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PASSIVE SAMPLING IN MONITORING OF NONYLPHENOL ETHOXYLATES AND NONYLPHENOL IN AQUATIC ENVIRONMENTS

BY

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Doctor of Philosophy

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ABSTRACT

The present practices for determining the concentration levels of various pollutants are in many respects insufficient and for this reason, there is an urgent need especially to develop more cost-effective sampling methods. In this study, a novel passive sampling tool (the Chemcatcher®) for monitoring nonylphenol ethoxylates (NPEOs) and nonylphenol (NP) in aqueous media was tested. These environmentally harmful substances have been widely used in different household and industrial applications and they affect aquatic ecosystems, for example, by acting as endocrine disrupting compounds.

The highest accumulation of NPEOs and NP in laboratory-scale tests was obtained when using an SDB-XC (standard styrene-divinyl benzene) Empore disk as a receiving phase (adsorbent) of the passive sampler. The accumulation of these compounds was then field tested by this technique for two or four weeks at two sampling sites which had received effluents from *e.g.* the pulp and paper industry for decades. In addition, the samplers were exposed in seawater conditions, although in these cases the results were, mainly due to a too long sampling time period, only approximate.

In all cases, NPEOs and NP were analysed by high-performance liquid chromatography coupled with an electrospray ionisation mass spectrometry (HPLC/ESI-MS). These compounds were also separated from water samples using solid phase extraction (SPE) pretreatment which showed to be a useful tool for this purpose. It could be concluded that passive sampling with Chemcatcher® offers an effective technique suitable for monitoring NPEOs and NP in watercourses. However, more accurate data (*e.g.*, obtained by LC/MS-MS) on various contaminants are still needed for further method development.

Keywords: alkylphenol ethoxylates, Chemcatcher®, passive sampling, high-performance liquid chromatography, non-ionic surfactants, nonylphenol, nonylphenol ethoxylates, solid phase extraction

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PREFACE

This research was carried out in cooperation between the University of Jyväskylä, Laboratory of Applied Chemistry, and the Finnish Environment Institute during the years 2006-2011.

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CONTENTS

ABBREVIATIONS

ACN	Acetonitrile
AEs	Alkyl ethoxylate
AO	Alcohol ethoxylate
AP	Alkylphenol
APCI	Atmospheric pressure chemical ionisation
APEC	Alkylphenoxy carboxylate
APEO	Alkylphenol ethoxylate
ASE	Accelerated solvent extraction
BDE-153	Hexabrominated diphenyl ether
BDE-47	Tetrabrominated diphenyl ether
BSI	British Standards Institution
C-18	Octadecyl silica phase; Empore disk with octadecyl silica adsorbent
CNPEC	Nonylphenol dicarboxylate
$C_{10}EO_6$	Decanol polyethoxylate
DCM	Dichloromethane
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EC	European Commission
ECD	Electron capture detector
EI	Electron ionization detector
ELY	Centres for Economic Development, Transport and the Environment
ESI	Electrospray ionisation
FIA	Flow injection analysis
FID	Flame ionisation detector
GC	Gas chromatography
GCB	Graphitised carbon black
HFA	Hexafluoroacetone
HPLC	High-performance liquid chromatography
ISO	International Organization for Standardization
LC	Liquid chromatography
LDPE	Low-density polyethylene
LLE	Liquid-liquid extraction
LOD	Limit of detection
$LogK_{OW}$	Log octanol/water partition coefficient
LOQ	Limit of quantification
MESCO	Membrane-enclosed sorptive coating sampler
MS	Mass spectrometry

MSD	Mass spectrometric detection, mass-selective detection, mass selective detector
MS-MS	Tandem mass spectrometry
MSPD	Matrix solid-phase dispersion
MTBE	Methyl *tert*-butyl ether
m/z	Mass-to-charge ratio
NaAc	Sodium acetate
NH_4Ac	Ammonium acetate
NP	Nonylphenol
NPEC	Nonylphenol carboxylate
$NPEC_2$	Nonylphenol ethoxy acetic acid
NPEO	Nonylphenol ethoxylate
$NPEO_{1-3}$	Mixture of nonylphenol mono-, di and triethoxylate
$NPEO_n$	Nonylphenol ethoxylate mixture, the average length of ethoxylate chain is n units
NP-HPLC	Normal-phase HPLC
OP	Octylphenol
OPEC	Octylphenol carboxylate
OPEO	Octylphenol ethoxylate
p.a.	pro analysis
PAH	Polyaromatic hydrocarbon
PCB	Polychlorinated biphenyl
PDMS	Poly(dimethylsiloxane)
PE	Polyethylene
PEG	Polyethylene glycol
PES	Polyethersulphone
PFB-Br	Pentafluorobenzyl bromide
PFOA	Perfluorooctanoate
PFOS	Perfluorooctanesulphonate
POCIS	Polar organic chemical integrative sampler
PRC	Performance reference compound
PS	Polysulphone
PTFE	Poly(tetrafluoroethylene)
REC	Regional Environment Centre
RP-HPLC	Reversed-phase HPLC
SBSE	Stir bar sorptive extraction
SDB-RPS	Styrene-divinylbenzene Empore disk with sulphonic acid functionality
SDB-XC	Standard styrene-divinylbenzene Empore disk
SEC	Size-exclusion chromatography
SIM	Selected ion monitoring
SPE	Solid phase extraction
SPMD	Semipermeable membrane device

STAMPS	Standardized aquatic monitoring of priority pollutants by passive sampling
TMAOH	Tetramethylammonium hydroxide
TWA	Time weighted average
UBL	Unstirred boundary layer
UHQ	Ultra high quality
UV	Ultraviolet
WFD	Water framework directive

1 INTRODUCTION

The European Union Water Framework Directive (2000/60/EC) (WFD) is an important piece of environmental legislation that protects rivers, lakes, coastal waters and groundwaters [EC, 2000]. Its objective is that by 2015 all the European surface waters have a 'good status', which means both 'good ecological status' and 'good chemical status'. The WFD classification for water quality consists of five status categories: high, good, moderate, poor and bad. The implementation of WFD requires extensive monitoring of the concentration levels of priority substances in waters, which would be time consuming using traditional sampling methods. The list of the priority pollutants includes 33 substances or substance groups, which have been indicated to be of major concern in European waters. Nonyl phenol ethoxylates (NPEOs) are harmful substances at a national level in Finland and nonylphenol (NP) is one of the priority pollutants at the community level.

The only legally accepted sampling method for monitoring levels of pollutants in water is spot sampling, in which the water is taken in bottles and the chemicals of concern are analysed [Allan *et al.*, 2006]. This method gives only a snapshot of the concentration levels at the time of sampling and, since the levels of many pollutants can fluctuate over a tidal cycle or with times of sporadic discharges of industrial or domestic effluents, spot samples can give misleading information on water quality. The implementation of the WFD requires the establishment and use of novel and low-cost monitoring programmes, which apply to all member states. Several methods have been developed to make the sampling process more representative compared to spot sampling, *e.g.*, on-line continuous monitoring, biomonitoring or passive sampling [Södergren, 1987; Huckins *et al.*, 1993; Koester *et al.*, 2003; Vrana *et al.*, 2006a].

National water quality monitoring in Finland is organised by the environmental authorities [Niemi and Heinonen, 2003]. It started in 1962 for river waters, in 1965 for lake depths and in 1963 for biological monitoring. The water samples are taken regularly at the same sampling sites to analyse physical and chemical water quality determinands using standardised methods. The local pollution control monitoring based on environmental legislation introduced the 'polluter pays' principle, which still applies. The monitoring plan is tailored for each polluter, such as fish farms, landfills and waste water treatment plants, and is accepted by the environmental authorities. The intensity of the sampling plan depends on the quality and quantity of waste water and the state of the receiving waters. The regional water quality monitoring, which completes national and local pollution control, was organised by the Regional Environment Centres (RECs). Since 2010, after the

closure of RECs, these tasks have become the responsibility of the Centres for Economic Development, Transport and the Environment (ELY).

The monitoring program of harmful substances in Finnish inland waters includes heavy metals and organochlorine compounds such as polychlorinated biphenyls as well as dioxins and furanes [Niemi and Heinonen, 2003]. The accumulation of these compounds is studied in fish and mussels. In addition, passive sampling methods have been developed alongside traditional procedures. The EU-funded project called STAMPS (Standardized Aquatic Monitoring of Priority Pollutants by Passive Sampling) produced a passive sampler, named the Chemcatcher®, which aims to be a formal standard in Europe [STAMPS, 2011]. The project was coordinated by the University of Portsmouth, and the Central Finland Regional Environment Centre (Research Professor S. Herve) was one of the partners. British standard BSI PAS 61:2006 [2006] and ISO standard 5667-23 [2011] concerning the method have been published.

Objectives of the study

The general objective of this work was to study the suitability of the Chemcatcher® passive sampler in the monitoring of NPEOs and NP in aquatic environments. The main purpose of this study was to focus on NPEOs instead of NP, since there already exist many research reports concerning NP. More precisely, the aims were as follows:

- Producing a novel SPE method for concentrating NPEOs and NP from water samples.
- Developing a HPLC/ESI-MS analysis method for determining NPEOs and NP.
- Developing a Chemcatcher® configuration suitable for monitoring NPEOs and NP in aquatic environments.
- Optimising the extraction procedure of NPEOs and NP from Empore disks used as the receiving phase of the sampler.
- Studying the accumulation of NPEOs and NP in different types of receiving phases with laboratory trials. The aim was also to test the effect of a diffusion-limiting membrane on top of the receiving phase.
- Preliminary testing of Chemcatchers® in inland and marine waters and the influence of sampling time. The aim was also to monitor the difference between exposure at heavily and less polluted sites.

2 PASSIVE SAMPLING

Several passive sampling techniques have been developed for monitoring harmful substances in aqueous media [Namieśnik *et al.*, 2005; Vrana *et al.*, 2005a; Seethapathy *et al.*, 2008]. According to various studies, the most time-consuming step in the analysis is sampling and sample preparation. Low aqueous concentrations of harmful compounds require a large sample volume, resulting in cumbersome sample handling. If the concentration of the contaminant varies over time, the resolution can be increased by taking multiple water samples [Stuer-Lauridsen, 2005]. This pseudo time-integrated sampling is both costly and laborious and is very seldom if at all used in large scale monitoring. The usual method for assessing long-term waterborne contamination is to take samples from sediment or biota. It is, however, difficult to estimate the effect of degradation and elimination rates, or biotransformation and resuspension of the contaminants. The passive sampling method combines sampling and the enrichment of the compounds of concern. Samplers measure only the freely dissolved fraction of the analyte, and therefore, conventional spot sampling may give higher concentrations [Kingston *et al.*, 2000; Aguilar-Martínez *et al.*, 2009]. It is evident that passive sampling can be used in highly polluted areas where biomonitoring using organisms would not be possible. The quantification of the compounds depends on the sampler type as well as the compounds studied. In general, after extracting the receiving phase (adsorbent), the further sample handling can be carried out similar to conventional spot sampling. Passive sampling is an effective tool which offers an assessment of the exposure of aquatic organisms to waterborne substances [Vrana *et al.*, 2005a; Kot-Wasik *et al.*, 2007].

2.1 Theory

All passive sampling techniques include a receiving phase, which can be a solvent, a chemical reagent or a porous adsorbent [Namieśnik *et al.*, 2005; Vrana *et al.*, 2005a]. Passive sampling can be defined as a sampling method based on the free flow of analyte molecules from the sampling media to a receiving phase of a sampling device that results from the difference between the chemical potentials of these analytes in the two media [Górecki and Namieśnik, 2002; Vrana *et al.*, 2005a]. In principle, the adsorption or absorption of pollutants in the passive sampler can be viewed as presented in Figure 1. The net flow of the analyte molecules continues until the system reaches equilibrium or until the sampling is stopped. The collected amount of analyte remains constant after reaching an equilibrium, assuming that the analyte concentration in the water does not fluctuate significantly. The analyte concentration in water can be determined from the ratio of its distribution between both phases, or from a calibration of the passive sampling device. The mass of pollutant accumulated

onto the receiving phase of a passive sampling device reflects either its equilibrium concentration or time-averaged concentration, depending on the sampler design.

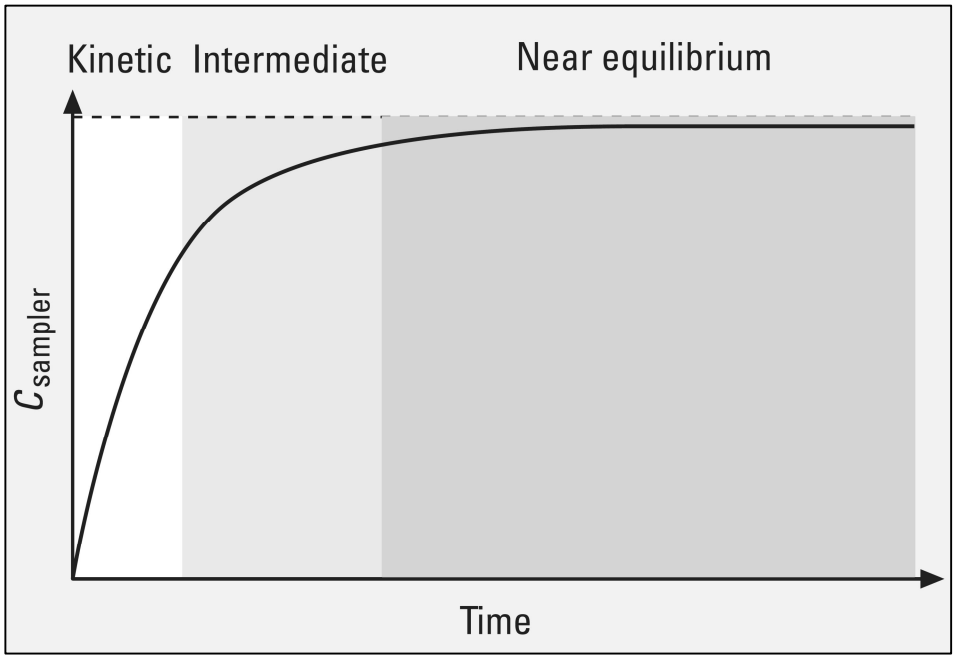

Figure 1 The passive sampler operates in three regimes: kinetic (white), intermediate (light grey), and near equilibrium (grey) [Mayer et al., 2003].

Mayer *et al.* [2003] described the exchange kinetics between the receiving phase and water by a first-order one-compartment model

$$C_S(t) = C_W \frac{k_1}{k_2}(1 - e^{-k_2 t}), \qquad (1)$$

where $C_S(t)$ is the analyte concentration in the passive sampler after an exposure time t and C_W is the analyte concentration in the aqueous environment. The uptake rate constant k_1 describes the accumulation of pollutant from aquatic phase to the receiving phase, whereas the offload rate constant k_2 stands for the offload of the contaminant from the receiving phase back to the aquatic environment. In equilibrium sampling the deployment time is long enough for the sampler to reach an equilibrium between the receiving phase and the water [Vrana *et al.*, 2005a]. Thus, the equation (1) reduces to

$$C_S(t) = C_W \frac{k_1}{k_2} = C_W K, \qquad (2)$$

where K can be defined as the phase-water partition coefficient (constant) for absorptive phases. For adsorbents K represents the distribution ratio, which depend on the concentration. In equilibrium sampling, the device senses a

volume that contains much more contaminant than the receiving phase [Mayer *et al.*, 2003]. Fast samplers have a high surface-area-to-volume ratio (A/V). If the diffusion through the unstirred boundary layer (UBL) appears to be the rate-limiting step, the uptake rate constant depends strongly on the sampling conditions. Turbulence reduces the thickness of the UBL, inducing a faster sampling rate.

In kinetic sampling, the rate of accumulation onto the receiving phase is assumed to be linearly proportional to the difference in the chemical activities of the contaminant in these two phases [Vrana *et al.*, 2005a]. At the beginning of the exposure, the desorption rate of the analyte from the receiving phase back to water is negligible, and the equation (1) reduces to

$$C_S(t) = C_W k_1 t \qquad (3)$$

and it can be further rearranged to

$$M_S(t) = C_W R_S t\,, \qquad (4)$$

where M_S (t) is the amount of accumulated analyte after time (t) and R_S is the sampling rate in litres per day (L d^{-1}). The R_S can be considered as the volume of water cleared of analyte per unit of exposure time by the device [Vrana *et al.*, 2005a].

The sampling rate is limited by the receiving phase, the aqueous boundary layer, the membrane (if used) and a layer due to biofouling [Kot-Wasik *et al.*, 2007]. The delay effect of these can be considered separately since they do not depend on one another. If the layer contributes more than 50% of the total resistance, it is considered as uptake rate-limiting. However, higher sampling rates shorten the linear uptake phase of the sampler [Gunold *et al.*, 2008].

2.2 Development of passive sampling

Sampling is the most critical step in determining water quality because unsuccessful sampling causes errors in further sample handling. Passive sampling usually combines sampling, the isolation of the analyte and its pre-concentration in a single step. Most techniques require little or no solvent, which remarkably reduces solvent costs [Górecki and Namieśnik, 2002]. Further sample handling steps are usually similar to those used in other sampling methods [Vrana *et al.*, 2005a].

The first report concerning passive sampling was released in 1927, when the technique was used for a semi-quantitative determination of CO in air [Gordon and Lowe, 1927]. A quantitative determination of NO_2 and SO_2 in air

was reported in 1973 [Palmes and Gunnison, 1973; Reiszner and West, 1973]. The first published passive sampling technique for determining organic compounds was a solvent-filled dialysis membrane developed by Södergren [1987]. It was designed for the monitoring of hydrophobic organic compounds in aquatic environments.

2.2.1 Semi-permeable membrane devices

Semi-permeable membrane devices (SPMDs) have been used in dialysis application to determine lipophilic organic compounds in water, sediment and air [Petty *et al.*, 2000; Namieśnik *et al.*, 2005]. They also act as biological membranes allowing the selective diffusion of organic compounds; thus the analytes can be concentrated to even higher levels than their octanol/water partition coefficients suggests [Huckins *et al.*, 1990; Namieśnik *et al.*, 2005]. The SPMD is considered to be a 'slow' technique, since it requires weeks, months or years to reach equilibrium for hydrophobic substances with an n-octanol-water partition coefficient larger than $K_{OW}>10^4$ [Mayer *et al.*, 2003].

Standard SPMDs for detecting lipophilic chemicals consist of a layflat low-density polyethylene LDPE tube 106 cm in length and 2.5 cm in width with a thickness of 75-90 µm. It contains 1 mL of ≥ 95% pure triolein [Petty *et al.*, 2000]. The surface-area-to-volume ratio (A/V) is about 80 cm^2 per mL, including both membrane and triolein, or about 460 cm^2 per mL of triolein. The mass of the system is about 4.5 g and its lipid content is approximately 20% (the triolein part). The existing calibration data are valid for all SPMDs with the properties mentioned above.

SPMDs should be stored frozen (≤-15°C) in vapour-tight cans before and after deployment [Petty *et al.*, 2000]. After exposure, the biofouling and particulate matter is removed, SPMDs are dialysed in an organic solvent (typically hexane) and the extract is cleaned by size-exclusion chromatography (SEC). The sample is further cleaned with Florisil, silica gel or alumina sorption chromatography. Detection and quantification can be performed with gas chromatography (GC) or liquid chromatography (LC), depending on the properties of the compounds studied.

Södergren [1987] developed a hexane-filled hydrophilic dialysis bag made from regenerated cellulose to determine non-polar pollutants in water. The accumulation was similar to that of aquatic organisms, since molecules with ≥1000 g mol^{-1} did not diffuse through the cellulose membrane. This mimics the behaviour of a biological membrane. When comparing cellulose and polyethylene (PE) membranes, the uptake was observed to be 24-84 times faster in the latter [Sabal iūnas and Södergren, 1996]. This indicates that the polar cellulose membrane restricts the accumulation of lipophilic compounds [Herve *et al.*, 1991]. Huckins *et al.* [1993] preferred triolein over hexane in passive membrane samplers, since low-molecular-mass solvents have high membrane

solubility and permeability, which causes diffusive losses into the surrounding aqueous media. In cases of higher-molecular-mass and larger molecules, the diffusion of the sequestering phase can be considered negligible. Also, isooctane-filled PE tubes have been used to determine hydrophobic compounds, such as chlorinated pesticides, in water with a concentration ratio of 200 to 300 [Peterson *et al.*, 1995; Namieśnik *et al.*, 2005].

Biofouling is the main problem with SPMDs, since it may reduce the uptake capacity of contaminants by 50% [Richardson *et al.*, 2002]. Rantalainen *et al.* [2000] observed slower uptake rates at low temperatures when monitoring polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and polychlorinated phenols with triolein-filled LDPE tubes in water and sediments. Changes in the salinity of the water phase have not been proven to have an influence on the sampling rates of SPMDs [Huckins *et al.*, 1999].

SPMDs have been used in several applications, such as monitoring organochlorine pesticides in river water and freshwaters [Ellis *et al.*, 1995; Verweij *et al.*, 2004; Wang *et al.*, 2009], polycyclic aromatic hydrocarbons in laboratory deep-well water and in seawater [Huckins *et al.*, 1999; 2004], penta- and hexachlorobenzene and hexachlorocyclohexane isomers in water (laboratory trial) [Vrana and Schüürmann, 2002], chlorobenzenes, polychlorinated biphenyls (PCBs) [Booij *et al.*, 1998; Meadows *et al.*, 1998], polyaromatic hydrocarbons (PAHs) in marine and estuarine sediments [Booij *et al.*, 1998] and NP in Mediterranean lagoons [David *et al.*, 2010]. Huckins *et al.* [1999] observed that the aqueous sampling rate for SPMDs was independent of the analyte concentration in the surrounding water.

2.2.2 Chemcatcher®

Most of the passive sampling techniques are suitable only for the determination of non-polar compounds. Many environmentally interesting compounds, *e.g.*, some pesticides, have low $\log K_{OW}$ values (less than 4) and are not classified as lipophilic according to the criteria of Noble [1993]. Thus, these polar compounds cannot be efficiently monitored with current, commercially available passive sampling systems.

The EU-funded pan-European project (STAMPS) produced a novel passive sampling device named Chemcatcher® for monitoring organic pollutants in aquatic environments [Kingston *et al.*, 2000]. It was coordinated by the University of Portsmouth; Central Finland Regional Environment Centre was one of the partners. There were two separate prototypes, one suitable for non-polar organic pollutants with $\log K_{OW}$ values greater that 4, and the other for polar organic pollutants with $\log K_{OW}$ values between 2 and 4. The sampler body supports a 47 mm C-18 Empore disk, which acts as a receiving phase and consists of octadecyl silica immobilised by PTFE fibrils. The disk is covered with a diffusion-limiting membrane material made of polysulphone (PS) for

polar analytes or PE for the non-polar analytes (Fig. 2). Using the diffusion-limiting membrane causes a lag in the accumulation time which derives from the fact that the compound must pass through the diffusive barriers (the water boundary layer, water in the membrane pores and the membrane) [Schäfer *et al.*, 2008].

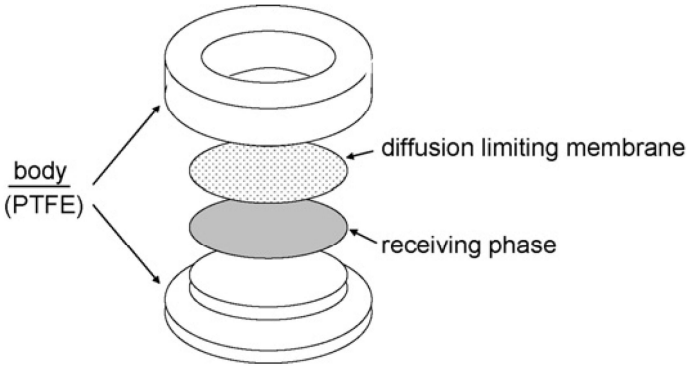

Figure 2 The general configuration of the Chemcatcher® [de la Cal *et al.*, 2008].

The previous type of Chemcatcher® sampler housing was made from polytetrafluoroethylene (PTFE) [Kingston *et al.*, 2000] and the next version from polycarbonate [Lobpreis *et al.*, 2008]. Both of these sampler bodies (Fig. 3) were tested with hydrophobic organic pollutants to find out whether the modification of sampler housing geometry affects the calibration parameters. The receiving phase of the old sampler design was located inside a 20 mm deep depression. It was observed that the cavity depth in the sampler housing had no effect on the correlation between the uptake and offload kinetics of polycyclic aromatic hydrocarbons. However, decreasing the depth increases the exchange kinetics. Schäfer *et al.* [2008] observed that the biofouling of uncovered Empore disks reduced the accumulation of the analyte compared to unfouled disks. The use of a diffusion-limiting PES membrane reduced the sampling rate, but then no biofouling was detected. Turbulence causes fluctuation in the aqueous boundary layer thickness and contributes to the sensitivity of the sampler [Lobpreis *et al*, 2008].

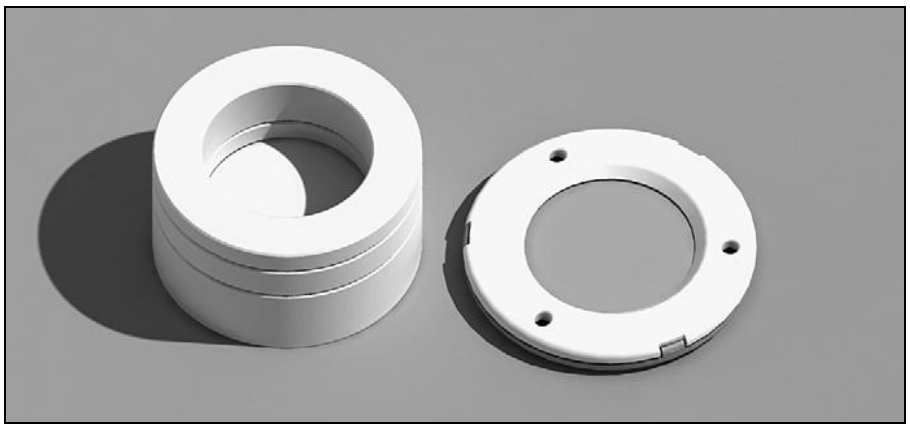

Figure 3 Old (left) and new (right) design of the Chemcatcher® sampler body [Lobpreis *et al.*, 2008].

The Empore disk, which acts as a receiving phase, is flexible and generally 0.5 mm thick [Fritz, 1999]. The sorbent material consists of non-spherical bonded silica or resin particles with sizes of 40 µm and 8 µm, respectively. According to the instructions, included with the disk, the C-18 Empore disk contains 90% (w/w) adsorbent particles (octadecyl bonded silica) and 10% (w/w) PTFE matrix which holds the membrane together [Fritz, 1999; Vrana *et al.*, 2006a]. The octadecyl bonded silica contains 17% (w/w) organic carbon so that 1 g of material contains 0.197 g of C-18. When the density of bonded C-18 is assumed to be the same as that of octadecane (0.78 g mL^{-1}), 1 g of C-18 Empore disk contains 0.227 mL of C-18 material. One 47 mm C-18 Empore disk weighs 572 mg and therefore contains 129.8 µL of C-18 [Verhaar *et al.*, 1995].

Preparation and extraction of samplers

Before deployment, the Empore disks are pretreated with different solvents to form a good interface between the sorbent and the sample matrix. C-18 and SDB-RPS Empore disks are commonly conditioned with methanol [Kingston *et al.*, 2000; Stephens *et al.*, 2005; Vrana *et al.*, 2005b; de la Cal *et al.*, 2008]. Vermeirssen *et al.* [2008] processed the SDB-RPS phase by dipping the whole disk in methanol and placing it in the sampler housing. The whole sampler was then immersed in methanol and UHQ water. In some studies, the C-18 Empore disk was saturated with *n*-octanol after conditioning with methanol [Vrana *et al.*, 2005b; Vrana *et al.*, 2006a; de la Cal *et al.*, 2008]. If a diffusion-limiting membrane is used, it is typically soaked in hexane before placing it on top of the disk.

After the deployment time the Empore disk is removed from the sampler body and extracted in an ultrasonic bath with solvents suitable for the compounds of concern. Acetone, followed by an ethyl acetate/2,2,4-trimethylpentane mixture, extracts from the C-18 phase both polar and nonpolar compounds with $LogK_{OW}$ between 2.21-6.90 and 1.2-2.85 [Kingston *et al.*, 2000; Stephens *et al.*, 2005, respectively]. Polar compounds can also be extracted from the SDB-RPS disk with acetone and methanol [Vermeirssen *et al.*, 2008]. Highly hydrophobic compounds have been extracted using acetone followed by a mixture of acetone/hexane [de la Cal *et al.*, 2008]. The extracts are combined, filtered, evaporated in a nitrogen stream, diluted with the appropriate solvent combination and finally submitted to analyses.

Although C-18 and SDB-RPS disks have similar partitioning and mass transfer coefficients, the latter have a longer kinetic sampling period due to a higher sorbent mass/surface area ratio. Stephens *et al.* [2005] observed that the equilibrium concentration for the SDB-RPS phase was two times higher than for the C-18 disk.

Calibration of Chemcatchers®

The accumulation of analytes to Chemcatchers® is usually tested in a constant concentration flow-through exposure system [Kingston *et al.*, 2000; Vrana *et al.*, 2005b; 2006a]. The system consists of a glass tank with an overflow to waste. UHQ water and a standard solution of the test analytes are delivered into the exposure tank at known and controlled rates using a peristaltic pump while the system is mixed with a stirrer. Prior to each exposure, the apparatus is operated without samplers to stabilise the concentration of analytes in water. The nominal concentration of the analytes remains constant during the test. The samplers are deployed in the tank for fixed time periods under controlled conditions such as analyte concentration, temperature and stirring speed.

Vrana *et al.* [2007; 2010] measured the time weighted average (TWA) concentration of hydrophobic pollutants using the Chemcatcher® with a receiving phase of a C-18 Empore disk saturated with n-octanol and covered by a PE membrane. Performance reference compounds (PRCs) were employed to calculate *in situ* sampling rates. The release of pre-loaded PRCs gave information for determining the TWA concentrations. However, PRCs should have similar properties as the studied analytes in order to simulate their uptake. Commonly, PRCs are deuterated or ^{13}C-labelled analytes [Vrana *et al.*, 2006a; Aguilar-Martínez *et al.*, 2009; Li *et al.*, 2010]. Herbicide levels have been determined using the Chemcatcher® with a SDB-RPS phase, which has a high affinity for polar organic compounds [Shaw *et al.*, 2009]. The offload of pre-loaded deuterated pesticides from the receiving phase back to water was not linear and therefore it could not be used to estimate the uptake of these compounds in varying natural conditions. Nevertheless, the laboratory exposure results indicated that the uptake of compounds was linear for 30 days when the disk was covered with a diffusion-limiting membrane. Without the membrane the uptake was linear for only 10 days. A one-day lag time was observed with diuron but not with other pesticides (*e.g.* atrazine, hexazinone and chlorpyrifos). The first samplers were retrieved after 24 hours, and it was possible that lag times shorter than that were not detected. The linear accumulation was observed for 15 days. Flow velocity had no significant effect on the sampling rates when observing the accumulation of polar pesticides in the Chemcatcher® with an SDB-XC (standard styrene-divinylbenzene) Empore disk as the receiving phase [Gunold *et al.*, 2008].

A channel system equipped with Chemcatchers® that provided river-like flow conditions using sewage treatment plant effluent or river water has also been reported [Vermeirssen *et al.*, 2008]. A box set on top of the channels served as a water dispenser. The water was passed through channels and dropped into a basin equipped with a pump that delivered the water back to the dispenser box. The water sample was refreshed continuously, meaning that more than

95% of the water was renewed within 10 minutes. The whole system could be placed in connection with the effluent stream of a waste water treatment plant.

The suitability of different Chemcatcher® configurations for determining the concentration of pharmaceuticals and biocides in water was compared by Vermeirssen *et al.* [2009]. The studied receiving phases were SDB-XC, SDB-RPS and SDB-RPS disks covered with PES diffusion-limiting membranes. The SDB-RPS disk sampled slightly higher amounts of the hydrophilic compounds during the trial, whereas the SDB-XC disk accumulated more hydrophobic compounds. It appeared that using the diffusion-limiting membrane on top of the SDB-RPS disk reduced the analyte flow to the sampler and extended the time for the receiving phase to reach equilibrium with the surrounding medium [Gunold *et al.*, 2008; Vermeirssen *et al.*, 2009].

Tetrabrominated diphenyl ether (BDE-47) and hexabrominated diphenyl ether (BDE-153), both used as flame retardants, as well as DDT and its main metabolites DDE and DDD have also been monitored with the Chemcatcher® [de la Cal *et al.*, 2008]. The receiving phase consisted of a C-18 Empore disk saturated with *n*-octanol and covered with an LDPE-diffusion-limiting membrane. Shaw and Mueller [2009] investigated the potential of Chemcatchers® to adapt to fluctuations of herbicide concentration. Low concentration levels were maintained for five days before introducing a tenfold amount of analytes. The concentration was halved repeatedly every 24 hours by dilution; the background concentration was achieved after about 72 hours and maintained until the end of the deployment time period. The measured uptake was compared with the predicted one. The Chemcatchers® without a diffusion-limiting membrane gave more accurate estimates of the fluctuating concentrations than the membrane covered ones. However, the rapid decrease of the concentration to the background level may lead to an equilibrium state in the receiving phase and thus the sampling is no longer kinetic.

A chelating Empore disk covered with a PES membrane provided the best performance for monitoring mercury in water [Aguilar-Martínez *et al.*, 2009]. The same disk covered with a cellulose acetate membrane has been utilised in Chemcatcher® configuration when monitoring metals (Cd, Cu, Ni, Pb and Zn) [Persson *et al.*, 2001; Björklund *et al.*, 2002; Allan *et al.*, 2007]. The chelating disk consists of polystyrene-divinylbenzene copolymer with iminodiacetate functional groups.

2.2.3 Other techniques

Stir bar sorptive extraction (SBSE) has been developed as a solventless technique for the pre-concentration of organic compounds in an aqueous matrix [Baltussen *et al.*, 1998]. The poly (dimethylsiloxane) (PDMS) coated stir bar is introduced into the water sample for a predetermined time, and thermally desorbed online with GC/MS. This method has been utilised in the

development of the MESCO (membrane-enclosed sorptive coating) passive sampler, consisting of a stir bar with a magnetic core coated with PDMS acting as the receiving phase (Gerstel-Twister bar) [Vrana *et al.*, 2001]. The stir bar is enclosed in a water-filled dialysis membrane bag of regenerated cellulose, and after exposure the coated stir bar can be directly analysed online with GC/MS. The method is suitable for monitoring hydrophobic organic pollutants [Vrana *et al.*, 2006b].

A passive sampling device for preconcentration of hydrophilic organic contaminants has also been developed [Alvarez *et al.*, 2004]. This polar organic chemical integrative sampler [POCIS, US Patent 6,478,961, Petty *et al.*, 2002] consists of a solid phase sorbent (receiving phase) sandwiched between two microporous PES membranes. The receiving phase and the membrane material vary depending on the application [Petty *et al.*, 2004]. After deployment, the sorbent mixture is eluted with suitable solvent combinations, evaporated to a smaller volume, and processed further as required by the analysis method. NP and NPEOs have also been monitored with POCIS [Writer *et al.*, 2010].

2.3 Passive sampling *vs.* biological incubation

The conventional sampling method for monitoring levels of pollutants in water is spot sampling. If the pollutants are present only at trace levels, this technique may not detect them at all. Long-term monitoring of waterborne contamination is possible by taking samples from sediment or biota. The mussel incubation method has been successfully used in monitoring low levels of organochlorine compounds in pulp and paper mill recipient water courses [Herve, 1991; Herve *et al.*, 1988; 1995; 2001; 2006] and persistent organic pollutants in freshwaters [Koistinen *et al.*, 2010]. To provide more information about harmful substances and to complete spot and biological sampling, alternative passive sampling techniques have been developed.

Most passive sampling techniques and biomonitoring systems are suitable only for the determination of non-polar compounds [Kingston *et al.*, 2000]. The uptake of contaminants in aquatic organisms can be processed directly from the gills or skin (bioconcentration) or through the consumption of contaminated food or suspended particles (biomagnification) [Verweij *et al.*, 2004]. The accumulation of compounds from water to fish fat tissue is in general a physical-chemical partitioning controlled by the relative affinities of the compound for both the water and the tissue [Gobas and Mackay, 1987]. If metabolic transformation can be considered negligible, a simple model with first order rate constants usually gives a satisfying description of the reaction kinetics. According to this model, the ratio of the analyte contents in fish and in water under steady-state conditions can be related to the octanol/water partitioning coefficient, K_{OW}. Elimination rate constants (k_2) tend to decrease as

K_{OW} increases, but the correlation with the uptake rate constant (k_1) is not as clear according to the literature. Gobas and Mackay [1987] have reported that the increase of lipid content in fish causes a proportional decrease in both k_1 and k_2. The accumulation of chemicals from the aqueous phase to the SPMDs is, however, controlled by simple partitioning between these two phases [Verweij *et al.*, 2004]. Booij *et al.* [2000] have observed that the sampling rates of LD PE membranes and SPMDs at a flow of 90 cm s^{-1} do not depend on $\log K_{OW}$.

The uptake of compounds to mussels takes place along both active and passive paths [Herve *et al.* 1991]. Mussels have been observed to collect lipophilic organochlorine compounds more efficiently than hexane-filled dialysis membranes, which is due to their efficient active uptake via food and the gills (bioconcentration). However, higher contents have been detected in SPMDs than in fish tissues, which may be due to a further clean up of fish extracts or metabolism of compounds [Ellis *et al.*, 1995]. The concentrations derived from the SPMDs and water samples correlated well.

Huckins *et al.* [2004] compared SPMDs and oysters when determining PAH concentrations in a laboratory trial mimicking seawater conditions. The accumulation was observed to be more efficient in oysters than in SPMDs. However, SPMDs were found to trap higher amounts of compounds with $\log K_{OW} \geq 5.6$. Also, PAH and organochlorine pesticide concentrations estimated with SPMDs and caged carp agreed better for compounds with lower K_{OW}, which implies an inaccuracy in the fish-water sorption equilibrium [Verweij *et al.*, 2004]. The comparison of PCB contents in SPMDs and brown trout revealed a similarity of the uptake rates when both were exposed to PCB-contaminated groundwater for 28 days [Meadows *et al.*, 1998]. The SPMDs had up to two times higher amounts of accumulated PCBs when compared to fish. The lower content in fish tissue may have been due to the metabolism of the monitored compounds.

El-Shenawy *et al.* [2009] compared biomonitoring to passive sampling for selected pesticides and PCBs. Mussels (*Mytilus edulis*) and Chemcatchers® were exposed in a flow-through exposure system with a nominal concentration of 100 ng L^{-1} for each analyte. The receiving phase (a C-18 Empore disk) was covered with either a PE or polysulphone (PS) membrane. PCB 52, PCB-153 and dieldrin were observed to accumulate in greater concentrations in mussel tissue. The Chemcatcher® with a PE membrane had a high affinity towards the non-polar compounds (phenanthrene, dieldrin, PCB 153 and PCB 52). The highest amounts of diuron, atrazine, irgarol and lindane were measured using the Chemcatcher® with a PS membrane.

The fact that the organisms are not passive causes issues when comparing them to passive samplers. Several things, such as selective feeding, have an effect on uptake kinetics [Kukkonen and Landrum, 1994; Bayen *et al.*, 2009]. In

both techniques a hig her surface-area-to-volume ratio (A/V) increases the uptake rate of hydrophobic chemicals.

2.4 Effect of humic substances

Dissolved organic matter (DOM), measured as dissolved organic carbon (DOC), is an important component in the freshwater environment [Porcal *et al.*, 2009]. It originates from various sources and due to its constituent acids it has an effect on the pH of the freshwater systems. Over 50% of DOC consists of humic and fulvic acids (referred to as humic substances), while the remainder consists of neutral acids, mono- and polysaccharides and amino acids [Kalff, 2001]. The structure of DOC is not readily recognized [De Paolis and Kukkonen, 1997; Kalff, 2001]. These substances have a variety of functional groups and therefore a potential to interact with freshwater organisms [Porcal *et al.*, 2009]. They also bind metals and nutrients, and they are able to pass biomembranes due to the low molecular mass of their building blocks.

ter Laak *et al.* [2009] studied the effect of humic acids on the transport of chemicals between the aqueous matrix and the sorbent phase of passive samplers. They observed that the presence of humic acid in water did not affect the accumulation of pyrene to PDMS, but it increased the accumulation of benzo[*b*]fluoranthene, which has a higher affinity towards humic acids. The humic material can only improve the transportation of chemicals if diffusion through the aqueous boundary layer is the rate limiting step. De Paolis and Kukkonen [1997] observed that humic acids bind hydrophobic substances more effectively than fulvic acids. In Finland the natural waters are humic- rich [Kortelainen, 1993].

3 NONYLPHENOL ETHOXYLATES (NPEOs) AND NONYLPHENOL (NP)

NP is used in the production of NPEOs, which have been widely used in different applications in households and industry. NPEOs are discharged to waste water treatment plants, where they degrade forming NP and short chain NPEOs [Thiele *et al.*, 1997; Di Gioia *et al.*, 2009]. These substances act as endocrine disrupting compounds and they therefore exert an effect on the aquatic ecosystem [Jonkers *et al.*, 2005a].

3.1 Chemical structure and properties

NPEOs consist of hydrophilic polyethylene glycol (ethoxylate) chains attached to phenolic oxygen and the hydrophobic nonyl group in an *ortho-*, *meta-* or *para*-position [John and White, 1998]. The number of ethylene oxide groups in the chain may vary from 1 to 50 or more, and it is expressed as a subscript ($NPEO_n$). The NPEOs with shorter ethoxylate chains (< 5) and NP are considered to be lipophilic, while higher ethoxymers are described as hydrophilic compounds [Ying *et al.*, 2002]. The majority of the monoalkylphenols (90%) are *para* isomers, while the remaining 10% consists of *ortho* isomers [Maguire, 1999]. The molecular formulae of NP and NPEOs are presented in Figure 4 [Jonkers *et al.*, 2001]. A typical NPEO surfactant is a complex mixture with an average of about 10 ethoxy units [Ferguson *et al.*, 2001]. The $\log K_{OW}$ values (Table 1) suggest that these compounds are attracted to organic matter in sediments [Ahel and Giger, 1993; Ying *et al.*, 2002].

In the experimental part of this study (Chapter 4) the NP used is a *para* substituted unbranched isomer, 4-*n*-NP. No other isomers are included, and therefore NP is discussed in the text using a singular form.

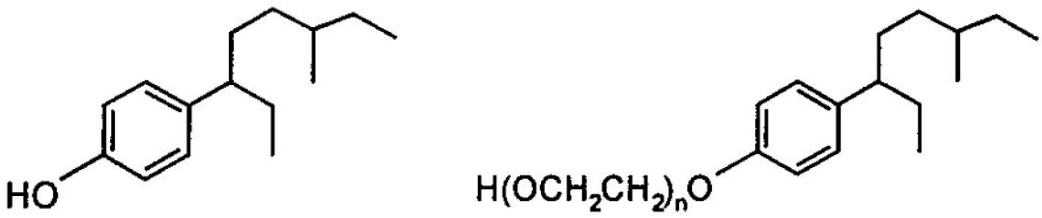

Figure 4 Molecular formulae of NP (left) and $NPEO_n$ [Jonkers *et al.*, 2001].

Table 1 $\log K_{OW}$ values of NP and $NPEO_{1\text{ to }3}$ [Ahel and Giger, 1993; Ying *et al.*, 2002]

Compound	Molecular weight	Log K_{OW}
NP	220.0	4.48
$NPEO_1$	264.0	4.17
$NPEO_2$	308.0	4.21
$NPEO_3$	352.0	4.20

3.2 Calculation of the Poisson distributions (Wt%) of NPEOs

Commercial NPEOs are complex mixtures of ethoxy homologues and alkyl isomers [Loos *et al.*, 2007a]. The percentage value of each oligomer from an NPEO mixture containing the average number of ethoxylates (n) can be calculated using the following equation [Weinheimer and Varineau, 1998]:

$$Wt\%(n) = \left(\frac{e^{-(n+1)}(n+1)^{x}}{x!} \right) \left(\frac{M_{x+1}}{M_{n}} \right) (100), \qquad (5)$$

where e = 2.71828

n = average number of ethoxylates in mixture (x+1)

x = 1, 2, 3,…

M_{x+1} = molecular weight of the $NPEO_n$

M_n = molecular weight of the product

The Poisson distribution (wt%) for NPEOs with mole ratios n=2 and n=10, are presented in Table 2. The calculated and analytically determined distributions correlate well, but are not exact due to impurities in the initial reactants. The calculated values, however, describe well the actual composition of the analytical mixture.

Table 2 Poisson distributions (wt%) for NPEOs [Weinheimer and Varineau, 1998]

$NPEO_n$ (n=1-22)	MW	Mole ratio (n)	
		2	10
1	264	31.53	
2	308	36.79	0.05
3	352	21.02	0.27
4	396	7.88	0.90
5	440	2.19	2.25
6	484	0.48	4.45
7	528	0.09	7.29
8	572	0.01	10.15
9	616		12.30
10	660		13.18
11	704		12.65
12	748		11.00
13	792		8.73
14	836		6.38
15	880		4.32
16	924		2.72
17	968		1.60
18	1012		0.89
19	1056		0.46
20	1110		0.23
21	1144		0.11
22	1188		0.05

3.3 Manufacturing and use of NP and NPEOs

Surfactant molecules may be divided into three main classes: anionic surfactants (*e.g.*, alkylphenol sulphonates), cationic surfactants (*e.g.*, quaternary ammonium salts) and non-ionic surfactants [Scullion *et al.*, 1996]. Alkylphenol ethoxylates (APEOs) have been used as nonionic surfactants for several decades since they are cost-effective and thereby very efficient for a number of applications due to their hydrophilic ethylene oxide and hydrophobic alkyl chains. The earliest documentation of APEOs was published in 1937 followed by academic studies in the 1940s [Weinheimer and Varineau, 1998].

APEOs are used as dispersing agents in the pulp and paper industry, as emulsifying agents in paints and pesticides, as flotation agents and industrial cleaners (metal surfaces, textile and leather processing) and as cleaning agents in household chemicals [Thiele *et al.*, 1997; Weinheimer and Varineau, 1998; Jonkers *et al.*, 2005c; Zgoła-Grześkowiak *et al.*, 2009; Ma and Cheng, 2010]. NPEOs are also widely used in wool processing to remove wax and dirt [Jones and Westmoreland, 1998] and as additives in aircraft anti-icers [Corsi *et al.*, 2006]. The common processing of 1 ton h^{-1} of raw wool corresponds to the organic load produced by the sewer system of a town populated by 30 000 people.

Among the APEOs, NPEOs are by far the most commonly used congener; 80% of the APEO surfactants used are NPEOs, while the remaining 20% are almost entirely octylphenol isomers (OPEOs). APEOs have been replaced in household applications in most western countries, mainly by alcohol ethoxylates (AOs). The use of NP and NPEOs was agreed to be diminished by the Paris Commission in 1992 [Ylä-Mononen, 1996]. The use of these compounds was phased out in 1995 in household products and in 2000 in industrial products. The use of NPEOs is only allowed if the organic fraction is completely removed by waste water treatment [EU regulation No. 1816, 2004; Di Gioia *et al.*, 2009]. In Finland their use in household products has been insignificant since 1993; in 1994 various areas of Finnish industry utilised 960 tons of NP and NPEOs [Ylä-Mononen, 1996]. At present, when their use is restricted, the main source of NPEOs are still industrial waste water treatment plants (municipal and industrial). The use of NPEOs has been restricted or banned in some European countries [Di Corcia *et al.*, 2000]. However, NPEOs can be used in closed systems and they are still used, for instance, in paint manufacturing in Finland [EDEXIM, 2010].

The production of NP begins with the oligomerisation of propene to nonene, which is further allowed to react with phenol, with sulphonated polystyrene-polydivinylbenzene acting as a catalyst. The temperature of the exothermic alkylation reaction is reduced by adding an excess of phenol, which favours the formation of the preferred monoalkylates. NPEOs are

manufactured from NP by a reaction with ethylene oxide (Fig. 5) [Weinheimer and Varineau, 1998]. The manufacturing is a semibatch process, where sodium hydroxide (NaOH) and potassium hydroxide (KOH) are the most commonly used catalysts in concentrations between 0.1% and 0.03%. The reaction pressure ranges from 5 to 6 bar and the temperature between 100°C and 180°C. The NP is heated with the catalyst and the water formed during the reaction is removed by adding nitrogen gas. Water removal minimises the formation of polyether glycol. Ethylene oxide is fed into the dried NP mixture in the reactor resulting in NPEOs (Fig. 5).

Figure 5 Reaction scheme of manufacturing NPEOs [Weinheimer and Varineau, 1998].

3.4 Degradation and biological effects of NPEOs

NPEOs are not formed in the environment but they are released into wastewater by different processes. In Finland $NPEO_1$ and $NPEO_2$ have been found in waste water in the range of 0.1-0.8 µg L^{-1} and in surface waters at 0.1-0.2 µg L^{-1} [Londesborough *et al.*, 2009] NPEOs are biologically degradable in conventional waste water treatment plants, and also in natural environments by both aerobic and anaerobic pathways [Jones and Westmoreland, 1998; Maguire, 1999; Bennie, 1999; Castillo *et al.*, 2001; González *et al.*, 2007]. The degree of degradation at the treatment plant depends on the amount of surfactants in the influent stream, on the plant design including operating conditions, and also on temperature [Bennie, 1999].

The degradation of NPEOs begins with oxidative hydrolytic shortening of the ethoxylate chain. This forms more toxic products such as nonylphenol mono- or diethoxylate ($NPEO_1$ or $NPEO_2$) and carboxylate (NPEC) under aerobic conditions. On the other hand, in an anaerobic environment the degradation product is NP. Some cleavage of the hydrophobic alkyl chain also occurs [Jones and Westmoreland, 1998]. Compared to the parent compounds, the resulting products are less water soluble, more toxic, lipophilic, estrogenic and persistent, and they can therefore be found in receiving waters [Bennie, 1999; Maguire, 1999].

Anaerobically digested sludge has been observed to contain high amounts of NP [Bennie, 1999]. The degradation of NPEOs appeared to be faster in the water phase under aerobic conditions than under the anaerobic conditions that prevail in sediment [Ying *et al.*, 2002]. APs and APEOs have been proven to be estrogenic in fish, avian and mammalian cells. The binding of these compounds to the oestrogen receptor mimics the effects of 17β-estradiol and results in

increased vitellogenin secretion [White *et al.*, 1994; Servos, 1999; Ying *et al.*, 2002; Loos *et al.*, 2007a].

3.4.1 Biological degradation

The biodegradation behaviour of NPEOs is still under debate. Jonkers *et al*. [2005a; 2005b] proposed two degradation paths of which the oxidative hydrolytic route takes place mainly in freshwaters only under aerobic conditions [Fig. 6 (A)], forming NPECs through ω-oxidation. The alkyl chain undergoes a further reaction forming doubly oxidised metabolites nonylphenol dicarboxylates (CNPEC). The non-oxidative hydrolytic pathway [Fig. 6 (B)] begins with a shortening of the ethoxylate chain, forming $NPEO_2$ and $NPEO_1$, which in anaerobic conditions further degrade to NP. The $NPEO_1$ and $NPEO_2$ may also be oxidised to NPECs if aerobic conditions are prevailing.

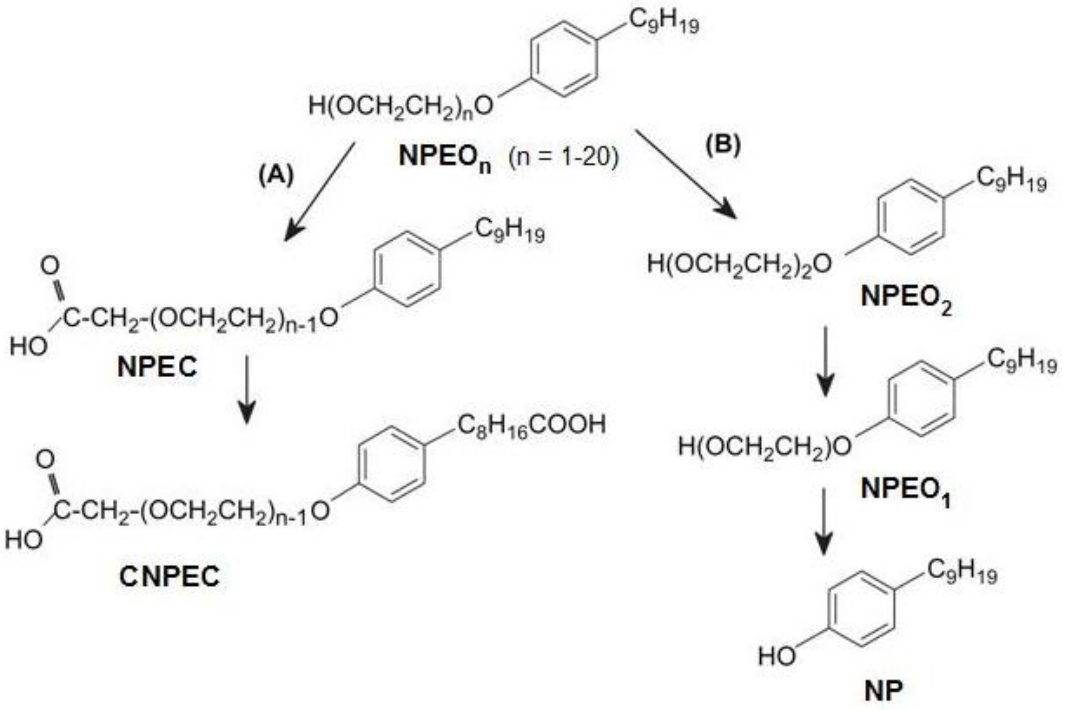

Figure 6 Biodegradation routes of NPEO: the oxidative hydrolytic pathway (A) and the non-oxidative hydrolytic pathway (B) [Jonkers *et al.*, 2005a; 2005b, revised by author].

In previous studies, Giger *et al.* [1984] observed higher NP contents in anaerobically stabilised sludge compared to aerobically stabilised ones. This indicates that anaerobic conditions increase the accumulation of NP in the sludge. During aerobic treatment microbial transformations shorten the polyethoxylate chains of APEOs, forming less biodegradable alkylphenol mono- and diethoxylates. Being less hydrophilic and water-soluble, $APEO_1$ and $APEO_2$ are partially accumulated in the lipophilic flocs of the sludge. After the sludge is stabilised, the alkylphenol mono- and diethoxylates are further degraded to alkylphenols. The transformation of NPEOs is strongly dependent

upon the temperature and therefore the degradation time during the winter can be significantly longer [Ahel *et al.*, 1994].

In full-scale treatment plants the primary biodegradation of NPEOs progresses easily [Maguire, 1999]. Fast primary degradation of NPEOs (>99%) was observed in laboratory scale trials after four days [Jonkers *et al.*, 2001]. NP and lower NPEOs and NPECs have been detected in groundwaters, and also in landfills under anaerobic conditions [Maguire, 1999]. Still, they seem to degrade in soil under aerobic conditions. A promising biofilm technique for degrading NP, and to some extent NPEOs, using an aerobic bacterial culture has been reported [Di Gioia *et al.*, 2009]. NPEO-contaminated effluents from activated sludge plants were treated with a culture of aerobic bacteria to develop a biotechnological process and complete mineralisation of NPEOs was observed.

3.4.2 Photodegradation

Based on the article published by Chen *et al.* [2007], $NPEO_{10}$ undergoes direct photolysis upon exposure to ultraviolet (UV) radiation. The complex degradation pathway includes shortening of ethylene oxide side chains, oxidation of alkyl chains and ethoxylate chains as well as hydrogenation of the benzene ring. Various lengths of both carboxylated and carbonylated ethoxylate and alkyl chains were detected as intermediates. An increasing humic acid concentration caused a decrease in photodegradation. The hydrogenation of the benzene ring due to UV irradiation could possibly reduce the persistence of toxic degradation products by generating less toxic cyclohexane compounds.

Castillo *et al.* [2001] studied the degradation products of $NPEO_9$ and decanol polyethoxylate ($C_{10}EO_6$) by irradiating them with a xenon arc lamp. The detected intermediates were polyethylene glycols (PEGs), $NPEO_2$ and nonylphenol ethoxy acetic acid ($NPEC_2$). Complete degradation could not be confirmed because polar organic acids, common to oxidation processes, were not detected.

3.4.3 Removal of NPEOs from sewage water

The fastest degradation rate of NPEOs was observed in plants under nitrifying conditions and low sludge loading rates [Bennie, 1999]. Small amounts of NP and NPEOs are still detected after waste water treatment, and they cause estrogenic activity in the effluents [Lamoree *et al.*, 2010].

A commercial NPEO mixture was observed to degrade under aerobic and anaerobic sewage treatment conditions during a laboratory trial [Zhang *et al.*, 2008]. Under the aerobic system the main metabolites were NPECs and $NPEO_{1-3}$, whereas under anaerobic treatment they were NP and $NPEO_{1-3}$. No NPECs were detected in anaerobic effluents and no NP was found in aerobic

ones. Thus, degrading enzymes and bacteria were present in both aerobic and anaerobic sludge.

The removal of NPEOs in sewage water treatment systems varies according to the treatment method. Conventional waste water processing begins with the removal of solid particles in sedimentation tanks, and the sludge is further processed, typically under anaerobic conditions and elevated temperatures [Scott and Jones, 2000]. Under aerobic conditions the NPEOs are degraded to shorter ethoxylate oligomers or NPECs, while in anaerobic conditions they are further degraded to NP [Clara *et al.*, 2005; Komori *et al.*, 2006; Zhang *et al.*, 2008]. Long chain NPEOs were almost completely removed during the waste water treatment process unlike short chain ones, which indicates a lower degradation rate for short chain NPEOs. About 50% of the long chain NPEOs were transformed to NPs and accumulated in the digested sludge [Brunner *et al.*, 1988]. However, the long chain ethoxylates remained in the water phase while the NP and the short chain ones were partitioned between the solid matter and the sludge [Céspedes *et al.*, 2008]. When studying six waste water treatment plants, González *et al.* [2004] observed that 93-96% of NPEOs were degraded.

More than 75 % of NPEOs were removed when waste water was treated in a wetland [Belmont *et al.*, 2006]. Also, the composting of sludge produced in wool processing decreased the NPEO amount by 96% in 14 weeks [Jones and Westmoreland, 1998]. Thus, once the sludge is used for landfill or soil, the surfactants are again readily biodegradable when introduced into an aerobic environment [Jones and Westmoreland, 1998; González *et al.*, 2007]. NPEOs have an amphiphilic character, and therefore some of them tend to pass through waste water treatment.

Esperanza *et al.* [2004] studied the removal of NPEOs between the influent and effluent in two pilot-scale municipal waste water treatment plants. The procedure consisted of a primary settling tank, three-stage aeration and final clarification. Synthetic waste water spiked with an NPEO mixture was introduced continuously into plants and the primary and waste-activated sludges were digested aerobically or anaerobically. The NPEO content between influent and effluent was reduced by 96% in both digestions. Biological waste water treatment appeared to be efficient as well, removing 92-97% of NPEOs [Fytianos *et al.*, 1997].

The degradation of NPEOs in two waste water treatment plants using mechanical settlement followed by activated sludge treatment has also been studied [Isobe and Takada, 2004] . During the treatment processes the $NPEO_1$ content decreased by 94% and 97%, whereas the $NPEC_1$ and $NPEC_2$ concentrations increased. This was due to the carboxylation of NPEOs that takes place under aerobic treatment conditions. Also, Loyo-Rosales *et al.* [2007b]

observed a 99% degradation of NPEOs during waste water treatment and an increase in short chain carboxylated derivatives during the process. The short-chain NPEOs appeared to have a higher affinity for particulate matter since they were observed in higher contents in solids.

The long chain NPEOs used in the textile industry are degraded to short chain metabolites with high concentrations of $NPEC_1$ and $NPEO_2$ in effluent samples after waste water treatment [Loos *et al.*, 2007a]. CNPECs were also observed to form in activated sludge sewage plants, representing 66% of all metabolites [Di Corcia *et al.*, 2000]. 87% of the CNPECs had octyl- and heptyl-groups with one ethoxy unit.

Loyo-Rosales *et al*. [2007a] observed that the removal efficiency of NPEOs in waste water treatment plants correlated with the water temperature, whereas the removal of suspended solids or organic carbon did not have an influence on the elimination of NPEOs. Effluents had higher concentrations of NPECs and NPEOs in the winter due to slower degradation at temperatures below 15°C. They also noticed that advanced waste water treatment did not necessarily improve the NPEO removal.

3.5 Determination of NP and NPEOs from water samples

NPEOs have been extracted from the water phase by many different techniques, such as solvent sublation, steam distillation, liquid-liquid extraction (LLE) and nowadays increasingly by SPE [Lee, 1999]. Longer chain ethoxylates ($NPEO_{3-10}$) may be adsorbed onto glassware. Martínez *et al.* [2004] added polyoxyethylene lauryl ether to act as a non-ionic surfactant into water samples covering the active spots of glassware and by this means prevented the adsorption of longer chain ethoxylates. Short chain NPEOs and NP tend to act less like surfactants and they are more likely to stay in the water phase. Before use, the glassware was rinsed with acetone and water and baked at 120°C for 8 hours. Rinsing glassware without detergent has also been used to avoid sample contamination [Datta *et al.*, 2002].

3.5.1 Sampling

The water samples have been conventionally collected in clean glass (amber) bottles or Pyrex borosilicate glass containers and kept at 4°C [Di Corcia *et al.*, 1994; Petrovic *et al.*, 2001; Rice *et al.*, 2003]. In some cases the sample was treated with formaldehyde to prevent microbial degradation [Ahel *et al.*, 1996; Bennie *et al.*, 1997; Maguire, 1999; Jonkers *et al.*, 2001; Shao *et al.*, 2002; Houde *et al.*, 2002; Jeannot *et al.*, 2002; Shao *et al.*, 2005; Zhang *et al.*, 2008;], or alternatively with 1% (v/v) formalin [Di Corcia *et al.*, 1994]. Preservation maintained a stable

surfactant concentration for 12 to 20 days [Houde *et al.*, 2002; Maguire, 1999, respectively]. When NPECs had been determined, the filtered water sample was acidified to pH 2 [Jonkers *et al.*, 2003] or even pH ≤ 1 [Isobe and Takada, 2004] with hydrochloric acid (HCl) to reduce microbial degradation. If the samples were immediately extracted, preservation was not performed. L-Ascorbic acid (1 g L^{-1}) has also been added to waste water samples to prevent oxidation [Komori *et al.*, 2006].

3.5.2 Determination of NP and NPEOs from water samples using SPE

In the literature, different SPE sorbents and conditioning procedures have been reported for concentrating NP, NPEOs, octylphenol (OP) and OPEOs from surface waters and analysing them by HPLC. The short-chain ethoxylates can be analysed by GC as well. The most commonly applied SPE sorbent appeared to be C-18, but other polymeric sorbents have also been used (see Table 3 and examples given below). The HPLC eluents varied depending on the column used, but the commonly applied solvents were methanol or acetonitrile (ACN). Aqueous ammonium acetate (NH_4Ac) and acetic acid solutions have been used as buffer in HPLC determinations.

C-18 cartridge (octadecyl silica sorbent)

The general SPE-extraction procedure using a C-18 phase for the isolation and concentration of NP, NPEOs and NPECs begins with the conditioning of the receiving phase with methanol and UHQ water [Jonkers *et al.*, 2001; Petrovic *et al.*, 2001; Martínez *et al.*, 2004; Hayashi *et al.*, 2005; Céspedes *et al.*, 2008]. For the analysis of NPECs the water sample was acidified with HCl to ensure that the carboxylates are in their protonated form [Jonkers *et al.*, 2001; Isobe and Takada, 2004]. After loading the sample, the sorbent was completely dried to avoid hydrolysis [Petrovic *et al.*, 2001]. The analytes were eluted with methanol [Jonkers *et al.*, 2001; Petrovic *et al.*, 2001; Isobe and Takada, 2004], methanol and dichloromethane (DCM) [Hayashi *et al.*, 2005; Céspedes *et al.*, 2008] or acetone followed by a methyl *tert*-butyl ether/methanol (MTBE/methanol) mixture [Martínez *et al.*, 2004].

Oasis HLB cartridge

The Oasis HLB cartridges (divinylbenzene-*N*-vinylpyrrolidone sorbent) have also been used in concentrating the APs, APEOs and alkylphenol carboxylates (APECs) from an aqueous matrix. The copolymer enables a reversed phase retention mechanism with a hydrophilic-lipophilic balance. The conditioning of the sorbent has been performed with methanol/UHQ water [Loos *et al.*, 2007a; 2007b], but in several cases additional solvents were used. According to the literature the conditioning solvent combinations have been MTBE/methanol (90:10, v/v) [Hu *et al.*, 2002]; MTBE followed by methanol and UHQ water [Waters, 2003]; diethyl ether, methanol and UHQ water

[Jeannot *et al.*, 2002] and DCM/methanol (80:20, v/v), methanol and finally UHQ water [Zhang *et al.*, 2008]. In most cases the cartridge was eluted with the same solvent combinations used in the conditioning step, excluding the final washing with UHQ water. Examples of solvents for conditioning and extracting steps were MTBE /methanol (90:10, v/v) [Hu *et al.*, 2002]; methanol/MTBE (10:90, v/v) [Waters, 2003]; methanol/diethyl ether (10:90, v/v) [Jeannot *et al.*, 2002] and DCM/methanol (80:20, v/v) [Zhang *et al.*, 2008]. Loos *et al.* [2007a] eluted the SPE-cartridge with a methanol/acetone/ethyl acetate (2:2:1) mixture that contained 0.1% formic acid. Without formic acid, different groups of environmental pollutants (polar herbicides, pharmaceutical products, perfluorooctanesulphonate (PFOS), perfluorooctanoate (PFOA)) were eluted simultaneously with NPEOs [Loos *et al.*, 2007b].

Other adsorbents

The SPE-procedures for other adsorbents and the solvents used in conditioning and elution steps varied with the receiving phase. Examples of the analysis are given below.

Loyo-Rosales *et al.* [2003] observed that water samples acidified to pH 4 and spiked with NPEOs gave very low recoveries (from 0% to 19%) with hydroxylated polystyrene-divinylbenzene copolymer sorbents compared to the octadecylsilica phase. They suggested that it was due to polar interactions between the phase and the APEOs via hydrogen bonds. The excess of free protons may cause the protonation of the oxygen atoms in the hydroxyl groups of the polymer and the ether bonds of the APEOs. Therefore, the hydrogen bonds between these two groups would not be favoured. The retention mechanism of the octadecylsilica phase is non-polar, and thus this phenomenon would not be observed with that solid phase. For this reason, acidification of the water sample when using a hydroxylated polystyrene-divinylbenzene copolymer should be carefully considered. The Isolute ENV+ (hydroxylated polystyrene-divinylbenzene) cartridge was conditioned with DCM, acetone and carbon-free water before loading the water sample, as performed by Rice *et al.* [2003]. The NPEOs were eluted with DCM . The same SPE phase has been conditioned with DCM, acetone and carbon-free deionised water [Loyo-Rosales *et al.*, 2003]. The elution was performed with DCM, methanol and acetone.

Komori *et al.* [2006] treated the residue of filtered waste water samples with supersonic extraction in acetone, and added the concentrated acetone extract to the filtrate. The sample was passed through a Sep-Pak Plus PS-2 (styrene-divinylbenzene copolymer) cartridge conditioned with methanol and UHQ water, and the NPEOs and NP were eluted with methanol. The further clean up was performed with a Sep-Pak Plus Silica cartridge conditioned with a chloroform/methanol mixture (4:1, v/v) and washed with hexane. The compounds were eluted with chloroform/methanol (4:1, v/v).

The GCB SPE cartridge was conditioned sequentially with tetramethylammonium hydroxide·5H_20 (TMAOH) in DCM/methanol (90:10, v/v), methanol and finally with distilled water (acidified to pH 2 with HCl) [Di Corcia *et al.*, 1994]. The water sample was also acidified to pH 3 with HCl and passed through the cartridge. The NP and NPEOs were eluted with DCM/methanol solution (70:30, v/v).

Crétier *et al.* [1999] determined aliphatic AOs in raw waste water using the styrene-divinylbenzene Empore disk. The disk was conditioned with acetone, methanol (twice) and UHQ water before sample processing. The AOs were extracted from the disk with methanol at ambient temperature or at 100°C. Some examples concerning the determination of NPEOs using SPE-pretreatment are presented in Table 3.

Table 3 Examples concerning the determination of NPEOs using SPE pretreatment

Compounds	Matrix	SPE phase material	Eluents	Method	Ref.
NP, NPEOs	Raw and treated waste water	GCB (prepared from 120-400 mesh size, Alltech Associates)	TMAOH·5H_2O in DCM/methanol (90:10, v/v), methanol, UHQ water (acidified to pH 2 with HCl); DCM/methanol	LC; Supelco C_8; 25 mM formic acid in DCM/methanol (90:10,v/v)/10 mM TMAOH in DCM/methanol (90:10, v/v)	Di Corcia *et al.*, 1994
NPEOs, AOs, PEGs	Waste water	Lichrolut RP-18	Methanol/UHQ water; CH_2Cl_2/methanol (70:30), CH_2Cl_2/methanol (90:10) acidified with 25 mM formic acid	LC-MS (APCI); Hypersil Green ENV; UHQ water acidified with 0.5% acetic acid/ACN methanol (1:1) acidified with 0.5% acetic acid	Castillo *et al.*, 1999
AOs	Raw waste water	SDB Empore disk	Acetone, 2×methanol, UHQ water; methanol	HPLC/MS (APCI, ESI); Kromasil C-18; UHQ water/methanol or UHQ water/ACN;	Crétier *et al.*, 1999
NPEOs, OPEOs and halogenated forms	Surface water, waste water	LiChrolut C-18	Methanol, UHQ water; methanol	LC/MS (ESI); LiChrospher 100 RP-18; ACN/UHQ water (positive ionisation), methanol/UHQ water (negative ionisation)	Petrovic *et al.*, 2001
NP and its reaction products	Drinking water	Oasis HLB	MTBE/methanol (90:10, v/v); washed with methanol/UHQ, UHQ water; MTBE/methanol (90:10, v/v);	GC/MS, HP-5MS	Hu *et al.*, 2002
OP, NP	Waste water	Oasis HLB	Diethyl ether, methanol, UHQ; washed with methanol/UHQ (40:60), UHQ and methanol/ammonia/ UHQ (10:2:88); methanol/diethyl ether (10:90)	GC/MS, GC/MS/MS; Varian-Chrompack CP-Sil 8 CB; SGE BPX-5	Jeannot *et al.*, 2002

Table 3 Examples concerning the determination of NPEOs using SPE pretreatment (continued)

Compound	Matrix	SPE phase material	Eluents	Method	Ref.
NP	Tap water	Oasis HLB	MTBE, methanol, UHQ water; washed with UHQ water/methanol (95:5); methanol/MTBE (10:90)		Waters, 2003
NP, NPEOs, OP, OPEOs	Surface water	Isolute ENV+	DCM, acetone, UHQ water; DCM, methanol, acetone	LC/MS-MS; MSpak GF-310 4D; 10 mM NH_4Ac in 50:50 v/v methanol/UHQ water and methanol	Loyo-Rosales *et al.*, 2003
NP, NPEOs, NPECs	Waste water	Isolute C-18	Methanol,UHQ water; washed with n-hexane; acetone, MTBE, methanol (9:1)	HPLC/MS (ESI); Hypersil APS 1; n-hexane/isopropanol (9:1); isopropanol/UHQ water (9:1); 80:20:0.1 water/isopropanol/formic acid with 10 mM NH_4Ac; Luna (Phenomenex); ACN; methanol 10 mM NH_4Ac; UHQ water, UHQ water with 10 mM NH_4Ac; 0.2% HFA	Martinez *et al.*, 2004
NP, OP, NPEOs, NPECs, NPECs	River water, waste water	Waters tC-18	Hexane, DCM, methanol, UHQ water; methanol	GC/MS (EI); HP-5MS fused silica	Isobe and Takada, 2004
NP, NPEOs, OP	Surface water, waste water	Lichrolut RP-18	methanol, UHQ water; methanol/DCM (9:1)	LC/MS (ESI); LiChrospher 100 RP-18; ACN, UHQ (positive ionisation), methanol, UHQ (negative ionisation)	Céspedes *et al.*, 2008

3.6 Determination of NP, $NPEO_1$ and $NPEO_2$ in water using multi-capillary trap extraction

The concentration of non-ionic surfactants from water samples with multi-capillary trap extraction is based on the fact that alkyl phenol ethoxylates are readily adsorbed onto the walls of plastic vessels [Szymański and Łukaszewski, 1990]. The general procedure is that the water sample is passed through the PTFE capillary and the analytes are adsorbed to the inner walls of the capillary. The compounds are eluted by introducing 1-2 mL of solvent to the trap and the sample is analysed using a suitable method. This has been utilised on river water, raw and treated sewage water [Morchalo *et al.*, 2005] and agricultural drains [Zgoła-Grześkowiak *et al.*, 2009]. The challenge is that all surfactants can be adsorbed to the PTFE trap. The recoveries of NP, $NPEO_1$ and $NPEO_2$ for spiked distilled water and drain water samples were between 75-93%.

3.7 Determination of NP and NPEOs in biological matrices (sample pretreatment)

The most challenging and laborious step in the extraction of biological tissue is lipid removal because it requires further cleaning of the sample, which reduces recovery. Some sample treatment procedures are described below.

Herve [1991] published a mussel incubation method for the monitoring of organochlorine compounds in recipient freshwater systems of the pulp and paper industry. The common lake mussel (*Anodonta piscinalis*) has proven to be a very resilient test animal, surviving even under heavily polluted conditions caused by effluents of the chemical pulp and paper industry. The mussels were incubated for four weeks at a sampling site and stored frozen until analysis. For extraction, homogenates of the soft tissue were prepared from five mussels with three replicate homogenate samples. Internal standards were added to the homogenates, and the samples were Soxhlet extracted with a hexane/acetone/diethyl ether/petroleum ether mixture for six hours. The solvent was evaporated first with a Rotavapor followed by a nitrogen stream.

NP and $NPEO_{1-16}$ were determined in incubated mussels (*Elliptio complanata*) [Cathum and Sabik, 2001; Sabik *et al.*, 2003] with recoveries between 24-111%. Mussels taken from a reference lake were placed in cages and incubated for 62 days upstream or downstream of a municipal waste water treatment plant. Homogenates of the mussel tissue were extracted with 50% acetone in hexane with a microwave extraction system, derivatised with pentafluorobenzyl bromide (PFB-Br) and cleaned up on a silica gel column [Cathum and Sabik, 2001]. No NP or $NPEO_1$ was detected in mussels despite the fact that their concentrations were above the detection limit in the sediment [Sabik *et al.*, 2003]. The contents of $NPEO_{5-8}$ were higher than those of $NPEO_{8-16}$, which was opposite to the results obtained from surface water samples. The concentrations of the target compounds determined in the mussels from the reference lake were equivalent to or slightly higher than those detected at upstream sites, indicating stronger biodegradation or biotransformation than bioconcentration.

The determination of NP, $NPEO_1$ and $NPEO_2$ from fish and shellfish began with the addition of 5 g of anhydrous sodium sulphate to 5 g of homogenised tissue sample [Tsuda *et al.*, 2000]. 30 mL of acetonitrile was added, the mixture was mixed twice in a high-speed homogeniser, and the organic layer was filtered through anhydrous sodium sulphate. The combined filtrate was evaporated just to dryness, the residue was dissolved in hexane and shaken twice with hexane-saturated acetonitrile for 5 min. The combined acetonitrile layers were evaporated, the residue was dissolved in hexane and passed through a glass clean-up column containing 5 g of hexane-rinsed Florisil PR and anhydrous sodium sulphate. The column was washed with hexane,

eluted with diethyl ether and hexane (1:9) for NP and with acetone and hexane (3:7) for $NPEO_1$ and $NPEO_2$. The solvents were evaporated nearly to dryness and diluted with the eluent. The recoveries of this method were good for both fish and shellfish matrices: NP (81.8–84.3%), $NPEO_1$ (83.5–84.3%) and $NPEO_2$ (90.5–96.2%). Using the same method, Mao *et al.* [2006] obtained average recoveries for fish and meat samples of 90.9%, 86.4% and 90.9%, for NP, $NPEO_1$ and $NPEO_2$, respectively.

The APs and APEOs were determined from carp. The homogenised fish were mixed with Na_2SO_4 (1:4, w/w) [Hesselberg, 1997; Datta *et al.*, 2002; Rice *et al.*, 2003]. Accelerated solvent extraction (ASE) was performed on a 35 g sample of this mixture with DCM as the eluent. The extract was evaporated to a small volume, diluted with hexane, and 0.5 mL of this extract was used for the determination of the lipid content. The rest of the extract was cleaned with aminopropyl silica cartridges, which were conditioned with acetone, DCM and hexane. The fish extract was passed through the cartridge, which was then washed with hexane and eluted with hexane/2-propanol (90:10, v/v). The recoveries were 74-125% ($NPEO_{1-5}$) [Datta *et al.*, 2002] and 78-93% [Rice *et al.*, 2003]. For NP, the recoveries were 103% and 96% for trout samples, but only 44% for carp samples. Several tests suggested that the variation in recoveries was due to differences in fish tissues [Rice *et al.*, 2003; Schmitz-Afonso *et al.*, 2003].

NP, $NPEO_1$, $NPEO_2$ and $NPEO_3$ have also been determined in tissue of laboratory raised goldfish (*Carassius auratus*) and validated with common carp (*Cyprinus carpio*) [Snyder *et al.*, 2001]. 20 g of homogenised fish tissue was blended with 350 mL of laboratory water, and the mixture was added to a boiling flask with sodium chloride, concentrated sulphuric acid, several glass boiling chips and a stir bar. *p*-Cumylphenol was added to the mixture to act as a surrogate standard. The mixture was steam distilled and the extract was fractionated with normal-phase HPLC. The recoveries were 69-76% for $NPEO_1$ and $NPEO_2$ but no more than 17% for $NPEO_3$. The fact that only NP and $NPEO_1$ were found in carp samples may have been due to the bottom feeder nature of the fish, since these compounds accumulate readily in the sediment.

Kannan *et al*. [2003] determined NP, $NPEO_1$, $NPEO_2$, $NPEO_3$ and $NPEC_1$ from bluegill sunfish and rock bass (*Ambloplites rupestris*) with the method developed by Snyder *et al.* [2001]. NP is likely to accumulate in the digestive and excretory systems, so only this part of fish was homogenised. Keith *et al.* [2001] extracted different fish species that reside in the middle depths of the water column and observed that NP was predominant. Alkylphenols and alkylphenol ethoxylates have also been extracted from fish tissue with steam distillation using cyclohexane as the solvent [Ahel and Giger, 1985; Lye *et al.*, 1999]. The recoveries were 104% for NP and 98% for $NPEO_1$ without column clean-up.

An NP and NPEO mixture with an average ethoxylate chain length of four units was isolated in biological tissue using matrix solid-phase dispersion (MSPD) [Zhao *et al.*, 1999]. In this procedure a small amount of spiked rainbow trout (*Oncorhynchus mykiss*) or zebra mussel (*Dreissena polymorpha*) tissue was blended with prewashed C-18 powder and transferred to the MSPD column on top of the 0.45-µm filter and Al_2O_3. Methanol was pressed out with a plunger twice and the fractions were concentrated with a nitrogen stream and analysed using HPLC with fluorescence detection. The fresh sample was only homogenised. The whole tissue was available for extraction due to its water sorption to the C-18 material, and therefore a small amount of solvent gave high recoveries (100-101%) at room temperature. Only spiked samples were tested.

As final example, Corsi and Focardi [2002] measured the concentrations of NP and $NPEO_{1-3}$ in fish samples. Two grams of grass goby (*Zosterisessor ophiocephalus*) fillets were Soxhlet extracted with *n*-hexane for 16 h and evaporated to a final volume of 5 mL. The extract was purified by percolating through an aminosilica column. The recoveries were higher than 90%.

3.8 GC determination of NP and NPEOs

GC determination requires that the analytes are volatile [Fendinger *et al.*, 1995]. A common procedure involves the derivatisation of the compounds studied to make the analytes easier to vaporize. With alkyl ethoxylates, the ethylene oxide groups are cleaved with hydrobromic acid or PFB-Br to create alkylbromides that can be determined by GC coupled with flame ionisation detection (FID), electron capture detection (ECD) or a mass spectrometric detector (mass selective detector, MSD) [Fendinger *et al.*, 1995; Lee, 1999; Cathum and Sabik, 2001; Aparicio *et al.*, 2007]. GC is considered to be suitable only for ethoxylates with a chain length of less than six units, because the longer chain ethoxylates cannot be derivatised to enter the vapour phase [Lee, 1999]. However, they can be determined with GC after an aluminium triiodide (AlI_3) treatment where the long chain NPEOs are converted to NP [Ma and Cheng, 2010]. NP, $NPEO_1$ and $NPEO_2$ can be determined by gas chromatography even without derivatisation [Aparicio *et al.*, 2007].

3.9 HPLC/MS determination of NPEOs

HPLC is commonly used for the analysis of APEOs [de Voogt *et al.*, 1997; Lee, 1999], and in particular for the long-chain ones, which are difficult or impossible to vaporize even with derivatisation. The major advantage of HPLC, compared to GC/MS, is its ability to separate and quantify the various homologues and oligomers by the length of their alkyl and ethoxylate chains. Reversed-phase HPLC (RP-HPLC) provides information on the alkyl chain

length, whereas normal-phase HPLC (NP-HPLC) resolves the ethoxylate oligomers [de Voogt *et al.*, 1997; Martínez *et al.*, 2004]. The UV, fluorescence and MSD detectors are increasingly used in the identification of NPEOs [Lee, 1999].

The NPEOs are capable of forming complexes with metal cations due to their flexible molecular structures [Crescenzi *et al.*, 1995]. NPEOs are commonly detected as their adducts, which they form with a buffer solution [Jonkers *et al.*, 2005a]. An adduct is formed when the NPEO molecule is wrapped around the positive ion, giving the negative oxygen ions optimal electrostatic interactions. The sodium adduct is most likely formed in small concentrations, but the cations of the buffer usually compete with this reaction. It is not recommended to use sodium or phosphorus buffers for an MS detector because of fouling of the device; therefore ammonium buffers are strongly recommended. The excess of ammonium prevents the formation of sodium adducts and enables the determination of NPEOs as their ammonium complexes [Cohen *et al.*, 2001; Martínez *et al.*, 2004].

The response of different NPEO oligomers in HPLC/MS varies, the short chain ethoxylates ($NPEO_{1\text{-}2}$) giving the lowest response [Jonkers *et al.*, 2005a]. Adducts are also formed with solvent molecules and an overlook of, *e.g.*, $[NPEO_1 + \text{methanol} + Na]^+$ leads to a decreased detection of the monoethoxylate oligomer content. Changing the organic solvent from methanol to ACN and using a sodium acetate (NaAc) buffer gave higher abundances of the adduct formed with the solvent molecule $[NPEO_{1,2} + ACN + Na]^+$ compared to $[NPEO_{1,2} + Na]^+$. Also *m/z* values presumably referring to $[NPEO_2 + 2\times ACN + Na]^+$ or $[NPEO_2 + NaAc + Na]^+$ adducts were found. However, changing the buffer to NH_4Ac did not produce similar adducts and thus $[NPEO_2 + 2\times ACN + NH_4]^+$ or $[NPEO_2 + NH_4Ac + NH_4]^+$ were not observed.

3.9.1 HPLC eluents

The mobile phase usually consists of an aqueous buffer and an HPLC-grade organic solvent, usually methanol or ACN. The buffer solution may include NH_4Ac, as recommended for the Acclaim Surfactant column used in the experimental section [Acclaim Surfactant Product Manual 2005; Liu *et al.*, 2006]. The resolution for individual OPEO oligomers with the Acclaim Surfactant column was compared to that in the conventional Symmetry C-18 column [Liu *et al.*, 2006]. The solvents used under isocratic conditions were ACN and NH_4Ac buffer (0.1 mol L^{-1} with pH 5.4). As assumed, the Acclaim Surfactant column provided improved peak resolution of oligomers.

Castillo *et al.* [1999] used HPLC/MS with atmospheric pressure chemical ionisation (APCI). Analytical separation of organic pollutants, including NPEOs, was performed with a Hypersil Green ENV column using gradient elution. Water acidified with 0.5% acetic acid and 50:50 ACN/methanol acidified with 0.5% acetic acid were used as eluents.

The role of methanol as a modifier with the C-18 column was studied and it appeared that the short oligomers of NPEO eluted earlier compared to longer chain ones [Jonkers *et al.*, 2005a]. The order of elution was reversed when ACN was used instead of methanol. ACN in the mobile phase is assumed to cause more linear orientation of the C-18 chains, which leads to more hydrophobic interactions with the analytes and causes the less polar short chain oligomers to elute last.

3.9.2 Ionisation techniques: APCI and ESI

The commonly used UV detectors are not suitable for the analysis of surfactants because of their lack of chromophores [Liu *et al.*, 2006]. Instead, an HPLC coupled to a mass-selective detector (HPLC/MS, HPLC/MS-MS) has become an important tool in environmental analysis. Both atmospheric pressure chemical ionisation (APCI) and electrospray ionisation (ESI) techniques have been used in the HPLC analysis of NPEOs. In the determination of AOs, APCI technique was less sensitive with short chain AOs than the ESI ionisation technique [Cassani *et al.*, 2004]. Some thermal degradation with high ethoxymers was also observed when using APCI [Cassani *et al.*, 2004]. ESI has proven to be a promising ionisation technique for analysing surfactants [Di Corcia, 1998]. It appeared to have a higher sensitivity and selectivity than APCI for NPEOs and their halogenated by products that are formed by prechlorination in the treatment process [Petrovic *et al.*, 2001].

When determining alkyl ethoxylates (AEs) with positive HPLC/ESI-MS as their sodium adducts $[M + Na]^+$, the detector response increased strongly with the length of the ethoxylate chain [Crescenzi *et al.*, 1995; Di Corcia, 1998; Cassani *et al.* 2004]. This refers to the better ability of long chain molecules to form more stable adducts. Since the ethoxylate part complexes the Na^+ ion, the effect of the alkyl chain length was weak.

4 EXPERIMENTAL

4.1 Materials and methods

Materials and chemicals used in this research are presented in Table 4. A commercial mixture of NPEOs was used in this study. The size of the peak in the HPLC/MS chromatogram is proportional to the concentration of the analyte in the mixture. The ionisation degrees of all ammonium adducts of the NPEOs were assumed to be the same.

Table 4 Materials and chemicals

Chemicals and deliverables	Purity	Manufacturer
ACN	HPLC-grade	J.T Baker
Acetic acid	99.8%	VWR
Acetone	p.a. 99.5%	Sigma-Aldrich
NH_4Ac	p.a. for mass spectroscopy	Fluka Analytical
Dichloromethane	p.a. 99.9%	Sigma-Aldrich
Ethylene glycol	99.5%	Riedel-de Haën
Hexane	HPLC-grade	Rathburn
Methanol	HPLC-grade	J.T Baker
4-*n*-NP(CAS: 104-40-5)	99.0%	Dr. Ehrenstorfer 15630000
$NPEO_{1-3}$	99.5%	Dr. Ehrenstorfer 15631000
$NPEO_{10}$ (CAS: 9016-45-9)		03853 Fluka
2-Propanol	99.8%	Merck KGaA
Empore disks, C-18		Varian
Empore disks, SDB-XC		Varian
Empore disks, SDB-RPS		Varian
Filter paper 42		Whatman, 10401712
Membrane filter 0.2 µm pore size, ME 24		Cronus Filter
SPE-cartridge Bond Elut C-18 LO, 200mg/3mL		Varian
SPE-cartridge ENVI Carb, 250mg/3mL		Supelclean
SPE-cartridge Oasis HLB, 60 mg/3mL		Waters
Titan PTFE-filter, 0.45 µm		Whatman

4.2 HPLC analysis

4.2.1 Apparatus

An HP Series 1100 binary pump, a vacuum degasser, a thermostatted column compartment and an autosampler were used for the chromatographic separations. These were performed on a Acclaim Surfactant column (250 mm × 4.6 mm I.D., d_p = 5 µm, Dionex, Sunnyvale, CA, USA) combined with a guard column (10 mm×4.3 mm) of the same stationary phase. Detection was carried out using an HP 1100 Series single quadrupole MS equipped with an ESI interface. The MS was tuned using an ESI calibration solution provided by Agilent to maximise its mass resolution and sensitivity. Data collection and processing were handled by an HP ChemStation (A06.03) chromatography data system.

4.2.2 Optimization of the HPLC/MS operating conditions

LC/MS has became the most widely used method in environmental analysis. Unlike GC/MS, there is no need to derivatise the non-volatile compounds, which makes sample treatment simpler. The compounds were detected using ESI-MS. Optimisation of the MS operating conditions was performed with flow injection analysis (FIA), where the standard solution was injected straight into the eluent stream and into the MS detector. The binary solvent system consisted of aqueous NH_4Ac (50 mM, pH 5.4, solvent A) and ACN (solvent B). The flow rate was kept constant at 1 mL min^{-1}. In the positive mode, the highest MS detection sensitivity was obtained with a nebuliser pressure of 50 psig, fragmentor value of 40 and capillary voltage of 4550 V. The optimal drying gas temperature was 350°C and the flow rate 11 L min^{-1}.

NPEOs were detected in the selected ion monitoring mode (SIM) as their ammonium adducts, which they form with the NH_4Ac buffer solution. The mass-to-charge (*m/z*) values for $[M+NH_4]^+$ (Table 5) were determined using the SCAN-mode in the molecular mass range of 200-1200 Da. In this study, the ionisation degrees of all ammonium adducts of NPEOs were considered to be the same. Combined SIM chromatograms of different NPEO oligomers are presented in Figure 7. It shows that the longer the ethoxylate chain, the shorter is the retention time.

Table 5 Detected m/z-values of NPEOs ammonium adducts ($NPEO_x$ - NH_4)

$NPEO_x$	*m/z*
$NPEO_1$	282.3
$NPEO_2$	326.3
$NPEO_3$	370.2
$NPEO_4$	414.2
$NPEO_5$	458.4
$NPEO_6$	502.4
$NPEO_7$	546.3
$NPEO_8$	590.5
$NPEO_9$	634.5
$NPEO_{10}$	678.5
$NPEO_{11}$	722.5
$NPEO_{12}$	766.5
$NPEO_{13}$	810.5
$NPEO_{14}$	854.5
$NPEO_{15}$	898.5
$NPEO_{16}$	942.5
$NPEO_{17}$	986.5
$NPEO_{18}$	1030.7
$NPEO_{19}$	1074.7

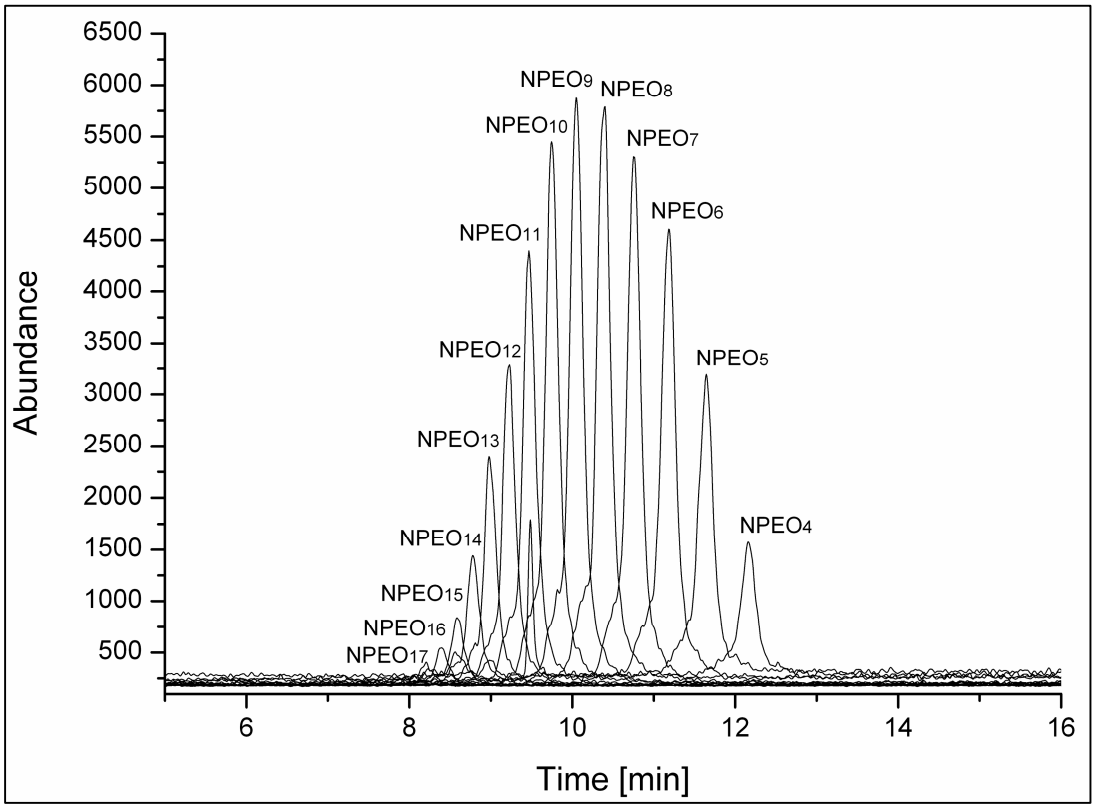

Figure 7 Overlayed HPLC/ESI-MS (SIM) chromatograms of NPEOs of different ethoxylate chain lengths. The compounds were recorded as their ammonium adducts $[M+NH_4]^+$.

The NPEO oligomers were separated using gradient elutions. The binary solvent system consisted of aqueous NH_4Ac (50 mM, pH 5.4, solvent A) and ACN (solvent B). To obtain the best separation and sharper peaks, different combinations of eluent were tested before selecting the final conditions. The

proportion of NH_4Ac was decreased from 50% to 15% during the first 20 min and then ramped back to 50% over a 3 min time period, and finally maintained at that level for 2 min before the next injection (Table 6). The eluent flow rate was 1 mL min^{-1}, the column oven temperature 30°C, and the injection volume 25 µL. The NPEOs were identified by matching their retention time with those of standard compounds. This method has already been published in a previous paper [Pessala *et al.*, 2009].

Table 6 Gradient of positive HPLC/ESI-MS -mode for determining NPEOs

Time (min)	50 mM NH_4Ac (%)	ACN (%)
0.01	50	50
20.00	15	85
23.00	50	50
25.00	50	50

NP was determined using the negative mode as its deprotonated [M^-] ion with m/z = 219. The operating conditions of the MS system were as follows: drying gas (N_2) at a flow rate of 11 L min^{-1} and at temperature of 350 °C, nebuliser pressure 50 psig, capillary voltage 2900 V, and fragmentor 60. In the negative mode, the ACN part was increased to 90% over 10 min and further to 100% over 8 min. The final eluent part consisting of 50% ACN was reached after 2 min and kept stable for 2 min (Table 7). The eluent flow rate was 1 mL min^{-1}, the column oven temperature was 30°C and the injection volume was 25 µL.

Table 7 Gradient of negative HPLC/ESI-MS -mode for determining NP

Time (min)	50 mM NH_4Ac (%)	ACN (%)
0.01	50	50
10.00	10	90
18.00	0	100
20.00	50	50
22.00	50	50

The standard stock solution was prepared from an $NPEO_{10}$ mixture, in which the length of the ethoxylate chain was on average 10 ethoxylate units, according to the manufacturer. However, the highest peak observed in the chromatogram was detected with an ethoxylate chain length of eight units, which refers to the highest concentration of that oligomer. Another standard mixture containing mainly $NPEO_1$, $NPEO_2$ and $NPEO_3$ was used to determine the short chain ethoxylates. Because there were also longer chain oligomers in this standard, and the $NPEO_{10}$ mixture also included some short chain oligomers, all tests were performed separately. Since NP did not interfere in the determination of NPEOs, it could be measured with either mixture. In almost all cases it was tested alongside $NPEO_{10}$.

4.2.3 Standards

The standard equations of NPEOs are presented in Table 8. The amount of each $NPEO_n$ oligomer in the standard mixture was calculated from the peak heights.

Table 8 Example of standard equations of deprotonated NP and ammonium adducts of NPEOs, y=ax (μg L^{-1})

$NPEO_n$	a	R^2
NP	0.88	0.985
$NPEO_1$	14.83	0.998
$NPEO_2$	14.89	0.998
$NPEO_3$	14.85	0.998
$NPEO_4$	56.96	0.998
$NPEO_5$	57.06	0.998
$NPEO_6$	57.25	0.998
$NPEO_7$	57.12	0.998
$NPEO_8$	57.25	0.998
$NPEO_9$	57.18	0.998
$NPEO_{10}$	57.13	0.998
$NPEO_{11}$	57.27	0.998
$NPEO_{12}$	57.14	0.998
$NPEO_{13}$	57.42	0.998
$NPEO_{14}$	56.95	0.998
$NPEO_{15}$	57.31	0.998
$NPEO_{16}$	57.05	0.998
$NPEO_{17}$	56.98	0.998
$NPEO_{18}$	57.87	0.997
$NPEO_{19}$	55.12	0.988

All the peak heights were added together to get the "total" height in the standard solution. This was considered as the concentration of the whole mixture including all oligomers. The ratio of the peak height of each oligomer to the total height was then calculated. This was considered to be the proportion of each individual oligomer in the mixture. These amounts were used as a content of each oligomer to calculate the calibration curve.

Only a few internal standards for NPEOs are presented in the literature. C^{13}–labelled NPEOs are only commercially available for $NPEO_1$ and $NPEO_2$. Also deuterated mono- and diethoxylates are commercially available, but they are expensive and therefore, using them would not be cost-efficient. The suitability of C^{13}–labelled mono- or diethoxylate to act as an internal standard for NPEOs with an average ethoxylate chain length of 10 units is poor. A more representative internal standard should have a longer ethoxylate chain and therefore, similar properties as the studied compounds. Suitable internal standards of low cost have not been found for NPEOs in the current literature and catalogues of chemical suppliers.

Several compounds with a chemical structure somewhat similar to NPEOs were tested when searching for a suitable internal standard. Most of the compounds could not be detected with a positive HPLC/ESI-MS. Methyl eicosanoate, 1-dodecanol, benzyl alcohol, acenaphthene-d_{10}, biphenyl-d_{10},

phenanthrene-d_{10} and anthracene-d_{10} were tested but they appeared to be unsuitable for this purpose. Because no proper internal standards were found, the concentrations of NPEOs and NP in the samples were calculated using an external calibration curve.

The NPEO amounts in the samples were calculated as a sum of $NPEO_{1-3}$ for short chain ethoxylates and as $NPEO_{5-15}$ for long chain ones. The results are expressed as an average of three replicates and the variability is determined by the standard deviation.

4.3 Pre-test of solid phase extraction (SPE)

The isolation and concentration of NPEOs and NP from water were performed using SPE. The general procedure begins with the selection of a suitable SPE sorbent. The SPE cartridge was conditioned with solvent(s) followed by UHQ water (internal resistance ≥ 18 MΩ·cm at 25°C) using a Vacuum Master sample processing manifold. The water sample was passed through the cartridge,which was then dried under vacuum for 30 minutes. If the sample contained interfering substances, the cartridge could be washed with a suitable solvent combination before eluting the compounds into a Kimax tube. The solution was evaporated until dryness under a nitrogen stream and the residue was diluted in the HPLC mobile phase ACN/50 mM NH_4Ac (v/v, 50:50). In this study three different phase materials were tested: octadecylsilica (Bond Elut C-18 LO), graphitised non-porous carbon (ENVI Carb) and divinylbenzene-*N*-vinylpyrrolidone (Oasis HLB). To avoid mixing, the recovery tests of $NPEO_{1-3}$ and $NPEO_{10}$ were performed separately. The most relevant SPE conditions tested are presented in Table 9. A known amount of the standard solution (300 µL, 3 mg L^{-1}) was added to UHQ water (50 mL or 2 L). The acetone eluent was also tested with the addition of 20 µL of ethylene glycol to act as a keeper and to see if the complete drying of the sample with a nitrogen stream had a negative effect on the recovery (SPE 7 and SPE 21). The glassware (bottles, Kimax tubes) was carefully cleaned by washing in the dishwasher and rinsing with HPLC-grade methanol. Also, the SPE cartridge holders (made of PTFE) were kept in HPLC-grade methanol overnight.

Table 9 SPE conditions used in pre-tests

Extract	Cartridge	Conditioning	Elution	Compounds eluted	Remarks
SPE 1	Bond Elut C-18 LO, 500 mg/3 mL	Methanol, UHQ water	DCM	$NPEO_{10}$	
SPE 2	Bond Elut C-18 LO, 500 mg/3 mL	Methanol, UHQ water	ACN	$NPEO_{10}$	
SPE 3	Bond Elut C-18 LO, 500 mg/3 mL	Methanol, UHQ water	Methanol/ACN (50/50, v/v)	$NPEO_{10}$	
SPE 4	Bond Elut C-18 LO, 500 mg/3 mL	Methanol, UHQ water	Methanol	$NPEO_{10}$	
SPE 5	Bond Elut C-18 LO, 500 mg/3 mL	Methanol, UHQ water	Methanol	$NPEO_{1-3}$	
SPE 6	Bond Elut C-18 LO, 500 mg/3 mL	Methanol, UHQ water	Acetone	$NPEO_{1-3}$	
SPE 7	Bond Elut C-18 LO, 500 mg/3 mL	Methanol, UHQ water	Acetone	$NPEO_{1-3}$	20 µL ethylene glycol
SPE 8	Oasis HLB, 60 mg/3mL	MTBE, methanol, UHQ water	MTBE/methanol, 90/10, v/v	$NPEO_{1-3}$	
SPE 9	Oasis HLB, 60 mg/3 mL	Methanol, UHQ water	Methanol	$NPEO_{1-3}$	
SPE 10	Bond Elut C-18 LO, 500 mg/3 mL	Methanol, UHQ water	Methanol	$NPEO_{1-3}$	
SPE 11	Bond Elut C-18 LO, 200 mg/3 mL	Ethyl acetate, acetone, methanol, UHQ water	Ethyl acetate, acetone, methanol	$NPEO_{1-3}$, NP	
SPE 12	Bond Elut C-18 LO, 200 mg/3 mL	Hexane, DCM, methanol, UHQ water	Hexane, DCM, methanol	$NPEO_{1-3}$, NP	
SPE 13	Bond Elut C-18 LO, 200 mg/3 mL	DCM, methanol, UHQ water	DCM, methanol	$NPEO_{1-3}$, NP	
SPE 14	Bond Elut C-18 LO, 200 mg/3 mL	Acetone, hexane, methanol, UHQ water	Methanol, hexane, acetone	$NPEO_{1-3}$, NP	
SPE 15	ENVI Carb 250 mg/3 mL	Ethyl acetate, acetone, methanol, UHQ water	Ethyl acetate, acetone, methanol	$NPEO_{1-3}$, NP	
SPE 16	ENVI Carb 250 mg/3 mL	Hexane, DCM, methanol, UHQ water	Hexane, DCM, methanol	$NPEO_{1-3}$	
SPE 17	ENVI Carb 250 mg/3 mL	DCM, methanol, UHQ water	DCM, methanol	$NPEO_{1-3}$	
SPE 18	ENVI Carb 250 mg/3 mL	Methanol	Methanol	$NPEO_{1-3}$	
SPE 19	ENVI Carb 250 mg/3 mL	Acetone, hexane, methanol	Methanol, hexane, acetone	$NPEO_{1-3}$	
SPE 20	Bond Elut C-18 LO, 500 mg/3 mL	Methanol, UHQ water	Acetone	$NPEO_{10}$	
SPE 21	Bond Elut C-18 LO, 500 mg/3 mL	Methanol, UHQ water	Acetone	$NPEO_{10}$	20 µL ethylene glycol

Table 9 SPE conditions used in pre-tests (continued)

Extract	Cartridge	Conditioning	Elution	Compounds eluted	Remarks
SPE 22	Oasis HLB, 60 mg/3 mL	MTBE, methanol, UHQ water	MTBE/methanol, 90/10, v/v	$NPEO_{10}$, NP	
SPE 23	Oasis HLB, 60 mg/3 mL	Methanol, UHQ water	Methanol	$NPEO_{10}$, NP	
SPE 24	Bond Elut C-18 LO, 500 mg/3 mL	Methanol, UHQ water	Methanol, hexane, acetone	$NPEO_{10}$, NP	pH=1.98 (HCl)
SPE 25	Bond Elut C-18 LO, 500 mg/3 mL	Methanol, UHQ water	Methanol, hexane, acetone	$NPEO_{10}$, NP	pH=6.78

4.4 Sampler design

The Chemcatcher® passive sampler consisted of a polycarbonate sampler housing (AlControl AB, Linköping, Sweden), which supports the receiving phase. Three different Empore disks were tested as a receiving phase (C-18, SDB-RPS and SDB-XC; Varian Inc.) and the suitability of the diffusion-limiting membrane on top of the disk was studied. The sampler housing was made of three pieces, two to attach the Empore disk and the membrane, and one to act as a transportation lid that protects the receiving phase (see Fig. 2, on page 8).

4.5 Preparation of the sampler

The method published by Vrana *et al.* [2006a] was modified in this study. The Empore disks were conditioned according to the instructions of the manufacturer; the detailed procedure is described in Chapters 4.5.1 and 4.5.2.

4.5.1 C-18 Empore disk

The sorbent in the C-18 Empore disk has an octadecyl silica phase, where the octadecyl group is bonded to the silica surface with an average particle size of 12 µm. Its retention mechanism is strongly non-polar and the average carbon percentage in the disk is 22.5%.

The C-18 Empore disk was first soaked in methanol for 20 min and placed in the filtration apparatus. 10 mL of methanol was passed through the disk, followed by 20 mL of UHQ water, and the disk was not allowed to dry out. The disk was fixed inside the sampler body and the cavity was filled with UHQ water. The lid was sealed and the sampler was placed in a zip-lock bag.

4.5.2 SDB-RPS and SDB-XC Empore disks

The sorbent of the SDB-XC disk is a standard styrene-divinylbenzene copolymer, which provides reversed-phase interactions in moderately polar

analytes. The phase material of the SDB-RPS disk is also a styrene-divinylbenzene copolymer, which has been made hydrophilic by adding sulphonic acid functional groups that may affect mass transfer, as Gunold *et al.* [2008] observed with pesticides. The reversed-phase and cation-exchange interactions provide selectivity towards polar organic analytes.

The SDB-RPS and SDB-XC Empore disks were conditioned by passing 20 mL of acetone through the disk, followed by 20 mL of isopropanol and 20 mL methanol. Finally, 20 mL of UHQ water was passed through and the disk was not allowed to dry out. The conditioned Empore disk was fixed onto the sampler housing, the cavity was filled with UHQ water and sealed with the transportation lid. The sampler was kept in a zip-lock bag until deployment.

4.6 Recovery tests of Empore disk extraction procedure

The recovery tests were made separately for both NPEO mixtures, because there were also longer chain oligomers in the $NPEO_{1-3}$ standard, and the $NPEO_{10}$ mixture also included some short chain oligomers. Since NP did not interfere in the determination of NP EOs it could be measured with either mixture. In almost all cases it was tested alongside $NPEO_{10}$.

The conditioned Empore disk (see Chapters 4.5.1 and 4.5.2) was spiked with a known amount of the standard solution. Subsequently, the disk was transferred to a Kimax tube and soaked in 3 mL of the first eluent in an ultrasonic bath for one minute (see detailed conditions in Table 10). The extract was poured into a syringe which had been cleaned by soaking it in methanol, and filtered through a 0.45-µm Titan PTFE filter into a new Kimax tube. The extraction was repeated with 3 mL of the second solvent in an ultrasonic bath, and the filtered eluent was combined with the first extract. If three eluents were used, this procedure was repeated once again. After the last solvent treatment, the Kimax tube was rinsed with 1 mL of methanol, which was filtered as well and combined with the extract. The extract was evaporated until dry under a nitrogen stream. The residue was dissolved in 300 µL ACN/50 mM NH_4Ac (v/v, 50:50) and analysed by HPLC/ESI-MS. The eluent combinations tested in this study are listed in Table 10.

Table 10 Empore disk extraction conditions used in pre-tests

Disk	Conditioning	Elution	Compounds eluted
SDB-RPS 1	Acetone, 2-propanol, methanol, UHQ water	Methanol	$NPEO_{1-3}$
SDB-RPS 2	Acetone, 2-propanol, methanol, UHQ water	Twice with methanol	$NPEO_{1-3}$
SDB-RPS 3	Acetone, 2-propanol, methanol, UHQ water	Methanol, acetone	$NPEO_{1-3}$, NP
SDB-RPS 4	Acetone, 2-propanol, methanol, UHQ water	Methanol	$NPEO_{10}$
SDB-RPS 5	Acetone, 2-propanol, methanol, UHQ water	Twice with methanol	$NPEO_{10}$, NP
SDB-RPS 6	Acetone, 2-propanol, methanol, UHQ water	Methanol (2 min), hexane, MTBE	$NPEO_{10}$, NP
SDB-RPS 7	Acetone, 2-propanol, methanol, UHQ water	Methanol (2 min), hexane, ethyl acetate	$NPEO_{10}$, NP
SDB-RPS 8	Acetone, 2-propanol, methanol, UHQ water	Methanol (2 min), hexane, DCM	$NPEO_{10}$, NP
SDB-RPS 9	Acetone, 2-propanol, methanol, UHQ water	Twice with methanol, hexane	$NPEO_{10}$, NP
SDB-RPS 10	Acetone, 2-propanol, methanol, UHQ water	Twice with methanol, hexane, acetone	$NPEO_{10}$, NP
SDB-XC 1	Acetone, 2-propanol, methanol, UHQ water	Methanol	$NPEO_{1-3}$
SDB-XC 2	Acetone, 2-propanol, methanol, UHQ water	Twice with methanol	$NPEO_{1-3}$
SDB-XC 3	Acetone, 2-propanol, methanol, UHQ water	Methanol, acetone	$NPEO_{1-3}$, NP
SDB-XC 4	Acetone, 2-propanol, methanol, UHQ water	Methanol	$NPEO_{10}$
SDB-XC 5	Acetone, 2-propanol, methanol, UHQ water	Twice with methanol	$NPEO_{10}$, NP
SDB-XC 6	Acetone, 2-propanol, methanol, UHQ water	Twice with methanol, hexane	$NPEO_{10}$, NP
SDB-XC 7	Acetone, 2-propanol, methanol, UHQ water	Methanol (2 min), hexane, acetone	$NPEO_{10}$, NP
C-18 1	Soaked in methanol for 20 min, UHQ water	Methanol (2 min), hexane, acetone	$NPEO_{1-3}$
C-18 2	Soaked in methanol for 20 min, UHQ water	Methanol (2 min), hexane, acetone	$NPEO_{10}$, NP
C-18 3	Soaked in methanol for 20 min, UHQ water	Methanol (2 min), hexane, DCM	$NPEO_{10}$, NP

The optimised procedures for extracting the Empore disks are presented in Table 11. These solvent combinations are used for disks deployed in the following laboratory and field trials.

Table 11 Optimised extraction procedure

Empore disk	Conditioning	Elution
SDB-RPS	Acetone, 2-propanol, methanol, UHQ water	Methanol (2 min), hexane, DCM
SDB-XC	Acetone, 2-propanol, methanol, UHQ water	Methanol (2 min), hexane, acetone
C-18	Soaked in methanol for 20 min, UHQ water	Methanol (2 min), hexane, DCM

4.7 Laboratory tests and field trials

The laboratory and field trials performed in this study are summarised in Table 12. The tests included laboratory trials for different receiving phases with and without a diffusion limiting membrane. Several field trials were conducted as well. The procedures are described in more detail in the following chapters.

Table 12 Summary of the laboratory tests and field trials

Trial	Receiving phase	Deployment time	Compounds	Remarks
Laboratory, pre-test	SDB-RPS, SDB-XC, C-18	6 days	$NPEO_{1-3}$, $NPEO_{10}$, NP	No membrane, membrane 1[a] or membrane 2[b]
Laboratory trials	SDB-RPS, SDB-XC, C-18	1, 2, 3, 5, 7 and 9 days	$NPEO_{1-3}$, $NPEO_{10}$, NP	
Laboratory trial in brackish water	SDB-RPS, C-18	5, 7, 9, 12, 14 and 16 days	$NPEO_{10}$	
Laboratory trial, without flow-through	SDB-RPS, SDB-XC	7, 14, 21 and 28 days	$NPEO_{10}$, NP	
Field trial 2007	C-18	2 + 4 weeks	NPEO	Kuusaankoski
Field trial 2008	SDB-RPS, SDB-XC, C-18	2 + 4 weeks	NPEO	Kuusaankoski
Field trial 2009	SDB-RPS, SDB-XC, C-18	2 + 4 weeks	NPEO	Kuusaankoski, Kymijoki
Field trial 2010	SDB-RPS, SDB-XC, C-18	2 + 4 weeks	NPEO	Kymijoki
Gulf of Finland	SDB-RPS, SDB-XC, C-18	3 weeks	NPEO	3 sites
Gulf of Bothnia	SDB-RPS, SDB-XC	3 months	NPEO	4 sites of which 2 reference sites

[a] 40 µm thick LDPE.

[b] 50 µm thick PE.

4.7.1 Exposure of passive samplers, UHQ water

The laboratory experiments were carried out in a controlled water temperature at 14°C. The trials were performed in separate glass tanks spiked

with a mixture containing long chain ethoxymers ($NPEO_{10}$) or with short chain oligomers ($NPEO_{1-3}$) and NP.

The Chemcatchers® were exposed in a constant concentration flow-through exposure system (Fig. 8). The system consisted of a 60 L glass tank with an overflow to waste. The UHQ water and the solution of test analytes dissolved in UHQ water were delivered into the exposure tank separately at known and controlled rates. Water was fed into the exposure tank using a peristaltic pump at 1.5 L h^{-1}. A standard solution of analytes in UHQ water was delivered into the exposure tank using a second peristaltic pump with a flow rate of 0.16 mL min^{-1}. The system was mixed with a stirrer. Prior to each exposure, the system was operated without samplers to stabilise the concentration of analytes in the tank. Three different receiving phases were deployed (SDB-RPS, SDB-XC and C-18) and the effect of diffusion-limiting membrane on the accumulation of compounds was also studied. The material of membrane 1 was 40 µm thick LDPE and membrane 2 was made of 50 µm thick PE. The membrane was placed on top of the receiving phase (Fig. 2) and the results were compared to the amounts detected in uncovered Empore disks.

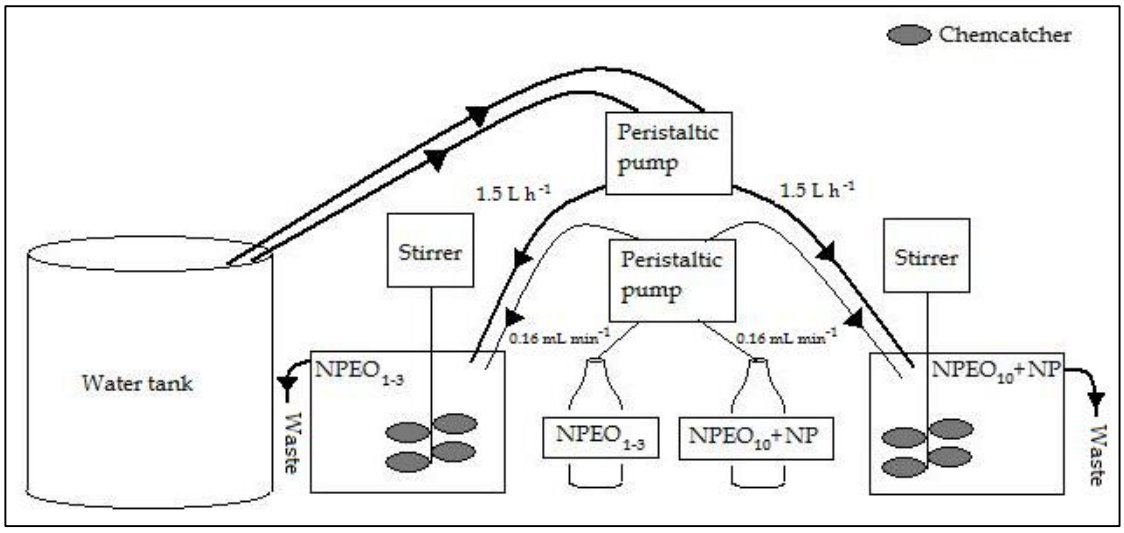

Figure 8 Exposure of Chemcatchers® in laboratory trials.

4.7.2 Exposure of passive samplers, brackish water

A laboratory test using brackish water was carried out at the Tvärminne Zoological Station. The samplers were placed into a flow-through system with standard solutions of $NPEO_{10}$ and NP as described in Chapter 4.7.1. Brackish water delivered from the Baltic Sea was used in this trial instead of UHQ water. Two types of Chemcatchers ® with different receiving phases, C-18 and SDB-RPS, were deployed in separate glass tanks. Three replicate samplers were retrieved at the same time. No diffusion-limiting membrane was used.

4.7.3 Exposure of passive samplers without flow-through conditions

The exposure test without a flow-through system was performed in laboratory. Two glass tanks were filled with UHQ water and spiked with standard solutions of $NPEO_{10}$ and NP. The system was stirred and kept in a temperature controlled room at 14°C. The trial was performed with SDB-RPS and SDB-XC disks and four samplers of each type were deployed in both tanks. At the beginning the concentration was equal in both tanks. Two replicate samplers were removed every week, in the first two weeks from tank 1 and in the last two weeks from tank 2. The results are expressed as the average of two replicates.

4.7.4 Field deployment of passive samplers

The Chemcatcher® passive samplers were also exposed in natural waters. Kuusaankoski (Fig. 9 A) was selected for the field trial site due to the influence of the pulp and paper industry, the chemical industry and treated waste water from the cities of Äänekoski and Suolahti. In central Finland, NPEOs are generally found at this sampling site. The second sampling site was located at the Kymijoki river in south-east Finland (Fig. 9 B), which receives discharges from the pulp and paper industry and treated waste water from municipal and industrial sources. The samplers were also exposed in three sites in the Gulf of Finland near the city of Kotka (Fig. 10). The deployment lasted for two or four weeks at the Kuusaankoski and Kymijoki sites, and for three weeks in the Gulf of Finland. The samplers were also exposed in the Gulf of Bothnia for three months at four sampling sites near the cities of Gävle and Sundsvall (Fig. 11). Two of these locations were reference sites, presumably with less pollution and further from the cities.

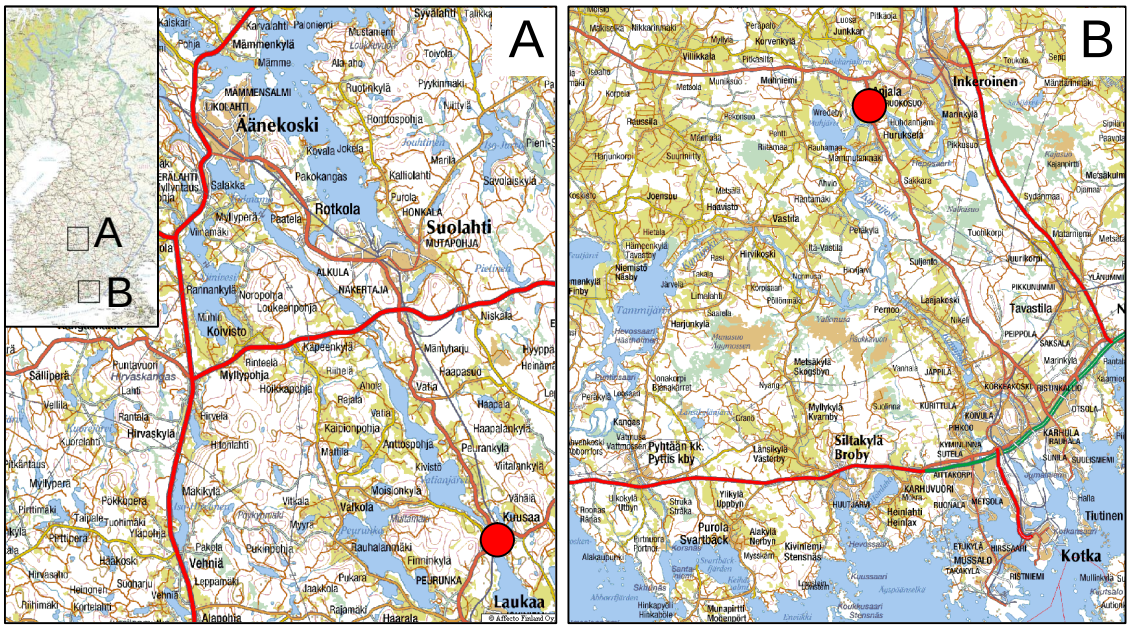

Figure 9 Location of the sampling sites of Kuusaankoski (A) and Kymijoki (B). (Map Service, 2011)

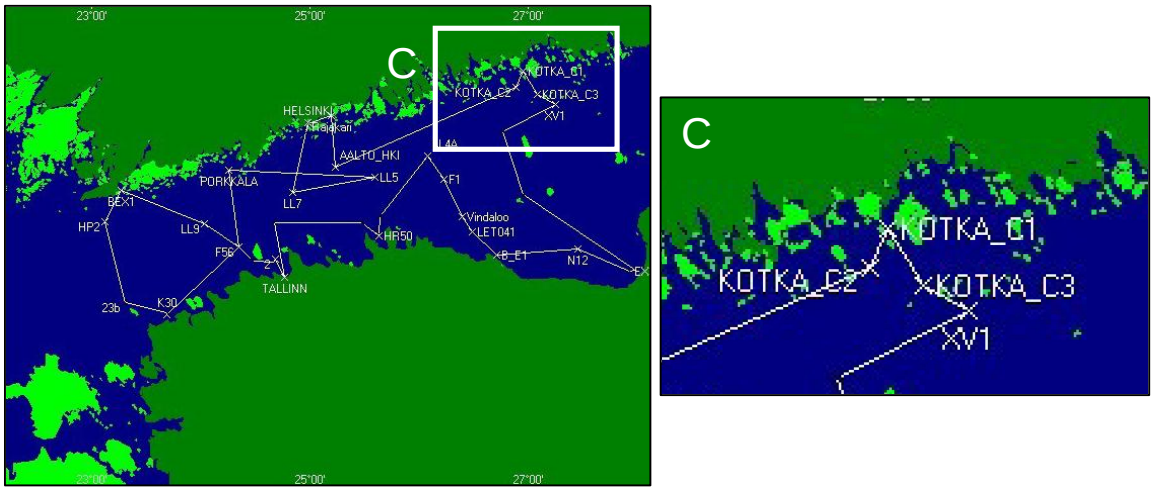

Figure 10 Sampling sites in Gulf of Finland [GOF-IA Cruise Report, 2009].

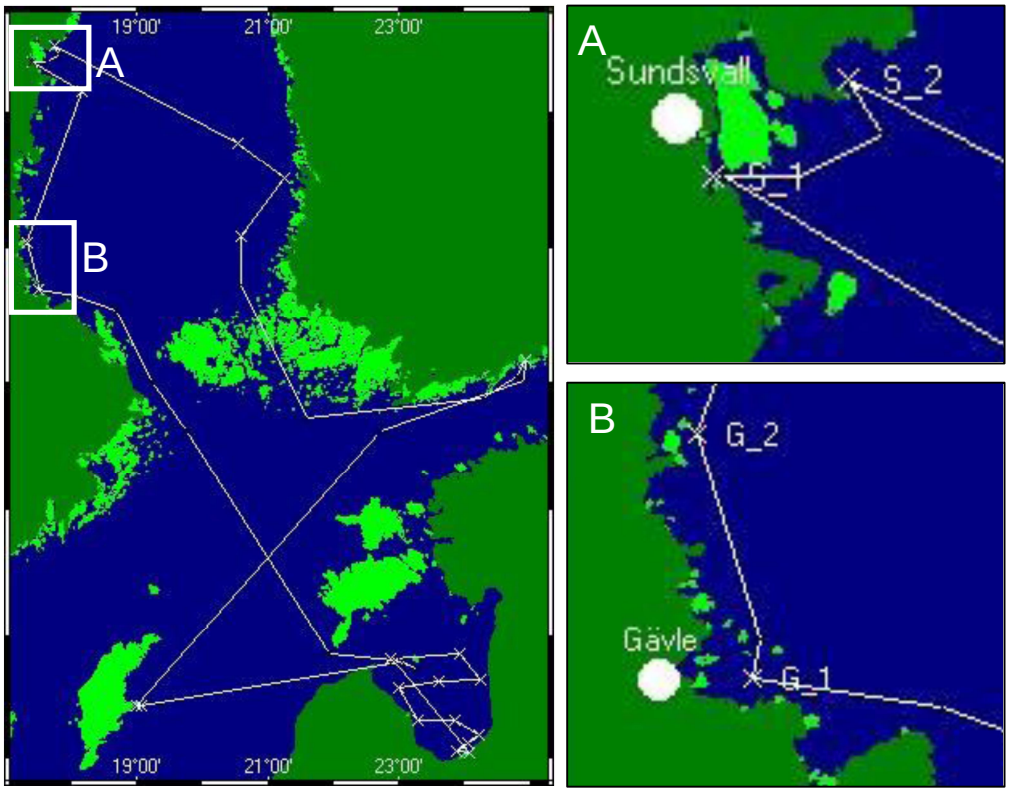

Figure 11 Sampling sites in Gulf of Bothnia [BEAST Cruise Report, 2010].

Before deployment the samplers were prepared as described in Chapter 4.5. Three replicate samplers were placed in Kuusaankoski for two or four weeks in August during the years 2007-2009. In 2007 only one type of receiving phase was used. Three replicate samplers were placed at the sampling site at the beginning of the field trial. Three of the samplers were collected after 14 days of exposure, the remaining three after 28 days.

In the years 2009-2010, three different receiving phases were deployed with three replicate samplers at the Kymijoki sampling site. In the Gulf of Finland there were three sampling sites in 2009 (Fig. 10). Site 1 was near the city of Kotka, site 2 was situated south-west of Kotka and site 3 was south-east of

Kotka. The sampling lasted three weeks and there were three replicate samplers of each phase at site 1. At sites 2 and 3 only one sampler of each type was deployed. The trial in the Gulf of Bothnia in 2010 lasted three months at two sampling sites and two reference sites (Fig. 11). The Sundsvall 1 and Gävle 1 sites were located close to cities, but the Sundsvall 2 and Gävle 2 sites were about 40 km away from urban centres. The reference sites were also located near the shore.

Before deployment the transportation lid was removed and the sampler was attached to a cage with a cable tie. The lid was stored in the zip-lock bag. After the sampling period the samplers were retrieved, closed with the transportation lid and enclosed in a zip-lock bag. They were stored in a cool box during transportation and kept at 4°C until analysis.

4.8 Extraction method for the C-18, SDB-RPS and SDB-XC receiving phases

After retrieval the sampler was disassembled and the Empore disk (C-18, SDB-RPS) was placed in a 12-mL Kimax tube using forceps. The disk was soaked in 3 mL of methanol in an ultrasonic bath for 2 min and the extract was filtered through a 0.45-µm PTFE syringe filter into another Kimax tube. The disk was then soaked in 3 mL of hexane in an ultrasonic bath for 1 min and the extract was filtered through the same syringe filter. Finally, the disk was soaked in DCM and the eluent was filtered and combined with previous extracts. The Kimax tube was rinsed with 1 mL of methanol and the solvent was added to the extract. The residue was evaporated under a nitrogen stream and dissolved in 300 µL ACN/50 mM NH_4Ac (v/v, 50:50). The disks exposed at the Kuusaankoski sampling site in 2007 were extracted with methanol only. The procedure with an SDB-XC disk was otherwise the same as described above, but the last extraction was performed with acetone instead of DCM.

4.9 Extraction of analytes from water samples

The spot samples were taken at the Kuusaankoski and Kymijoki sites. The water samples were filtered with What man 42 filter paper before SPE extraction. The SPE cartridges (Bond Elut C-18 LO, 500mg/3 mL, Varian) were conditioned with 3 mL of methanol and 3 mL of UHQ water in a vacuum manifold. After the sample was passed through the cartridge the sorbent was vacuum-dried for 30 minutes. The analytes were extracted sequentially with 3 mL each of methanol, acetone and hexane, and the combined extract was evaporated under a nitrogen stream. The residue was dissolved in 300 µL of ACN/50 mM NH_4Ac (v/v, 50:50) and the sample was analysed by HPLC/ESI-MS.

5 RESULTS AND DISCUSSION

5.1 Pre-test of SPE

The isolation and concentration of NPEOs and NP from water samples were performed using SPE. A pre-test of the SPE extraction was carried out under various eluent conditions presented in Table 9. This rough and rapid screening was conducted without replicate measurements. The recoveries are presented in three groups: long chain NPEOs ($NPEO_{10}$; Fig. 12), short chain NPEOs ($NPEO_{1-3}$; Fig. 13) and NP (Fig. 14). Based on these tests the most suitable SPE treatment procedure was selected and used in further experiments.

Extracting the Bond Elut C-18 LO cartridge with methanol (SPE 4 in Fig. 12) gave good recoveries for $NPEO_{10}$, but the recovery was much lower with the Oasis HLB sorbent (SPE 23 in Fig. 12). In both SPE 3 and SPE 4 (Fig. 12), with good recoveries, the Bond Elut C-18 LO cartridge was eluted using methanol. A sequential elution with methanol, hexane and acetone gave the highest recoveries, and the adjustment of pH did not significantly affect the results (SPE 24 and SPE 25 in Fig. 12). An experiment was performed where ethylene glycol was added to the extract before nitrogen evaporation to observe if the NPEOs were vapourised during the procedure. However, the addition of this keeper did not improve the results (SPE 20 and SPE 21 in Fig. 12). Good recoveries were obtained when the longer chain ethoxymers are eluted with methanol from a Bond Elut C-18 LO cartridge.

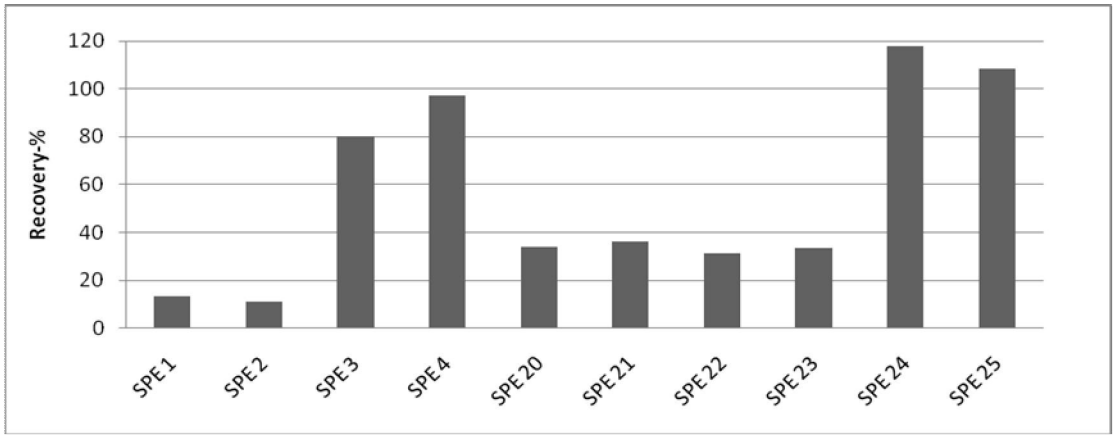

Figure 12 $NPEO_{10}$ recoveries obtained by the SPE pre-tests (see Table 9).

For the short chain ethoxymers, the acetone elution from the Bond Elut C-18 LO phase appeared to be more efficient (SPE 6 in Fig. 13) when compared to methanol treatment alone (SPE 5 in Fig. 13). Again, the addition of ethylene glycol did not improve the recoveries (SPE 6 and SPE 7 in Fig. 13), which indicates the non-volatile character of $NPEO_{1-3}$ during nitrogen evaporation.

The extraction gave good recoveries when acetone was one of the solvents (SPE 6-7, SPE 11, SPE 14-15 and SPE 19 in Fig. 13).

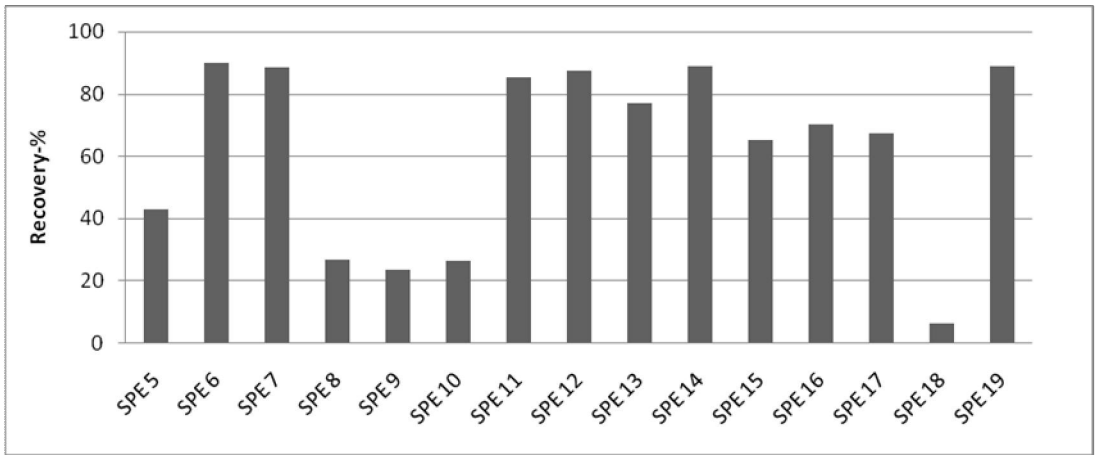

Figure 13 $NPEO_{1-3}$ recoveries obtained by SPE pre-tests (see Table 9).

The SPE treatment using Bond Elut C-18 LO phase gave the best recoveries for NP (SPE 11-14, SPE 24-25, Fig. 14). A pH adjustment of the water sample did not influence the recoveries (SPE 24 and SPE 25 in Fig. 14). There was some background interference in natural water samples in the NP determination. Because this study was mainly focused on the monitoring of NPEOs, the recoveries of NP remained at the low level.

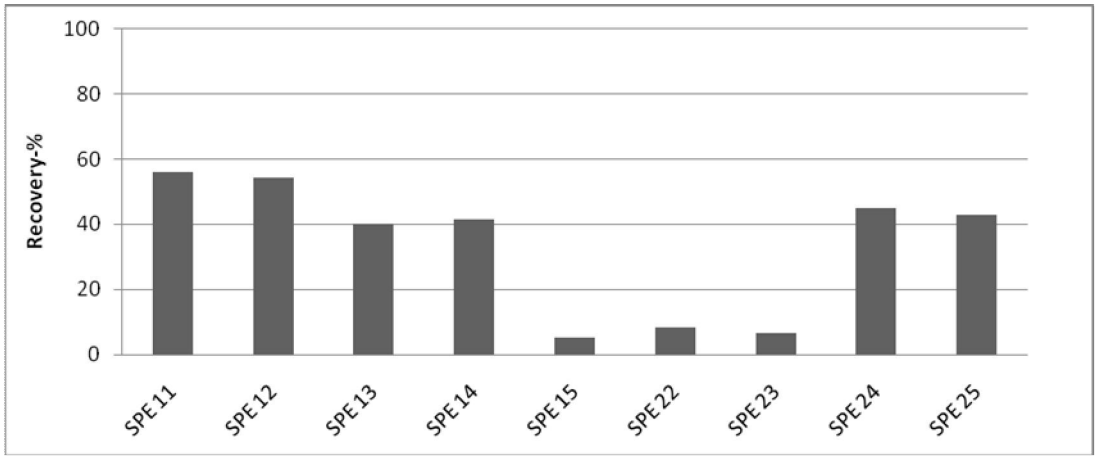

Figure 14 NP recoveries obtained by SPE pre-tests (see Table 9).

In conclusion, the most suitable SPE method for the isolation and concentration of all these analyte groups appeared to be an extraction of a Bond Elut C-18 LO cartridge with methanol followed by hexane and acetone. This procedure was selected for further extractions.

5.2 Pre-test of Empore disk extraction

A conditioned Empore disk, used as a receiving phase of the Chemcatcher®, was spiked directly with a known amount of standard solution

(see Chapter 4.6). The recoveries in the Empore disk extraction tests are presented in three groups: long chain NPEOs ($NPEO_{10}$; Fig. 15), short chain NPEOs ($NPEO_{1-3}$; Fig. 16) and NP (Fig. 17). The eluent conditions of these extractions are presented in Table 10.

The extraction SDB-RPS 8 (Fig. 15) gave the best recovery for $NPEO_{10}$. This procedure included the treatment of the disk with methanol (2 min), hexane and finally DCM. If the final elution step was performed with MTBE (SDB-RPS 6 in Fig. 15), ethyl acetate (SDB-RPS 7 in Fig. 15) or acetone (SDB-RPS 10 in Fig. 15) the recoveries were significantly lower.

The recovery of $NPEO_{10}$ from the SDB-XC adsorbent was the highest when extracted with methanol (2 min), hexane and acetone (SDB-XC 7 in Fig. 15). Sequential methanol processing increased the recovery remarkably (*cf.* SDB-XC 4 and SDB-XC 5 in Fig. 15). The addition of hexane elution further improved the results (SDB-XC 6 in Fig. 15).

When extracting the long chain oligomers from the C-18 phase, the results indicated that DCM treatment (C-18 3 in Fig. 15) gave better recoveries than acetone extraction C-18 2 (in Fig. 15). In both experiments mentioned above the disk was first extracted with methanol and hexane.

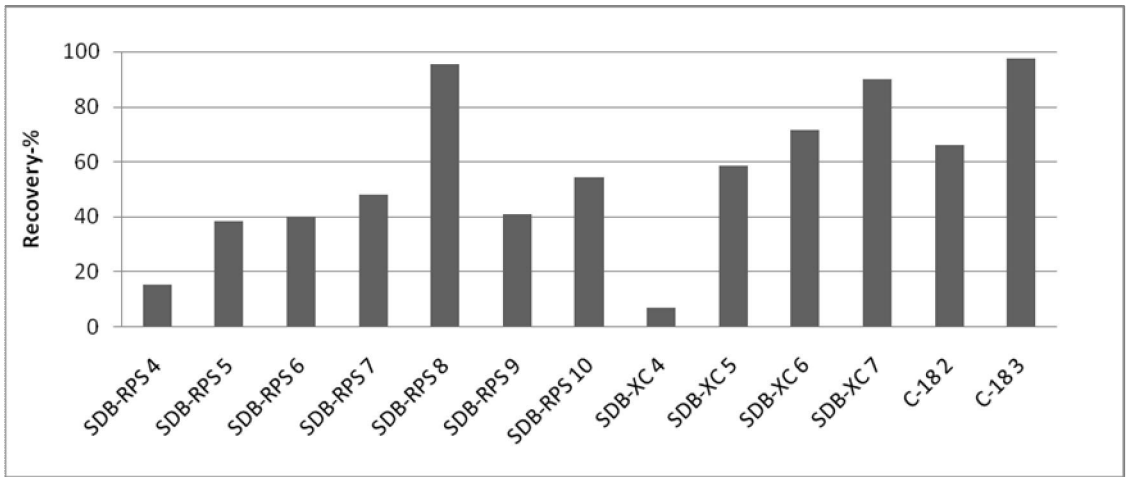

Figure 15 The recoveries of $NPEO_{10}$ extractions from Empore disks (see Table 10).

The extraction of short chain NPEOs ($NPEO_{1-3}$; Fig. 16, Table 10) was the most efficient when the disk was treated twice or for two minutes with methanol. The best recovery was obtained when the C-18 phase was extracted with methanol, hexane and acetone (C-18 1 in Fig. 16). Good recoveries were also achieved in extractions SDB-RPS 2 and SDB-XC 2 where the disks were eluted twice with methanol.

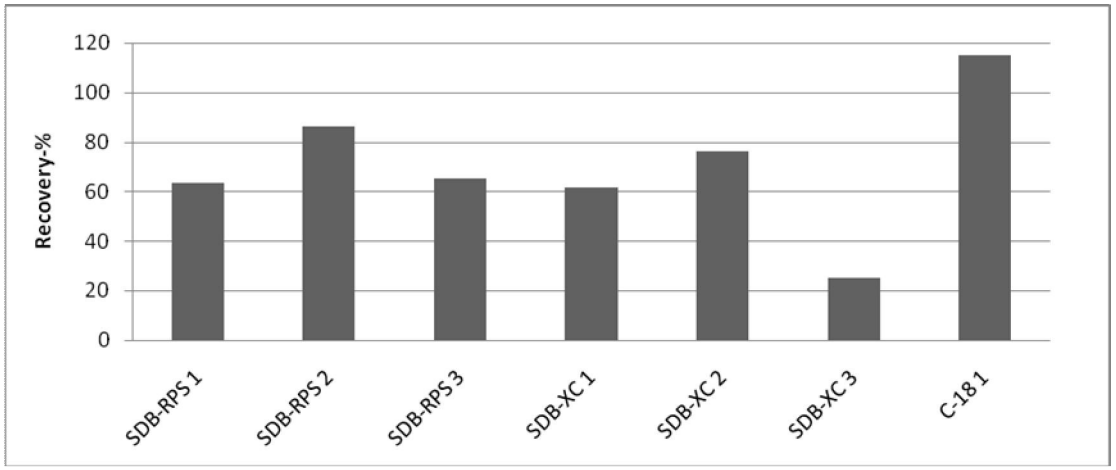

Figure 16 The recoveries of $NPEO_{1-3}$ extractions from Empore disks (see Table 10).

The best NP recoveries were obtained when the SDB-RPS disk was treated with methanol followed by hexane (SDB-RPS 7-10 in Fig. 17, see also Table 10). DCM or acetone as the third solvent gave the best results (SDB-RPS 8 and SDB-RPS 10). Eluting the SDB-XC disk twice with methanol and a further treatment with hexane (SDB-XC 6) followed by acetone (SDB-XC 7) improved the recovery significantly. For the C-18 disk the treatment with DCM (C-18 3) appeared to be more efficient than the acetone elution (C-18 2), when the disk was first extracted with methanol for 2 minutes followed by hexane elution.

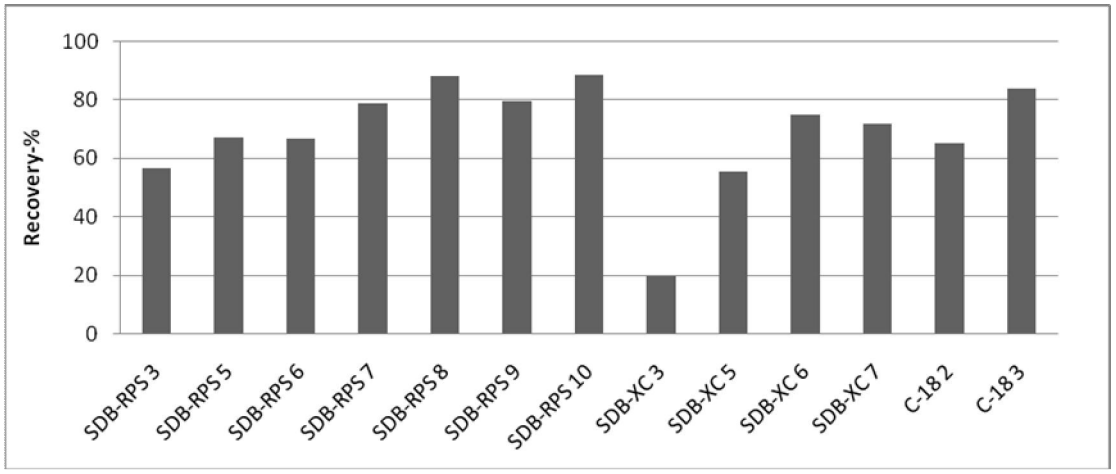

Figure 17 The recoveries of NP extractions from Empore disks (see Table 10).

The results presented in Figs. 15-17 show that a double extraction with methanol improved the recovery of NPEOs, whereas the treatment with hexane improved the recovery of NP. The highest recovery for SDB-RPS disks was obtained when the adsorbent was first extracted twice with methanol, then with hexane and finally with DCM. The same procedure was used for eluting the compounds from C-18 disks. The data obtained from the extraction of SDB-XC disks showed that a repeated methanol treatment improved the recovery of all tested compounds. Still, the highest recoveries for NPEOs were obtained when the duplicate methanol extraction was followed with hexane and acetone

treatment; this practice was used as a general procedure for the extraction of SDB-XC disks. Treating the disk twice with methanol gave the same recoveries as doubling the extraction time (2 min); the latter procedure was used in the following experiments in this study.

The optimised extraction conditions for determining all three groups of compounds are presented in Table 11 and the obtained recoveries are presented in Figure 18. The recoveries were calculated as the average of three replicate measurements and the results suggested that the extraction procedure for SDB-XC disks was less effective than the treatment of the other two disks (Fig. 18). In particular, the recovery of NP was lower with the SDB-XC adsorbent.

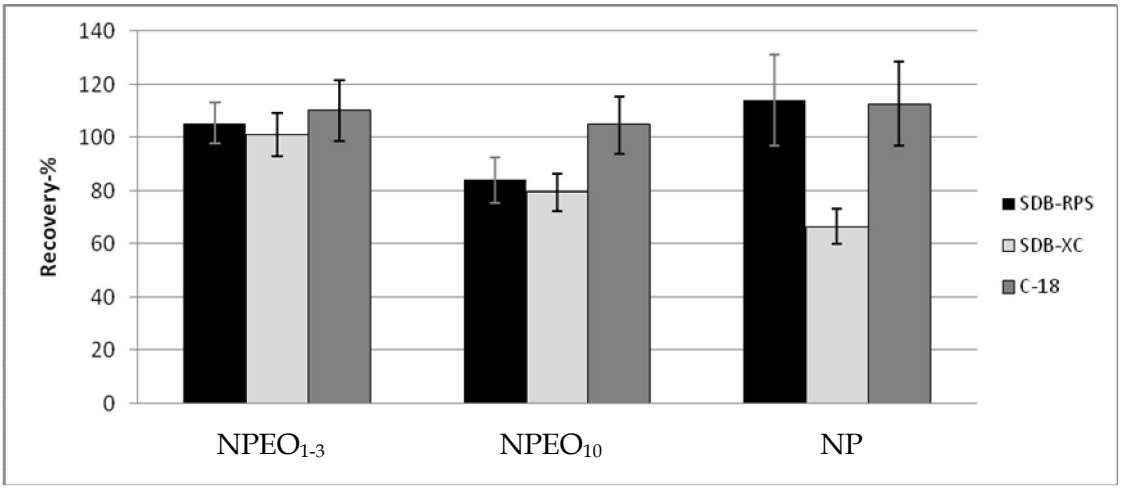

Figure 18 The recoveries of $NPEO_{1-3}$, $NPEO_{10}$ and NP obtained with the optimised extraction procedure (see Table 11).

5.3 Laboratory trials of passive samplers with and without a diffusion-limiting membrane

The passive samplers were exposed in glass tanks for six days, after which the amount of NPEOs and NP accumulated on each sampler was analysed. Samplers for $NPEO_{10}$ and NP were exposed in one tank and the ones for $NPEO_{1-3}$ in another to avoid the mixing of NPEO standards. Two replicate samplers were deployed in the following tests. The setup of the laboratory tests is described in Chapter 4.7.1 and Figure 8.

The data handling was done separately for three groups: $NPEO_{1-3}$, $NPEO_{10}$ and NP. As this was a single laboratory test and the samplers with or without diffusion limiting membranes were deployed simultaneously in the same tank, the results were normalized. This means that the highest amount is considered to be 100% and all the other results are presented with respect to that. This makes the comparison of the figures of one analyte group more convenient.

5.3.1 Accumulation of $NPEO_{1-3}$

The accumulation of $NPEO_{1-3}$ in uncovered Empore disks (without a diffusion limiting membrane) is presented in Figure 19. The highest amount of $NPEO_{1-3}$ was found in the SDB-RPS phase.

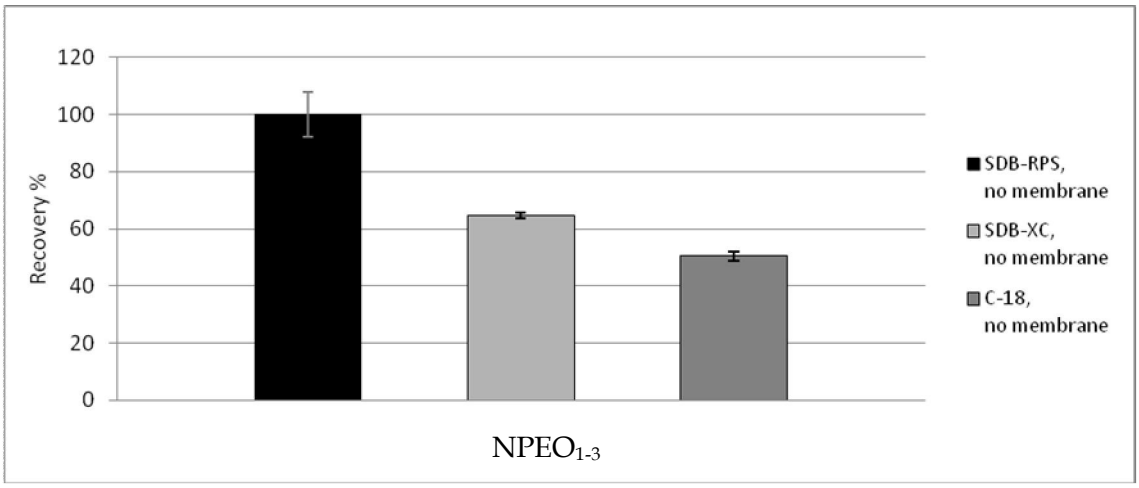

Figure 19 Accumulation of $NPEO_{1-3}$ in uncovered Empore disks.

Adding the diffusion-limiting membrane on top of the Empore disk reduced the uptake rate (Fig. 20), and the amount of the compounds in the disk was lower than in the uncovered ones. Among the membrane-covered receiving phases, the highest content of $NPEO_{1-3}$ was found in the SDB-RPS adsorbent covered with membrane 2 (Fig. 20). The second highest amount was determined in the SDB-XC disk with membrane 1 on top of it. Also, for the C-18 phase, the disk covered with membrane 2 had higher contents than those for the disks with membrane 1 (Fig. 20).

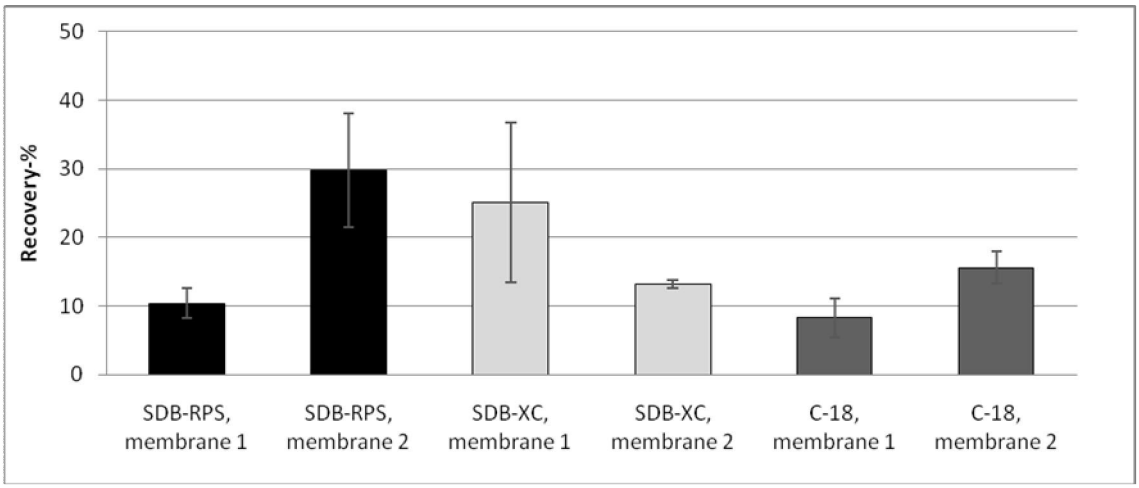

Figure 20 Accumulation of $NPEO_{1-3}$ in Empore disks covered with diffusion-limiting membranes. Membrane 1 was 40 µm thick LDPE and membrane 2 was 50 µm thick PE.

5.3.2 Accumulation of $NPEO_{10}$

The same laboratory trials as shown in Chapter 5.3.1 were performe d for a $NPEO_{10}$ standard mixture; the results showed that these oligomers accumulated best in the SDB-RPS disk (Fig. 21). The SDB-RPS disk overlaid with membrane 1 collected the highest amount of the studied compounds among the membrane-covered disks (Fig. 22). Also, when the disk was covered with membrane 2, the highest contents were found in the SDB-RPS phase (Fig. 22). The results indicated that the SDB-RPS disk would be the most sui table for monitoring $NPEO_{10}$ compounds.

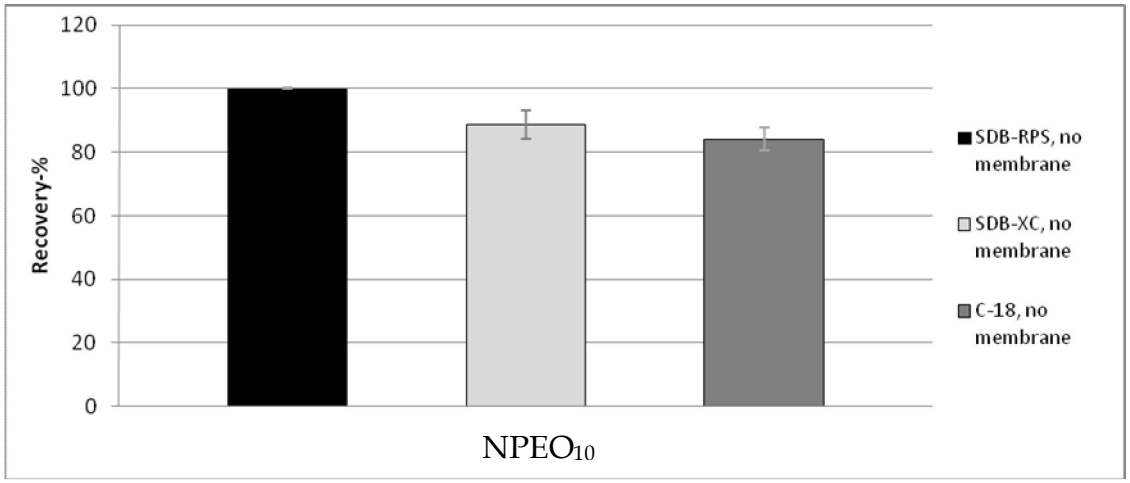

Figure 21 Accumulation of longer chain $NPEO_{10}$ in uncovered Empore disks.

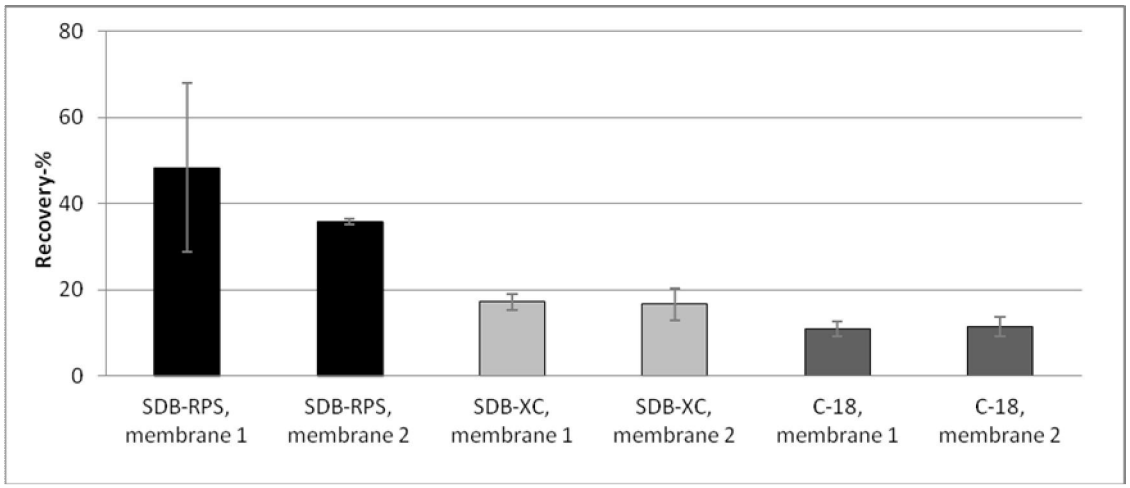

Figure 22 Accumulation of longer chain $NPEO_{10}$ in Empore disks overlaid with diffusion-limiting membranes. Membrane 1 was 40 µm thick LDPE and membrane 2 was 50 µm thick PE.

5.3.3 Accumulation of NP

SDB-RPS disks overlaid with membrane 2 collected NP m ost efficiently (Fig. 23). High contents were also found in uncovered C-18 disks (Fig. 23).

A high amount of NP in membrane-covered disks was not expected due to the diffusion-limiting character of the membrane. Otherwise, the trend was that uncovered disks had more NP accumulated than membrane-covered disks.

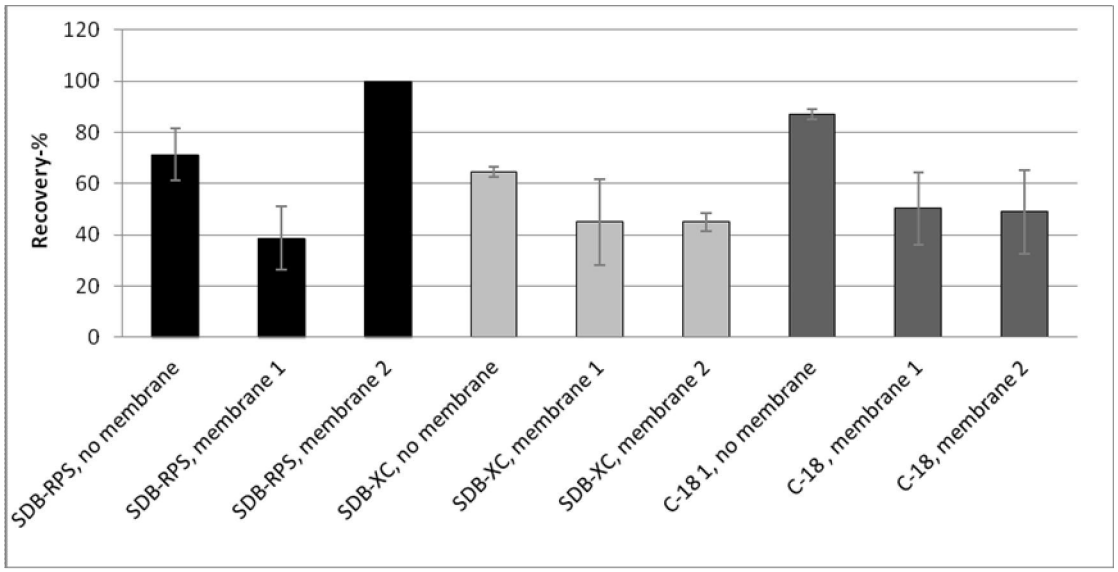

Figure 23 Accumulation of NP in Empore disks with or without diffusion-limiting membranes. Membrane 1 was 40 µm thick LDPE and membrane 2 was 50 µm thick PE.

5.4 Laboratory exposure of passive samplers

The procedure in laboratory exposure experiments with the flow-through of UHQ water and standard solutions is described in Chapter 4.7.1 and Figure 8. Three replicate samplers of each type were retrieved simultaneously after deployment and the results were expressed as an average of those measurements. No diffusion-limiting membrane was used in this study. The test was performed with each disk type for all compound groups simultaneously, $NPEO_{1-3}$ in one tank and $NPEO_{10}$ and NP together in another (Fig. 8).

The concentration of the standards delivered in the tanks were 210 µg L^{-1} for $NPEO_{1-3}$, 162 µg L^{-1} for $NPEO_{10}$, expressed as total amount of mixture, and 141 µg L^{-1} for NP. The calculated concentrations in the tanks were 1335 ng L^{-1} for $NPEO_{1-3}$, 1030 ng L^{-1} for $NPEO_{10}$ and 890 ng L^{-1} for NP.

5.4.1 Accumulation of $NPEO_{1-3}$

The uptake of short chain NPEOs ($NPEO_{1-3}$) was the fastest with the SDB-RPS disk, in which the compounds were accumulated almost linearly (see Figs. 24, 26 and 28). The $NPEO_{1-3}$ concentrations in water samples were

measured only twice, but they were slightly higher than in the trials carried out with the other two adsorbents (Figs. 25, 27 and 29).

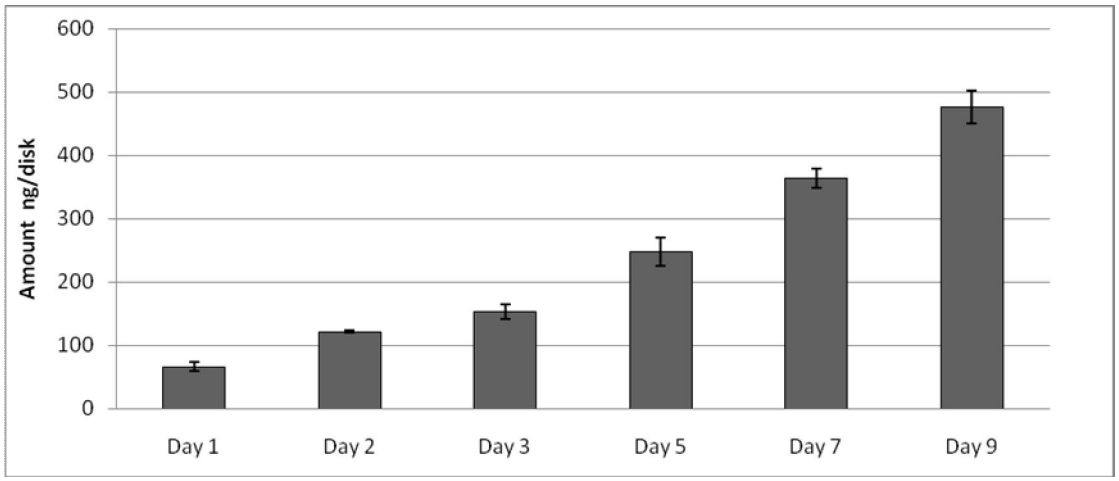

Figure 24 Accumulation of $NPEO_{1-3}$ in the SDB-RPS disks during the laboratory trial

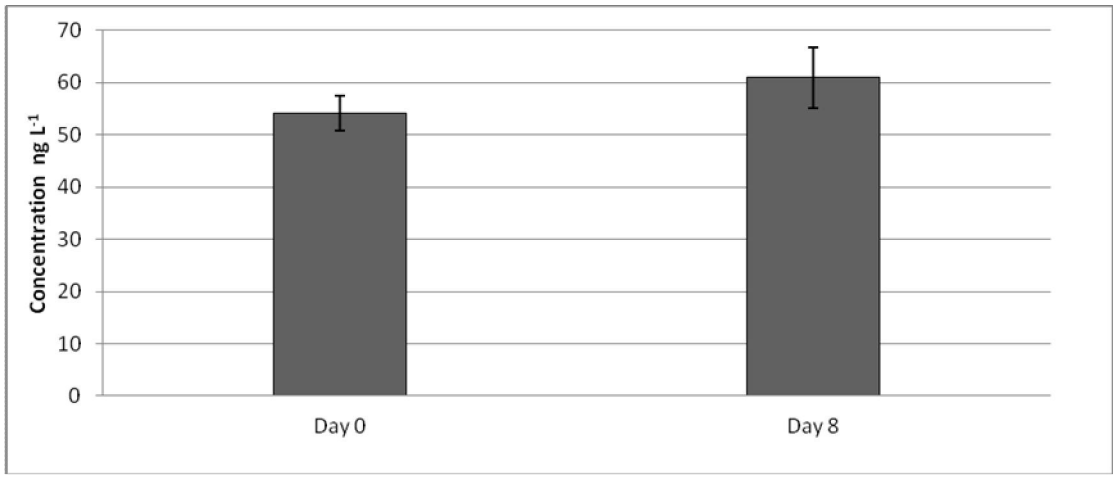

Figure 25 $NPEO_{1-3}$ concentration in water samples during the laboratory trial with the SDB-RPS Empore disks.

The SDB-XC disk appeared to collect the ethoxymers with increasing trend except for the last samplers retrieved (Fig. 26). The lack of one oligomer in the sample had a large effect on the results since the amounts were calculated as a sum of only three oligomers $NPEO_{1-3}$. The contents found in the water samples seemed to be higher at the end of the test (Fig. 27). This suggested that the samplers placed in the tank on day 0 trap a large amount of NPEOs at first, which caused a temporary drop in their water concentration (Fig. 27). This will be balanced due to the flow-through conditions. In general, the concentrations of the short chain ethoxylates in water samples appeared to be approximately at the same level in both SDB-RPS (Fig. 25) and SDB-XC (Fig. 27) trials when compared to the contents in the water samples where the C-18 disks were tested (Fig. 29).

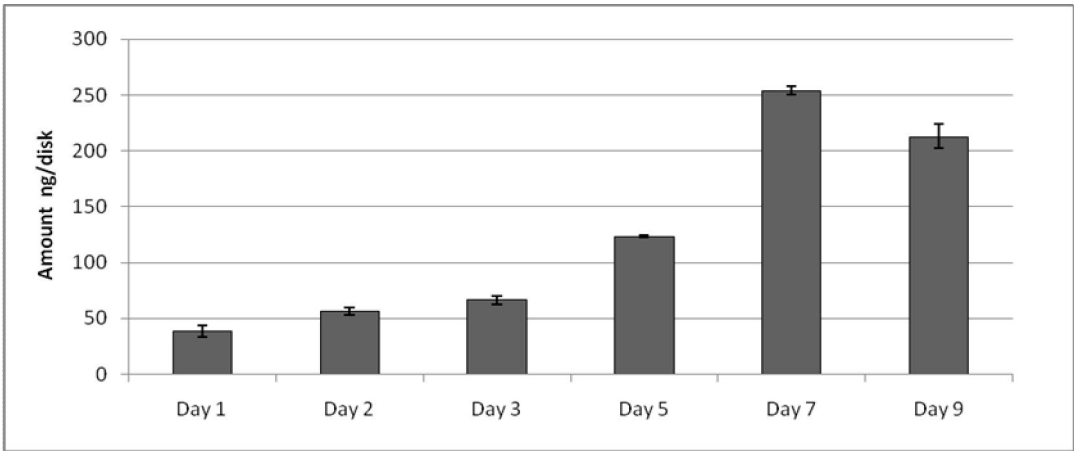

Figure 26 Accumulation of $NPEO_{1-3}$ in the SDB-XC disks during the laboratory trial.

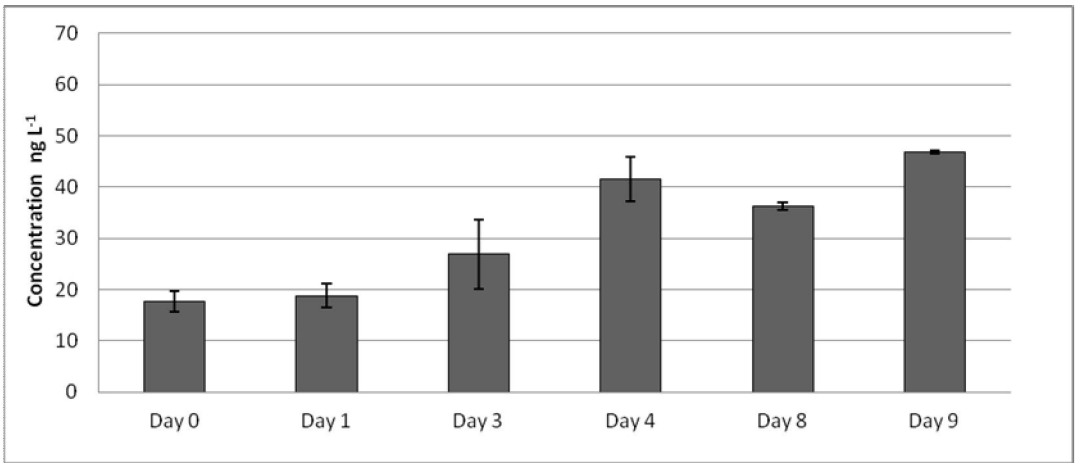

Figure 27 $NPEO_{1-3}$ concentration in water samples during the laboratory trial with the SDB-XC Empore disks.

The content of $NPEO_{1-3}$ adsorbed in the C-18 disks increased with exposure time (Fig. 28). During the first three days of sampling the content found in the C-18 phase was lower than that measured in other disk types (Figs. 24, 26 and 28). However, the concentrations measured in the tank water were low during this test (Figs. 25, 27 and 29).

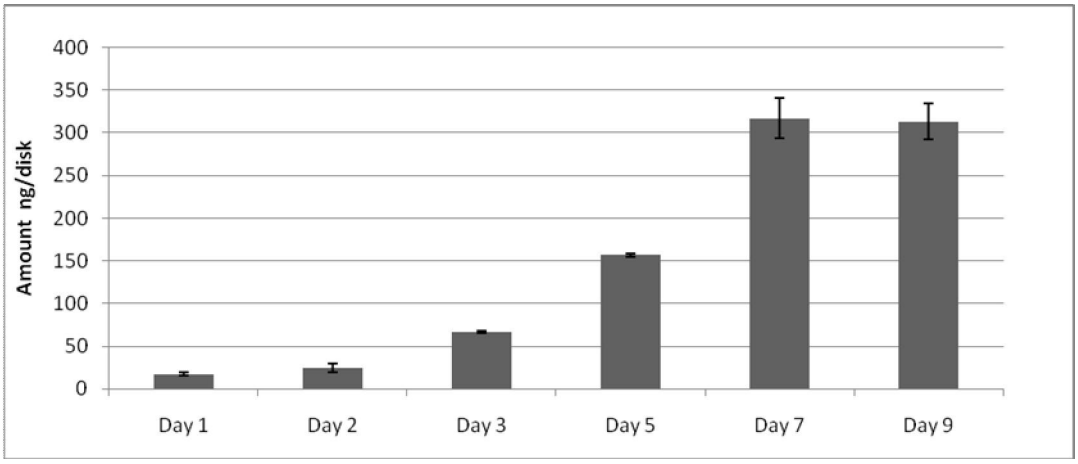

Figure 28 Accumulation of $NPEO_{1-3}$ in the C-18 disks during the laboratory trial.

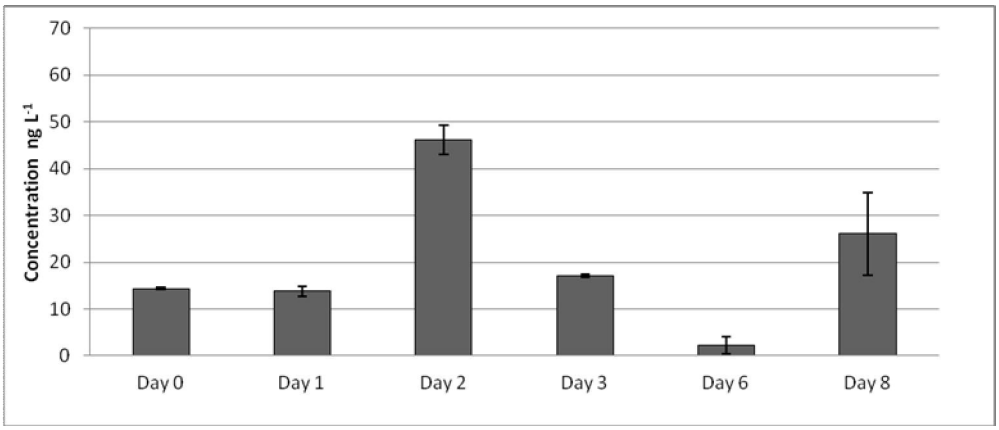

Figure 29 $NPEO_{1-3}$ concentration in water samples during the laboratory trial with the C-18 Empore disks.

5.4.2 Accumulation of $NPEO_{10}$

The $NPEO_{10}$ amounts found in the SDB-RPS disks increased with time during the entire sampling period (Fig. 30), as observed also in the trial with short chain ethoxylates (Fig. 24). Water samples were taken only twice during the laboratory test (Fig. 31).

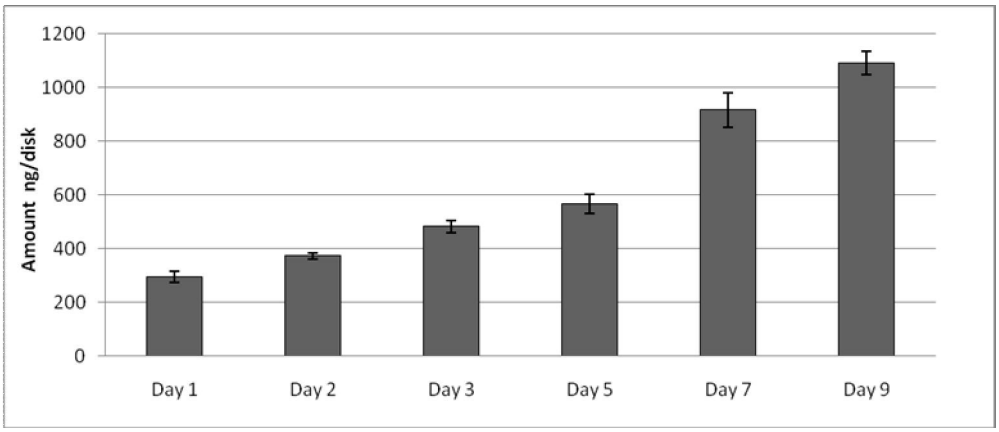

Figure 30 Accumulation of $NPEO_{10}$ in the SDB-RPS disks during the laboratory trial.

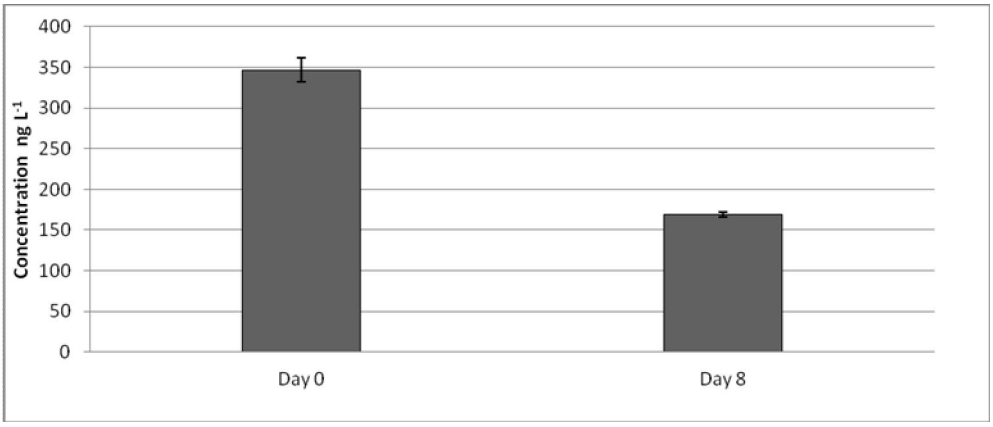

Figure 31 $NPEO_{10}$ concentration in water samples during the laboratory test with the SDB-RPS Empore disks.

The accumulation of $NPEO_{10}$ in the SDB-XC receiving phase increased as well (Fig. 32), and the amounts determined in the disks were higher than those in the trials with the other two disk types (Figs. 30 and 34). The $NPEO_{10}$ concentration in water was also slightly higher than that in other trials (Figs. 33, 31 and 35), but the fluctuations of water concentration were not observed to have an effect on the sampling (Figs. 32 and 33).

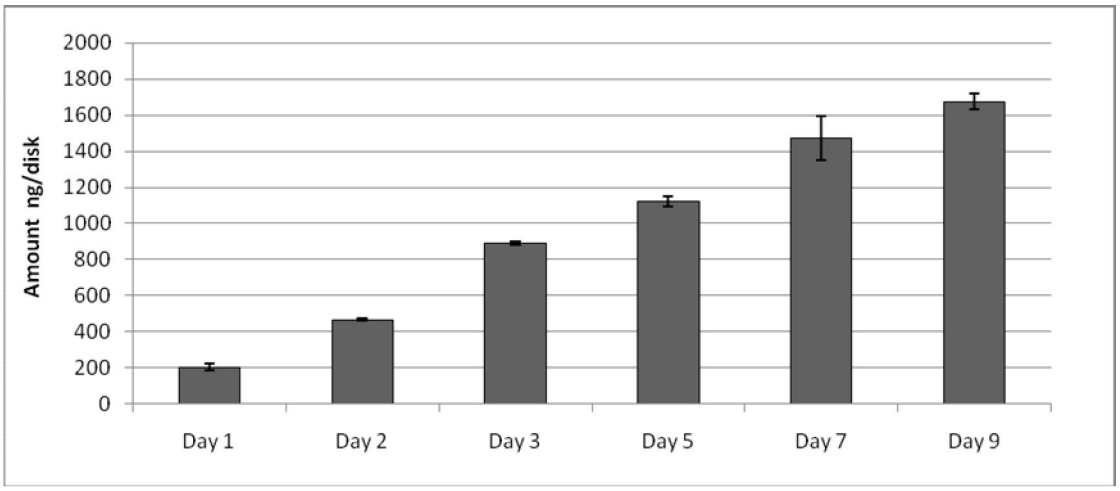

Figure 32 Accumulation of $NPEO_{10}$ in the SDB-XC disks during the laboratory trial.

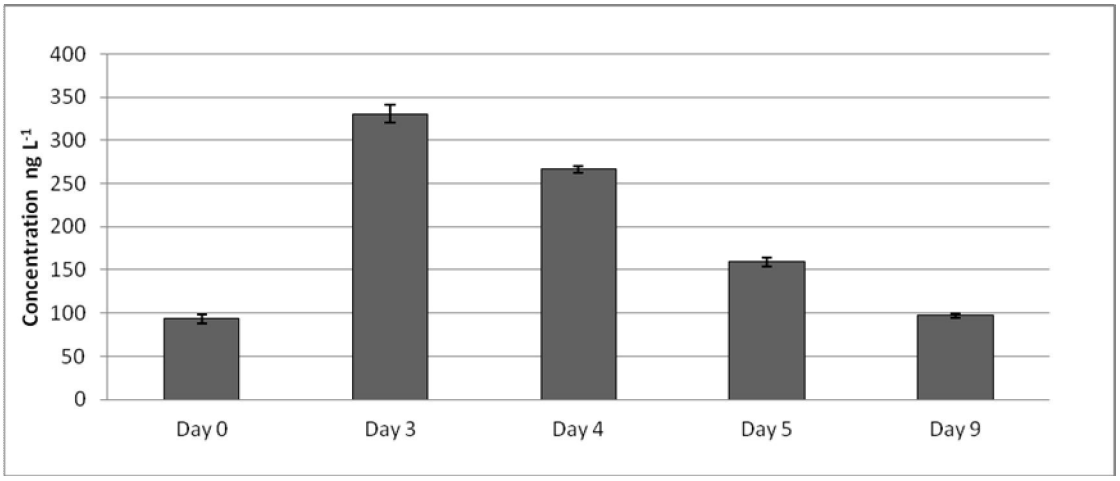

Figure 33 $NPEO_{10}$ concentration in water samples during the laboratory test with the SDB-XC Empore disks.

The content of $NPEO_{10}$ in the C-18 adsorbent increased with exposure time (Fig. 34). The highest $NPEO_{10}$ concentration in water was measured on day 8 when it had almost doubled compared to the three previous sampling times (Fig. 35). Of these laboratory tests the highest $NPEO_{10}$ concentration in water was measured in the SDB-XC trial (Fig. 33) and the lowest content during the C-18 trial (Fig. 35).

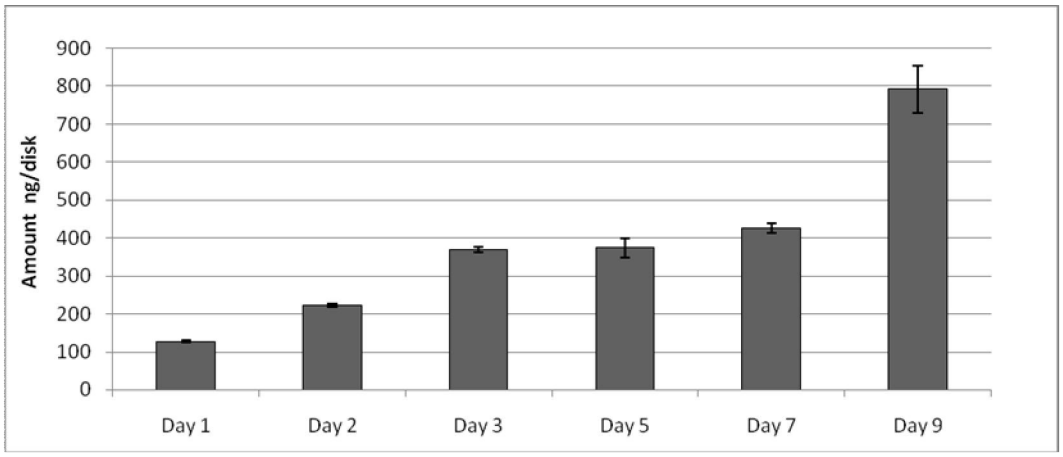

Figure 34 Accumulation of $NPEO_{10}$ in the C-18 disks during the laboratory trial.

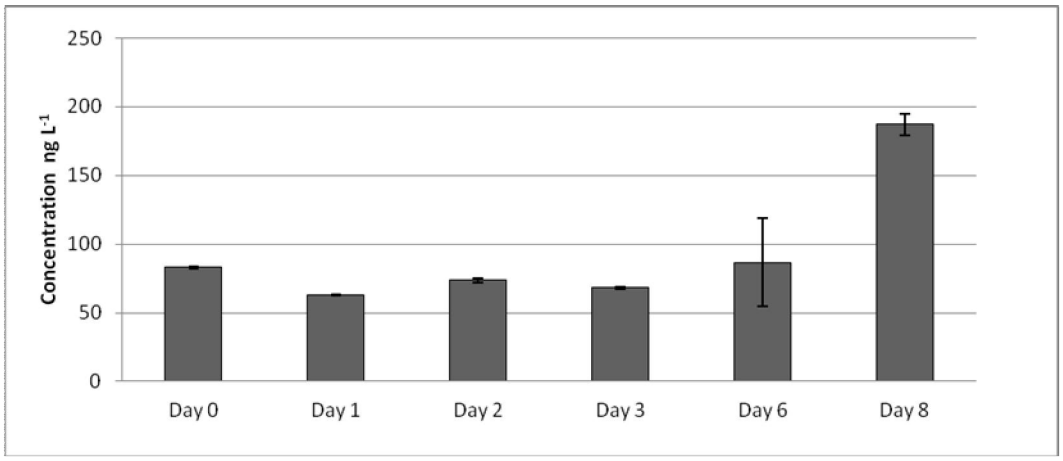

Figure 35 $NPEO_{10}$ concentration in water samples during the laboratory test with the C-18 Empore disks.

5.4.3 Accumulation of NP

The accumulation of NP in SDB-RPS disks was slow in the first four sampling batches, but it increased towards the end of the trial (Fig. 36). The water samples were taken only twice during the test (Fig. 37).

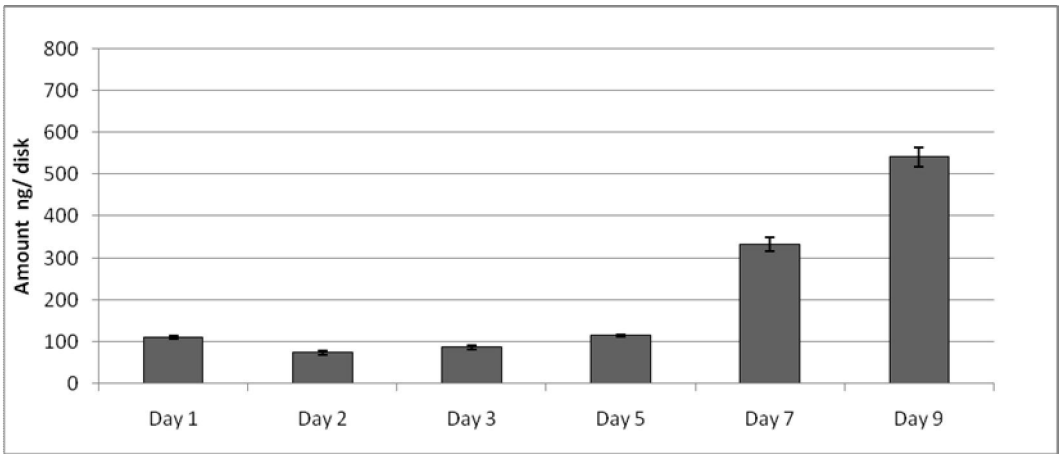

Figure 36 Accumulation of NP in the SDB-RPS disks during the laboratory trial.

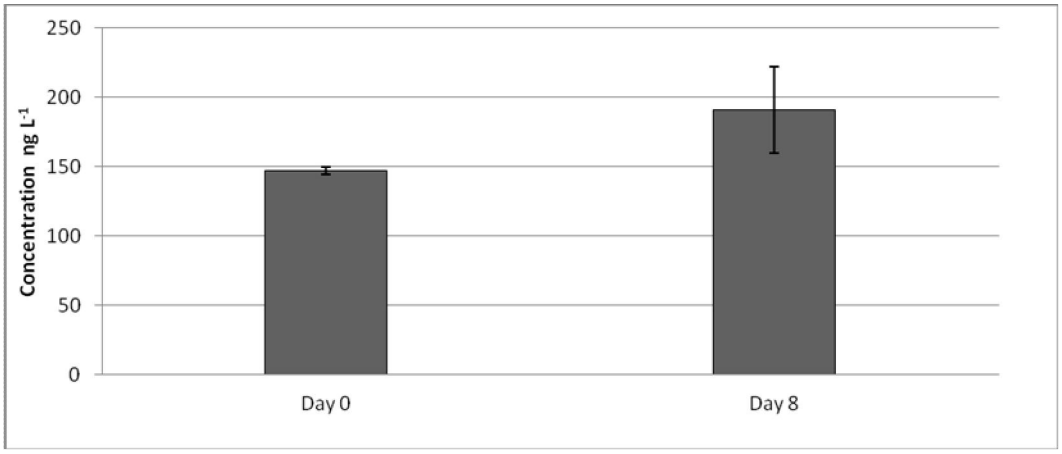

Figure 37 NP concentration in water samples during the laboratory test with the SDB-RPS Empore disks.

The test carried out with SDB-XC receiving phase showed more efficient accumulation than with the SDB-RPS disks (Figs. 38 and 36). The water concentration of the studied compound decreased in the last two spot sampling sets (Fig. 39).

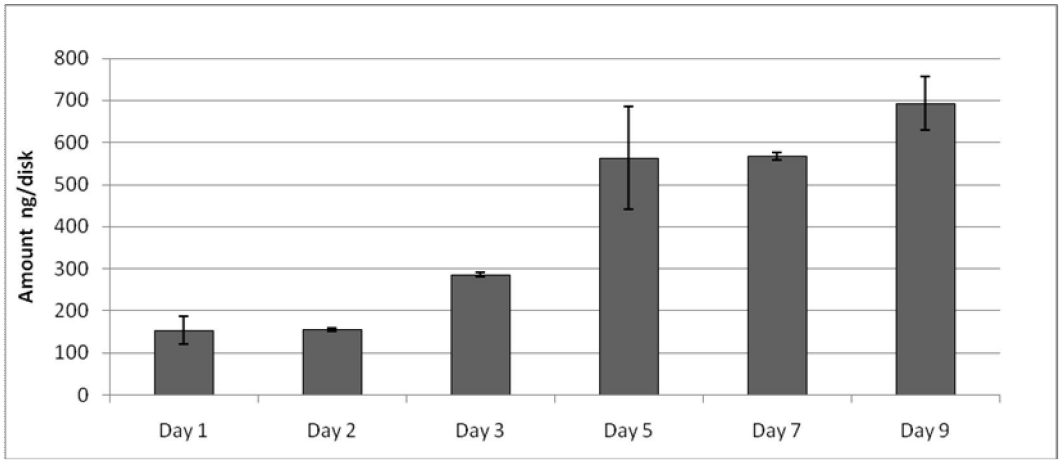

Figure 38 Accumulation of NP in the SDB-XC disks during the laboratory trial.

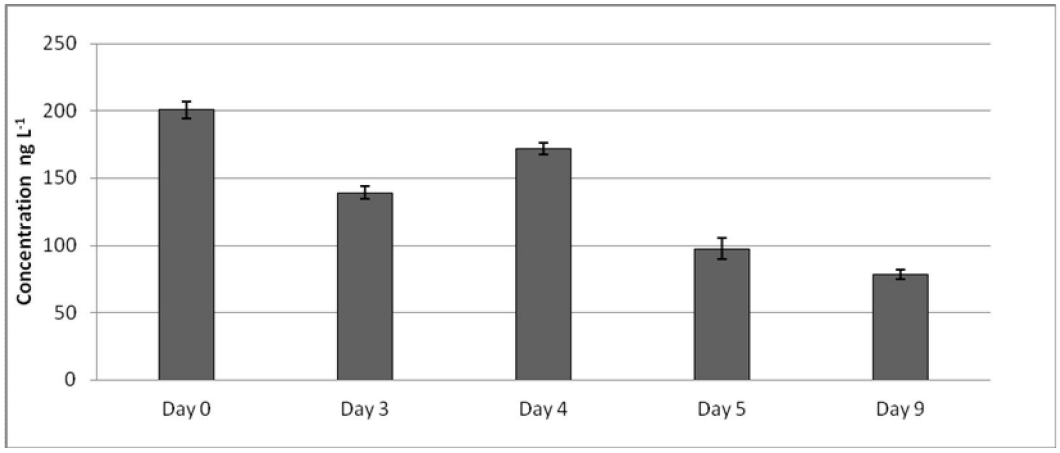

Figure 39 NP concentration in water samples during the laboratory test with the SDB-XC disks.

The accumulation of NP in the C-18 disks was slow in the first four sampler batches (Fig. 40). However, in the last two sets the contents measured were at about the same level as the other two disks (Figs. 40, 38 and 36). The trend was similar in SDB-RPS exposure. The concentration of NP in water during the C-18 exposure test was slightly lower (Fig. 41) than in the other trials (Figs. 37 and 39).

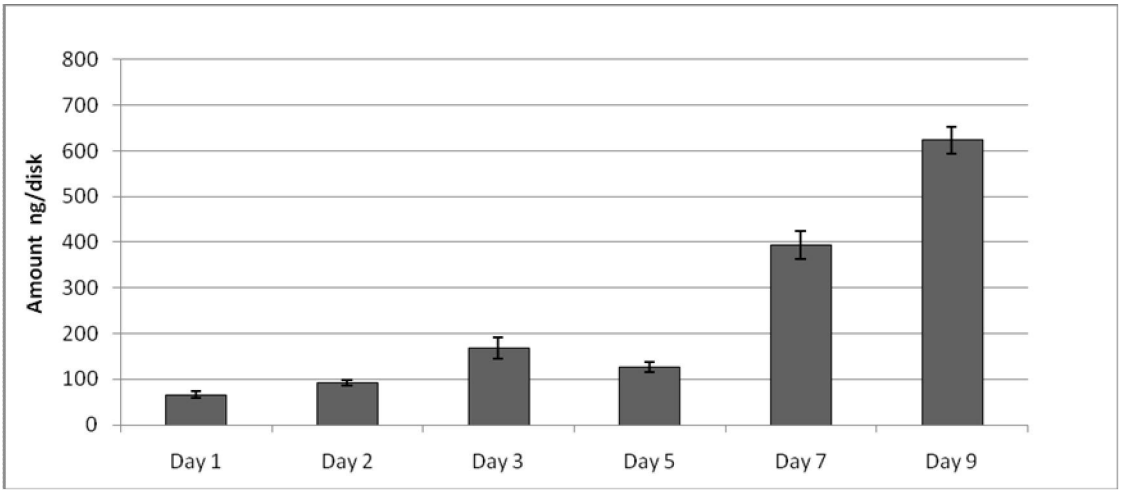

Figure 40 Accumulation of NP in the C-18 disks during the laboratory trial.

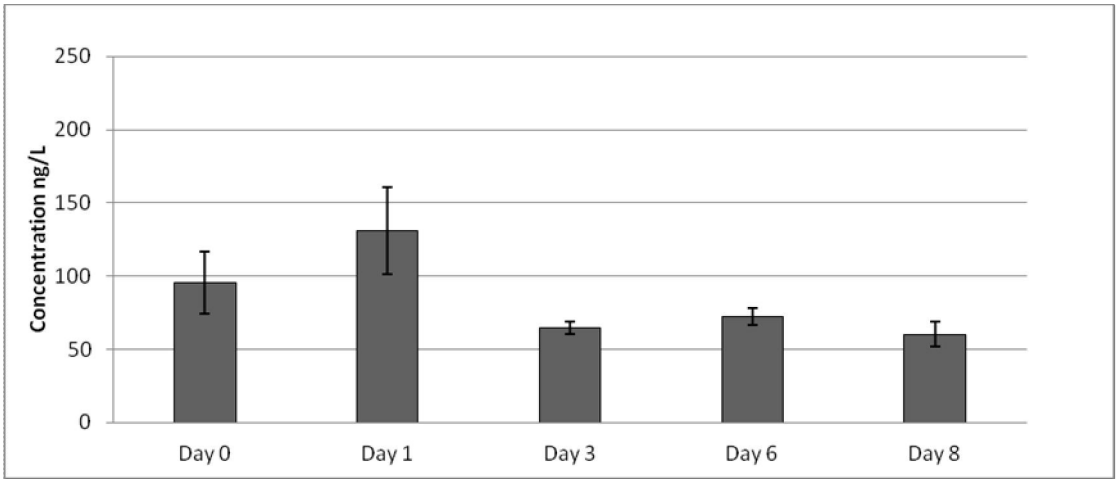

Figure 41 NP concentration in water samples during the laboratory test with the C-18 disks.

To summarise, the uptake of $NPEO_{1-3}$ was the most efficient in SDB-RPS disks, but after the trial the highest content of $NPEO_{10}$ and NP was found in the SDB-XC adsorbent. The $NPEO_{10}$ and NP concentrations in water during the C-18 trials were the lowest (Figs. 35 and 41) and therefore the contents found in C-18 disks were also low (Figs. 34 and 40).

5.5 Laboratory trial of passive samplers in brackish water

The laboratory trials in brackish water were performed with two receiving phases, SDB-RPS and C-18, at Tvärminne Zoological Station. The samplers were exposed under flow-through conditions in separate glass tanks. The replicate samplers of each type were retrieved simultaneously and the amounts accumulated were expressed as the average of three measurements. Increased accumulation was observed with the SDB-RPS phase but not with the C-18 phase (Fig. 42). The increase in the $NPEO_{10}$ content in the tank, due to small differences in water inflow, may have caused a saturation of the C-18 disks. The other explanation could be that the C-18 disk trapped the compounds more efficiently and at a higher rate, which further caused the saturation effect. During the first two weeks the $NPEO_{10}$ concentration in water was higher in the C-18 trial than in SDB-RPS test (Fig. 43).

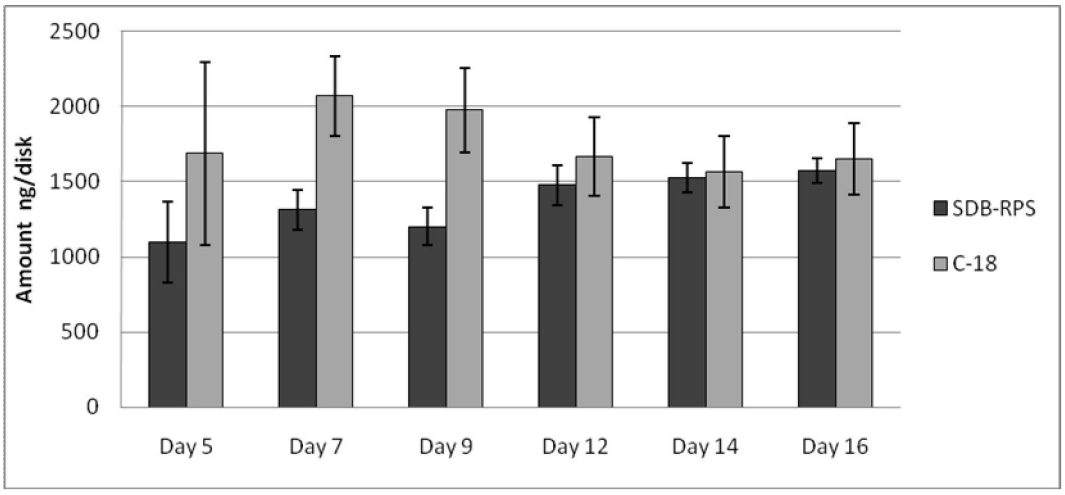

Figure 42 Accumulated amounts of $NPEO_{10}$ in C-18 and SDB-RPS disks exposed in brackish water.

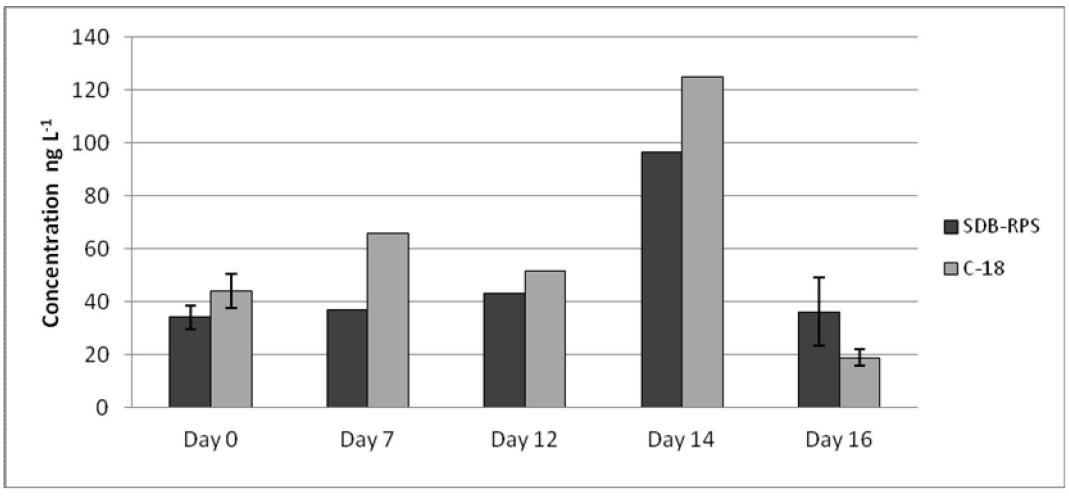

Figure 43 $NPEO_{10}$ concentration in water during the laboratory test with C-18 and SDB-RPS Empore disks in brackish water.

5.6 Laboratory test without flow-through conditions

The laboratory trial without flow-through of standard solution and UHQ water showed that the $NPEO_{10}$ or NP content in Empore disks did not increase in the same way as in the trials carried out under flow-through conditions (Figs. 44 and 46). The deployment time of four weeks affected the accumulation as well, since the disks reached an equilibrium with the surrounding media. Since chemicals were not added to the water phase, the concentration of analytes was reduced during the test and therefore, the content in the disk did not increase further (Figs. 44 and 46). The accumulation of $NPEO_{10}$ in the SDB-XC disks had an increasing trend for three weeks, but the last batch showed lower amounts (Fig. 44). Since the Chemcatchers® with different receiving phases, were deployed under identical conditions and retrieved after the same sampling time, the results indicated a higher affinity for $NPEO_{10}$ in the SDB-XC disks. As was expected, the concentration of the studied compounds in water decreased strongly during the trial (Figs. 45 and 47).

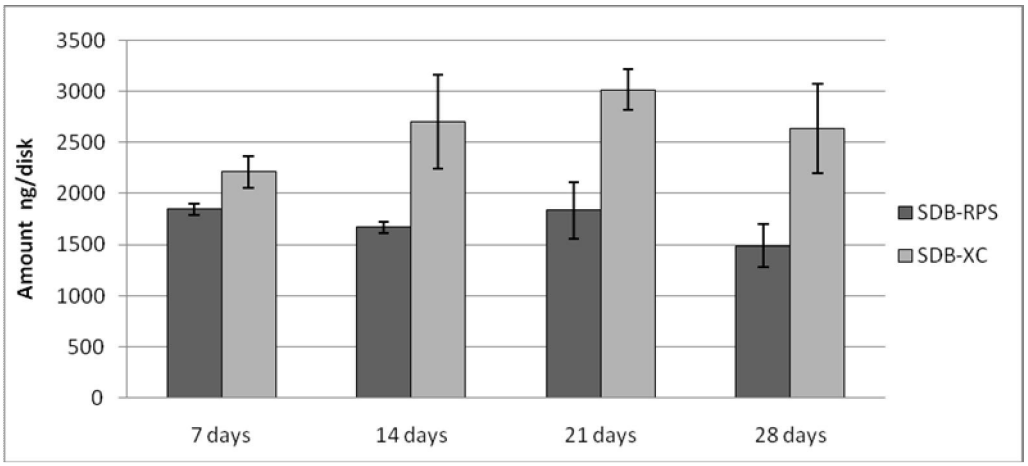

Figure 44 Accumulated amounts of $NPEO_{10}$ in the SDB-XC and SDB-RPS disks in a stable laboratory system.

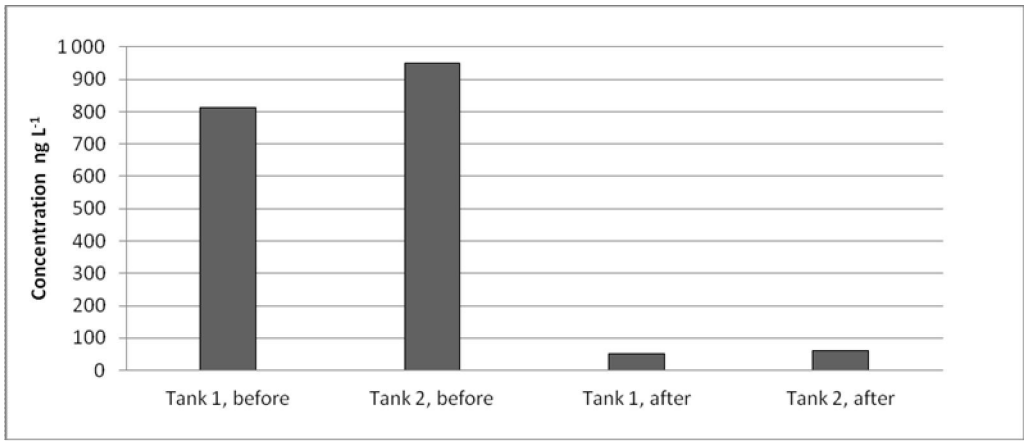

Figure 45 Concentration of $NPEO_{10}$ in the water tank before and after the deployment in the stable laboratory system trial.

Higher contents of NP were found in the SDB-RPS phase after the first week, but as the test continued , higher amounts were measured in the SDB-XC disks (Fig. 46). The samplers exposed for four weeks contained ab out the same amount of NP, which suggested that the receiving phase attained equilibrium with the surrounding aquatic media.

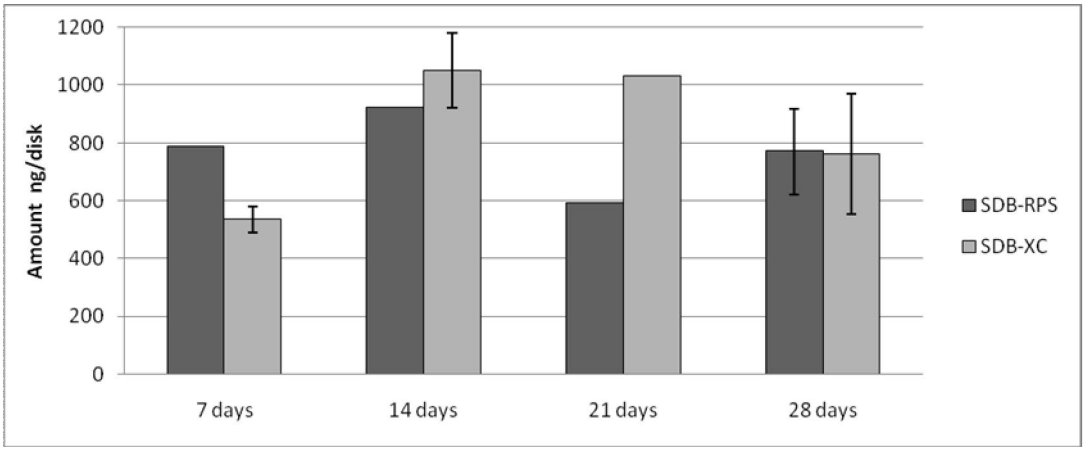

Figure 46 Accumulated amounts of NP in the SDB-RPS and SDB-XC disks in a stable laboratory system.

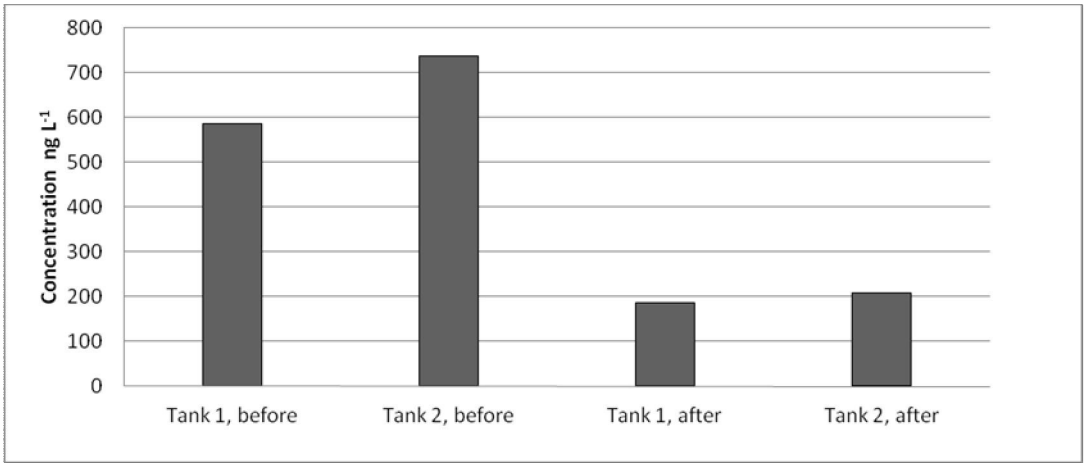

Figure 47 Concentration of NP in the water tank before and after the deployment in the stable laboratory system trial.

5.7 Waterflow and temperature during the field trials of Chemcatchers®

Water flow can affect the concentrations of contaminats in aquatic media by leaching more contaminants into the w atercourses. Flows were assessed at the nearest hydrological monitoring sites, which were S araavesi for the Kuusaankoski site and Anjala for the Kymijoki site. The water discharge values at the Kuusaankoski and Kymijoki sampling sites indicated that the water flow was the highest in the year 2008 (Figs. 48 and 49). At the Kuusaankoski site the

waterflow was higher in June but it decreased in August (Fig. 48), and this happened every summer except in 2008. At Kymijoki the water flow decreased during the summer in 2006 and 2010 (Fig. 49). In other years of the monitoring period it either remained at the same level (2009) or increased (2007-2008).

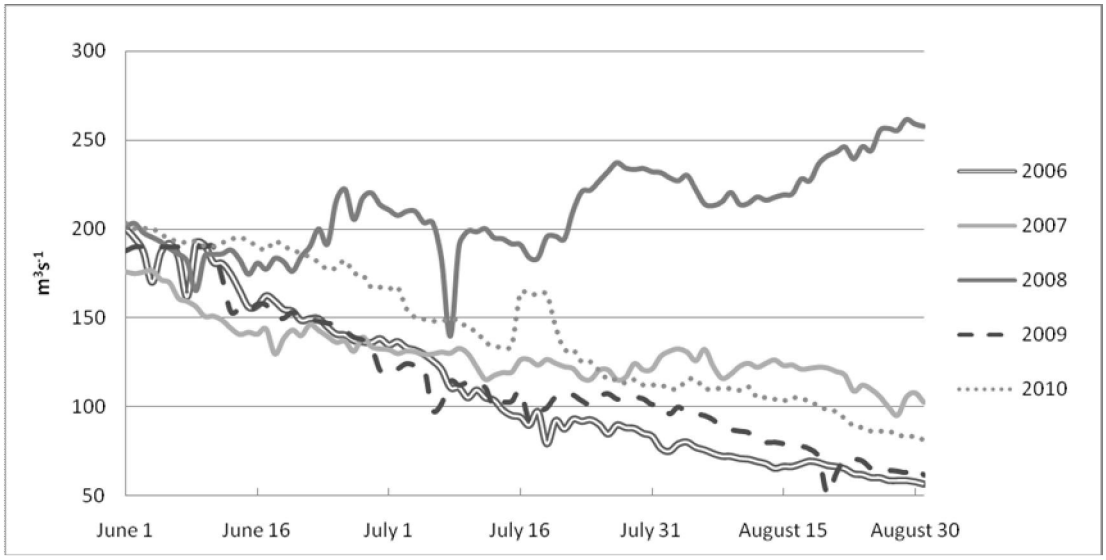

Figure 48 Water flow near the Kuusaankoski sampling site (Saraavesi) [HERTTA, 2011].

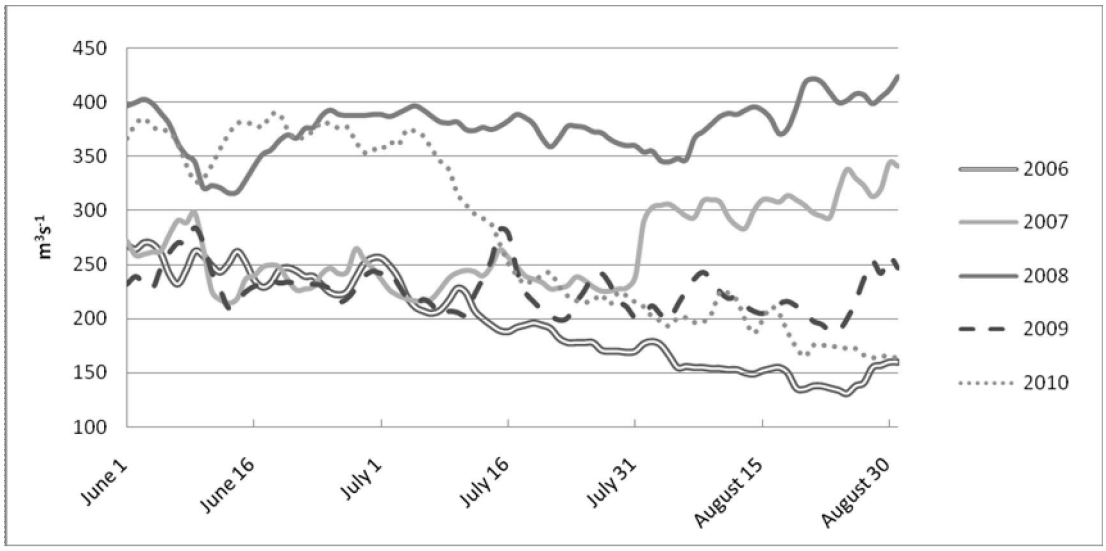

Figure 49 Water flow near the Kymijoki sampling site (Anjala) [HERTTA, 2011].

The observed water temperatures were at the same level at both sampling sites during the trials carried out in years 2006 and 2007 (Fig. 50). In 2008 and 2009 the temperatures were higher at the Kymijoki sampling site, whereas in summer 2010 they were higher at Kuusaankoski. Overall, the temperatures at both sites started to increase after summer 2008 by a few degrees per year.

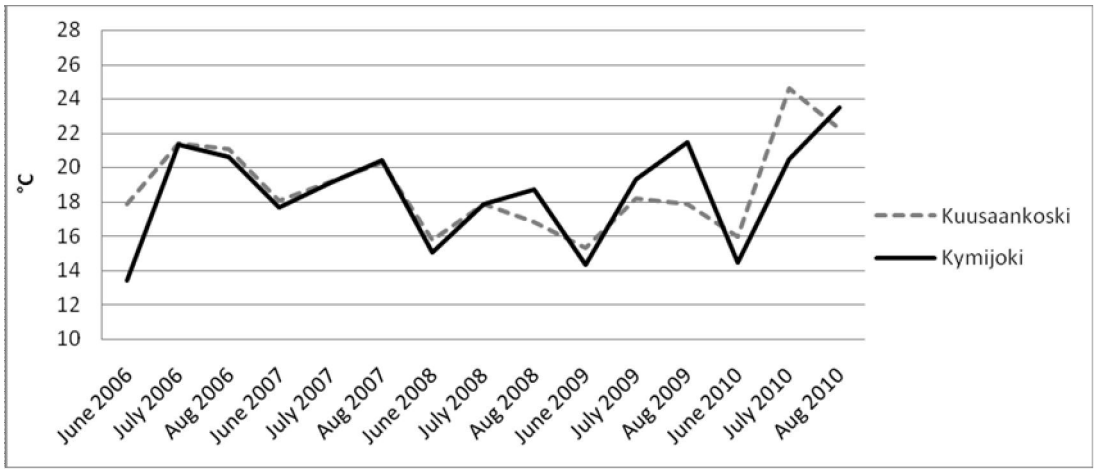

Figure 50 Temperatures near the sampling sites of Kuusaankoski and Kymijoki [HERTTA, 2011].

5.7.1 In 2007

The accumulation of analytes in passive samplers at the Kuusaankoski sampling site (Fig. 9) is presented in Figure 51. The amount of NPEOs ($NPEO_{5\text{-}15}$, in Chapter 4.2.3) in samplers deployed for four weeks was more than double that in samplers exposed for only two weeks. The NPEO concentrations determined by spot sampling remained approximately the same after the first measurement (Fig. 52). This year the disks were extracted with methanol only, and due to that the NPEO contents were lower than in the following years.

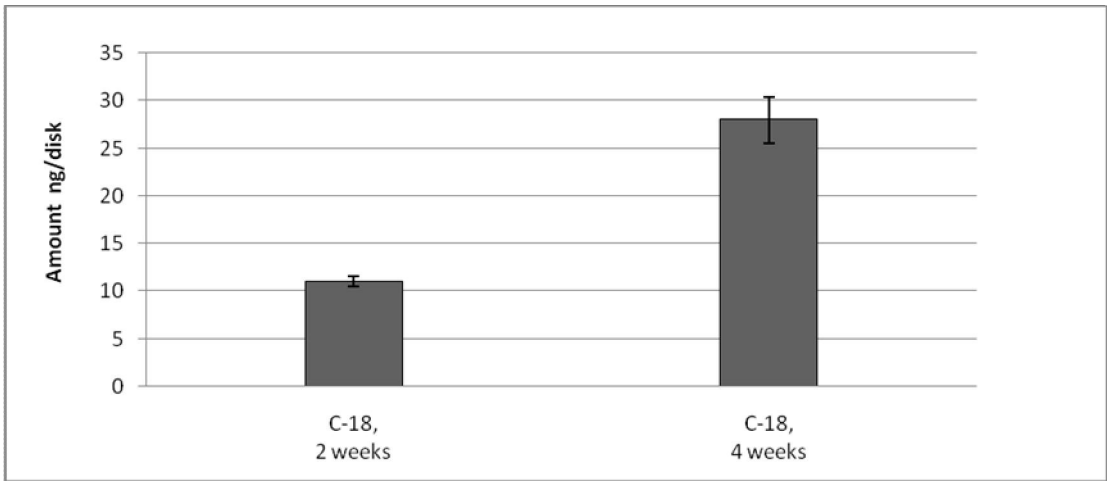

Figure 51 Accumulation of NPEOs ($NPEO_{5\text{-}15}$) in uncovered C-18 Empore disks at the Kuusaankoski sampling site, 2007.

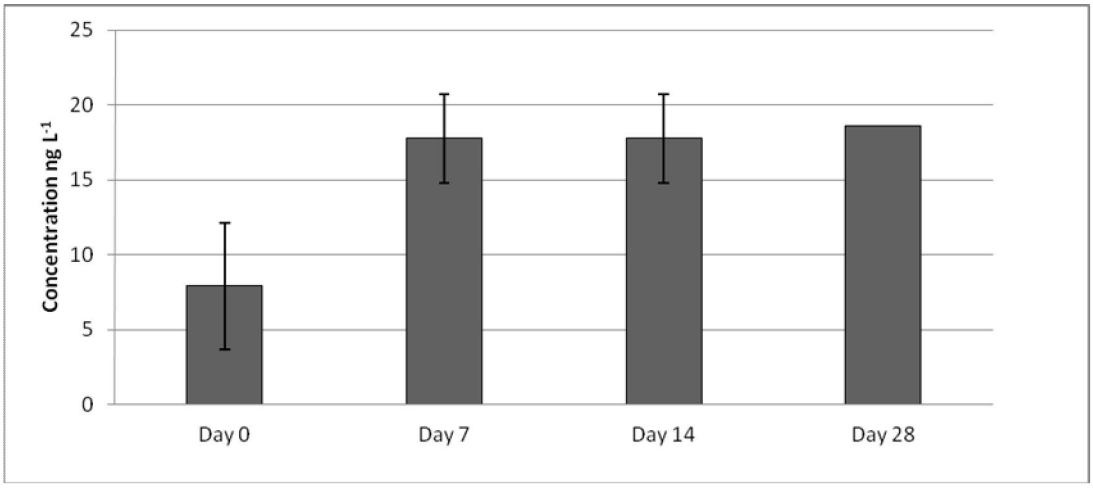

Figure 52 NPEO ($NPEO_{5-15}$) concentrations in spot samples during the field trial in Kuusaankoski in 2007.

5.7.2 In 2008

The samplers were exposed at the Kuusaankoski sampling site for 14 or 28 days (Fig. 9) and the amounts of NPEOs in different receiving phases are presented in Figure 53. It appeared that the SDB-XC phase collected NPEOs most efficiently after two weeks (Fig. 53). However, at the end of the experiment SDB-XC and C-18 gave si milar results. This year the water temperature was the lowest during the study period 2006-2010 (Fig. 50).

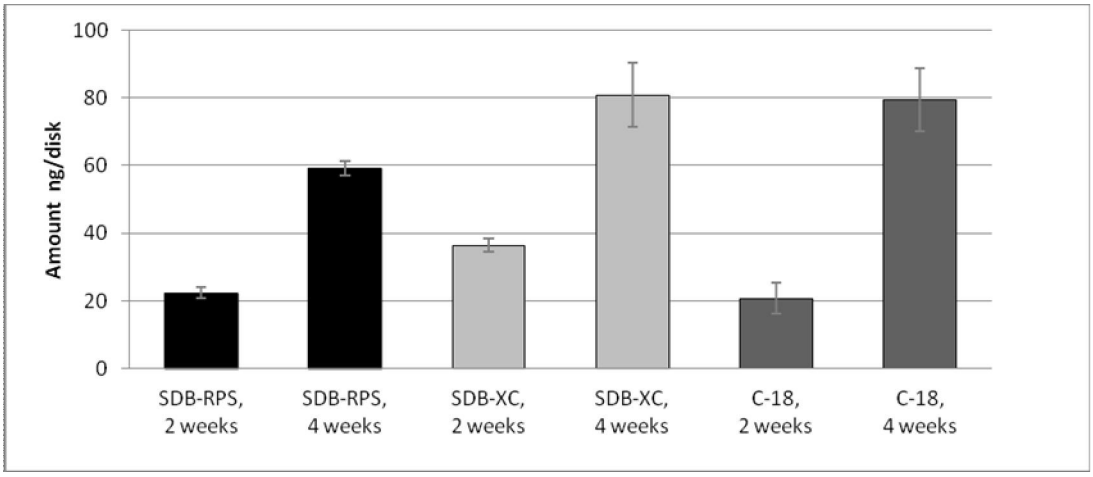

Figure 53 Accumulation of N PEOs ($NPEO_{5-15}$) in uncovered Empore disks at the Kuusaankoski sampling site in 2008.

The amount of NPEOs measured in spot samples suggested that the concentration fluctuated during the study period (Fig. 54).

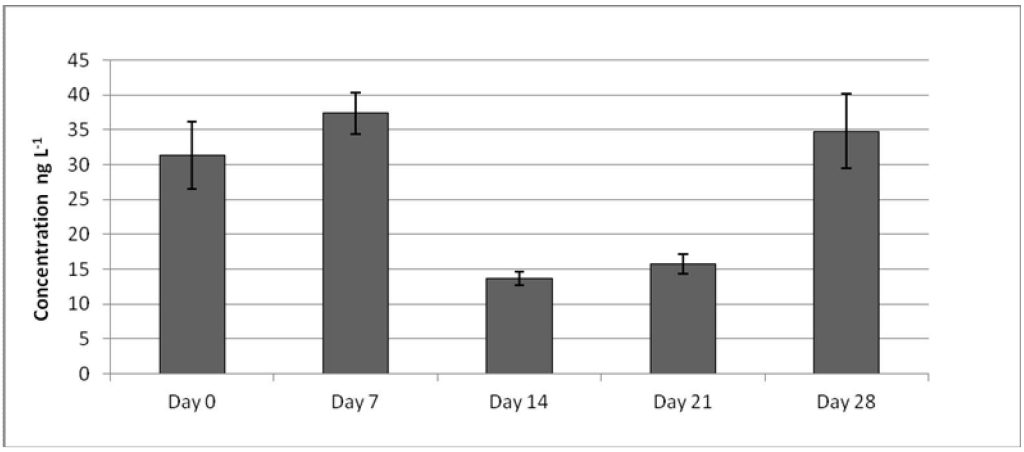

Figure 54 NPEO ($NPEO_{5-15}$) concentrations in spot samples during the field trial at the Kuusaankoski site, in 2008.

5.7.3 In 2009

The field trials were carried out at the Kuusaankoski and Kymijoki sampling sites (Fig. 9) using three different receiving phases. Differences between the NPEO ($NPEO_{5-15}$) contents found in the disks after two or four weeks were not significant as only small increases were observed in samplers deployed for four weeks (Figs. 55 and 56). The highest accumulations were measured in the SDB- RPS phase at both sites. The samplers exposed at the Kuusaankoski sampling site gave unexpected results (Fig. 55). The amounts found in SDB-RPS disks were much higher than those measured in the other two receiving phases, and the samplers deployed for two weeks had even higher contents than the ones exposed longer (C-18 in Fig. 56). One explanation could be the saturation of the receiving phase with other compounds in natural waters. The water discharge did not vary dramatically in Kymijoki, but at Kuusaankoski it decreased during the summer period (Figs. 48 and 49). The water temperature was higher than in previous years (Fig. 50). The first water sample in Kuusaankoski gave lower NPEO concentrations than the rest of the samples (Fig. 57). At Kymijoki the water samples were taken only twice (Fig. 58).

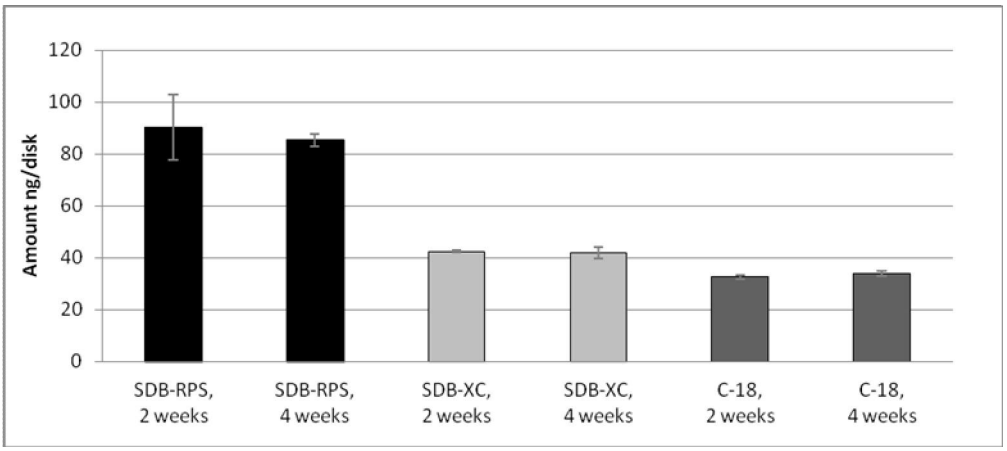

Figure 55 Accumulation of NPEOs ($NPEO_{5-15}$) in uncovered Empore disks at Kuusaankoski, 2009

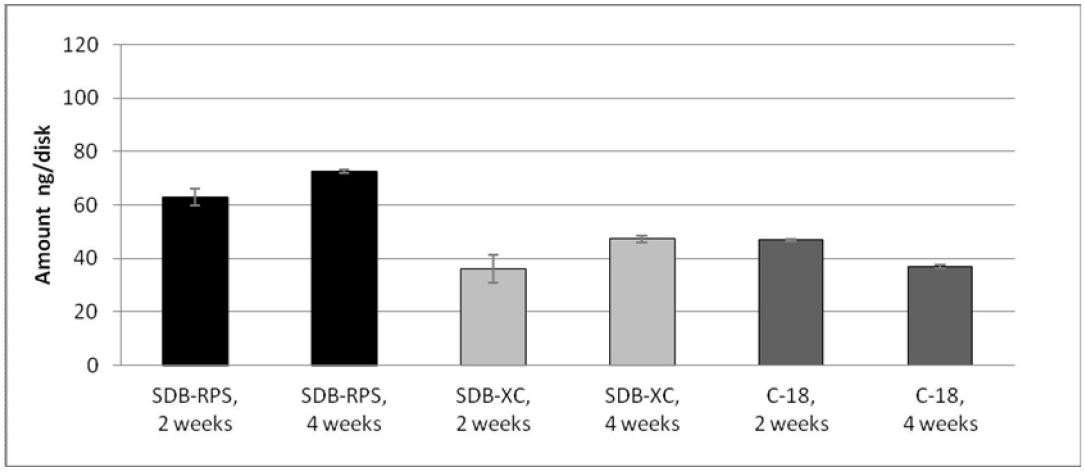

Figure 56 Accumulation of NPEOs ($NPEO_{5-15}$) in uncovered Empore disks at the Kymijoki sampling site, 2009.

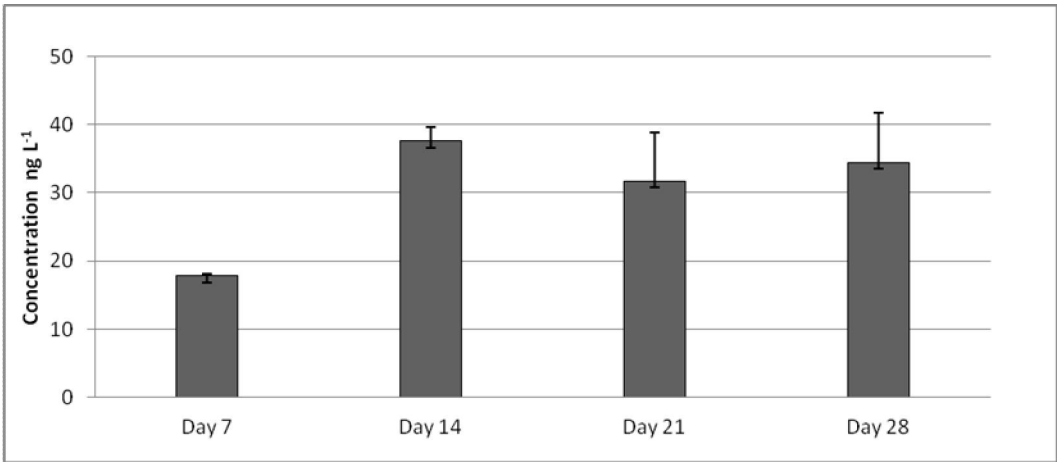

Figure 57 NPEO ($NPEO_{5-15}$) concentrations in spot samples during the field trial at Kuusaankoski, 2009.

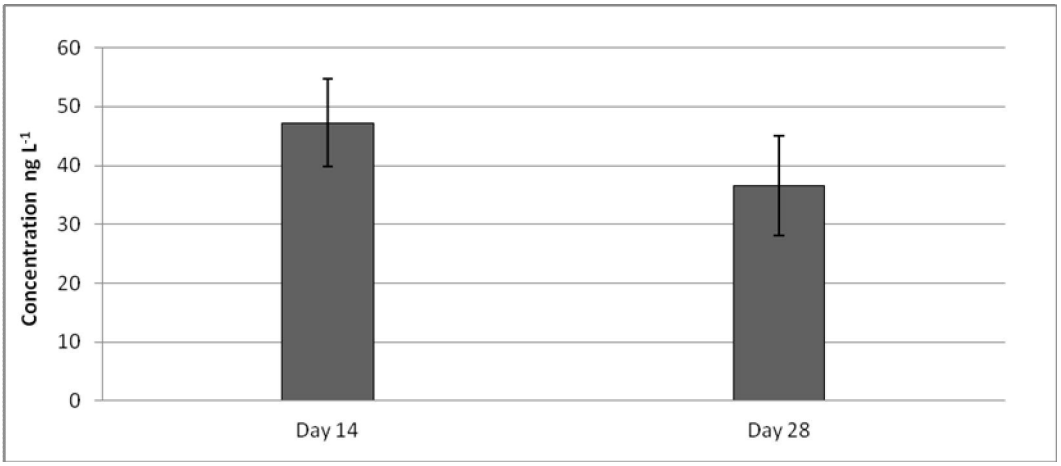

Figure 58 NPEO ($NPEO_{5-15}$) concentrations in spot samples during the field trial at Kymijoki, 2009.

5.7.4 In 2010

The samplers kept at the K ymijoki sampling site indicated that the SDB-RPS disks accumulated the highest amounts during the trial (Fig. 59). On the other hand, the SDB-XC disks collected the lowest amounts of NPEOs.

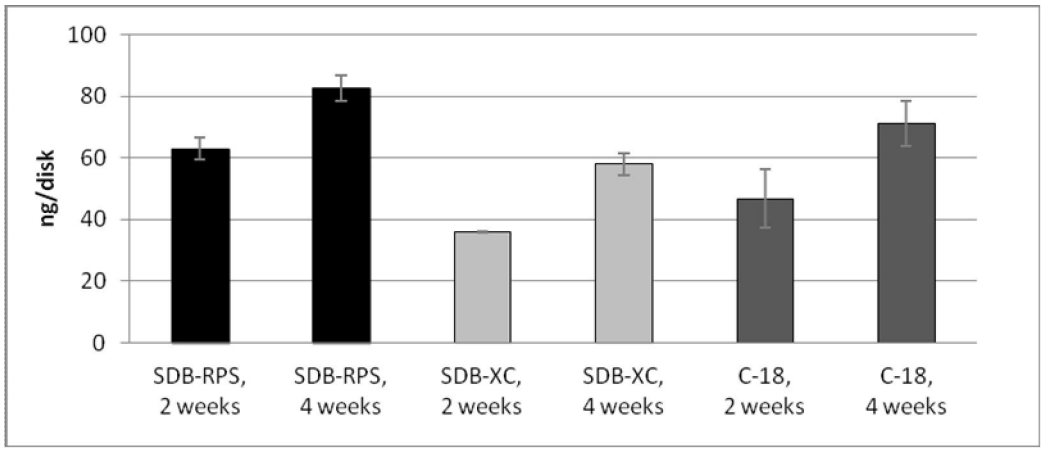

Figure 59 Accumulation of NPEOs ($NPEO_{5-15}$) in uncovered Empore disks at the Kymijoki sampling site, 2010.

The concentration of NPEOs measured in water samples showed that at the beginning of the trial the amou nts in the aquatic phase were higher than at the other two sampling times (Fig. 60).

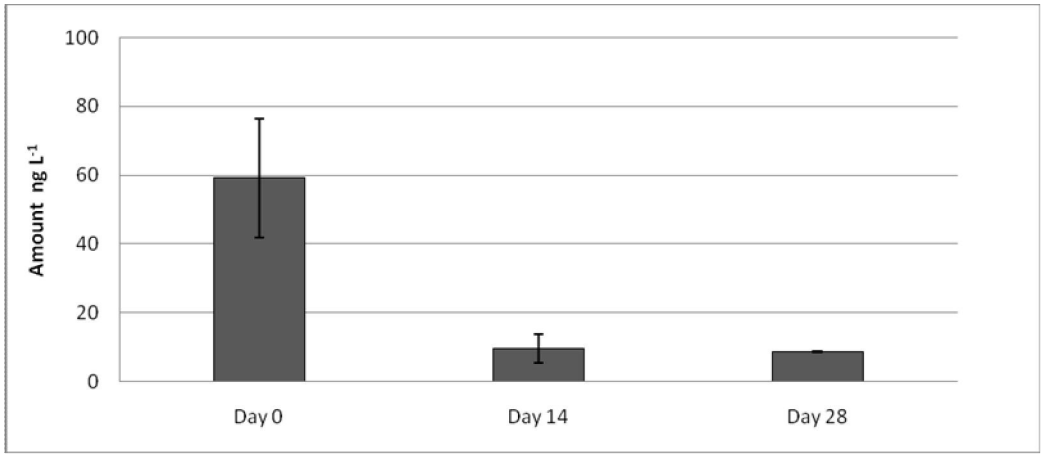

Figure 60 NPEO ($NPEO_{5-15}$) concentrations in spot samples during the field trial at the Kymijoki sampling site, 2010.

5.7.5 Gulf of Finland

The passive samplers were exposed at three sites in the Gulf of F inland near the city of Kotka (Fig. 10). The results were as expected, since the accumulated amounts found in samplers expo sed at site 1 we re slightly higher than at sites 2 and 3 (Fig. 61). Site 1 received discharges from the Kymijoki river, but due to dilution, the NPEO contents are lower further from the shore. Based on the results obtained by all three phases the highest amounts were found a t site 1.

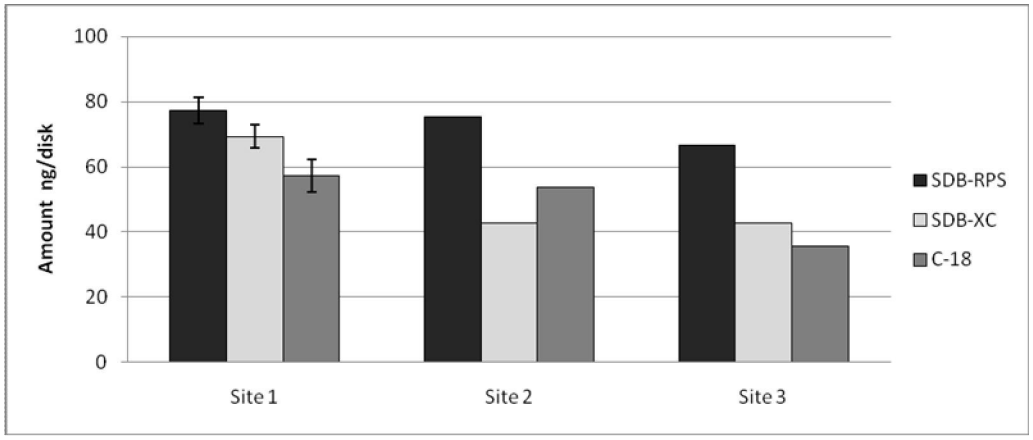

Figure 61 Accumulation of NPEOs ($NPEO_{5-15}$) in uncovered Empore disks in the Gulf of Finland.

5.7.6 Gulf of Bothnia

A preliminary field trial was carried out in the Gulf of Bothnia (Fig. 11), near the cities of Gävle and Sundsvall. The differences between NPEO contents found in the samplers exposed at the reference and sampling sites were negligible (Fig. 62). Notably at the site Gävle 2 (reference), the SDB-XC sampler collected higher contents of NPEOs than at site Gävle 1. The amounts of NPEOs found in the samplers placed near the city of Sundsvall were equal at both locations. The results indicated that the sampling period was too long, which caused a balance between the concentration in the receiving phase and that in the surrounding aquatic media. In addition, natural waters contain a number of harmful substances and compounds with a high affinity for the receiving phase. These substances are also trapped in the disk, while they are not studied in this work. During the sampling time of three months those compounds filled the receiving capacity of disk. However, this has not been studied here more extensively and should be verified in separate experiments.

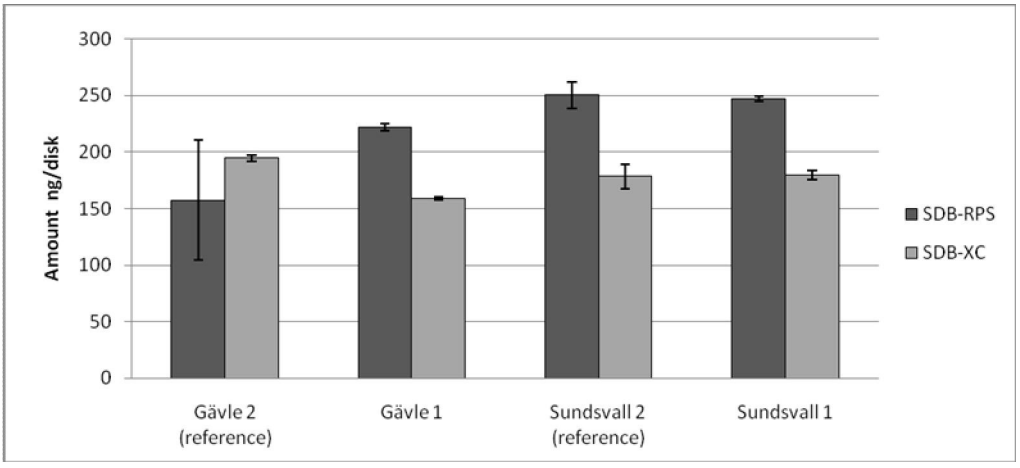

Figure 62 Accumulation of NPEOs ($NPEO_{5-15}$) in uncovered Empore disks in the Gulf of Bothnia, 2010.

5.8 Inaccuracy of the analysis

NPEOs were detected with HPLC/ESI-MS using SIM-mode. In all samples the long chain NPEOs were separated well. However, the determination of NP in natural samples, both in water and the receiving phase, was not straightforward due to background interference caused by sample matrix. Especially Finnish waters, which are rich of humic substances brought also challenges to the determination.

The limits of detection (LOD) and quantification (LOQ) concentrations were calculated as three and ten times the signal-to-noise ratio, respectively. The standard solutions used for calculating the calibration curves were measured with samples, and because of the variation of the MSD response the equations differed. As an example, for determining $NPEO_8$ in water samples the LOD concentration was 2 ng L^{-1} and LOQ concentration 7 ng L^{-1}. For NP the LOD and LOQ were 24 ng L^{-1} and 78 ng L^{-1}, respectively.

The NPEO amounts in samples were calculated as a sum of $NPEO_{1-3}$ for short chain ethoxylates ($NPEO_{1-3}$) and $NPEO_{5-15}$ for long chain ones ($NPEO_{10}$). The lack of one oligomer in the sample had a large effect on the results since the amounts were calculated as a sum of only three oligomers $NPEO_{1-3}$. All the results were calculated using peak heights since it was observed to have better repeatability.

In this study, the ionisation degrees of all ammonium adducts of NPEOs were considered to be the same. Repeated analyses of a standard solution were performed to study the errors of the HPLC/MS instrument (Fig. 63). In general, the accuracy of the measurements was 5-10%. Averages and standard errors were also calculated from the peak heights and the results are reported in Figure 63.

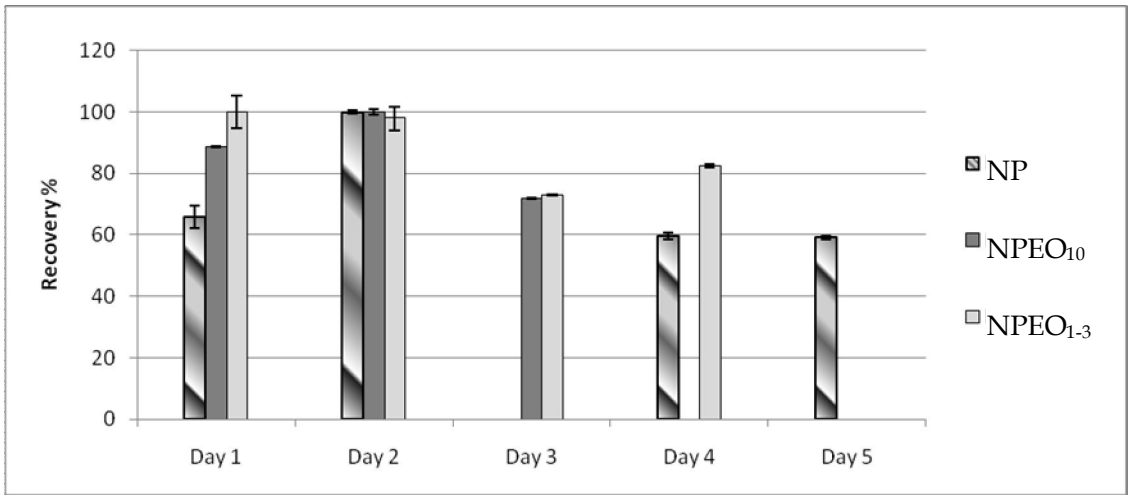

Figure 63 Normalized averages and standard deviations.

The day-to-day variation of HPLC/MS measumements was greater than expected. During the analysi s the s tandards were not stored in the frid ge. Instead, they were kept on the autosampler t ray at room temperature between the replicate inje ctions. The standards me asured in this tes t could have changed, due to the phenomena such as adsorption on the sample vial walls, caused by the prolonged wai ting time bet ween the analyses. The i ntra-day variation was still quite small (5-10%) between the analyses, which also refers to changes in the standard solutions within time (Fig. 63).

Due to t he matrix effect, the an alysis of NP from natu ral waters was difficult. The base line noise level was rather high and this made the determination challenging by lowering the sensitivity. Another analysis method including an ef ficient pretreatment step or a more sensitive and sel ective detection procedure, such as HPLC instrument coupled with a tandem mass spectrometry (MS-MS) would have been more suitable for NP studies.

6 CONCLUSIONS

The aim of this work was to improve the analysis methods suitable for NPEOs in aqueous media and to test the proper Chemcatcher® configurations. The results showed that SPE pretreatment followed by HPLC/ESI-MS analysis was a useful tool for the concentration and identification of NPEOs. For NP, the procedure gave slightly lower recoveries. First, the procedure for extracting NPEOs and NP from Empore disks was optimised. The laboratory exposure tests clearly indicated that the best accumulation of these substances was observed when the SDB-XC disk acted as a receiving phase. Also, SDB-RPS gave promising results for the monitoring of short chain NPEOs.

As expected, the use of a diffusion-limiting membrane on top of the receiving phase diminished the accumulation rate in the disk. Concentration levels of NP and NPEOs in Finnish waters are quite low, and for this reason further experiments and field trials were performed with the uncovered receiving phases. The deployment time of two weeks is recommended, since the long exposure time increases the biofouling. The deployment of samplers in three sites in Gulf of Finland near the city of Kotka confirmed that the site located nearest to the city was the most polluted one.

The present Chemcatcher® monitoring method is a simple, versatile method for the monitoring of contaminants in watercourses. Unfortunately, it is sensitive to many interfering effects. Therefore, the method is still under development and more samples should be used in determination. The use of PRC in disk would improve the reliability of the method. In addition, this procedure should be compared with other passive sampling methods or methods based on the bioaccumulation of NP and NPEOs in biological material, *e.g.* in mussels in laboratory and, finally, natural water systems.

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