Fate of artificial sweeteners and perfluoroalkyl acids in aquatic environment

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Academic Dissertation

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following four publications, which are referred to in the text by their Roman numerals:

I. Perkola N and Sainio P (2014) Quantification of four artificial sweeteners in Finnish surface waters with isotope-dilution mass spectrometry. Environmental pollution 184:391–396.

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III. Vaalgamaa S, Vähätalo AV, Perkola N and Huhtala S (2011) Photochemical reactivity of perfluorooctanoic acid (PFOA) in conditions representing surface water. Science of the Total Environment 409(16):3043–3048.

IV. Perkola N., Vaalgamaa S., Jernberg J. and Vähätalo A.V. Degradation of artificial sweeteners via direct and indirect photochemical reactions. Manuscript.

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THE AUTHOR’S CONTRIBUTION

I. Noora Perkola was responsible for planning the experiment, conducted the experimental work, and is the corresponding author.

II. Noora Perkola carried out the experimental laboratory work and data processing, and is the corresponding author.

III. Noora Perkola took part in planning the experiments and writing the article, and was responsible for the analysis of perfluorooctanoic acid and its phototransformation products.

IV. Noora Perkola took part in planning the experiments, was mainly responsible for the experimental laboratory work, and is the corresponding author.

ABSTRACT

The newly detected chemicals, the environmental distribution, fate, and effects in the environment of which are not well known, are called emerging compounds. Artificial sweeteners are one group of emerging compounds. The consumption of artificial sweeteners is high, and because they do not significantly metabolise, all that is consumed finds its way to wastewater treatment plants. Two artificial sweeteners, acesulfame and sucralose, do not degrade in wastewater treatment either, leading to elevated concentrations in the receiving water bodies. Another group of emerging compounds is perfluoroalkyl acids. They have been used both in industry and consumer products since the 1950s. The fluorine-carbon chain of perfluoroalkyl acids makes them extremely resistant to biological, chemical and physical degradation. They are ubiquitous in the environment and are suspected to be carsinogenic, immunotoxic and to interfere with reproduction.

The aim of this study was to add to knowledge about the environmental distribution, fate and effects of artificial sweeteners and perfluoroalkyl acids. The occurrence of artificial sweeteners and perfluoroalkyl compounds was surveyed in surface waters. Wastewater effluents and sludge, storm water, and landfill leachate were analysed to evaluate the fluxes of perfluoroalkyl acids into the aquatic environment. Artificial sweeteners and perfluorooctanoic acid were irradiated under artificial sun to investigate their potential to transform via direct and indirect photochemical reactions in surface waters and ultraviolet radiation and during germicidal ultraviolet water treatment. Furthermore, *Daphnia magna* were exposed to artificial sweeteners to evaluate the ecotoxicological effects.

It was discovered that artificial sweeteners and perfluoroalkyl acids are ubiquitous in the Finnish aquatic environment. Of the studied emission sources, wastewater effluents were the most important source of perfluoroalkyl acids in environmental waters. Based on the irradiation of perfluorooctanoic acid with a solar simulator, perfluorocarboxylic acids do not transform via direct or indirect photochemical reactions in the environment. Although a decrease in acesulfame was observed under irradiation with artificial sun, the photolytic half-life in surface water is at least one year. The photolytic half-lives of the other sweeteners were estimated to be 3 to 6 years. The ecotoxicological tests suggest that cyclamic acid might hinder the reproduction of *Daphnia magna*. Perfluorocarboxylic acids, sucralose and acesulfame are persistent and ubiquitous in surface waters.

TIIVISTELMÄ

Kemikaalien määrä on lisääntynyt merkittävästi viime vuosikymmeninä, ja ympäristöstä löydetään jatkuvasti kemikaaleja, joita ei ole aikaisemmin havaittu. Näitä kemikaaleja, joiden levinneisyydestä, kohtalosta ja vaikutuksista ympäristössä tiedetään hyvin vähän tai ei lainkaan, kutsutaan nouseviksi aineiksi. Tällaisia yhdisteitä ovat muun muassa keinotekoiset makeutusaineet ja perfluoratut alkyylihapot. Kalorittomat makeutusaineet asesulfaami, sukraloosi, syklaamihappo ja sakariini eivät juurikaan metaboloidu elimistössä. Asesulfaami ja sukraloosi eivät myöskään hajoa jätevedenpuhdistamoilla. Koska makeutusaineiden käyttömäärät ovat suuria, niitä päätyy puhdistettujen jätevesien mukana merkittäviä määriä vastaanottaviin vesistöihin. Perfluorattuja alkyylihappoja on käytetty teollisuudessa ja kuluttajatuotteissa 1950-luvulta lähtien. Perfluoratut yhdisteet ovat erittäin pysyviä vahvan fluori-hiilisidoksen ansiosta. Reagoimattomuudestaan huolimatta niiden epäillään olevan muun muassa karsinogeenisia, immunotoksisia ja lisääntymistervettä häiritseviä aineita.

Tämän väitöskirjan tarkoitus oli selvittää keinotekoisten makeutusaineiden ja perfluorattujen alkyylihappojen esiintymistä, pysyvyyttä ja vaikutuksia vesiympäristössä. Yhdisteiden levinneisyydestä Suomessa on hyvin vähän tietoa, minkä vuoksi työssä kartoitettiin niiden pitoisuuksia pintavesissä. Lisäksi selvitettiin jätevesien, lietteen, kaatopaikan suotoveden ja huleveden merkitystä perfluorattujen alkyyliyhdisteiden päästölähteinä. Auringonvalon kykyä hajottaa pintavesiin päätyneitä perfluorattuja karboksyylihappoja ja keinotekoisia makeutusaineita tutkittiin keinoauringon avulla. Tutkittavat yhdisteet altistettiin myös ultraviolettisäteilylle, jotta saatiin tietoa niiden käyttäytymisestä vedenpuhdistuksessa. Lisäksi arvioitiin keinotekoisten makeutusaineiden vaikutuksia vesikirpuille ekotoksikologisten kokeiden avulla.

Keinotekoisia makeutusaineita ja perfluorattuja alkyylihappoja esiintyy laajalti pintavesissä. Perfluorattujen alkyylihappojen merkittävimmät päästöt tulevat Suomessa yhdyskuntajätevedenpuhdistamoilta. Keinoauringolla tehtyjen kokeiden perusteella perfluoratut karboksyylihapot ja makeutusaineista sukraloosi, syklaamihappo ja sakariini eivät hajoa suoran tai epäsuoran valokemiallisen reaktion avulla ympäristössä. Asesulfaamin valokemialliseksi puoliintumisajaksi pintavedessä arvioitiin vähintään yksi vuosi. Ekotoksisuustestien perusteella keinotekoisista makeutusaineista syklaamihappo saattaa haitata vesikirppujen lisääntymistä.

ABBREVIATIONS

AA-EQS	Annual average environmental quality standard
ACS	Acesulfame
BOD_7	Seven-day biochemical oxygen demand
CDOM	Chromophoric dissolved organic material
CYC	Cyclamic acid
FASA	perfluoroalkyl sulphonamide
FASE	Perfluoroalkyl sulfonamido ethanol
Fe(III)	Ferric iron
FTOH	Fluorotelomer alcohol
HPLC	High performance liquid chromatography
IWWTP	Industrial wastewater treatment plant
LC	Liquid chromatography/liquid chromatograph
MAC-EQS	Maximum allowable concentration
MS	Mass spectrometry/mass spectrometer
MWWTP	Municipal wastewater treatment plant
NOEC	No observed effect level
NORMAN	Network of reference laboratories, research centres and related organisations for the monitoring of emerging environmental substances
PAP	Perfluoroalkyl phosphate
PE	Population equivalent
PFAA	Perfluoroalkyl acid
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutanesulfonic acid
PFCA	Perfluorocarboxylic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexanesulfonic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PFPA	Perfluorophosphonic acid
PFPIA	Pwerfluorophosphinic acid
PFSA	Perfluoroalkyl sulfonic acid
PFDA	Perfluorodecanoic acid
Q	Flow rate (m^3/s)
RSD	Relative standard deviation
SAC	Saccharin
SCL	Sucralose
SD	Standard deviation
SPE	Solid phase extraction
TOF MS	Time-of-flight mass spectrometer
TQ MS	Triple quadrupole mass spectrometer
UHPLC	Ultra-high performance liquid chromatography
UV	Ultraviolet
UVC	Ultraviolet-C
WWTP	Wastewater treatment plant

INTRODUCTION

1.1 Emerging compounds

The amount of chemicals used today in industry, farming and households is plentiful. Due to the vast consumption of chemicals and more sophisticated detection techniques, many of these anthropogenic chemicals are being detected in the environment and in humans – a phenomenon called chemicalisation. Chemicals that have recently been detected in the environment, often at higher concentrations than expected, and whose source, distribution or fate in the environment may not be known or fully understood, are called emerging compounds. Emerging compounds include a wide variety of substances such as industrial, agricultural and household chemicals, and pharmaceuticals. They are often released into the aquatic environment in wastewater effluents, or from farming, but in some cases the sources of a chemical are not known. There are many statements on which compounds are emerging or which are the most important. The NORMAN network has listed "the currently most frequently discussed" emerging compounds (NORMAN 2011) and a list of candidate emerging substances (NORMAN 2013). In this study, the environmental occurrence, sources and fate of two groups of emerging substances are studied: artificial sweeteners and perfluoroalkyl acids.

1.1.1 Artificial sweeteners

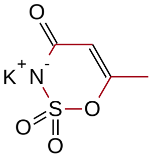
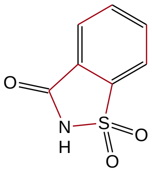
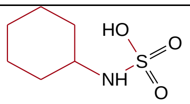
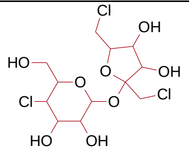
Artificial sweeteners are a group of anthropogenic compounds that are used as additives in foodstuffs, personal care products, pharmaceuticals and animal feed (Community Register of Feed Additives; Regulation 2008/1333/EC). Acesulfame, saccharin, cyclamic acid and sucralose are artificial sweeteners with very limited metabolism in the human digestive system (Renwick 1986; Roberts et al. 2000). Therefore, they pass through the human body virtually unchanged, ending up in wastewaters. Most acesulfame and sucralose pass through the wastewater treatment as well (Brorström-Lunden et al. 2008; Minten et al. 2011; Scheurer et al. 2009; Torres et al. 2011). Although the majority of saccharin and cyclamic acid is removed in the wastewater treatment plants, they are still detectable in the effluents and receiving waters. The emerging status of artificial sweeteners was recently reviewed by Lange et al. (2012). All the four sweeteners have been detected in wastewater-contaminated surface (Berset and Ochsenbein 2012; Buerge et al. 2009; Ferrer and Thurman 2010; Morlock et al. 2011; Müller et al. 2011) and marine water (Green et al. 2008; Mead et al. 2009). As very water-soluble and relatively persistent compounds, acesulfame and sucralose may permeate to groundwater (Buerge et al. 2009; van Stempvoort et al. 2011a; Wolf et al. 2012). They can also persist through conventional water treatment, thus ending up in drinking water (Mawhinney et al. 2011; Scheurer et al. 2010).

The degradation of sweeteners in natural water environments is related to their behaviour in WWTPs. Sucralose and acesulfame are regarded as persistent in water. Mineralisation processes such as hydrolysis of sucralose in aquatic environments are slow, and the half-life of sucralose in water is estimated to be several years (Richardson and Ternes, 2011). The degradation of acesulfame and sucralose in water or flooded soil is negligible (Labare and Alexander 1993, 1994; Soh et al. 2011). In addition, river bank filtration can efficiently remove saccharin and cyclamic acid from wastewater effluent, but the degradation of sucralose and acesulfame is very slow (Scheurer et al. 2010). On the other hand, the rapid microbial degradation of artificial sweeteners has been observed under aerobic conditions in dry soil. Buerge et al. (2011) observed degradation of more than 90% of saccharin, cyclamic acid, sucralose and acesulfame over three weeks of aerobic soil incubation. In addition, Soh et al. (2011) observed 45% loss of sucralose in dry soil in four weeks. The photochemical transformation of acesulfame and sucralose with high-intensity radiation has been presented in several papers (Coiffard et al. 1999; Sang et al. 2014; Scheurer et al. 2014; Soh et al. 2011). However, to my knowledge, transformation under environmentally relevant UV radiation has not been studied.

Sucralose and acesulfame can be used as indicator compounds for other compounds such as herbicides, pharmaceuticals or perfluoroalkyl acids (PFAAs) in water systems (Mawhinney et al. 2011, Müller et al. 2011). Through differences in the degradation rate, the analysis of more than one sweetener provides information on contamination sources. Müller et al. (2011) observed a linear correlation between acesulfame and population (population/Q; persons/m^3s^{-1}) with $R^2 = 0.94$. Therefore, the ratio of acesulfame to other contaminants can be used to distinguish municipal discharge from industrial or other point sources. The ratios for the four sweeteners could also be used in the estimation of the distance from the source or the time elapsed since discharge, as saccharin and cyclamic acid degrade more rapidly in the environment than acesulfame and sucralose do (van Stempvoort et al. 2011a).

The environmental effects of artificial sweeteners are largely unknown, although several reports on the effects of sucralose have been published. Bioaccumulation in aquatic organisms has not been observed with this hydrophilic compound (Lillicrap et al. 2011). Sucralose does not show acute or chronic toxicity to survival and reproduction of laboratory-cultured cladocera *Daphnia magna* (Eriksson-Wiklund et al. 2012; Hjorth et al. 2010; Huggett and Stoddard 2011). However, the behavioural effects on some aquatic species have been seen. Hjorth et al. (2010) observed alterations in the feeding rate and egg production of *Calanus glacialis* with exposure to sucralose in increased concentrations. Also, Eriksson-Wiklund and colleagues (2012) observed changes in the locomotive behaviour and respiration of gammarids and in the swimming height of *Daphnia magna*. They found a bell-shaped response, i.e. the greatest effects at median, environmentally relevant concentrations (5 µg/L; >0.5 µg/L and <500 µg/L). A recent study suggests that sucralose may affect neurological and oxidative mechanisms on *Daphnia magna* (Eriksson-Wiklund et al. 2014). Ecotoxicology of acesulfame, saccharin, and cyclamic acid has not been studied. Based on their water solubility and octanol-water partition coefficients (log$K_{o/w}$) (Table 1), bioaccumulation is not expected. As non-toxic chemicals they are not likely to cause a considerable health hazard. However, some subtle effects might occur.

Table 1. Properties of artificial sweeteners, including acceptable daily intake (ADI), and removal by metabolism and in wastewater treatment plants (WWTP).

	Acesulfame-K	**Saccharin**	**Cyclamic acid**	**Sucralose**
Molecular structure				
E-code	E950	E954	E952	E955
ADI (mg/kg/vrk)	9	5	7	15
Metabolism	No	No	1-60%	Max 5%
Removal at WWTPs (%)	0-40	70-100	80-100	0-20
pK_a	2.0	1.6	1.9	-
Solubility in H_2O (g/L)	270	3.5	soluble	283
log$K_{o/w}$	-0.18	0.45	0.61	-0.47

1.1.2 Perfluoroalkyl acids

Perfluoroalkyl acids (PFAAs) are carboxylic, sulfonic, sulfinic, phosphonic, and phosphinic acids, where all the hydrogens of the carbon chain are substituted by fluorine. The general formula of perfluoroalkyl carboxylic acids (PFCAs) is $C_nF_{2n+1}COOH$, and perfluoroalkyl sulfonic acids (PFSAs) $C_nF_{2n+1}SO_3H$. They have been used in several products such as firefighting foams, wetting agents, lubricants, corrosion inhibitors, and stain-resistant treatment for fabric, leather and paper, or released as by-products (Giesy and Kannan 2002). The most studied compounds are perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), as they and their precursors have been the main products manufactured in the fluorochemical industry since the 1950s. Traditionally, these two compounds have been the most abundant PFAAs in nature and humans (Galatius et al. 2013; Giesy and Kannan 2001; Loos et al. 2009). In 2006 the European Union restricted the production and use of PFOS, its salts and perfluorooctane sulfonyl fluoride (Directive 2006/122/EC), and they were added to Annex B of the Stockholm Convention on Persistent Organic Pollutants in 2009. The restriction of PFOA is also being discussed at the EU (ECHA 2014). PFSAs and PFCAs are strong or relatively strong acids and therefore present in anionic form in natural waters (Goss 2008; Rayne and Forest, 2009). The vapour pressure and air-water partitioning coefficients of the deprotonated PFAAs are low (Rayne and Forest, 2009), and the strong C-F bonds make perfluorinated compounds extremely persistent and resistant to natural degradation (Giesy and Kannan 2002). The longer the fluorinated carbon chain, the more bioaccumulative and biomagnificable the PFAAs are (Conder et al. 2008). PFSAs are more bioaccumulative than PFCAs of the same fluorinated carbon chain length.

PFAAs are ubiquitous in the environment. As persistent and relatively water-soluble compounds, they have been detected in groundwater (Loos et al. 2010a) and the open sea (Ahrens et al. 2010; Yamashita et al. 2004). PFAAs are found in Arctic mammals (Kannan et al. 2001), as well as human tissues, blood and breast milk (Fromme et al. 2009). Based on their stability, perfluorinated compounds were first considered to be metabolically inert and therefore non-toxic (Sargent and Seffl 1970), an assumption that has been invalidated since. For example, elevated serum concentrations have been linked to breast cancer (Bonefeld-Jorgensen et al. 2011). As endocrine disrupting compounds (Benninghoff et al. 2011; Henry and Fair 2013; Kjedsen and Bonefeld-Jorgensen 2013; Long et al. 2013; Maras et al. 2006), PFAAs are suspected to have adverse effects on human reproduction (Fei et al. 2009; Fei and Olsen 2011; Hoffman et al. 2010; Joensen et al. 2009) and development (Apelberg et al. 2007; Fei et al. 2008; Lopez-Espinosa et al. 2011; Washino et al. 2009).

The main sources of PFAAs in the water environment are WWTPs and contaminated soils, although other point sources may occur. Current WWTP processes do not remove PFAAs. In contrast, the concentrations can rise during the treatment process due to degradation of precursors. PFCAs with a chain length of more than seven carbons and PFSAs with more than six carbons tend to partition into sludge (Higgins et al. 2005; Rayne and Forest 2009; Schultz et al. 2006; Sinclair and Kannan 2006; Suominen et al. 2014). If sludge is spread on fields as fertiliser or to improve soil quality, it may serve as a source of PFAAs in surface waters via run-off, or the contaminants may permeate to groundwater. Air deposition of volatile precursors such as fluorotelomer alcohols (FTOHs), perfluoroalkyl sulfonamides (FASAs), and perfluoroalkyl sulfonamido ethanols (FASEs) plays an important role in (remote) areas where point sources do not exist (Ahrens et al. 2010; Dreyer and Ebinghaus 2009; Martin et al. 2006; Rhoads et al. 2008; Scott et al. 2006; Shoeib et al. 2006). The long-distance transport of volatile precursors may also become a more important source in populated areas if local emissions can be controlled, but the production and use of these chemicals continues elsewhere.

The aquatic toxicity of PFAAs has been studied extensively. As the most prevalent PFAAs, PFOS and PFOA have gained the most attention (Hekster et al. 2003). Both PFCAs and PFSAs have been observed to affect the body size and weight, cholesterol levels, and reproduction in rotifers and fish, for example (Zhang et al. 2012 and 2013). The aquatic toxicity of PFOS and perfluorobutanesulfonic acid (PFBS) has been reviewed by Giesy et al. (2010). According to the acute and long-term toxicity tests with algae, invertebrates, amphibians and fish, marine invertebrates seem to be more sensitive to PFOS than freshwater species, and PFBS is far less toxic to aquatic organisms than PFOS (Giesy et al. 2010). The lowest PFOS' no observed effect level (NOEC) concentration of 0.05 mg/L has been defined for the freshwater invertebrate *Chironomus tentants* in a 10-day survival and growth test (MacDonald et al. 2004). Although PFBS – a substitute of the restricted PFOS – shows much lower acute and long-term toxicity, it has recently been shown to have adverse effects on the hepatohistology and sexual development of the amphibian *Xenopus laevis* (Lou et al. 2013). The European Union has proposed an annual average environmental quality standard (AA-EQS) of PFOS to be 0.65 ng/L in inland surface waters, and 0.13 ng/L in coastal waters. The corresponding maximum allowable concentrations (MAC-EQS) are 36 and 7.2 µg/L.

1.2 Photoreactions

Photolytic transformation can be an important degradation method in the environment, especially for microbially recalcitrant compounds. The sun emits the whole range of ultraviolet (UV) and visible radiation, from 100 to 700 nm, but the Earth's atmosphere blocks the most energetic wavelengths (Figure 1). Therefore <290 nm radiation does not reach the Earth surface. The compounds that have chromophores, i.e. conjugated π-bonds, can absorb solar radiation and have great potential to undergo direct photochemical reactions in surface water, soil surface, etc. (Zepp and Cline 1977).

The compounds that do not absorb solar radiation may undergo indirect photochemical reactions through chemical transients (e.g. hydroxyl radicals) produced by natural photoreactive substances such as ferric iron (Fe(III)), nitrate, and chromophoric dissolved organic material (CDOM). Fe(III) has an important role as an oxidative agent since it may

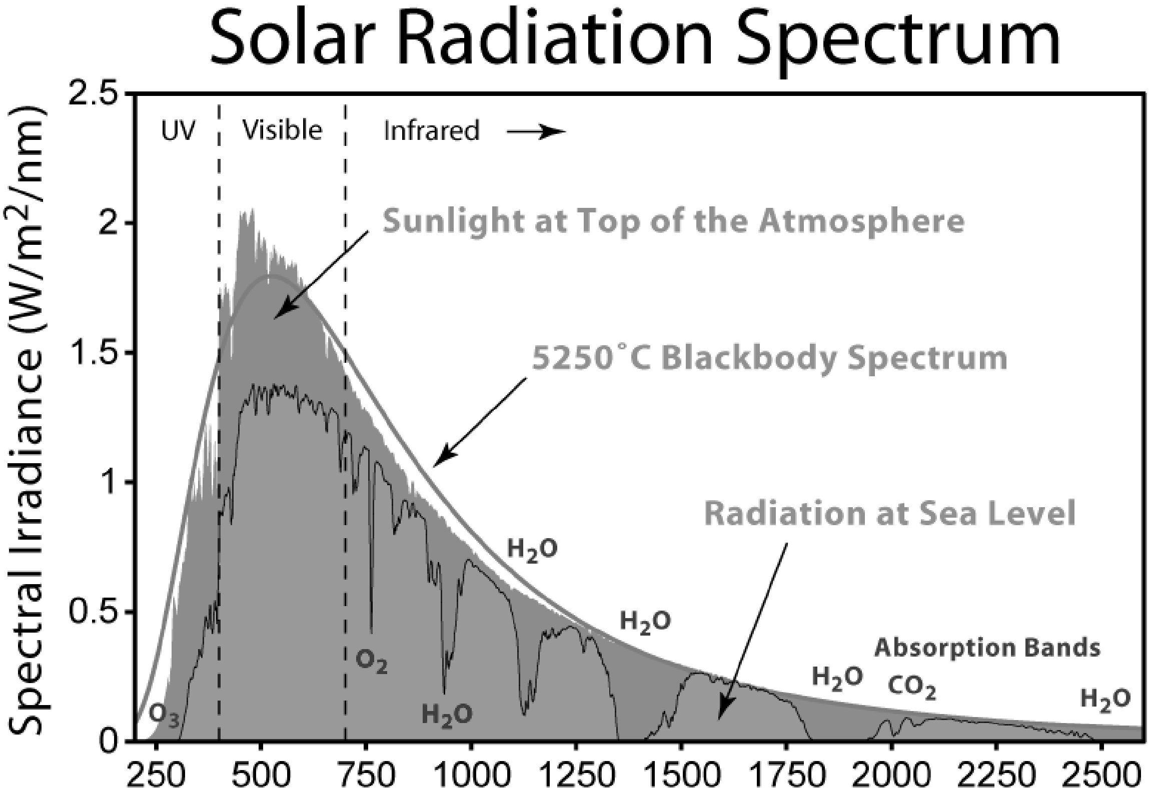


Figure 1. Solar radiation spectrum at the top of the atmosphere and at sea level. Figure: Robert A. Rohde

undergo photo-Fenton reactions to produce hydroxyl radicals in water (White et al. 2003). Nitrate absorbs sunlight in the UV range at a maximum of 302 nm (Machando and Boule 1995). This leads into photolysis and eventually to the formation of hydroxyl radicals under UV light irradiation (Zuo et al. 2006). CDOM is present in many natural water bodies, and can contribute to the photolysis of organic compounds through the production of free radicals (Hoigne et al. 1988; Paul et al. 2004). However, since CDOM absorbs radiation, it also confines the spectral exposure to a narrow surface layer (Wetzel 2003). CDOM can also maintain Fe in solution through complex formation (Shapiro 1966). These complexes affect water colour and therefore enhance both ultraviolet and visible light absorption in natural waters (Maloney et al. 2005; Xiao et al. 2013).

1.3 Ecotoxicological tests

Many emerging compounds like artificial sweeteners and short-chain PFAAs are not bioaccumulative, and the environmentally relevant concentrations are not considered to be acute toxic. However, they are either persistent or continually released into the environment and many are ubiquitous. Therefore, there is a need to evaluate their long-term ecotoxicological impacts on various species. The evaluation of the potential effects of these kinds of chemicals may be more complex than traditional pollutants such as heavy metals. They are not likely to cause direct mortality, at least in environmentally relevant concentrations. Therefore, sub-lethal effects i.e. changes in fitness-related features such as reproduction, growth, development, behaviour and physiology of individuals, should be studied. These effects may be subtle and may not increase or decrease linearly with increasing concentrations. In addition, there are individual and interspecies variations in tolerance to environmental stressors. Therefore, observation of the potentially occurring subtle effects over individual differences can be challenging. The individual variability can be diminished by using laboratory strains. In order to overcome the individual alteration, an adequate amount of replicates is needed.

Daphnia magna is a freshwater species that is commonly used to study the ecotoxicological effects of chemicals in an aquatic environment. They reproduce by continued parthenogenesis, enabling a laboratory population with a similar genetic strain. In addition, *Daphnia magna* are small, easy to culture, and their lifespan is short. Therefore, both lethal and sub-lethal effects can be studied with different life stages and in a reasonable time period. From an ecological perspective, daphnids play an important role in aquatic ecosystems. They are often the primary grazers of microorganisms such as algae and the primary prey for zooplanktivorous predators (Tessier et al. 2000).

2. OBJECTIVES OF THE STUDY

The objective of this study was to add to the knowledge about emerging substances. More specifically, the purpose was to study the occurrence and fate of artificial sweeteners and perfluoroalkyl acids in the aquatic environment. The first aim was to study the distribution of these chemicals in surface waters and to evaluate their sources. Several river and lake water samples were collected to study the occurrence of artificial sweeteners (I) and perfluoroalkyl acids in surface waters. In addition, perfluoroalkyl acids were analysed from wastewater effluent and sludge, landfill leachate and storm water, and mass flows from these sources were evaluated (II). The second aim was to investigate the fate of these substances in the aquatic environment. The potential of artificial sweeteners (IV) and perfluorooctanoic acid (III) to undergo direct and indirect photochemical degradation in surface water as well as in ultraviolet germicidal water treatment was studied with laboratory experiments. The microbial degradation of artificial sweeteners in surface water and wastewater was evaluated using closed bottle incubation. The third aim was to evaluate the effects of artificial sweeteners on aquatic organisms with a laboratory breed.

3. MATERIAL AND METHODS

A summary of the material and methods that were used in this study are presented in this chapter. A more detailed description on the samples, sample preparation, and instrumental analysis and procedures are presented in the original publications.

3.1 Sampling

For sweetener analysis, surface water samples were collected from twelve rivers and three lakes in 2011 and 2012 (Tables 2 and 3). The rivers and lakes are known or suspected to be affected by wastewater effluents, wastewater pumping stations, on-site wastewater treatment, or diffuse load from agriculture (I). Six rivers were sampled five or six times during one year, whereas one or two samples were grabbed from the other six rivers (Table 3). All river samples, except those from Tuusulanjoki and single samples from the Vantaa and Kokemäki, were taken at the national water quality monitoring sites which represent the overall contamination of the given river. Sampling depths were 0.1, 0.5, or

1.0 m below surface in rivers, depending on the river depth and flow rate at the sampling site. Lake water was collected at 1.0 m below surface. Lake Tuusula, leading to the Tuusulanjoki, is exposed to scattered load and potentially leaking sewers or wastewater pumping stations. Lake Vanajavesi was sampled in a bay where a wastewater treatment plant discharges effluents close to a river estuary. Lake Päijänne is a large lake which acts as the source of tap water for the Helsinki metropolitan area. It was sampled during the autumn overturn at two locations: near a wastewater effluent discharge point and at a distance of five kilometres.

To estimate the inputs of PFAAs into the aquatic environment, wastewater effluents and sludge, landfill leachate and storm water in an industrial area were sampled in 2009–2010 (Table 4) (II). Effluent samples were collected from one industrial and three municipal WWTPs six times. Municipal WWTP sludge, landfill leachate and storm water were sampled twice. Additionally, wastewater from the metal plating industry was sampled four times. Wastewater and landfill leachate were 24-h composite samples. Municipal wastewater effluents were adjusted to flow rate, whereas industrial wastewater effluents and landfill leachate were adjusted to time. Sludge, storm water and metal plating industry wastewater were collected as crab samples. The occurrence of PFAAs in surface waters was studied from five rivers (Porvoo, Vantaa, Vanjoki, Väänteenjoki and Nummenjoki) located in southern Finland (Table 3). The samples were taken as crab samples in June and July 2010.

Table 2. Sampling site information on lakes: lake area, depth, residence time and catchment area, and the population equivalents of the wastewater treatment plants that discharge effluents into given lake. (I)

Lake	Tuusula	Vanajavesi	Päijänne
Sampling time	6/11	6/12	8/11
Area (km^2)	6	120	1,118
Depth (m) (average; max)	3.2; 10	8.0; 24	16; 95
Residence time (months)	7	12	35
Catchment area (km^2)	92	2,700	26,460
Population equivalent	-	200,000	320,000
Other remarks	Wastewater pumping stations near the shore		Source of drinking water for Helsinki metropolitan area

Table 3. Sampling site information on rivers: river length, average, minimum and maximum currents and catchment area, and sum of the population equivalents of the wastewater treatment plants that discharge effluents into given river.

River	**Length (km)**	**Current (m^3/s)**	**Catchment area (km^2)**	**Population equivalent**	**Other remarks**
Aura	70	7.4 (0.00–286)	874	22,600	
Kapeenkoski	1.5	83 (15–343)	-	< 10,000	Rapids on Kymi waterway
Kokemäki	121	240 (52–600)	27,046	762,500	
Kymi	240	282 (65–816)	37,159	122,900	
Kyrö	200	43 (2.3–528)	4,923	160,000	
Mustio	25	19 (9–43)	-	35,000	Natura 2000 site
Nokianvirta	10	147 (0.09–419)	-	710,000	Part of Kokemäki waterway
Nummenjoki	19	6.1 (0.4–28)	-	-	
Oulu	107	250 (18–848)	22,840	6,800	
Porvoo	143	11 (1–200)	1,237	345,500	
Tuusula	15	1.0 (0.03–15)	125	-	Part of Vantaa waterway
Vanjoki	25	4.6 (0.1–26)	-	-	
Vantaa	101	16 (2.1–131)	1,686	161,700	
Vuoksi	150	596 (150–1,115)	68,501	60,000	
Väänteenjoki	5	7.5 (1–39)	-	-	

Table 4. Information on the wastewater, sludge, storm water and landfill leachate sampling sites. (II)

Sample type	**Sampling location**	**Type of treatment**	**Flow rate m^3/d**	**Population equivalent**	**Industrial wastewater proportion (%)**	**Discharge**
Municipal effluent (MWWTP1)	Turku	Primary, chemical, biological, improved N removal	120 000	280 000	7	Harbour near coastline
Municipal effluent (MWWTP2)	Espoo	Primary, chemical, biological, improved N removal	100 000	295 000	8	Approx. 7.5 km from coastline
Municipal effluent and sludge (MWWTP3)	Helsinki	Primary, chemical, biological, improved N removal	270 000	780 000	15	Approx. 7.8 km from coastline
Industrial effluent (IWWTP1)	Porvoo	Primary, chemical, biological	564	–	100	Coastline
Industrial wastewater	Vantaa	Chemical, mechanical	–	–	100	MWWTP3
Storm water	Porolahti creek, Helsinki	None	–	–	–	Via brook to coastline
Landfill leachate	Espoo	None	~1300	–	–	MWWTP2

3.2 Sample pre-treatment

Environmental water samples were extracted using solid phase extraction (SPE). Sweetener samples were filtered with glass fibre filters (GF/A 1.6 µm; Whatman, Kent, UK). 100 mL of each sample were spiked with surrogate standard (deuterated asesulfame, cyclamic acid and sucralose, and ^{13}C-labelled saccharin). When necessary, the PFAA samples were centrifuged to diminish the solid material and to avoid blocking the SPE cartridges. 200 mL of wastewater, storm water and surface water, and 50 mL of landfill leachate were spiked with a ^{13}C-surrogate mixture. Sample pH was adjusted to 2-3 with formic acid. The PFAA wastewater, storm water, and river samples were extracted with reversed-phase SPE cartridges (Varian Bond Elut Plexa (II) or Waters Oasis HLB), whereas the artificial sweetener samples (I) and landfill leachate for PFAA analysis (II) were extracted with weak anion-exchange SPE (Waters Oasis WAX). All cartridges were conditioned with 20 mL of methanol and 5 mL of deionised water. The samples were passed through, and the sweeteners were extracted with 6 mL of methanol and 8 mL of 1% NH_3 in acetonitrile that were collected in separate tubes and evaporated to dryness with an EZ-Envi Personal Evaporator (Genevac; Ipswich, UK), and redissolved in 0.5 mL of deionised water. The PFAAs were extracted from both types of cartridges with 3 mL of 1% NH_3 in methanol, and the extracts were cleaned with activated carbon (Supelclean ENVI-Carb 120/400), where necessary (II). Freeze-dried sewage sludge samples (1 g) were spiked with PFAA surrogates and left to soak in 1.0 mL of 200 mM NaOH solution for 30 min (II). The samples were extracted with methanol according to a method described by de Voogt et al. (2006), based on a method by Powley et al. (2005). The PFAA extracts were diluted 1:1 with deionised water. All samples were analysed with liquid chromatography mass spectrometry (LC-MS).

3.3 Liquid chromatography mass spectrometry

Liquid chromatography mass spectrometry (LC-MS) is a commonly used technique to analyse environmental contaminants. High performance liquid chromatography (HPLC) is a technique where compounds are separated based on their partitioning between a liquid mobile phase and a solid stationary phase, i.e. their mobility through a column. Ultra-high performance liquid chromatography (UHPLC) is an HPLC technique where the column internal diameter and stationary particle size are very small, resulting in better resolution, sensitivity and faster analysis than with conventional techniques. In reversed-phase LC, where the mobile phase is polar and the stationary phase is nonpolar, polar compounds such as PFAS and artificial sweeteners can be separated. In this study, an Acquity UPLC (Waters, Milford, MA, USA) was utilised with a nonpolar C18 column stationary phase: Acquity UPLC BEH C18 and Acquity UPLC HSS C18. The latter is a little more effective in retaining very polar compounds, such as acesulfame and saccharin, and was used in analysing sweeteners in surface water samples (I). BEH C18 was used for PFAAs (II, III), and sweeteners in the photolysis study (IV).

Mass spectrometry is an analysis technique whereby molecules are ionised and the mass-to-charge ratios of molecule ions or ionised fragments are measured. The ability of a mass spectrometer to separate two ions with different mass-to-charge ratios (*m/z*) is called mass resolution. The ability of a mass spectrometer to measure *m/z* accurately is called mass accuracy. Mass spectrometers can be divided into two categories: the ones with high mass resolution and the ones with low mass resolution. For example, triple quadrupole mass spectrometers (TQ MS) are low mass resolution instruments that are very effective in the quantitation of known molecules even at low concentrations, whereas time-of-flight mass spectrometers (TOF MS) can be high mass resolution instruments with higher mass accuracy, and often used to search and identify unknown components of the sample. In this study, a Xevo TQ MS (Waters, Milford MA, USA) was used to quantitate PFAS (II, III) and sweeteners (I, IV). A Micromass LCT Premier XE (Micromass MS Technologies, Manchester, UK) TOF MS was used to search for photochemical transformation products of artificial sweeteners (IV). The details on instrumental analysis are presented in references I, II, III, and IV.

3.4 Photochemical experiments

A photoreactor can be used to study phototransformation reactions in a laboratory. The reactor that we used in our studies (III, IV) was Atlas Suntest CPS+, which is designed to study the effects of solar radiation in particular on molecules and materials. A xenon arc lamp is a gas discharge lamp which produces a continuous spectrum across the UV-visible spectral region, and is very similar to the spectrum of sunlight. In this study, a xenon arc lamp was used in solar simulation tests. The Earth’s atmosphere blocks the most energetic UV rays, below 290 nm wavelength UV radiation. Therefore, to simulate the solar radiation at sea level, the xenon arc lamp was equipped with a UV filter. In addition, as the lamp also emits infrared radiation, the samples to be radiated were placed in a tempered water bath (+20 °C) to avoid heating the samples. The emission of the solar simulator system at 290–500 nm was 302 W/m^2 when measured with a Macam SR991 spectroradiometer. The global terrestrial photolytic solar radiation at that range contributes 27.3 W/m^2 to the net surface shortwave radiation (Chu and Liu 2009). Therefore, the solar simulator emitted eleven times more intense radiation at 290–500 nm than the mean solar radiation at the Earth’s surface. Artificial sweeteners were irradiated with a solar simulator in deionised and surface waters, and $Fe_2(SO_4)_3$ solution for 96 and 168 hours. PFOA was irradiated in deionised water, $Fe_2(SO_4)_3$ solution, NO_3 solution, fulvic acid solution, and filtered sea water for 66 and 165 hours. The irradiations correspond to 44.2, 77.4, 30.3, and 75.6 days of average solar radiation on Earth, respectively.

UV disinfection is a water treatment technique where short wavelength UVC radiation is applied. Low-pressure mercury lamps emitting mainly at 254 nm are commonly used in both drinking water and wastewater treatment. To investigate the behaviour of PFOA (III) and artificial sweeteners (IV) during this purification step, the xenon arc lamp was replaced with a low-pressure mercury lamp, and the UV filters were removed. PFOA was irradiated with UVC (254 nm) in deionised water, Fe(III) solution, nitrate solution, fulvic acid solution, and filtered sea water for 48 and 144 hours. Artificial sweeteners were irradiated in Fe(III) solution, nitrate solution, and deionised water for 24 hours. In that experiment, acesulfame was fully degraded and thus an additional 1–240-min irradiation of acesulfame in deionised water was performed in order to calculate a reaction rate constant and half-life.

To study the indirect photolysis of sweeteners (IV) and PFOA (III), $Fe_2(SO_4)_3$ solution with 2.79 mg/L of Fe(III), and NO_3 solution with 1 mg/L of N were prepared. Indirect phototransformation of PFOA was also studied in Nordic aquatic fulvic acid solution (15 mg/L of C). The sensitiser concentrations were selected to correspond to the upper range present in natural surface waters. In addition, filtered natural lake and river water (sweeteners), and sea water (PFOA) were used as sensitisers. The spiked samples were divided into *initial*, *irradiation*, and *dark control* samples. Samples other than *irradiation* samples were wrapped in aluminium foil. The *initial* samples were stored at +4 °C, whereas the *dark controls* were kept in the photoreactor (+20 °C) during irradiations. In addition, blank samples were prepared for each solution. Irradiations were performed in quartz tubes. The tubes were completely filled to avoid air-water interface in the tube.

The sweeteners were spiked into deionised laboratory water (Milli-Q®, Merck Millipore, Darmstadt, Germany), Fe(III) solution, nitrate solution, and filtered river and lake water at concentrations of 84–460 µg/L. Nitrate solution was exposed only to UVC, whereas lake and river water was exposed to simulated solar radiation. PFOA was spiked into deionised water, Fe(III) solution, nitrate solution, fulvic acid solution, and filtered sea water at concentrations of 100 ng/L. All solutions were exposed to both UVC and simulated solar radiation.

The rate constants (k) and half-lives ($t_{1/2}$) were calculated using the following equations:

$$k = ln\left(\frac{C_0}{C_t}\right)t^{-1} \qquad (1)$$

and

$$t_{1/2} = \ln 2t(\ln C_0 - \ln C_t)^{-1} \tag{2a}$$

where C_0 is the initial analyte concentration before exposure to radiation, and C_t is the concentration after irradiation of time t. When the removal of the parent compound could not be detected, the photolytic half-lives were estimated with the standard deviation of the method (SD), as follows.

$$t_{1/2} = \ln 2t(\ln C_0 - \ln(C_0 - SD))^{-1} \tag{2b}$$

The term C_0–SD represents the potential amount of parent compound that could have been removed during the irradiation tests but could not be verified because it was within the method SD.

3.5 Microbial degradation

The microbial persistence of artificial sweeteners in fresh, unfiltered surface water and wastewater effluent was studied by incubation in closed bottles. Surface water and deionised water was spiked with 100–500 ng/L of acesulfame, saccharin, cyclamic acid, and sucralose. Wastewater effluent was not spiked. Each sample type was divided into ten bottles; one sample was extracted immediately, one was stored in freezer, and the rest were kept in dark conditions at 4 or 15 °C for up to four weeks. The samples were extracted and analysed according to the method described in sections 3.2 and 3.3.

3.6 Ecotoxicological tests

The ecotoxicological effects of artificial sweeteners were studied with a multi-generational test on *Daphnia magna* Straus. The neonates (F0) were cultured in the test solutions for 21 days according to OECD Technical Guideline number 211 (OECD 211). The test temperature was 20±2 °C. The test solutions were renewed three times a week, and the offspring (F1) were collected and calculated. The exposure was continued with the F1 generation: ten animals were randomly selected from each replicate (5–10) and transferred to a 500 mL vessel containing similar test solution where the first exposure was performed, and the offspring (F2) was collected and calculated. Furthermore, the second generation (F2) was cultured until adult in pure nutrient solution.

First, *Daphnia magna* were exposed to the four sweeteners and their mixture at two concentrations: 10 and 1000 µg/L solutions of acesulfame, saccharin, cyclamic acid and sucralose, and a mixture of the four were prepared in an Elendt M7 medium (Elendt 1990; OECD 211). Controls were cultured in pure medium. Five replicates of each solution were prepared, as well as ten controls. Based on the results of the first test, another exposure with the more concentrated solutions (1000 µg/L) of acesulfame and cyclamic acid, and ten replicates was performed. The sweetener concentrations were monitored during the experiments and no decrease in concentrations was observed.

4. RESULTS AND DISCUSSION

The presentation of the results of this study is divided into three parts. Section 4.1 presents the occurrence and sources of artificial sweeteners (4.1.1) and PFAAs (4.1.2) into the aquatic environment. In addition, section 4.1.1 introduces the results of the incubation study on artificial sweeteners. In section 4.2 the results of photochemical degradation experiments of artificial sweeteners and PFAAs under UVC (4.2.1) and solar radiation (4.2.2) are discussed. Section 4.3 shows the outcome of ecotoxicological studies that were performed on artificial sweeteners.

4.1 Environmental occurrence

The emerging compounds acesulfame, saccharin, cyclamic acid, sucralose, PFCAs and PFSAs are ubiquitous in Finnish surface waters. They are persistent in surface water: microbial and photochemical degradation is slow. Although microbes in wastewater can efficiently degrade saccharin and cyclamic acid, their microbial degradation in surface water is sluggish. Saccharin and cyclamic acid are also degraded by soil microbes. However, as water-soluble compounds, they tend to stay in the water phase.

The main sources of artificial sweeteners and PFAAs into surface waters are municipal wastewater effluents. Artificial sweeteners originate from human consumption, whereas PFAAs are released from products and chemicals used in households and industry, and finally from waste disposal sites where they are dumped. Saccharin can also be released from farming if certain herbicides are used, or from pig manure if pigs are fed with saccharin-containing feedstuffs. Further sources of PFAAs include air deposition and run-off from urban areas, firefighting sites, or other PFAA-contaminated areas.

4.1.1 Artificial sweeteners

Artificial sweeteners were detected in all lakes and rivers (Table 5) (I). Acesulfame was the most abundant sweetener in the surface waters. The highest concentrations in the lake samples, 780 ng/L of acesulfame, were obtained in Lake Vanajavesi where wastewater effluents are discharged near the sampling point. The concentrations of artificial sweeteners in lakes were similar to the ones reported by Berset and Ochsenbein (2012) but considerably lower than the maximum concentrations, 2800, 180 and 130 ng/L of acesulfame, saccharin, and cyclamic acid reported by Buerge et al. (2009) in Swiss lakes. Considerable dilution of wastewater effluents during the autumn overturn occurs in Lake Päijänne, as the sweetener concentrations were similar close to the discharge point and five kilometres downstream, and relatively low (200 and 210 ng/L of acesulfame). Surprisingly, the sample from Lake Tuusula (and the adjacent Tuusulanjoki) included almost as much sweeteners as was found in Lake Päijänne. This might be caused by on-site wastewater treatment, or substantial leaking of untreated wastewater through leaking sewers or wastewater pumping stations.

In rivers, the acesulfame concentration ranged between 28 and 9,600 ng/L, and the maximum sweetener concentrations were obtained in the Vantaa and Porvoo rivers (Table 5). Acesulfame and sucralose concentrations are inversely proportional to river flow rate (Q), i.e. the more dilution or higher Q, the smaller the concentrations. Another factor affecting the sweetener concentrations is population equivalent (PE), i.e. the greater the population load, the higher the concentrations. PE is the average wastewater load of one person per day. It is defined as the organic biodegradable load with a seven-day biochemical oxygen demand (BOD_7) of 70 g oxygen/day. The approximate PEs of the WWTPs releasing effluents into rivers are given in Table 3. Linear correlation was obtained between acesulfame and sucralose concentrations and PE/Q (Figure 2). Consequently, the most contaminated surface waters were the Vantaa and Porvoo rivers, located in a densely populated area in southern Finland. They are relatively small rivers with an average flow rate of only 16 and 11 m^3/s, respectively, but which receive wastewater effluents of over 160,000 and 340,000 population equivalent WWTPs (Table 3). Similarly, the sweetener concentrations were the lowest in the rivers with high currents and low wastewater burdens, i.e. the Oulu, Vuoksi, and Kymi rivers. Compared to reported sweetener concentrations in European and North American rivers, the Porvoo and Vantaa rivers are among the highest contaminated. During dry seasons when the river flow rates are at their lowest (3.3–8.1 m^3/s), the proportion of wastewater effluents considerably increase. Although the river water is diluted with water from an upstream lake at low discharge, 30% of the water in the River Porvoo can be wastewater. In a European study, up to 14,200 ng/L of acesulfame have been detected in the Swiss rivers Aa and Glatt, with high effluent burden and low discharge (Müller et al. 2011).

According to our unpublished study, the ratio of saccharin and acesulfame (SAC/ACS) is on average 0.6 in untreated municipal wastewater and 0.06 in treated effluent. The average SAC/ACS ratio was 0.24 in the samples from Tuusulanjoki and Lake Tuusula, indicating the introduction of untreated or aged wastewater into the lake. The SAC/ACS ratio in the River Vantaa sample, where the highest saccharin and cyclamic acid concentrations were detected, was 0.06, similar to the ratio in WWTP effluents. Sweetener concentrations in the other sample from the same river collected at the water quality monitoring site downstream were also elevated but the SAC/ACS ratio was only 0.01. This indicates that effluent (or aged untreated wastewater) release has occurred close to the sampling point where the maximum saccharin and cyclamic acid concentrations were detected, but was thereafter diluted by the side streams Tuusulanjoki and Keravanjoki. In fact, there are many wastewater pumping stations in the River Vantaa catchment area which comprises the Tuusulanjoki and Lake Tuusula, and leakages of untreated wastewater caused by malfunctions in the pumping stations are not uncommon (HSY 2011). These findings show the potential of the sweeteners and their ratios as tracers of wastewater emissions.

The microbial persistence of artificial sweeteners was studied by incubation in closed bottles. All sweeteners appeared to be microbially recalcitrant in surface water during a four-week incubation period at 4 and 15 °C as the sweetener concentrations did not change. Acesulfame and sucralose did not decrease in wastewater effluent either. However, the saccharin and cyclamic acid concentrations approximately halved during four-day incubation in wastewater effluent at both temperatures. The concentrations of saccharin reduced by 42% at 4 °C and 52% at 15 °C in four days, and the corresponding values for cyclamic acid were 52 and 62%. Previous studies of the microbial degradation of artificial sweeteners in environmental waters support our findings. Lange and Alexander (1994) obtained only slow metabolism of sucralose in lake water in a 93-day incubation, and van Stempvoort et al. (2011b) did not observe any degradation of sucralose or acesulfame but complete removal of saccharin or cyclamic acid in refrigerated (wastewater-contaminated) groundwater samples after 13.5 months. Wastewater microbes are known to efficiently degrade saccharin and cyclamic acid, and over 90% removal efficiency can be obtained in WWTPs (Scheurer et al. 2009). Conversely, van Stempvoort et al. (2011b) did not observe any degradation of any of the four sweeteners in refrigerated wastewater after 20 days. They did their experiments with field-filtered (0.45 µm) samples, and the wastewater samples were frozen before the 20-days storage period in a refrigerator, which has most likely affected the microbe count of the samples and may explain the divergent results.

Table 5. Lake and river water sampling sites, number of the samples (n), sample depths, and the results obtained for acesulfame (ACS), saccharin (SAC), cyclamate (CYC) and sucralose (SCL). If n ≥ 2, the minimum and maximum concentrations are presented. (I)

Sampling site	***n***	**Depth (m)**	**ACS (ng/L)**	**SAC (ng/L)**	**CYC (ng/L)**	**SCL (ng/L)**
Lake Päijänne 1	1	1.0	210	<25	<13	n.d.
Lake Päijänne 2	1	1.0	200	<25	<13	n.d.
Lake Tuusula	1	1.0	150	25	<13	n.d.
Lake Vanajavesi	1	1.0	780	27	28	<200
River Aura	6	1.0	56–400	30–96	<13–100	n.d. –<200
River Kapeenkoski	1	0.5	34	<25	<13	<200
River Kokemäki 1	6	1.0	180–410	<25–42	<13–14	n.d.–<200
River Kokemäki 2	1	1.0	490	27	35	n.d.
River Kymi	6	0.1	89–150	<25	<13	n.d.–<200
River Kyrö	1	0.1	530	<25	16	<200
River Mustio	1	1.0	310	<25	<13	<200
River Nokianvirta	1	1.0	300	<25	<13	<200
River Oulu	1	1.0	28	<25	<13	<200
River Porvoo	6	1.0	810–9,600	37–150	14–120	<200–1,000
River Tuusula	2	0.5	140, 210	35, 62	25, 27	n.d.
River Vantaa 1	5	1.0	490–4,300	<25–46	16–44	<200–440
River Vantaa 2	1	1.0	7,800	490	210	730
River Vuoksi	6	0.1	65–110	<25	n.d.–<13	n.d.–<200

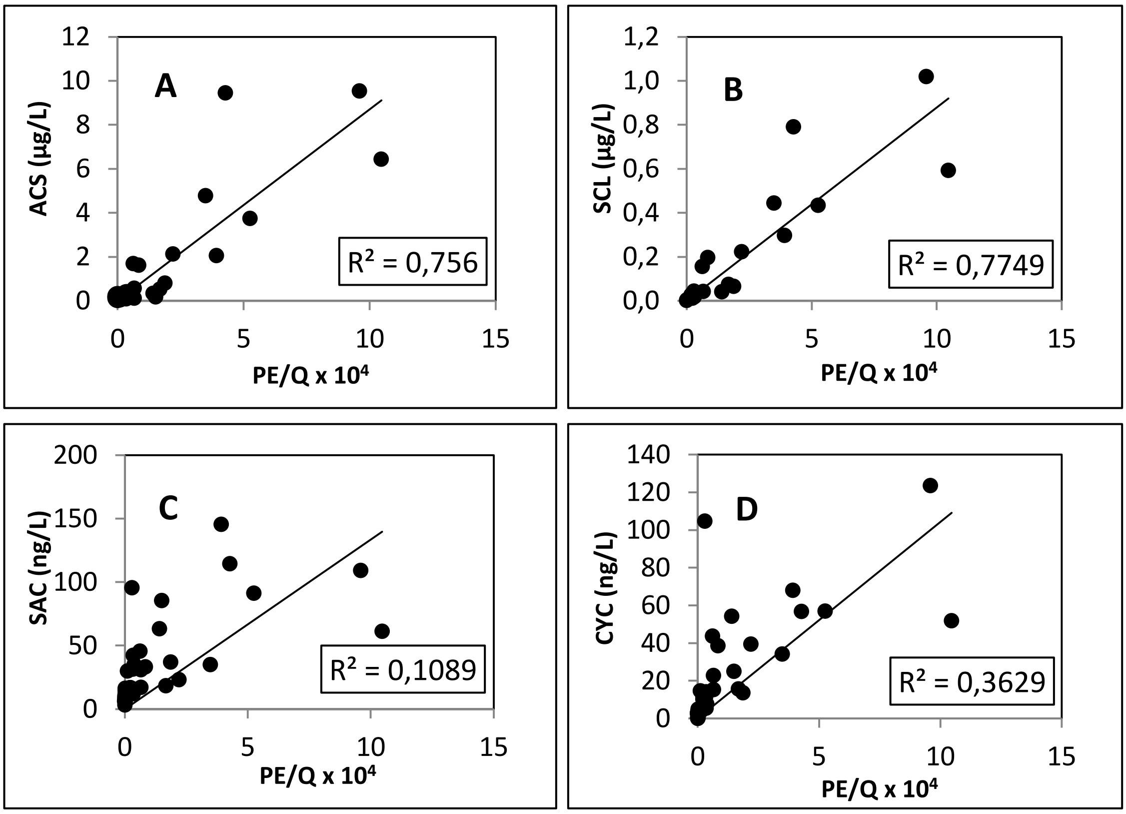


Figure 2. Linear correlation of the sweetener concentrations versus population equivalent (PE) and river flow rate (Q). (I)

4.1.2 Perfluoroalkyl acids

PFAAs were detected in all studied wastewater effluents, landfill leachate, storm water, and sludge. The highest concentrations in water samples were obtained in metal plating industry wastewater, followed by landfill leachate and industrial effluent (Table 6) (II). PFOA and PFOS were the most prevalent in most samples. However, in landfill leachate and storm water the concentration of the very water-soluble PFHxA was higher than PFOA and PFOS. Concentrations in municipal wastewater treatment plant (MWWTP) effluents ranged from <0.5 ng of perfluorodecanoic acid (PFDA) to 640 ng of PFOS per litre (Table 6) (II). On average, PFOS and PFOA were the most abundant PFAAs. According to an EU-wide monitoring survey (Loos et al. 2013), the PFAA contents in our study are close to the median concentrations of European effluents. An exception is MWWTP3, where high concentrations of PFOS were quantified occasionally in effluent and sludge, up to 640 ng/L and 110 µg/kg d.w., respectively. The PFOS concentrations in MWWTP3 effluent are as much as fifty times higher than the median (12.2 ng/L) and ten times the average (62.5 ng/L) reported in European effluents (Loos et al. 2013). This indicates a specific source of PFOS to MWWTP3. The average PFAA concentrations in industrial WWTP effluent were higher than in municipal effluents. Up to 1,300 ng/L of

PFOS and 100 ng/L of PFOA were detected. The metal plating industry wastewater, which is led to MWWTP3, contained 1.4–18 mg/L PFOS. Despite the high concentrations, the wastewater flux from the metal plating industry is too low to be the sole explanation to the elevated PFOS concentrations in MWWTP3.

The mass flows of PFAAs into the Baltic Sea were evaluated based on the obtained average concentrations and WWTP flow rates (II). The MWWTPs were the major sources of PFAAs. Although the PFAA concentrations were highest in industrial wastewater effluents and landfill leachate, the flow rates are much smaller than in MWWTP effluents, resulting in lower mass flows of PFAAs. The mass flows of industrial storm water could not be calculated, but given the large surface areas and relatively high precipitation (or

Table 6. The minimum, maximum, average and median concentrations of perfluoroalkyl acids in municipal (MWWTP) and industrial wastewater effluent (IWWTP), municipal sludge, storm water and landfill leachate. The unit for water samples is ng/L and µg/kg d.w. for sludge. (II)

		PFHxA	PFOA	PFDA	PFOS
MWWTP1	min-max	2.5–4.9	7.0–11	<0.5–0.9	7.8–14
n=6	average	3.4	8.0	0.50	12
	median	3.1	7.4	<0.5	13
MWWTP2	min-max	4.6–11	9.0–15	<0.5–1.3	3.8–20
n=6	average	6.2	12	1.0	10
	median	6.4	12	1.1	10
MWWTP3	min-max	2.8–9.5	6.6–13	<0.5–1.4	8.0–640
n=6	average	4.7	9.8	0.88	140
	median	3.9	9.8	0.90	60
IWWTP1	min-max	11–75	8.7–100	<0.5–1.7	320–1300
n=6	average	40	49	1.0	880
	median	36	55	1.0	1000
Target industry	min-max	9.0	27	<0.5	1.4E+6–18E+6
n=4	average	-	-	-	7.9E+6
	median	-	-	-	6.1E+6
Sludge	min, max	0.35, 0.72	0.65, 0.91	0.75, 0.97	16, 110
n=2	average	0.54	0.78	0.86	63
Storm water	min, max	3.5, 17	3.5, 5.1	<0.5, 0.6	6.5, 9.9
n=2	average	10	4.3	<0.5	8.2
Landfill leachate	min, max	49, 200	76, 270	2.0, 3.7	87, 140
n=2	average	120	170	2.8	110

PFHxA: Perfluorohexanoic acid; PFOA: Perfluorooctanoic acid; PFDA: Perfluorodecanoic acid; PFOS: Perfluorooctanesulfonic acid melting waters), surface run-off from industrial as well as urban areas might be an important source of PFAAs in Finland as well. The mass flows from MWWTPs were 0.15–0.46 kg of PFHxA, 0.35–0.99 kg of PFOA, 0.03–0.10 kg of PFDA, and 0.37–11 kg of PFOS per year. The PFOS mass flows were exceptionally high in the MWWTPs 1 and 3 in the 2009–2010 investigations. In North American WWTPs, the mass flow of PFOA has been reported to be the highest (0.13–7.7 kg/year), whereas PFOS emissions were considerably lower (0.03–0.62 kg/year) (Loganathan et al. 2007; Sinclair and Kannan 2006). The MWWTP3 sludge contained relatively high concentrations of PFOS, as well. Sludge can be modified to soil to improve its fertility and physical properties. Although the concentration in MWWTP3 sludge was well below the 500 µg/kg proposed as a low concentration limit for PFOS in sewage sludge in EU (ESWI 2011), the flux of PFOS to soil via the application of sludge as soil enrichment was evaluated to be 0.8–16 kg/year in Finland (Mehtonen et al. 2012).

In general, the most abundant compounds in the studied rivers were PFOS and PFHxA, and the least abundant was PFBA (Table 7). However, the PFAA concentration patterns appeared to be river-specific, indicating differences in the contamination sources. For example, perfluorononanoic acid (PFNA) concentration was the second lowest in the River Porvoo, whereas it was the second or third highest in the Vantaa, Nummenjoki, and Väänteenjoki. The river-specific patterns have been reported in literature: in European studies PFOA and PFHxA were the most abundant PFCAs, and river-specific patterns and concentrations were obtained (Loos et al. 2010b; McLachlan et al. 2007). Based on the median and maximum concentrations in 122 European rivers, PFOS was the most abundant compound, followed by PFOA (Loos et al. 2009). In our study, the PFAA concentrations were the highest in the River Vantaa and there must have been a specific source of PFAAs. However, compared to other European rivers, the PFAA concentrations were relatively low, even in the most contaminated rivers. In the River Vantaa the PFOS concentration (8.95 ng/L) was close to the median (6 ng/L) of the 122 rivers in an EU-wide survey (Loos et al. 2009). On the other hand, even if the levels are lower than in the most contaminated rivers, they show a widespread contamination of surface waters. In addition, the annual average environmental quality standard (AA-EQS) of PFOS – 0.65 ng/L (EU) – is very likely exceeded in the Vantaa (8.95 ng/L) and Porvoo (1.07 and 2.88 ng/L). These rivers flow into the Gulf of Finland and are also the most contaminated according to the sweetener concentrations. Riverine discharges are calculated to be the major source of PFAAs into the Baltic Sea (Ahrens et al. 2010; Filipovic et al. 2013).

In 2009 and 2010, when the samples were collected, the use of PFOS-containing firefighting foams was allowed, but has been banned since. Thus the PFOS mass flows and environmental concentrations should be smaller these days. However, PFAAs still leak to surface and groundwater from fire-training areas where PFOS-containing firefighting foams have been used extensively (Ahrens et al. 2014; Moody et al. 2003; de Solla et al. 2012). Moreover, there are certain industrial applications such as metal plating where the use of PFOS and related substances is permitted. As an example, the metal plating industry wastewater contained on average 7.6 mg of PFOS/L. Therefore, the PFOS concentrations in WWTP effluents and receiving waters can also be high today. Another emitter of PFAAs into the environment is air deposition. Volatile fluorinated compounds, such as fluorotelomer alcohols and their esters, and perfluoroalkyl sulfonamidoethanols that degrade to PFCAs and PFSAs, respectively, can drift long distances (Buck et al. 2011, Schenker et al. 2008, Xie et al. 2013). Therefore, air deposition may be significant compared to local point sources. Furthermore, the largest European and North American fluorochemical manufacturers have shifted to products based on perfluorobutane sulfonyl chemistry since 2002 (Renner 2006). Although there are still other manufacturers producing PFOS and its precursors (especially in China), PFOS levels in biota and human samples are decreasing (Ahrens et al. 2011; Kato et al. 2011; Olsen et al. 2012), whereas the amount of long-chain PFCAs ($> C_8$) keep increasing. On the other hand, short-chain PFCAs show an increasing trend in alpine snow samples between 1997 and 2007 (Kirchgeorg et al. 2013). Therefore, emissions of PFCAs appear to be increasing, and further monitoring of environmental levels of PFAAs is needed.

Table 7. Perfluoroalkyl acid concentrations (ng/L) in river water samples.

River	Date	PFBA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFOS	SUM
Väänteenjoki	7/2010	<0.1	0.27	0.12	0.12	0.19	<0.05	0.15	0.86
Nummenjoki	7/2010	<0.1	0.19	0.10	0.08	0.14	<0.05	0.28	0.79
Vanjoki	7/2010	nd	0.23	0.11	0.13	0.12	<0.05	0.13	0.72
Vantaa	7/2010	nd	4.21	0.87	1.51	1.79	0.08	8.95	17.34
Porvoo	6/2010	nd	1.56	0.34	0.63	0.10	<0.05	1.07	3.70
Porvoo	7/2010	nd	1.95	0.36	0.67	0.19	<0.05	2.88	6.05

PFBA: Perfluorobutanoic acid; PFHxA: Perflurohecanoic acid; PFHpA: Perfluoroheptanoic acid; PFOA: perfluorooctanoic acid; PFNA: Perfluorononanoic acid; PFDA: Perfluorodecanoic acid; PFOS: Perfluorooctanesulfonic acid; SUM: Sum of all perfluoroalkyl acids

4.2 Photochemical transformation

UV treatment with high-intensity irradiation can be applied to remove chemical and microbial contaminants from water. In water treatment, the most commonly used wavelengths are in the low UVC range. In this study, the potential of UV treatment to remove artificial sweeteners and perfluoroalkyl acids was evaluated. The chemicals were irradiated with 254 nm UVC and phototransformation products were searched for. The atmosphere blocks the most energetic wavelengths of sunlight and radiation below 290 nm. Therefore, UVC radiation does not occur naturally on the Earth's surface and UVC irradiations do not answer the question whether photochemical reactions of sweeteners and PFAAs occur in nature. The direct and indirect photochemical degradation of artificial sweeteners and PFOA was investigated with simulated solar radiation, and the photochemical half-lives in surface water were calculated.

4.2.1 Ultraviolet-C radiation

Phototransformation of acesulfame under UVC irradiation is rapid (see Figure 3) (IV). According to our tests, the half-life is approximately 1.2 min (IV). Our reaction rate constant was $k = 9.9*10^{-3}\ s^{-1}$ in deionised water (IV), similar to $6.3*10^{-3}\ s^{-1}$ obtained by Scheurer et al. (2014) at acesulfame concentration 100 µg/L with a 5 W low pressure mercury lamp. The elimination rate is strongly reliant on the intensity of radiation. Therefore, the half-life depends on the spectrum and output of the light source. Scheurer at al. (2014) stated that the photodegradation reaction followed pseudo-first order kinetics when C_0 was between 1 and 10,000 µg/L with their flow-through system, whereas at higher concentrations the reaction rate was limited by the photons emitted by the light source. The typical acesulfame concentrations in surface and groundwater and even wastewater fall well below 10,000 µg/L (Buerge et al. 2009; Loos et al. 2013; Scheurer et al. 2009; Wolf et al. 2012). Two recent papers have demonstrated that UV disinfection in water treatment works can eliminate acesulfame only partially (Nödler et al. 2013; Scheurer et al. 2014). However, it can be concluded that acesulfame can be removed with UV treatment if the exposure time is sufficient.

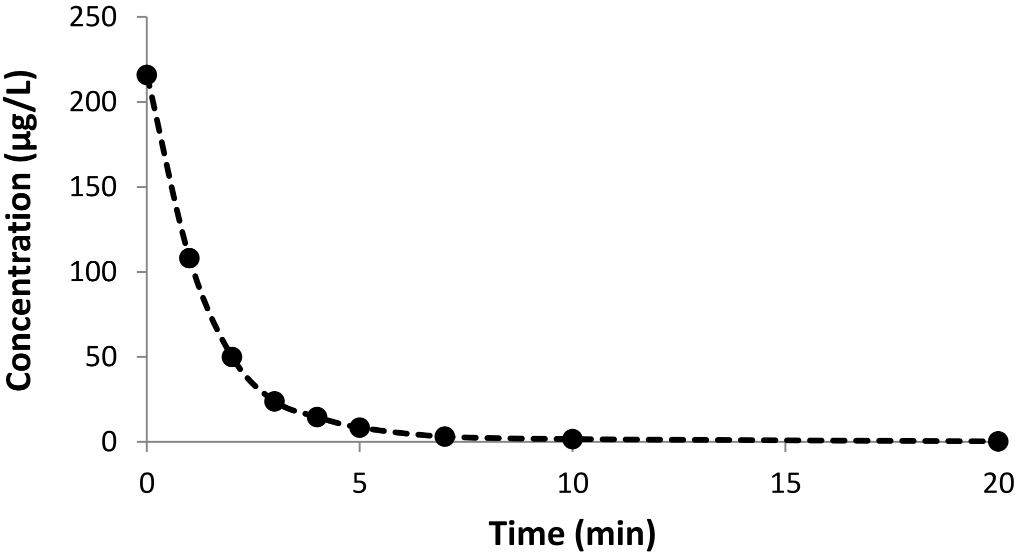


Figure 3. Reduction of 216 µg/L acesulfame under UVC irradiation. The samples were analysed with LC-TQ MS. (IV)

In our experiments, cyclamic acid and saccharin were also transformed under UVC irradiation. If the phototransformation was expected to follow first-order kinetics, the rate constants were k=$3.6*10^{-6}$ and $9.2*10^{-6}$ s^{-1} in deionised water, respectively. However, direct photolysis of cyclamic acid and saccharin during UV water treatment is not relevant because the half-lives were 53 and 21 hours in deionised water, respectively. Only 4% of sucralose was transformed in deionised water during a 24-hour UVC irradiation in our experiment (k=$4,7*10^{-7}$ s^{-1}) (IV). This is in accordance with earlier studies (Soh et al. 2011; Torres et al. 2011). Although Sang et al. (2014) were able to completely degrade sucralose (20 mg/L) in less than 200 minutes under photocatalytic conditions and sixteen lamps (254 nm) with total power of 128 W, the photolytic degradation of sucralose is not likely to occur during water treatment. Ferric iron enhanced the phototransformation of all sweeteners and the transformation rate of cyclamic acid and sucralose approximately doubled. Rate constants or half-lives could not be calculated for saccharin and acesulfame in Fe(III) solution, because they were completely degraded in the 24-hour experiment when Fe(III) was present. Nitrate did not induce the phototransformation of artificial sweeteners at 254 nm irradiation. The absorption coefficient of the 1 mg/L nitrate solution is only 0.34 m^{-1} at 254 nm (Vaalgamaa et al. 2011) making it a rather inefficient sensitiser at the given wavelength.

Three phototransformation products of acesulfame were detected with LC-TOF MS: iso-acesulfame (*m/z* 161.987), hydroxylated acesulfame (*m/z* 179.998), and hydroxypropanyl sulphate (*m/z* 155.001) (IV). The molecular structures of the product ions were estimated based on their potential elemental composition and fragmentation on TQ MS. Figure 4 shows the formation and decrease of the three product ions during UVC irradiation. All three transformation products were also detected in recently published studies: Sang et al. (2013) reported a total of ten ions including *m/z* 180 and 154 that appeared under photocatalytic reaction, whereas Scheurer et al. (2014) detected five ions including *m/z* 161.9867 and 179.9972 with conventional UVC irradiation. In addition, we obtained two product ions from the recent studies, amidosulfonic acid (*m/z* 96) and ion *m/z* 137, as MS fragmentation ions of hydroxylated acesulfame and hydroxypropanyl sulphate, respectively (IV). Significantly higher acesulfame concentrations were applied in the other studies than in our experiments, which may explain why we were able to detect only the most prevalent transformation products. Interestingly, Sang et al. (2013) observed increased toxicity of photolysis products of both acesulfame and sucralose with an acute toxicity bioassay. However, their finding should be further experimented and validated to be able to identify which transformation products are responsible for the increased toxicity and to be able to make safety assessments.

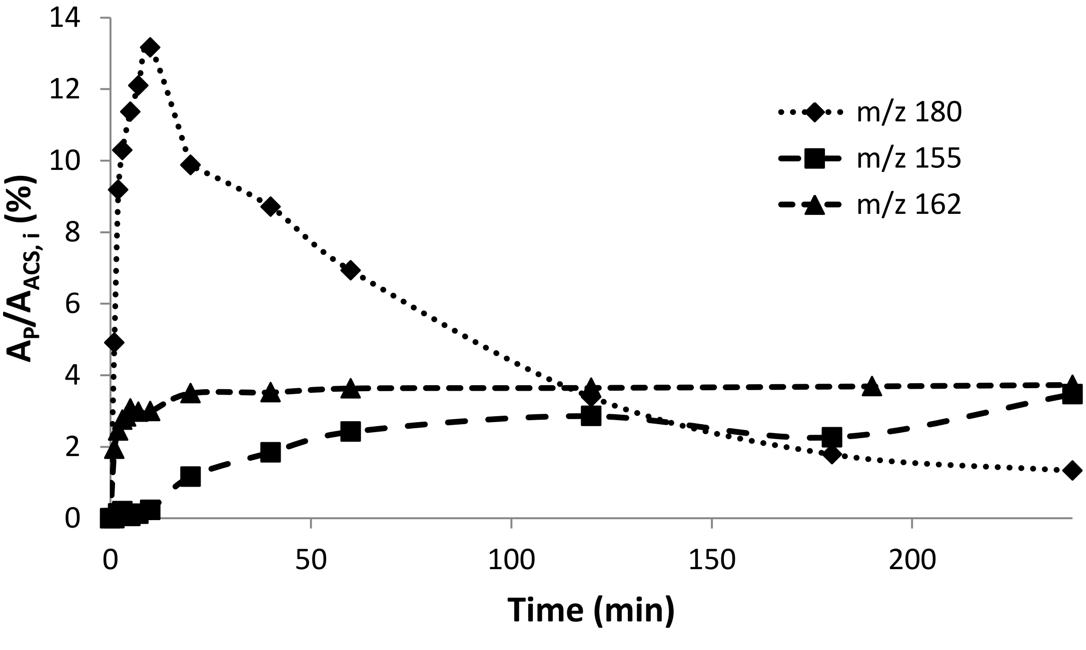


Figure 4. Formation and elimination of acesulfame phototransformation products under UVC irradiation presented as the peak area of the product ion (A_P) versus the peak area of acesulfame before irradiation ($A_{ACS,\ i}$) in percent. *m/z* 155, 162, and 180 are hydroxypropanyl sulphate, iso-acesulfame, and hydroxylated acesulfame, respectively. The samples were analysed with LC-TQ MS. (IV)

PFOA degraded slowly into shorter-chained PFCAs under UVC irradiation (Figure 5) (III). PFHpA and PFHxA were detectable after 48-hour irradiation, and after 144-hour irradiation perfluoropentanoic acid (PFPeA) was also detected. This is in agreement with earlier studies where PFOA was decomposed to fluoride ions and several smaller PFCAs via dealkylation (Wang et al. 2008b; Qu et al. 2010). The detected decomposition products encompassed up to 88% of the observed PFOA reduction. In other words, more PFOA was decomposed than PFHxA, PFHpA, and PFPeA was formed. Hence, some other decomposition products such as inorganic fluoride, perfluorobutanoic acid (PFBA), trifluoroacetic acid, or perfluoroalcohols were expected to form (Lee et al. 2009; Wang et al. 2008a). In addition, the products were likely to decompose further under irradiation. However, the concentration of PFHpA appeared to increase linearly during the exposures (Figure 6), indicating that more PFHpA was formed than decomposed. Therefore, the photoreactivity of short-chain PFCAs also seems to be low. The decomposition of PFOA was most efficient in the seawater sample, where 45% of PFOA was removed in relation to the initial concentration in 144 hours, followed by fulvic acid (31%), Fe(III) (28%), and nitrate (26%) solutions. In pure water the phototransformation of PFOA was weakest, as 24% was removed in 144 hours. This indicates that sensitisers increased the degradation of PFOA, although it was mainly caused by direct photolysis.

Efficient photolysis of PFOA and other PFCAs has been achieved with combined UVC irradiation and photocatalytic conditions, and often rigorous conditions such as high pressure or acidity (Cao et al. 2010; Estrellan et al. 2010; Hori et al. 2005; Qu et al. 2010). Wang et al. (2008a, 2008b) reported 80% removal of PFOA under 254-nm irradiation in aerated Fe(III) solution in 250 min. In our study, degradation of PFOA was not significantly different in Fe(III) solution compared to degradation in pure water. The difference may be explained by aeration, which brings oxygen into the solution, thus maintaining the photo-Fenton process i.e. oxidation of Fe^{2+} back to Fe^{3+}.

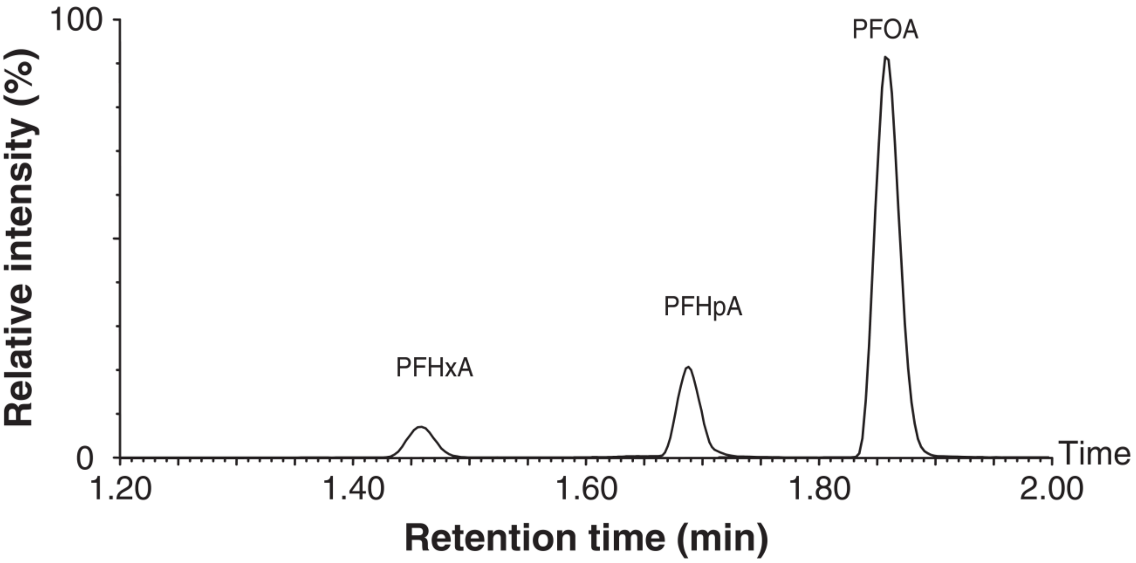


Figure 5. Phototransformation products of PFOA after 144-hour irradiation with UVC (254 nm) (III).

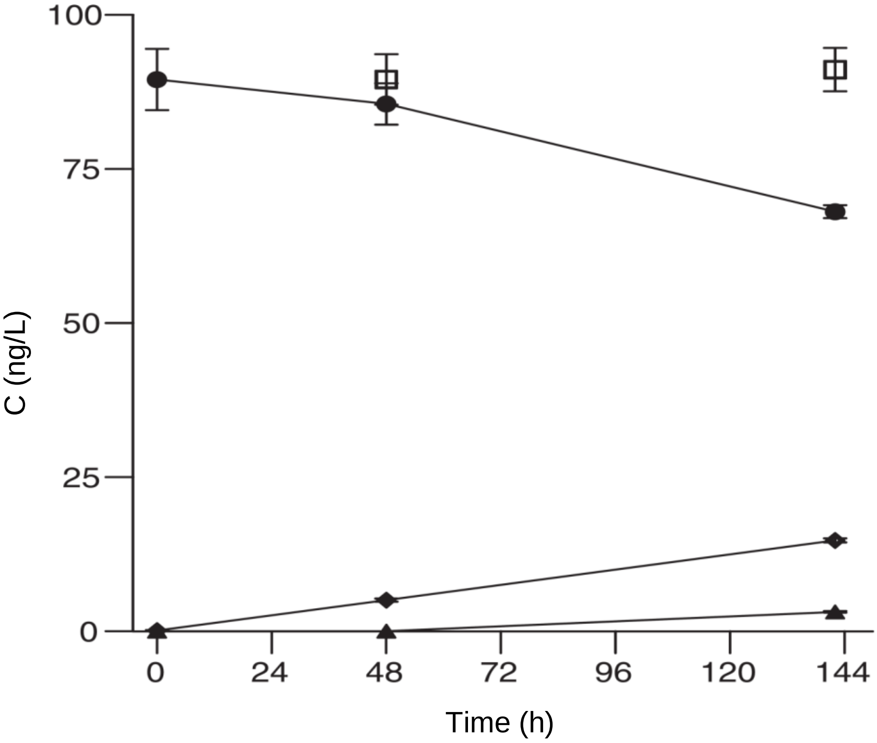


Figure 6. Concentrations of PFOA (●), PFHpA (◆), and PFHxA (▲) in deionised water during the irradiation at 254 nm, and the concentration of PFOA in dark control samples (□). Error bars represent the standard deviation of replicated (n=3) measurements (III).

4.2.2 Solar radiation

According to our tests, acesulfame can transform via direct photolysis under solar radiation in surface water (Figure 7) (IV). Fe(III) enhanced the photolytic transformation of acesulfame under solar radiation. However, the enhancement of photolysis was not observed in natural lake and river water in relation to the pure water sample (Figure 7). The degradation rate constant (k) of acesulfame was $5.5*10^{-11}$ s^{-1} in deionised water, $5.6*10^{-11}$ in lake water, and $4.6*10^{-11}$ s^{-1} in river water. The difference in rates may be caused by the shading effect of the dissolved material in river water. Based on average solar radiation on the Earth's surface, the photolytic half-life of acesulfame on the surface of water is approximately 12 months (Equation 2a). Also saccharin was decreased in Fe(III) solution with a half-life of 3.6 months.

Simulated solar radiation did not affect the concentrations of PFOA, cyclamic acid, and sucralose in any solution, or saccharin in other solutions than Fe(III) (III, IV). These chemicals do not absorb solar radiation and direct photolysis was not expected. According to our experiments, indirect photolysis is also unlikely under solar radiation in water. The radicals that are formed by photosensitisers do not efficiently break the strong C-F bonds

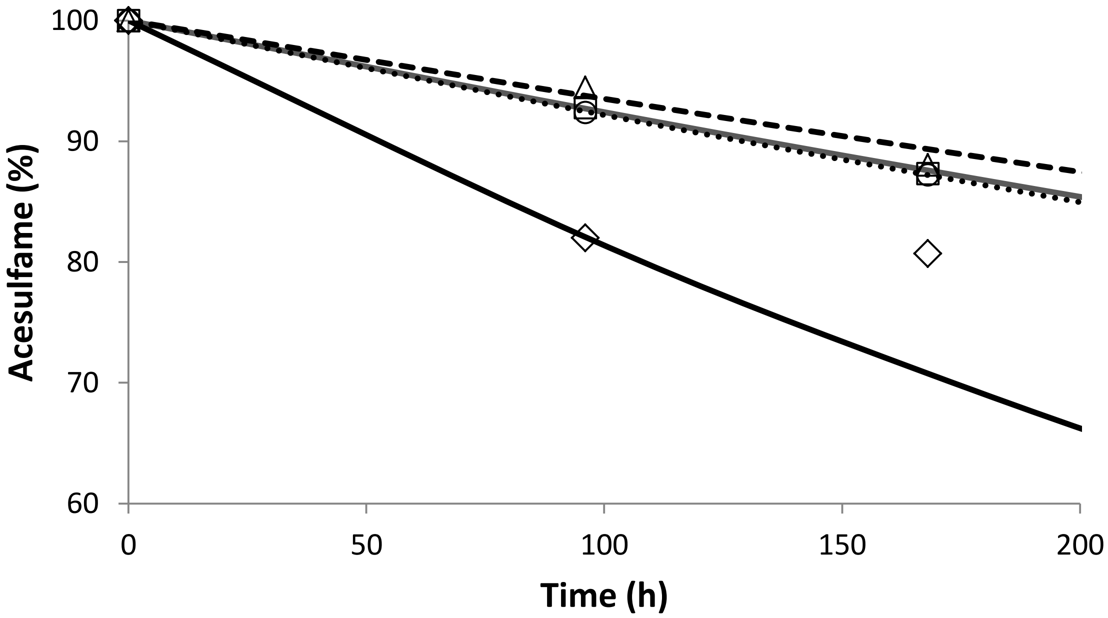


Figure 7. Decrease of acesulfame in lake (•••; ○), river (---; △), deionised water (—; □), and ferric iron solution (—; ◇) under simulated solar radiation. The markers show the measured reductions and the lines represent reduction over time (hours) (IV).

of PFOA in an aquatic environment, and neither do they mediate degradation of cyclamic acid and sucralose.

When degradation of the parent compound was not observed, the solar photolytic half-lives were calculated on the basis of measurement uncertainty, i.e. the standard deviation (SD) in the quantification of the exposed concentrations (Equation 2b). This is based on the fact that losses smaller than SD cannot be observed. The relative standard deviation (RSD) was determined from the initial samples (n=15) (III), or the initial, dark, and irradiated samples (n=11–12) (IV). Using the RSD as a potential unobservable loss of the parent compound, photolytic half-lives in surface water were at least 4.5, 3.0, and 6.0 years for PFOA, cyclamic acid, and sucralose, respectively. The photolytic half-life of saccharin in other solutions than Fe(III) was at least 3.6 years based on RSD. The half-life can also be calculated through the instrumental LOD of the main product (Equation 2c). If PFHpA was assumed to be the main degradation product of PFOA, the photolytic half-life in water surface was even longer, 256 years.

In a 3M industrial report, no direct or indirect phototransformation of PFOA was observed under environmental conditions and the photolytic half-life of PFOA was estimated to be over 349 days (0.96 years) (Hatfield 2001). Conversely, in a recent study PFCAs and PFSAs were reported to undergo relatively rapid photolysis under natural conditions (Taniyasu et al. 2013). However, that study has received criticism as the results may be explained by adsorption of PFCAs and PFSAs into test vials, and at least some results are within the method uncertainty (Wang et al. 2014). On the whole, photochemical degradation of PFOA is not likely in the environment. Contrariwise, 8:2 fluorotelomer alcohol and perfluoroalkanesulfonamides degrade under environmental conditions to produce PFCAs (Chu and Liu 2009; Plumlee et al. 2009). Consequently, solar radiation is more likely a source of PFCAs in the environment rather than a removal mechanism.

According to our experiments, only acesulfame can be degraded photochemically in nature and it may be an important degradation method for this microbially recalcitrant compound. The other three artificial sweeteners and perfluorooctanoic acid do not transform via solar radiation in surface water. Microbial degradation is a more important removal method for saccharin and cyclamic acid. Sucralose and PFCAs are extremely persistent in water, as they can resist both microbial and photochemical degradation as well as other mechanisms such as hydrolysis. The photolytic half-lives presented here represent the potential degradation rate in the surface layer of lakes, rivers, and oceans. Because the sweeteners and PFCAs are mixed in the whole water layer, the true photochemical half-lives are longer. In addition, the half-lives vary depending on latitude, elevation and season. More solar radiation reaches the equator than the poles, the atmospheric absorption is weaker at high altitudes, and the radiation is highest during sunny summer days. For example, Finland is a relatively flat country located between 60°N and 70°N, where ice and snow covers lakes and rivers in winter, blocking solar radiation. In addition, solar radiation meets the Earth's surface at a slighter angle near the poles. Even on sunny summer days the total solar (UV) radiation is less effective in southern Finland than in the Mediterranean. Therefore, the photochemical half-lives are longer in Finland than in Mediterranean countries.

4.3 Ecotoxicological test on artificial sweeteners

The long-term multi-generational tests with *Daphnia magna* did not have any effect on reproduction in acesulfame, saccharin, sucralose, or sweetener mixture solutions. However, cyclamic acid appeared to have a small negative impact on reproduction, and the effect increased slightly from 10 µg/L to 1000 µg/L solution (Figure 8). In the first test, a statistically significant difference ($F_{2,17,\alpha=0.05} = 4.107$; $p = 0.035$) was obtained with one-way ANOVA in the number of offspring (F1) between the control and cyclamic acid concentration 1000 µg/L. However, the mean live offspring per parent was only 47, while it should be at least 60 (RSD 18%) according to the OECD Technical Guideline number 211 (OECD 211). Therefore, the second test with ten replicates and one concentration level (1000 µg/L) was performed to ensure the result of the first test. In the second test, the average number of offspring was 66 (RSD 15%), and there were slightly fewer offspring in cyclamic acid solution compared to the control, although the variance between controls and cyclamic acid solution was not statistically significant ($F_{1,18,\alpha=0.05} = 2.188$; $p > 0.05$). The decrease in offspring production was not observed in the daphnids that were cultivated in the mixture of all four sweeteners. This indicates that some other sweetener(s) counteracts or diminishes the effect of cyclamic acid.

Even small changes in the fitness or reproduction of daphnids may be ecologically important. The effects tend to multiply in an ecosystem if the energy transfer from trophic level to another is disturbed. To be able to fully understand the potential effects of artificial sweeteners on aquatic organisms, further experimental studies on behavioural effects and biochemical responses are needed. Studies with sucralose indicate that the effects are subtle (Eriksson-Wiklund et al. 2012). The capability to cope with environmental stressors varies between species and populations. For example, sucralose affects the feeding rate and egg production of a natural species while there was no effect on the laboratory strain of copepods (Hjorth et al. 2010). There is no information available on the ecotoxicology of acesulfame, saccharin and cyclamic acid. Acesulfame is very persistent in the aquatic environment, and saccharin and cyclamic acid are introduced continually into surface water in wastewater effluents. Hence, the organisms of the receiving waters are continuously exposed to elevated saccharin and cyclamic acid concentrations.

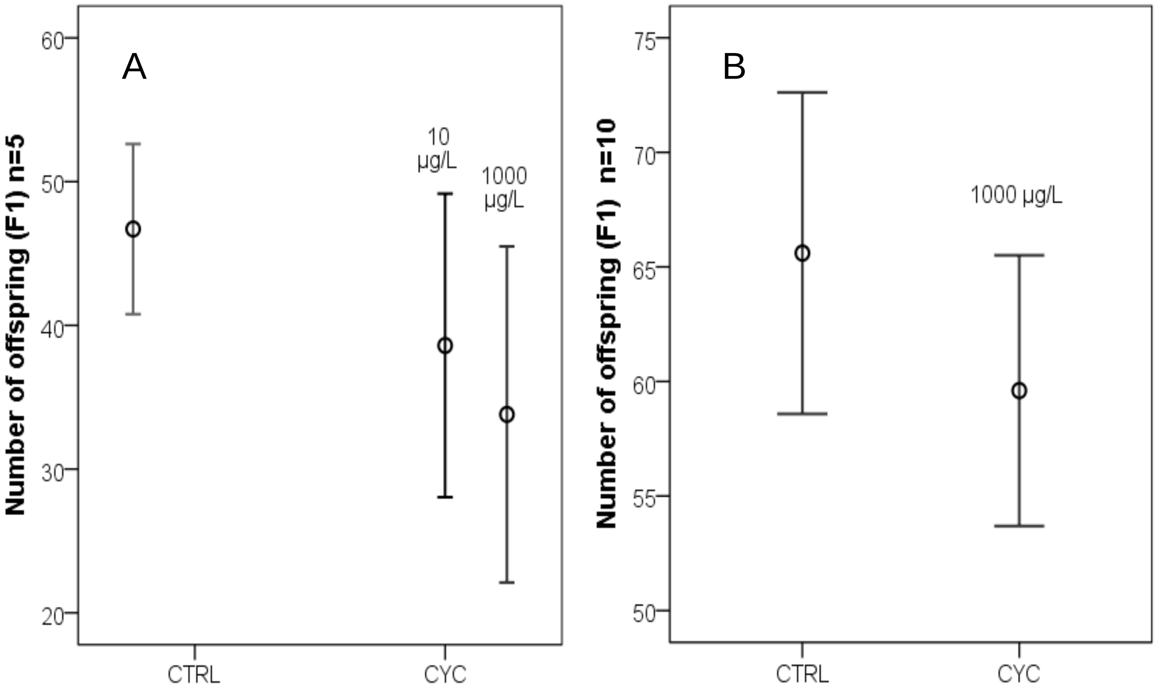


Figure 8. Number of *Daphnia magna* offspring after 21-day cultivation in control (CTRL) and cyclamic acid (CYC) solutions. The circles represent the mean values, and the vertical lines are the 95% confidence intervals for Test 1 (A) and Test 2 (B).

5. CONCLUSIONS

New emerging compounds are being introduced continuously due to the synthesis of new chemicals, and more sophisticated techniques that enable the detection of small concentrations and the identification of new contaminants. A lot of research is needed to evaluate the fate and effects of this vast group of chemicals. This thesis adds to the knowledge on the environmental distribution, sources and fate of artificial sweeteners and perfluoroalkyl acids in an aquatic environment, and provides information for risk assessment and policy-making.

Artificial sweeteners and PFAAs were ubiquitously present in wastewater-contaminated surface waters. PFAAs travel long distances in the air and can be found in pristine waters as well. PFAAs and artificial sweeteners acesulfame and sucralose are very persistent in aquatic environments as they do not change physically or chemically and are not degraded by microbes. The photochemical degradation of artificial sweeteners and PFAAs is very slow in surface waters, and only acesulfame can be degraded during germicidal UV water treatment. Artificial sweeteners are viable tracers of municipal wastewater. Acesulfame in particular persists through conventional wastewater and drinking water treatment and is not degraded in the environment. In addition, the consumption of acesulfame is wide and it can therefore be traced all the way to groundwater and drinking water. The detection of artificial sweeteners indicates that other water-soluble, environmentally-persistent emerging compounds may also be present. The removal of pharmaceuticals such as carbamazepine and dichlofenac, for example, is poor in wastewater treatment plants, and they tend to stay in the water phase.

The aquatic ecotoxicity of both PFAAs and artificial sweeteners should be investigated further. Most ecotoxicological studies on PFAAs consider only the two historically most prevalent compounds, PFOA and PFOS, and are conducted with environmentally irrelevantly high concentrations. The effects of other PFAAs and PFCAs, as well as other per- and polyfluorinated compounds, are not known or fully understood. Sucralose is the only artificial sweetener on which some ecotoxicological data is available in literature. Experimental studies indicate that sucralose and cyclamic acid might affect the behaviour or breeding of aquatic crustaceans. Even subtle effects can impact overall community structure. The challenge to finding the potential ecotoxicological and ecological effects of water-soluble, non-acutely toxic chemicals like artificial sweeteners, (short-chain) PFAAs, or their mixtures is that other endpoints than survival and reproduction should be applied.

In addition to aquatic ecotoxicology studies, the monitoring of perfluorinated organic substances is needed. Although the PFOS concentrations in biota and humans show a decreasing trend because of restrictions and phase-out of production in Western countries in the last decade, the concentrations of other PFCAs and PFSAs continue to rise. For example, recent studies show that the two possible short-chain replacement compounds for PFOS and PFOA – PFBS and PFBA – are becoming the dominant PFAAs in the environment, and also the concentrations of long-chained PFCAs are growing. In addition to PFCAs and PFSAs, other PFAAs such as perfluorophosphonic acids (PFPA) and perfluorophosphinic acids (PFPIA) have been detected in wastewater effluents, surface water and drinking water. Polyfluoroalkyl phosphates (PAPs) often used in grease-resistant food packaging have been detected in both the environment and human blood. PFAAs are known to percolate to groundwater. High concentrations of PFOS and other PFAAs have been detected in contaminated groundwater and drinking water, causing health risks. In particular, fire drill areas where PFOS-containing firefighting foams have been used extensively are a major source of PFAAs into groundwater. In Finland, groundwater is the main source of tap water. Therefore, the occurrence of these chemicals in groundwater and particularly in areas close to water intake should be screened.

Emerging compounds are receiving a lot of attention. There are numerous studies on environmental occurrence, fate and effects of various emerging substances. On the other hand, there is a lot to study. More compounds enter the list of emerging substances than are removed. New chemicals are synthetised at a fast pace, potentially including new candidates to the list of emerging substances. The prioritisation of which emerging compounds are the most emerging or important to study is challenging. Some assumptions may be made on the basis of the physical and chemical properties of compounds. However, it would be naive to presume that our knowledge of chemistry and especially toxicology is implicit. PFAAs or DDT are perfect examples of compounds which were assumed to be safe based on their inertness, until proven otherwise.

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