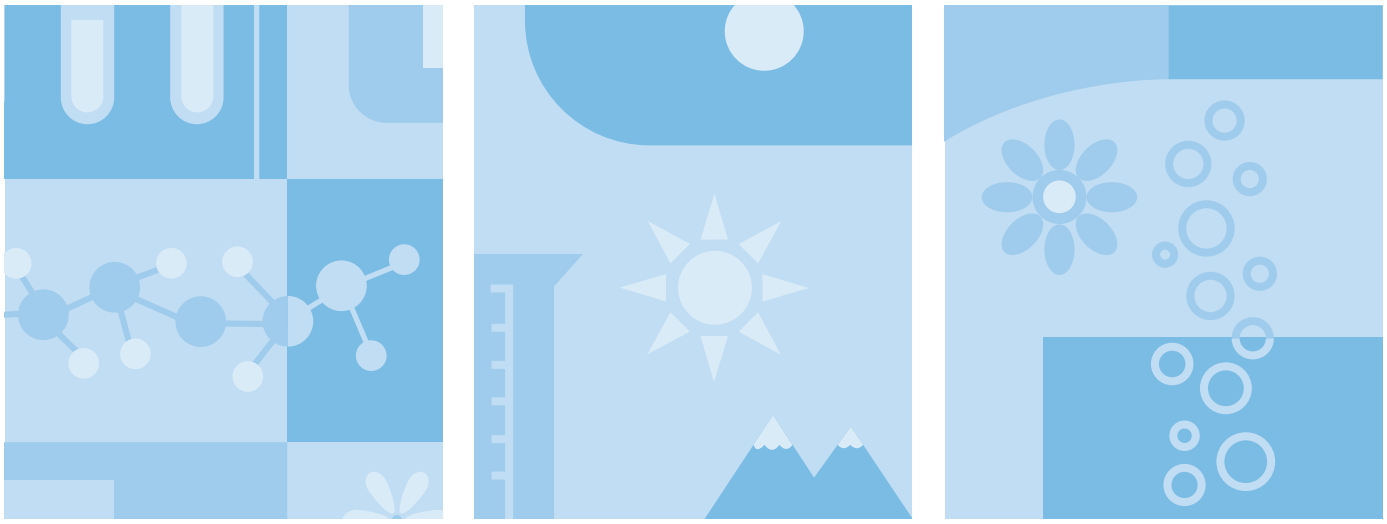
PM
17/12

Temporal trends of perfluorinated alkyl acids in eggs, milk and farmed fish from the Swedish food production



Anders Glynn, Per Ola Danerud and Tuija Pihlström, National Food Agency, Urs Berger, Robin Vestergren, Ian T. Cousins and Jana Johansson, Stockholm University, Anders Bignert, Swedish Museum of Natural History.

Swedish Chemicals Agency – www.kemi.se

KEMI
Swedish Chemicals Agency

Temporal trends of perfluorinated alkyl acids in eggs, milk and farmed fish from the Swedish food production

Anders Glynn, Per Ola Danerud and Tuija Pihlström, National Food Agency, Urs Berger, Robin Vestergren, Ian T. Cousins and Jana Johansson, Stockholm University, Anders Bignert, Swedish Museum of Natural History.

Order No. 511 079
Sundbyberg, December 2012
Publisher: Swedish Chemicals Agency©
Order address: CM Gruppen, P.O. Box 11063, SE-161 11 Bromma, Sweden
Phone: +46 8 5059 33 35, Fax +46 8-5059 33 99, E-mail: kemi@cm.se
The report is available as a downloadable pdf on www.kemi.se

Preface

The Swedish Chemicals Agency (KemI) has been assigned by the Swedish Government to produce a national action plan for a toxic-free everyday environment: Action plan for a toxic-free everyday environment 2011 – 2014 – protect the children better.

Efforts are now going on in several areas, both in Sweden, within the EU and internationally and often in cooperation with other authorities. Reducing chemical risks in the everyday environment is one step towards attaining the Swedish Parliament´s environment quality objective A Non-Toxic Environment, which is the objective that KemI is responsible for.

Within the framework of the action plan, KemI compiles knowledge in KemI´s report and PM series elaborated by experienced colleagues, researchers or consultants. In this way, KemI presents new and essential knowledge in publications which can be downloaded from the website www.kemikalieinspektionen.se

Perfluorinated substances are persistent and some of them are also bioaccumulative and toxic to reproduction. The substances are widely used by industry, for example for grease and dirt-repellent impregnation of textiles and carpets, oil-resistant coatings for paper products approved for contact with food, fire fighting foams, and surfactants in the mining and oil industry. An important sub-group is (per) fluorinated organic surfactants, including the substances perfluoroctanoic sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). The extensive use has led to spread in the environment and direct and indirect exposure of the population.

To increase awareness of food as a source of exposure to perfluorinated substances, the National Food Agency has been commissioned to conduct a time-trend analysis of perfluorinated alkyl acids in eggs, milk and farmed fish from the Swedish food production. The analysis is part of the joint work of the Swedish Chemicals Agency and the National Food Agency to identify sources which contaminate food with dangerous chemical substances.

Content

Summary

Temporal trends of perfluorinated alkyl acids (PFAAs), i.e. perfluoroalkane sulfonates (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs) in egg yolks, raw milk and farmed fish between 1999 and 2010 were studied. The aim was to follow up consequences of changes in production, emissions and use of PFSAs, PFCAs and related chemicals since the beginning of the 2000s. Since 1999 the National Food Agency has every year banked samples from the official food control program, for future studies of temporal trends of contaminants in Swedish food production. Samples of raw milk, hen´s egg yolk, and muscle from farmed brackish water rainbow trout were used in the temporal trend study.

Analyses of PFAAs was done by ultra performance liquid chromatography coupled to tandem mass spectrometry after extraction and clean up using a variety of analytical techniques for the different matrices. Of the 11 PFAAs analysed, temporal trends could be studied for perfluorooctanoic acid (PFOA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorohexane sulfonate (PFHxS), and PFOS in at least one of the investigated matrices. Regression analyses showed that levels of PFOA, PFHxS and perfluorooctane sulfonate (PFOS) in egg yolks declined during the study period, whereas no significant temporal trends were observed among PFCAs with perfluorinated carbon chains longer than seven carbons. In farmed rainbow trout levels of PFOS and PFHxS decreased, whereas no change in PFUnDA levels was seen. In milk no temporal trend of PFOS could be detected.

The phase out of production and use of PFOS-related compounds by its main global manufacturer and the efforts to reduce emissions of PFOA could contribute to the observed declining trends of PFOS and PFOA in eggs and of PFOS fish. Studies of temporal trends of PFOS and PFOA body burdens among young women in Sweden also show decreasing trends since late 1990s. The decrease in PFHxS levels in eggs and farmed fish is opposite to the increase in PFHxS exposure of young women from the Uppsala area during the last decade.

Moreover, market basket studies between 1999 and 2010 suggest that PFHxS contamination of staple foods has not increased in Sweden. Therefore the observed increase in PFHxS exposure in young women is most probably due to increased exposures from other sources than staple foods. No temporal trends of PFDA, PFUnDA, PFDoDA and PFTrDA levels in eggs, or of PFUnDA in farmed fish, were observed.

Market basket studies indicate that long-chain PFCA intakes from staple foods have increased between 1999 and 2010, which could, at least partly, be due to a combination of increased levels in fish and increased fish consumption in the general Swedish population during the last decade.

Sammanfattnig

Denna studie undersökte tidstrender hos perfluorerade alkylsyror (PFAA), dvs. perfluoralkansulfonater och perfluoroalkylkarboxylsyror (PFCA) i äggulor, obehandlad mjölk och odlad fisk mellan 1999 och 2010. Syftet var att följa upp konsekvenserna av förändringar i produktion, utsläpp och användning av PFSA, PFCA och liknande kemikalier sedan början av 2000-talet. Livsmedelsverket har varje år sedan 1999 arkiverat prover från det officiella livsmedelskontrollprogrammet för framtida studier av tidstrender av föroreningar i den svenska livsmedelsproduktionen. Prover av obehandlad mjölk, hönsäggula och muskler från odlad bräckvatten-regnbåge användes i den tidstrendstudien.

Analyser av PFAA:er gjordes genom ultravätskekromatografi kopplad till tandemmasspektrometri efter extraktion och rening genom en mängd olika analytiska tekniker för de olika matriserna. Av de analyserade 11 PFAA:erna kunde tidstrender studeras för perfluoroktansyra (PFOA), perfluorodecanoic syra (PFDA), perfluoroundecanoic syra (PFUnDA), perfluorododecanoic syra (PFDoDA), perfluorotridecanoic (PFTrDA) syra, perfluorohexansulfonat (PFHxS) och PFOS i åtminstone en av de undersökta matriserna. Regressionsanalyser visade att halterna av PFOA, PFHxS och perfluoroktansulfonat (PFOS) i äggulorna minskade under observationstiden, medan inga signifikanta tidstrender observerades bland PFCA:er med perfluorerade kolkedjor längre än sju kolatomer. I odlad regnbåge minskade halterna av PFOS och PFHxS, medan ingen förändring i PFUnDA-nivåer observerades. I mjölk kunde ingen tidsmässig tendens av PFOS påvisas.

Genom att den huvudsakliga tillverkaren fasar ut sin produktion och användning av PFOS-relaterade föreningar och genom insatser för att minska utsläppen av PFOA kan detta bidra till den observerade nedåtgående trenderna av PFOS och PFOA i ägg och av PFOS i fisk. Studier av tidstrender av PFOS och PFOA i kroppen hos unga kvinnor i Sverige visar också minskade trender sedan slutet av 1990-talet. Minskningen av PFHxS-nivåer i ägg och odlad fisk är motsatt ökningen PFHxS-exponering hos unga kvinnor från Uppsala-området under det senaste decenniet.

Dessutom tyder studier av den s.k. varukorgen mellan 1999 och 2010 på att PFHxS-kontamination av baslivsmedel inte har ökat i Sverige. Därför beror den observerade ökningen av PFHxS-exponering hos unga kvinnor mest sannolikt på ökad exponering från andra källor än baslivsmedel. Inga tidstrender observerades för PFDA, PFUnDA, PFDoDA och PFTrDA-nivåer i ägg, eller PFUnDA i odlad fisk.

Varukorgsstudier tyder på att långkedjiga PFCA-intag från baslivsmedel har ökat mellan 1999 och 2010, vilket åtminstone delvis kan bero på en kombination av ökade nivåer i fisk och ökad fiskkonsumtion i den allmänna svenska populationen under det senaste decenniet.

Introduction

In 2010 the Swedish government commissioned the Swedish Chemicals Agency (KEMI) to develop a national action plan for a toxic-free everyday environment (KEMI, 2011). The aim of the action plan is to reduce human health risks in the general population in Sweden posed by hazardous chemicals, with a special focus on children. Implementation of the action plan involves many parties, among others several governmental agencies, such as the National Food Agency (NFA). One important early activity is to identify important sources of hazardous chemical contamination of food.

In KEMI's action plan poly- and perfluorinated alkyl substances (PFASs) are prioritized as one of the hazardous chemical groups that should be investigated (KEMI, 2011). The perfluorinated alkyl acids (PFAAs) such as perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonates (PFSAs), are of particular interest to scientists and regulators worldwide. The pathway of human exposure to PFAAs are still poorly defined but food has been identified to be important for perfluorooctane sulfonate (PFOS) and long-chain PFCAs (Vestergren, 2011).

The phase-out of PFOS and related perfluorooctane sulfonyl fluoride (POSF)-based chemistry, that was initiated by the main manufacturer 3M in the beginning of the 2000s (3M, 2000), has resulted in a decline in human PFOS exposure in Sweden (Glynn et al., 2011; Jönsson et al., 2008; Sundstrom et al., 2011). A consequence of this phase-out was that 3M ceased to manufacture perfluorooctanoic acid (PFOA) as well as products based on C-6 and C-10 homologues of POSF. Concurrently, manufacture of PFOA continued and increased by other manufacturers because it was needed as a processing aid in the manufacturer of polytetrafluoroethylene (PTFE) (Prevedouros et al., 2006). Nevertheless, the phase-outs by 3M, as well as recent measures to reduce production, use, and emissions of PFOA (EPA, 2010) by other manufacturers, have caused a decline in human PFOA exposure in Sweden (Glynn et al., 2011; Sundstrom et al., 2011). Concomitantly, relatively rapidly increasing trends of human exposure to perfluorinated sulfonates with 4 and 6 carbons, perfluorobutane sulfonate (PFBS) and perfluorohexane sulfonate (PFHxS), were observed in a population of young women from the Uppsala area (Glynn et al., 2011). Moreover, human exposure to perfluorinated carboxylates with 8-10 perfluorinated carbons has slowly increased during the beginning of the 2000s (Glynn et al., 2011; Jönsson et al., 2008).

As a part of the collaboration between KEMI and NFA, a project was initiated with the aim to improve the knowledge about food as a source of human exposure to PFAAs. Temporal trends of PFAAs in eggs, milk and farmed fish between 1999 and 2010 were studied. These types of foods may be important sources of PFAA exposure for children, especially among those with high consumption of the foods in question. The results can be used to determine if the changes in production and use of PFAAs that have occurred since the beginning of the 2000s have had an impact on levels of PFAAs in food.

Materials and methods

Samples

Rainbow trout, cow´s milk and hen's egg (yolk) samples were collected annually from 1999 to 2010 within the Swedish National Food Agency's official food control program. In the present study a total of 36 pooled egg samples, 36 pooled milk samples and 36 individual fish samples were analyzed.

Eggs

Sweden has about 200 egg packaging facilities. Sampling of eggs was spread out over the country with emphasis on the largest packaging plants in the southernmost 1/3 of Sweden. Every year samples from 20-25 producers were banked for future contaminant analyses. Each banked sample consisted of a pool of 10-12 eggs from one producer. The yolks were separated from the egg whites before homogenization and freezing. The samples were stored at -20^{o}C until analysis. In the present study all available egg yolk samples were analyzed. They were divided into 3 pools per year. The pools were weighted with regard to fat content (16.4-34%) and geographical origin.

Milk

Between 1999 and 2009, raw milk was sampled from the tanks of milk transport vehicles. The tanks generally contained milk from 10 dairy farms. From 2010 samples were taken from the milk storage tanks on individual dairy farms. In 1999 all samples were taken in the province of Värmland in the western part of Central Sweden, and in 2000 in the southern provinces Skåne and Halland. From 2001 sampling was more evenly spread over the country, but still with a sampling predominantly in the southernmost 1/3 of Sweden where most of the milk production occurs. Each year milk 10-25 samples from the food control were banked for future analyses of contaminants. All available samples were divided into 3 pools per year. The pools were weighted with regard to fat content (1.27-6.8%) and geographical origin.

Fish

Rainbow trout was collected from fish farms along the Swedish Baltic Sea coast (brackish water). All individuals included in the present study were estimated to be older than 12 months. The banked samples were cut out as cutlets between the dorsal fin and the tail fin, and were stored at -20^{o}C. Muscle samples were taken from each of the cutlets and analyzed individually.

Chemicals and standards

All native and isotope labeled PFCA and PFSA standard compounds were purchased from Wellington Laboratories (Guelph, ON, Canada) in 2 µg mL^{-1} solution mixtures. The 11 target analytes were perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexane sulfonic acid (PFHxS) and perfluorooctane sulfonic acid (PFOS). Internal standards were $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFUnDA, $^{13}C_2$-PFDoDA, $^{18}O_2$-PFHxS and $^{13}C_4$-PFOS. $^{13}C_8$-PFOA and $^{13}C_8$-PFOS were used as volumetric standards in the

calculation of total method recovery of the internal standards. All isotope labeled standards were certified to contain <0.5% of their native analogues.

All reagents were analytical reagent grade. Tetrabutyl ammonium hydrogen sulfate (TBA) was purchased from MERCK, sodium hydroxide (NaOH) from Akzo Nobel, sodium carbonate (Na_2CO_3) from Riedel-de Haën, sodium hydrogen carbonate ($NaHCO_3$) and ammonium acetate (CH_3COONH_4) from KEBO, formic acid (CH_2O_2) from Fluka, potassium hydroxide (KOH) from BDH, ammonium sulfate ($(NH_4)_2SO_4$) from Sigma-Aldrich, and anhydrous granulated sodium sulfate (Na_2SO_4) from Scharlau. Florisil sorbent (60/100 mesh) and Supelclean graphitized carbon (ENVI-carb) were obtained from SUPELCO. The water used in the method was HPLC grade (PROLABO Chromanorm). Acetonitrile (Chromasolv grade) was purchased from Riedel-de Haën. Methyl *tert*-butyl ether (MTBE) was purchased from Rathburn chemicals. Methanol (MeOH, LiChrosolv grade) was supplied by MERCK. All solvents were checked for residual PFAAs prior to use.

Sample preparation methods

Eggs

The sample preparation method for egg yolk is described in detail elsewhere (Vestergren, 2011). In short, isotope labeled internal standards were spiked to 2.5 g of egg yolk sample and left to equilibrate with the sample material at room temperature overnight. To release analytes from the sample matrix, aqueous NaOH was added and the sample was left for 30 min. Na_2CO_3/$NaHCO_3$ buffer (adjusted to pH 10 with NaOH) and TBA-solution (both in pre-cleaned HPLC water) as well as 5 mL of MTBE were added and the mixture was vortex mixed for 30 s. The sample was extracted in an ultrasonic bath at room temperature for 10 min and the phases were separated by centrifugation. The top MTBE layer was transferred to a 15 mL polypropylene tube and the extraction was repeated twice with 5 mL MTBE. The combined extracts were evaporated to a final volume of approximately 3 mL under a gentle stream of dry nitrogen gas. SPE clean-up was performed using a manually packed column with a Florisil and ENVI-carb sorbent mixture consisting of 1.5 g Florisil and 25 mg ENVI-carb. To remove remaining moisture from the extract, 1 g of anhydrous granular Na_2SO_4 was applied on top of the column. The cartridge was rinsed with MeOH and conditioned with MTBE before the sample extract was loaded. The cartridge was then washed with 10 mL of MTBE that was discarded. The target compounds were eluted with 6 mL of a 30/70 MeOH/MTBE mixture. The extract was evaporated to ~100 µL under nitrogen and the volumetric standards (100 pg of both standards in 50 µL of MeOH) and 100 µL aqueous ammonium acetate were added. Prior to analysis, the extracts were cooled overnight to -18 °C and subsequently centrifuged. An aliquot of 100 µL of the clear final extract was transferred to an auto-injector vial.

Milk

The milk was extracted using a modified version of the method originally published by Olsen et al. (2007). Isotope labeled internal standards were spiked (100 pg of each standard in 50 µL of methanol) to 2.5 g of milk in a 15 mL polypropylene tube. To release analytes from the sample matrix, 600 µL of 1 mol L^{-1} formic acid in water was added. The sample was vortex mixed and left to equilibrate at room temperature overnight. After addition of 600 µL of saturated ammonium sulfate in water the sample was vortex mixed. A volume of 5 mL acetonitrile was added and the mixture was vortex mixed again for 30 s. The sample was then extracted in an ultrasonic bath at room temperature for 15 min. Subsequently, the phases were

separated by centrifugation for 10 min at 2,000 rpm. The top acetonitrile layer was transferred to a 15 mL polypropylene tube and the extraction was repeated twice with 3 mL acetonitrile. The combined extracts were evaporated to incipient dryness under a gentle stream of dry nitrogen gas. The extract was reconstituted in 300 µL of water. After addition of 500 µL of aqueous KOH (1 mol L^{-1}) and 500 µL of a 0.5 mol L^{-1} TBA solution in water the mixture was vortex mixed thoroughly. A volume of 5 mL MTBE was added and the sample was vortex mixed and put into an ultrasonic bath for 15 minutes. After centrifugation (2000 rpm, 5 min) the top MTBE layer was transferred to another 15 mL PP tube. The extraction was repeated twice with 3 mL MTBE. The extract was evaporated to incipient dryness under nitrogen. A volume of 50µL methanol was added, as well as the volumetric standards (100 pg of both standards in 50 µL of MeOH) and 100 µL aqueous ammonium acetate (4 mmol L^{-1}). The extract was then sonicated for 5 min. An aliquot of 100 µL of the clear final extract was transferred to an auto-injector vial.

Fish

The extraction method used for fish samples is based on a method developed by Powley and Buck (2005) with modifications described by Berger et al. (2009). An aliquot of 0.5 g of fish muscle was spiked with isotope labeled internal standards (1 ng of each standard in 50 µL of methanol) and 5 mL acetonitrile was added. After homogenisation in the acetonitrile extraction solvent using a blender the sample was additionally extracted in an ultrasonic bath for 15 min and then centrifuged (2000 rpm, 5 min). The extract was transferred to a 15 mL polypropylene tube. The extraction using the blender and sonication was repeated with 5 mL acetonitrile. The extracts were combined and concentrated to 1 mL under a gentle stream of dry nitrogen gas. The concentrated extract was then subjected to dispersive clean-up on graphitized carbon (25 mg ENVI-Carb) and 50 µL glacial acetic acid in an Eppendorf centrifuge tube. The tube was vortex-mixed thoroughly and centrifuged (10,000 rpm, 10 min). A volume of 500 µL of the extract was transferred to another Eppendorf tube and the volumetric standards (1 ng of both standards in 50 µL of MeOH) and 500 µL aqueous ammonium acetate (4 mmol L^{-1}) were added before cooling the extract to 4 °C in the fridge. Prior to analysis, the extract was allowed to warm to room temperature and centrifuged at 10,000 rpm for 10 min. An aliquot of 100 µL of the clear final extract was transferred to an auto-injector vial.

Instrumental analysis and quantification

The instrumental analysis and quantification is described in detail elsewhere (Vestergren, 2011). The purified sample extracts were analyzed using an Acquity ultra performance liquid chromatography (UPLC) system coupled to a Xevo TQS tandem mass spectrometer (MS/MS) with an electrospray ionization (ESI) interface operated in the negative ion mode (all from Waters Corp.). A “PFC isolator column” obtained from Waters (“PFC kit”) was inserted in the UPLC system prior to the injector to trap and delay contamination originating from the UPLC instrument and solvents. The analytical separation column was a BEH C18 (1.7 µm particles, 50 × 2.1 mm, Waters). A binary mobile phase gradient consisting of 10% MeOH in water and pure MeOH, both containing 2 mmol L^{-1} ammonium acetate, was applied for separation of the target compounds. The mass spectrometer was operated in multiple reaction monitoring mode.

Quantification was performed using the internal standard method (isotopic dilution) for all target analytes. For PFHpA, PFTrDA and PFTeDA, which did not have corresponding isotope labeled standards, $^{13}C_2$-PFHxA (for PFHpA) and $^{13}C_2$-PFDoDA were used for

quantification, respectively. All quantified concentrations given in this report are on a sample fresh weight (fw) basis and were not blank corrected.

Quality assurance

Solvent injections were performed regularly to monitor the instrumental background. The injections did not reveal instrumental background signals from the HPLC/MS/MS system for any of the target analytes.

A series of procedural blank samples were extracted along with every batch of samples for assessment of the method detection limits (MDLs) of the different target analytes. If the procedural blank chromatograms contained detectable signals, the MDL was derived from the arithmetic mean plus three times the standard deviation of the analyte signal in the procedural blanks. The milk samples were extracted in two batches with 5 procedural blank samples accompanying each batch. The fish samples were extracted in one batch including 3 blanks. The egg samples were extracted in 4 batches including 5 blanks per batch. In the cases where procedural blank samples did not contain a detectable signal, the MDLs were set equal to the lowest quantified concentration in a sample with an analyte signal displaying a signal-to-noise ratio of at least 3. PFHxS, PFOS, PFNA, PFUnDA, PFDoDA, PFTrDA and PFTeDA were typically non-detectable in the procedural blank chromatograms for all methods, which is reflected in lower MDLs compared to the other analytes.

Method precision and accuracy were evaluated by extraction and analysis of subsamples (n = 3) from a larger control sample of fish muscle homogenate from an interlaboratory comparison study (ILC) (van Leeuwen et al., 2009). The precision (%RSD) of the triplicate analysis was between 1% and 6% for all analytes. The quantified mean concentrations in the samples deviated with 9-23% from the mean concentrations reported in the ILC study.

$^{13}C_8$-PFOA and $^{13}C_8$-PFOS were used as volumetric standards to calculate recoveries of the internal standards in all samples analyzed. The mean recoveries of the different internal standards were in the ranges 34-64%, 43-70% and 51-157% for the methods for egg yolk, fish and milk, respectively.

Statistics

To test for significant changes in individual PFAA concentrations over time, log-linear regression analyses were carried out on the geometric means of pooled samples (eggs and milk) or individual samples (fish) each year. In cases when the PFAA level was below the method detection limit (MDL) of the analytical method, the level was set to the value at MDL. Statistical analyses of temporal trends were done when levels of a given PFAA were above LOD in more than 40% of the samples.

Results and discussion

Levels of PFAAs in milk, eggs and fish

In milk the levels of the analyzed PFAAs were in most cases below the MDL. Only PFOS was present at levels above MDL in enough samples to make statistical analyses of temporal trends meaningful (N above MDL=21, range: 3.5-7.3 pg/g fw). A few samples had measurable levels of PFHpA (N=5, range: 2.3-3.0 pg/g fresh weight), PFDA (N=3, range: 2.7-3.3 pg/g fw), PFUnDA (N=3, range: 1.4-1.8 pg/g fw), and PFHxS (N=4, range: 1.0-1.1 pg/g fw). The low PFAA levels are in accordance with levels observed in dairy products on the Swedish market reported by Vestergren (Vestergren, 2011).

All PFAAs could be measured in at least one pooled egg yolk sample from 1999 to 2010. Levels above MDL in only a few samples were observed for PFHxA (N=4, range: 8-13 pg/g fw), PFHpA (N=1, 5 pg/g fw), PFNA (N=10, range: 20-143 pg/g fw), and PFTeDA (N=9, range: 5-11 pg/g fw). The median level of PFOA was 21 pg/g fw (range:<14-225 pg/g fw), of PFDA 10 pg/g fw (<6-67 pg/g fw), PFUnDA 27 pg/g fw (<8-241 pg/g fw), PFDoDA 7 pg/g fw (<6-52 pg/g fw), PFTrDA 19 pg/g fw (<4-102 pg/g fw), PFHxS 12 pg/g fw (<10-128 pg/g fw), and PFOS 375 pg/g fw (<26-6478 pg/g fw). The pattern with considerable higher levels of PFOS than of other PFAAs is similar to the pattern of PFAA levels in eggs reported from market basket studies 1999, 2005 and 2010 (Vestergren, 2011).

In farmed brackish water rainbow trout all samples had levels of PFHxA, PFNA, and PFTeDA below the MDL. A few samples had levels above MDL for PFHpA (N=1, 11 pg/g fw), PFOA (N=1, 84 pg/g fw), PFDA (N=1, 102 pg/g fw), PFDoDA (N=5, range: 15-88 pg/g fw), and PFTrDA (N=6, range:32-51 pg/g fw). Meaningful analyses of temporal trends could be performed for PFUnDA (N=12, range: 24-72 pg/g fw), PFHxS (median 13 pg/g fw, range: <11-40 pg/g fw), and PFOS (median: 121 pg/g fw, range: <37-795 pg/g fw). The median PFOS level in farmed rainbow trout was considerably lower than the PFOS level in the fish samples of the market baskets 1999-2010, representing the fish consumed by the average fish consumer in Sweden (Vestergren, 2011).

Table 1. *Annual change in concentrations of PFAAs in eggs, milk and farmed rainbow trout, 1999 - 2010*[a].

Compound	N	Change per year (%)		R^2 (%)	p
		mean	95% CI Lower/upper		
Eggs					
PFHxS	36	-11	-16/-6.4	72	<0.001
PFOS	36	-31	-42/-20	80	<0.001
PFOA	36	-12	-21/-2.5	45	<0.001
PFDA	36	ns			
PFUnDA	36	ns			
PFDoDA	36	ns			
PFTrDA	36	ns			
Milk					
PFOS	36	ns			
Rainbow trout					
PFHxS	36	-4.3	-8.1/-0.42	38	<0.032
PFOS	36	-18	-27/-8.2	63	<0.002
PFUnDA	36	ns			

[a]CI=confidence interval, ns=not significant.

Temporal trends

Linear regression analyses of temporal trends of log-transformed PFAA levels in eggs showed declining trends of PFHxS, PFOS and PFOA levels between 1999 and 2010 (Fig. 1, Table 1). No significant trends were observed for PFCAs with perfluorinated carbon chains longer than 7 carbons (Fig. 1, Table 1). In milk no significant temporal trend of PFOS levels was observed (Fig. 1, Table 1). The low levels close to the MDL increase the uncertainty of the

trend. Significant declining trends of PFHxS and PFOS levels were seen in farmed brackish water rainbow trout.

Food is most probably an important source of human exposure to some PFAAs, such as PFOS and PFOA (Vestergren, 2011). For other PFAAs, such as PFHxS and PFCAs with chain lengths higher than 7 perfluorinated carbons, little is known about the contribution of different sources to the total exposure. Using samples from a market basket study, Vestergren (2011) observed that the per capita intake of PFSAs and PFCAs from staple foods collected in 1999, 2005 and 2010 was dominated by PFOS. Intake of PFOA was 2-4 times lower, whereas intakes of PFHxS, PFDA, PFUnDA, PFDoDA and PFTrDA were 5-37 times lower. The intake calculations showed that fish consumption in 2010 contributed over 50% to the intake of PFOS, PFUnDA, PFDoDA and PFTrDA. For PFOA and PFHxS fish contributed less than 30% to the intake. Consumption of eggs and dairy products gave a minor contribution to the intakes in 2010, except in the case of PFHxS (dairy ~20%) and PFOA (dairy ~20%) (Vestergren, 2011).

Temporal trends of PFOS

The PFOS levels in egg yolk decreased with about 30% per year during the study period (Fig. 1, Table 1). In 1999 a relatively high PFOS level was detected in eggs in the market basket study (1300 pg/g fw), and the level decreased dramatically to less than 100 pg PFOS/g fw in 2005 and 2010 (Vestergren, 2011). Concomitantly, the contribution of egg consumption to the per capita intake of PFOS decreased from about 30% to less than 5% (Vestergren, 2011). Although the market basket study only comprised one pooled sample of eggs from the Swedish market (8-10 food stores) per year, the decrease in PFOS levels in eggs between 1999 and 2005 is in line with the declining temporal trend of PFOS in egg yolk between 1999 and 2010 observed in the present study (Fig. 1).

The relatively large contribution of egg consumption to the per capita intake of PFOS estimated in the market basket study in 1999 (Vestergren, 2011), in combination with the rapid decline in PFOS levels in eggs between 1999 and 2010, could at least partly play a role in the observed decline in PFOS levels in blood serum/plasma of women from Lund and Uppsala, and in mother´s milk from Stockholm during the same time period (Glynn et al., 2011; Jönsson et al., 2008; Sundstrom et al., 2011).

There could be several sources of PFOS contamination of eggs. In Sweden, eggs are produced mainly indoors by free-range hens or caged hens. The most likely source of chemical exposure of hens in the conventional production is the feed, but hens can be exposed to chemicals via several other sources, such as the drinking water, floor covering materials, building/equipment materials, and biocides/veterinary drugs. Hens in organic production can spend time outdoors, and thus contamination of the soil and food organisms could contribute to chemical exposure. Between 2000 and 2007 the production of organic eggs increased from 2-3% to 7% (Jordbruksverket, 2010).

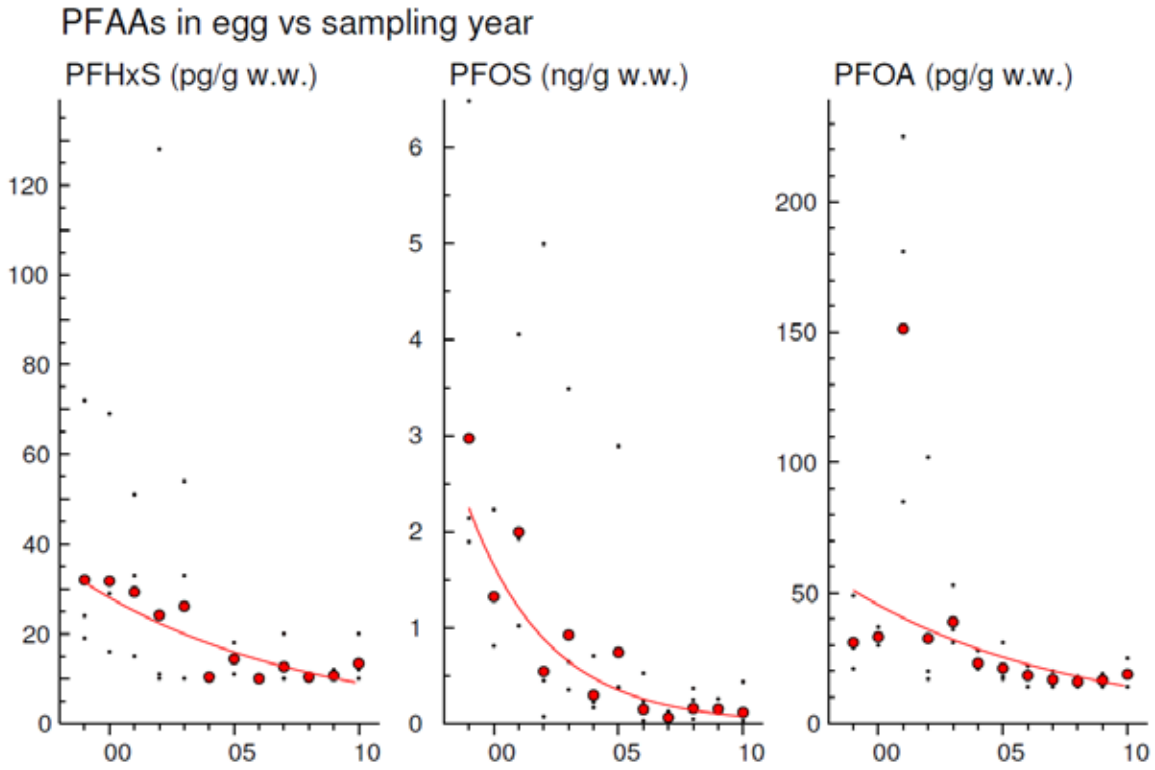


Figure 1. *Temporal trends of PFHxS, PFOS and PFOA in hen´s egg yolks, sampled on the Swedish market between 1999 and 2010. Sampling occurred within the National Food Agency´s food control program. Pooled samples of egg yolk were prepared by mixing 6-9 food control samples, resulting in 3 pooled samples per year. Each food control sample contained 10-12 eggs from one egg producer. Red dots represent the geometrical mean of PFAA levels each year. Red line is the log-normal regression line of the temporal trend (simple regression analysis, $p \leq 0.05$).*

The decreased PFOS contamination of hen´s eggs is most probably due to the phase-out of PFOS-related production and use, resulting in a decreased contamination of the hen´s feed. Similarly decreased contamination of the fish feed may explain the decreased PFOS contamination of farmed rainbow trout. In the case of farmed rainbow trout a decline in bioaccumulation of PFOS from the water, due to decreased PFOS emissions into the aquatic environment, could contribute to the observed temporal trend (Martin et al., 2003a; Martin et al., 2003b).

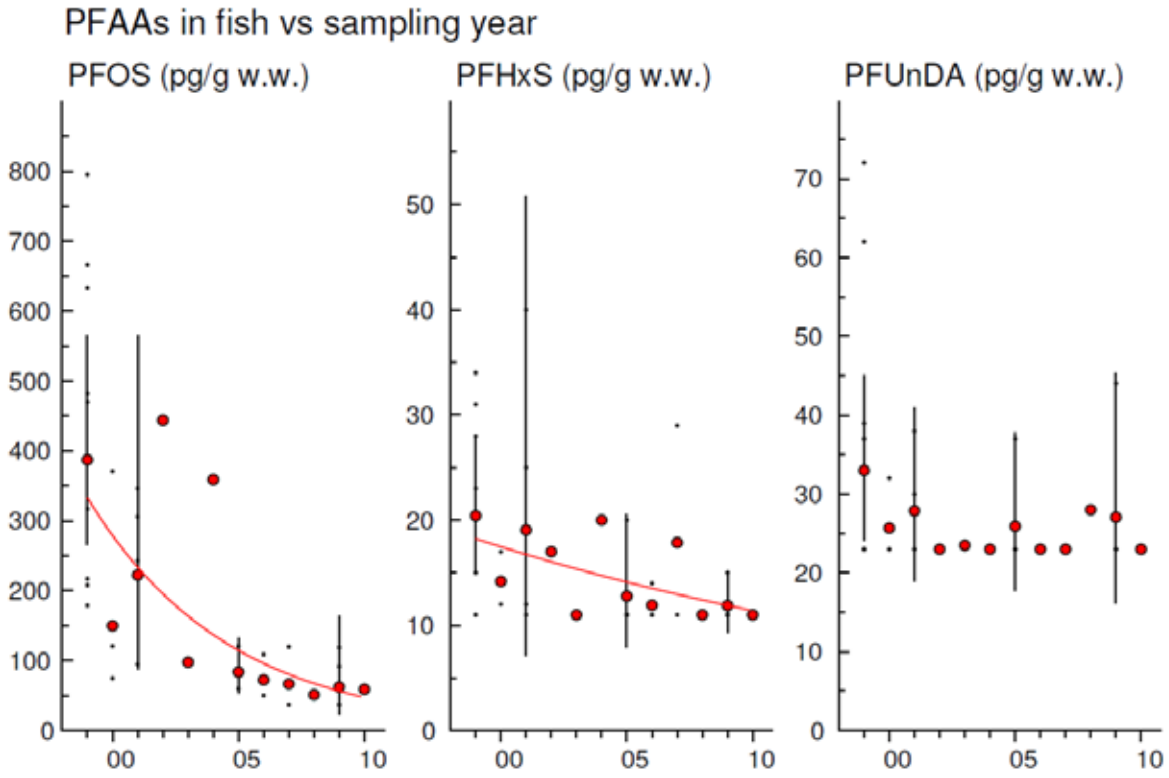


Figure 1. *Temporal trends of PFHxS, PFOS and PFUnDA in farmed brackish water rainbow trout, sampled on the Swedish market between 1999 and 2010. Sampling occurred within the National Food Agency´s food control program. Individual samples of fish were analyzed. Red dots represent the geometrical mean of PFAA levels each year. Red line is the log-normal regression line of the temporal trend (simple regression analysis, $p \leq 0.05$).*

In the market basket study the total per capita intake of PFOS declined 30-40% between 1999 and 2005-2010 (Vestergren, 2011). This decline was mostly due to the decline in PFOS intake from eggs and to some extent from meat and meat products. The other large contributor to per capita PFOS intake in 1999 was fish and fish products (~60%), and the contribution of fish consumption to the PFOS intake increased to over 80% in 2005. No temporal trend of PFOS levels in the fish and fish product market baskets was indicated (Vestergren, 2011).

Most of the fish consumed in Sweden is caught in the wild. Little is known about temporal trends of PFOS in wild fish on the Swedish food market. The guillemot, which is a fish-eating sea bird, can be used as an indicator of temporal trends of PFOS in Baltic Sea fish (Bignert et al., 2011). An increasing trend in PFOS in guillemot eggs was observed between the late 1960s and 2009 (Bignert et al., 2011). However, PFOS levels seem to level out after 2000, though relatively large variations between sampling years obscure a clear recent trend.

In raw milk PFOS was the only PFAA that was present in high enough levels to make an analysis of temporal trends meaningful. No significant temporal trend could be observed (Table 1). The PFOS levels in raw milk were close to the MDL, which makes it difficult to detect a temporal trend in concentrations. Moreover, in the beginning of the study period the sampling of milk was restricted to one region of Sweden, which may to some extent have confounded the results.

Temporal trends of PFHxS

PFHxS levels decreased in egg yolks and farmed rainbow trout between 1999 and 2010 (Fig. 1 and 2, Table 1), which could be a result of a declined production and use of PFHxS-related compounds during the last decade (3M, 2002). The decline in PFHxS levels in eggs and farmed fish is in contrast to the observation of an increase in PFHxS exposure of young women from the Uppsala area (Glynn et al., 2011). An increasing human exposure to PFHxS between the late 1980s and early 2000s has also been suggested in a study of middle-aged women from the Lund area and nursing women from Stockholm (Jönsson et al., 2008; Sundstrom et al., 2011). Due to a low number of samples in later years, temporal trends after year 2000 are uncertain in these latter studies (Jönsson et al., 2008; Sundstrom et al., 2011). In the market basket study, the PFHxS level in eggs was more than 10 times lower in 2010 than in 1999 (Vestergren, 2011). As a consequence, the contribution of egg consumption to the per capita intake of PFHxS declined from about 25% to less than 5% between 1999 and 2010 (Vestergren, 2011), which is in line with a declined PFHxS level in eggs in Sweden shown in our study.

The PFHxS level in the fish market basket was about 2-fold higher in 1999 than in 2010 (Vestergren, 2011), corroborating the declining PFHxS levels in farmed fish observed in the present study. The total per capita intake of PFHxS was halved between 1999 and 2010.

Taken together the results of the market basket study and our study of temporal trends of PFHxS in eggs and farmed fish suggest that the human exposure of PFHxS from staple foods in Sweden has decreased since the late 1990. Consequently, the increase in human PFHxS exposure, as suggested among young Uppsala women between 1996 and 2010 (Glynn et al., 2011), most probably is due to an increased exposure from other sources than staple foods.

Temporal trends of PFOA

PFOA levels in eggs also declined during the study period (Fig. 1, Table 1), which at least partly could be a result of a recent decrease in industrial emissions of PFOA, as a result of stewardship programmes introduced by many manufacturers (EPA, 2010). The decline of PFOA levels was slower than the decline of PFOS. To some extent this could be due to the fact that PFOA production is still ongoing in industrialized countries. Temporal trend studies of human exposure show that exposures have declined during the last decade, but the decrease has been slower than for PFOS (Glynn et al., 2011; Sundström et al., 2011).

Temporal trends of PFDA, PFUnDA, PFDoDA and PFTrDA

In contrast to the declining trends of PFHxS, PFOS and PFOA levels in hen´s eggs, no significant temporal trends were observed of levels of PFCAs with perfluorinated carbon chain lengths higher than 7 carbons (Table 1). Furthermore, no change of the levels of the carboxylic acid with 10 perfluorinated carbons (PFUnDA) was observed in farmed rainbow trout (Table 1). In the market basket study consumption of hen's eggs did not contribute much to the total per capita intake of PFDA, PFUnDA, PFDoDA and PFTrDA (Vestergren, 2011). Fish consumption dominated with contributions between 40-100%. In total the per capita intake of the compounds tended to increase between 1999 and 2010, mainly due to an increase in levels in the fish baskets (Vestergren, 2011). An increased per capita consumption of fish in Sweden between 1999 and 2010 could also contribute to the increasing human exposures to long chain PFCAs in Sweden during the last decade (Glynn et al., 2011; Jönsson et al., 2008).

Conclusions

The decreasing levels of PFOS and PFOA in eggs and PFOS in farmed fish is in line with the observed decline in human PFOS and PFOA exposure in Sweden. The phase out of production and use of PFOS-related compounds and the efforts to decrease the emissions of PFOA most probably contribute to the observed trends. PFHxS levels in eggs and farmed fish decreased between 1999 and 2010, which is opposite to the increase in PFHxS exposure of young women from the Uppsala area. Three market basket studies between 1999 and 2010 suggest that PFHxS-contamination of staple foods has not increased in Sweden. Therefore the observed increase in PFHxS exposure seen in young women is most probably due to increased exposures from other sources than staple foods. No temporal trends of PFDA, PFUnDA, PFDoDA and PFTrDA levels in eggs, or of PFUnDA in farmed fish, were observed. The market basket studies indicate that exposures to some long chain PFCAs from staple foods have increased between 1999 and 2010, which could, at least partly, be due to a combination of increased levels in fish and an increased fish consumption in the general population during the last decade in Sweden.

Acknowledgements

Ingalill Gadhasson is thanked for skillful laboratory work. Anne-Sofie Kärsrud is acknowledged for help in sample extraction.

References

3M. (2000) Letter, with attachments, from William Weppner, 3M, to Charles Auer, EPA OPPT, regarding the phase-out plan for POSF-based products. AR 226-0600.

3M. (2002) Identification of fluorochemicals in human sera. III. Pediatric participants in a group A Streptococci clinical trial investigation. Final Report from the Epidemiological Department, 3M Company.

Berger U., Glynn A., Holmstrom K.E., Berglund M., Ankarberg E.H., Tornkvist A. (2009) Fish consumption as a source of human exposure to perfluorinated alkyl substances in Sweden - analysis of edible fish from Lake Vattern and the Baltic Sea. Chemosphere 76:799-804.

Bignert A., Boalt E., S D., J H., Johansson A.K., Miller A., Nyberg E., Berger U., Borg H., Eriksson U., Holm K., Nylund K., Haglund P. (2011) Comments concerning the National Swedish Contaminant Monnitoring Programme in Marine Biota, 2011. Naturhistoriska riksmuseet. http://www.nrm.se/download/18.42129f1312d951207af800025998/Marina+programmet+2011.pdf.

Butt C.M., Berger U., Bossi R., Tomy G.T. (2010) Levels and trends of poly- and perfluorinated compounds in the arctic environment. Sci Total Environ 408:2936-65.

Eskhult G., Aune M. (2007) Results from the Swedish monitoring program 2003-2005 for PCDD/F and dioxin-like PCBs in foodstuffs. Organohalogen Compounds 69:2323-2326.

EPA. (2010) Perfluorooctanoic acid (PFOA) and fluroinated telomers. PFOA Homepage http://www.epa.gov/opptintr/pfoa/index.html.

Glynn A. (2010) Riskvärdering: PFAS i mälarfisk. PM, Livsmedelsverket.

Glynn A., Berger M.L., Lignell S., Aune M., Darnerud P.O. (2011) Perfluorerade organiska ämnen i serum från förstföderskor i Uppsala - tidstrend 1996-2010. Sakrapport till Naturvårdsverkets Miljöövervakning.

Holmstrom K.E., Johansson A.K., Bignert A., Lindberg P., Berger U. (2010) Temporal Trends of Perfluorinated Surfactants in Swedish Peregrine Falcon Eggs (Falco peregrinus), 1974-2007. Environ Sci Technol. DOI: 10.1021/es100028f.

Jorbruksverket. (2005) Äggproduktion i ekologiskt lantbruk. Jordbruksverket. http://www2.jordbruksverket.se/webdav/files/SJV/trycksaker/Pdf_jo/jo05_21.pdf.

Jordbruksverket. (2010) Ekologisk produktion. Varför matchar inte utbudet efterfrågan? Jordbruksverket. http://www.sjv.se/download/18.677019f111ab5ecc5be80001855/10_08kortversion.pd.

Jönsson B., Axmon A., Axelsson J., Lindh C. (2008) Retrospektiva studier av halter av perfluorerade ämnen i plasma hos kvinnor mellan 1987 och 2007. Rapport till Naturvårdsverket. http://www.imm.ki.se/Datavard/Rapporter/Rapport%20NVV%20PFCs%202008.pdf.

KemI. (2011) Handlingsplan för en giftfri vardag. Kemikalieinspektionen. http://www.kemi.se/Documents/Publikationer/Trycksaker/Handlingsplan-Giftfri-vardag.pdf.

Martin J.W., Mabury S.A., Solomon K.R., Muir D.C. (2003a) Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (Oncorhynchus mykiss). Environ Toxicol Chem 22:196-204.

Martin J.W., Mabury S.A., Solomon K.R., Muir D.C.G. (2003b) Dietary accumulation of perfluorinated acids in juvenile rainbow trout (Oncorhyncuchus mykiss). Environ Toxicol Chem 22:189-195.

Olsen G.W., Burris J.M., Ehresman D.J., Froehlich J.W., Seacat A.M., Butenhoff J.L., Zobel L.R. (2007) Half-life of serum elimination of perfluorooctanesulfonate,perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ Health Perspect 115:1298-305. DOI: 10.1289/ehp.10009.

Powley C., Buck R.C. (2005) Matrix-effect free analytical methods for determination of perfluorinated carboxylic acids in biological samples. Poster presented at the Society of Environmental Toxicology and Chemistry (SETAC), 15th Annual Meeting of SETAC Europe, Lille, France, May 22−26., 2005.

Prevedouros K., Cousins I.T., Buck R.C., Korzeniowski S.H. (2006) Sources, fate and transport of perfluorocarboxylates. Environ Sci Technol 40: 32-44.

Sundstrom M., Ehresman D.J., Bignert A., Butenhoff J.L., Olsen G.W., Chang S.C., Bergman A. (2011) A temporal trend study (1972-2008) of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in pooled human milk samples from Stockholm, Sweden. Environ Int 37:178-83.

van Leeuwen S.P., Swart C.P., van der Veen I., de Boer J. (2009) Significant improvements in the analysis of perfluorinated compounds in water and fish: results from an interlaboratory method evaluation study. J Chromatogr A 1216:401-9. Vestergren R. (2011) Human exposure to perfluoroalkyl acids. Doctoral thesis in Applied Environmental Science, Department of Applied Environmental Science (ITM), Stockholm University. http://su.diva-portal.org/smash/record.jsf?pid=diva2:451723.

Vestergren R. (2011) Human exposure to perfluoroalkyl acids. Doctoral thesis in Applied Environmental Science, Department of Applied Environmental Science (ITM), Stockholm University. http://su.diva-portal.org/smash/record.jsf?pid=diva2:451723.

www.kemikalieinspektionen.se

Swedish Chemicals Agency, P.O. Box 2, SE-172 13 Sundbyberg
Phone: +46 8 519 41 100, Fax: +46 8 735 76 98, E-mail: kemi@kemi.se