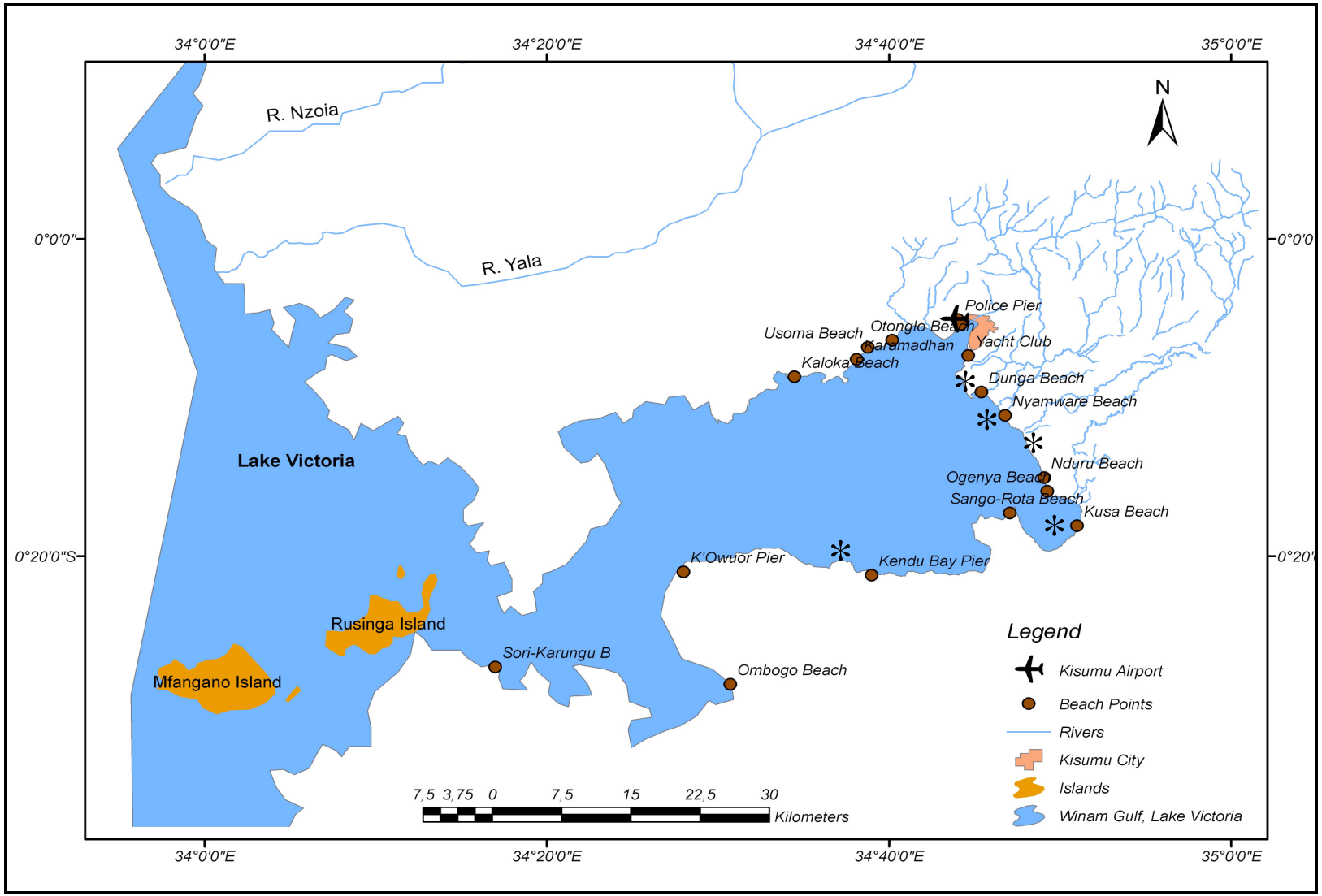


Figure 12. A map showing fish sampling beach locations (indicated by *) within Winam gulf of Lake Victoria.

All fish samples were frozen after collection (in all cases within 24 hours of having been caught) and stored frozen. Samples were immediately transported to the KMFRI laboratories at Kisumu in insulated boxes packed with artificial ice packs or dry ice. All samples were verified as being still frozen on arrival at our laboratory, from which they were dispatched to Laboratory for analysis. In order to minimize the possibility of introducing PFCs contamination into samples, fluoropolymer materials were avoided. Liver samples were obtained after dissection of the fish in our laboratory.

3.3 **Chemicals**

*3.3.1 **Analytical Standards***

Perfluorinated compounds and an internal standard perfluoro-n-[1, 2, 3, 4,-$^{13}C_4$] octanoic acid (MPFOA) (Wellington Laboratories, Canada), were dissolved in methanol at a concentration of 50 µg/ml. Perfluorobutane sulfonate (PFBS) standard (98 %) was obtained from Sigma-Aldrich (Germany). Fluoroaliphatic ester NOVEC ™ FC4430 {90 % polymeric fluorochemicalactives, 8 % non-fluorochemicalactives and 2 % co-solvents}, Fluoroaliphaticester NOVEC ™ FC4432 {87 % polymeric fluorochemicalactives, 7 % non-fluorochemicalactives, 5 % 1-methyl-2-pyrudidinone and < 1 % toluene} and fluoroethylene polymer (Zonyl) {100 % polytetrafluoroethylene}, fluorosurfactant (Zonyl) {25 % water and 25 % DipropylenGlycol Methylether} were obtained from 3M and DuPont respectively. 10-(trifluoromethoxy) decane-sulfonate {concentration: 91 mg L^{-1}} a prototype was obtained from Merck (Germany). Test compounds for degradation were obtained from our laboratory stocks as supplied by the manufacturers.

*3.3.2 **Analytical Reagents and other Chemicals used***

The stock solutions were prepared weekly. Methanol, (all suprasolv), ammonium acetate (p.a.), acetonitrile, acetone, methylene chloride (all suprasolv) were obtained at the highest commercially available purity (HPLC-grade), the counter-ion solution, 0.5 M tetrabutylammonium (TBA) hydroxide, was prepared by dissolving TBA hydrogen sulphate in water; the pH value of the solution was adjusted to 10 with 2M NaOH-solution, benzyl

bromide, 100 mM, was dissolved in acetone and other chemicals were obtained from Merck (Darmstadt, Germany). Chemicals used for the preparation of the mineral medium for biodegradation tests and Winkler method were all analytical grade reagents: Potassium dihydrogen orthophosphate, Di-potassium hydrogen orthophosphate, Di-sodium hydrogen orthophosphate dehydrate, ammonium chloride, calcium chloride, anhydrous, calcium chloride dehydrate, magnesium sulphate heptahydrate, Iron (III) chloride hexahydrate, ammonium acetate (p.a.) hydrochloric acid (p.a) , ethylene-diaminetetra-acetic acid (EDTA disodium salt), manganese sulphate monohydrate, sodium hydroxide, sodium iodide, sulfuric acid (98 %), starch and sodium thiosulfate were all obtained from Merck Darmstadt, Germany.

3.3.3 Instruments used

- Ultra pure Milli-Q water was processed by a Millipore-Q-system (Millipore, S.A. Molsheim France).
- Separation were carried out with HPLC (HP 1090) interfaced with an Ion Trap MS (Thermo LCQ-Duo).
- GC-MS analyses were carried out with a Saturn Varian 4D, Version 5.2 Column: Capillary SGE BPX35 (30 m length, 0.25 mm i.d, 0.25 mm film thickness).
- Software Tiamo (with sample changer- Metrohm) using an ion selective electrode (ISE) for fluoride or a gold electrode were granted by Hessenwasser Zentralllabor, Darmstadt.
- Measurements in manometric mehod were taken by probe; model OxiTop OC110 with WTW OxiTop IS 12 inductive stirring system. Temperature kept constant using WTW model TS 606 1006-i incubation cabinet.
- Analysis of total organic carbon (TOC) was performed by elementar high TOC equipment and the analysis of COD for each compound was measured according to EPA Method 410.4 by the software Tiamo using a gold electrode.

3.4 Sample Analysis

3.4.1 Analytical procedure for water analysis

For 1 L of sample, an internal standard was added to the water sample in the sample bottle prior to solid phase extraction (SPE). 1000 ml sample was let to run through water Oasis HLB SPE cartridges (60mg) previously conditioned, at a flow rate 3 ml/min to 6 ml/min. Cartridges were washed with 40 % methanol (6 mL) and then completely dried. Target analytes were then eluted with 6 ml of methanol. The eluent was evaporated with a gentle stream of nitrogen gas to a final volume of 1000 µl for analysis by HPLC-MS/MS.

Separation were carried out with HPLC (HP 1090) interfaced with an Ion Trap MS (Thermo LCQ-Duo). Solvents: 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 at 20 minutes. Flow rate was 0.3 ml/min. Separation was done by a Betasil C18 column. MS conditions: Type: quadruple, Ionization: ESI negative. MS instrument tuning was conducted for each analyte (parent ion and fragment ions) by direct-infusion of a ~ 1 µg/mL standard solution at a flow rate of 10 µL min^{-1}. MS/MS conditions were type quadruple; ionization ESI negative; MRM mode; capillary voltage 1 kV, desolvation temperature 450 °C; cone gas flow 60 L h^{-1}; desolvation gas flow 740 L h^{-1}. Analyte identification was verified by comparison of chromatographic retention time and by mass spectral daughter characterization. The following ion transitions were monitored for PFOA (SRM ms/ms 412.9 and 369.0, Cone voltage 35 kV, Collision energy 10 eV) and PFOS (SRM ms/ms 499.0 and 99, Cone voltage Collision energy 35 eV).

3.4.2 Analytical procedure for fish analysis

The extraction method was based on ion pairing as described initially by Ylinen et al. (1985) which is further elaborated by Hansen et al. (2001). 5 g fish muscles and about 1 g of fish liver were homogenised with 5 times its weight of water. 1g of the homogenate to which an internal standard 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 1ml and methylene chloride (1ml) 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. Separation was carried as described in section 3.4.1 above.

3.4.3 Analytical procedure for Sediments analysis

The method used in analysis of PFOS and PFOA in sediments were adopted from Higgins et al (2005) and Senthilkumar et al (2007) with slight modifications. The method involves extraction and clean up method consisting of liquid solvent extraction followed by cleanup and concentration via solid phase extraction (SPE) for the analysis of anionic PFCs in sediments. Approximately 10 g of homogenized dry sediment was weighed in a clean 50-mL polypropylene tube, to which 20 mL of a 1 % acetic acid solution was added and then spiked with an internal standard. The mixture was then vortexed, placed in the preheated sonication bath, and sonicated for 15 min. After sonication, acetic acid solution was decanted into a clean flask. The acidified sediment residue was mixed with 25 mL of an aliquot of extraction solvent mixture (90:10 (v/v) methanol and 1 % acetic acid in Milli-Q water). The sample was shaken in an orbital shaker for 20 min at 300 rpm, followed by ultra sonificated for 30 min. The methanol/acetic acid layer was collected in the clean flask for further analysis. Another 25 mL of extraction solvent mixture was added again to the residue sediment and the extraction procedure was repeated again. Combined extracts were then rotary evaporated and transferred to a clean 10-mL polypropylene tube. In some cases, multiple10-mL polypropylene tubes were used. To reduce solid phase extraction (SPE) cartridge from clogging during the subsequent cleanup step, the final vials containing sludge extracts and washes were centrifuged for 1 hr at 3000 rpm. Five blanks were also analyzed. To concentrate the extracts and to remove potential matrix interferences, samples were passed through water Oasis HLB SPE cartridges (60mg) previously conditioned with 10 mL of methanol followed by 10 mL of 1 % acetic acid, at a flow rate 3 ml/min to 6 ml/min. Cartridges were washed with 40 % methanol (6 mL) and then completely dried. Target

analytes were then eluted with 6 ml of methanol. The eluent was concentrated under nitrogen to 1 ml for instrumental analysis. Instrumental analysis was performed using a high-performance liquid chromatograph interfaced with a tandem mass spectrometer (HPLC-MS/MS). Separation were carried out with HPLC (HP 1090) interfaced with an Ion Trap MS (Thermo LCQ-Duo). Solvents used were: A = 2 mmol/l Ammonium acetate in methanol and B: 1 mmol/l Ammonium acetate in water. Gradient program used was identical to that of water described in section 3.4.1, except that the gradient was kept at maximum level until 20 minutes before reversion to original conditions at 25 minutes

3.4.4 Extraction and derivatization of samples with GC analysis

For the sample preparation of fish, more than 5 g muscles and about 1 g of liver were homogenised with 5 times its weight of water. 1 g of the homogenate, to which an internal standard 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 was repeated a further two times. The MTBE fractions were all combined, concentrated to 1 ml and dichloromethane (1 ml) was added before proceeding to the extract clean-up. Sample extracts were cleaned up by low pressure chromatography using silica. For this, 2 g of activated silica were mixed with dichloromethane and poured in a glass column. The sample extract was then added to the column and first eluted with 15 ml of dichloromethane to remove fat, followed by 30 ml of acetone which contained all target analytes. The acetone fraction was evaporated to dryness at room temperature under a stream of nitrogen. The residue was dissolved in 0.5 ml of acetone to which then, 0.1 ml of benzyl bromide solution was added. The tube was heated at 80 °C for 15 minutes, cooled, and evaporated to dryness with nitrogen. The residue was dissolved in 1 ml of dichloromethane for GC analysis. For water samples, 1 L was loaded for solid phase extraction of which an internal standard was already added in the sample bottle prior to solid phase extraction. Sample was let to run through water Oasis HLB SPE cartridges (60mg), which was previously conditioned at a flow rate of one drop per second (3 ml/min to 6 ml/min). Target analytes were then eluted with 6 ml of methanol. The eluate was evaporated with a gentle stream of nitrogen gas to a final volume of 1000 µl. For Afterwards, derivatisation was conducted. Blanks were treated with exactly the same manner as the samples, except that the

sample is being replaced by the appropriate amount of water (MilliQwater). Procedural blanks were analyzed with each batch of samples (a maximum of 10 samples). GC-MS analyses were carried out with a Saturn Varian 4D, Version 5.2 Column: apillary SGE BPX35 (30 m length, 0.25 mm i.d, 0.25 mm film thickness). Separation: the temperature program was 30 °C isothermal for 10 min, then 10°C/min to 120 °C, 30 °C/min to 280 °C.

3.4.5 Degradation study analysis

The stability of the substitutes of perfluorinated surfactants was tested by employing advanced oxidation processes (AOP), followed by conventional tests, among them an automated method based on the manometric respirometry test [OECD 301 F] – OxiTop, closed bottle test- CBT [OECD 301 D] and standardized fix bed bioreactor [FBBR] on Perfluorobutane sulfonate standard, two fluoroaliphatic esters NOVEC ™ FC4430 and fluoroaliphatic ester NOVEC ™ FC4432, fluoroethylene polymer, 50 % fluorosurfactant (Zonyl) and 10-(trifluoromethoxy) decane-sulfonate. Test compounds for degradation were obtained from our laboratory stocks as supplied by the following manufacturers; 3M, DuPont and Merck. Some of these surfactants are well-established in the market and have been used in several applications as alternatives to PFOS and PFOA compounds. Table 3 shows the general summary of the test method applied, substances concentration, inoculum used, and test duration in degradation experiments.

Table 3. Summary of methods used in degradation tests for each compound and concentration in each experiment.

Test Substance	Method	Inoculum	Concentration (mg L^{-1})	Reference substance	Test duration
PFBS	CBT Oxi-Top AOP FBBR	Rhine river water Activated sludge Surface water	73 100	 NaAC	28 days 40 days 120 mins 28 days
NOVEC ™ FC4430	CBT Oxi-Top AOP	Rhine river water Activated sludge	3 100	 NaAC	28 days 40 days 120 mins
NOVEC ™ FC4432	CBT Oxi-Top AOP	Rhine river water Activated sludge	5 100	 NaAC	28 days 40 days 120 mins
Fluoroethylene polymer Fluorosurfactant	CBT Oxi-Top AOP	Rhine river water Activated sludge	0.03 100	 NaAC	28 days 40 days 120 mins
10-(trifluoromethoxy) decane-sulfonate	CBT Oxi-Top AOP FBBR	Rhine river water Activated sludge Surface water	0.03 91 91	 NaAC	28 days 40 days 120 mins 28 days

3.4.5.1 Degradation using advanced oxidation process

In advanced oxidation process (AOP) reagents UV/H_2O_2 and only UV radiation was applied to test substances dissolved in ultrapure water at concentration of 100 mg L^{-1} (standard solution). Samples were taken for analysis every 20 min during the treatment period of 120 minutes to monitor degradation. The effect of dilution on concentration of surfactants was tested by preparing samples of 50 and 25 mg L^{-1} diluted solutions. 100 µL of 30 % H_2O_2 was added to each vial sample. The samples were analysed using the software Tiamo (with sample changer- Metrohm) and an ion selective electrode (ISE) for fluoride.

3.4.5.2 Measurement of BOD using Closed Bottle Test and Manometric Respirometry Test

The manometric respirometry test was done by adopting the method from Roppola et al. (2006) and OECD (1993) with slight modifications. Inoculum was pre-conditioned to the experimental conditions, but not pre-adapted to the test substance. Pre-conditioning consisted of aerating activated sludge in a secondary effluent for 7 days at the test temperature, aimed at improving the precision of the test method by reducing blank values (OECD, 1993). The following flasks were used: containing test substance and inoculum (test suspension); containing only inoculum (inoculum blank); containing reference compound and inoculum (procedure control). The inoculum was sewage effluent which was collected from a sewage treatment plant at Darmstadt, Germany, directly from the aeration tank of a sewage treatment plant. Coarse particles were removed by filtration through a fine sieve and the sludge was kept aerobic thereafter. A measured weight of 16.4 mg of samples and control standards was used for all chemical tested and put in round bottle flasks. The measuring bottles were rinsed with sample. Exactly 1.6 mL of raw water was measured and put in a 164 ml round bottle flask, which is filled to the brim using the mineral medium. The mineral medium solution was prepared using KH_2PO_4, K_2HPO_4, $Na_2\ HPO_4 \cdot 2H_2O$, NH_4Cl, $CaCl_2$, $MgSO_4 \cdot 7H_2O$, and $FeCl_3 \cdot 6H_2O$. The bottles were sealed with a rubber sleeve containing three tablets of NaOH as CO_2 absorber. Magnetic stirring rods were put into the bottle. Samples were analysed in duplicates. The measuring heads were screwed onto the bottles and the samples were stabilised in the incubation cabinet (20.0 ± 0.2◦C) for six hours before the measurement was started. The experiment was carried out for 40 days and sodium acetate (NaAC) was used as reference compound.

3.4.5.3 The Closed Bottle Test (CBT)

The closed bottle test (CBT) is recommended for the first, simple test for the assessment of the biodegradability of organic compounds. The CBT was performed according to test guidelines OECD 301 D (OECD, 1993) in the dark at room temperature (20 ± 1 °C), as described in detail elsewhere (Kümmerer et al., 1996). The standard test period for the CBT was 28 days. Each test vessel contained the same mineral salt solution (mineral medium) prepared the same way as for the manometric respirometry test. Inoculum used in this method was water from Rhine River instead of the domestic or industrial sludge that is commonly used. At least duplicate BOD bottles of all series were prepared for dissolved oxygen measurement at weekly intervals over the 28 days incubation. Aerobic biodegradation

progress was monitored by measuring the oxygen concentration using the Winkler Method (Titration method). The amount of oxygen taken up by the microbial population during biodegradation of the test substance was corrected for uptake by a blank run, and expressed as a percentage of Theoretic Oxygen Demand (ThOD) or Chemical Oxygen Demand (COD). Analysis of total organic carbon (TOC) and COD for each compound was measured following the method by EPA Method 415.1 and 410.4 respectively.

3.4.5.4 Biodegradation simulation using a fixed- bed bioreactor

A fixed-bed bioreactor (FBBR) was developed in order to determine the biodegradability of single compounds under aerobic conditions. A glass column filled with glass beads (18 cm filling level) forms the main part of an FBBR, enabling micro organisms to accumulate on the surface. The micro organisms derived from the Rhine water (used as inoculum), circulated with a flow rate of 16 mL min^{-1} in a closed loop. A membrane pump aerates the water in the storage bottle. Three FBBRs were run with 5 L surface water each taken from a pristine creek (Biebesheim, Germany). Perfluorobutane sulfonate and 10-(trifluoromethoxy) decane-sulfonate biodegradability was tested using this method. The duration time of the degradation experiment was 28 days at room temperature with a neutral pH. To avoid photodegradation the experiment was set up in the dark. For PFBS analysis 1mL sample was taken weekly and diluted to 1 L with MilliQ water for SPE analysis using previously conditioned Oasis HLB cartridges (60mg), at a flow rate of one drop per second (3 ml/min to 6 mL min^{-1}). Target analytes were then eluted with 6 mL of methanol. The eluate was evaporated with a gentle stream of nitrogen gas to a final volume of 1000 µL. This final was then taken for HPLC-MS/MS analysis.

3.5 Quality control and assurance measures applied in this study

When this current study commenced, there was no international standard method to be referred to as a method for analysis of perfluorinated compounds in environmental matrices. However, accepted and internationally recognised analytical methods are available. Differences in methodology are in details as applied by individual analysts and laboratories. In this current study, **method validation** was performed using **ISO 17025** perspective and reliable **standard operating procedures (SOP)** were used which were specific to our working conditions and laboratory. This SOPs ranged from field sampling, sample pre-treatment, extraction to instrumental analysis. For water samples extraction and instrumental analysis, the validation method generally employed was **ISO/DIS 25101:** *Water quality -- Determination of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) -- Method for unfiltered samples using solid phase extraction and liquid chromatography/mass spectrometry.* Perfluorinated compounds analysis methods differ depending on the matrices of samples. All measuring instruments and equipments were subjected to quality control processes with **quality control charts** used to check their performance.

To evaluate the accuracy of the method as a whole, spike/ recovery were performed for sediment, water, and fish samples. A known concentration of target compounds was spiked into an aliquot of the sample matrix (i.e., matrix spikes) and then run through the analytical procedure as a check for matrix effects, through calculation of the recoveries. External calibration standards were prepared in methanol at concentrations reaching up to 1000 ng (absolute amount). Calibration standards were injected daily before and after a batch of samples was analyzed. A midpoint calibration standard was injected after every 10 samples, throughout the instrumental analysis, to check for instrument response and drift. Procedural blanks were analyzed by passage of water and reagents through the entire analytical procedure, to monitor for contamination in reagents and glassware.

The reliability and accuracy of the digestion and analytical procedures was assessed by extraction and analysis of spiked samples whose concentrations were previously determined. Repeatability of results were also done in intervals of time, weekly, where a sample of known concentration was passed through the whole analytical procedure to verify the method. The accuracy of the methods used in fish, water and sediments analysis was evaluated using sequential extraction experiments, standard additions, and spike/recovery experiments. Each experiment was performed to evaluate different components of the method's accuracy. First,

to evaluate whether the method's extraction procedure was capable of completely removing the analyte from the biota and abiotic matrixes, sequential extraction experiments were performed. Sequential (exhaustive) extractions of both unspiked (native) and spiked samples matrices ranging from 0.25 to 1000 ng/g or ng/L for solid and liquid were conducted by extracting these samples and analyzing each extraction step separately.

To determine the accuracy of the LC/MS/MS analysis step, standard addition experiments were performed. Because these experiments were performed with cleaned-up sample extract, they only reflect the accuracy of the LC/MS/MS analysis step and account for any matrix-induced ion suppression or enhancement. Reproducibility tests and in this case interlaboratory comparison tests were performed

Sampling containers consisted of materials that do not change the composition of the sample during sample storage. Any type of fluoropolymer plastics including polytetrafluoroethene (PTFE) and fluoroelastomer (e.g. Viton®) materials were avoided during sampling, sample storage or extraction. Glassware was also avoided for sampling due to potential analyte loss caused by sorption. Sample containers were rinsed thoroughly with water (Milli Qwater) and methanol (HPLC grade) prior to use. Sample containers were also checked for possible background contamination before use.

Interferences from instruments are significant for normal LC systems because many parts are made of PTFE and other fluoropolymer materials. It is necessary to check for possible blank contamination from individual parts such as, tubing, solvent inlet filters, seals for valves, degasser and replace these with materials such as stainless steel and polyetheretherketone (PEEK) where possible. The HPLC-vial caps should be free of fluoropolymer material. The procedural blank including the instrumental blank should be at least 10 fold less than the expected concentrations in real samples.

In summary, the following elements of Analytical Quality Management (AQM) have been applied during the performance of this study:

I. Applying standard operation procedures (SOP) as part of internal quality management system.

II. Inter-laboratory comparison analysis in collaboration with the chemistry department of the University of Mainz and IWW (Rheinisch-Westfaelisches Institut für Wasser, Mülheim), both in Germany.
III. Quality control charts for the used instruments and equipments
IV. Instrumental calibrations with external and internal standards
V. Proper methodology for results recording and use of acceptable statistical analysis for results interpretation.

3.6 Statistical analysis methods applied in this study

Standard statistical methods were applied for this work. Comparisons were made between samples collected from different sites. **Regression analysis** was used for prediction, inference, hypothesis testing and modeling of relationships among different sets of data. **Correlation**, (often measured as a correlation coefficient), indicates the strength and direction of a linear relationship between two random variables. A number of different coefficients are used for different situations. Pearson product-moment correlation coefficient, which is obtained by dividing the covariance of the two variables by the product of their standard deviations, was applied in this study. Statistical differences between PFOS and PFOA concentrations in fish, water and sediments were correlated, and varied according to different sampling locations. For fish samples, the concentrations of the two species in these studies were correlated. Furthermore, correlation between muscles and liver concentrations of PFOS and PFOA was statistically analysed using **SPSS** and **Excel Statistical package**. Statistical analyses using step-wise regression and analysis of variance (**ANOVA**) was done. Null hypothesis of homogeneity was tested using SPSS. Homogeneity of variance was established using one-way analysis of variance. When a difference was significant ($p < 0.05$), Schéffe's multiple range test was used as a post-test. Linear regression analysis was performed to evaluate the correlation between two parameters.

CHAPTER FOUR

4 RESULTS AND DISCUSSION

4.1 Concentration of PFOA and PFOS in water samples

Eight calibration curve points from enriched standards gave a value of $r^2 > 0.998$ for both PFOA and PFOS concentrations and was prepared routinely for quantification of water samples and to check for linearity. The LOQ of target chemicals in water samples was evaluated for each sample based on the average blank concentrations plus five times its standard deviation of ten blanks. Procedural blanks were used to check for possible sources of contamination during extraction procedures. They were prepared following the same procedures as the real samples, with the difference of using Milli-Q water instead. Concentrations of target analytes in most of the blank samples were lower than the limit of quantification (LOQs), exceptions showed PFOA concentrations values a little above LOQ, which were considered in the future calculations. Linearity of matrix matched those of standard calibration curves. 10 µl out of 1mL of the final sample injected in the splitless mode, measured a LOQ of 4 pg (absolute amount) for HPLC/MS/MS. It corresponds to a concentration of 0.4 ng/L in water samples. The monitored ions in MS were m/e 371, 413 for PFOA and 499 for PFOS. Water samples were spiked and analyzed to test the precision of the method. Overall, mean matrix spike recoveries for water analysis ranged from 72.0 % to 111.6 % (n = 16). Mean recoveries of PFOA and PPFOS were 92 ±11 % (Table 4). Results of accuracy and precision of analyses for the present investigation were found to be satisfactory. Repeatability of the results (as shown in Table 5) done after every third day for (n = 9) number of times gave average concentrations 31.11ng/L and 8.30 ng/L with percentage standard deviation 5.2 % and 10.0 % for PFOA and PFOS respectively.

Table 4. Recoveries of PFOS and PFOA in river water samples from Rhine and tributaries.

Samples	Analyte compounds	Concentration in water sample (ng L^{-1})	Spike amount (ng)	Concentration after being spiked (ng L^{-1})	Recovery (%)
Rhine river	PFOA	3.06	2	5.04	98.81
(Gernsheim)	PFOS	2.97		4.93	98.10
Rhine river	PFOA	2.71	5	7.41	94.11
(Mainz)	PFOS	< 0.40		4.65	92.92
Main river	PFOA	1.41	5	5.90	89.80
	PFOS	1.06		5.86	96.10
Rhine river	PFOA	1.84	10	11.08	92.43
(Gernsheim)	PFOS	< 0.4		7.30	73.03
Rhine river	PFOA	4.80	20	24.27	97.40
(Gernsheim)	PFOS	3.29		23.22	99.40
Rhine river	PFOA	3.17	50	43.24	80.00
(Mainz)	PFOS	1.43		37.4	72.00
Main river	PFOA	1.51	50	45.78	88.52
	PFOS	1.44		41.60	80.31
Rhine river	PFOA	1.49	100	113.0	111.61
(Mainz)	PFOS	1.26		108.3	107.02

Analysis of perfluorinated compounds in Rhine river and its tributaries has been done by several workers in Germany. During investigations of PFC concentrations in the water surface in Germany, Skutlarek et al. (2006), observed remarkably high PFOA-concentrations not only in the rivers Ruhr (tributary of the Rhine, up to 177 ng L^{-1}) and Moehne (tributary of the Ruhr, up to 7070 ng L^{-1}), but also in public water supplies, which use river water to produce drinking water by bank filtration or artificial recharge. In July 2006, waterworks Moehnebogen installed activated-charcoal filters, which efficiently decreased PFC-concentrations in drinking water (Hölzer et al., 2008). By tracking down the PFC-contamination on the Moehne and Ruhr rivers, the drainage of agricultural land, which is treated with a fertilizer containing a mixture of food-industry sewage sludge, was located as the main source of contamination (Skutlarek et al. 2006). Indeed, the polluted fertilizer was

applied to several hundred fields in Germany, which might eventually leach into groundwater (BfR, 2006).

Table 5. Repeatability of PFOA and PFOS concentrations obtained by measurements taken in different periods for a water sample

Date	PFOA (ng L^{-1})	*PFOS (ng L^{-1})*
30.08.07	32.50	8.25
03.09.07	30.25	7.85
28.09.2007	31.00	9.25
01.10.2007	33.25	8.85
02.10.2007	28.90	7.00
05.10.2007	30.30	8.60
08.10.07	33.40	7.40
08.01.08	29.35	7.85
17.01.08	31.00	9.45

In Kenya, PFOA was detected in most sampling locations in the Winam Gulf of Lake Victoria and within the rivers draining into Lake Victoria at Kisumu City. Figure 13 shows concentrations (ng/L) of PFOA (black) and PFOS (white) represented by bars at each sampling points within the Kisumu city region. Concentrations of PFOS less than 1 ng/L are not of concern and therefore not shown. The maximum concentration of PFOA measured within the lake was 11.65 ng/L at the dock/car wash sampling location. These concentrations are approximately three orders of magnitude higher than those observed in other sampling locations within Lake Victoria. Many cottage industries and the main ship dock in Kenya's western region is located at this area and they could act as a source for these elevated concentrations relative to PFOA concentration in ambient water. According to Taniyasu et al (2003), the general PFOS and PFOA concentrations in ambient waters, with no point source of pollution, are less than 5 ng/L. Relatively higher concentrations were also obtained along Auji river with an average of 17.0 ng/L at point A2 (see figure 13). Domestic waste from a Nyalenda slums and also inputs from the Kibos river catchment can be considered as the source of these perfluoroalkylated compounds along Auji river. Kibos and Auji rivers are interconnected within Nyakach swamp around the area. Auji river drains its water in Lake Victoria at Hippo point and Yatch club. This point is of interest because water for domestic use in Kisumu City is drawn near this point. The highest PFOA concentration detected was

96.40 ng/L (point K3) at Nyalenda Municipal Waste Treatment Ponds along the Kibos river catchment. This high concentration could be attributed to Municipal Waste which is drained into the Kibos river catchment at this point (see figure 13). Relatively low concentrations of PFOA and PFOS were obtained along Kisat river with mean of 0.58 ng/L. A municipal sewage treatment plant is located downstream of Kisat river before it discharges into Kisumu bay of the Winam Gulf.

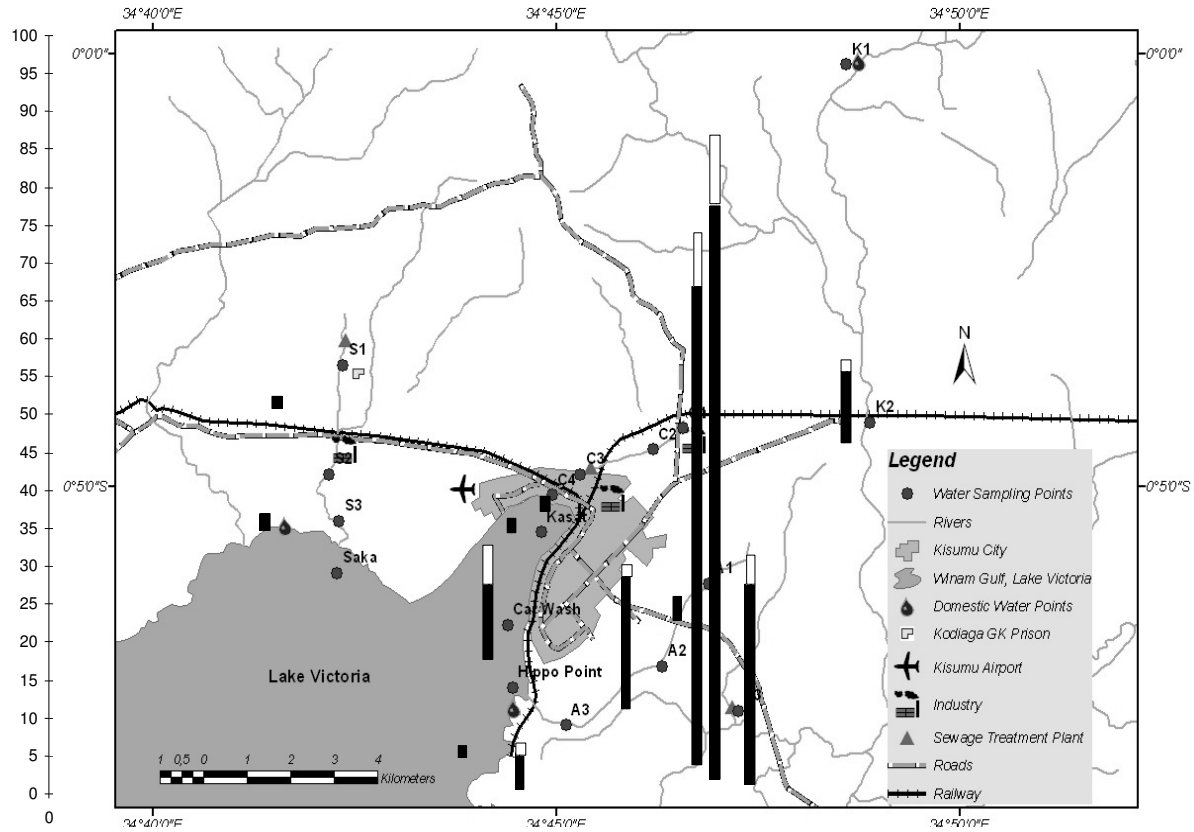


Figure 13. Concentrations (ng/L) of PFOA (black) and PFOS (white) represented by bars at each sampling points within Kisumu city region in Kenya.

There are several industries and a textile mill located in this area which may also be responsible as a source for these concentrations. Saka river system flows through a residential populated area, and along it, is a molasses industry and small cottage industries. Lower concentrations of PFOA were determined along Saka river with mean of 0.71 ±0.18 ng/L. Table 6 shows the results of concentration range of analytes and mean (in brackets) obtained from each locations. Results obtained from the other sampling locations not presented in Table 6 were generally below the limit of quantification. PFOA was the predominant fluorochemical found in all the samples analysed in this study. Variability in the

concentrations of PFOA or PFOS in River waters samples of Kibos, Kisat, Auji and Saka was higher than for the Winam Gulf water samples, suggesting generalized point source such as domestic and industrial waste.

Lake water samples showed the presence of all investigated perfluorinated compounds at pg/L levels while most samples from river waters studied are in ng/L range for PFOA concentrations. Both concentrations of PFOA and PFOS in the Lake are several orders of magnitude lower than the concentrations at the outlets of the mentioned rivers into the Lake Victoria waters. These lower values appear to be the control point PFC concentration values in pg/L levels for remote lake water far from local sources. These can be represented by range of values and mean concentration (in brackets) obtained at the following locations: Nyamware Beach < 0.40 – 1.55 (0.82 ±0.10), Nduru beach < 0.40 – 0.10 (0.80 ±0.10), Ogenya Beach < 0.40 –0.85 (0.68 ±0.10), Songa-Rota beach < 0.40 – 0.80 (0.64 ±0.08) ng/L. PFOS concentration values were < 0.40 ng/L for samples from these locations.

Table 6. Concentrations range and mean (in brackets) in ng/L for PFOS and PFOA obtained from various sampling locations.

Sampling Location	N	PFOS	PFOA
Kisat	9	<0.40 – 0.45 (0.42 ±0.28)	0.41 – 0.73 (0.58 ±0.12)
Auji	6	1.20–1.72 (1.33 ±0.23)	3.76–4.56(4.06 ±0.30)
Kibos	8	1.34–8.25 (3.87 ±2.40)	7.97–32.50(17.66 ±8.55)
Saka	5	<0.40 – 0.75 (0.56±0.23)	0.45 – 0.92 (0.71 ±0.18)
Police Pier	6	<0.40	<0.40 – 0.62 (0.40 ±0.20)
Nyalenda MWSP	11	5.67–13.23 (8.88 ±2.73)	44.89 – 96.40 (65.70±13.90)
Pipeline	6	<0.40	<0.40 – 0.60 (0.41 ±0.19)
Dock/Car wash	6	<0.40–2.53 (2.00 ±0.67)	<0.40–11.65 (5.10 ±4.90)
Hippo Point/Yatch Club	4	<0.40 – 0.65 (0.55 ±0.14)	0.44 – 1.05 (0.75 ±0.25)
Dunga Beach	5	<0.40– 0.95 (0.54 ±0.09)	<0.40 – 1.95 (0.84 ±0.09)
Nyamware Beach	5	<0.40	<0.40 – 1.55 (0.82 ±0.10)
Nduru beach	6	<0.40	<0.40–0.10* (0.81 ±0.10)
Ogenya Beach	3	<0.40	<0.40 – 0.85* (0.68 ±0.10)
Songa-Rota beach	3	<0.40	<0.40 –0.80* (0.64 ±0.08)

Only one to three samples are above the limit of quantification. Values below LOQ are donated by ' < '. Values below the limit of detection were not included in the estimation of the mean.

4.2 Concentration of PFOA and PFOS in fish samples

The method of analysis for this sample 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 $r^2 = 0.997$, which 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 from the final sample volume of 1 ml injected in the splitless mode, the LOQ was 5 pg (absolute amount) corresponding to concentration of 0.5 ng/g for HPLC-MS/MS. 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 77.1 % to 106.1 % and results of recovery are shown in Table 7.

Table 7. Recoveries of PFOS and PFOA in fish samples.

Samples	Analyte compounds	Concentrations (ng L^{-1})	Spike absolute amount (ng)	Concentrations after being spiked (ng L^{-1})	Recovery (%)
Trout muscles	PFOA	0.41	2	2.04	84.6
Trout liver	PFOS	0.33		2.00	85.8
	PFOA	2.18	5	7.22	100.6
Roach muscles	PFOS	3.02		7.65	95.4
Trout muscles	PFOA	0.17	5	5.45	105.4
Trout liver	PFOS	0.28		5.60	106.1
	PFOA	1.84	10	11.00	92.9
Roach muscles	PFOS	2.07		9.30	77.1

Results of accuracy and precision of analyses for the present investigation were found to be satisfactory. Repeatability of the results (as shown in Table 8) done after every third day for (n = 4) number of times gave average concentration of 0.41 ng/g and 0.33 ng/g for PFOA and PFOS which gave percentage standard deviation 10 % and 18.2 % respectively. 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 %.

Table 8. Repeatability of PFOA and PFOS concentrations obtained by measurements taken in different periods for trout muscles

Date	PFOA (ng L^{-1})	PFOS (ng L^{-1})
08.01.08	0.41	0.33
14.01.08	0.38	0.28
18.01.2008	0.45	0.42
23.01.2008	0.35	0.3

4.2.1 Perfluorinated compounds in Rhine River, German and its tributaries

High levels of perfluorinated organic surfactants, particularly PFOS, have been detected in farmed trout from one of two pools examined near Arnsberg in studies by the regional authorities in North Rhine-Westphalia. Up to 1.180 µg PFOS per gram fish flesh were measured (BfR, 2006). Table 9 shows the results of obtained mean concentrations of PFOS and PFOA in fish liver and muscles samples (ng g^{-1} wet wt.) from the Tout and Roach fish of the Rhine river. The two fish species, namely *Rutilus rutilus* (Roach, family Cyprinidae) and *Salmo trutta fario* (Trout/ Forelle, family Salmoninae), obtained from Rhine River (Lamperheim) and Main River (Taunus), respectively, were taken as representative samples.

Table 9. Concentrations of PFOS and PFOA in fish liver and muscles samples (ng g-1 wet wt.) from the Rhine river.

Samples	Replicates	PFOA (ng g^{-1})	PFOS (ng g^{-1})
Trout muscles	4	0.41 ± 0.06	0.33 ± 0.03
Trout liver	4	2.18 ± 0.13	3.09 ± 0.42
Roach muscles	3	0.17 ± 0.02	0.28 ± 0.04
Roach liver	3	1.84 ± 0.05	2.07 ± 0.16

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 of PFOS in Nile perch muscles ranged from 0.90 ng/g to 10.50 ng/g and 1.40 to 35.70 ng/g for liver samples (Table 10). Table 10 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 for muscle and liver ranged from 0.90 ng/g 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. 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 elsewhere.

Giesy and Kannan (2001b) 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) who worked on archived lake trout samples obtained concentrations of upto 180 ng/g mean whole body PFOS concentrations. Hoff et al. (2005) conducted PFOS assessment on gibel carp (*Carassius auratus gibelio*), carp (*Cyprinus carpio*), and eel (*Anguilla anguilla*) in Flanders (Belgium). The liver PFOS concentrations in fish from the Ieperlee 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 the current 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 are 18 cm and 24 cm total length (TL) for male and female fish, respectively. For Nile perch, only fishes of more than 40 cm TL in length were obtained for further analysis.

Table 10. 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 in Lake Victoria.

		PFOS (ng/g)		PFOA (ng/g)	
Sampling Location	**Fish species**	**Muscles**	**Liver**	**Muscles**	**Liver**
Dunga Beach	*Lates niloticus*(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)
	Oreochromis niloticus(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	*Lates niloticus*(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)
	Oreochromis niloticus(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	*Lates niloticus*(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)
	Oreochromis niloticus(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	*Lates niloticus*(n=5)	1.00 – 2.20 (1.83 ±0.21)	2.20 – 5.60 (4.20 ±0.58)	<0.50	<0.50
	Oreochromis niloticus(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	*Lates niloticus*(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)
	Oreochromis niloticus(n=4)	1.20 – 2.40 (1.80 ±0.21)	2.30 – 5.60 (4.15 ±0.55)	<0.50	<0.50

*Only one to two samples are above the limit of quantification. Values below LOQ are donated by ` < `. Values below the LOQ were not included in the estimation of the mean.

Assumption was made that Nile perch almost reached maturity at 40 cm TL. 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 the current work, 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 has also been observed that the perfluorinated compounds concentration does not correlate significantly between the two fish species, for both muscles and liver samples. This indicates that the concentration of such compounds 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 to be another potential source given the near similarities in quantities observed. Even though the environmental fate of PFOS is not completely understood, there is an accepted mechanism by which it occurs in remote areas, which is the assistance of other more volatile PFCs such as perfluoroalkyl sulfonamides, acting as precursors which then 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) on the food for Nile tilapia introduced in Lake Victoria showed that Nile tilapia known originally to be herbivorous, feeding mostly on algae.It has diversified its diet to include insects, fish, algae and plant materials. The shift in it´s diet could be caused by 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 the Lake Victoria ecosystem. According to Njiru et al. (2000), feeding patterns observed in *Oreochromis niloticus* may be as a result of the species plasticity and a response to changed ecological conditions prevailing in the lake following water hyacinth infestation. Due to this changed feeding behaviour in *Oreochromis 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). 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 14. 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 14 (B). The most 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 14 (A).

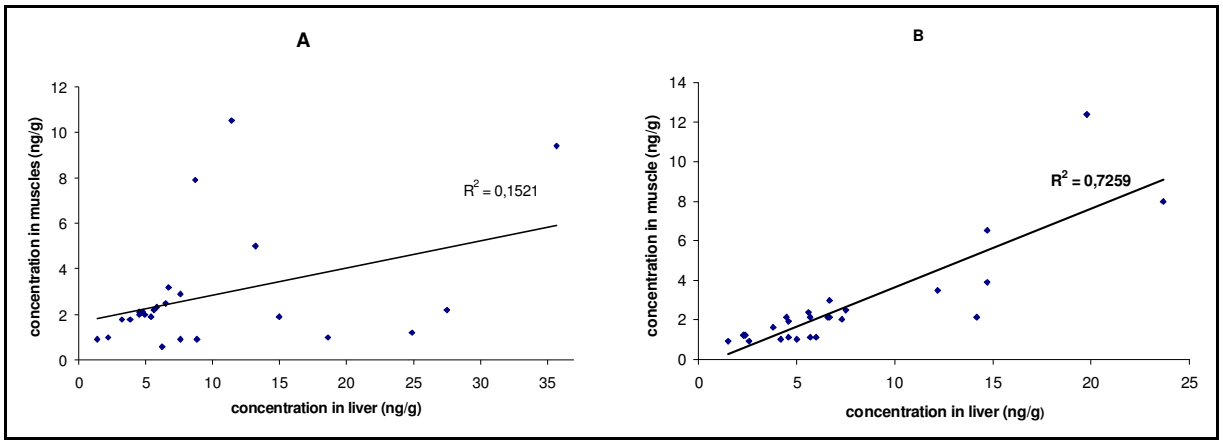


Figure 14. A scatter diagram showing correlation between Nile perch muscles and liver (A) and Nile tilapia muscles and liver (B).

4.3 PFOS and PFOA in sediments from the Gulf of Lake Victoria´s catchment area

Sediment samples were spiked and analyzed to test the precision of the method used. Recovery range was between 75 % and 114 % for both PFOA and PFOS. 13 C labelled internal standards were spiked into samples for the calculation of recoveries. Mean internal standard recoveries of internal standards was 76 ± 8 % (n = 6). Concentrations were not corrected for the recoveries of internal standards, because recoveries were within an acceptable level of 70 % for all compounds. Table 11 shows the recovery experiment summary and percentage recoveries obtained for spiked sediment samples. Repeatability analysis results are presented in Table 12.

Table 11. Recoveries of PFOS and PFOA in sediment samples.

Samples	Analyte compounds	Concentration in sediment (ng L^{-1})	Spike amount (ng)	Concentration after being spiked (ng L^{-1})	Recovery (%)
Alt Rhein 1	PFOA	< 1	2	1.5	75
	PFOS	< 1		1.8	90
Alt Rhein 2	PFOA	< 1	5	4.7	94
	PFOS	< 1		5.3	114
Alt Rhein 3	PFOA	0.21	5	5.7	105.4
	PFOS	< 1		4.5	90
Alt Rhein 4	PFOA	< 1	10	8.9	89
	PFOS	< 1		9.4	94

Table 12. Repeatability of PFOA and PFOS concentrations (ng g^{-1} dry weight) obtained by measurements taken in different periods from River Auji upstream location.

Date	PFOA (ng g^{-1})	PFOS (ng g^{-1})
02.07.08	38.9	6.5
04.07.08	40.1	7.0
07.07.08	43.6	7.5
18.07.08	42.5	7.0
02.07.08	39.0	6.6

Repeatability of the results done after every third day for (n = 5) number of times gave average concentrations of 6.9 and 40.8 ng/g with percentage standard deviation 5.8 and 5.2 % for PFOS and PFOA respectively. Results of accuracy and precision of analyses for the present investigation were found to be satisfactory. Figure 15 shows intergrated chromatogram peaks of standard (A) and sample (B).

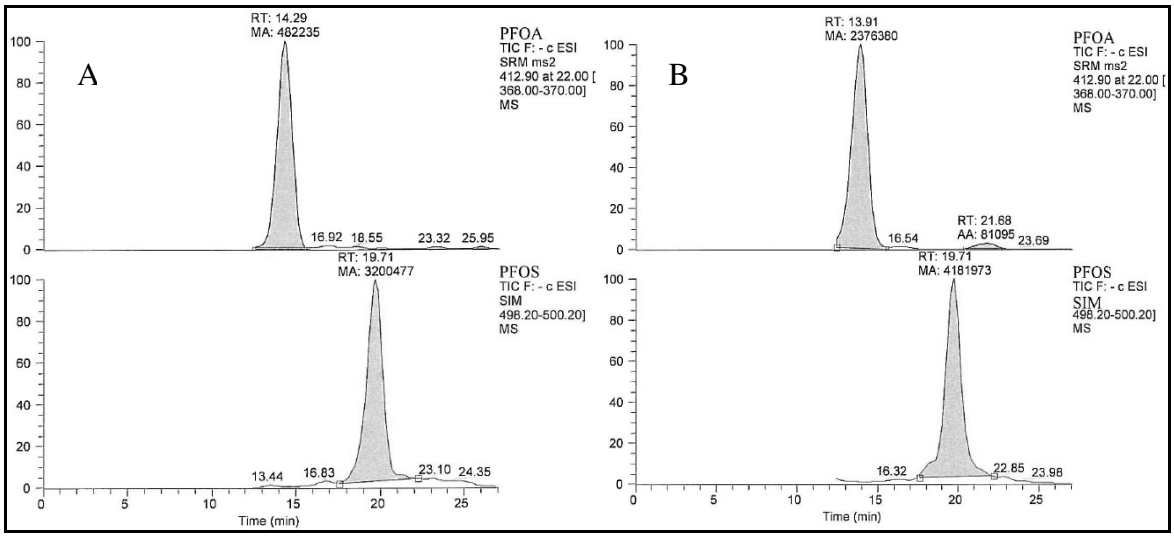


Figure 15. Intergrated chromatogram peaks obtained from analysis of standard (designated A) and sample (designated B) showing retention time.

Perfluorooctanoic acid was detected in most sampling locations in Winam Gulf of lake Victoria and within the rivers draining into Lake Victoria at Kisumu City. The highest PFOA concentration detected was 99.1 ng/g (point K3) at Nyalenda Municipal Waste Treatment Ponds along the Kibos river catchment. This high concentration can be attributed to Municipal Waste which is drained into the Kibos river catchment at this point (see figure 16). Relatively low concentrations of PFOA were obtained along Kisat river with mean value of 1.9 ng/g at Kisat Kotur sampling location. A municipal sewage treatment plant is located in the final part of Kisat river (point C3) before it discharges into Kisumu bay of the Winam Gulf. There are several industries located in this area, including a textile mill, which may provide a source for mean concentration (18.1 ng/g). However, PFOS concentration value at this point was < 1 ng/g. The mean concentration obtained at Kisat downstream sampling site were 14.6 ng/g and 3.8 ng/g for PFOA and PFOS respectively. Saka river system flows through a residential populated area. A molasses industry and small cottage industries are also located along this river. Lower concentrations of PFOA were determined along Saka river with mean of 2.5 ±0.9 ng/g at the downstream location. Table 13 shows the results of concentration range of analytes and mean (in brackets) obtained from each location. Results obtained from the other sampling locations not presented in Table 13 were generally below the limit of quantification. Perfluorooctanoic acid was the predominant fluorochemical found in all the samples analysed in this study. Variability in the concentrations of PFOA or PFOS in River sediment samples of Kibos, Kisat, Auji and Saka was higher than for Winam Gulf sediment samples, suggesting generalized point source such as domestic and industrial waste.

Table 13. Concentrations range and mean (in brackets) in ng/L of PFOS and PFOA in sediments obtained from various sampling locations within Lake Victoria basin.

Sampling Location	PFOA	PFOS
Nyalenda Ponds (n = 4)	67.8 - 99.1 (94.8 ±5.1)	46.7 - 57.5 (51.6 ±5.5)
Nyalenda ponds exit (n = 4)	68.8 - 80.6 (76.1 ±6.4)	40.8 - 51.7 (45.4 ±5.6)
Kasat Kotur (n = 3)	1.4 - 2.4 (1.9 ±0.5)	< 1
Kasat (n = 3)	16.7 - 19.8 (18.1 ±1.6)	< 1
Kisat downstream (n = 4)	12.5 - 16.4 (14.6 ±2.0)	3.2 - 4.3 (3.8 ±0.6)
Saka upstream (n = 3)	< 1	< 1
Saka downstream (n = 4)	1.4 - 3.2 (2.5 ± 0.9)	< 1
Kibos bridge (n = 4)	2.3 - 3.4 (2.8±0.6)	< 1
Kibos (n = 5)	5.0 – 7.9 (6.7±1.5)	< 1
Auji upstream (n = 5)	39.7 - 46 (43.6 ±3.4)	6.6 – 8.7 (7.5 ±1.1)
Auji downstream (n = 5)	34.5 - 38.9 (36.7 ±2.2)	1.8 - 2.9 (2.3 ±0.6)
Pipeline (n = 4)	2.7 - 3.4 (3.1 ±0.4)	< 1
Dock/Car wash (n = 4)	21.3 - 24.1 (23.1 ±1.5)	2.6 - 4.0 (3.3 ±0.7)
Hippo Point/Yatch Club (n = 4)	13.3 - 15.4 (14.1 ±1.2)	2.9 - 3.2 (3.0 ±0.2)
Dunga Beach (n = 4)	3.7 - 5.0 (4.3 ±0.6)	< 1 - 1.5 (1.4 ±0.2) *
Nyamware Beach (n = 4)	< 1	< 1
Nduru beach (n = 4)	< 1	< 1
Ogenya Beach (n = 4)	< 1	< 1

* Only one to three samples are above the limit of quantification. Values below LOQ are donated by ` < `. Values below the limit of detection were not included in the estimation of the mean.

Lake and river sediment samples showed the presence of all investigated perfluorinated compounds at ppb levels while most samples from river sediment studied are higher in concentration than in those from the Lake. Figure 16 shows concentrations (ng/g) of PFOA (black) and PFOS (white) represented by bars at each sampling points within Kisumu city region. Concentrations of PFOS less than 1 ng/g are not shown in Figure 16. The maximum concentration of PFOA measured within the lake was 24.1 ng/g at the dock/car wash followed by 15.4 ng/g at Hippo point sampling location. These concentrations are approximately three orders of magnitude higher than those observed in other sampling locations in Lake Victoria. Many cottage industries and the main ship dock in Kenya's western region are all located in

this area and they could possibly be responsible as sources for these elevated concentrations relative to PFOA concentration in other sampling locations within the Lake. Relatively higher concentrations were also obtained along Auji river with an average of 43.6 ng/g at point A2 and 36.7 ng/g (see figure 16). Domestic waste from Nyalenda slums and also inputs from the Kibos river catchment can be considered as the source of these perfluoroalkylated compounds along Auji river. Kibos and Auji rivers are interconnected within Nyakach swamp around the area. Auji river drains its water in Lake Victoria at Hippo point and Yatch club. This point is of interest because water for domestic use in Kisumu City is drawn near this point. A fishing commercial activities centre and also a residential area are located at the Dunga beach sampling location. The mean concentrations of PFOA and PFOS obtained in the sediments were 4.3 and 4.1 ng/g respectively. Both concentrations of PFOA and PFOS in the Lake are several orders of magnitude lower than concentrations at the outlets of the rivers studied into the Lake Victoria sediments. These lower concentration values are in the levels of < 1 ng/g for remote Lake sediments far from local sources. Such background values can be represented by the following locations; Nyamware Beach, Nduru beach, Ogenya Beach and Songa-Rota beach.

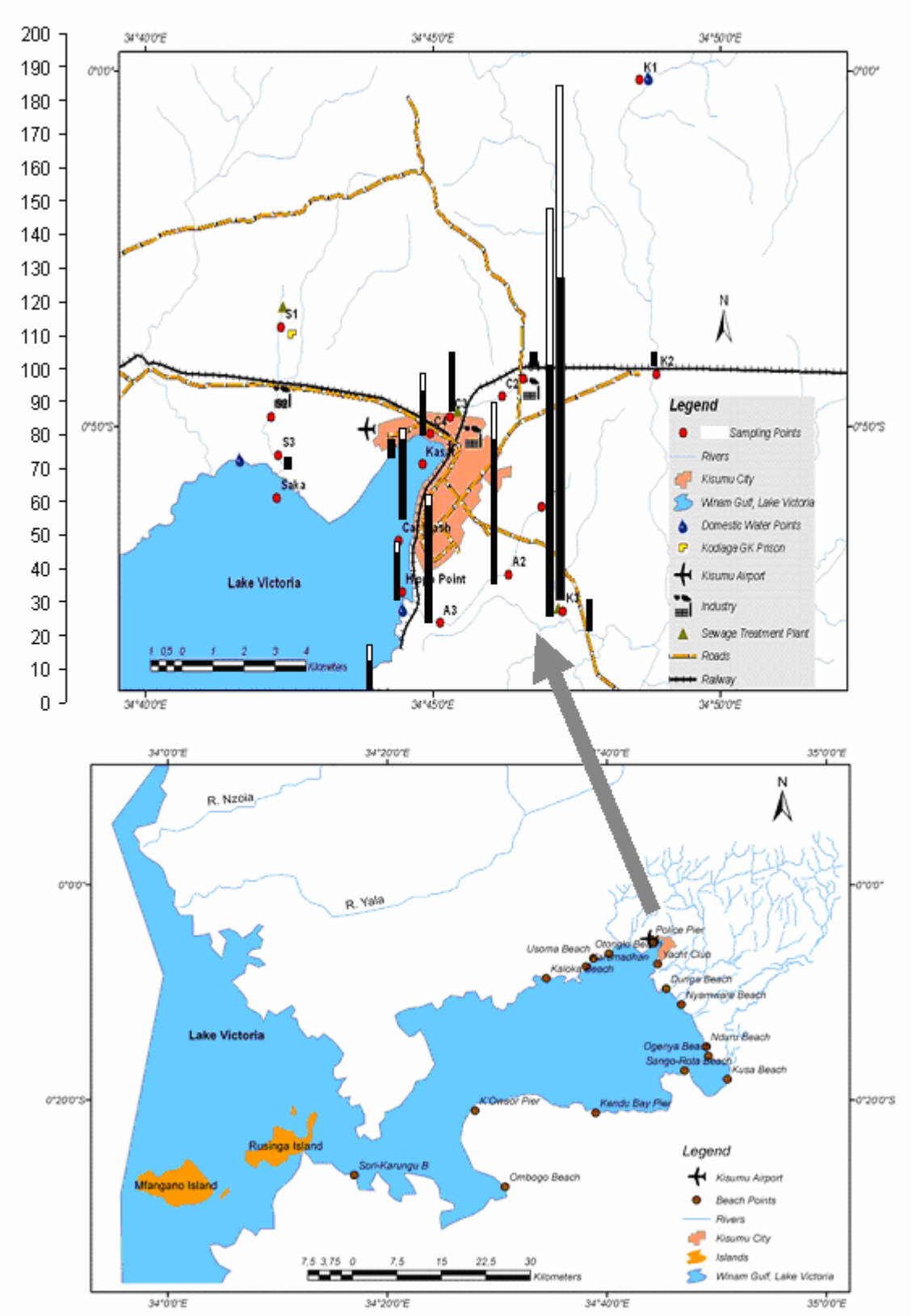


Figure 16. Concentrations in ng/g (dry wt) of PFOA (black) and PFOS (white) in sediments represented by bars at each sampling locations within Winam gulf of lake Victoria and sampling points along rivers draining into the gulf.

4.4 Derivatisation for GC analysis

Spiked PFOA and PFOS were determined in matrix of fish muscles, fish liver and water by GC/MS and LC/MS methods. The obtained results indicate that GC analysis can be used as an alternative to LC Method for quantification in contaminated areas or where high concentrations of the analztes are expected. Calibration curve for both perfluoro alkyl acids analysed gave a $r^2 > 0.98$ as shown in Figures 17 and 18.

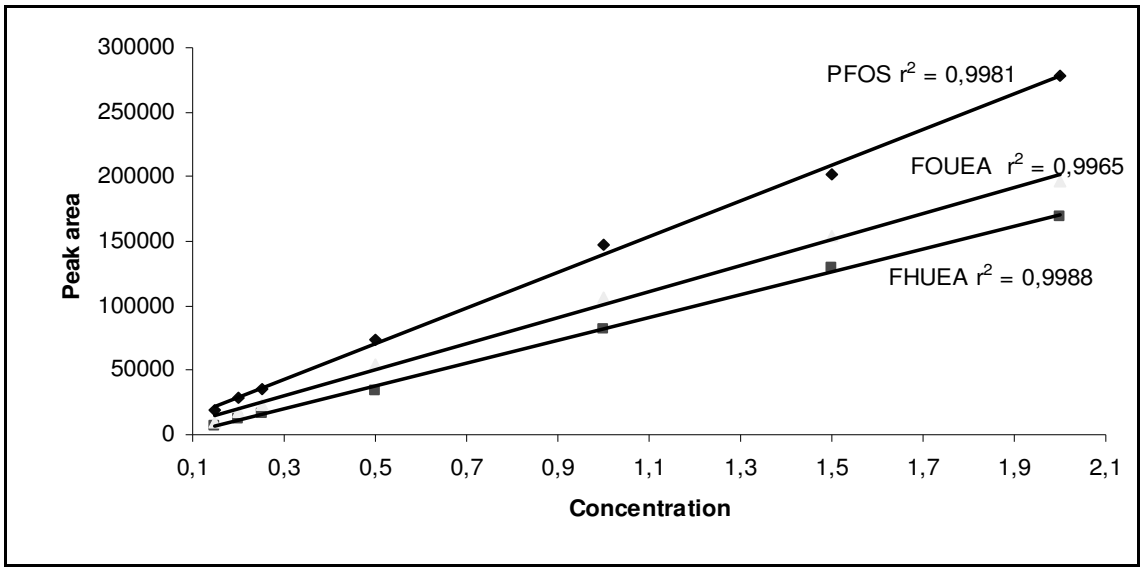


Figure 17. Calibration curve for PFOA, FHUEA and FDUEA using GC/MS

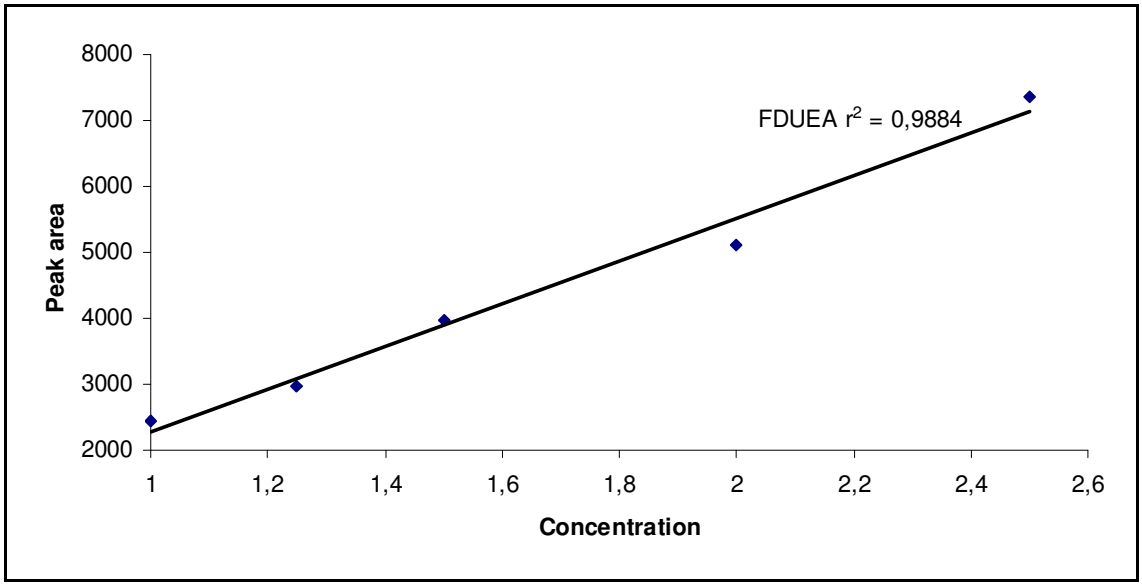


Figure 18. Calibration curve for FDUEA using GC/MS

The ion pair extraction with tetrabutylammonium (TBA) hydroxide is selective for acidic organic compounds allowing efficient transfer of the anion of PFCs into the organic phase. The ratio of the derivatizing agent to that of perfluorinated acid ratio is $2.2 * 10^4$: 1 for the GC method. Instrumental analysis allowed the LOD of 200 ng/L in gas chromatography. Results of recoveries obtained in perfluorinated acid derivatisation experinment are shown in Table 14.

Table 14. Recoveries for fish and water samples using gas chromatography derivatisation method and liquid chromatography.

Sample	Initial	Added	GC Recovered ug/L	GC Recovery %	LC Initial	LC Recovered	LC Recovery %
Nyalenda WSP 1	Below LOQ	1.0 µg	1.01 ±0.15	101.1	0.017 ug/L	0.98 ug/L n=2	96
Nyalenda Auji 3	Below LOQ	0.50 µg	0.44 ±0.04	88.0	1000.4 ng/L	950.3 ng/L	94.9
Liver sample	Below LOQ	1.0 µg	1.05 ±0.12	104.6	9.5 ng/g	1.01µg/g	100*
Muscles sample	Below LOQ	0.50 µg	0.47 ±0.07	94.4	0.75 ng/g	0.5 µg/g	99.8*

LOQ: Limit of quantification. Results rounded to one decimal place after calculation

Analytical curves of perfluoro-n-octanoic acid (PFOA), 2H-perfluoro-2-octenoic acid (FHUEA), 2H-perfluoro-2-decenoic acid (FOUEA) and 2H-perfluoro-2-dodecenoic acid (FNUEA) enrichment standards in water and fish from concentration of 0 – 2.5 µg/L were measured by GC/MS/MS. The linear coefficient of the curves were higher than 0.98 (see Figures 17 and 18). The mass spectrometry mornitored ions m/e were 504, 448, 548 and 648 for benzyl- (perfluorooctanoate, 2H-perfluoro-2-octenoate, 2H-perfluoro-2-decenoate and 2H-perfluoro-2-dodecenoate) esters formed respectively. Their corresponding chromatograms are indicated in Figure 21. Other monitored ions m/e originating from the elimination of the esters fragments were 91, 107, 108, 135, and 137 corresponding to the fragments $C_7H_7^+$, $C_7H_7O^+$, $C_7H_7OH^+$, $C_7H_7CO_2^+$ and $C_8H_9O_2^-$ as shown in Figure 19 and 20.

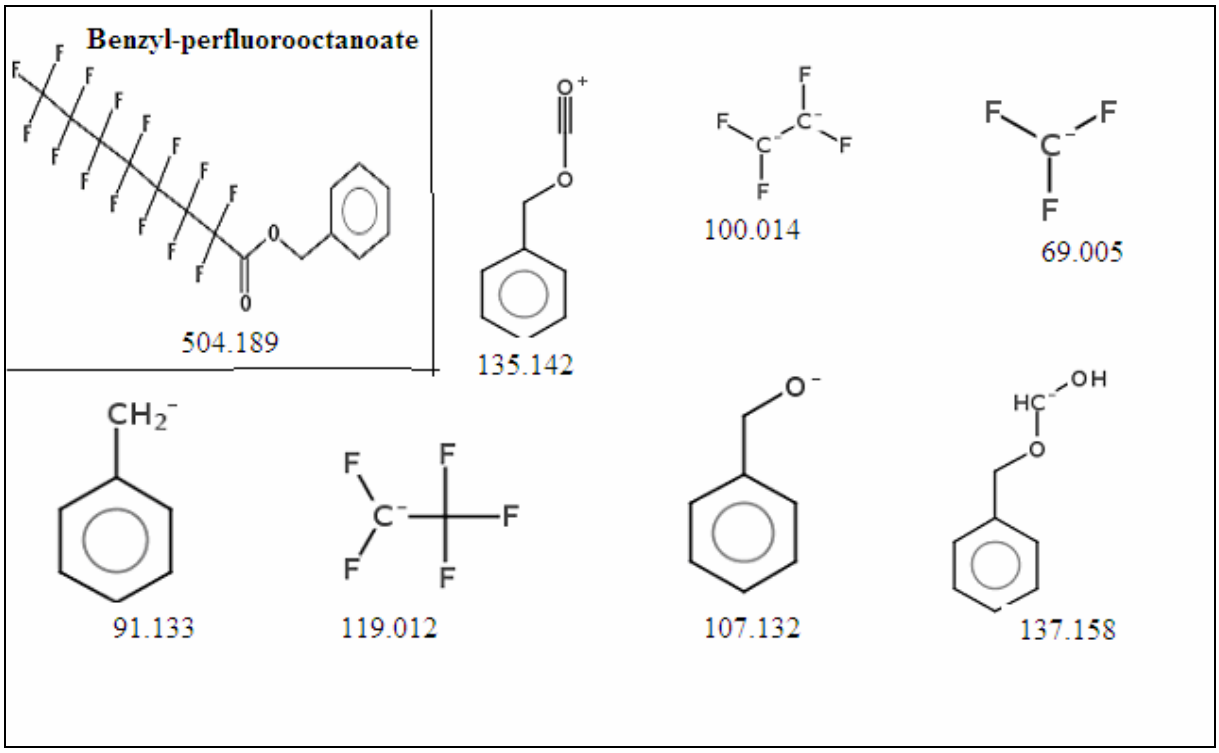


Figure 19. Fragment ions obtained in mass spectrum of $C_{15}H_7F_{15}O_2$. The structure of benzyl-(perfluorooctane)-ester is shown.

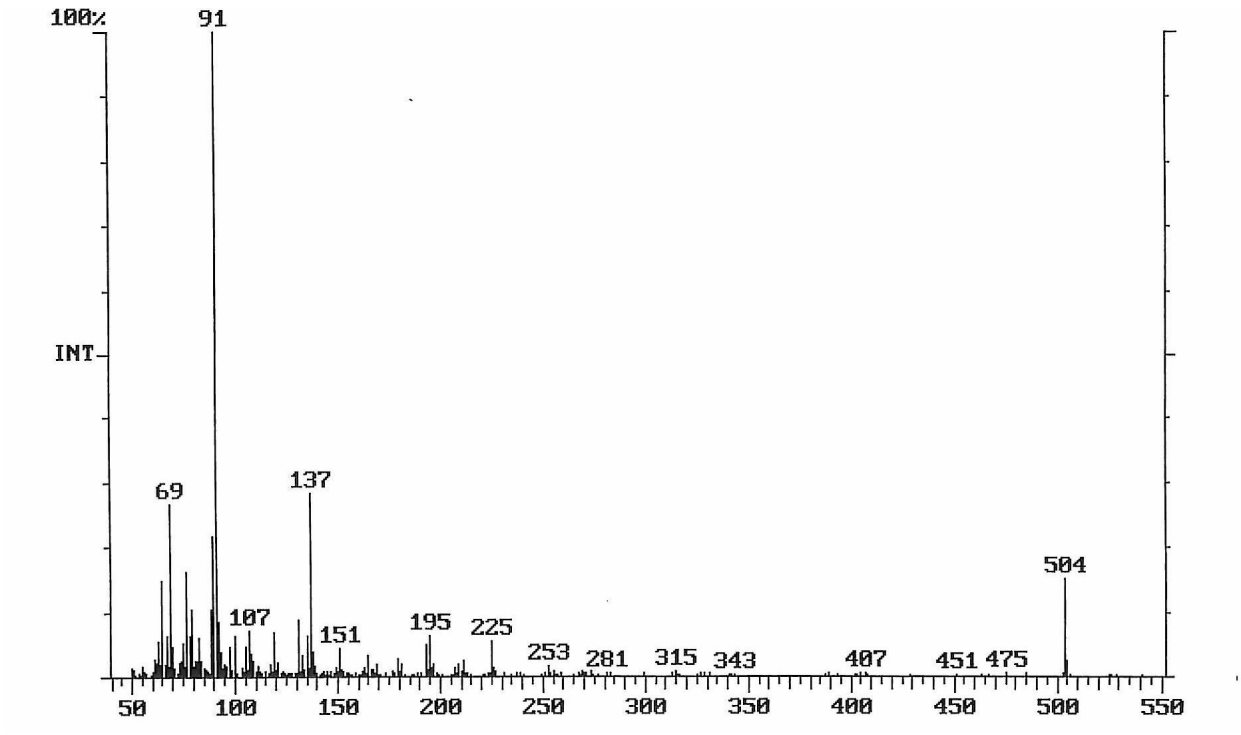


Figure 20. MS Spectrum for PFOA showing 504 m/e ($C_{15}H_7F_{15}O_2$)

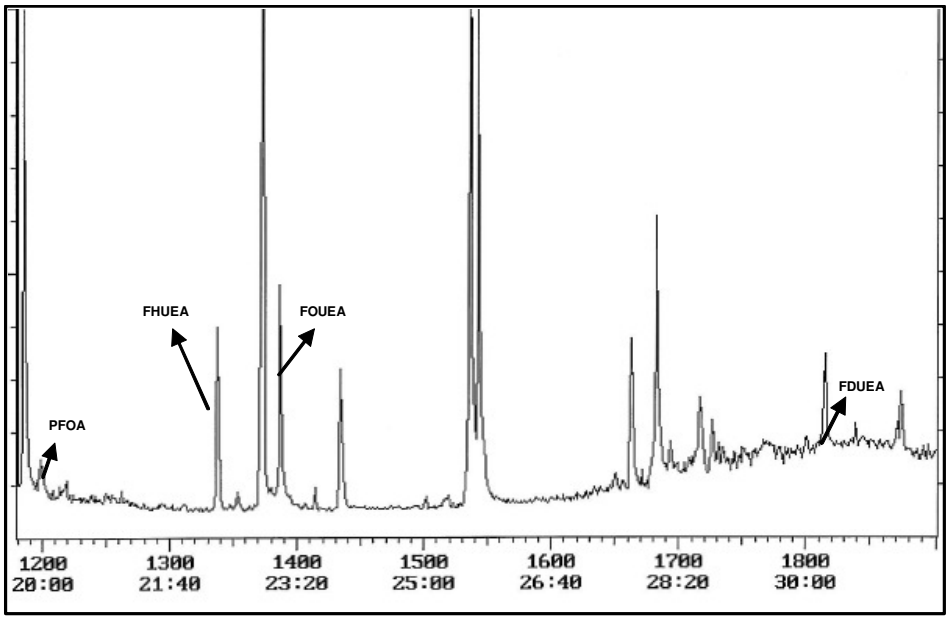


Figure 21. GC-MS/MS Chromatogram for PFOA, FHUEA, FOUEA, FNUEA

Fish and water samples were spiked and analyzed to test the precision of the method. Results of accuracy and precision of analyses for the present investigation were found to be satisfactory as shown in Table 15. Recoveries ranging from 87.7 to 104.6 % of spiked samples of fish and water samples were obtained. The concentrations of the water, fish liver and muscles were previously determined. Repeatability in GC ranged from 88 % to 104 %. Results of experiment´s repeatability are shown in Table 16. Typical values for precision obtained were 0.14 – 3.7 % for GC/MS with concentrations ranging from 0.1 µg/ml to 2.5 µg/ml). Instrumental limit of quantification (LOQ) of ≈ 0.2, 0.15, 0.5 µg/L for PFOA, FHUEA and FOUEA, FDUEA in GC/MS/MS respectively. Precision range of 6.38 % to 10.97 % from interlaboratory comparison. The LOQ in the GC/MS method does not have the sensitivity to study potential human exposure to PFCs, but it is applicable to contaminated environmental samples. Long chain perfluorinated acids have been determined in biota (Fish) and abiota (water) by derivatisation and subsequent analysis by GC/MS. The ion pair extraction with TBA is selective for acidic organic compounds allowing efficient transfer of the anion of perfluorinated acids into the organic phase. Results demonstrate that GC/MS can be a alternative to LC/MS method for quantification of perfluorinated acids in contaminated areas, and where higher concentration of the analyte is expected. In overall, the methods developed in this study for the measurement of poly- and perfluorinated acids, can also be applied to measure fluorotelomer acids, and polyfluorosulfonamides. The gas

chromatography method can be applied in the analysis of water, and biological matrices, so that we can better understand the fate of per- and polyfluorinated compounds in the environment.

Table 15. Recovery concentrations of perfluorocarboxylic acids of spiked samples from fish and water samples in GC/MS and HPLC/MS. Initial concentrations (IC) in ug/L are indicated.

Sample	Initial concentration	Spike amount (ug L^{-1})	Recovered PPFOA (ug L^{-1})	Recovered FHUEA (ug L^{-1})	Recovered FOUEA (ug L^{-1})	Recovered FDUEA (ug L^{-1})
Nyalenda WSP 1	Below LOQ	1.0	1.01 ±0.12	1.1±0.10	0.95±0.15	0.95 ±0.10
Nyalenda Auji 3	Below LOQ	0.50	0.44 ±0.04	0.55 ±0.07	0.45 ±0.05	0.5 ±0.05
Liv DP36	Below LOQ	1.0	1.05 ±0.12	1.05 ±0.15	0.95 ±0.15	1.05 ±0.10
MusTP 28	Below LOQ	0.50	0.47 ±0.07	0.45 ±0.05	0.45 ±0.05	0.52 ±0.07
MusTP 28	Below LOQ	0.25	0.21 ±0.03	0.25 ±0.05	0. 2±0.05	ND

LOQ = Limit of quantification ND = Not detectable

Table 16. Repeatability results obtained using gas chromatography

Date	Sample	Peak Area percentage ratio
19.07.2007	Fish liver sample (LivDP31)	1.04
20.07.2007	Fish liver sample (LivDP31)	0.88
23.07.2006	Fish liver sample (LivDP31)	1.01

4.5 Results of Degradation studies of emerging PFC substitutes

For advanced oxidation processes (AOP), it was observed that only a small fraction represented by 1.5 % of original perfluorobutane sulfonate (PFBS), was degraded by using UV or UV/ H_2O_2 system for all tested concentrations (Figure 22). This demonstrates that even in a more oxidative environment PFBS is not readily degradable. From the original concentration of 100 mg/L PFBS, slightly lower concentration (96.5 ± 2.0 mg/L) was obtained after the experiment. This represented about 3.35×10^{-6} moles of F- removed from a concentration of 3.35×10^{-4} moles of F-. Insignificantly higher F- concentrations were obtained when the original PFBS concentration was diluted to 50 and 25 mg/L as observed in figure 20. For fluoroaliphatic esters NOVEC ™ FC4430 and NOVEC ™ FC4432, significant degradation was observed both with time of exposure to UV radiation and even more accelerated degradation when in association with H_2O_2 (Figure 23) as observed. The F- concentrations in the final solution was six and ten orders of magnitude for NOVEC ™ FC4430 and NOVEC ™ FC4432 respectively, which are more than that observed for PFBS.

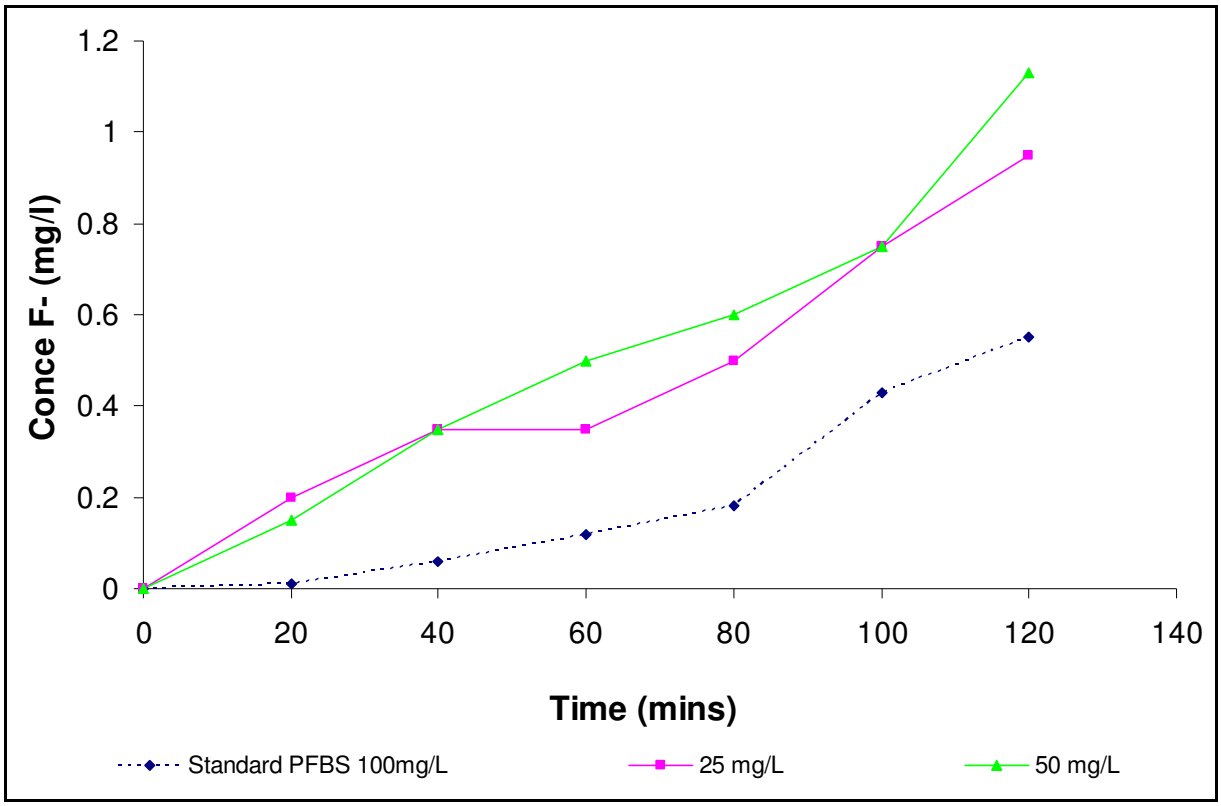


Figure 22. Degradation of PFBS using UV/ H_2O_2 at different concentrations

The final step of the AOP was accompanied by a lag phase (the plateau) of the fluoride concentration in solution at 120 minutes in all experiments. Fluoroaliphatic ester NOVEC ™ FC4430 and NOVEC ™ FC4432 demonstrated a degradation plateau at 6 mg L^{-1} and 10 mg L^{-1} of fluoride concentration respectively, when using UV radiation together with H_2O_2. It can be concluded that fluoroaliphatic ester NOVEC ™ FC4432 degrades more than NOVEC ™ FC4430 as observed in figure 22. However addition of H_2O_2 to both substances resulted to equal margin of F- yield for both substances (approximately 2 mg/L as seen in the figure 22).

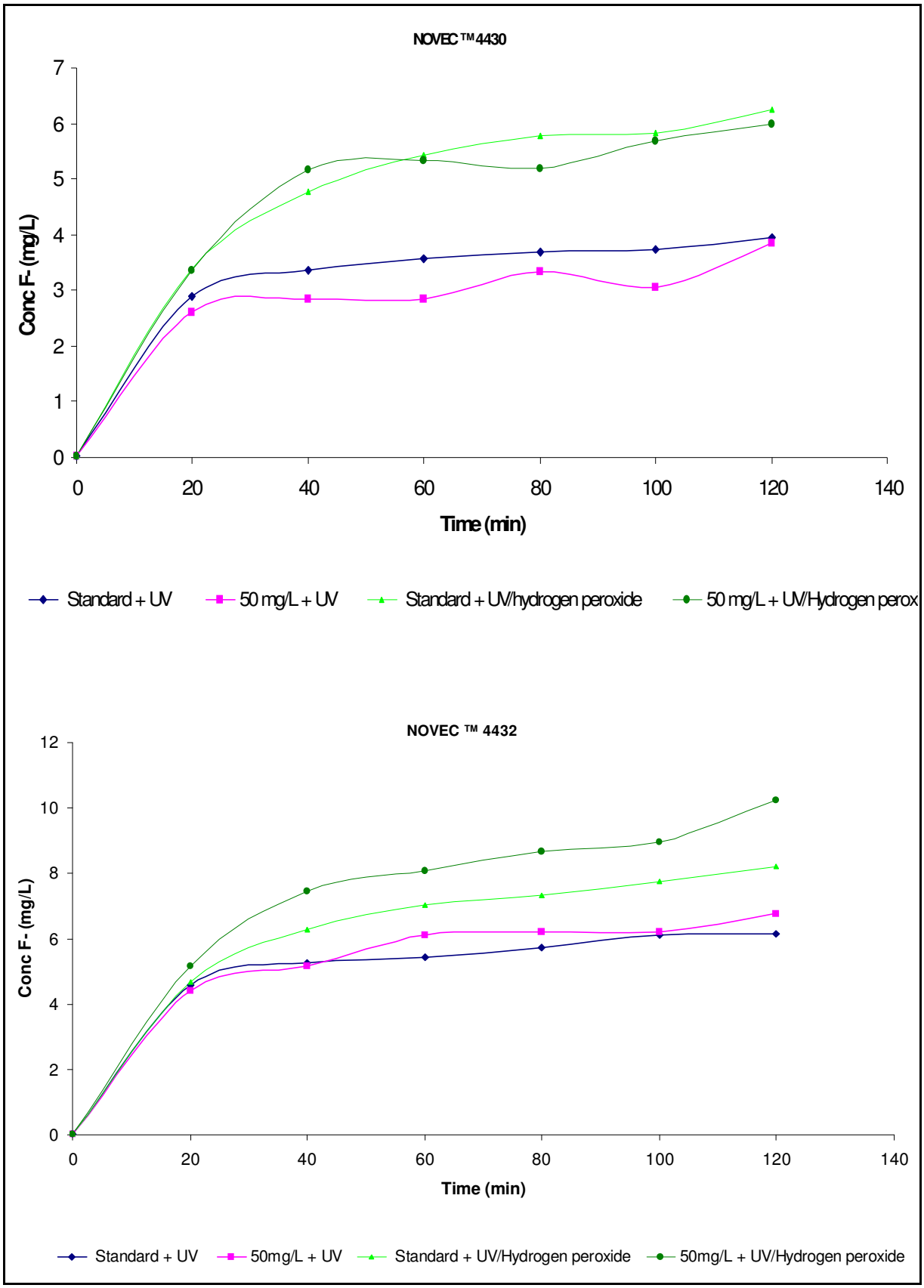


Figure 23. Degradation of fluoroaliphatic esters NOVEC ™ FC4430 and NOVEC ™ FC4432 at 2 different concentrations when submitted to UV radiation and UV/ H_2O_2 System.

By reducing the concentration of both ester substances, a slight increase in F- concentration in resultant solution was noted, though this was insignificant for NOVEC ™ FC4432. Figure 24 and 25 show more elevated concentration of F- in the resultant concentration after degradation using standard + UV for 10-(trifluoromethoxy) decane-sulfonate (plateau at 18 mg L^{-1}) that represents 3.16×10^{-4} moles of the theoretical 3.28×10^{-4} moles of F- in the initial solution. Furthermore a complete degradation is observed when oxidation is applied (112 % calculated value) as well as for diluted solution (50mg/L) which gave 21mg/L F- concentration. Fluorosurfactant Zonyl plateau was at 18 mg L^{-1}. Also observed was the decrease in TOC measurements, confirming the proportional degradation of the carbon chain. This could be an indication of biodegradation potential by micro organisms for 10-(trifluoromethoxy) decane-sulfonate and fluorosurfactant Zonyl. Results in AOP experiment also showed that there was no significant influence of the compounds` concentrations on degradation. Calculated relative standard deviation from the mean of concentration differences resulting from AOP experiments gave values ≤ 20 %.

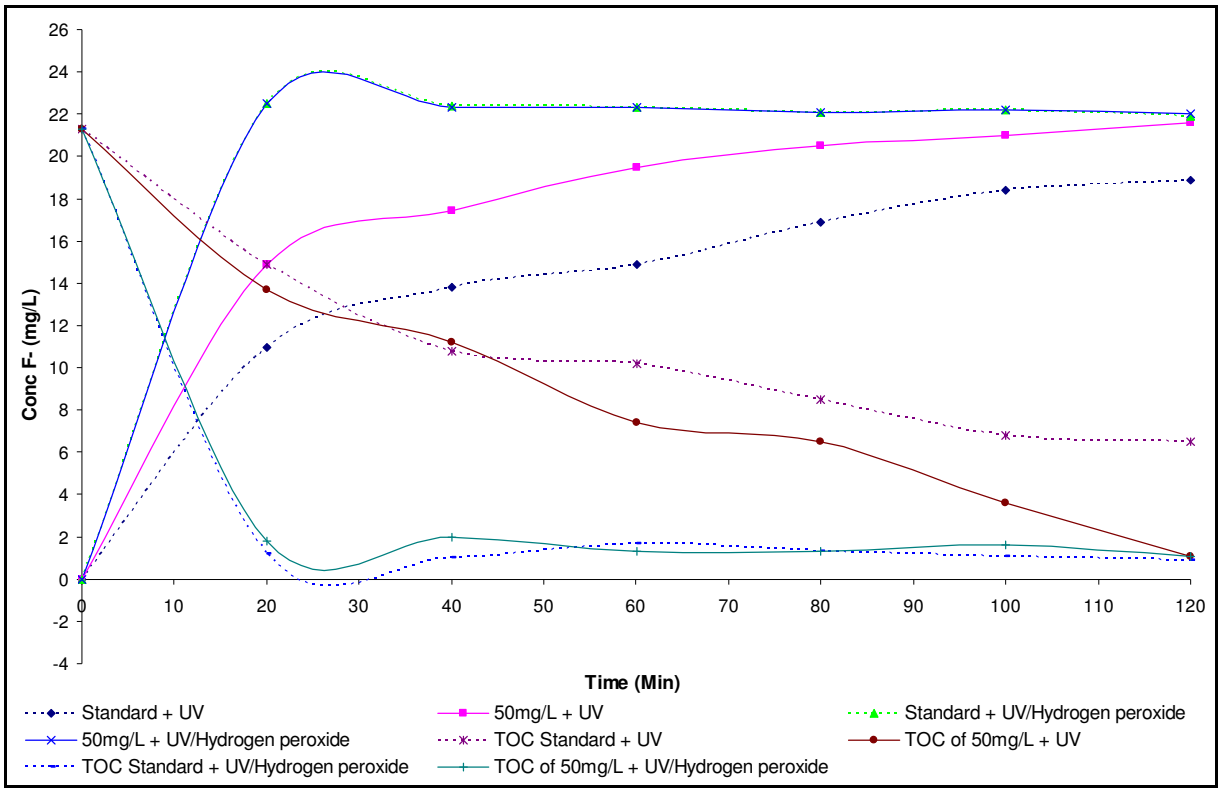


Figure 24. Degradation of trifluoromethoxy alkly sulfonate at different concentrations when submitted to UV radiation and UV/ H_2O_2 System, followed by the TOC analysis.

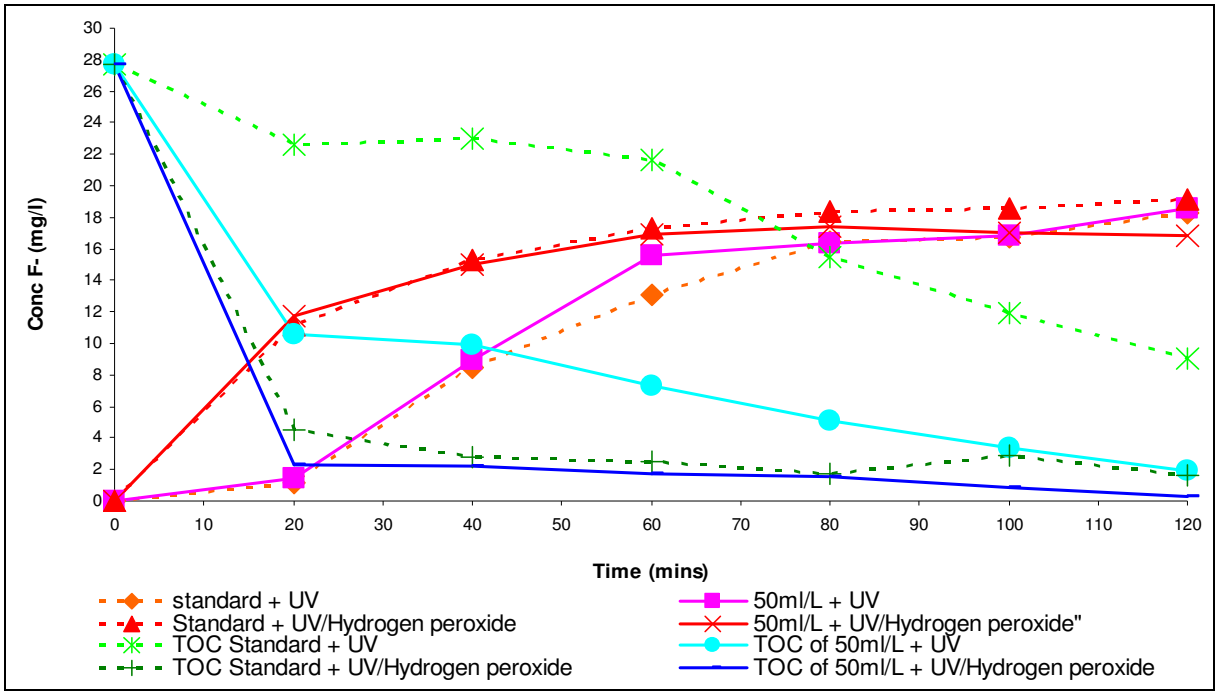


Figure 25. Degradation of fluorosurfactant Zonyl at different concentrations when submitted to UV radiation and UV/ H_2O_2 system, followed by the TOC analysis.

Ready biodegradation results are shown in Figure 26. The reference compound was > 60 % biodegradable in the 10 day window, validating the test criteria. Ready biodegradation test of fluoroaliphatic esters NOVEC ™ FC4430 and NOVEC ™ FC4432, the fluorosurfactant Zonyl and 10-(trifluoromethoxy) decane-sulfonate using Oxi-Top test was not complete in the 10-day criterion. Results of Oxi-top measurements show fluoroaliphatic esters NOVEC ™ FC4430, fluoroaliphatic ester NOVEC ™ FC4432, fluorosurfactant Zonyl and 10-(trifluoromethoxy) decane-sulfonate percentage biodegradation of 25 %, 28 %, 13 % and 40 % respectively within the experimental duration. Meanwhile fluoroethylene polymer and PFBS ($C_4F_9SO_3$) were observed to biodegrade < 10 % of total biodegradation within the experimental duration. Results obtained in the Manometric Respirometry Test compared well to those obtained in the Closed Bottle Test.

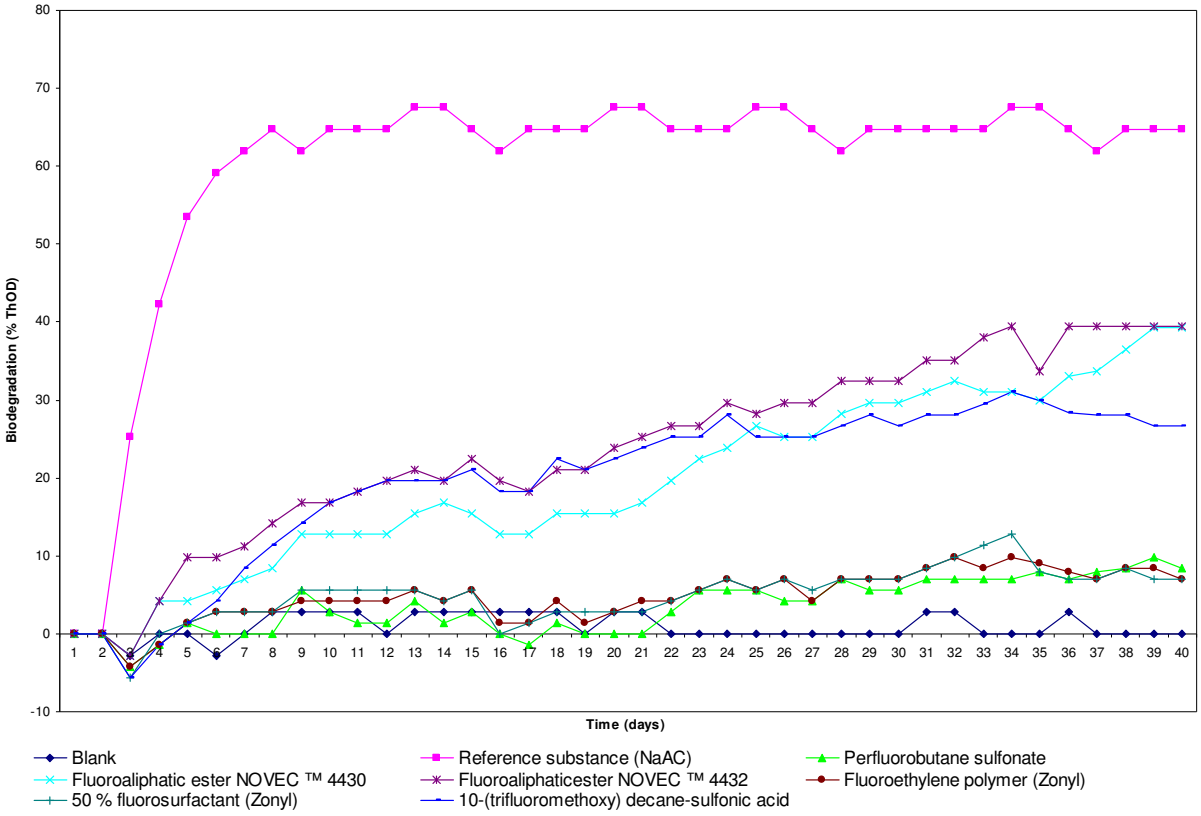


Figure 26. Oxi-Top BOD values resulting from biodegradation of studied surfactants compounds, using pre-conditioned activated sludge as inoculum obtained from sewage works Darmstadt, German.

For Closed Bottle Tests (CBT), biodegradation curves were plotted as percentage of biodegradation and expressed as a percentage of Theoretic Oxygen Demand (ThOD) versus time for each vessel. Mean value of replicates was calculated and average biodegradation curves were plotted (Figure 27). No significant biodegradation of PFBS of less than biodegradation < 3 % was observed. Fluoroethylene polymer was not tested by CBT because it was not soluble in the test media; in addition the results from ready degradability test did not show degradability. Figure 27 show that the compounds fluorosurfactant Zonyl, fluoroaliphatic ester NOVEC ™ FC4430 and NOVEC ™ FC4432 presents 47 %, 22 % and 19 % biodegradation respectively for Closed Bottle Test. Hence, biodegradadtion was not completed in the 28 days test duration time. 10-(trifluoromethoxy) decane-sulfonate showed > 80 % biodegradation in the period of 28 days of test. In this way, 10-(trifluoromethoxy) decane-sulfonate present ready biodegradation when using Rhine river water as inoculum for bacteria culture. Biodegradation values for fluoroaliphatic ester NOVEC ™ FC4430 and NOVEC ™ FC4432 (22 % and 19 % respectively) and PFBS (< 3 %) compared well to those obtained for Oxi-Top test (25 %, 28 % and < 10 % respectively).

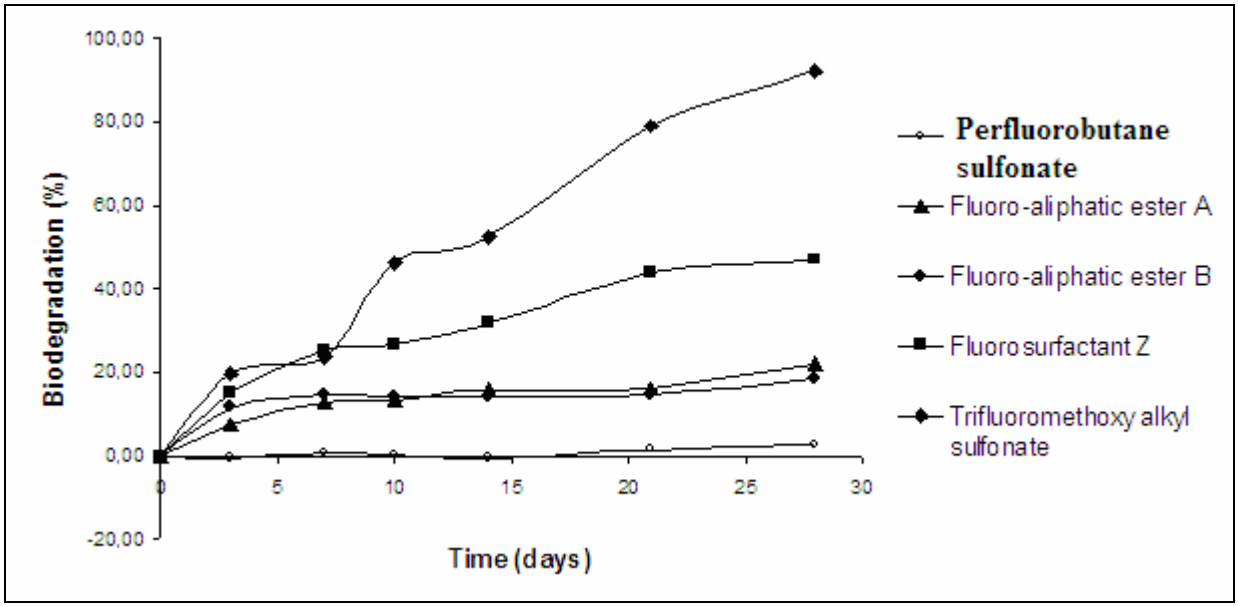


Figure 27. Biodegradation values (%) and expressed as a percentage of Theoretic Oxygen Demand (ThOD) of studied surfactants compounds in CBT, using Rhine river water as inoculum.

Fixed-bed bioreactor (FBBR) was performed for PFBS and 10-(trifluoromethoxy) decane-sulfonic acid. During the degradation test carried out with trifluoromethoxy alkyl sulfonate in an FBBR primary degradation was accomplished already after six days. Primary degradation accompanies desulfonation and oxidation of the alkyl chain at different positions. In the previous study done by Peschka et al. (2008b), on the prototype substance 10-(trifluoromethoxy) decane-sulfonic acid itself had shown biomineralization to fluoride by ubiquitous microorganisms in which gave an intense signal LC/MS/MS analysis at RT = 27.7 min and exhibited two characteristic fragment ions (m/z = 85 and m/z = 219) next to its molecular ion $[M-H]^-$ with m/z = 305. Fragments were formed by the cleavage of the fluorinated methoxy group. 10-(trifluoromethoxy) decane-sulfonate was found to meet the 60 % or greater biodegradability criteria in 28 days and can therefore be considered as ultimately biodegradable. In the current study, a fixed-bed bioreactor (FBBR) experiment was also conducted for PFBS in duplicate at concentration of 100 mg L^{-1}. Samples were extracted by SPE using Oasis HLB cartridges and prepared for LC/MS/MS analysis. The monitored m/z was 299 (mass spectrum in figure 28). For the period of 28 days experiment, weekly analysis were performed, but no significant difference (relative standard deviation < 7 %) in the area of the PFBS peak was observed, indicating that it did not biodegrade in the experiment conditions.

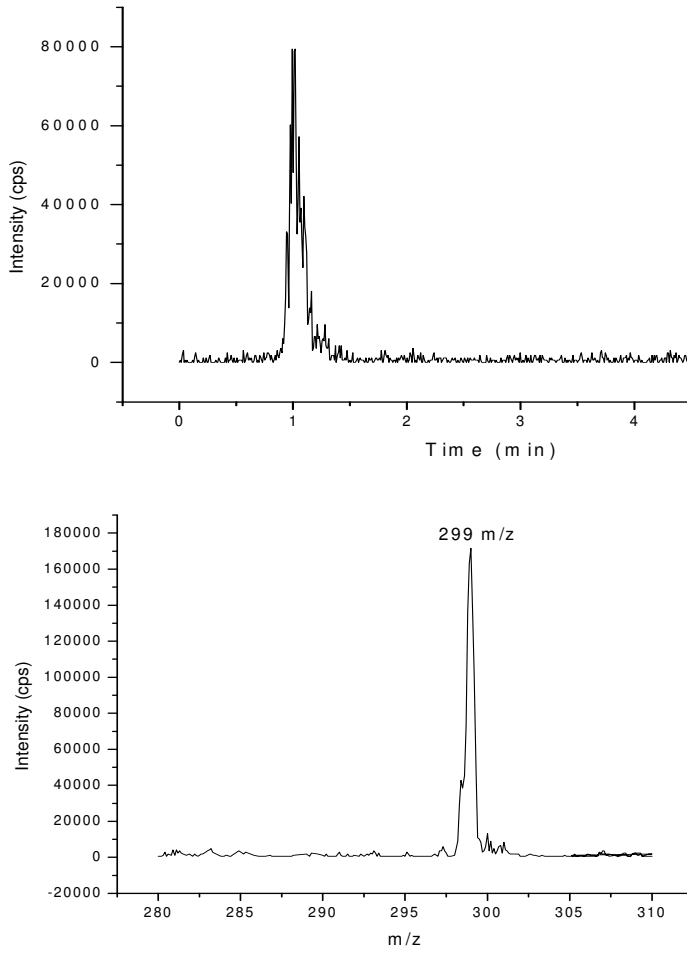


Figure 28. LC chromatogram and mass spectra of perfluorobutane sulfonate (PFBS).

Test conditions, however, are not comparable to those in natural environment which includes seasonal and daily changes in hydraulic flows, nutrient concentrations, or in physicochemical conditions. A systematic procedure to translate the results of a laboratory test to transformation rates under real circumstances is still lacking, and this is the reason why biodegradation of a substance in the natural environment does not always match laboratory results (Žgajnar Gotvajn and Zagorc-Končan, 2003). Investigations using substance-specific analytical methods, i.e. chromatography and/or mass spectrometry, are difficult to perform and the results are often difficult to interpret. Schroeder (1991) concluded from such experiments that the investigated fluorinated surfactants are not mineralised. Complete mineralisation of fluorinated surfactants requires the cleavage of the carbon-fluorine bond. Although the carbon-fluorine bond is very stable against physical and chemical attack, biochemical cleavage and release of fluoride have recently been reported (Alexy et al., 2004). In principle, in the OECD confirmatory test the degradability of fluorinated surfactants is

given as > 80 % with a few exceptions (Remde and Debus, 1996). Here, we observe based on the results obtained that only 10-(trifluoromethoxy) decane-sulfonate presented biodegradability > 80 %, when tested by CBT. Studies have shown that fluorinated compounds with fluoride content in the order of more than 50 % will not biodegrade over a period of 60 days (Remde and Debus, 1996), which explains the fact that the fluorosurfactant Zonyl showed only 47 % and 13 % biodegradation in CBT and Oxi-Top test respectively. In conclusion, a substance attaining the 60 % mineralisation threshold level would be expected to undergo fast and virtually complete ultimate biodegradation in the aquatic environment. Most importantly, this implies that the biodegradation processes could bring about removal of the test substances and their degradation intermediates in sewage treatment plants. Indeed, it has already been shown that there is a good correlation between positive results in ultimate biodegradability screening tests and the extent of carbon removal in sewage plant simulation tests (Gerike and Fischer, 1979). Struijs and Stoltenkamp (1994) concluded that a positive result obtained for a surfactant in a ready biodegradability test can be safely extrapolated to environments in regions where domestic waste water is processed by sewage treatment plants. Hence, surfactants failing to reach the 60 % threshold pass level within the 28-day period will require additional evidence to prove their environmental safety. Further biodegradation studies using FBBR should be performed for fluoroaliphatic ester NOVEC ™ 4430, NOVEC ™ 4432 and fluorosurfactant Zonyl in order to identify potential metabolites which could be generated from the biodegradation of the compounds and while elucidating the possible degradation pathway of the surfactants.

4.6 Perfluorinated compounds correlation in water and sediments within the Winam gulf of Lake Victoria catchment at Kisumu city.

This section presents the correlations between perfluorooctanoic acid and perfluorooctane sulfonate levels in the analysed water and sediment samples. Analysis data presented are representative for samples from sampling locations where the results were above quantification limit. This is applicable to both sediment and water samples which had LOQ of more than 1 ng/L for water and 1 ng/g for sediment samples. Data consisting of concentration means obtained from 12 sampling locations within the study area were analyzed by statistical methods. Between the two compounds analyzed (PFOA and PFOS), the statistical results show that PFOA is primarily more concentrated in sediments and water than PFOS. On the other hand, results obtained in this study also indicate that PFOS is primarily more

concentrated in fish samples than PFOA. Figures 29 and 30 represent observed levels of PFCs analysed in water as compared to sediment matrices.

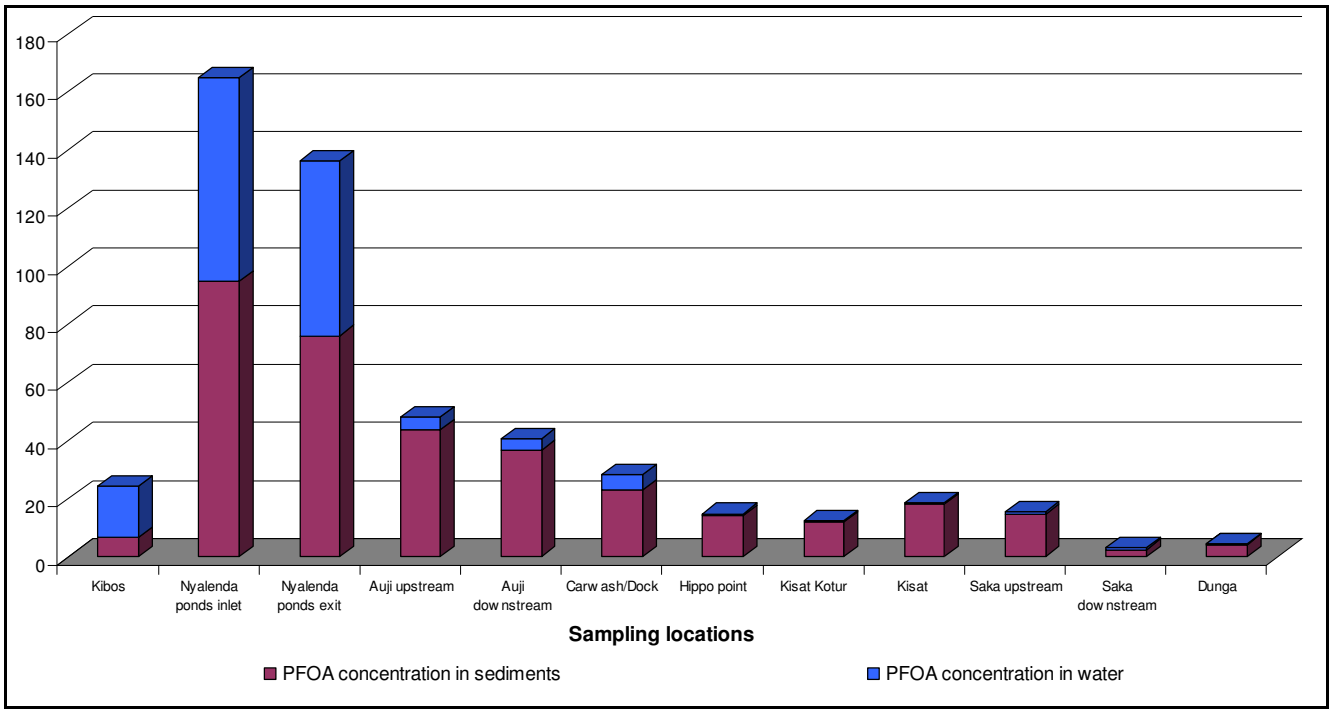


Figure 29. PFOA concentration in sediments as compared to water for various sampling locations

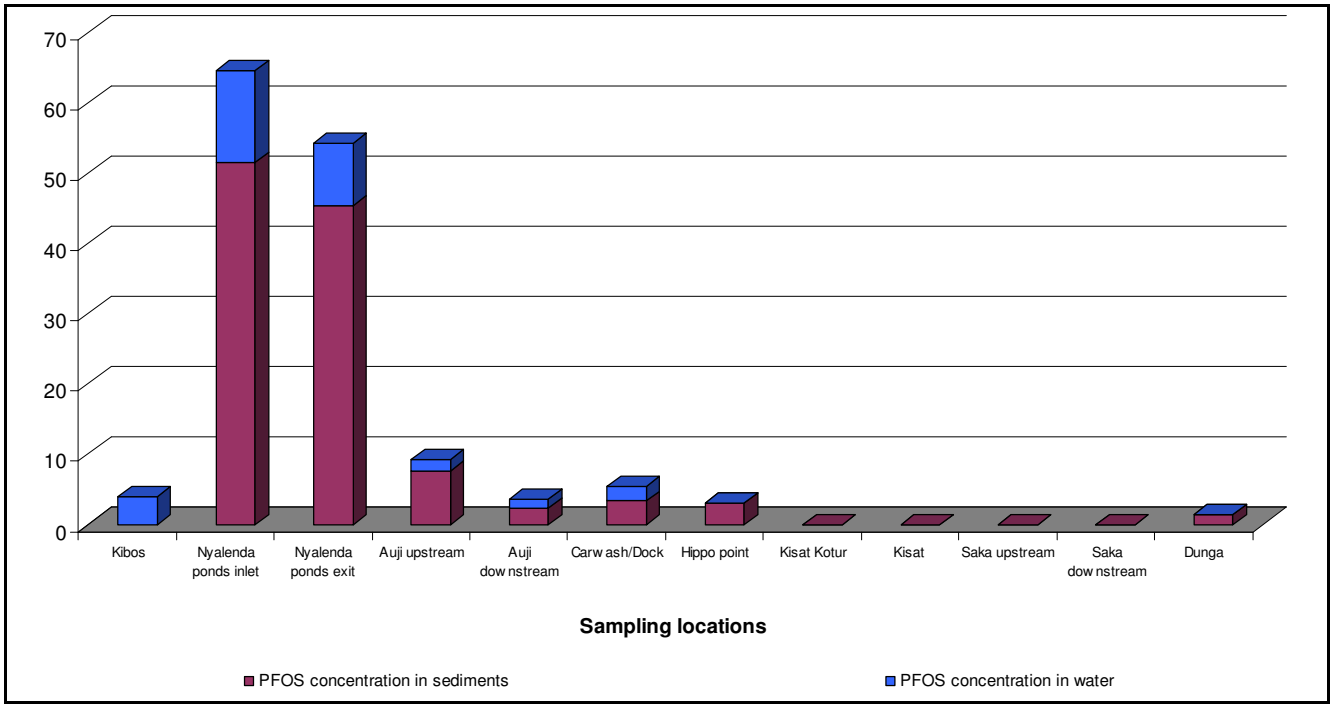


Figure 30. PFOS concentration in sediments as compared to water for various sampling locations around the Winam gulf.

From the linear regression results, the levels of the two PFCs analysed in water samples are moderately dependent on the levels obtained in the sediments within our sampling locations. Significant correlation between PFOA in both sediments and water was observed, which according to slope values of linear regression gave a value of r = 0.891. Figure 31 and 32 show scatter diagrams for PFOA and PFOS concentrations in water tested against those in sediments from all sampling locations.

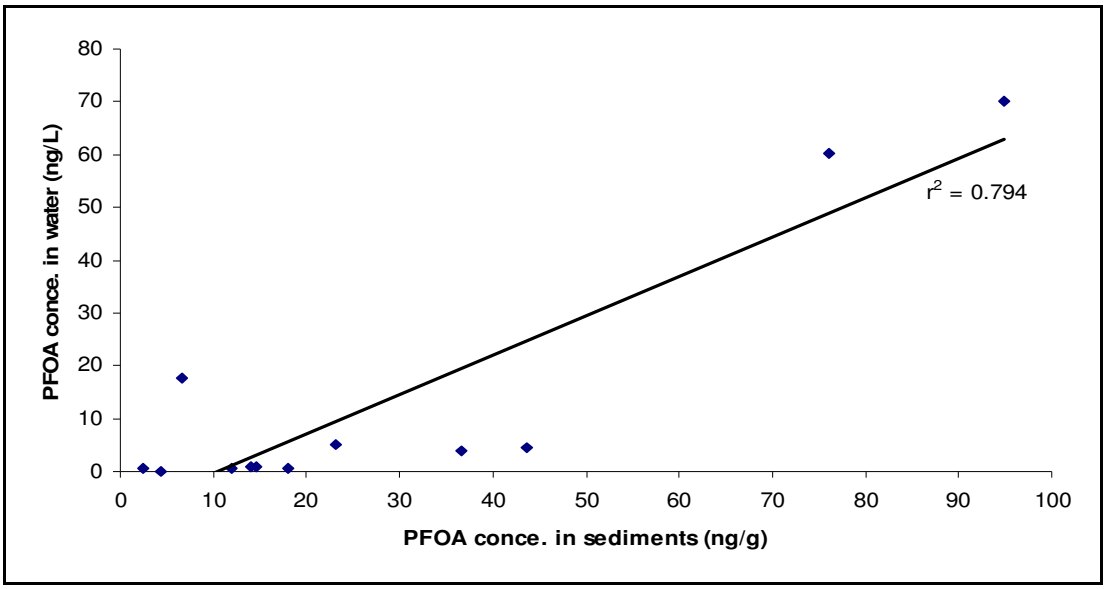


Figure 31. Scatter diagram for PFOA concentrations in water tested against those in sediments

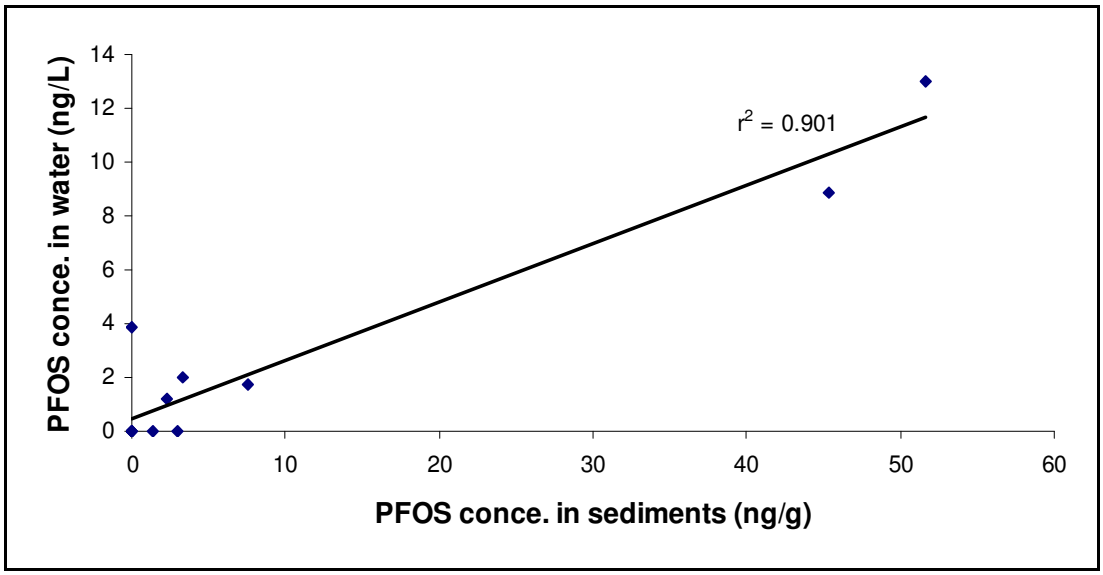


Figure 32. Scatter diagram for PFOS concentrations in water tested against those in sediments

Table 17 shows the results of the Pearson Correlation Test between the concentrations of PFOA and PFOS in water and sediments for the 12 sampling locations. Correlation test using Pearson correlation test (2-tailed) for PFOS alone in water and in sediments samples gave r = 0.949 indicating significant correlation in these two matrices. Although correlation between PFOA and PFOS levels in water and in sediments was positive (r = 0.566 and r = 0.230 respectively), it was not significant.

This positive correlation could suggest that there exist similar sources of these pollutants. Moreso, when correlation and regression was tested for individual PFOA and PFOS in sediments and water samples from Kibos-Nyalenda-Auji river system, significant correlation was observed (r = 0.944 for sediments and r = 0.984 for water).

The obtained results imply that the major source of PFCs analysed in this study in Lake Victoria within Kisumu city is through the Nyalenda-Auji river system within the catchment. The observed increase in mass flow levels of PFCs at Nyalenda ponds may have resulted from biodegradation of precursor compounds such as fluorotelomer alcohols, which is supported by significant correlations in the mass flow of PFOA in water and sediments. PFOS is the stable end product of the degradation of most perfluorooctanesulfonylfluoride (POSF)-based perfluorochemicals. Although the production of POSF-based fluorochemicals is now being regulated, these compounds have been manufactured for use in a number of industrial and commercial products for over 40 years (Giesy and Kannan, 2002). Furthermore, the levels of PFOA and PFOS were significantly correlated after the Nyalenda waste treatment ponds. This can be adequately used as a reference in future studies and in management of similar pollutants within the Winam gulf of Lake Victoria.

Table 17. Correlation results between the concentrations of PFOA and PFOS in water and sediments.

		PFOA in water	PFOA in sediments	PFOS in water	PFOS in sediments
PFOA in water	Pearson Correlation	1	0.891**	0.566	0.326
	Sig. (2-tailed)		0.000	0.055	0.301
	N	12	12	12	12
PFOA in sediments	Pearson Correlation	0.891**	1	0.465	0.230
	Sig. (2-tailed)	0.000		0.127	0.473
	N	12	12	12	12
PFOS in water	Pearson Correlation	0.566	0.465	1	0.949**
	Sig. (2-tailed)	0.055	0.127		0.000
	N	12	12	12	12
PFOS in Sediments	Pearson Correlation	0.326	0.230	0.949**	1
	Sig. (2-tailed)	0.301	0.473	0.000	
	N	12	12	12	12

** Correlation is significant at the 0.01 level (2-tailed). Only data of concentration results above 1 ng/l for water and 1 ng/g for sediment samples was used.

CHAPTER FIVE

5 MANAGEMENT IMPLICATION

This chapter provides information and solutions on the sources, loadings and fate of pollutants mainly perfluorinated compounds and similar organic pollutants to Lake Victoria and their management implications based on the information that existed as of December 2006 and in this current study. The highlights of the research findings are based on the following;

- Information on the concentrations, sources, loadings and pathways of perfluorinated compounds and other non conventional pollutants evaluation, with the aim of identifying source reduction actions.
- Available regulatory monitoring information that includes all pollutants especially non conventional micro-pollutants. Previously, research studies and routine monitoring used methods that cannot detect low levels of contaminants of concern. Qualitative information is acknowledged as an important component of the pollutant source identification process and decision making.
- Identification and highlights of remedial and other regulatory program efforts that contribute to pollutant reduction goals on which strategies can build.
- Critical pollutants from the upstream connecting channels that enter Lake Victoria via its catchment rivers and by atmospheric deposition. Restoring beneficial uses in Lake Victoria depends partly on the successful implementation of upstream, and out of basin programs that reduce emissions of critical pollutants.

5.1 Sources and loads of perfluorinated compounds in Winam Gulf of Lake Victoria.

Perfluorooctanoic acid, perfluooctane sulfonate and other non conventional Pollutants enter Lake Victoria via a number of pathways, including its tributaries, precipitation, point sources (e.g., sewage treatment plants, industrial facilities, waste sites) and non-point sources (e.g., urban stormwater, agricultural runoff, atmospheric deposition). Lake Victoria receives some of its known contaminant loadings from upstream of its inlet rivers. The major sources of PFCs to Lake Victoria as observed in this study were via the Kibos-Nyalenda-Auji river system. The pollutants were mainly detected in the municipal waste water system (accounting

for more than 50 % of PFOA and PFOS loads in the Winam gulf within Kisumu city) and load into Lake Victoria through Nyalenda waste water treatment ponds. Other significant sources were observed to be through the Kisat river catchment, and the main source attributed to inefficient sewage treatment plant and waste water or effluent from numerous small industries that exist and existed in the area. During sample collection, it was observed that River Kisat and Kibos-Nyalenda-Auji river system surface water were highly turbid, brownish in color except at upstream areas of Kisat River (near municipal sewage work) which was dark/grey colored with a permanent unpleasant smell, due to discharges of sewage waste effluents. The dark/grey color was also observed at the Nyalenda waste water treatment ponds inlet. Contamination of the Kasat region is a consequence of the effluents emanating from industries, and other miscellaneous urban wastes. During this current study, it was observed that untreated sewage flows into the lake daily from sources around it.

In Tanzania and Uganda, industrial wastewater treatment facilities are generally absent, but in Kenya a majority of factories operate separate treatment plants (Scheren et al., 2000). Only a few industries are connected to an urban sewage system (Scheren et al., 2000). Growth in industries has taken place against a backdrop of no infrastructure development for disposal of effluents. The currently existing sewage infrastructure has not been expanded or improved for decades. However, some of the industries are being allowed to establish their operations in areas that have been designated as “non-industrial”, so they lack the infrastructure to handle their own waste products. Some studies have shown that fishes in Lake Victoria contain varying levels of organochlorine pesticide residues (Mitema and Gitau, 1990), reflecting the transport of agrochemical residues from farms within the catchment, through rivers to the lake.

5.2 Hazard assessment and safety recommendation regarding PFOA and PFOS in human dietry intake

An evaluation of the ecological risk to aquatic animals associated with exposure to PFOS was performed by comparing the measured concentrations of PFOS in water with water-quality (guideline) values that are protective of aquatic organisms (Beach et al., 2005). Currently, no guidelines are specifically derived for saltwater, but guideline values have been developed following the procedures outlined in the U.S. Environmental Protection Agency Great Lakes Initiative (U.S. EPA 1995) based on the results of toxicity with tests done on freshwater

organisms (Beach et al., 2005). The screening value of PFOS for the protection of aquatic plants was determined to be 2.3 mg/L. Tier II water-quality values for aquatic animals are represented by the secondary maximum concentration (SMC) and secondary continuous concentration (SCC). The PFOS values for SMC and SCC were 8,500 and 1,200 ng/L, respectively. These values represent concentrations at which aquatic organisms would not be unacceptably affected if the 4 days average and 1hour average concentrations of PFOS did not exceed 1,200 or 8,500 ng/L more than once every three years respectively. Based on laboratory animal feeding studies, the No Observed Adverse Effect Level (NOAEL) of PFOS was estimated to be 0.1 mg/kg/day and the lowest observed adverse effect level (LOAEL) was estimated to be 0.4 mg/kg/day (U.S. EPA, 2000).

The U.S. EPA's Science Advisory Board suggested that PFOA is a possible human carcinogen. However, a final risk assessment by the U.S. EPA is not completed yet. The Health Protection Agency (HPA, 2007) advises that the maximum acceptable concentration of perfluorooctane sulfonate (PFOS) in drinking water is 0.3 μg/l, and that the maximum acceptable concentration of perfluorooctanoic acid (PFOA) in drinking water is 10 μg/l. This follows a request for advice from the Drinking Water Inspectorate for England and Wales (DWI). The advice incorporates a recent review of toxicological evidence by the UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), an independent scientific committee that gives advice to government departments and agencies. It also takes note of a recent study of dietary intakes by the Food Standards Agency (FSA). Recommendations such as these are always subject to review in the light of new information. The COT statements indicate in particular the need for more precise estimates of the dietary intake of PFOS, consideration of the impact of other perfluorinated chemicals in the diet on total PFOS exposure, better understanding of the way the body handles PFOS and PFOA, and clarification of the mechanism by which PFOA at high doses causes tumours in the liver and pancreas in rats.

The PFOS concentrations are almost invariably higher than PFOA concentrations and the PFOS concentrations in fish liver are consistently higher than those in muscles (fillet). PFOS has been shown to bioaccumulate in fish and a kinetic bioconcentration factor has been estimated to be in the range 1000 – 4000. The time to reach 50 % clearance in fish has been estimated to be around 100 days (EFSA, 2008). Fish seems to be an important source of human exposure to PFOS, although the data might be influenced by results of studies in

relatively polluted areas, which is likely to over-estimate exposure from commonly consumed fish. There are very few data, especially for Europe, that can serve as reliable indicators of the relative importance of most other kinds of food. Based primarily on the available data for fish and fishery products, indicative estimates of dietary exposure to PFOS were 60 ng/kg (body weight) per day for average consumers, and 200 ng/kg (body weight) per day for high consumers of fish. However, recent studies have indicated much lower exposures, demonstrating the uncertainty in the assessments. From a subchronic study in Cynomolgus monkeys, the Scientific Panel on Contaminants in the Food Chain (CONTAM) identified 0.03 mg/kg (body weight) per day as the lowest no-observed adverse-effect level (NOAEL) and considered this a suitable basis for deriving a Tolerable Daily Intake (TDI). The CONTAM Panel established a TDI for PFOS of 150 ng/kg (body weight) per day by applying an overall Uncertainty Factor (UF) of 200 to the NOAEL (EFSA, 2008). An UF of 100 was used for inter and intra-species differences and an additional UF of 2 to compensate for uncertainties in connection to the relatively short duration of the key study and the internal dose kinetics.

The CONTAM Panel noted that the indicative dietary exposure of 60 ng/kg (body weight) per day is below the TDI of 150 ng/kg b.w. but the highest exposed people within the general population might slightly exceed this TDI. Drinking water is estimated to contribute less than 0.5 % of the indicative exposure. The importance of fish is, however, not supported by all studies. It is possible that additional exposure to PFOS could result from precursors and other sources (EFSA, 2008). For PFOA, the total contribution from the non-food sources, mainly indoor exposure, could be as high as 50 % compared to the estimated average dietary exposure to PFOA. Drinking water is estimated to contribute less than 16 % to the indicative exposure. Based on the limited data, the CONTAM Panel identified the indicative average and high level dietary exposures of 2 and 6 ng/kg (body weight) per day, respectively. Persons with higher fish consumption do not always show higher levels of PFOA in blood compared to persons with “normal” fish consumption. It is possible that additional exposure to PFOA could result from non food sources and precursors. PFOA is readily absorbed. Elimination is dependent on active transport mechanisms which vary between different species, and between sexes in some species. The CONTAM Panel established a TDI for PFOA of 1.5 µg/kg body weight) per day. The CONTAM Panel noted that the indicative human average and high level dietary exposure for PFOA of 2 and 6 ng/kg (body weight) per day, respectively, are well below the TDI of 1.5 µg/kg b.w. per day (EFSA, 2008).

5.3 Factors contributing to the pollution of the lake

In addition to the specific anthropogenic threats to the world's lake basins, the lack of proper coordination of management activities and institutional fragmentation are perhaps the greatest problems facing lake basins around the world (Borre et al., 2001). The factors contributing to the pollution of the lake and influent rivers include: poor urban planning against the backdrop of population and industrial growth, the use of old, dilapidated and inappropriate technologies, poor maintenance of treatment plants, lack of waste treatment and disposal mechanisms, poor sanitation infrastructure, poor agricultural/land-use practices, and inappropriate use and disposal of chemicals such as pesticides, fertilizers, and industrial wastes. Currently, there are poor standards for industrial operations, and there are no incentives to encourage the industries to engage clean technologies. In addition to this, the authorities concerned have failed to provide services such as sewage treatment and waste disposal in both urban and rural areas. There is a lack of monitoring and enforcement of existing regulations and legislation, and, in most sectors, lack of resources and will on the part of the governments to mitigate the environmental problems. The major concerns (issues) that emerged from the environmental assessment (Odada et al., 2003) using the Global International Waters Assessment (GIWA) transboundary diagnostic analysis approach were: unsustainable exploitation of fisheries (overexploitation, excessive by-catch and discards, and destructive fishing practices), and pollution (microbiological, eutrophication, chemical and suspended solids).

There is no enforcement of existing regulations regarding chemicals use and their disposal, and the current legislations are out-dated and in need of revision. Lack of monitoring and poor scientific knowledge has led to the use of inappropriate or obsolete technologies to the detriment of the environment. The current water treatment processes do not remove PFCs and other micro pollutants. From the results of this study, the observation can be confirmed for water treatment plants at Nyalenda and along Kisat river. Furthermore, the concentration of PFCs in raw water can influence the concentration in insufficiently treated tap water.

5.4 Actions and Progress for management of Lake Victoria catchment

The action to be taken for restoration and remedation of Lake Victoria should consider the following factors for;

Evaluation of critical pollutants

There is a need to evaluate critical pollutant information and relate actions in all watersheds within its portion of the basin based on available ambient monitoring information and emissions data from industrial, municipal and other non-point source discharges (such as combined sewer overflows and waste sites). Local strategies should be developed to address identified sources of critical pollutants in these watersheds.

Policy option

In the policy part, it is observed that in several cases, more than one policy ought to be taken up at once in order to obtain the desired impact. This is true with quotas for fishing and processing. Also, the successful implementation of these policy options will not be achieved without involving, in a participatory manner, the communities living on the lakeshores who are involved in fishing as a source of their subsistence livelihood and income generation. Capacity building in terms of civic education and leadership and management skills will help enhance this empowerment.

Ecological factor

Although attempts to improve the quality of the Lake should generally focus on chemical pollution, other factors are important and should be considered Ecological factors, such as invasion of the lakes by foreign species, habitat changes, overfishing, and random variations in organism populations, are especially influential. Lack of appreciation of the significance of ecological factors stems partly from the inappropriate application of the concept of eutrophication to Lake Victoria (Odada et al., 2004). Emphasis on ecological factors is not intended to diminish the seriousness of pollution, but rather to point out that more cost-effective management, as well as more realistic expectations of management efforts by the public, should result from an ecosystem management approach in which ecological factors are carefully considered.

Remedation and removal

Removal efficiencies of PFOA, PFOS and other organic pollutants in water treatment has been demonstrated elsewhere to be achievable. The removal rate of PFCs of over 90 % was achieved in a plant using activated carbon which was exchanged once or twice a year (Takagi et al 2008). Therefore, activated carbon treatment in water treatment plants and frequent

exchange of the adsorbent efficiently removes PFOS and PFOA in water treatment. A negative removal rate in certain plants at certain time periods suggest formation of PFOS and PFOA during the treatment. This may be due to the breakdown of certain precursor compounds to PFOS and PFOA during the treatment process (Takagi et al., 2008).

5.4.1 Nyalenda River Catchment Management Plan

Nyalenda River Catchment contributes to more than a half of all PFCs load into Lake Victoria. Because of the critical link between Lake Victoria and the Nyalenda waste water treatment facility, this work suggests to implement the Nyalenda waste water management plan which will work to reduce toxic chemical concentrations from the Nyalenda ponds by reducing trace pollutants with a goal of achieving water quality that will protect human health, aquatic life, and wildlife, and while doing so, improve and protect water quality in Lake Victoria as well. To be able to achieve this, the management plan should be committed to: reduce point and non-point sources of pollution to the river, by completing site specific clean-up activities, controlling point source discharges, encouraging pollution prevention techniques and restoring critical habitat areas along the river. Therefore, efficient treatment of waste water prior to re-introduction to the river system should be a priority. A modern waste water treatment facility which has micro organic pollutants treatment unit using oxidation or activated carbon among other techniques should be incoperated along side the municipal waste water treatment ponds.

5.5 Pollution and Management Policy Options

In the broarder outlook, the pollution management of Lake Victoria in general and Winam Gulf basin in particular require an integrated approach more like the European Water Framework Directive (EWFD). Apart from the scientific technological approach as the basis of pollution control, social and political aproach is nessecary. The following options are recommendations as the basis of the pollution and management policy for Winam Gulf of Lake Victoria basin.

- *Accreditation of analytical laboratories for standards enforcement.* In order to facilitate water-quality standards enforcement conveniently and relatively cheaply, accreditation of regional and national water-quality laboratories is essential.

Implementation of this policy option will go a long way in reducing health costs and increase labor productivity.

- *Water Framework Directive.* A water framework directory such as the European Water Framework Directive (**Directive 2000/60/EC of the European Parliament)** is recommended as a good approach to integrated Lake Victoria basin management. In order to have a consistent and smooth policy implementation for the management of the Lake Victoria basin, harmonization of policies, regulations and laws is vital. This work is underway within the auspices of East African Community for fisheries, environment and natural resources management (Odada et al., 2004).
- *Stronger vetting of technologies that are being promoted by the national and international agencies.* Many factories are using old, obsolete and/or inappropriate technologies that do not adequately reduce the pollutant load in effluents before discharge to rivers and the lake. In some cases, prohibited chemicals such as DDT are used (Odada et al., 2004). Clean technologies should be promoted alongside better economic incentives. Stronger vetting of prohibited chemicals (recently also some PFCs) will reduce the risk of the adverse effects of pollution on human health and the environment.
- *Enforcement of regulations for effective treatment in municipalities and industries.* Factory inspectorate requires all processing plants to have waste-treatment facilities from their factories. However, few industries have "working" treatment plants or ponds for that matter. Wastewater and solid waste is left to spread to streams and residential areas where they affect the health of inhabitants living in the vicinity of these areas or those who use contaminated water from streams and rivers. In other places, the disposal of industrial and municipal effluent results in huge economic losses to the economy through destruction of tourist attractions such as at the yatch club and the Hippo point area. With the enactment of environmental policies and frame law and establishment of environmental protection agencies, this policy option has a high probability of succeeding.
- *International conventions governing and regulating the use of chemical products should be complied to and enforced.* Ratification of many international conventions dealing with chemical products such as PFCs, personal care products and pesticides should be enforced. Although some have been implemented, not all the ratified conventions are applied as desired. The result of such a state of affairs is to exacerbate environmental degradation and biodiversity decline among others with disastrous

effects. With proper awareness, mobilization and commitment, popular participation seems to be one way of facilitating the objectives by putting pressure on relevant authorities.

- *Incorporate all stakeholders in drafting of regulations and in monitoring and enforcing agreed upon regulations.* Participatory approaches have been found to be effective in implementation of policies and decisions, which require the input of the community and where the communities in turn stand to benefit from the process. This is because the involvement of the beneficiaries inculcates a sense of responsibility and ownership among others. Because the cost of implementation and the benefit accrues to them, they become effective partners ensuring proper and successful implementation. This policy option has a high probability of success. It provides high dividends in terms of high success rates, as has been experienced across the region in several project implementations.
- *Improvement of waste disposal activities by involving the private sector and communities.* Private sector participation in waste disposal activities is important in order to fill the void left by the public institutions which have failed to render these services. The feasibility and effectiveness of this policy option is that it is a business venture with the capability of generating income. In some places there are established community youth groups that engage in waste collection and disposal from residential areas.
- *Improve monitoring and enforcement and revise regulations in urban planning to also cater for environmental issues.* Current urban-planning regulations and plans are outdated. Town-planning services have not taken into account the increasing populations which have far outstripped projections made in the 1960s and 1970s. Lack of monitoring and enforcement of building and new settlement developments have resulted in urban centers evolving haphazardly with poor sanitation and lack of essential services. However, the capacity to undertake the revision and corrective measures exists within relevant land offices.

CHAPTER SIX

6 GENERAL CONCLUSION

Although there is no drinking water quality criteria established for PFOS and PFOA in Kenya, there are some guideline values reported for other countries. The action level of PFOA agreed between the U.S. EPA and E.I. (Eleuthère Irénée) Dupont de Nemours is 0.50 µg/L (U.S. EPA, 2006). Compared to this value, PFOA concentrations in our water samples from Lake Victoria catchment are lower. Perfluorooctane sulfonate and perfluorooctanoic acid were detected in all samples analysed in this study. Therefore, it is of greater concern to monitor PFOS and PFOA in raw and tap water regularly to check the levels considering the health effects associated with such exposures. PFOA was detected in approximately 50 % of the samples analyzed at concentrations generally higher than those found for PFOS in water and sediments. Variability in the concentrations of PFOA or PFOS in river waters (ranges of PFOA 0.40 – 96.40 and PFOS < 0.40 – 13.23 ng/L) was higher than for lake waters (ranges of PFOA 0.40 – 11.65 and PFOS < 0.40 – 2.53 ng/L respectively) suggesting generalized point sources such as domestic and industrial waste. Perfluorooctane sulfonate was detected in all species analyzed. Concentration values of PFOS in muscles of Nile perch (*Lates niloticus*) of up to 10.50 and 35.70 ng/g for liver samples were obtained. Nile tilapia (*Oreochromis niloticus*) concentration values were measured up to 12.40 and 23.70 ng/g for muscles and liver samples respectively. Generally, these levels (ng/g) are not high and do not pose any immediate danger to consumers when compared to results obtained in similar studies globally. However, measures should be taken to control the PFC inputs in Lake Victoria ecosystem. The results of this study can be used as pioneer 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.

A positive linear relationship was found between PFOA concentrations in water and sediment in most samples analysed, however, the correlation was not significant but an indication of similar pollution sourses. Moreso, when correlation and regression was tested for individual PFOA and PFOS in sediments and water samples from Kibos-Nyalenda-Auji river system, significant correlation was observed (r = 0.944 for sediments and r = 0.984 for water. The findings indicated lead point sources such as Kibos-Nyalenda-Auji river system. Results suggested that the PFCs analysed demonstrated measurable relationships in water and

sediments of the study catchment area, and could be used as indicators to assess the water quality of rivers flowing in Winam gulf and to project the sources of similar pollutants within the Lake Victoria. Quantifying PFCs in sediments has provided insight into their sources, distribution, and mobility in the environment.

Fluoroethylene polymer and perfluorobutane sulfonate are not biodegradable. However, microorganisms are capable of degrading the trifluoromethoxy alkly sulfonate when using the Closed Bottle Test. Moreover biodegradation of tested surfactants showed to be < 60 % within the period of 28 days of the experiment using Closed Bottle Test when Rhine river water was used as an inoculum for bacteria culture. Fluoroaliphatic ester NOVEC ™ FC4430, NOVEC ™ FC4432 and fluorosurfactant Zonyl in both methods did not meet the criterion to satisfy ready biodegradation, however they showed slow degradation throughout the test duration. Fluoroethylene polymer demonstrated no biodegradation potential by ubiquitous microorganisms in the Manometric Respirometry Test for ready biodegradability. The same results were observed for PFBS, which degradation due to UV and oxidation exposure was not significant within 120 minutes. Biodegradability test carried out with trifluoromethoxy alkly sulfonate in a fixed-bed bioreactor primary degradation was accomplished already after six days showing biomineralisation to fluoride while no biodegradation was observed for PFBS in this test conditions. Ready biodegradability test of fluoroaliphatic esters using Manometric Respirometry Test was not complete in the 10-day criterion for ready degradation, however, biodegradation continued to 40 days mark. The same results were observed for all compounds tested. Insignificant degradation was observed for PFBS in AOP method using UV or UV/ H_2O_2 system. Other compounds showed increased degradation with time of exposure to UV radiation and even more accentuated degradation when applied together with H_2O_2.

The analytical method used in this study is capable of detecting PFCs at the sub-ng/g level in urban sediments. The method includes methanol extraction of the analytes from the sediment, a reversed phase solid phase extraction (SPE) clean-up procedure, and an analysis procedure via liquid chromatography tandem mass spectrometry (HPLC/MS/MS). Long chain perfluorinated acids namely; perfluoro-n-octanoic acid (PFOA), 2H-perfluoro-2-octenoic acid (FHUEA), 2H-perfluoro-2-decenoic acid (FOUEA) and 2H-perfluoro-2-dodecenoic acid (FNUEA) has been determined in biota (fish) and abiota (water) by derivatisation and

subsequent analysis by GC/MS. The ion pair extraction with TBA is selective for acidic organic compounds allowing efficient transfer of the anion of PFCs into the organic phase. Results demonstrate that GC/MS can be a alternative to the LC/MS method for quantification of PFOA in contaminated areas, where expected higher concentration of the analyte is expected.

Treatment of municipal and industral waste should be emphasized in order to minimise input of organic pollutants from domestic sources into Lake Victoria. Urban refuse collection should be improved to reduce the insidences where surface drainage carries refuse to the rivers and lake. Drainage of raw sewage that is not treated adequately into Winam gulf via inlet rivers such as Kisat should be stopped. Urgent rehabilitation of sewage works at Kisat is recommended. In addition, modern treatment of waste water using organic pollutants adsobents, such as activated carbon or other oxidation processes should be used. The removal of PFCs by ozonation and activated carbon treatment can be applied to reduce the loading not only of PFCs but other micro organic pollutants. River Auji should be rechanneled to avoid pouring waste water near Hippo point where water works is situated. Incoporation of monitors in the drainage systems in complementing the conventional chemical analysis is vital. It is anticipated that the present research findings will be put into use by research organisations such us FAO, LVEMP and KMFERI, UNEP, Governmental and Non Governmental Organisations (NGOs).

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APPENDICES

Table 18. Concentrations (pg/g) of PFOA and PFOS in fish liver and muscles from different sampling locations within Lake Victoria beaches.

Nile Perch (*Lates Niloticus*)				
Dunga Beach (DP)	**Liver**		**Muscles**	
	PFOS	**PFOA**	**PFOS**	**PFOA**
	6240	580	580	<LOQ
	11380	1780	10450	2160
	8740	<LOQ	7900	<LOQ
	7620	<LOQ	890	<LOQ
	8790	<LOQ	940	<LOQ
Kusa beach (KP)				
	PFOS	**PFOA**	**PFOS**	**PFOA**
	5620	<LOQ	2220	<LOQ
	2220	<LOQ	1030	<LOQ
	4530	<LOQ	2080	<LOQ
	3760	<LOQ	1800	<LOQ
	4860	<LOQ	2030	<LOQ
Kendu bay pier beach (KbP)				
	PFOS	**PFOA**	**PFOS**	**PFOA**
	3230	<LOQ	1780	<LOQ
	6740	1020	3200	<LOQ
	4500	<LOQ	2000	<LOQ
	5430	<LOQ	1920	<LOQ
	4840	<LOQ	2090	<LOQ
Nyamware beach (NP)				
	PFOS	**PFOA**	**PFOS**	**PFOA**
	15030	1780	1870	<LOQ
	35720	2340	9410	890
	18600	<LOQ	980	<LOQ
	27500	<LOQ	2150	<LOQ
	24880	<LOQ	1150	<LOQ
Ndura beach (Nr P)				
	PFOS	**PFOA**	**PFOS**	**PFOA**
	1350	<LOQ	930	<LOQ
	13190	1200	5010	<LOQ
	5780	<LOQ	2340	<LOQ
	6540	<LOQ	2460	<LOQ

	7560	<LOQ	2880	<LOQ

Nile Tilapia (*Oreochromis niloticus*)

Nyamware beach (NP)

PFOS	**PFOA**	**PFOS**	**PFOA**
2400	<LOQ	1200	<LOQ
23740	980	8020	<LOQ
14680	<LOQ	3860	<LOQ
12230	<LOQ	3460	<LOQ
5680	<LOQ	2120	<LOQ

Dunga Beach (DP)

PFOS	**PFOA**	**PFOS**	**PFOA**
19670	1890	12440	<LOQ
1460	<LOQ	890	<LOQ
6700	<LOQ	2140	<LOQ
7500	<LOQ	2490	<LOQ
14700	<LOQ	6490	<LOQ

Ndura beach (Nr P)

PFOS	**PFOA**	**PFOS**	**PFOA**
2640	<LOQ	910	<LOQ
14230	1020	2140	<LOQ
4580	<LOQ	1140	<LOQ
6570	<LOQ	2130	<LOQ
6730	<LOQ	3000	<LOQ

Kusa beach (KP)

PFOS	**PFOA**	**PFOS**	**PFOA**
4210	<LOQ	980	<LOQ
7320	<LOQ	2000	<LOQ
5670	<LOQ	1100	<LOQ
6030	<LOQ	1050	<LOQ
4960	<LOQ	1020	<LOQ

Kendu bay pier beach (KbP)

PFOS	**PFOA**	**PFOS**	**PFOA**
2310	<LOQ	1210	<LOQ
5620	<LOQ	2440	<LOQ
4590	<LOQ	1890	<LOQ
3750	<LOQ	1580	<LOQ
4500	<LOQ	2100	<LOQ

Table 19. Concentrations (ng/g) of PFOA and PFOS in sediments from different sampling locations within Lake Victoria Catchment Rivers.

Sampling site	PFOA			SD	Average	PFOS			SD	Average
Nyalenda ponds 4A	96.2	89.2	99.1	5.1	94.8	57.5	46.7	50.5	5.5	51.6
Nyalenda ponds 4B	78.9	68.8	80.6	6.4	76.1	40.8	43.8	51.7	5.6	45.4
Nyalenda ponds 1	24.8	26.2	25.8	0.7	25.6	5.3	5.9	5.2	0.4	5.5
Nyalenda ponds 2	19.3	21.3	20.8	1.0	20.5	2.6	3.6	2.9	0.5	3.1
Kasat Kotur s2	1.4	1.8	2.4	0.5	1.7	<1	<1	<1		
Kasat Kotur s1	16.7	19.8	17.7	1.6	18.1	<1	<1	<1		
Kasat Kotur	11.4	15.4	13.6	2.0	13.5	5.6	4.4	4.8	0.6	4.9
Nyalenda ponds 3B	18.3	16.8	17.3	0.8	17.5	1.2	2.1	2.6	0.7	2.0
Nyalenda ponds 3A	14.5	16.1	17.5	1.5	16.0	3.4	2.9	3.1	0.3	3.1
Nyalenda ponds 4B	18.5	20	23.5	2.6	20.6	2.6	2.8	2	0.4	2.5
Kisat sediment 2	14.8	12.5	16.4	1.9	14.6	3.9	4.3	3.2	0.6	3.8
Kodiaga prisons 1	<1	<1	<1			27.4	30.5	28.8	1.5	28.9
Saka 1	<1	<1	<1			<1	<1	<1		
saka 2	<1	<1	<1			<1	<1	<1		
Saka 3	2.8	3.2	1.4	0.9	2.5	<1	<1	<1		
Kibos bridge	2.3	3.4	2.7	0.6	2.8	<1	<1	<1		
Kibos 1	5	7.3	7.9	1.5	6.7	<1	<1	<1		
Auji 1	46	39.7	45.1	3.4	43.6	8.7	6.6	7.3	1.1	7.5
Auji 2	41	38.6	39.6	1.2	39.7	5.7	6.3	6.5	0.4	6.2
Auji 3	34.5	38.9	36.7	2.2	36.7	2.3	2.9	1.8	0.5	2.3

Table 20. Concentrations (ng/g) of PFOA and PFOS in sediments from different sampling locations within Lake Victoria gulf water.

Sampling site	PFOA			SD	Average	PFOS			SD	Average
Pipeline	3.2	2.7	3.4	0.4	3.1	<1	<1	<1		
Dock/Car wash	21.3	23.8	24.1	1.5	23.1	3.3	2.6	4	0.7	3.3
Hippo Point/Yatch Club	13.3	13.5	15.4	1.2	14.17	3.2	3	2.9	0.2	3.0
Dunga Beach	5	4.3	3.7	0.7	4.3	1.5	1.2	<1	0.2	1.4
Nyamware Beach	<1	<1	<1			<1	<1	<1		
Nduru beach	<1	<1	<1			<1	<1	<1		
Ogenya Beach	<1	<1	<1			<1	<1	<1		

Table 21. Concentrations (ng/L) of PFOA and PFOS in Nyalenda municipal waste treatment ponds water

Sample	Location	Concentration (ng/L)	
		PFOA	**PFOS**
NMWP I1	Ponds inlet	75.38	6.89
NMWP I2		63.12	10.36
NMWP I3		96.35	5.69
NMWP I4		70.02	10.26
NMWP I5		58.37	12.56
NMWP O1	Ponds	69.5	8.45
NMWP O2		65.68	9.27
NMWP O3		53.56	13.23
NMWP O4		44.89	6.45
NMWP O5		60.25	5.67

Table 22. Concentrations (ng/L) of PFOA and PFOS in Kisat River (ng/L) water

Sample	Location	Concentration (ng/L)	
		PFOA	**PFOS**
KK I	Kisat Kotur	BLQ	BLQ
KK 2		BLQ	BLQ
KK 3		BLQ	BLQ
KK 4		BLQ	BLQ
K UI	Kisat upstream	0.4	BLQ
K U2		0.73	0.4
K U3		0.83	0.4
K U4		0.63	BLQ
K M1	Kisat middlestream	0.54	BLQ
K M2		0.5	BLQ
K M3		0.52	BLQ
K M4		0.7	BLQ
K D1	Kisat downstream	0.67	BLQ
K D2		0.45	BLQ

K D3	0.56	0.45
K D4	BLQ	BLQ

Table 23. Concentrations (ng/L) of PFOA and PFOS in Nyalenda Auji River system water

Sample	Location	Concentration (ng/L)	
		PFOA	**PFOS**
NA 1	Nyalenda Auji	4.56	1.2
NA 1		4.12	1.23
NA 2		4.22	1.05
NA 3		3.9	1.72
NA 4		3.82	1.45
NA 5		3.76	1.3
NA 6		4.1	1.3
NA 7		BLQ	BLQ
NA 8		3.82	0.4

Table 24. Concentrations (ng/L) of PFOA and PFOS in Kibos River water

Sample	Location	Concentration (ng/L)	
		PFOA	**PFOS**
Kibos B1	Kibos Bridge 1B	8.11	2.45
Kibos B2		7.97	1.38
Kibos B3		8.3	1.34
Kibos B4		12.78	5.26
Kibos C1	Kibos Bridge 1C	21.99	4.02
Kibos C2		19.65	2.59
Kibos C3		32.51	8.25
Kibos C4		29.94	5.69

Table 25. Concentrations (ng/L) of PFOA and PFOS in Saka River water

Sample	Location	Concentration (ng/L)	
		PFOA	**PFOS**
Saka	Saka upstream	BLQ	BLQ
Saka i		0.78	BLQ
Saka ii		BLQ	BLQ

Saka iii		0.56	BLQ
Saka 2ai	Saka	0.78	0.38
Saka 2aii		0.78	0.42
Saka 2aiii		0.92	BLQ
Saka 2aiv		0.86	0.51
Saka 3i		0.56	0
Saka 3ii		0.45	0
Saka Di	Saka downstream	0.68	0.75
Saka Dii		BLQ	BLQ

Table 26. Concentrations (ng/L) of PFOA and PFOS in water samples from different sampling locations within Lake Victoria beaches.

Sample	**Location**	**Concentration (ng/L)**	
		PFOA	**PFOS**
LVK I	LV Kemfri	BLQ	BLQ
LVK 2		BLQ	BLQ
LVK 3		BLQ	BLQ
LVC 1	LV 2 car wash	0.78	BLQ

LVC 2		0.88	BLQ
LVC 3		0.78	BLQ
LVD 1	LV Dock	7.05	2.23
LVD 2		0.88	BLQ
LVD 3		9.52	1.25
LVD 4		0.78	BLQ
LVD 5		11.65	2.53
LVHP 1	LV Hippo Point	1.05	0.45
LVHP 2		0.82	0.65
LVHP 3		0.68	BLQ
LVHP 4		0.44	BLQ
OB1	Ombogo Beach	BLQ	BLQ
OB 2		BLQ	BLQ
OB 3		BLQ	BLQ
N 1	Nyamware	BLQ	BLQ
N 2		BLQ	BLQ
N 3		BLQ	BLQ

D 1	Dunga	0.78	BLQ
D 2		0.8	BLQ
D 3		0.95	BLQ
O 1	Otonglo	BLQ	BLQ
O 2		BLQ	BLQ
O 3		BLQ	BLQ

Table 27. Oxi-Top BOD values resulting from biodegradation of studied surfactants compounds with time in minutes, using pre-conditioned activated sludge as inoculum.

Time in Minutes	Blank	Ref. subs (NaAC)	PFBS	NOVEC ™ 4430	NOVEC ™ 4432	Fluoro-Ethylene	50 % fluoro-surfactant	$C_{11}H_{20}F_3SO_4^-$
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
1520	-2.8	25.3	-4.2	-2.8	-2.8	-4.2	-5.6	-5.6
2888	0	42.2	-1.4	4.2	4.2	-1.4	0	-1.4
4408	0	53.5	1.4	4.2	9.85	1.4	1.4	1.4
5776	-2.8	59.1	0	5.6	9.85	2.8	2.8	4.2
7296	0	61.9	0	7	11.25	2.8	2.8	8.4
8664	2.8	64.7	0	8.4	14.1	2.8	2.8	11.3
10184	2.8	61.9	5.6	12.7	16.9	4.2	5.6	14.1
11552	2.8	64.7	2.8	12.7	16.9	4.2	5.6	16.9
13072	2.8	64.7	1.4	12.7	18.3	4.2	5.6	18.3
14440	0	64.7	1.4	12.7	19.7	4.2	5.6	19.7
15960	2.8	67.5	4.2	15.5	21.1	5.6	5.6	19.7
17328	2.8	67.5	1.4	16.9	19.7	4.2	4.2	19.7
18848	2.8	64.7	2.8	15.5	22.5	5.6	5.6	21.1
20216	2.8	61.9	0	12.7	19.7	1.4	0	18.3
21736	2.8	64.7	-1.4	12.7	18.3	1.4	1.4	18.3
23104	2.8	64.7	1.4	15.5	21.1	4.2	2.8	22.5
24320	0	64.7	0	15.5	21.1	1.4	2.8	21.1
25992	2.8	67.5	0	15.5	23.9	2.8	2.8	22.5
27512	2.8	67.5	0	16.9	25.3	4.2	2.8	23.9
28880	0	64.7	2.8	19.7	26.7	4.2	4.2	25.3
30400	0	64.7	5.6	22.5	26.7	5.6	5.6	25.3

31768	0	64.7	5.6	23.9	29.55	7	7	28.1
33288	0	67.5	5.6	26.7	28.15	5.6	5.6	25.3
34656	0	67.5	4.2	25.3	29.55	7	7	25.3
36176	0	64.7	4.2	25.3	29.55	4.2	5.6	25.3
37544	0	61.9	7	28.15	32.35	7	7	26.7
39064	0	64.7	5.6	29.55	32.35	7	7	28.1
40432	0	64.7	5.6	29.55	32.35	7	7	26.7
41952	2.8	64.7	7	30.95	35.15	8.4	8.4	28.1
43320	2.8	64.7	7	32.35	35.15	9.85	9.85	28.1
44840	0	64.7	7	30.95	38	8.45	11.3	29.5
46208	0	67.5	7	30.95	39.4	9.85	12.7	30.95
47576	0	67.5	8	30	33.75	9	8	30
49096	2.8	64.7	7	33	39.4	8	7	28.3
50464	0	61.9	8	33.75	39.4	7	7	28.1
51984	0	64.7	8.45	36.55	39.4	8.45	8.4	28.1
53352	0	64.7	9.85	39.35	39.4	8.4	7	26.7
54720	0	64.7	8.4	39.35	39.4	7	7	26.7

Table 28. Degradation experiment with UV Light/ H_2O_2.

Compound: PFBS Concentration : 100 mg/L
Vials 1 to 7 - 20 mL of PFBS Solution
Only UV -Lampe
Total time : 2 hours

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
	0	0.01	0.8	0.01
1	20	0.05	0.8	0.06
2	40	0.07	0.6	0.12
3	60	0.11	0.6	0.18
4	80	0.17	0.4	0.43
5	100	0.18	0.4	0.45
6	120	0.11	0.2	0.55
7	140	0.11	0.2	0.55

Vials 1 to 6 - PFBS 25ml/L
Vials 7 to 12 - PFBS 50ml/L

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
1	20	0.04	0.2	0.20
2	20	0.04	0.2	0.20
3	40	0.07	0.2	0.35
4	60	0.07	0.2	0.35
5	80	0.1	0.2	0.50
6	100	0.15	0.2	0.75
7	120	0.19	0.2	0.95

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration

8	20	0.06	0.4	0.15
9	40	0.14	0.4	0.35
10	60	0.2	0.4	0.50
11	80	0.24	0.4	0.60
12	100	0.3	0.4	0.75
	120	0.45	0.4	1.13

Vials 1 to 6 - 20 mL of PFBS Solution + 100 µL H_2O_2. UV -Lampe + H_2O_2
Vials 7 to 12 - PFBS 50ml/L + 100 µL H_2O_2
Total time : 2 hours

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
1	20	0.03	0.8	0.04
2	40	-0.02	0.8	-0.03
3	60	-0.04	0.8	-0.05
4	80	0.1	0.8	0.13
5	100	0.17	0.8	0.21
6	120	0.15	1	0.15

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
7	20	0.03	0.4	0.08
8	40	-0.03	0.4	-0.08
9	60	-0.05	0.4	-0.13
10	80	0.07	0.4	0.18
11	100	0.07	0.4	0.18
12	120	0.11	0.4	0.28

Vials 1 to 6 - PFBS 25ml/L + 100 µL H_2O_2

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
1	20	0.01	0.2	0.05
2	40	-0.04	0.2	-0.20
3	60	-0.08	0.2	-0.40
4	80	-0.04	0.2	-0.20
5	100	-0.08	0.2	-0.40
6	120	-0.02	0.2	-0.10

Compound: Novec ^{TM} 4430 Concentration : 100 mg/L
Vials 1 to 6 - 20 mL of Novec Solution
Only UV -Lampe
Total time : 2 hours

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
	0	0.02	0.8	0.03
1	20	2.32	0.8	2.90
2	40	2.69	0.8	3.36
3	60	2.86	0.8	3.58
4	80	2.95	0.8	3.69
5	100	2.98	0.8	3.73
6	120	3.15	0.8	3.94

Vials 7 to 12 - Novec 50ml/L

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
	0	0.02	0.8	0.03
7	20	1.04	0.4	2.60
8	40	1.14	0.4	2.85
9	60	1.14	0.4	2.85
10	80	1.33	0.4	3.33
11	100	1.22	0.4	3.05
12	120	1.54	0.4	3.85

Vials 1 to 6 25ml/L

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
	0	0.02	0.8	0.03
1	20	0.79	0.2	3.95
2	40	0.67	0.2	3.35
3	60	0.73	0.2	3.65
4	80	0.81	0.2	4.05
5	100	0.88	0.2	4.40
6	120	0.9	0.2	4.50

Vials 7 to 12 - 20 mL of Novec Solution + H_2O_2. UV -Lampe + H_2O_2

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
	0	0.02	0.8	0.03
7	20	2.71	0.8	3.39
8	40	3.82	0.8	4.78
9	60	4.34	0.8	5.43
10	80	4.62	0.8	5.78
11	100	4.66	0.8	5.83
12	120	4.99	0.8	6.24

Vials 1 to 6 - Novec 50ml/L + H_2O_2

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
	0	0.02	0.8	0.03
1	20	1.34	0.4	3.35
2	40	2.07	0.4	5.18
3	60	2.13	0.4	5.33
4	80	2.08	0.4	5.20
5	100	2.27	0.4	5.68
6	120	2.4	0.4	6.00

Vials 7 to 12 25ml/L + H_2O_2

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
	0	0.02	0.8	0.03
7	20	0.81	0.2	4.05

8	40	1.05	0.2	5.25
9	60	0.98	0.2	4.90
10	80	1	0.2	5.00
11	100	1.11	0.2	5.55
12	120	1.14	0.2	5.70

Compound: Novec ^{TM} 4432 Concentration : 100 mg/L
Vials 1 to 6 - 20 mL of Novec Solution
Only UV -Lampe
Total time : 2 hours

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
	0	0.02	0.8	0.03
1	20	3.67	0.8	4.59
2	40	4.21	0.8	5.26
3	60	4.33	0.8	5.41
4	80	4.57	0.8	5.71
5	100	4.89	0.8	6.11
6	120	4.92	0.8	6.15

Vials 7 to 12 - Novec 50ml/L

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
	0	0.02	0.8	0.03
7	20	1.77	0.4	4.43
8	40	2.06	0.4	5.15
9	60	2.44	0.4	6.10
10	80	2.48	0.4	6.20
11	100	2.48	0.4	6.20
12	120	2.71	0.4	6.78

Vials 1 to 6 - 20 mL of Novec Solution + H_2O_2. UV -Lampe + H_2O_2

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
	0	0.02	0.8	0.03
1	20	3.75	0.8	4.69
2	40	5.03	0.8	6.29
3	60	5.63	0.8	7.04
4	80	5.85	0.8	7.31
5	100	6.21	0.8	7.76
6	120	6.57	0.8	8.21

Vials 7 to 12 - Novec 50ml/L + H_2O_2

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
	0	0.02	0.8	0.03
7	20	2.07	0.4	5.18
8	40	2.98	0.4	7.45
9	60	3.23	0.4	8.08
10	80	3.46	0.4	8.65

11	100	3.58	0.4	8.95
12	120	4.1	0.4	10.25

Compound:PFBS Concentration : 100 mg/L
PFBS *sauer* mit 1mL of *Salpetersäure* 65 % in 1L solution
Vials 1 to 6 - 20 mL of PFBS Acid Solution
Only UV -Lampe
Total time : 2 hours

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
	0		0.8	0.00
1	20	0.02	0.8	0.03
2	40	0.02	0.8	0.03
3	60	0.02	0.8	0.03
4	80	0.03	0.8	0.04
5	100	0.02	0.8	0.03
6	120	0.03	0.8	0.04

Vials 7 to 12 - PFBS Acid 50ml/L

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
	0		0.8	0.00
7	20	0.03	0.4	0.08
8	40	0.03	0.4	0.08
9	60	0.03	0.4	0.08
10	80	0.03	0.4	0.08
11	100	0.04	0.4	0.10
12	120	0.03	0.4	0.08

Vials 1 to 6 - 20 mL of PFBS Acid Solution + H_2O_2. UV -Lampe + H_2O_2

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
	0		0.8	0.00
1	20	-0.01	0.8	-0.01
2	40	-0.01	0.8	-0.01
3	60	-0.01	0.8	-0.01
4	80	-0.05	0.8	-0.06
5	100	-0.03	0.8	-0.04
6	120	-0.03	0.8	-0.04

Vials 7 to 12 - PFBS Acid 50ml/L + H_2O_2

Vials	Time (min)	Concentration F- (mg/L)	Coef. Dilution	Real Concentration
	0		0.8	0.00
7	20	0	0.4	0.00
8	40	0.01	0.4	0.03
9	60	0	0.4	0.00
10	80	-0.05	0.4	-0.13
11	100	-0.01	0.4	-0.03
12	120	-0.03	0.4	-0.08

Table 29. Degradation of trifluoromethoxy alkly sulfonate at different concentrations when submitted to UV radiation and UV/ H_2O_2 System, followed by the TOC analysis.

		Without H_2O_2	Without H_2O_2	With H_2O_2	With H_2O_2
Analyte	**Time in minutes**	**50ml/L**	**25ml/L**	**50ml/L**	**25ml/L**
F-	20	11	14.9	22.5	22.5
F-	40	13.8	17.4	22.4	22.3
F-	60	14.9	19.5	22.3	22.3
F-	80	16.9	20.5	22.1	22.1
F-	100	18.4	21	22.2	22.2
F-	120	18.9	21.6	21.9	22
TOC	20	14.9	13.7	1.2	1.8
TOC	40	10.8	11.2	1	2
TOC	60	10.2	7.4	1.7	1.3
TOC	80	8.5	6.5	1.3	1.3
TOC	100	6.8	3.6	1.1	1.6
TOC	120	6.5	1.1	0.9	1.1

Table 30. Degradation of fluorosurfactant Zonyl at different concentrations when submitted to UV radiation and UV/ H_2O_2 system, followed by the TOC analysis.

		Without H_2O_2	Without H_2O_2	With H_2O_2	With H_2O_2
Analyte	**Time in minutes**	**50ml/L**	**25ml/L**	**50ml/L**	**25ml/L**
F-	20	1.15	1.45	11.2	11.7
F-	40	8.45	8.9	15.3	15
F-	60	13.1	15.6	17.3	16.9
F-	80	16.4	16.3	18.4	17.4
F-	100	16.7	16.8	18.6	17
F-	120	18.3	18.6	19.1	16.8
TOC	20	22.6	10.6	4.5	2.3
TOC	40	23	9.9	2.8	2.2
TOC	60	21.6	7.3	2.5	1.7
TOC	80	15.5	5.1	1.7	1.5
TOC	100	11.9	3.4	2.9	0.9
TOC	120	9	1.9	1.6	0.3

Table 31. Degradation OECD 301 D - 10-(trifluoromethoxy) decane-sulfonate in closed sample bottle.

	VT	Volume 1	Volume 2	OD (mg/L)
Merck 0	100	4	0.3	4.30
	100	3.85	0.8	4.65
Merck 1	100	3.7	0.3	4.00
	100	3.98	0.3	4.28
Merck 2	100	3.5	0.5	4.00
	100	3.3	0.5	3.80
Merck 3	100	3.6	0.6	4.20
	100	3.6	0.5	4.10
Merck 4	100	3.7	0.6	4.30
	100	3.4	0.5	3.90
Merck 5	100	3.2	0.7	3.90
	100	3.2	0.7	3.90
Merck 6	100	3.2	0.6	3.80
	100	3.3	0.55	3.85

Table 32. Degradation closed bottle OECD 301 D - Novec ™ FC4430 and FC4432

Days	(mL)	VT	Volume 1	Volume 2	OD (mg/L)		VT	Volume 1	Volume 2	OD (mg/L)
19-Mar	Blank 0	100	3.8	0.75	4.55	4430 0	100	3.85	0.7	4.55
		100	3.8	0.6	4.40		100	3.95	0.6	4.55
25-Mar	Blank 1	100	3.85	0.95	4.80	4430 1	100	3.3	1.1	4.40
		100	3.7	1.3	5.00		100	3.3	1.3	4.60
28-Mar	Blank 2	100	3.5	1	4.50	4430 2	100	2.8	0.8	3.60
		100	3.6	0.8	4.40		100	2.8	0.7	3.50
02-Apr	Blank 3	100	3.3	0.5	3.80	4430 3	100	3.2	0.4	3.60
		100	3.4	0.6	4.00		100	3.1	0.5	3.60
08-Apr	Blank 4	100	3.5	0.7	4.20	4430 4	100	2.4	0.8	3.20
		100	3.7	0.4	4.10		100	2.5	0.5	3.00
15-Apr	Blank 5	100	2.9	0.7	3.60	4430 5	100	2.2	0.4	2.60
		100	3.7	0.5	4.20		100	2.3	0.5	2.80
18-Apr	Blank 6	100			0.00	4430 6	100	4.1	0.6	4.70
		100			0.00		100	3.5	4.00	
25-Mar	Blank 1	200	7.8	1.55	9.35	4432 0	200	8.1	0.7	8.80
		95	3.45	0.8	8.95		85	2.9	0.35	7.65
28-Mar	Blank 2	200	4.5	0.85	5.35	4432 1	200	1.75	1.2	2.95
		100	2.05	0.45	5.00		95	0.9	0.3	2.53
02-Apr	Blank 3	200	5.05	1	6.05	4432 2	200	2.5	0.65	3.15
		95	2.4	0.4	5.89		95	1.1	0.25	2.84
08-Apr	Blank 4	200	5	1.2	6.20	4432 3	200	2.25	0.5	2.75
		104	2.65	0.65	6.35		92	1.05	0.2	2.72
15-Apr	Blank 5	200	4.5	0.85	5.35	4432 4	200	1.6	0.55	2.15
		100	2.4	0.5	5.80		100	0.85	0.3	2.30
18-Apr	Blank 6	200	3.9	0.85	4.75	4432 5	200	1	0.2	1.20
		100	1.9	0.4	4.60		100	0.6	0.05	1.30

Table 33. TOC - Total Organic Carbon using Elementar High TOC Instrument.

	TIC	Sda	SDr	NPOC	Sda	SDr	TC	Sda	SDr	Mean (TOC)
Novec ™ FC4430	0.318			44.72			45.038			45 mg/L
	0.312			45.306			45.618			
Mean	0.315	0.005	1.438	45.013	0.414	0.92	45.328	0.41	0.904	
Novec ™ FC4432	0.21			44.733			44.942			44,5 mg/L
	0.201			44.253			44.453			
	0.211			44.368			44.579			
Mean	0.207	0.006	2.791	44.451	0.25	0.563	44.658	0.255	0.571	
PFBS	0.301			16.069			16.37			15,5 mg/L
	0.307			15.321			15.629			
	0.302			15.005			15.307			
Mean	0.304	0.003	1.062	15.465	0.546	3.532	15.769	0.545	3.457	

TOC = total organically bound carbon, NPOC = non purgeable organic carbon, TC = total carbon, TIC= total inorganically bound carbon

Table 34. Recoveries: Fish and Water samples using GC - MS and LC - MS.

			GC	GC	LC	LC	LC
Sample	**Initial**	**Added ug/L**	**Recovered ug/L**	**Recovery %**	**Initial**	**Recovered**	**Recovery %**
Nyalenda WSP 1	Below LOQ	1.0	1.01 ±0.15	101.1	0.017 ug/L	0.98 ug/L n=2	96
Nyalenda Auji 3	Below LOQ	0.50	0.44 ±0.04	88.0	1000.4 ng/L	950.3 ng/L	94.9
Liv DP36	Below LOQ	1.0	1.05 ±0.12	104.6	ND	ND	ND
MusTP 28	Below LOQ	0.50	0.47 ±0.07	94.4	ND	ND	ND
MusTP 28	Below LOQ	0.25	0.21 ±0.03	87.8	ND	ND	ND

Table 35. Reproducibility from GC method

Date	Sample	Peak Area percentage ratio
19.07.2007	LivDP31	1.04
20.07.2007	LivDP31	0.88
23.07.2006	LivDP31	1.01

Table 36. Monitored Levels of PFOS in aquatic biota (data from selected studies, based on OECD, 2002).

Researcher	Organism, matrix and concentration	Study area
Global monitoring survey of marine mammals (Florida, California, Alaska, northern Baltic Sea, Mediterranean Sea, Arctic, Sable Island (Canada)	Bottlenose dolphin (liver, n = 26): Max: 1520 ng/g wet wt. Mean: 420 ng/g wet wt.	Florida
	Ringed seal (liver, n = 81): Max: 1100 ng/g wet wt. Mean: 240 ng/g wet wt.	Northern Baltic Sea
Survey of fish (US, Europe, North Pacific Ocean, Antarctic)	Fish (muscle, n = 172): Max: 923 ng/g wet wt. Mean. 40 ng/g wet wt.	Belgian estuary
	Carp (muscle, n = 10): Max: 296 ng/g wet wt. Mean: 120 ng/g wet wt.	US Great Lakes
Survey of oysters in the US (Chesapeake Bay & Gulf of Mexico)	Oyster (Whole body, n =77) Max: 100 ng/g wet wt. Mean: 60 ng/g wet wt.	US
Fish samples upstream and downstream of 3M facility in Decatur, Alabama, US	Fish (whole body): Mean (upstream): 59.1 µg/kg wet wt. Mean (downstream): 1,332 µg/kg wet wt.	Decatur, US
Swedish urban and background fish samples	Perch: 3 - 8 ng/g (urban sites in the vicinity of municipal STPs); 20-44 ng/g in Lake Mälaren and near Stockholm	Sweden (Lake Mälaren)

Table 37. Results from Interlaboratory Comparison (AQM).

Sample	Analysis centre		
	IWW (HP)	**Uni Mainz (Saturn)**	**Uni Mainz (Agilent)**
Nyalenda WSP 1	15 ng/L	16.42 ±0.64 ng/L (n = 4)	17 ng/L
Nyalenda Auji 3	3.5 ng/L	3.85 ±0.44 ng/L (n = 4)	4.22 ng/L
Liver sample	9.87 ng/g	8.45 ± 2.10 ng/g (n = 3)	9.5 ng/g
Muscle sample	< 1 ng/g	0.83 ng/g	0.75 ng/g

NA: Not analysed, IWW Rheinisch-Westfaelisches Institut für Wasser Moritzstr. Precision 6.38 % to 10.97 % from interlaboratory comparison

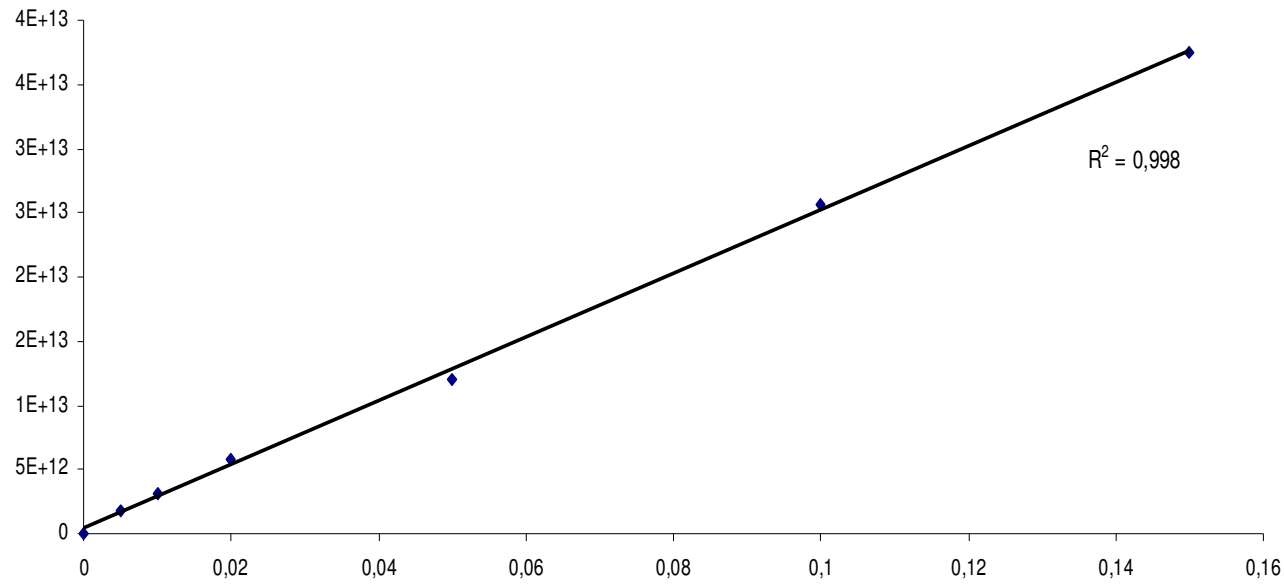
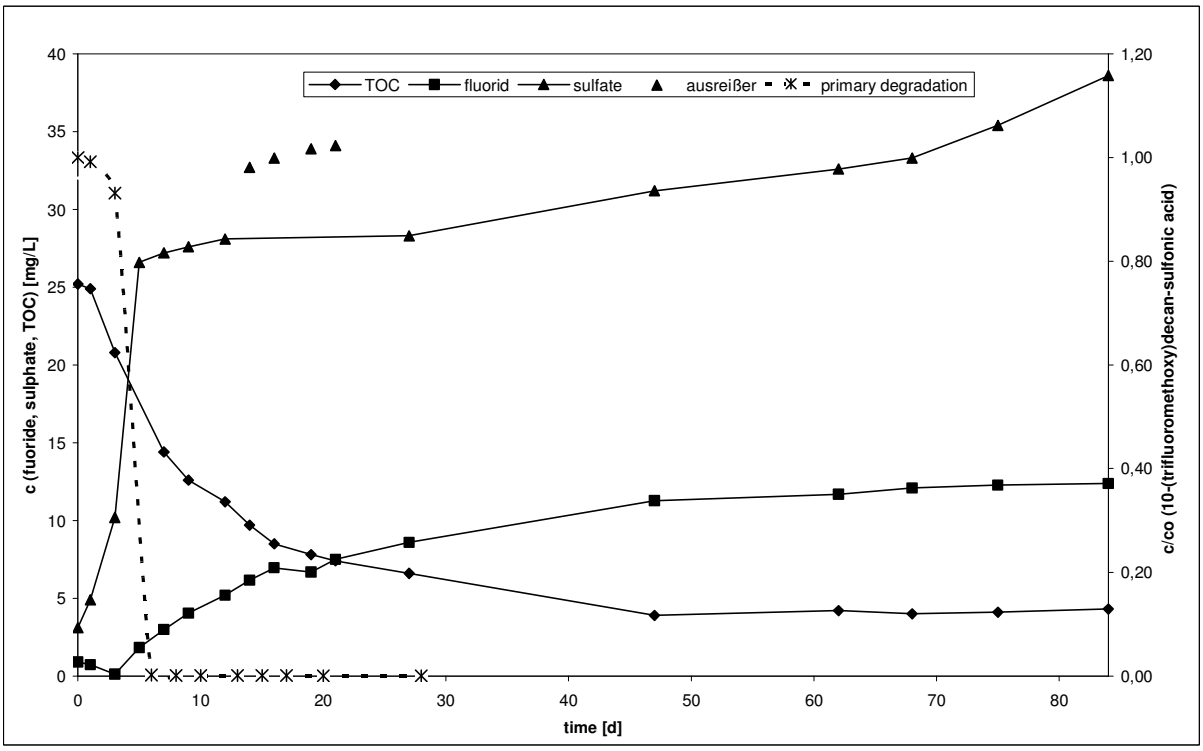


Figure 33. Calibration curves used in water instrumental analysis.



Adapted from Peschka et al. (2008b)

Figure 34. TOC, generation of F- and SO_4^{2-} in an FBBR and the primary degradation of 10-(trifluoromethoxy)decan-sulfonate spiked at a concentration of 100 mg/L in surface water

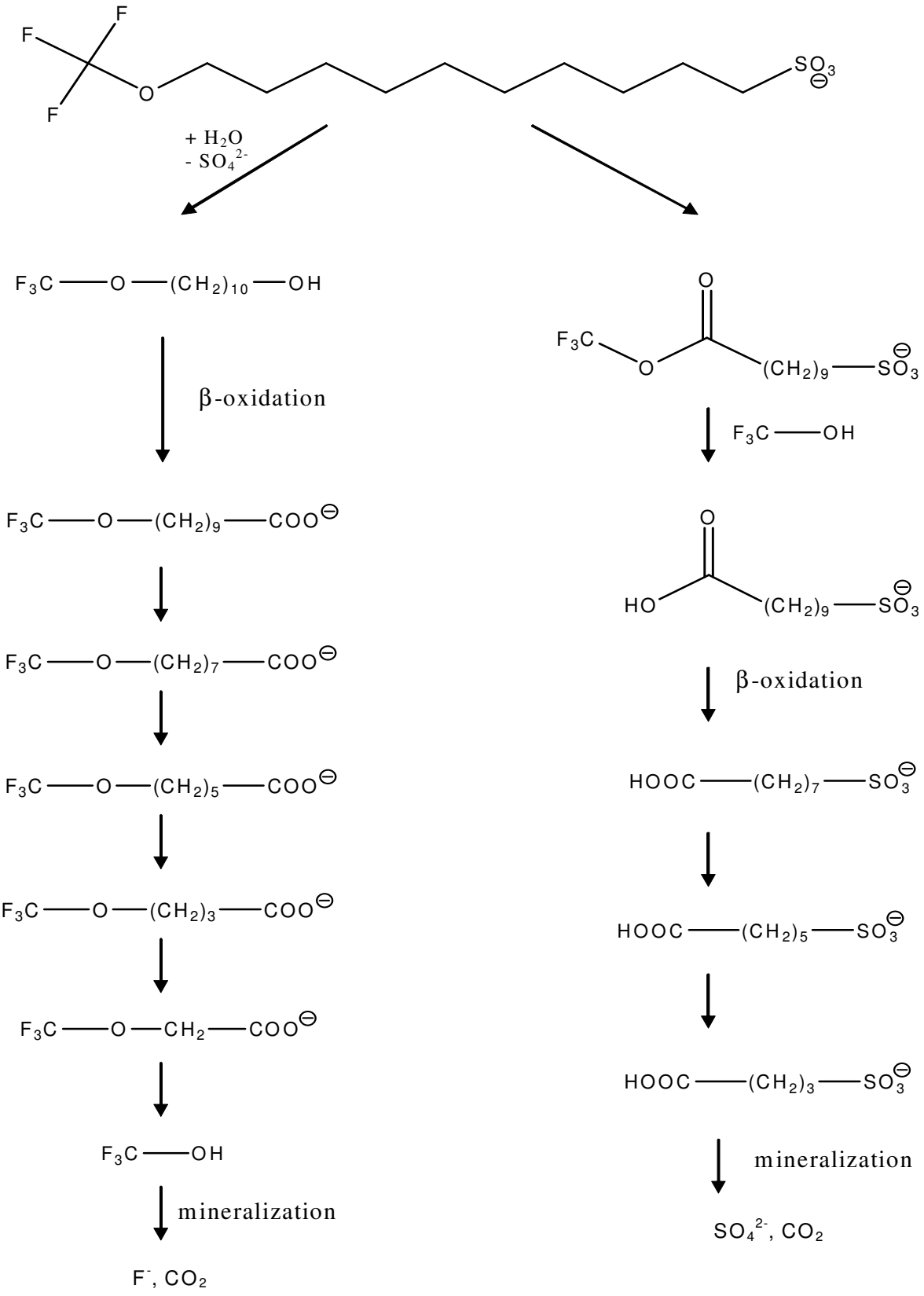
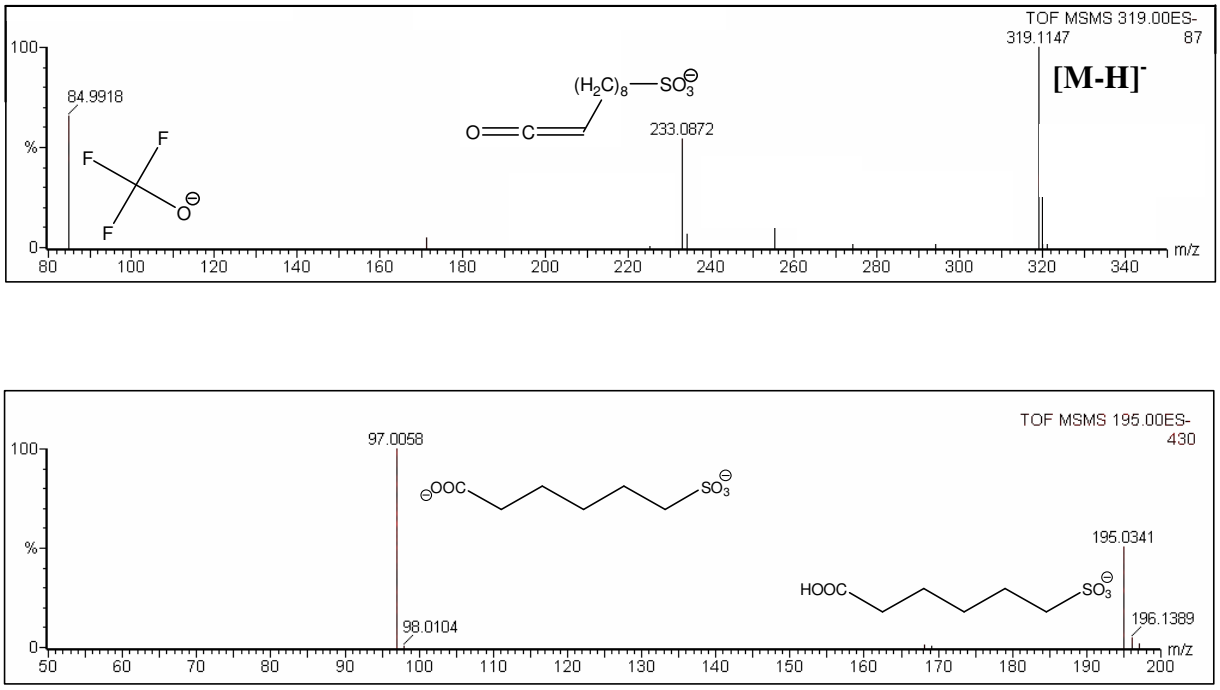


Figure 35. Degradation pathway of 10-(trifluoromethoxy) decane-sulfonate. Adopted from Peschka et al (2008b).



Adapted from Peschka et al. (2008b)

Figure 36. Mass spectrum of 10-(trifluoromethoxy) decane-sulfonate. Showing mass formular ($C_{11}H_{20}F_3SO_4$- 305); metabolites ($C_{11}H_{18}F_3SO_5$ 319; $C_6H_{11}SO_5^-$ 195; CF_3O^- 85) and mass of fragments ($C_{10}H_{17}SO_4^-$ 233).

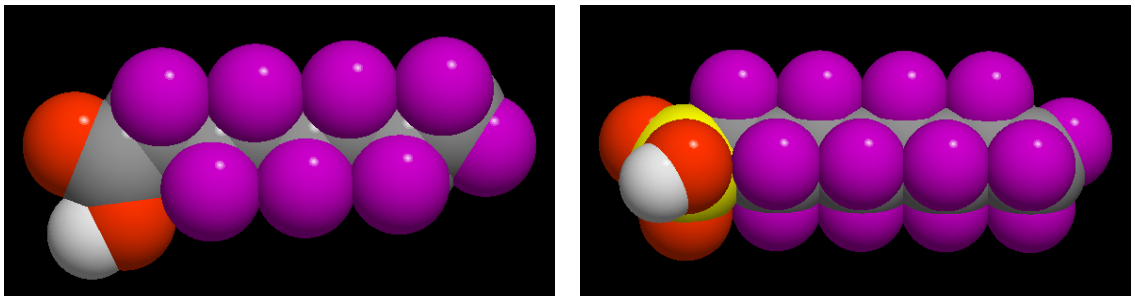


Figure 37. Structures of perfluorooctanoic acid and perfluorooctane sulfonate.

Figure 38. Section of the inlet waste water flow into Nyalenda waste water treatment ponds.

Figure 39. One of the waste water treatment ponds at Nyalenda along the Kibos-Nyalenda-Auji river system. The picture shows water hyacinth (Eichhornia crassipes*)* infestetion in the ponds mainly due to nutrients inloads.

Figure 40. Sections of Auji river meanders into Lake Victoria (above) and the outlet from Nyalenda ponds into Auji River (below).

Figure 41. Nile Tilapia (*Oreochromis niloticus*) at Dunga sampling location (above) and Nile Perch (*Lates niloticus*) at Ndura beach sampling location (below).

Figure 42. Waste water outlet to winam gulf of Lake Victoria from a factory along Saka River

Figure 43. Sampling location along Winam Gulf of Lake Victoria shore at Hippo point/Yatch club (above) and showing Pipeline (below).