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report

2006

PRESENCE OF PERFLORINATED CHEMICALS IN EELS FROM 11 EUROPEAN COUNTRIES

Investigating the contamination of the European eel with PFCs, substances used to produce non-stick and water-repellant coatings for a multitude of products



SLIPPING AWAY

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published by Greenpeace International

date September 2006

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preface



This report investigates the contamination of the European eel (*Anguilla anguilla*) with perfluorinated chemicals (PFCs), substances used to produce non-stick and water-repellant coatings for a multitude of products. The study looks at eels from 21 rivers and lakes in 11 European countries, ranging from remote rural to urban industrial areas in Belgium, Czech Republic, Denmark, France, Germany, Ireland, Italy, Netherlands, Poland, Spain and the UK. Our findings show that there is widespread contamination of eels across Europe with these hazardous substances. This study completes a project, in which the first report documented the contamination of the species with other groups of hazardous chemicals, namely brominated flame retardants and PCBs (polychlorinated biphenyls). Our intent with this project is to provide a “snapshot” that will broaden existing knowledge on the extent to which commonly used hazardous substances are contaminating freshwater ecosystems across Europe, to raise concern for their potential impact on a vulnerable species in decline, and to stress the need for effective solutions that will promote a healthy and sustainable environment.

Also known as the common eel, the European eel has long been recognized as a creature able to tolerate high levels of pollution and to survive in oxygen-depleted environments, storing high concentrations of hazardous substances in its body during its long life span. A common citizen of estuaries across Europe, the species has gained recognition as a bio-monitor, revealing the pollution of its local freshwater environment. Crashing populations across the continent now raise alarm among conservationists that something is going terribly wrong for the eel. Where once abundant, its populations are fast diminishing. The reasons are uncertain, but it is thought that habitat destruction, global warming and overfishing are all likely to play a role. And now, increasingly, scientists are beginning to suspect that hazardous chemicals may also be impacting the life cycle of the species. Knowledge on the eel life cycle is extremely limited by the fact that they travel long distances to breed far from land, possibly in the Sargasso Sea. Recent toxicology studies cited in this report shed new light and suggest that the chemical body burden of the adult eel may be transferred from adult to offspring. Concentrations tolerated by the adult in its immature ‘yellow eel’ life stage may damage the developing gonads as fat reserves are consumed,

and their chemical burden mobilized, in the final migratory ‘silver eel’ stage. The chemicals may also become a lethal dose to eel embryos after spawning. In short the adults may be poisoning their young by passing over the hazardous substances accumulated during a lifetime of swimming in contaminated waters.

Perfluorinated compounds, or PFCs, are chemicals with non-stick and water repellent properties in which natural carbon-hydrogen bonds have been replaced with short and very strong carbon-fluorine bonds. They are used to manufacture non-stick coatings for cookware and clothing, stain-resistant carpets, coatings for food packaging (e.g. fast food wrappings, the linings of microwave popcorn bags), firefighting foams, paints and adhesives. These man-made substances persist in the environment and can accumulate in body tissue and bio-magnify through the food chain to varying degrees, sometimes to remarkable proportions. Some of the most highly mobile PFCs travel to polar regions where they may degrade into highly persistent and bioaccumulative breakdown products. So widespread is their distribution, PFCs are found in almost all environmental media, and in animals from invertebrates, fish and amphibians to birds and mammals. PFCs are known to accumulate in blood and growing evidence points to their toxicity, particularly to the liver. PFCs are now known to contaminate human blood and to pass from pregnant mother to unborn children *via* the umbilical cord.

There is growing acceptance among scientists and policy-makers that chemicals that persist and bioaccumulate should be phased out and replaced, or substituted, with safer alternatives, applying what is known as the Substitution Principle. Existing laws based on setting acceptable levels of exposure are generally acknowledged to have failed and the substitution principle is slowly but steadily gaining regulatory acceptance. For example, both the European Parliament and Council of Ministers have voted for the proposed EU legislation, REACH (Registration, Evaluation, Authorisation of CHemicals), to require that persistent and bioaccumulative substances be replaced with safer alternatives whenever they exist. The European Parliament has gone one step further by voting to apply the substitution principle to all substances that REACH will identify to be of Very High Concern because of their toxicity, ability to cause cancer, birth defects or reproductive illnesses or to disrupt hormonal

function, thereby requiring the development of safer alternatives and their ultimate replacement. However, the Council of Ministers rejected this measure in their first round of decision making on the legislation, arguing that such substances could still be authorized for use as long as companies can convince authorities that 'safe' thresholds can be established and that the risks of the substances can be 'adequately controlled'.

When chemicals such as perfluorinated chemicals are in widespread use they inevitably find their way into the environment from production, use and disposal. Hazardous substances commonly used in products pollute house dust, rainwater, human blood, and unborn infants. The effects of these chemicals in combination with each other also make it virtually impossible to establish safe levels of exposure. For example, even if a perfluorinated chemical is not overtly toxic at the concentration present, it may nevertheless damage cell permeability, possibly leading to the cellular penetration of other toxic compounds which may be present.

Rising concern for the impacts of hazardous substances on reproduction and the next generation highlight the difficulty of establishing safe thresholds for hazardous substances. Increasingly hazardous chemicals are being shown to disrupt the hormone system which regulates growth and development. Studies indicate that some PFCs affect growth and reproduction in aquatic plants and invertebrates, impacting hormone levels in some fish. This issue is of particular relevance as it is now under debate by the EU institutions, as is the use of effect thresholds for regulatory purposes under REACH. To take the case of the eel, it is evident that assumptions made by basing thresholds on effects seen in adults of a species could be invalid when considering what doses could affect the embryos at the earliest stage of life.

Greenpeace urges the European Union to heed the warnings posed by widespread contamination of our freshwater ecosystems, and in particular of a species that may be threatened with extinction, with yet another group of persistent organic pollutants. We urge the European Parliament and the Council of Ministers to take precautionary measures that will control chemical contamination at the source by requiring that all chemicals of Very High Concern under REACH, including those that may disrupt hormonal function, be authorised only on a time-limited basis and replaced with safer alternatives whenever they exist.



executive summary



Over the past 50 years, man has manufactured, used and unwittingly released to the environment a wide range of perfluorinated chemicals (PFCs), a group of substances which do not occur naturally. PFCs have been used for many applications, including as stain repellent surface coatings on carpets, leather and textiles; water, grease and oil-repellents on disposable paper and board products; non-stick treatments on saucepans; paints, adhesives and cleaning agents; in semiconductor manufacture, industrial photographic applications and in fire-fighting foams. The diverse use of PFCs, together with the volatile nature of some, has unfortunately resulted in a number of these chemicals becoming very widespread environmental contaminants.

PFCs are highly stable compounds, with any break-down normally limited to formation of other, more stable perfluorinated by-products, a property which means they are very persistent (long-lived) in the environment. Numerous studies have reported the presence of PFCs in living tissue of aquatic invertebrates, fish, birds, mammals and humans, a phenomenon exacerbated by their propensity to bioaccumulate (build up) in blood and liver tissue. Of further concern is that some laboratory studies indicate that PFCs are capable of causing toxic effects in a number of different animal species. Because of their widespread environmental presence, coupled with their inherent properties of persistence, bioaccumulation and toxicity, PFCs are a fast-growing concern.

The present study was designed to determine the concentrations of 3 perfluoroalkyl sulphonates, including the formerly widely used PFOS (perfluorooctane sulphonate), and 6 perfluorocarboxylic acids, including PFOA (perfluorooctanoic acid), in the liver and muscle tissues of European eels (*Anguilla anguilla*) caught from 21 different locations within 11 European countries (Belgium, Czech Republic, Denmark, France, Germany, Ireland, Italy, Netherlands, Poland, Spain and the UK). This was the second phase of a two part study, in which we previously determined the concentrations of PCBs and certain brominated flame retardants in muscle tissue from the same eels.

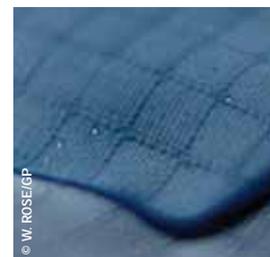
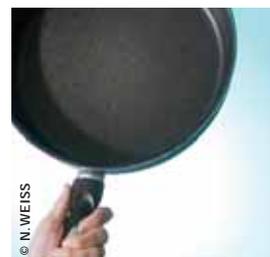
Two perfluoroalkyl sulphonates, namely PFOS and PFHxS (perfluorohexane sulphonate) were found in 10 and 11 of the 16 pooled eel liver samples analysed respectively. Concentrations for PFOS ranged from <16 ng/g wet weight to 498 ng/g wet weight, and for PFHxS from <21 ng/g wet weight to 583 ng/g wet weight. With regard to the perfluorocarboxylic acids, PFDA (perfluorodecanoic acid) was recorded in 3 of 16 liver samples at levels between 34 and 92 ng/g wet weight while PFOA was only found in one of the 16 liver samples at a concentration only just above the limit of quantification. Testing of a limited number (four) of muscle samples from eels as part of the present study showed that two were contaminated with PFHxS and one with PFOS. While the concentrations recorded for PFOS fall within the broad range reported in other studies for this compound in liver tissue from freshwater fish, those for PFHxS appear unexpectedly high. The possibility that these apparently anomalous levels result from analytical interferences by other, so far unidentified, compounds cannot be excluded but is thought to be small. Further investigations will be necessary to draw firm conclusions for this substance.

The results of this study provide a preliminary overview of the extent of contamination of the European eel by PFCs across Europe. It is not known for certain whether the levels found in these eels are representative of all eels in the catchments in which they were collected, nor whether they are capable of causing detrimental health impacts and therefore acting as a further contributory factor in the decline of the European eel. Indeed, it is likely that observed declines in eel populations across many parts of Europe result from a complex interaction of a number of factors, including habitat disruption, overfishing, pathogens and climate change, though exposure to chemical contaminants such as persistent organic pollutants may well be an important additional stressor. Other studies have identified adverse effects on reproductive success in eels relating to body burdens of dioxin-like compounds, including some PCBs, for example. With regard to perfluorinated chemicals, a recent study of eels from Belgium reported an association between liver PFOS concentration and elevated levels of the enzyme ALT (alanine aminotransferase), indicative of liver stress. The levels of PFOS found in the livers of eels in the present study were within the lower end of the range of those which correlated with elevated ALT levels, suggesting that the potential for quite widespread impacts on European eels at current environmental levels of PFOS cannot be ruled out. Furthermore, PFOS has been shown to biomagnify to higher concentrations through the food chain. Predators of the eel, such as herons and even man, may therefore also be in danger of accumulating PFOS in their tissues to levels even higher than in the eels themselves.

Despite the widespread distribution of PFCs in wildlife and humans, legislative measures to control their use remain at a preliminary stage in most parts of the world. Proposed restrictions under the EU Marketing and Use Directive will phase out some (though not all) uses of PFOS but will not control uses of fluorinated polymers, perfluorinated carboxylic acids or of other perfluorinated chemicals, including the increasingly widely used telomer alcohols, which are thought to undergo long-range transport before breaking down to perfluorinated carboxylic acids.

There is hope that future chemicals legislation currently under development within Europe, namely REACH (Registration, Evaluation, Authorisation of CHemicals), will bring in stricter controls for persistent and bioaccumulative chemicals, as well as those that are toxic and can cause cancer, reproductive illnesses and defects, including a requirement for their substitution with safer alternatives wherever possible. However, current differences of opinion between the European Parliament and the Council of Ministers threaten to weaken the legislation. Even though many PFCs are persistent and bioaccumulative, it is not yet known whether REACH will allow their continued use or have mechanisms in place to drive the development and use of safer materials or substances. In the meantime, the continued use of PFCs combined with their inherent resistance to degradation inevitably means that the exposure of wildlife and humans to these highly persistent, bioaccumulative and potentially toxic chemicals will continue well into the future.

PFCs are used to manufacture non-stick and water repellent coatings for a variety of products such as food wrappings, clothing, carpets and kitchenwear.



introduction



The European eel (*Anguilla anguilla*) is under threat and in serious decline. Over the last few decades, sharp decreases in eel populations in inland waterways have been reported from many parts of Europe, with current populations in some catchments estimated to be as low as 1% of historical levels. It is likely that a number of different factors have contributed to this decline, including overfishing, habitat loss, the spread of parasites, poor water quality and even climate change, which may be interfering with the eels' long-distance migration to the deep Atlantic for spawning and the subsequent eastward drift of larvae to replenish Europe's waterways with young eels.

The presence of toxic chemicals in European rivers and lakes, from direct industrial discharges or, increasingly significantly, from households and municipal treatment works, has long been a concern regarding the health and reproductive success of many freshwater species, including eels. Some recent research has indicated that pollution of waterways with dioxin-like chemicals, including the now banned industrial chemicals PCBs (polychlorinated biphenyls), may still be having a profound impact on reproductive development and spawning success in the European eel. Even if not solely responsible for population declines, such chemical pollution could well be a substantial contributory factor and may limit recovery from other stresses. Further investigation of the distribution of these and other persistent organic pollutants in eel populations across Europe is therefore vital.

In a report published in November 2005, we documented the presence of PCBs in samples of eels collected from 20 locations in 10 European countries, confirming that contamination levels in muscle tissue, though varying over a wide range, were still remarkably high in some waterways. At the same time, we were able to quantify the presence of another less well studied group of persistent organic pollutants, the brominated flame retardants, providing one of the broadest geographical surveys to date of the environmental spread and accumulation of these chemicals, still used in a wide range of consumer products, in a key freshwater predator species.

In the current study, we have investigated the presence in the same eel samples of another group of environmentally persistent chemicals which continue in widespread use, namely perfluorinated chemicals or PFCs. Knowledge of the environmental fate and distribution of these chemicals, though rapidly increasing, nevertheless remains limited.

However, some early indications suggest that these chemicals too, even at current environmental levels, may be capable of damaging the health of fish species including the European eel.

What are perfluorinated chemicals?

Just as carbon-based chemicals can form chemical bonds with chlorine and bromine, they can also bind with the halogen fluorine, forming so-called 'organofluorine' chemicals. Around 30 organofluorines have been reported to occur in nature (Hekster *et al.* 2003), each containing only a single fluorine atom in each molecule. In contrast, many of the organofluorine chemicals produced by man for use as ingredients in pesticides, industrial chemicals and consumer goods contain several fluorine atoms per molecule. Those in which all the carbon-hydrogen bonds present in the organic chemical backbone have been replaced by carbon-fluorine bonds are known as perfluorinated chemicals, or PFCs. These chemicals are not produced by natural processes and hence never occur in nature other than through human activities.

The direct chemical bond between carbon and fluorine is very short and very strong, making it highly resistant to chemical, biological and thermal degradation (So *et al.* 2004). It is these characteristics, along with their unusual solubility properties, which have made perfluorinated chemicals so attractive to commerce over the last 50 years (Paustenbach *et al.* 2005). However, they also confer upon PFCs one of their major environmental down-sides, namely their long persistence in the environment once they are released, whether from manufacturing or disposal operations or during the useful lifetime of a product (Key *et al.* 1997).

As noted earlier, as well as their high stability, PFCs also have some highly unusual solubility and surface active properties. The chemistry of many PFCs means that they have relatively low solubilities in both water and oils, unique properties which have underpinned their development and widespread use as water, grease and stain-repellent finishes on textile and paper products, as well as for specialised solvents and surfactants used in industry and as components of cosmetics and plastic products (OECD 2002, Hekster *et al.* 2003). Their resistance to breakdown even at high temperatures has led to their use also in fire-fighting foams and in lubricants for high temperature applications (OSPAR 2006).

Manufacture and use of perfluorinated chemicals

The PFCs which have been manufactured over the past 50 years fall into four broad categories:

- * **Perfluorinated alkyl sulphonates (PFAS)** - including the well known compound PFOS (perfluorooctane sulphonate) and a wide range of PFOS-related chemicals (such as PFOSA¹, a chemical intermediate in the manufacture of fire-fighting foams, industrial cleaners, floor polish, mist suppressants for metal plating baths and in circuit board manufacture, FOSA², used in photographic papers, medical applications and in pesticides, and FOSE³, used to make a whole range of water, grease and stain-repellent finishes for carpets, clothing and disposable food cartons, among others) (OECD 2002). More recently, commercial attention has switched to the smaller chain length perfluorobutane sulphonate (PFBS) as a result of growing environmental concerns surrounding PFOS (see below).
- * **Perfluorocarboxylic acids (PFCAs)** - especially PFOA (perfluorooctanoic acid), used as a polymerisation aid in the manufacture of the well known fluorinated polymer PTFE (polytetrafluorethylene).
- * **Fluoropolymers** - the best known being PTFE, marketed as Teflon and widely used for 'non-stick' cookware.
- * **Fluorotelomer alcohols (FTOH)** - perfluorinated carbon chains of various lengths linked to an alcohol group, used in the manufacture of surface-coating polymers, paints, adhesives and cleaning agents, and conferring similar water and stain repellent properties to textiles and paper products as the PFOS-related chemicals (Dinglasan *et al.* 2004).

A more detailed description of historic and current trends in the manufacture and use of a whole range of perfluorinated chemicals is provided by Walters & Santillo (2006).

1. Perfluorooctane sulphonic acid
2. N-alkylperfluorooctanesulfonamide
3. N-alkylperfluorooctanesulfonamidoethanol

Until 2000, the most widely used perfluoroalkyl sulphonate (PFAS) chemicals were those based on PFOS. At that time, the annual production of all PFOS-related chemicals in the USA alone was 3000 tonnes (Stock *et al.* 2004), with an estimated 37% used in surface treatment preparations for leather, carpets and other textiles and around 42% used as water, grease and oil repellents in paper and board products, especially those used for disposable food packaging (Kannan *et al.* 2002b). Although there were a large number of different PFOS-related chemicals in use, which were designed to optimise specific properties conferred on the products, the majority share the common property of eventual degradation back to PFOS itself, a compound so resistant to further degradation that it is expected to persist for very long periods in the environment (Kannan *et al.* 2002a). PFOS also has a very high potential to bioaccumulate, to build up in the tissues of living organisms and accumulate through the food chain to reach highest body-burden concentrations in top predators. Through a combination of bioconcentration from water into body tissues of up to 1000 times and biomagnification up the food chain of between 2 and 20 times per trophic level, overall bioaccumulation factors in excess of 6000 for top predators have regularly been reported for this chemical (Hekster *et al.* 2003, Martin *et al.* 2004a, Kannan *et al.* 2005a, Sinclair *et al.* 2006). This, combined with its chemical stability, known and suspected toxicity and widespread use in open applications, has meant that PFOS has become a global environmental concern (Geisy & Kannan 2001, Houde *et al.* 2006).



PFOS - a widespread persistent organic pollutant

Although PFOS itself has a relatively low tendency to partition to the atmosphere and undergo long-range transport, many of the PFOS-related compounds which have seen common use are far more volatile. It is thought that the transport of these precursor chemicals on air currents and their subsequent degradation to form PFOS could explain the very widespread presence of PFOS as an environmental contaminant, even in remote parts of the world such as the high arctic (see below). Unlike the more fat soluble organochlorine and organobromine chemicals (such as PCBs, chlorinated pesticides and many brominated flame retardants, which have long been recognised as persistent organic pollutants (POPs) and associated with bioaccumulation) PFOS accumulates in the bodies of animals by binding to proteins in the blood, thereby building up to particularly high levels in liver tissue (Martin *et al.* 2003a, b). Some of the highest environmental levels of PFOS recorded have been in the livers of polar bears, seals and fish at the top of the food chain (Martin *et al.* 2004a, b, Bossi *et al.* 2005, Kannan *et al.* 2005b, Smithwick *et al.* 2005, Sinclair *et al.* 2006).

In 2000, following long-standing concerns expressed by the US EPA regarding the growing evidence of widespread contamination and bioaccumulation, one of the world's major manufacturers of PFOS-related compounds, 3M, announced a cessation in production, shifting its well-known 'Scotch' brand of stain-repellent surface treatments to perfluorobutane sulfonate (PFBS) chemistry instead (Olsen *et al.* 2003). Evidence to date indicates that, while PFBS may be equally persistent, it has far less propensity to bioaccumulate (Hekster *et al.* 2003) and may exhibit lower toxicity also. Despite 3M's decision, and the various national and regional legislative initiatives which have followed (see below), PFOS remains a major environmental concern. Firstly, some uses of PFOS-related chemicals have been allowed to continue, most notably in certain photographic, metal plating and electronics manufacturing processes, in stocks of aqueous fire fighting foam (AFFF) and in industrial hydraulic fluids use in aviation (EC 2005). Of these, the fire fighting foams represent the largest remaining stock of these compounds within Europe, at around 122 tonnes (OSPAR 2006). Secondly, however, even if all uses were discontinued, the high persistence of PFOS will inevitably mean that it will continue as an environmental contaminant for a long period.

In addition to the perfluorinated alkyl sulphonates, many other perfluorinated compounds have been manufactured over time, many of which remain in use. Of particular concern for the environment are the perfluorinated carboxylic acids, including the well known PFOA, and a host of other compounds which may well be acting as sources of these acids through partial degradation once they are released to the environment.

PFOA and other perfluorinated carboxylic acids

Best known of the perfluorinated carboxylic acids (PFCAs) is PFOA (perfluorooctanoic acid), used as a non-reactive polymerisation aid in the production of fluoropolymers, especially the classic PTFE (polytetrafluoroethylene), made famous by the 'Teflon' brand of 'non-stick' kitchenware and, more recently, by expanded PTFE membranes such as 'Gore-Tex'. Although it has been reported that only very small proportions of the PFOA used are carried over as residues in the finished polymer, this is because the vast majority is lost to atmosphere during the polymer curing process (Washburn *et al.* 2005). Although less bioaccumulative than PFOS, PFOA is nevertheless highly persistent and is now also recognised as a very widespread environmental contaminant, especially in water (Taniyasu *et al.* 2004, Caliebe *et al.* 2004, Yamashita *et al.* 2005). A recent study in Germany documented the presence of remarkably high concentrations of perfluorinated chemicals in the waters of the rivers Ruhr and Möhne (up to 446 and 4385 ng/l respectively), as well as in drinking water (up to 598 ng/l), with PFOA being the major component (Skutlarek *et al.* 2006). PFOA has also been found to be the predominant perfluorinated chemical in landfill leachates in a study of the distribution of PFCs in the Nordic environment (Kallenborn *et al.* 2004).

Aside from its deliberate production, PFOA along with other shorter and longer-chain PFCAs can be generated as unintended byproducts in the manufacture of the fourth general group of perfluorinated chemicals, the extensively used perfluorinated telomer alcohols, or FTOHs (Poulsen & Jensen 2005), which may increasingly be used in applications for which PFOS-related chemicals were formerly used (ENDS 2004). A major use of FTOHs is in the onward manufacture of polymers for use as stain-repellent surface coatings on carpets, leather and textiles (such as DuPont's 'Zonyl' and 'Stainmaster' brands and Lanxess' 'Baygard') and as water, grease and oil-

repellent treatments for disposable paper and board products (also including 'Zonyl', along with Atofina/DuPont's 'Foraperle' and Clariant's 'Cartafluor'). For the period 2002-2003, annual global production of fluorotelomer alcohols was estimated at 5000 tonnes (Dinglasan *et al.* 2004).

Although these fluorotelomer-based polymers ostensibly make the textile products they are used to treat more durable, they are susceptible to wear over time, leading to loss of the fluorotelomers themselves back to the environment (Stock *et al.* 2004). Loss from packaging during use and following disposal may also represent a significant environmental source of these chemicals (Begley *et al.* 2005). In a process analogous to that of the PFOS-related compounds, it is thought that the fluorotelomer alcohols may be acting as a long-distance transport mechanism for their ultimate breakdown products, the perfluorinated carboxylic acids. For example, the commonly used fluorotelomer alcohol 8:2 FTOH is thought to degrade to PFOA itself, either through chemical oxidation in the atmosphere (Ellis *et al.* 2003, 2004), microbial degradation (Dinglasan *et al.* 2004), metabolism in higher organisms (Martin *et al.* 2005) or, more likely, a combination of all three. Therefore, even if residual concentrations of perfluorinated carboxylic acids in fluorotelomer preparations are relatively low, the products themselves may ultimately be acting as substantial source.

Although PFOS and PFOA are by far the most widely studied perfluorinated chemicals to date, in terms of both their environmental distribution and toxicity, they are by no means the only PFCs which can be detected in the environment, including in biota. For example, PFOS may be accompanied by PFHxS (perfluorohexane sulphonate), a known contaminant in many PFOS-related formulations, though generally found at lower levels than PFOS itself (Kannan *et al.* 2002a, Taniyasu *et al.* 2003). Moreover, some perfluorinated carboxylic acids with chain lengths slightly longer than PFOA, especially PFNA, PFDA and PFUnDA, have higher bioaccumulative potentials than PFOA and are frequently detected in top predators such as polar bears (Martin *et al.* 2004b, Smithwick *et al.* 2005), sea turtles (Keller *et al.* 2005), harbour porpoises (van de Vijver *et al.* 2004) and even dolphins and sperm whales (van de Vijver *et al.* 2003). Other PFCs, including PFOSA, have also been reported in some species (Kannan *et al.* 2002 a,c,d, Martin *et al.* 2004b, Kallenborn *et al.* 2004, Sinclair *et al.* 2004), though relatively few studies to date have included specific analyses for compounds such as these.

Perfluorinated chemicals - an emerging concern

Perfluoroalkyl sulphonates (especially PFOS) and perfluorocarboxylic acids (especially PFOA) have, therefore, been reported as contaminants in almost all environmental media, including freshwater, groundwater and seawater, sediments and soils and in animals from aquatic invertebrates, fish and amphibians to birds, turtles and mammals, especially marine and aquatic mammals. More recently, they have become increasingly widely reported as contaminants in human blood (Kannan *et al.* 2004a, b), including the USA (Olsen *et al.* 2003, Kuklennyik *et al.* 2004), northern Canada (Tittlemeir *et al.* 2004), Sweden (Karrman *et al.* 2004) and Japan (Masunaga *et al.* 2002, Taniyasu *et al.* 2003). In a recent study of human serum from volunteers in the Netherlands, PFOA was found in all 39 maternal blood samples analysed, as well as in 16 of 17 samples of umbilical cord blood from newborn babies, confirming its ability to cross the placenta and expose the developing child in the womb (Greenpeace/WWF 2005). In the same study, PFOS was found in 38 of 39 maternal samples, and in 7 of 17 cord blood samples. More recently still, a pilot study in Germany has revealed the widespread presence of PFOA and PFHxA in human breast milk (Suchenwirth *et al.* 2006).

Although historical data sets are inevitably limited by the relatively recent realisation of their environmental significance, there is growing evidence for increasing trends in tissue concentration of PFOS in wildlife from a number of regions around the globe from the mid 1990s onwards, including in lake trout (Martin *et al.* 2004a), guillemots (Holmstrom *et al.* 2005), ringed seals (Bossi *et al.* 2005) and polar bears (Houde *et al.* 2006). Despite the rapidly expanding interest in this and other perfluorinated chemicals, however, data on concentrations and trends in a range of other regions and key species remain scarce.

Besides concerns relating to the persistence of PFCs and, in the case of at least some, their bioaccumulative properties, PFCs are also of concern because of their known or suspected toxicity to plants and animals. For example, PFOS, PFOA, PFOSA and other similar compounds with chain lengths between 6 and 15 carbon atoms have been shown to interfere with communication between cells in body tissues through so-called 'gap junctions' (Hu *et al.* 2002). These effects appear particularly strong in the case of PFOS, perhaps related to observed impacts on the fluidity



and permeability of cell membranes. In this way, exposure to PFOS may also increase the cellular penetration of other toxic compounds which may be present, including dioxins, thereby potentially augmenting their effects (Hu *et al.* 2003), in a manner analogous to that previously reported for the herbicide 2,4-D when in combination with chemical agents able to damage the cell membrane (Jacobi *et al.* 1996).

Both PFOS and PFOA have also been reported to have adverse effects on the liver in both rodents and monkeys, with PFOA being a particularly strong hepatic tumour promoter in rats (Kawashima *et al.* 1995, Adinehzadeh *et al.* 1999, Berthiaume & Wallace 2002). PFOA has also been reported to suppress immune system function in mice, albeit at relatively high exposures (Yang *et al.* 2002). At rather lower exposures, including those in ranges currently reported for wildlife populations in some regions, Austin *et al.* (2003) reported that PFOS was able to cross the blood-brain barrier in rats and disrupt the normal oestrous cycle, perhaps through acting as a neuroendocrine disruptor.

Evidence relating to the toxicity of perfluorinated chemicals to humans is far less conclusive. This is not least because the few epidemiological studies to date have necessarily focused on relatively small cohorts of workers in the perfluorinated chemical industry, from which the identification of significance for small excesses in, for example, deaths from certain forms of cancer (Gilliland & Mandel 1993, Alexander *et al.* 2003) is inevitably statistically difficult. Much attention has focused on contamination of groundwater with PFOA from a DuPont facility in West Virginia (USA) and the consequent risks for local communities relying on the aquifer for drinking water (ENDS 2004). The United States Environmental Protection Agency (US EPA) published a draft risk assessment for PFOA early in 2005, which identified potential concerns for human health, especially for workers exposed occupationally, relating to observations of PFOA's reproductive, developmental and immunotoxicity in animals (for announcement in public register, see USEPA 2003). The draft assessment, which remains subject to review, also stressed the many substantial data gaps which limit the drawing of firm conclusions, including unexplained patterns of exposure among human populations as determined in biomonitoring programmes.

Although studies of the toxicity of perfluorinated chemicals to aquatic organisms, including fish, are also limited, there is some evidence for acute and chronic toxicity of PFOS in particular. At PFOS concentrations of tens of milligrammes per litre in water, such as may occur locally in water courses following a spill of PFOS-related chemicals or use of AFFFs, effects on growth and reproduction have been recorded in aquatic plants and invertebrates (Boudreau *et al.* 2003). Similar concentration ranges of PFOA have been reported to impact on circulating levels of testosterone and other hormones in fish (Oakes *et al.* 2004). However, reduction in the survival, growth and emergence behaviour of midge larvae, a vital food source for many species of fish and birds, have been recorded by MacDonald *et al.* (2004) at PFOS concentrations one to two orders of magnitude lower (down to 50 µg/l). Low level exposures were also found to have a profound impact on reproductive success over many generations in nematode worms, a key food organism for many other aquatic species (Tominaga 2004). There is some evidence that elevated levels of PFOS in the liver of carp and eels correlates with increases in activity of the enzyme alanine aminotransferase, an early indicator of liver damage (Hoff *et al.* 2005). In the case of most fish species, however, the toxicological significance of the contamination of marine and freshwater bodies with perfluorinated chemicals simply remains unknown.

More detailed reviews of the environmental fate and effects of a range of perfluorinated chemicals are provided by Geisy & Kannan (2002), Allsopp *et al.* (2005) and Houde *et al.* (2006). What is clear from the synopsis above, however, is that the manufacture, use and release of a diverse array of perfluorinated chemicals, many of which seem ultimately to be degrading only partially to form highly persistent and bioaccumulative break-down products, is a rapidly emerging concern.

The UK and Swedish governments, the OECD, US EPA and a number of other bodies have all adopted positions on the future regulation of PFOS and related substances. In the US, the use of these chemicals must be notified to the EPA. During 2005, the UK government prepared draft legislation for the phase out of PFOS by 2010, which it intended to implement from April 2006 unless the European Commission came forward with a draft measure in the meantime (DEFRA 2005).

In the latter part of 2005, the European Commission did propose controls, under the so-called (Restrictions on) Marketing and Use Directive (76/769/EEC), as laid out in paper COM (2005) 618 (EC 2005). However, the major part of the uses prohibited under this draft measure have already been discontinued within Europe, including use in carpets, upholstery and other textiles and in paper packaging products. Moreover, evidence exists that many of the remaining uses which would be permitted under the Commission's proposed amendment, such as use in semiconductor manufacture, industrial photographic applications, chrome-plating and in fire-fighting foams, could also be phased-out within the next five years. Indeed, proposals for national measures in the UK and Sweden explicitly expressed the desirability and feasibility of such programmed phase-outs addressing all uses other than as components of hydraulic fluids used in aviation, setting out, in the case of the UK, a series of application-specific phase-out dates ranging from 2007-2010.

The restrictions proposed by the Commission also fall short in other ways, such as the allowance for 0.1% by weight of PFOS in preparations and finished articles, which may ultimately result in continued or future use in a far broader range of product groups in which deliberate use at concentrations below 0.1% is feasible (OECD 2004).

Environmental releases may be expected to continue for as long as preparations containing PFOS-related chemicals, perfluorinated telomer alcohols and other PFCs remain in use. Moreover, chemicals such as PFOS and PFOA are effectively resistant to natural degradation processes. It is therefore vital that, in addition to introducing strict measures to curb and ultimately eliminate their use and release, efforts also continue to develop analytical methods and build data sets which can be used to monitor trends in the use and environmental distribution of these chemicals.

Fish as biomonitors of water pollution - the case of the European eel

In November 2005, we published a report describing the widespread occurrence of some more traditional POPs, namely PCBs and brominated flame retardants, in eels (*Anguilla anguilla*) collected from locations in 10 countries across Europe (Santillo *et al.* 2005). The rationale for that study was two-fold. Firstly, eels in their 'yellow eel' life stage have long been proposed as valuable biomonitors of water quality and pollution status in freshwater systems, because of their relatively long life-cycle, localised habit, diverse prey selection and ability to live in a wide range of conditions (Feunteun 2002, Versonnen *et al.* 2004). The 'yellow eel' phase is by far the longest stage in the lifecycle (3-15 years) and the phase in which eels grow substantially, accumulating large fat reserves but remaining sexually immature. It is in this phase, therefore, that the major part of chemical bioaccumulation may be expected to occur. Nevertheless, eels had been studied to date for the presence of only a sub-set of known environmental contaminants, and then only in a small number of countries. Secondly, eel populations are in rapid decline in many parts of Europe (Wirth & Bernatchez 2002, Laffaille *et al.* 2005, van Ginneken & Maes 2005), rendering the species absent or severely under threat in many rivers and lakes in which it was previously common (Dekker 2003). There are undoubtedly a number of different factors contributing to this observed decline (Feunteun 2002), including overfishing, habitat destruction, introduced diseases and even climate change, which may be impacting in particular on the complex breeding cycles of the eels in the deep Atlantic Ocean. However, it seems likely that chemical pollution, including heavy metals and persistent organic pollutants, may also be contributing to the decline (Robinet & Feunteun 2002, Palstra *et al.* 2006), perhaps by reducing the eels' ability to cope with the other environmental stresses they are facing.



Our report from November 2005 (Santillo *et al.* 2005) confirmed the widespread presence of several brominated flame retardants, including tetra- and pentabrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD), in the muscle tissue of eels from a total of 20 locations in 10 countries, from the west of Ireland and the UK, across central Europe to Spain, Italy and Poland. The results also confirmed that the well known organochlorine pollutants PCBs remained present at remarkably high levels in some eels, despite the long-standing prohibition on manufacture and use of these chemicals. This serves as a reminder of the very long-term consequences which can result from the use of highly persistent and bioaccumulative man-made chemicals. Some of the eel samples analysed, in each case represented by a 'pooled' or composite sample of muscle tissue from between 2 and 5 eels collected at each location, were relatively free of pollution, especially in remote areas of Ireland and Poland. Others, such as the specimens collected from the River Thames in the UK, the River Main in Germany, the Hollandsdiep in the Netherlands and the Tevere River as it passes through the centre of Rome were more heavily contaminated with PCBs and/or brominated chemicals.

It is not possible to deduce from these results that these chemical pollutants have been responsible for adverse effects or decline in eel populations in those areas. Nor is it possible to state that the contaminant levels reported are typical or representative of the entire regions or countries in which the samples were collected. Rather the data provide a snapshot of the range of tissue levels for these particular contaminants which can be found in eels across Europe as a whole, reflecting differing degrees of pollution of freshwater systems with these chemicals. Overall, the data add significantly to the existing knowledge base on contamination of this key and increasingly threatened species, and provide data for several countries for the first time.

Perfluorinated chemicals - a further threat to a species in decline?

As discussed above, perfluorinated chemicals such as PFOS and PFOA are expected to accumulate through rather different physicochemical processes than organochlorine and organobromine chemicals, building up in the blood and liver rather than primarily in fat deposits in the flesh. Nevertheless, there is reason to believe that these chemicals may well be accumulating in eel tissues and that eels may once again, therefore, provide a valuable source of biomonitoring data. Chemicals including PFOS, PFHxS and PFOSA have previously been reported as contaminants in the blood, livers or whole body homogenates of a number of different freshwater fish species in Europe, North America and Japan, including Chinook salmon, whitefish, brown trout (Sinclair *et al.* 2004), pike (Berger *et al.* 2004), bluegill and carp (Taniyasu *et al.* 2003). To date, only one other published study has been identified in which the presence of PFOS has been determined in eels, reporting some markedly high concentrations (up to 9031 ng/g wet weight) in the livers of specimens collected from certain canals and ponds in Flanders (Belgium), downstream from urban and/or industrial centres (Hoff *et al.* 2005).

Following the analysis of the 20 composite eel muscle samples for PCBs and brominated flame retardants described above, the liver tissue was retained from as many of the samples as possible. This gave the opportunity to use the same sample set as for our November 2005 report in order to obtain a similar 'snapshot' of the distribution of various perfluorinated compounds, including PFOS, PFOA and others, in eels from a range of locations across Europe. In addition to those samples, a single pooled sample from Køge Bay in Denmark was also made available for this second phase of the survey.

materials and methods



Study design

As described in the previous study, samples of freshly caught European eels (*Anguilla anguilla*), in the “yellow eel” life stage, were initially obtained from a total of 20 locations across 10 countries in Europe (Belgium, Czech Republic, France, Germany, Ireland, Italy, Netherlands, Poland, Spain and UK). Subsequent to that study, an additional sample of five eels was obtained from Koge Bay in Denmark, collected during July 2005 and bringing the total number of discrete sampling locations to 21.

The catchments and types of water body from which the samples were collected ranged from rural and relatively undeveloped sites (such as the single site selected in Poland) to sites within urban and/or industrial zones (such as the sample collected from the Tevere River in the centre of Rome and some of the samples collected in Germany and the Netherlands). A list of the sample sites and their descriptions is given in Table 1.

Given that perfluorinated compounds have a tendency to accumulate in blood and liver tissue rather than in muscle or fat tissue, it was initially intended that samples of liver (again pooled from all individual eels caught at each location) would be used for this second phase of the analyses. Unfortunately this was not possible in every case, as for three of the four samples from Germany (samples Germany 1, 3 and 4) and for the single sample from Denmark, the eels had been gutted prior to being forwarded to our laboratory. In these cases it was therefore necessary to use pooled muscle tissue (fillet or flesh) samples for analysis for perfluorinated compounds. It is hoped that it will be possible to conduct comparative analyses of liver and muscle tissue for these same compounds in the future, using samples for which both tissue types are available, but this has not been possible to date. We therefore present in this report the results from analysis of 17 pooled liver samples and 4 pooled muscle samples.

In each case, pooled samples were analysed for:-

- * 3 perfluoroalkyl sulphonates (PFAS) - PFOS, PFHxS and PFBS, and
- * 6 perfluorocarboxylic acids (PFCA) - PFOA, PFHxA, PFBA, PFDA (perfluorodecanoic acid), PFUnDA (perfluoroundecanoic acid) and PFDoDA (perfluorododecanoic acid)

Sample collection

Other than the two samples from Ireland and the single sample from the UK, all sample collections were arranged by staff from the respective national offices of Greenpeace. The two pooled samples from the Republic of Ireland were kindly provided by staff at the Marine Institute, Galway. The single pooled sample from the River Thames in the United Kingdom was kindly supplied by staff at the Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Burnham-on-Crouch.

In all cases, samples of freshly caught eels were provided by local anglers or retailers, taking all due care to verify the precise catch location. In order to avoid contamination or cross-contamination of the samples, eels were wrapped either individually or as a pooled sample in sheets of new, clean aluminium foil and placed inside transparent polyethylene bags. All samples were frozen as soon as possible after collection (and in all cases within 24 hours of the eels having been caught) and stored frozen and in the dark.

Samples were transported by courier to the Greenpeace Research Laboratories, University of Exeter (UK), in insulated boxes packed with artificial ice packs or dry ice. All samples were verified as being still frozen on arrival at our laboratory, from which they were dispatched (again by courier) to the CEFAS Burnham Laboratory for analysis.



Analytical methods

Sample preparation

Defrosted eels were measured, weighed and gutted and the intact livers were weighed and combined to obtain one composite sample per location. Where the eels were delivered gutted, a composite sample per location was prepared from fillets. Target analytes were extracted using an ion-pair extraction method (Hansen *et al.* 2001). No more than 5g of sample, to which an internal standard (perfluoro-n-[1,2-¹³C₂] decanoic acid) was added, were homogenised with 5 times its weight of water. 1g of the homogenate was thoroughly mixed with 1 ml of 0.5 M tetrabutylammonium hydrogen sulphate solution (adjusted to pH10) and 2 ml of 0.25 M sodium carbonate buffer. Target analytes were then extracted by adding 5 ml of methyl-*tert* butyl ether (MTBE) to the aqueous sample mixture and shaking for 20 min. The MTBE supernatant was then recovered and the extraction repeated a further two times. The MTBE fractions were all combined, concentrated to 1ml and dichloromethane (1ml) was added before proceeding to the extract cleanup.

Sample extracts were cleaned up by low pressure chromatography using silica. For this, 1.8 g of activated silica were mixed with dichloromethane and poured in a glass column. The sample extract was then added to the column and eluted using 15 ml of dichloromethane to remove fats, followed by 30 ml of acetone which contained all target analytes. The acetone fraction was then evaporated just to dryness and reconstituted in an aliquot of methanol for LC-MS analysis.

Sample analysis

Samples extracts were analysed by high performance liquid chromatography-electrospray-ion trap mass spectrometry (LC-ESI-ITMS) with perfluorinated sulphonates being detected in MS mode and perfluorinated acids in MS/MS mode. Analytes were separated on a FluoroSep-RP Octyl (150mmx3.2mm i.d., 5µm particle size) protected by a 10mm guard column of the same material using a 2mM aqueous ammonium acetate/methanol gradient over 40 minutes.

Quality assurance/quality control (QA/QC)

Quality control for analysis of PFOS, PFHxS, PFOA, PFDA and PFDoA in fish liver was provided by parallel analysis of a certified reference material (fish liver extract), confirming acceptable recoveries in all cases (114-140%). Analysis of PFOS in the muscle samples also yielded acceptable recoveries against a certified reference material (fish tissue, recover 117%). For all other analyte and tissue combinations, the assigned (or certified) value in the respective certified reference materials were below the limits of detection of the current methods, such that recovery efficiencies could not be determined.

Limits of quantification for the perfluoroalkyl sulphonates ranged from 16-21 ng/g wet weight, and for the majority of the perfluorocarboxylic acids, from 18-36 ng/g wet weight. An exception was the perfluorinated acid PFUnDA, for which substantially higher limits applied (83-162 ng/g wet weight).



TABLE 1: DETAILS OF SAMPLING DATES AND LOCATIONS FOR THE POOLED EEL TISSUE SAMPLES ANALYSED IN THE CURRENT STUDY

SAMPLE CODE	DATE OF COLLECTION	LOCATION
1. Belgium 1	01-03/08/05	Canal Charleroi-Bruxelles, near Arquennes (S of Brussels), Central Belgium
2. Czech 1	31/07/05	River Elbe, at Hrensko (N of Děčín, near border with Germany), N Czech Republic
3. Czech 2	03/08/05	River Otava, at junction of Otava and Vltava Rivers (S of Prague), Central Czech Republic
4. France 1	26/07/05	Etang de Thau, between cities of Meze and Sete (SW of Montpellier), S France
5. France 2 ⁴	30/07/06	Nantes, W France
6. Germany 1	27/07/05	River Elbe, near Hoopte (S of Hamburg), N Germany
7. Germany 2	01/08/05	River Main, near Bamberg (N of Nürnberg), S Germany
8. Germany 3	25-27/07/06	River Weser, at Nienburg (between Bremen and Hannover), N Germany
9. Germany 4	02/08/05	River Rhein, Riedstadt (near Darmstadt), S Germany
10. Ireland 1	11/08/05	Lake Furnace (partially tidal brackish lough), Newport (County Mayo), W Ireland
11. Ireland 2	11/08/05	Owengarve River, Glenthomas, near Newport (County Mayo), W Ireland
12. Italy 1	01/08/05	Tevere River, central Rome, Central Italy
13. Italy 2	03/08/05	Bracciano Lake (Anguillara), Central Italy
14. Netherlands 1	27/07/05	Haringsmakanaal, Leeuwarden, N Netherlands
15. Netherlands 2	19/07/05	Noordzee Kanaal, IJmuiden, W Netherlands
16. Netherlands 3	03/07/05	Hollandsdiep, Hoge Zwaluwe, S. Netherlands
17. Poland 1	26/07/05	Lake Druglin Duły, close to small village of Rołyńsk (Great Mazurian Lakes region), NE Poland
18. Spain 1	25/07/05	River Miño, at Forchadela Tomiño, near A Guarda (Pontevedra region), NW Spain
19. Spain 2 ⁵	21/07/06	River Ebro, 800 metres from the mouth of the Ebro River (SW of Tarragona), E Spain
20. UK 1	16/08/06	River Thames, off Canvey Island (E of London), SE England
21. Denmark 1	26/07/05	Køge Bay

4. was labeled France 3 on arrival - renumbered as France 2 as only two samples were provided

5. was labeled Spain 3 on arrival - renumbered as Spain 2 as only two samples were provided

locations of samples collected



1. Belgium 1
2. Czech 1
3. Czech 2
4. France 1
5. France 2⁴
6. Germany 1
7. Germany 2
8. Germany 3
9. Germany 4
10. Ireland 1
11. Ireland 2
12. Italy 1
13. Italy 2
14. Netherlands 1
15. Netherlands 2
16. Netherlands 3
17. Poland 1
18. Spain 1
19. Spain 2⁵
20. UK 1
21. Denmark 1

See table 1 on page 17 for details.

results and discussion



The main results of the analyses of both the liver and muscle samples are summarised in Table 2, with all values expressed as ng/g wet weight⁶ of liver or muscle tissue (parts per billion, or ppb).

The perfluorocarboxylic acids PFBA, PFHxA, PFUnDA and PFDoDA were not found in any of the samples at levels which could be quantified and so these compounds are not listed in Table 2.

TABLE 2: CONCENTRATIONS OF THE PERFLUOROALKYL SULPHONATES AND PERFLUOROCARBOXYLIC ACIDS DETECTED IN AT LEAST ONE OF THE POOLED EEL LIVER OR MUSCLE TISSUE SAMPLES ANALYSED. ALL OTHER PERFLUORINATED COMPOUNDS INCLUDED IN THE ANALYSES WERE EITHER BELOW LIMITS OF QUANTIFICATION FOR THE METHOD EMPLOYED (ND) OR NOT QUANTIFIABLE BECAUSE OF ANALYTICAL INTERFERENCES (N/A).

SAMPLE CODE	NUMBER IN POOLED SAMPLE	ng/g WET WEIGHT				
		PFOS	PFHxS	PFOA	PFDA	
LIVER SAMPLES						
Belgium 1	4	201	51	nd	nd	
Czech 1	2	126	21	nd	nd	
Czech 2	2	nd	583	nd	nd	
France 1	5	nd	219	23	nd	
France 2	5	76	50	nd	nd	
Germany 2	5	498	nd	nd	44	
Ireland 1	5	nd	147	nd	nd	
Ireland 2	5	nd	nd	nd	nd	
Italy 1	5	16	nd	nd	nd	
Italy 2	2	n/a	n/a	n/a	n/a	
Netherlands 1	2	98	nd	nd	34	
Netherlands 2	2	102	89	nd	nd	
Netherlands 3	2	290	110	nd	nd	
Poland 1	5	nd	307	nd	nd	
Spain 1	4	nd	nd	nd	nd	
Spain 2	5	36	412	nd	nd	
UK 1	5	248	83	nd	92	
MUSCLE SAMPLES						
Germany 1	5	18	35	nd	nd	
Germany 3	5	nd	nd	nd	nd	
Germany 4	5	nd	nd	nd	nd	
Denmark 1	5	nd	175	nd	nd	

6. ng/g wet weight - nanogrammes of PFC per gramme of whole fresh liver or muscle tissue



It should also be noted that, due to analytical interferences:

- * PFBS could not be quantified in any of the samples analysed, and
- * none of the target analytes could be quantified in sample Italy 2; hence the results are discussed hereinafter on the basis of 16 rather than 17 liver samples.

These are unfortunate limitations, but do not detract from the significance of the remaining data.

What is clear from Table 2 is that the two most commonly found and abundant perfluorinated compounds detectable in the eel liver samples were the perfluoroalkyl sulphonates PFOS and PFHxS (found in 10 and 11 of the 16 liver samples analysed respectively). In contrast, PFOA was recorded in only one of the 16 liver samples (France 2), and then only marginally above the limit of quantification. PFDA (perfluorodecanoic acid), a perfluorinated carboxylic acid of slightly longer chain length, was found in 3 samples (Netherlands 1, Germany 2 and the single sample from the UK) at levels between 34 and 92 ng/g wet weight.

For PFOS, concentrations ranged from <16 ng/g wet weight (in samples Czech 2, France 1, Ireland 1 and 2, Poland 1 and Spain 1) to a maximum of 498 ng/g wet weight in sample Germany 2 (mean 106 ng/g, median 56 ng/g, skewed downwards by the significant number of non-detects). After Germany 2, the next highest PFOS concentrations were found in liver samples Netherlands 3 (290 ng/g), UK (248 ng/g) and Belgium (201 ng/g). Relatively low PFOS levels were recorded in Italy 1 and Spain 2 (16 and 36 ng/g respectively).

PFHxS concentrations in the liver samples were unexpectedly of a similar order, ranging from <21 ng/g wet weight (in Germany 2, Ireland 2, Italy 1, Netherlands 1 and Spain 1) to a maximum of 583 ng/g wet weight, recorded for sample Czech 2 (mean 130 ng/g, median 67 ng/g). Relatively high concentrations of PFHxS were also recorded in sample Spain 2 (412 ng/g), followed by Poland 1 (307 ng/g) and France 1 (219 ng/g).

In terms of the muscle tissue samples, PFOS and PFHxS were found in only one of the three samples from Germany (sample Germany 1), and then at levels close to detection limits (18 and 35 ng/g wet weight respectively). While no PFOS residues could be detected in the single sample from Denmark, a surprisingly high concentration of PFHxS was recorded for this muscle sample (175 ng/g wet weight). None of the four muscle samples analysed revealed detectable levels of PFOA or PFDA.

Taken together, the concentrations of PFOS determined for the 16 eel livers in the current study are of a similar order to those previously reported for certain other freshwater fish species by other authors (see Table 3). Although the concentrations recorded here are substantially lower than in the most contaminated eels collected from the Ieperlee canal (at Boezinge, downstream from the industrial zone of Ypres) and the Blokkerdijk pond (downstream from Antwerp) in the Flanders region of Belgium by Hoff *et al.* (2005), they are nevertheless in a similar range to levels recorded in eels from the Zuun basin (Sint-Pieters-Leeuw, 11.2-162 ng/g wet weight), the Oude Maas pond (Dilsen-Stokkem) and the Watersportbaan basin (Ghent) (212-857 ng/g wet weight) by the same authors.

As noted above, the rather high levels of PFHxS recorded in several of the samples in the current study were unexpected, and do not appear to have parallels in data published for other species; in other studies, levels of PFOS are generally far higher than corresponding concentrations of other perfluoroalkyl sulphonates, including PFHxS. The reason for this unusual result is not known. While it cannot be ruled out that the apparent concentrations for this analyte have been elevated by the presence of another interfering, but so far unidentified, compound eluting from the analytical system at the same time as PFHxS, the probability of this is felt to be very low given the quality control measures applied.

If these results are confirmed, it could possibly reflect differences in the accumulation and metabolism of this compound in eels compared to other fish species. Alternatively it could be an indication that the distribution of different perfluoroalkyl sulphonate compounds in inland waterways around Europe is more complex than may initially be expected, though again the underlying reasons for this are not known. PFHxS is known to occur as a contaminant in some PFOS-based formulations (Taniyasu *et al.* 2003), but this does not seem a likely source in this case as concentrations would then be expected to be consistently rather lower than for PFOS itself. Although in the case of PFOS there appears to be some relationship between the concentrations recorded here and the proximity of the sampling locations to urban and/or industrial centres in the various countries, no such relationship is apparent for PFHxS. Indeed, concentrations of these two chemically-related compounds appear to vary independently in the eel liver samples collected. All that can be concluded at this stage is that these apparently anomalous results deserve further investigation and explanation.

Although no other comparable data are available for eels, it is worth noting that the complexity of the relationship between PFOS and PFHxS concentrations has been noted in liver and blood samples from other species. In the case of polar bears, for example, liver tissue collected from bears from seven different locations in the North American and European Arctic yielded mean concentration ratios of PFHxS to PFOS ranging from 0.022:1 (South Hudson Bay) to 2.28:1 (Svalbard) (Smithwick *et al.* 2005). Furthermore, while analysis of blood from human donors from Michigan (USA) and Poland showed correlation between concentrations of PFOS and PFHxS, no such correlation was apparent for equivalent samples from Kentucky (USA), Colombia or Japan (Kannan *et al.* 2004b), once again indicating that any relationships between the distribution of these two chemicals may be quite complex. Similarly, no correlation was apparent between these two chemicals in blood samples from a total of 83 human volunteers in Sweden; while PFHxS was generally less abundant than PFOS, in a small number of samples concentrations of the two chemicals fell in a similar range (Kärman *et al.* 2004).

The small numbers of liver samples in which perfluorocarboxylic acids could be detected in the current study probably reflects the higher detection limits achievable for these compounds relative to other studies, as levels reported for other fish species tend to fall in the low ng/g wet weight range (i.e. below limits for quantification in the current study). Nevertheless, it is interesting to note that PFDA was found slightly more frequently than the more commonly recognised contaminant PFOA, and at higher concentrations, perhaps a result of the higher propensity for bioaccumulation of the former, longer-chain compound.

It is unfortunate that liver tissue was not available for the remaining three samples from Germany and the single sample from Denmark. The relatively low concentrations of PFOS and PFHxS in muscle samples Germany 1, 3 and 4 may be expected from knowledge that these chemicals have a tendency to accumulate in the blood and liver in preference to other tissues (although the data of Sinclair *et al.* 2004 reproduced in Table 3 for accumulation of PFOS in fish suggest that this assumption may not always be valid). Against this background, the value of 175 ng/g wet weight recorded here for PFHxS in the single pooled muscle sample from Denmark seems remarkably high. However, given that results are currently available for only one pooled sample from this location, any inferences drawn on this basis would be purely speculative. Further sampling and analysis would be essential in order to discern PFHxS distribution patterns in more detail and to test the consistency of ratios between different tissue types from the same species.



TABLE 3: COMPARISON OF CONCENTRATIONS OF PERFLUORINATED CHEMICALS IN LIVER AND MUSCLE TISSUE OF EELS IN THE CURRENT STUDY WITH PREVIOUSLY PUBLISHED DATA FOR EELS AND OTHER FRESHWATER SPECIES FROM AROUND THE WORLD. [- INDICATES THAT THE ANALYTE WAS NOT INCLUDED IN THE STUDY.]

SPECIES	LOCATION	TISSUE	SAMPLE NUMBER	ng/g WET WEIGHT				SOURCE
				PFOS	PFHxS	PFOA	PFDA	
Eel	Locations in 11 European countries	liver muscle	16	<16-498	<21-583	<19-23	<18-92	This study
			4	<16-18	<21-175	<19	<18-<36	
Blue gill	Lake Biwa (Japan)	liver	2	254-310	<5.2	-	-	Taniyasu <i>et al.</i> 2003
Largemouth bass		liver	2	159-309	<5.8	-	-	
Chinook salmon	Michigan lakes (USA)	liver	6	32-173	-	-	-	Sinclair <i>et al.</i> 2004
		muscle	6	<7-189	-	-	-	
Lake whitefish		liver	5	33-81	-	-	-	
		muscle	5	97-168	-	-	-	
Brown trout		liver	10	<17-26	-	-	-	
		muscle	10	<7-46	-	-	-	
White sucker	Canadian arctic lakes	liver	3	6.5-8.6	-	<2	1.7-3.1	Martin <i>et al.</i> 2004
Brook trout		liver	2	29-50	-	<2	2.3-2.8	
Lake whitefish		liver	2	12	-	<2	1.2-1.8	
Eel	Ieperlee canal (Belgium)	liver	28 in total	250-9031	-	-	-	Hoff <i>et al.</i> 2005
	Blokkersdijk pond (Belgium)	liver		633-1822	-	-	-	
	Zuun Basin (Belgium)	liver		11.2-162	-	-	-	
	Oude Maas & Watersport-baan basin (Belgium)	liver		212-857	-	-	-	
Largemouth bass	New York State (USA)	liver	28	9-315	-	<1.5-6.7	-	Sinclair <i>et al.</i> 2006
Smallmouth bass		liver	38	10-120	-	<1.5-7.7	-	

For purposes of comparison, concentrations of PFOS in the 16 liver samples analysed are shown alongside those previously reported for PCBs (sum of ICES 7) and the brominated flame retardant HBCD (hexabromocyclododecane, sum of three isomers) for muscle samples from the same pooled specimen samples in Table 4. While there is some tentative indication, as noted above, that PFOS concentrations may be higher in eels collected close to urban or industrial centres in central European countries, no numerical correlation is observed between concentrations of the different contaminants reported in Table 4 across the data

set as a whole. Given the rather different source origins for PCBs and PFOS, the latter arising far more significantly from recent or ongoing uses in consumer products, as well as from industrial applications, this lack of apparent correlation is perhaps not surprising. What these data do suggest, however, is that levels of contamination by one chemical group in body tissues of eels cannot be reliably predicted on the basis of concentrations of others. In other words, different water bodies across Europe may be characterised by very different patterns of chemical contamination.

TABLE 4: COMPARISON OF PFOS CONCENTRATIONS IN POOLED EEL LIVER SAMPLES FROM THE CURRENT INVESTIGATION WITH CONCENTRATIONS OF PCBs (SUM OF ICES 7) AND HBCD (SUM OF THREE ISOMERS) IN POOLED MUSCLE TISSUE FROM THE SAME EEL SAMPLES AS DETERMINED IN OUR PREVIOUS STUDY (SANTILLO *ET AL.* 2005). [N/A – NOT QUANTIFIED BECAUSE OF ANALYTICAL INTERFERENCES. ND – BELOW LIMITS OF QUANTIFICATION FOR THE METHOD EMPLOYED.]

SAMPLE CODE	NUMBER IN POOLED SAMPLE	ng/g WET WEIGHT		
		Σ-HBCD	Σ-PCBs (ICES 7)	PFOS
Belgium 1	4	5	97	201
Czech 1	2	4	184	126
Czech 2	2	nd	66	nd
France 1	5	3	29	nd
France 2	5	2	5	76
Germany 2	5	15	566	498
Ireland 1	5	nd	4	nd
Ireland 2	5	3	5	nd
Italy 1	5	26	483	16
Italy 2	2	4	120	n/a
Netherlands 1	2	9	16	98
Netherlands 2	2	2	165	102
Netherlands 3	2	9	1512	290
Poland 1	5	1	2	nd
Spain 1	4	7	54	nd
Spain 2	5	4	123	36
UK 1	5	>50	136	248

conclusions - the results in context



The present study provides a first broad overview of the extent of contamination of eels with PFOS and other perfluorinated chemicals, extending existing knowledge to cover a wider geographical area. However, as stated at the outset, this rather limited study cannot be assumed to have provided anything other than a 'snapshot' of the presence and concentrations ranges of various perfluorinated chemicals in this key freshwater species from a number of locations across Europe. The concentrations reported here should not be assumed to be representative of the water bodies sampled as a whole, nor (more importantly) of the regions or even countries from which the samples were collected. In addition, the rather unusual results recorded here for PFHxS clearly warrant further investigation before they can be confirmed.

Nevertheless, the results reinforce the importance of biomonitoring for the presence of this novel group of persistent and bioaccumulative chemicals in sensitive aquatic species. It is only through such programmes that trends in contaminant levels and the significance of possible point and diffuse sources may be determined. More detailed sampling and analysis programmes than that possible within the remit of the current study would be required to determine representative concentration ranges for these contaminants in any one country.

Moreover, given that this study focused on the quantification of chemical contaminants only in tissue samples, it is not possible to deduce information concerning the health status of the eels sampled, nor therefore the possible links between chemical exposure and observed declines in eel populations in many parts of Europe. While their demise is undoubtedly the result of a complex interaction between many different factors, including major pressures from overfishing and habitat disruption, it is nevertheless possible that exposure to a range of chemical pollutants may also be an important contributory factor.

A role for perfluorinated chemicals in eel population decline?

For example, Corsi *et al.* (2005) reported that the presence of persistent organochlorine contaminants, including pesticides and PCBs, in eels sampled from the Orbetello lagoon in Tuscany (Italy) may, in concert with other types of man-made contaminants, be acting to reduce fitness and ultimate reproductive success in those populations. More recently still, it has been demonstrated by Palstra *et al.* (2006) that dioxin-like contaminants (including some PCBs) are capable of 'devastating effects' on the development and survival of eel embryos as a result of the high proportion of the contaminant-laden fat reserves being mobilised during spawning, potentially damaging the gonads of the adult fish and even the eggs themselves. Palstra *et al.* (2006) go on to note that the current gonadal levels of dioxin-like contaminants alone in European eels from many locations are capable of interfering with normal embryonic development, and suggest that such contaminants may have contributed directly to the observed decline in populations.

Is it possible that other groups of contaminants, including the perfluorinated compounds, may also have contributed to the decline or at least be acting to limit recovery? Mechanisms certainly exist by which this could be the case. For example, Hoff *et al.* (2005) describe a 'strongly significant' correlation between liver PFOS concentrations and blood serum concentrations of the enzyme ALT (alanine aminotransferase) in eels collected from various locations in Belgium, a relationship which has not been reported previously for other species, notably marine and estuarine species (Hoff *et al.* 2003). Elevated concentrations of ALT are a strong indication of liver stress and damage in fish, as well as in mammals. Furthermore, the liver concentrations of PFOS observed to correlate with elevated ALT in the Belgian eels were well below the no-effect levels previously reported for this effect in rats, for example.

While it cannot be confirmed that this correlation indicates a cause-effect relationship, nor ruled out that other chemical agents may have contributed to the liver stress in the eels, their results certainly raise direct cause for concern relating to the widespread presence of PFOS and other perfluorinated chemicals in eels from various locations across Europe. Indeed, the concentrations we recorded in the majority of the eel liver samples in our study fall within the range (albeit at the lower end) reported by Hoff *et al.* (2005) for the possible stimulation of serum ALT. Studies in other species have demonstrated that PFOS can accumulate in fish eggs to concentrations more than double those in maternal tissues (Sinclair *et al.* 2004), indicating a substantial propensity for intergenerational transfer of this contaminant and the potential therefore for PFOS to impact on the very earliest and most sensitive developmental life stages. No comparable information exists for eels, symptomatic of the more general paucity of data on the potential impacts of perfluorinated chemicals on development and reproduction in this species. Some basic information on the mechanisms of immune response in eels, whether to pathogens or chemical stresses, also remains unavailable (Nielsen & Esteve-Gassent 2006).

Passing the burden on - concerns for predators and consumers

Bearing in mind the high potential for PFOS and certain other perfluorinated chemicals to biomagnify through the food chain, accumulating to particularly high levels in top predators such as birds of prey, cetaceans, seals and polar bears, it is important to consider also the additional concerns relating to species feeding on the eels, including humans. In a study of the transfer of perfluorinated chemicals, including PFOS, between different trophic levels in the Great Lakes (USA), Kannan *et al.* (2005a) estimated that bald eagles are accumulating PFOS at concentrations between 5 and 10 times higher than those in one of their key prey species, chinook salmon (based on relative concentrations detected in liver). In turn, the biomagnification factor (BMF) between the salmon and its principal prey species, the round goby, was between 10 and 20. By analogy, it is reasonable to suggest that predators such as herons which feed on eels may also be accumulating burdens of PFOS in their livers and other body tissues in excess of those afflicting the eels themselves. The presence of PFOS and PFHxS in the eels is, therefore, an indicator of what could well be a much broader phenomenon of perfluorinated chemical contamination in Europe's aquatic environments.

The significance of seafood as a contributor of perfluorinated chemicals to the human diet has recently been noted both in the Baltic states (Falandysz *et al.* 2006) and in China (Gulkowska *et al.* 2006). However, the toxicological significance of such intake remains uncertain. In Germany, the Agency for Risk Assessment (BfR 2006) has suggested a tolerable daily intake for PFOS in humans of 0.1 ug/kg weight and have suggested, therefore, that for a 60 kg person eating 300g of fish per day, the fish consumed should contain no more than 20 ng PFOS /g wet weight of fish (or 0.02 mg/kg). This is close to the limit of detection of the analytical method employed in the current study. Nevertheless, one of the muscle samples from Germany (Germany 1) contained PFOS at a concentration of 18 ng/g, close to the proposed guidance level. Although concentrations in muscle may be expected to be somewhat below levels in liver, our study provides no means of direct comparison. Some other published work suggests that concentration ranges on a wet weight basis may actually be quite similar in the two tissue types in certain other freshwater fish species. In any case, considering the range of concentrations in liver tissue (<16 - 498 ng/g wet weight), it seems likely that at least some of the eels sampled in our current study could yield muscle (fillet or flesh) PFOS concentrations above the proposed German guidance level for regular fish eaters.

Dealing with the emerging legacy

Despite growing recognition of the widespread distribution of perfluorinated chemicals in wildlife and humans, and despite voluntary moves away from the large-scale manufacture and use of PFOS-based substances, legislative measures to control or prevent the use of such chemicals remain at a preliminary stage over much of the globe. The proposed amendment to the EU Marketing and Use Directive (EC 2005) will prohibit many former uses of PFOS and related chemicals, though principally only those uses which have in effect already been subject to voluntary phase out, as recognised explicitly in the European Commission's justification for the measure itself. At the same time, the proposal envisages permitting many of the ongoing uses of these chemicals, such as in the semiconductor and photographic industries, chromium-plating operations, fire-fighting foams and any number of unspecified 'controlled closed systems', to continue, at least for several years to come.



Furthermore, the manufacture and use of other perfluorinated chemicals, including the fluorotelomer alcohols which may be acting as a conveyor for long-range transport of perfluorocarboxylic acids like PFOA, PFDA and PFUnDA, will remain unaddressed. The manner and extent to which fluorotelomer compounds can still be used in consumer goods, for example, was recently demonstrated by a study of perfluorinated chemicals used as water-proofing agents in all-weather clothing on sale in the Nordic countries (SSNC 2006). While the four jackets analysed in that study yielded only traces of PFOS (<0.04-0.24 ug/m²), with PFOA present at somewhat higher levels (0.8-24.6 ug/m²), by far the majority of the overall perfluorinated chemical content of the fabric comprised the fluorotelomer alcohol 8:2 FTOH, a chemical which can act as a long-range source of perfluorinated carboxylic acids.

Initiatives such as the proposed restrictions on PFOS-related substances under the existing Marketing and Use Directive, and the far broader ongoing efforts to include PFOS among the list of POPs targeted for global phase-out under the 2001 Stockholm Convention (KemI 2005), are certainly a step forward. Nevertheless it has to be recognised that, to some extent at least, the damage has already been done. For years, if not decades, to come, we will be dealing with the legacy of the manufacture, use and release of yet another persistent organic pollutant whose intrinsic properties could well have predicted its widespread and uncontrollable passage through the environment and accumulation through the food chain. Once again it seems we have been rather slow to learn the lessons from past mistakes.

Stronger regulation within REACH?

Within Europe, at least, there is some greater promise for the future that persistent and bioaccumulative chemicals, among other 'substances of very high concern' will be subject to far stricter controls. The proposed REACH legislation (Registration, Evaluation, Authorisation of CHemicals) remains under development and subject to final discussion, amendment and agreement by both the European Parliament and the Council of Ministers in late 2006 or early 2007. Already there is a broad consensus among both bodies that very persistent, very bioaccumulative (vPvB) substances, in common with persistent, bioaccumulative and toxic (PBT) substances, should not be granted authorisation for continued use if effective and less hazardous alternatives are available (the substitution principle). However, substantial differences remain between the Parliament and Council approaches which, when combined with the overall weakening of data requirements for initial registration of substances since the initial proposal and the apparent failure of technical criteria to capture a necessarily wide array of PBT and vPvB substances in the first place, threaten at the very outset to undermine the effectiveness of REACH to protect the environment and human health from even the most hazardous substances in use.

It is far from certain, for example, that PFOS will be identified as a vPvB or PBT substance using the criteria established under REACH, despite the overwhelming scientific evidence for its persistence and propensity to bioaccumulate. In the case of the other perfluorinated chemicals marketed and used within Europe, their fate under REACH is yet more uncertain. It is likely that, for some, the very limited requirements under REACH for data which need to be submitted before a chemical can be registered and put on the market will inevitably mean that detailed consideration of possible fate and effects will remain practically impossible. Information on long-term, low dose effects, such as developmental and reproductive toxicity and disruption of hormonal communication, is likely to be particularly lacking.

Should sufficient data be available and lead to the conclusion, for example, that one or more perfluorinated chemicals are carcinogenic or toxic to reproduction, the outstanding differences between the Parliament and Council approaches will become all too apparent. Under the Parliament approach, this would be sufficient for the substance to be refused authorisation should suitable alternatives be available. According to the Council, it would be a signal to try to establish a 'safe level' of exposure, a threshold below which the risks of continued use and release would be deemed to be 'adequately controlled'. Likewise for any perfluorinated chemicals which may exhibit properties which, despite not meeting the strict criteria for PBT or vPvB, nevertheless give rise to an equivalent level of concern (as a result of evidence for extensive environmental contamination, for example). On the basis of existing criteria, it is conceivable that even a substance like PFOS might fall into this 'equivalent concern' bracket, and therefore also be subject to the possibility of authorisation under the 'adequate control' route envisaged by the Council. To date, 'adequate control' of risk is not clearly defined under REACH. What is clear, however, is that under such a mechanism, some scale of use and release of persistent perfluorinated chemicals may be allowed to continue well into the future.

The differences between the Parliament and Council approaches for substances of very high concern under REACH are explored in more detail elsewhere (Santillo & Johnston 2006) and remain to be resolved. In the mean time, the presence of PFOS and other perfluorinated chemicals in the body tissues of eels collected from many parts of Europe adds to the growing body of evidence for the environmental legacy that their ongoing manufacture and use is creating. Although substantial data gaps and uncertainties inevitably remain, it seems inconceivable that the risks presented by such chemicals, either directly or indirectly through partial degradation, could ever be considered to be 'adequately controlled' for as long as their release to our environment is allowed to continue.

Unless we are prepared to accept widespread, long-term and effectively irreversible contamination of our environment and our own body tissues with perfluorinated chemicals, it would appear that the only sustainable strategy will be to replace them progressively with less hazardous, preferably non-hazardous, alternatives wherever and whenever they are available, the so-called substitution principle. Of course, in implementing such an approach, it will be essential to ensure that one problematic chemical is not simply replaced with another, with an environmental fate perhaps even less well understood. In this context, it is of concern that the majority of products currently marketed as alternatives to existing PFCs also rely on perfluorinated chemistry (see, for example, Walters & Santillo 2006), a testament to the somewhat unique properties that this basic structure confers. Hence, as part of the substitution process, it will undoubtedly also be vital to consider the fundamental necessity for the diversity of perfluorinated chemicals and materials on which we have increasingly come to rely. In other words, are disposable grease-proof hamburger wrappers, microwavable popcorn bags and stain-resistant carpets truly essential to support our quality of life? Or could we live without them?



- Adinehzadeh M., Reo N.V., Jarnot B.M., Taylor C.A. and Mattie D.R. (1999). Dose-response hepatotoxicity of the peroxisome proliferator, perfluorodecanoic acid and the relationship to phospholipid metabolism in rats. *Toxicology* 134: 179-195.
- Alexander B.H., Olsen G.W., Burris J.M., Mandel J.H. and Mandel J.S. (2003). Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility. *Occup Environ Med* 60: 722-729.
- Allsopp M, Santillo D, Walters A & Johnston P (2005) Perfluorinated Chemicals: an emerging concern Technical Note: 04/2005 Available from: http://www.greenpeace.to/publications_pdf/perfluorinated_chemicals_2005.pdf
- Austin M.E., Kasturi B.S., Barber M., Kannan K., MohanKumar P.S. and MohanKumar S.M.J. (2003). Neuroendocrine effects of perfluorooctane sulphonate in rats. *Environmental Health Perspectives* 111 (12): 1485-1489
- Begley, T.H., White, K., Honigfort, P., Twaroski, M.L., Neches, R. & Walker, R.A. (2005) Perfluorochemicals: Potential sources of and migration from food packaging. *Food Additives & Contaminants* 22(10) 1023-1031
- Berger U., Järnberg U. and Kallenborn R. (2004). Perfluorinated Alkylated substances (PFAS) in the European Nordic environment. *Organohalogen Compounds* 66: 4046-4052.
- Berthiaume J and Wallace K.B. (2002). Perfluorooctanoate, perfluorooctanesulfonate, and N-ethyl perfluorooctanesulfonamido ethanol; peroxisome proliferation and mitochondrial biogenesis. *Toxicology Letters* 129: 23-32.
- BfR (2006) Hohe Gehalte an perfluorierten organischen Tensiden (PFT) in Fischen sind gesundheitlich nicht unbedenklich. Stellungnahme Nr. 035/2006 des BfR vom 27. Juli 2006, Bundesinstitut für Risikobewertung: 4 pp.
- Bossi, R., Riget, F.F., Dietz, R., Sonne, C., Fauser, P., Dam, M. & Vorkamp, K. (2005) Preliminary screening of perfluorooctane sulfonate (PFOS) and other fluorochemicals in fish, birds and marine mammals from Greenland and the Faroe Islands. *Environmental Pollution* 136: 323-329
- Boudreau T.M., Sibley P.K., Mabury S.A., Muir D.G.C. and Solomon K.R. (2003). Laboratory evaluation of the toxicity of perfluorooctane sulphonate (PFOS) on *Selenastrum capricornutum*, *Chlorella vulgaris*, *Lemna gibba*, *Daphnia magna*, and *Daphnia pulex*. *Archives of Environmental Contamination and Toxicology* 44: 307-313.
- Caliebe C., Gerwinski W., Hühnerfuss H. and Theobald N. (2004). Occurrence of perfluorinated organic acids in the water of the North Sea. *Organohalogen Compounds* 66: 4074-4078.
- Corsi, I., Mariottini, M., Badesso, A., Caruso, T., Borghesi, N., Bonacci, S., Iacocca, A. & Focardi, S. (2005) Contaminants and sub-lethal toxicological effects of persistent organic pollutants in the European eel (*Anguilla anguilla*) in the Orbetello lagoon (Tuscany, Italy). *Hydrobiologia* 550: 237-249
- DEFRA (2005) UK plan national action on perfluorooctane sulphonate (PFOS) and substances that degrade to it. DEFRA Press release October 2005 available from <http://www.defra.gov.uk/environment/chemicals/pdf/pfos-note0510.pdf>
- Dekker, W. (2003) Status of the European eel stock and fisheries. Eel Biology (Proceedings of International Symposium on Advances in Eel Biology, Tokyo (Japan), Sept 28-30 2001: 237-254
- Dinglasan-Panlilio M.J.A., Ye Y., Edwards E.A. and Mabury S.A. (2004). Fluorotelomer alcohol biodegradation yields poly- and perfluorinated acids. *Environmental Science and Technology* 38 (10): 2857-2864.
- Dinglasan-Panlilio MJ, Mabury SA (2005) Evidence of 8:2 FTOH production from the biodegradation of 8:2 telomer methacrylate under aerobic conditions. Poster ENV023 presented at Fluoros symposium 2005
- Dinglasan-Panlilio MJ, Mabury SA (2006) Significant residual fluorinated alcohols present in various fluorinated materials. *Environmental Science & Technology* 40(5): 1447-1453
- EC (2005) Proposal for a Directive of the European Parliament and of the Council relating to restrictions on the marketing and use of perfluorooctane sulfonates (amendment of Council Directive 76/769/EEC), COM(2005) 618 final, 5.12.2005
- Ellis D.A., Martin J.W. and Mabury S.A. (2003). Atmospheric lifetime of fluorotelomer alcohols. *Environmental Science and Technology* 37 (17): 3816-3820.
- Ellis D.A., Martin J.W., De Silva A.O., Mabury S.A., Hurley M.D., Sulbaek Andersen M.P. and Wallington T.J. (2004). Degradation of fluorotelomer alcohols: a likely atmospheric source of perfluorinated carboxylic acids. *Environmental Science and Technology* 38 (12): 3316-3321.
- ENDS, Environmental Data Services Ltd. (2004). Perfluorinated chemicals: jumping from the frying pan to fire? ENDS Report 354 p28-31
- Falandysz, J., Taniyasu, S., Gulkowska, A., Yamashita, N. & Schulte-Oehlmann, U. (2006) Is fish a major source of fluorinated surfactants and repellents in humans living on the Baltic coast? *Environmental Science & Technology* 40(3): 748-751
- Feunteun, E. (2002) Restoration and management of the European eel: an impossible bargain? *Ecological Engineering* 18: 575-591
- Giesy J.P. and Kannan K. (2002). Perfluorochemical surfactants in the environment. *Environmental Science and Technology* 36 (7): 147A-152A.
- Giesy J.P. and Kannan K. (2001). Global distribution of perfluorooctane sulphonate in wildlife. *Environmental Science and Technology* 35 (7): 1339-1342.
- Gilliland F.D. and Mandel J.S. (1993). Mortality among employees of a perfluorooctanoic acid production plant. *Journal of Occupational Medicine* 35 (9): 950-954.
- Greenpeace/WWF (2005) A present for life: hazardous chemicals in umbilical cord blood, September 2005: 59 pp. <http://www.greenpeace.org/toxics/bloodcordreport>
- Gulkowska, A., Jiang, Q.T., So, M.K., Taniyasu, S., Lam, P.K.S. & Yamashita, N. (2006) Persistent perfluorinated acids in seafood collected from two cities in China. *Environmental Science & Technology* 40(12): 3736-3741
- Hansen, J.C., Clemen, L.A., Ellefson, M.E. & Johnson, H.O. (2001) Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. *Environmental Science & Technology* 35(4): 766-770
- Hekster F.M., Laane R.W.P.M. and de Voogt P. (2003). Environmental and toxicity effects of perfluoroalkylated substances. *Reviews of Environmental Contamination and Toxicology* 179: 99-121.
- Hoff, P.Y., van de Vijver, K., van Dongen, W., Esmans, E.L., Blust, R. & de Coen, W.M. (2003) Perfluorooctane sulphonic acid in bib (*Trisopterus luscus*) and plaice (*Pleuronectes platessa*) from the Western Scheldt and the Belgian North Sea: Distribution and biochemical effects. *Environmental Toxicology & Chemistry* 22(3): 608-614

- Hoff, P.T., van Campenhout, K., van de Vijver, K., Covaci, A., Bervoets, L., Moens, L., Huyskens, G., Goemans, G., Belpaire, C., Blust, R. & de Coen, W. (2005) Perfluorooctane sulphonic acid and organohalogen pollutants in liver of three freshwater fish species in Flanders (Belgium): relationships with biochemical and organismal effects. *Environmental Pollution* 137: 324-333
- Holmström K.E., Järnberg U. and Bignert A. (2005). Temporal trends of PFOS and PFOA in guillemot eggs from the Baltic Sea, 1968-2003. *Environmental Science & Technology* 39 (1): 80-84.
- Houde, M., Martin, J.W., Letcher, R.J., Solomon, K.R. & Muir, D.C.G. (2006) Biological monitoring of polyfluoroalkyl substances: a review. *Environmental Science & Technology* 40(11): 3463-3473
- Hu W., Jones P.D., Upham B.L., Trosko J.E., Lau C. and Giesy J.P. (2002). Inhibition of gap junctional intercellular communication by perfluorinated compounds in rat liver and dolphin kidney cells *in vitro* and Sprague-Dawley rats *in vivo*. *Toxicological Sciences* 68 (2): 429-436.
- Hu W., Jones P.D., DeCoen W., King L., Fraker P., Newsted J. and Giesy J.P. (2003). Alterations in cell membrane properties caused by perfluorinated compounds. *Comparative Biochemistry and Physiology Part C* 135: 77-88.
- Jacobi, H., Leier, G. & Witte, I. (1996) Correlation of the lipophilicity of xenobiotics with their synergistic effects on DNA synthesis in human fibroblasts. *Chemosphere* 32(7): 1251-1259
- Kallenborn, R., Berger, U., Järnberg, U., Dam, M., Glesne, O., Hedlund, B., Hirvi, J.-P., Lundgren, A., Bügel Mogensen, B. & Sigurdsson, A.S. (2004) Perfluorinated alkylated substances (PFAS) in the Nordic environment, Sft: 107 pp. [http://www.sft.no/nyheter/dokumenter/pfas_nmr2004.pdf?search=%22Perfluorinated%20alkylated%20substances%20\(PFAS\)%20in%20the%20Nordic%20environment%22](http://www.sft.no/nyheter/dokumenter/pfas_nmr2004.pdf?search=%22Perfluorinated%20alkylated%20substances%20(PFAS)%20in%20the%20Nordic%20environment%22)
- Kannan K., Corsolini S., Falandysz J., Oehme G., Focardi S. and Giesy J.P. (2002a). Perfluorooctanesulfonate and related fluorinated hydrocarbons in marine mammals, fishes, and birds from coasts of the Baltic and the Mediterranean Seas. *Environmental Science and Technology* 36 (15): 3210-3216.
- Kannan K., Hansen K.J., Wade T.L. and Giesy J.P. (2002b). Perfluorooctane sulfonate in oysters, *Crassostrea virginica*, from the Gulf of Mexico and the Chesapeake Bay, USA. *Archives of Environmental Contamination and Toxicology* 42: 313-318.
- Kannan K., Choi J-W., Iseki N., Senthilkumar K., Kim D.H., Masunaga S. and Giesy J.P. (2002c). Concentrations of perfluorinated acids in livers of birds from Japan and Korea. *Chemosphere* 49: 225-231.
- Kannan K., Newsted J., Halbrook R.S. and Giesy J.P. (2002d). Perfluorooctanesulfonate and related fluorinated hydrocarbons in mink and river otters from the United States. *Environmental Science and Technology* 36 (12): 2566-2571.
- Kannan K., Corsolini S., Falandysz J., Fillman G., Kumar K.S., Loganathan B.G., Mohd M.A., Olivero J., Van Wouwe N., Yang J.H. and Aldous K.M. (2004). Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environmental Science and Technology* 38 (17): 4489-4495.
- Kannan, K., Tao, L., Sinclair, E., Pastva, S., Jude, D.J. & Geisy, J.P. (2005a) Perfluorinated compounds in aquatic organisms at various trophic levels in a Great Lakes food chain. *Arch. Environ. Contam. Toxicol.* 48: 559-566
- Kannan, K., Yun, S.H. & Evans, T.J. (2005b) Chlorinated, brominated and perfluorinated contaminants in livers of polar bears from Alaska. *Environmental Science & Technology* 39(23): 9057-9063
- Kärnman A., van Bavel B., Järnberg U., Hardell L. and Lindström G. (2004a). Levels of perfluoroalkylated compounds in whole blood from Sweden. *Organohalogen Compounds* 66: 4058-4062.
- Kärnman, A., van Bavel, B., Hardell, L. & Lindström, G. (2004b) Perfluoroalkylated compounds in whole blood and plasma from the Swedish population. Programområde Hälsorelaterad miljöövervakning Kontrakt nr 215 0213, HÄMI 040404, 215 0213, dnr 721-4007-02 Mm: 17 pp.
- Kawashima Y., Kobayashi H., Miura H. and Kozuka H. (1995). Characterization of hepatic responses of rat to administration of perfluorooctanoic and perfluorodecanoic acids at low levels. *Toxicology* 99: 169-178.
- Keller, J.M., Kannan, K., Taniyasu, S., Yamashita, N., Day, R.D., Arendt, M.D., Segars, A.L. & Kucklick, J.R. (2005) Perfluorinated compounds in the plasma of loggerhead and Kemp's ridley sea turtles from the southern coast of the United States. *Environmental Science & Technology* 39(23): 9101-9108
- KEMI (2005) Proposal for listing Perfluorooctane sulfonate (PFOS) in Annex A of the Stockholm Convention on Persistent Organic Pollutants, Prepared by the Swedish Chemicals Inspectorate (KemI), Sweden, June 2005: 4 pp. http://www.pops.int/documents/meetings/poprc/meeting_docs/en/POPRC1-INF9-b.pdf
- Key B.D., Howell R.D. and Criddle C.S. (1997). Fluorinated organics in the biosphere. *Environmental Science and Technology* 31 (9): 2445-2454.
- Kuklennyik Z., Reich J.A., Tully J.S., Needham L.L. and Calafat A.M. (2004). Automated solid phase extraction and measurement of perfluorinated organic acids and amides in human serum and milk. *Environmental Science and Technology* 38 (13): 3698-3704.
- Laffaille, P., Briand, C., Fatin, D., Lafage, D. & Lasne, E. (2005) Point sampling the abundance of European eel (*Anguilla anguilla*) in freshwater areas. *Archiv für Hydrobiologie* 162(1): 91-98
- MacDonald M.M., Warne A.L., Stock N.L., Mabury S.A., Solomon K.R. and Sibley P.K. (2004). Toxicity of perfluorooctane sulfonic acid and perfluorooctanoic acid to *Chironomus tentans*. *Environmental Toxicology and Chemistry* 23 (9): 2116-2123.
- Martin J.W., Mabury S.A., Solomon K.R. and Muir D.C.G. (2003a). Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout. *Environmental Toxicology and Chemistry* 22 (1): 196-204.
- Martin J.W., Mabury S.A., Solomon K.R. and Muir D.C.G. (2003b). Dietary accumulation of perfluorinated acids in juvenile rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry* 22 (1): 189-195.
- Martin J.W., Whittle D.M., Muir D.C.G. and Mabury S.A. (2004a). Perfluoroalkyl contaminants in a food web from Lake Ontario. *Environmental Science and Technology* 38 (20): 5379-5385.
- Martin J.W., Smithwick M.M., Braune B.M., Hoekstra P.F., Muir D.C.G. and Mabury S.A. (2004b). Identification of long-chain perfluorinated acids in biota from the Canadian Arctic. *Environmental Science and Technology* 38 (2): 373-380.
- Martin JW, Mabury SA and O'Brien PJ. (2005) Metabolic Products and Pathways of Fluorotelomer Alcohols in Isolated Rat Hepatocytes. *Chemico-Biological Inter.* In press.
- Masunaga S., Kannan K., Doi R., Nakanishi J. and Giesy J.P. (2002). Levels of perfluorooctane sulfonate (PFOS) and other related compounds in the blood of Japanese people. *Organohalogen Compounds* 59: 319-322



Nielsen, M.E. & Esteve-Gassent, M.D. (2006) The eel immune system: present knowledge and the need for research. *Journal of Fish Diseases* 29: 65-78

Oakes K.D., Sibley P.K., Solomon K.R., Mabury S.A., Van Der Kraak G.J. (2004). Impact of perfluorooctanoic acid on fathead minnow (*Pimephales promelas*) fatty acyl-CoA oxidase activity, circulating steroids, and reproduction in outdoor microcosms. *Environmental Toxicology and Chemistry* 23 (8): 1912-1919.

OECD (2002) Hazard Assessment of perfluorooctane sulfonate (PFOS) and its salts Organisation for Economic Co-operation and Development ENV/JM/RD(2002)17/FINAL , JT00135607

OECD (2004) Results of survey on production and use of PFOS, PFAS and PFOA, related substances and products/mixtures containing these substances. OECD Environment, Health and Safety Publications Series on Risk Management No. 19, JT00176885, Paris 2004: 59 pp. Available from: [http://www.oelis.oecd.org/olis/2005doc.nsf/LinkTo/env-jm-mono\(2005\)1](http://www.oelis.oecd.org/olis/2005doc.nsf/LinkTo/env-jm-mono(2005)1)

Olsen G.W., Church T.R., Miller J.P., Burris J.M., Hansen K.J., Lundberg J.K., Armitage J.B., Herron R.M., Medhdizadehkashi Z., Nobiletti J.B., O'Neill E.M., Mandel J.H. and Zobel L.R. (2003). Perfluorooctanesulfonate and other fluorochemicals in the serum of American red cross adult blood donors. *Environmental Health Perspectives* 111 (16): 1892-1901.

OSPAR (2006) Hazardous Substances Series: OSPAR Background Document on Perfluorooctane Sulphonate (PFOS), 2006 Update, publ. OSPAR Commission, ISBN 1-905859-03-1, Publication Number 269/2006: 46 pp.

Palstra, A.P., van Ginneken, V.J.T., Murk, A.J. & van den Thillart, G.E.E.J.M. (2006) Are dioxin-like contaminants responsible for the eel (*Anguilla anguilla*) drama? *Naturwissenschaften* 93: 145-148

Paustenbach DJ, Panko JM, Scott P, Unice K (2005) Retrospective modelling of potential residential exposure to perfluorooctanoic acid (PFOA) releases from a manufacturing facility. Poster EVN019 presented at Fluoros symposium 2005

Poulsen P.B. & Jensen, A.A. (2005) More environmentally friendly alternatives to PFOS-compounds and PFOA Danish Environmental Protection Agency Environmental Project No. 1013 Miljøprojekt

Robinet, T.T. & Feunteun, E.E. (2002) Sublethal effects of exposure to chemical compounds: A cause for decline in Atlantic eels? *Ecotoxicology* 11(4): 265-277

Santillo, D. & Johnston, P. (2006) Effect thresholds and "adequate control" of risks: the fatal flaws in the Council position on Authorisation within REACH. *Greenpeace Research Laboratories Technical Note 01/2006*, publ. Greenpeace International, April 2006: 20 pp. http://www.greenpeace.to/publications_pdf/effect-thresholds-and-adequat.pdf

Santillo, D., Johnston, P., Labunska, I. & Brigden, K. (2005) Widespread presence of brominated flame retardants and PCBs in eels (*Anguilla anguilla*) from rivers and lakes in 10 European countries. *Greenpeace Research Laboratories Technical Note 12/2005*, publ. Greenpeace International, October 2005: 56 pp. <http://www.greenpeace.org/raw/content/international/press/reports/pollutionPCBBFReels.pdf>

Sinclair E., Taniyasu S., Yamashita N. and Kannan K. (2004). Perfluorooctanoic acid and perfluorooctane sulfonate in Michigan and New York waters. *Organohalogen Compounds* 66: 4069-4073.

Sinclair, E., Mayack, D.T., Roblee, K., Yamashita, N. & Kannan, K. (2006) Occurrence of perfluoroalkyl surfactants in water, fish and birds from New York State. *Arch. Environ. Contam. Toxicol.* 50: 398-410

Skutlarek, D., Exner, M. & Färber, H. (2006) Perfluorierte Tenside (PFT) in der aquatischen Umwelt und im Trinkwasser. *Fortschritte in Umweltchemie und Ökotoxikologie (UWSF - Z Umweltchem)* 18(3): 151 - 154

Smithwick, M., Mabury, S.A., Solomon, K.R., Sonne, C., Martin, J.W., Born, E.W., Dietz, R., Derocher, A.E., Letcher, R.J., Evans, T.J., Gabrielsen, G.W., Nagy, J., Stirling, I., Taylor, M.K. & Muir, D.C.G. (2005) Circumpolar study of perfluoroalkyl contaminants in polar bears (*Ursus maritimus*). *Environmental Science & Technology* 39(15): 5517-5523

So M.K., Taniyasu S., Yamashita N., Giesy J.P., Zheng J., Fung Z., Im S.H. and Lam P.K.S. (2004). Perfluorinated compounds in coastal waters of Hong Kong, South China, and Korea. *Environmental Science and Technology* 38 (15): 4056-4063.

SSNC FoEN (2006) Fluorinated pollutants in all-weather clothing. Norwegian Society for the Conservation of Nature/Friends of the Earth Norway and Swedish Society for Nature Conservation, January 2005, ISBN 91 558 0721 6: 43 pp. <http://www.snf.se/pdf/rap-hmv-allvadersklader-eng.pdf>

Stock N.L., Lau F.K., Ellis D.A., Martin J.W., Muir D.C.G. and Mabury S.A. (2004). Polyfluorinated telomer alcohols and sulfonamides in the North American troposphere. *Environmental Science and Technology* 38 (4): 991-996.

Suchenwirth, R.H.R., Jüring, H., Huppmann, R. & Bücking, M. (2006) Perfluorierte Alkyl-Substanzen (PFAS) in der Muttermilch Ergebnisse und vorläufige Bewertungen einer Pilotstudie des Nds. Landesgesundheitsamtes, Niedersächsisches Landesgesundheitsamt, Umwelt und Gesundheit Report 3: 11 pp. http://cdl.niedersachsen.de/blob/images/C23217451_L20.pdf

Taniyasu S., Kannan K., Horii Y., Hanari N. and Yamashita N. (2003). A survey of perfluorooctane sulfonate and related perfluorinated organic compounds in water, fish, birds, and humans from Japan. *Environmental Science and Technology* 37 (12): 2634-2639.

Taniyasu S., Yamashita N., Kannan K., Horii Y., Sinclair E., Petrick G. and Gamoto T. (2004). Perfluorinated carboxylates and sulfonates in open ocean waters of the Pacific and Atlantic Oceans. *Organohalogen Compounds* 66: 4035-4039.

Tittlemier S., Ryan J.J. and Van Oostdam J. (2004). Presence of anionic perfluorinated organic compounds in serum collected from northern Canadian populations. *Organohalogen Compounds* 66: 4009-4014.

Tominaga N., Kohra S., Iguchi T. and Arizono K. (2004). Effects of perfluoro organic compound toxicity on nematode *Caenorhabditis elegans* fecundity. *Journal of Health Science* 50 (5): 545-550.

US EPA (2003) Perfluorooctanoic Acid (PFOA), Fluorinated Telomers; Request for Comment, Solicitation of Interested Parties for Enforceable Consent Agreement Development, and Notice of Public Meeting, Federal Register: April 16, 2003, Volume 68, Number 73, Notices: 18626-18633 <http://www.epa.gov/fedrgrstr/EPA-TOX/2003/April/Day-16/t9418.htm>

van de Vijver K.I., Hoff P.T., Das K., Van Dongen W., Esmans E.L., Jauniaux T., Bouqueneau J.M., Blust R. and De Coen W.M. (2003). Perfluorinated chemicals infiltrate ocean waters: link between exposure levels and stable isotope ratios in marine mammals. *Environmental Science and Technology* 37 (24): 5545-5550.

van de Vijver K.I., Hoff P.T., Das K., Van Dongen W., Esmans E.L., Siebert U., Bouqueneau J.M., Blust R. and De Coen W.M. (2004). Baseline study of perfluorochemicals in harbour porpoises (*Phocoena phocoena*) from Northern Europe. *Marine Pollution Bulletin* 48: 986-1008.

van Ginneken, J.T. & Maes, G.E. (2005) The European eel (*Anguilla anguilla*, Lineus), its lifecycle, evolution and reproduction: a literature review. *Rev. Fish Biol. Fisheries* 15: 367-398

Versonnen, B.J., Goemans, G., Belpaire, C. & Janssen, C.R. (2004) Vitellogenin content in European eel (*Anguilla anguilla*) in Flanders, Belgium. *Environmental Pollution* 128(3): 363-371

Walters, A. & Santillo, D. (2006) Uses of perfluorinated substances. Greenpeace Research Laboratories Technical Note 06/2006: 18 pp. http://www.greenpeace.to/publications_pdf/uses-of-perfluorinated-chemicals.pdf

Washburn, S.T., Bingman, T.S., Braithwaite, S.K., Buck, R.C., Buxton, L.W., Clewell, H.J., Haroun, L.A., Kester, J.E., Rickard, R.W. & Shipp, A.M. (2005) Exposure assessment and risk characterization for perfluorooctanoate in selected consumer articles. *Environmental Science and Technology* 39:3904-3910

Wirth, T. & Bernatchez, L. (2003) Decline of North Atlantic eels: a fatal synergy? *Proceedings of the Royal Society of London Series B - Biological Sciences* 270(1516): 681-688

Yamashita, N., Kannan, K., Taniyasu, S., Horii, Y., Petrick, G. & Gamo, T. (2005) A global survey of perfluorinated acids in oceans. *Marine Pollution Bulletin* 51(8-12): 658-668

Yang Q., Abedi-Valugerdi M., Xie Y., Zhao X-Y., Möller G., Nelson B.D. and DePierre J.W. (2002). Potent suppression of the adaptive immune response in mice upon dietary exposure to the potent peroxisome proliferator perfluorooctanoic acid. *International Immunopharmacology* 2: 389-397.

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