Aquatic Toxicology of Perfluorinated Chemicals

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1 Introduction

Perfluorinated compounds (PFCs) are fluorinated at all of the valence electrons of the carbon atoms in organic molecules, or at least a portion of the molecule is perfluorinated (Fig. 1). All PFCs are synthetic and many have

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been used in commercially available products or released as byproducts. A partial list of the compounds that are known to have been manufactured and/or released into the environment is given in Table 1. These compounds vary in structure, and thus exhibit different environmental fates and toxicities. Unfortunately, there is presently little information on the chemical–physical properties of most PFCs, and even less toxicity information is available for these compounds. There is some information available on the mechanisms of toxic action and acute and chronic toxicity for a few compounds. Most such information is for the two primary PFCs that have been found as residues in the environment: perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA).
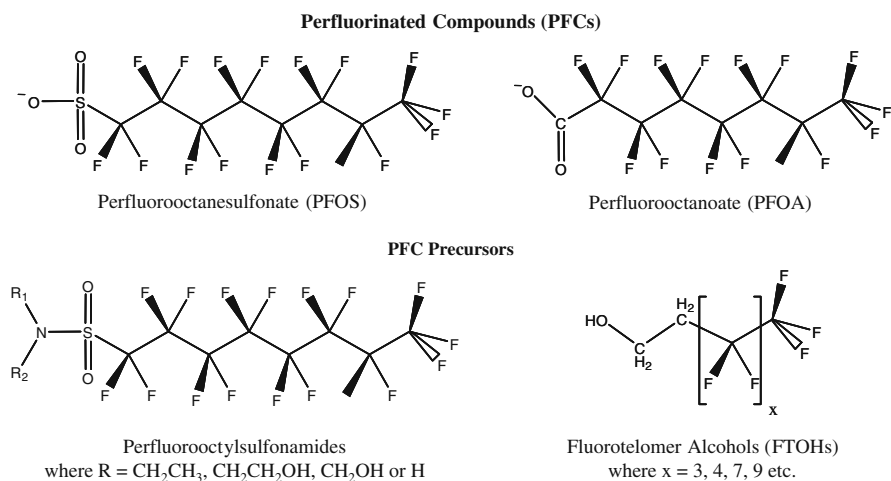
Perfluorinated Compounds (PFCs)

Perfluorooctanesulfonate (PFOS)   Perfluorooctanoate (PFOA)

PFC Precursors

Perfluorooctylsulfonamides
where R = CH₂CH₃, CH₂CH₂OH, CH₂OH or H

Fluorotelomer Alcohols (FTOHs)
where x = 3, 4, 7, 9 etc.

Fig. 1 Structure of perfluorinated compounds (PFCs) and some of their precursors

Among the more prominent PFCs that have been used in the production of commercial or industrial products, and released into the environment, are the perfluorinated fatty acids (PFFAs). The PFFAs are synthetic, fully fluorinated, fatty acid analogues that are characterized by a perfluoro-alkyl chain and a terminal sulfonate or carboxylate group. The high-energy carbon–fluorine (C–F) bond renders these compounds resistant to hydrolysis, photolysis, microbial degradation, and metabolism by animals, which makes them environmentally persistent (Giesy and Kannan 2002). PFCs have been manufactured for over 50 yr and have been used in materials such as wetting agents, lubricants, corrosion inhibitors, stain-resistant treatments for leather, paper and clothing, and in foam fire extinguishers (Sohlenius et al. 1994; Giesy and Kannan 2002). The global environmental distribution, bioaccumulation, and biomagnification

Table 1 Perfluorinated compounds (PFCs) and their precursor molecules

Compound (synonyms)	CAS number	Molecular structure	Molecular wt
PFCs			
Perfluorobutanesulfonate (C4, PFBS)	29420-49-3	$C_4F_9SO_3^-$	299
Perfluorohexanesulfonate (C6, PFHxS)	432-50-7	$C_6F_{13}SO_3^-$	399
Perfluorooctanesulfonate (C8, PFOS)	2795-39-3	$C_8F_{17}SO_3^-$	499
Perfluorooctanesulfonic acid	1763-23-1	$C_8F_{17}SO_3H$	500
Tridecafluoroheptanoate (C7, PFHpA)	–	$C_6F_{13}COO^-$	363
Perfluoroheptanoic acid	375-85-9	$C_6F_{13}COOH$	364
Perfluorooctanoate (C8, PFOA)	–	$C_7F_{15}COO^-$	413
Perfluorooctanoic acid	335-67-1	$C_7F_{15}COOH$	414
Heptadecafluoronoate (C9, PFNA)	–	$C_8F_{17}COO^-$	463
Perfluorononanoic acid	375-95-1	$C_8F_{17}COOH$	464
Nonadecafluorodecanoate (C10, PFDA)	–	$C_9F_{19}COO^-$	513
Perfluorodecanoic acid	335-76-2	$C_9F_{19}COOH$	514
Perfluoroundecanoate (C11, PFUnA)	–	$C_{10}F_{21}COO^-$	563
Perfluoroundecanoic acid	2058-94-8	$C_{10}F_{21}COOH$	564
Perfluorododecanoate (C12, PFDoA)	–	$C_{11}F_{23}COO^-$	613
Perfluorododecanoic acid	307-55-1	$C_{11}F_{23}COOH$	614
Perfluorotridecanoate (C13, PFTrA)	–	$C_{12}F_{25}COO^-$	663
Perfluorotetradecanoate (C14, PFTA)	–	$C_{13}F_{27}COO^-$	713
Perfluorotetradecanoic acid	376-06-7	$C_{13}F_{27}COOH$	714
Perfluoropentadecanoate (C15, PFPA)	–	$C_{14}F_{25}COO^-$	763
PFC precursors			
Perfluorooctane sulfonamide (PFOSA)	754-91-6	$C_8F_{17}SO_2NH_2$	499
n-Methyl perfluorooctane sulfonamidoethanol (n-MeFOSE)	24448-09-7	$C_8F_{17}SO_2N(CH_3)$ C_2H_4OH	557
n-Ethyl perfluorooctane sulfonamidoethanol (n-EtFOSE)	1691-99-2	$C_8F_{17}SO_2N(C_2H_5)$ C_2H_4OH	571
n-Ethyl perfluorooctane sulfonamidoacetic acid (PFOSAA)	2991-51-7	$C_8F_{17}SO_2N(C_2H_5)$ CH_2CO_2H	585
n-Ethyl perfluorooctane sulfonamide (n-EtFOSA)	4151-50-2	$C_8F_{17}SO_2NH(C_2H_5)$	528

Table 1 (continued)

Compound (synonyms)	CAS number	Molecular structure	Molecular wt
Perfluorooctane sulfonylfluoride (POSF)	307-35-7	$C_8F_{17}SO_2F$	502
6:2 Fluorotelomer alcohol (6:2 FTOH)	647-42-7	$CF_3(CF_2)_5C_2H_4OH$	364
8:2 Fluorotelomer alcohol (8:2 FTOH)	678-39-7	$CF_3(CF_2)_7C_2H_4OH$	464
10:2 Fluorotelomer alcohol (10:2 FTOH)	865-86-1	$CF_3(CF_2)_9C_2H_4OH$	564

of several perfluoro-compounds have recently been studied (Giesy and Kannan 2001). PFOS is the most commonly found perfluorinated compound in the tissues of wildlife.

Since PFFAs are chemically stabilized by strong covalent C–F bonds, they were historically considered to be metabolically inert and non-toxic (Sargent and Seffl 1970). Accumulating evidence has demonstrated that PFFAs are actually biologically active and can cause peroxisomal proliferation, increased activity of lipid and xenobiotic metabolizing enzymes, and alterations in other important biochemical processes in exposed organisms (Obourn et al. 1997; Sohlenius et al. 1994). In wildlife, the most widely distributed PFFA, PFOS, accumulates primarily in the blood and in liver tissue (Giesy and Kannan 2001). Therefore, the major target organ for PFFAs is presumed to be the liver. However, this does not exclude other possible target organs such as the pancreas, testis, and kidney (Olson and Anderson 1983). Until recently, most toxicological studies have been conducted on PFOA and perfluorodecanoic acid (PFDA), rather than on the more environmentally prevalent PFOS. However, PFOS appears to be the ultimate degradation product of several commercially used perfluorinated compounds, and the concentrations of PFOS found in wildlife are greater than those of other PFCs (Giesy and Kannan 2002; Kannan et al. 2001a,b).

A large body of ecotoxicological information, generated over a period of more than 20 yr, exists for various salts of PFOS. However, until recently, definitive information was not available on chemical purity, and validated analytical methodology did not exist to measure exposure concentrations in many of the early studies. Therefore, data generated prior to 1998 were less reliable as to the nature of substance(s) tested, and exposure concentrations were not measured as part of these studies. Because it is the most prominent of all the PFOS salts produced, the potassium salt of PFOS was chosen for many of the laboratory studies that have been cited in this chapter. The commercially prepared potassium product was available as a full-strength salt. For example, in 1997, PFOS-K^+ accounted for >45% of all PFOS salts produced

(US EPA 2001). The primary ecotoxicological data used in this chapter are based in a series of studies utilizing a well-characterized sample of PFOS potassium salt. Although the lithium, ammonium, diethanolamine, and dide-cyldimethylammonium salts have been tested, many of the studies on these salts utilized mixtures containing only 25–35% active ingredients. The majority of these studies were conducted in accordance with US EPA and/or OECD Good Laboratory Practices. Older studies have also been included where more recently generated data were not available for various species. In addition, in this assessment we also examine recent studies published in the open literature that pertain to ecological presence and biochemical modes of action of PFFAs.

2 Environmental Fate

2.1 Physical/Chemical Properties

PFOS is moderately water soluble, non-volatile, and thermally stable. The potassium salt of PFOS has a reported mean solubility of 680 mg/L in pure water. However, PFOS is a strong acid, and in water at a neutral pH it will completely dissociate into ionic forms. Thus, the PFOS anion can form strong ion pairs with many cations, which results in salting out in natural waters that contain relatively great amounts of dissolved solids (Table 2). For example, as the salt content increases, the solubility of PFOS decreases such that PFOS solubility in salt water is approximately 12.4 mg PFOS/L. PFOS has a reported mean solubility of 56 mg PFOS/L in pure octanol. However, due to the surface-active properties of PFOS, when it is added to octanol and water in a standard test system to measure K_{ow}, it forms three layers. Thus, an octanol/water partition coefficient has not been directly measured for PFOS, but has been estimated from its water and octanol solubilities. Other physio-chemical properties for this molecule such as bioconcentration factor and soil adsorption coefficient cannot be estimated with conventional quantitative structure activity relationship (QSAR) models. The use of K_{ow} is not appropriate to predict these other properties because PFOS does not partition into lipids, but instead binds to certain proteins in animals (Jones et al. 2003). As a result, use of either water solubility or predicted K_{ow} values may underestimate the accumulation of PFOS into organisms and other environmental media. PFOS is not expected to volatilize, based on its vapor pressure and predicted Henry's Law Constant. OECD (2002) classified PFOS as a type 2, non-volatile chemical that has a very low or possibly negligible volatility. Available physical/chemical properties for the potassium salt of PFOS are presented in Table 2.

Table 2 Physical/chemical properties of the potassium salt of perfluorooctanesulfonate (PFOS)

Parameter	Value	Reference
Melting point	$\geq 400°C$	Jacobs and Nixon (1999)
Boiling point	Not calculable	OECD (2002)
Specific gravity[a]	~ 0.6 (7–8)	OECD (2002)
Vapor pressure	3.31×10^{-4} Pa @ 20°C	Van Hoven et al. (1999)
Water solubility		
Pure water	680 mg/L	Ellefson (2001c)
Fresh water	370 mg/L	OECD (2002)
Sea water	12.4 mg/L	Ellefson (2001a)
Octanol solubility	56 mg/L	Ellefson (2001b)
Log K_{ow}[b]	–1.08	OECD (2002)
Henry's law constant[c]	4.34×10^{-7}	OECD (2002)

[a] pH values in parentheses
[b] Log K_{ow} calculated from PFOS solubility in water and *n*-octanol
[c] Henry's law constant calculated at 20°C using solubility in pure water

2.2 Photolysis

No experimental evidence of direct or indirect photolysis of PFOS is yet available (Hatfield 2001a). The indirect photolytic half-life for PFOS, using an iron oxide photo-initiator matrix model, was estimated to be ≥ 3.7 yr at 25°C. This model was chosen because it minimized the experimental error in this matrix. This half-life is based on the analytical method of detection.

2.3 Hydrolysis

Under experimental conditions (50°C and pH conditions of 1.5, 5, 7, 9, or 11) no hydrolytic loss of PFOS was observed in a 49-d study (Hatfield 2001b). Based on mean values and precision measures, the hydrolytic half-life of PFOS was estimated to be ≥ 41 yr at 25°C. However, it is important to note that this estimate was influenced by the analytical limit of quantification, and no loss of PFOS was detected in the study.

2.4 Biodegradation

Biodegradation studies in which PFOS was monitored analytically for loss of parent compound have been conducted using a variety of microbial sources and exposure regimes (Lange 2001; Gledhill and Markley 2000a,b,c). In one study with activated sludge, no loss or biotransformation of PFOS was observed over a 20-wk period under aerobic conditions, nor were there any losses observed in

a study conducted for 56 d with activated sludge under anaerobic conditions. The findings from these studies are supported by the results from a MITI-I test (Kurume Laboratory 2002) that showed no biodegradation of PFOS after 28 d, as measured by net oxygen demand, loss of total organic carbon, or loss of parent material. In addition, no losses of PFOS were observed in a biodegradation study conducted with soil under aerobic conditions. Therefore, to date, no laboratory data exist that demonstrates that PFOS undergoes significant biodegradation under environmental conditions.

2.5 Thermal Stability

Several studies suggest that PFOS would have relatively low thermal stability. This conclusion is based on the fact that the carbon–sulfur (C—S) bond energy is much weaker than the carbon–carbon (C—C), or the carbon–fluorine (C—F) bond energies, and as a result, would more easily break under incineration conditions (Dixon 2001). This conclusion is supported by a study by Yamada and Taylor (2003) indicating that PFOS should be nearly completely destroyed when incinerated.

2.6 Adsorption/Desorption

PFOS appears to adsorb strongly to soil, sediment, and sludge (Table 3) with an average distribution coefficient (K_d) greater than 1 ml/g, and an organic carbon normalized adsorption coefficient (K_{oc}) greater than 10,000 ml/g (Ellefson 2001d). Based on these values, PFOS would not be classified as qualitatively mobile, as defined by OECD guidelines. Once adsorbed to these matrices, PFOS does not readily desorb, even when extracted with an organic solvent. The average desorption coefficient (K_{des}) for soils was determined to be less than 0.001 L/g. In these matrices, adsorption and desorption equilibria were achieved in less than 24 hr; moreover, in more than 50% of cases, equilibria

Table 3 Adsorption and desorption of PFOS to sediments and soils[a]

Soil type	Adsorption kinetics			Desorption kinetics	
	K_d (L/g)	K_{oc} (L/g)	K_{adsF}[b]	K_{des} (L/g)	K_{desF}[b]
Clay	0.0183	70.4	0.0560	0.000471	0.222
Clay loam	0.00972	37.4	0.0421	0.0000158	0.082
Sandy loam	0.0353	126	0.0919	0.0000349	0.104
River sediment	0.00742	57.1	0.0094	0.0000100	0.039
Domestic sludge	<0.120	NC[c]	0.0568	<0.000237	29.5

[a]Values of K_d, K_{oc}, and K_{des} are averaged values
[b]Freundlich coefficient
[c]NC = not calculable

were achieved after approximately 1 min of contact with the test adsorbents. As a result, PFOS exhibited little mobility in all matrices tested and would not be expected to migrate any significant distance. The shape of the adsorption isotherm (H-type) indicates a very strong chemical/adsorption interaction. Since PFOS is a strong acid, it probably forms strong bonds in soils, sediments, and sludge via a chemisorption mechanism.

2.7 Bioconcentration

The potential of PFOS to bioaccumulate and bioconcentrate into fish and the relative importance of dietary and waterborne sources of PFOS to fish accumulation have been evaluated. In a bioaccumulation study with juvenile rainbow trout (*Oncorhynchus mykiss*), fish were exposed to 0.54 μg PFOS/g in the diet for 34 d, followed by a 41 d depuration phase (Martin et al. 2003a). PFOS was accumulated in and depurated from the liver and carcass in a time-dependent manner. The predicted time to reach 90% steady state would be 43 d, which was approximately the same as the exposure duration in the study. The liver and carcass depuration rate constants were 0.035 and 0.054/d, representing depuration half-lives of 20 and 13 d, respectively. The assimilation efficiency was $120 \pm 7.9\%$, which indicates efficient absorption of PFOS from ingested food. This assimilation efficiency is greater than that observed with chlorinated contaminants such as polychlorinated biphenyls (PCBs), where efficiencies in trout can range from 20 to 60% (Fisk et al. 1998). In addition, this assimilation efficiency of PFOS is indicative of enterohepatic recirculation, which could affect the disposition of PFOS in fish. Evidence of enterohepatic recirculation in rats has been demonstrated to affect the rate of elimination (Johnson et al. 1984). As a result, this process may also be an important mechanism that helps to maintain PFOS concentrations in fish beyond what is predicted from K_{ow} or water solubility values. The bioaccumulation factor (BAF) for PFOS was 0.32 ± 0.05, which indicates that dietary exposure did not result in biomagnification in trout. This small BAF probably resulted from several factors, including a relatively low experimental feeding rate ($F = 1.5\%$ body wt) coupled with a relatively rapid rate of depuration. Taken together, these data show that under these experimental conditions, the diet would not be a major route of PFOS exposure for fish.

Studies conducted with other fish species have shown that PFOS will bioconcentrate in tissues from waterborne exposures (Table 4). Bluegill exposed to 0.086 or 0.87 mg PFOS/L in a flow-through system accumulated PFOS into edible and non-edible (fins, head, and viscera) tissues in a time-dependent manner (Drottar et al. 2001). In this bluegill study, fish were exposed to 0.086 mg PFOS/L for 62 d, but were only exposed to 0.87 mg PFOS/L for 35 d, because of excessive mortality. At the end of the exposure phase of both treatments, PFOS tissue concentrations appeared to still be increasing. As a

Table 4 Kinetic parameters and bioconcentration factors (BCF) of PFOS in fish

Species	Tissue	Apparent BCF[a]	K_u (L/kg × d)	K_d (L/d)	BCF_K[b] (L/kg)	Half-life (d)
Bluegill	Edible	484	8.9	0.0047	1,866	146
	Unedible	1,124	22	0.0052	4,312	133
	Whole	856	16	0.0045	3,614	152
Rainbow trout	Carcass	–	53	0.048	1,100	15
	Blood	–	240	0.057	4,300	12
	Liver	–	260	0.050	5,400	14

[a]Apparent BCF was calculated as the concentration in fish at the end of the exposure phase divided by the average water concentration
[b]BCF_K was estimated as K_u/K_d

result, kinetic analyses of the data were conducted to calculate the kinetic bioconcentration factor (BCF_K) from estimated uptake and depuration rate constants. Fish exposed to 0.87 mg PFOS/L were not used to estimate these parameters. The BCF_K values for edible, inedible, and whole fish tissues were calculated to be 1,866, 4,312, and 3,614, respectively. During the elimination phase of the study, PFOS depurated slowly and the time to reach 50% clearance for edible, non-edible, and whole fish tissues were 146, 133 and, 152 d, respectively.

Tissue distribution and accumulation kinetics were determined in rainbow trout exposed to 0.35 µg PFOS/L (Martin et al. 2003b). The magnitude of PFOS concentrations in tissues were in the order of blood > kidney > liver> gall bladder. The least concentrations of PFOS were observed in the gonads, followed by adipose, and then muscle tissue (Table 4). In blood, approximately 94–99% of the PFOS was associated with plasma, and only a minor amount was associated with the cellular fraction. PFOS also accumulated in the gills, indicating their importance in the uptake and depuration in trout. In general, the depuration rate constants determined for carcass, blood, and liver showed that PFOS was more rapidly depurated than are some organochlorine contaminants (PCBs and toxaphene) but the rate is slower than that observed for other surfactants (Fisk et al. 1998; Tolls and Sijm 1995). When compared to other surfactants, the uptake rate constants were greater than expected and were directly related to greater tissue concentrations (Tolls et al. 1997). BCF_K were 1,100, 4,300, and 5,400 for carcass, blood, and liver, respectively. As was observed for bluegill, steady state PFOS concentrations in tissues were not achieved at the end of the exposure period. The 12-d accumulation ratios (BCF divided by tissue concentration at the end of the exposure period) for carcass, blood, and liver were greater than 600 indicating that the tissue concentrations were far from steady state. However, values of the BCF_K, calculated for rainbow trout, were well within the range of values observed for other species such as bluegill and carp.

In a flow-through bioconcentration study conducted with carp (*Cyprinus carpio*), fish were exposed to 2 or 20 μg PFOS/L, and water and fish tissue samples were collected throughout testing (Kurume laboratory 2001). Upon sampling, fish were separated into parts that included integument (skin except head, scales, fins, alimentary canal, or gills), head, viscera (internal organs except for alimentary canal and liver), liver, and carcass, and then analyzed for concentrations of PFOS. Kinetic analysis was not conducted because the study was not designed to examine uptake from water; rather, BCFs were calculated in all fish tissues at steady state. Steady state was assumed when three or more consecutive sets of tissue PFOS concentrations were not statistically different. In fish exposed for 58 d, the BCFs in carp from the 2 μg PFOS/L treatment ranged from 200 to 1,500. In fish from the 20 μg PFOS/L exposure, BCFs ranged from 210 to 850. PFOS depurated slowly and the time to reach 50% clearance for fish in the 20 μg PFOS/L treatment was 49 d, whereas 152 d was required for fish in the 2 μg PFOS/L treatment to reach 50% clearance.

To date, laboratory studies have demonstrated that PFOS accumulates into fish in a time- and concentration-dependent manner. In addition, these studies suggest that the primary route of accumulation of PFOS into fish is from exposure to aqueous PFOS. Dietary sources of PFOS are secondary and may not significantly enhance the overall accumulation of PFOS by fish. However, what actually happens under natural environmental conditions is yet to be tested. The reason for this is that discrepancies exist between accumulation factors as measured in the laboratory and those estimated in field studies. For example, bioaccumulation factors calculated from liver and surface water PFOS concentrations ranged from 6,300 to 125,000 in the common shiner (*Notropis cornutus*) collected in a Canadian creek (Moody et al. 2001). In contrast, the bioconcentration factor for rainbow trout, based on liver concentrations was 5,400, approximately 23-fold less than the maximal value derived in shiners (Martin et al. 2003b). The discrepancy between laboratory and field accumulation values has also been observed for fish collected from Tokyo Bay, Japan (Taniyasu et al. 2003). In that study, PFOS concentrations in fish livers were similar to those observed in the Great Lakes region of the United States and resulted in bioaccumulation factors that ranged from approximately 1,260 to 19,950. Again, the estimated BAFs were greater than those measured in laboratory studies. In a field study conducted in a reservoir in the Tennessee River, near Decatur Alabama, fish and surface water samples were collected and analyzed for PFOS. Bioconcentration factors from surface water PFOS concentrations and whole body PFOS concentrations in catfish and largemouth bass ranged from 830 to 26,000 (Giesy and Newsted 2001). Although BCF values determined in the laboratory are within the lower range of these values, they are also approximately four-fold less than the greater values estimated with fish from this location. The determination of BCF values from field exposures is complicated by the fact that less polar, PFOS-containing compounds could have been accumulated and then degraded to PFOS. Thus, while there is good agreement between the results of laboratory studies, BCFs and BAFs estimated

from field data vary greatly, and in many cases exceed values calculated from studies conducted under laboratory conditions. Factors contributing to variation in values of BAF and BCF developed from field observations may include interspecies and sex-dependent variation in accumulation. In addition, dietary sources of PFOS may be more important in the accumulation of PFOS by fish over their life cycle than would be expected based on results from laboratory studies conducted with rainbow trout. Finally, the accumulation of PFOS precursors, and their subsequent biotransformation into PFOS, may also be a contributing factor to the greater than expected PFOS concentrations in fish collected from the field. Overall, additional studies will have to be conducted to evaluate the relative importance of different accumulation pathways of PFOS by fish populations under natural environmental conditions.

3 Ecotoxicology

Recently, the toxicity of several PFFAs has been intensively studied, although most work has been limited to either PFOS or PFOA (Hekster et al. 2003). Among the PFFAs, PFOS is the most commonly found perfluorinated compound in environmental samples; this compound is particularly prevalent in the tissues of aquatic organisms (Giesy and Kannan 2001). The finding of such residues, in recent years, has resulted in primary efforts to investigate the toxicity of PFOS to aquatic organisms. From laboratory toxicity studies, the PFOS is known to be moderately acute and slightly chronically toxic to aquatic organisms, in general. In this chapter, the acute and chronic toxicity of PFOS to aquatic organisms, both for freshwater and marine species, is reviewed.

The use of PFOS-based products, or those compounds that can degrade to PFOS, was discontinued in 2000. This was done, in part, because it was possible to substitute the less accumulative and less toxic PFFA, perfluorobutanesulfonate (PFBS). Although PFBS is a widely used replacement for PFFA in many products, and we do review available information, there is considerably less toxicology information on it.

3.1 Acute Toxicity of PFOS to Aquatic Organisms

3.1.1 Aquatic Macrophytes

Data on the acute toxicity of PFOS to aquatic plants are somewhat limited (Table 5). The acute toxicity of PFOS to duckweed (*Lemna gibba*) has been reported; the number of fronds or biomass produced during the 7-d exposure served as an index to relative toxicity. There were two primary conclusions: First, the 7-d IC$_{50}$ was found to be 108 mg PFOS/L, with a 95% confidence interval of 46–144 mg PFOS/L, and a no observable effect concentration

Table 5 Acute toxicity of PFOS to aquatic organisms (95% confidence intervals in parentheses)

Trophic level	Test organism/species	Test duration	End point	NOEC (mg/L)	LOEC (mg/L)	$EC_{50}/LC_{50}/IC_{50}$ (mg/L)	Reference
Freshwater							
Macroalgae	*Lemna gibba*	7 d	Frond number	15		108 (46–144)	Desjardins et al. (2001c)
		7 d	Frond number	29.2		59.1 (51.5–60.3)	Desjardins et al. (2001c)
		7 d	Biomass	6.6		31.1 (22.2–36.1)	Boudreau et al. (2003a)
Invertebrate	*Daphnia magna*	48 hr	Survival	33.1 (32.8–34.1)		130 (112–136)	Boudreau et al. (2003a)
		48 hr	Immobility	0.8 (0.6–1.3)		67.2 (31.3–88.5)	Boudreau et al. (2003a)
		48 hr	Survival/immobility	32		61 (33–91)	Drottar and Krueger (2000b)
		48 hr	Survival			58 (46–72)	Robertson (1986)
		48 hr	Survival			67 (48–92)	Robertson (1986)
		48 hr	2nd-generation survival	12			Drottar and Krueger (2000f)
	Daphnia pulicaria	48 hr	Survival	46.9 (33.1–65.3)		169 (136–213)	Boudreau et al. (2003a)
		48 hr	Immobility	13.6 (2.2–33.1)		134 (103–175)	Boudreau et al. (2003a)
	Unio complanatus	96 hr	Survival	20		59 (51–68)	Drottar and Krueger (2000c)
Amphibians	*Xenopus laevis*	96 hr	Growth	4.82	7.97	15.6	Palmer and Krueger (2001)
Fish	*Pimephales promelas*	96 hr	Survival	3.2	5.4	9.1 (7.7–11)	Drottar and Krueger (2000h)

Table 5 (continued)

Trophic level	Test organism/ species	Test duration	End point	NOEC (mg/L)	LOEC (mg/L)	EC$_{50}$/LC$_{50}$/IC$_{50}$ (mg/L)	Reference
	Oncorhynchus mykiss	96 hr	Survival			7.8 (6.2–9.8)	Robertson (1986)
		96 hr	Survival			9.9 (7.5–13.4)	Robertson (1986)
		96 hr	Survival	6.3	13.0	22 (18–27)	Palmer et al. (2002a)
Marine							
Invertebrate	*Artemia salina*	48 hr	Survival			9.4 (7.4–12.1)	Robertson (1986)
		48 hr	Survival			9.4 (7.3–12.2)	Robertson (1986)
		48 hr	Survival			8.9 (6.7–11.9)	Robertson (1986)
	Mysidopsis bahia	96 hr	Survival	1.1		3.6 (3.0–4.6)	Drottar and Krueger (2000d)
		96 hr	Second-generation survival	0.53			Drottar and Krueger (2000g)
	Crassostrea virginica	96 hr	Shell growth	1.8		>3.0	Drottar and Krueger (2000)e
Fish	*Oncorhynchus mykiss*	96 hr	Survival			13.7 (10.7–17.7)	Robertson (1986)
		96 hr	Survival			13.7 (10.7–17.8)	Robertson (1986)
	Cyprinodon variegatus	96 hr	Survival	<15		>15	Palmer et al. (2002b)

(NOEC) of 15 mg PFOS/L, based on frond number (Desjardins et al. 2001c). The sub-lethal effects noted in *L. gibba,* exposed to concentrations $\geq$31.9 mg PFOS/L, included root destruction and/or cupping of the plant (fronds) downward (convex) on the water surface. There was a concentration-dependent increase in dead, chlorotic, and necrotic fronds at greater PFOS concentrations (147 and 230 mg PFOS/L). A recovery period was not evaluated in this study. Second, *L. gibba* exposed to PFOS showed a 7-d IC_{50} of 59 mg PFOS/L (52–60 mg PFOS/L) based on the frond number and a 7-d IC_{50} of 31 mg PFOS/L (22–36 mg PFOS/L) based on the biomass, wt/wt (Boudreau et al. 2003a). The values, based on frond number and biomass were 29 and 6.6 mg PFOS/L, respectively. At the greatest concentration tested (160 mg PFOS/L), the plant fronds exhibited both high percentages of chlorosis and necrosis.

3.1.2 Invertebrates

Several studies on the acute toxicity of PFOS have been conducted with the cladoceran *Daphnia magna* (Table 5). *D. magna* is known to be a representative species among the aquatic invertebrates that are commonly used in standardized toxicity testing. In these acute toxicity studies, cladocerans were exposed to various concentrations of PFOS for 48 hr, and survival (mortality) and immobility were used as end points to calculate LC_{50} or EC_{50} values. Several earlier studies had reported that the LC_{50} for *D. magna* was 58–67 mg PFOS/L (Robertson 1986; Drottar and Krueger 2000b; Boudreau et al. 2003a). However, water concentrations of PFOS were not verified in these studies. Later in 2000, a similar finding was observed in a separate acute toxicity test with *D. magna*, where the 48-hr LC_{50} was reported to be 61 mg PFOS/L with a 95% confidence interval of 33–91 mg PFOS/L (Drottar and Krueger 2000b). The NOEC, based on survival/immobility, was 32 mg/L in that study.

Recently, additional acute toxicity tests with *Daphnia* species have been performed following ASTM guidelines (Boudreau et al. 2003a) (Table 5). In these studies, the 48-hr LC_{50} for *D. magna* was determined to be 130 mg PFOS/ L, and the 48-hr LC_{50} for *D. pulicaria* was determined to be 169 mg PFOS/L. Based on immobility of the cladocerans, the 48-hr EC_{50} values for *D. magna* and *D. pulicaria* were determined to be 67.2 and 134 mg PFOS/L, respectively. NOEC values for *D. magna* (0.8 mg PFOS/L) and *D. pulicaria* (13.6 mg PFOS/L) significantly differed from each other. The differences between studies in reported LC_{50} and NOEC values for PFOS-exposed *Daphnia* species could result from uncertainty in differentiating between the immobility and the lethality end point. *D. magna* appeared to be more sensitive than *D. pulicaria* where the end point was 48-hr immobility (Boudreau et al. 2003a).

In another acute toxicity test with the freshwater mussel (*Unio complanatus*), the mussels were exposed to various concentrations of PFOS for 96 hr (Table 5). The 96-hr LC_{50} was determined to be 59 mg PFOS/L (51–68 mg PFOS/L), whereas the 96-hr NOEC, based on mortality, was 20 mg PFOS/L (Drottar and Krueger 2000c). Mussel tissues were analyzed for PFOS

content in this study. Chemical analysis of tissue showed that there was no mortality associated with 96-hr PFOS exposure of <7.3 mg/kg, wt/wt. In contrast, 90% mortality was observed in mussels containing >88 mg PFOS/ kg, wt/wt after 96 hr of exposure.

In addition to freshwater invertebrate toxicity testing, PFOS toxicity to marine species has also been evaluated (Table 5). In a series of acute toxicity tests with brine shrimp (*Artemia salina*), the average ($n = 3$) 48-hr LC_{50} was 9.2 ± 0.29 mg PFOS/L (Robertson 1986). In an acute toxicity test with the salt-water mysid (*Mysidopsis bahia*), the 96-hr LC_{50} was 3.6 mg PFOS/L, and the NOEC was determined to be 1.1 mg PFOS/L, based on mortality (Drottar and Krueger 2000d). The effect of PFOS exposure on a benthic marine invertebrate has also been reported. Shell deposition in the eastern oyster (*Crassostrea virginica*) was examined in this study; shell growth was inhibited at a concentration of 1.8 mg PFOS/L, by 20% compared to controls (Drottar and Krueger 2000e). However, an EC_{50} could not be calculated in this study because growth was only inhibited by 28% at the greatest PFOS concentration of 3.0 mg/L tested. In summary, the acute invertebrate toxicity data indicated that, in short-term exposures, marine invertebrates are more sensitive to PFOS exposure than are freshwater ones.

3.1.3 Amphibians

The developmental effects of PFOS on the African-clawed frog (*Xenopus laevis*) have been investigated by the Frog Embryo Teratogenesis Assay-Xenopus (FETAX) (Palmer and Krueger 2001). In this assay, frog embryos and tadpoles were exposed to various concentrations of PFOS (0.0–24 mg PFOS/L) for 96 hr, and the end points of survival, growth, and developmental anomalies were examined during early stages of development. Significant mortality occurred at concentrations >14.4 mg PFOS/L and the 96-hr LC_{50} was found to be 14–18 mg PFOS/L, for the three replicate assays. There was a correlation between PFOS exposure and malformations in each of the three assays, and the most commonly observed malformations were improper gut coiling, edema, as well as notochord and facial abnormalities. The 96-hr EC_{50} for malformations was 12–18 mg PFOS/L. Finally, tadpole growth was affected in the second and third assays, and the minimum concentrations inhibiting growth were determined to be 8.0 and 8.3 mg PFOS/L. The NOEC for growth was determined to be 5.2 mg PFOS/L.

3.1.4 Fish

Several acute toxicity studies with PFOS have been conducted on fish including fathead minnows (*Pimephales promelas*), sheepshead minnows (*Cyprinodon variegatus*), bluegill sunfish (*Lepomis macrochirus*), and freshwater and marine rainbow trout (*Oncorhynchus mykiss*) (Table 5). Of the freshwater fish exposures, the fathead minnow was the most sensitive species with a 96-hr LC_{50} of

9.1 mg PFOS/L and an NOEC of 3.2 mg PFOS/L. After 96 hr of exposure, the sub-lethal effect of erratic swimming was noted in fathead minnows exposed to concentrations >5.6 mg PFOS/L (Drottar and Krueger 2000 hr).

Two acute toxicity tests with PFOS have been performed with rainbow trout in freshwater (Robertson 1986; Palmer et al. 2002a). Although the 96-hr LC_{50} values for PFOS in rainbow trout differed more than two-fold between these two studies, the LC_{50} of 22 mg PFOS/L, as reported in the Palmer et al. (2002a) study, is more reliable than that reported in Robertson (1986), because the LC_{50} value in the Palmer et al. (2002a) study was calculated with measured PFOS concentrations rather than being based on nominal concentrations.

The sheepshead minnow, a brackish-marine species, has also been tested for PFOS acute toxicity, but was exposed to only one concentration of PFOS, 15 mg PFOS/L. This was the greatest concentration attainable in saltwater and required the addition of methanol (0.05%). No mortality was observed at this concentration after 96 hr of exposure, thus the 96-hr LC_{50} was reported as >15 mg PFOS/L, and the NOEC for sub-lethal effects was reported to be <15 mg PFOS/L (Palmer et. al 2002b). In another study, freshwater rainbow trout were acclimated over 5 d to a final salinity of 30% and were exposed to PFOS for 96 hr (Robertson 1986). For rainbow trout exposed to PFOS in saltwater, the 96-hr LC_{50} was calculated as 14 mg PFOS/L, and no sub-lethal effects were observed among rainbow trout at any PFOS concentration tested in this study. It should be noted that PFOS concentrations were not measured in this study and some of the nominal exposure concentrations were greater than the solubility of PFOS in saltwater.

3.2 Chronic Toxicity of PFOS to Aquatic Organisms

3.2.1 Microorganisms

The potential effects of PFOS on microorganisms in activated sludge have been determined by exposing microbes from a municipal wastewater treatment plant to various concentrations of PFOS (0.9–870 mg PFOS/L) (Schafer and Flaggs 2000). After 3 hr of exposure, there was a 39% inhibition of the respiration rate, compared to controls, at the greatest concentration. However, the test concentration in this study exceeded the water solubility for PFOS, and as a result, based on known environmental concentrations PFOS would not be expected to cause any effects to microorganism communities (Table 6).

3.2.2 Microalgae

Many studies have been conducted to determine the toxicity of PFOS to aquatic microalgal species including phytoplankton and diatoms (Table 6). Since the life cycle of most of these species is quite short (ranging from hr to d), these studies represent the measurement of chronic effects on multiple generations,

Table 6 Chronic toxicity of PFOS to aquatic organisms (95% confidence intervals in parentheses)

Trophic level	Test organism/ species	Test duration	End point	NOEC (mg/L)	LOEC (mg/L)	$EC_{50}/LC_{50}/IC_{50}$ (mg/L)	Reference
Freshwater							
Microorganisms	Microorganism community	96 hr	Respiratory inhibition			>870	Schaefer and Flaggs (2000)
Microalgae	*Selenastrum capricornutum*	96 hr	Growth (cell density)	42		68 (63–70)	Drottar and Krueger (2000a)
		96 hr	Inhibition of growth rate	42		121 (110–133)	Drottar and Krueger (2000a)
		96 hr	Growth (cell density)	5.3 (4.6–6.8)		48.2 (45.2–51.1)	Boudreau et al. (2003)
		96 hr	Growth (chlorophyll a)	16.6 (8.5–28.1)		59.2 (50.9–67.4)	Boudreau et al. (2003)
	Navicula pelliculosa	96 hr	Growth (cell density)	150		263 (217–299)	Sutherland and Krueger (2001)
		96 hr	Inhibition of growth rate	206		305 (295–316)	Sutherland and Krueger (2001)
	Chlorella vulgaris	96 hr	Growth (cell density)	8.2 (6.4–13.0)		81.6 (69.6–98.6)	Boudreau et al. (2003)
	Zooplankton community	35 d	Community structure	3.0			Boudreau et al. (2003b)
Macroalgae	*Myriophyllum spicatum*	42 d	Biomass (dwt)	11.4		12.5 (6–18.9)	Hanson et al. (2005)
		42 d	Root length (cm)	11.4		16.7 (10.8–22.5)	Hanson et al. (2005)
	Myriophyllum sibiricum	42 d	Biomass (dw)	2.9		3.4 (1.6–5.3)	Hanson et al. (2005)
		42 d	Root length (cm)	0.3		2.4 (0.5–4.2)	Hanson et al. (2005)
Invertebrate	*Daphnia magna*	21 d	Adult survival	5.3 (2.5–9.2)		42.9 (31.7–56.4)	Boudreau et al. (2003a)

Table 6 (continued)

Trophic level	Test organism/species	Test duration	End point	NOEC (mg/L)	LOEC (mg/L)	EC$_{50}$/LC$_{50}$/IC$_{50}$ (mg/L)	Reference
	Chironomus tentans	10 d	Survival	0.05		>0.15	MacDonald et al. (2004)
		10 d	Growth (chlorophyll a)	0.05		0.087	MacDonald et al. (2004)
		20 d	Survival	0.0		0.092	MacDonald et al. (2004)
		20 d	Growth (chlorophyll a)	0.0		0.094	MacDonald et al. (2004)
Amphibians	*Rana pipiens*	16 wk	Partial life cycle	0.3	3	6.21 (5.12–7.52)	Ankley et al. (2004)
Fish	*Pimephales promelas*	28 d	Microcosm	0.3	3.0	7.2 (5.2–9.2)	Oakes et al. (2005)
		47 d	Early life stage	0.29	0.58		Drottar and Krueger (2000i)
Marine							
Microorganisms	*Anabaena flos-aquae*	96 hr	Growth (cell density)	93.8		131 (106–142)	Desjardins et al. (2001a)
		96 hr	Inhibition of growth rate	93.8		176 (169–181)	Desjardins et al. (2001a)
Microalgae	*Skeletonema costatum*	96 hr	Growth (cell density)	>3.2		>3.2	Desjardins et al. (2001b)
Invertebrate	*Mysidopsis bahia*	35 d	Growth, no. of young produced	0.24			Drottar and Krueger (2000g)

even when the exposure period of these tests are short (72–96 hr). The toxicological end points that have been evaluated in these studies include growth (measured in terms of cell density or chlorophyll a content) and/or area under the growth curve over the test duration. Reported 96-hr EC_{50} values for freshwater microalgae (growth end point as measured by cell density) ranged from 48 to 263 mg PFOS/L. The 96-hr NOEC values for biomass ranged from 5.3 to 150 mg PFOS/L. Using biomass as the end point, the most sensitive species was *Selenastrum capricornutum* (NOEC = 5.3 mg PFOS/L), whereas the diatom *Navicula pelliculosa* was the least sensitive species (NOEC = 150 mg PFOS/L) (Boudreau et al. 2003a; Sutherland and Krueger 2001). When growth rate was evaluated as the test end point, 96-hr EC_{50} values ranged from 121 to 305 mg PFOS/L, and NOEC values ranged from 42 to 206 mg PFOS/L. Again, *S. capricornutum* was the most sensitive species, and *N. pelliculosa* was the least sensitive, using growth rate as the end point. The effects of PFOS on these microalgal species were algistatic, since growth resumed when microalgae from the greatest PFOS treatments were placed in fresh growth media at the end of the exposure period. Furthermore, signs of aggregation or adherence of the cells to the flask were not observed, nor were there any noticeable changes in cell morphology at the end of the studies for any concentration evaluated.

Although concentration–response relationships for growth have been developed for freshwater algae, the marine diatom, *Skeletonema costatum*, was not affected by exposure to PFOS. In this study, a 96-hr EC_{50} could not be determined because at the greatest dissolved concentration attained under test conditions (3.2 mg PFOS/L), growth was not significantly inhibited. As a result, an analysis of the sensitivity between freshwater and marine algae could not be conducted.

In addition to evaluating PFOS toxicity in individual species of microalgae, the effects of PFOS has also been evaluated at the community level. In a controlled freshwater microcosm study, 0, 0.3, 3.0, 10, or 30 mg PFOS/L were administered to a zooplankton community for a total of 35 d. Results indicated that the zooplankton community structure was significantly altered by exposure to 10 or 30 mg PFOS/L. By day 35, the total number of zooplankton species decreased by an average of 45.1 and 74.3% in the 10 and 30 mg PFOS/L treatments, respectively. Thus, the NOEC based on changes in zooplankton community structure was determined to be 3.0 mg PFOS/L (Boudreau et al. 2003b).

3.2.3 Aquatic Macrophytes

The chronic toxicity of PFOS was evaluated for two aquatic macrophytes, *Myriophyllum sibiricum* and *M. spicatum,* in a microcosm study (Hanson et al. 2005; Table 6). Both species were exposed to PFOS concentrations ranging from 0.03 to 30 mg PFOS/L for 42 d; measured test end points were plant length, root number and length, node number, and biomass, expressed as dry weight (dwt). Toxicity was observed at PFOS concentrations of >3 mg PFOS/L

for *M. spicatum*, with the 42-d EC_{50} exceeding 12 mg PFOS/L. The NOEC was found to be consistently >11 mg PFOS/L. Toxicity for *M. sibiricum* was observed at PFOS concentrations of >0.1 mg PFOS/L, and the 42-d EC_{50} value was greater than 1.6 mg PFOS/L. The NOEC values of 2.9 and 0.3 mg PFOS/L were based on biomass and root length, respectively. In general, *M. sibiricum* was more sensitive than *M. spicatum*, regardless of the test end point.

3.2.4 Invertebrates

Life-cycle tests with *D. magna* have been conducted to evaluate the chronic toxicity of PFOS to freshwater aquatic invertebrates (Table 6). In one study, the 21-d LC_{50} was determined to be 43 mg PFOS/L, and the NOEC, based on adult survival, was estimated to be 5.3 mg PFOS/L (Boudreau et al. 2003a). In a separate life-cycle toxicity test of *D. magna*, the LC_{50} and NOEC, based on adult survival, were reported as 12–13 mg PFOS/L, respectively (Drottar and Krueger 2000f). In another life-cycle toxicity test with the saltwater mysid, the 35-d NOEC, based on growth and number of young produced, was found to be 0.24 mg PFOS/L (Drottar and Krueger 2000 g). In the course of the life-cycle tests with both *D. magna* and the saltwater mysid, the young produced were briefly exposed to the same concentrations to which the respective first-generation adults were exposed. Survival was monitored for 48 hr (*D. magna*) or 96 hr (*M. bahia*). After 48 hr of exposure, the results of a daphnid second-generation acute exposure produced an NOEC of 12 mg PFOS/L. The second-generation mysid shrimp were exposed to negative control, 0.055, 0.12, 0.24, or 0.53 mg PFOS/L for 96 hr. Survival was >95% for all second-generation mysids exposed to these test concentrations. The mysid second-generation acute exposure NOEC was 0.53 mg PFOS/L. These results indicated that the saltwater mysid may be more sensitive to PFOS than is freshwater *D. magna*. However, additional studies would need to be conducted to better evaluate the toxicity of PFOS to second-generation organisms. Specifically, a greater range of PFOS concentrations is needed to further define the NOAEC (no observable adverse effect concentration) for second-generation mysid shrimp.

3.2.5 Amphibians

The survival and development of northern leopard frogs (*Rana pipiens*), from early embryogenesis through complete metamorphosis, has been investigated in a water exposure study with PFOS (Ankley et al. 2004; Table 6). In tadpoles exposed to 0.03, 0.1, 0.3, 1.0, 3.0, or 10 mg PFOS/L, mortality was observed within 2 wk of study initiation in the 10 mg PFOS/L treatment; >90% mortality was observed by week 4. Tadpole survival was not affected in any other treatment group. The mean LC_{50} at week 5 was 6.2 mg PFOS/L (5.1–7.5 mg PFOS/L). No statistically significant effects were observed for tadpoles exposed to <1.0 mg PFOS/L. However, there was a slight increase in time to metamorphosis and a decrease in total length of tadpoles at levels >3.0 mg PFOS/L. In

addition, there was a slight increase in the incidence of thyroid follicle cell atrophy that was subtle and difficult to quantify. The PFOS-related chronic effects in leopard frogs occurred within a concentration range that has been shown to cause effects in fish and invertebrates.

3.2.6 Fish

Chronic toxicity data, from an early-life stage toxicity test, are available for fathead minnows (*P. promelas*; Drottar and Krueger 2000i; Table 6). In this study, eggs and larvae were exposed to PFOS in a flow-through system for 47 d. Measured water concentrations of PFOS in the various treatments were <LOQ (limit of quantification), 0.15, 0.30, 0.60, 1.2, 2.4, or 4.6 mg PFOS/L. Fish exposed to PFOS at concentrations <0.30 mg PFOS/L showed no significant reduction in time to hatch, hatching success, and survival or growth. The PFOS did not affect percent hatch or growth of fry at any of the concentrations tested. Survival was the most sensitive end point in this study. Compared to controls, percent survival was significantly reduced among fathead minnows exposed to concentrations >0.60 mg PFOS/L. Thus, the NOEC and LOEC (lowest observable effect concentration) for fathead minnows were determined to be 0.30 and 0.60 mg PFOS/L, respectively (Drottar and Krueger 2000i).

3.3 Toxicity of PFBS

3.3.1 Microorganisms

Effects of PFBS on activated sludge microorganisms have been evaluated by exposing microbes to concentrations of 1–1,000 mg PFBS/L for up to 3 hr (Wildlife International 2001a). The maximal inhibitory effects on respiration was observed at 300 mg PFBS/L, but there was no clear dose response because effects at 1,000 mg PFBS/L were less than that observed at 300 mg PFBS/L. As a result, PFBS was not considered to be inhibitory to sewage microorganisms and had a 3-hr EC_{50} of >1,000 mg PFBS/L.

3.3.2 Aquatic Plants

To date, only one study has been conducted with an aquatic plant, the freshwater alga *S. capricornutum* (Wildlife International 2001e). In this study, PFBS was found to be practically non-toxic with a 96-hr EC_{50} of 2,347 mg PFBS/L, and a NOEC of 1,077 mg PFBS/L, based on reductions in biomass. Using growth of exposed cells during the recovery phase of the study, as the end point, PFBS was found to be algistatic.

3.3.3 Aquatic Organisms

To date, the effects of PFBS have been investigated in only a few freshwater aquatic organisms including the bluegill (*L. macrochirus*), the fathead minnow (*P. promelas*), and the water flea (*D. magna*; Table 7). For the bluegill, the 96-hr LC_{50} was 6,452 mg PFBS/L, and the NOEC was 6,452 mg PFBS/L (Wildlife International 2001a). For fathead minnow, the 96-hr LC_{50} was 1,938 mg PFBS/ L, and the NOEC was 888 mg PFBS/L (Wildlife International 2001b). For *D. magna*, the 48-hr LC_{50} was 2,183 mg PFBS/L, and the NOEC was 886 mg PFBS/L. One chronic toxicity test has been conducted with a freshwater organism, *D. magna*. In this study, the 21-d NOEC for reproductive end points was 502 mg PFBS/L, and the LOEC was 995 mg PFBS/L. This resulted in a chronic value of 707 mg PFBS/L. These data indicate that PFBS is not very toxic to freshwater organisms, with effect levels only being observed at a concentration greater than 700 mg PFBS/L.

3.4 Water Quality Criteria for the Protection of Aquatic Life

Multiple approaches are available to derive water quality values; the specific approach will depend upon the regulatory agency involved in the calculation of these values and their environmental policies (US EPA 1985; RIVM 2001; CCME 1999). In the United States, three types of water quality can be derived including numeric, narrative, and operational (US EPA 1985). However, for the purposes of this chapter, only numeric criteria were considered. Numeric criteria are scientifically based numbers that are intended to protect aquatic life from adverse effects of contaminants without consideration of societal values, economics, or other non-scientific considerations.

To derive numeric water quality values for those PFCs that have sufficient and appropriate toxicity data, we have relied on the US EPA Great Lakes Initiative (GLI; US EPA 1995). The GLI provides specific procedures and methodologies for utilizing toxicity data to derive water quality values that are protective of aquatic organisms. The GLI presents a two-tiered methodology (Tier I and Tier II), in which the Tier I procedures are essentially the same as the procedures for deriving national water quality criteria (NWQC; US EPA 1985). The Tier II aquatic life methodology is used to derive values when limited toxicity data are available. Because greater uncertainties are associated with limited toxicity data, the Tier II methodology generally produces more stringent values than do the Tier I methodology. EPA has indicated that Tier II values are not intended to be adopted as state water quality standards (US EPA 1995). The guidance provided by the GLI is intended to provide both acute and chronic criteria for the protection of fish, invertebrates, and other aquatic organisms. The final acute value (FAV) is a semi-probabilistic approach that requires data for a range of specified taxa and produces the concentration deemed to be protective of approximately 95% of tested genera. The FAV is

Table 7 Summary of acute and chronic toxicity values for aquatic organisms and plants exposed to perfluorobutanesulfonate (PFBS)

Organism	Genus/species	Test duration	Media	NOEC (mg/L)	LOEC (mg/L)	LC$_{50}$ (mg/L)	Reference
Acute							
Water flea	*Daphnia magna*	48 hr	FW	886	1,707	2,183 (1,707–3,767)	WLI[c] (2001b)
Fathead minnow	*Pimephales promelas*	96 hr	FW	888	1,655	1,938 (888–3,341)	WLI (2001c)
Bluegill	*Lepomis macrochirus*	96 hr	FW	2,715	5,252	6,452 (5,252–9,433)	WLI (2001d)
Algae[a]	*Selenastrum capricornutum*	96 hr	FW	1,077	2,216	2,347 (2,018–2,707)	WLI (2001e)
Mysid	*Mysidopsis bahia*	96 hr	SW	127	269	372 (314–440)	WLI (2001f)
Chronic							
Water flea[b]	*Daphnia magna*	21 d	FW	502	995		WLI (2001 g)

[a]Reported data are based on biomass measurements
[b]Reported data based on reproduction and length measurements
[c]WLI = Wildlife International
FW = Fresh Water
SW = Salt Water

used to establish an acute criterion or criteria maximum concentration (CMC), which is equivalent to one-half the FAV. The chronic criterion or criteria continuous concentration (CCC) represents a concentration of a chemical such that 95% of the genera tested have greater chronic values. The purpose and use of these numerical criteria are not to provide concentrations of a chemical that will be protective of all aquatic species in a specific ecosystem, but to provide reasonable protection to ecologically and commercially important species under most circumstances such that overprotection or under-protection of aquatic species is avoided (US EPA 1985).

The Tier I procedures in the GLI utilize a semi-probabilistic method that requires, at a minimum, results from acceptable acute toxicity tests, with at least one species of freshwater animal in at least eight different families. These families should include the following:

1. the family *Salmonidae* in the class *Osteichthyes*;
2. a second family in the class *Osteichthyes*, preferably a cyprinid;
3. a third family in phylum *Chordata*;
4. a planktonic crustacean (e.g., cladoceran, copepod);
5. a benthic crustacean (e.g., ostracod, isopod, amphipod);
6. an aquatic insect;
7. a family in a phylum other than *Arthropoda* or *Chordata* (e.g., *Annelida*, *Mollusca*); and
8. a family in any order of insect or phylum not already represented.

The guidance for a Tier I assessment also requires data from at least one toxicity test with an alga or a vascular plant and at least one acceptable BCF. In addition, chronic toxicity data are needed from at least three different aquatic animals and should include a planktonic crustacean and a fish species.

When sufficient toxicity data are not available for calculating a national water quality criterion pursuant to EPA Tier I methodology, the GLI guidance provides for calculation of Tier II values. The derivation of Tier II criteria is based on the application of an assessment factor that is used to offset the absence of sufficient toxicity data. However, because of the greater uncertainties associated with limited toxicity data, the Tier II methodology generally produces more stringent values than does the Tier I methodology. Furthermore, EPA has indicated that Tier II values are not intended to be adopted as state water quality standards (US EPA 1995). In both Tier I and Tier II methodologies, water quality criteria that protect against chronic effects can be calculated using available acute data, and an acute–chronic ratio, when sufficient chronic data are not available.

A critical step in deriving either Tier I or II water quality criteria is the evaluation of data usability based on criteria outlined by US EPA (1985) guidance. In this chapter, data were screened to determine usability based on the following: (1) Only data from tests with freshwater organisms were used; (2) Only data on organisms resident in North America were used; species that do not have reproducing wild populations in North America were rejected; (3) Data

with pre-exposed organisms were not used; and (4) Data lacking controls or control treatments, or with unacceptable control results, were not used. Finally, for those studies that meet the requirements listed above, toxicity data were further screened for certain experimental conditions (i.e., water quality considerations, life stage, and measured end points). The water quality criteria values for selected PFCs have been presented and are summarized in Fig. 2.
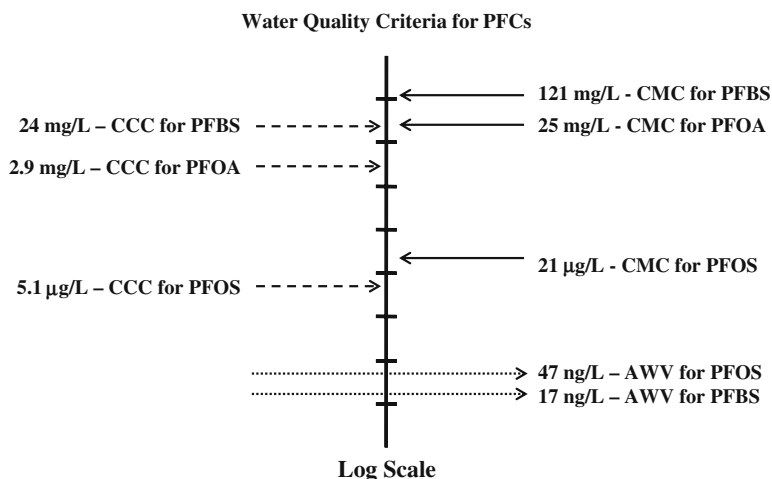
Water Quality Criteria for PFCs

121 mg/L - CMC for PFBS

24 mg/L – CCC for PFBS
25 mg/L - CMC for PFOA

2.9 mg/L – CCC for PFOA

21 µg/L - CMC for PFOS

5.1 µg/L – CCC for PFOS

47 ng/L – AWV for PFOS
17 ng/L – AWV for PFBS

Log Scale

Fig. 2 Comparison of water quality criteria values for the protection of aquatic organisms (CMC: criteria maximum concentration; CCC: criteria continuous concentration) and wildlife (AWV: avian wildlife value) for PFCs, including PFOS, PFBS, and PFOA

3.4.1 PFOS

Collectively, the data on acute toxicity of PFOS meet the GLI species requirements for using Tier I methodology (Table 5). The genus mean acute values (GMAV) for aquatic species are used to calculate a FAV. The GLI Tier I approach for calculating a FAV utilizes a subset of the data nearest the fifth centile of a statistical population of acute toxicity values, in which the data are fitted to a log-triangular distribution, wherein only the four least acute toxicity values nearest the fifth centile are used (US EPA 1995). The specific steps used to calculate the FAV include (1) ranking the GMAV from the greatest to least value and (2) assigning each GMAV a cumulative probability calculated as $P_R = R/(N+1)$, where R is the rank and N is the number of GMAVs in the data set (Table 8). By using the four GMAVs, which have the cumulative probabilities closest to 0.005, one can calculate the FAV as follows (Eqs. 1–4):

$$S^2 = \frac{\sum \left((\ln \text{GMAV})^2\right) - \left(\left(\sum (\ln \text{GMAV})\right)^2 / 4\right)}{\sum (P) - \left(\left(\sum (\sqrt{P})\right)^2 / 4\right)} \tag{1}$$

Table 8 Summary of genus mean acute toxicity values (GMAV) for aquatic organisms exposed to PFOS[a]

Organism	Genus/species	SMAV (mg/L)	GMAV (mg/L)	Rank	Cumulative probability[b]
Water flea	*Daphnia magna*	61	71	10	0.9091
	Daphnia pulicaria	134			
Mussel	*Unio complanatus*	57	57	9	0.8182
Spring peeper	*Pseudacris crucifer*	38	38	8	0.7273
Planarian	*Dugesia japonica*	17	17	7	0.6364
Amphipod	*Hyalella azteca*	15	15	6	0.5455
Rainbow trout	*Oncorhynchus mykiss*	14	14	5	0.4545
Leopard frog	*Rana pipiens*	6.2	6.2	4	0.3636
Oligocheate	*Lumbriculus variegatus*	5.6	5.6	3	0.2727
Fathead minnow	*Pimephales promelas*	2.5	2.5	2	0.1818
Midge	*Chironomus tentans*	0.089	0.089	1	0.0909

[a]Species mean acute values (SMAV) and genus mean acute values (GMAV) calculated as the geometric mean of LC_{50} values from acceptable studies
[b] Cumulative probability calculated as $P = (\text{Rank}/N + 1)$ where N is the number of GMAV

$$L = \sum (\ln \text{GMAV}) - S\left(\sum(\sqrt{P})\right)/4 \tag{2}$$

$$A = S(\sqrt{0.005}) + L \tag{3}$$

$$\text{FAV} = e^A \tag{4}$$

Using the GLI methodology, the FAV for the effects of PFOS on aquatic organisms was calculated to be 42 µg PFOS/L (Table 9). This value represents the 95% protection level for aquatic organisms and relies on currently available acute toxicity data. Because the GLI method puts greater emphasis on the four least LC_{50} values used, this criterion is skewed if one genus or species tested is more sensitive than the others. In fact, this is the case for PFOS, wherein there is a difference of approximately 40-fold between the most sensitive genus (*Chironomus*), and the next most sensitive genus (*Pimephales*). In addition, *Chironomus tentans* appear to be uniquely sensitive in that other small, non-predatory white midges, exposed to 30 mg PFOS/L for 10 d, were unaffected by PFOS, whereas at >300 µg PFOS/L all *C. tentans* died. As a result, inclusion of the *C. tentans* acute data in the derivation of the FAV probably results in a conservative water quality value that would be protective of most aquatic organisms.

Depending upon the availability of chronic toxicity data, a final chronic value (FCV) can be calculated in the same manner as is the FAV, or it can be

Table 9 Calculation of a freshwater final acute value (FAV) for PFOS[a]

Rank	GMAV (mg/L)	P	Sqrt (P)	Ln (GMAV)	(Ln GMAV)^2
4	6.2	0.3636	0.6030	1.825	3.3290
3	5.6	0.2727	0.5222	1.723	2.9679
2	2.5	0.1818	0.4264	0.916	0.8396
1	0.089	0.0909	0.3015	−2.414	5.8269
	Sum	0.9091	1.8532	2.0497	12.9634

$S^2 = 235.7512$

$S = 15.3542$

$L = -6.60101$

$A = -3.1667$

Final acute value (FAV) $= 42$ µg PFOS/L

[a]Only the four most sensitive genera were used in the calculation of the FAV, because the total number of acceptable toxicity results was less than 59

calculated by dividing the FAV by a final acute–chronic ratio (ACR). An ACR can be derived by dividing a species-specific chronic value from an acceptable chronic toxicity test by an LC_{50} from the same species. A chronic value is calculated as the geometric mean of the lower (NOEC) and upper (LOEC) limit from a chronic toxicity test. According to the GLI, the final acute–chronic ratios is calculated as the geometric mean of acute–chronic ratio from at least three different species, a fish, a daphnid, and one other sensitive species. For PFOS, acceptable chronic toxicity studies are available for two freshwater species, *D. magna* and *P. promelas* and one saltwater species, *M. bahia,* for which acute toxicity data are also available. Although saltwater species cannot be used in the derivation of freshwater water quality criteria, these data can be used to calculate an acute–chronic ratio. For *D. magna*, a species mean acute value (SMAV) from acceptable toxicity data was 61 mg PFOS/L, while a chronic value was calculated as 17 mg PFOS/L. This resulted in an ACR of 3.6. For the fathead minnow (*Pimephales promelas*), the SMAV was 8.1 mg PFOS/L, while the chronic value was calculated as 0.48 mg PFOS/L; this resulted in an ACR of 16.9. Finally, for *M. bahia* the SMAV was 3.5 mg PFOS/L, while the chronic value was 0.37 mg PFOS/L and resulted in an ACR of 9.5. The final ACR for PFOS, based on these three ACRs, was 8.3. The FCV was calculated by dividing the FAV (42 µg PFOS/L) by the ACR (8.3) and resulted in a FCV of 5.1×10^{-3} mg PFOS/L (or 5.1 µg PFOS/L).

GLI guidelines require the review of aquatic plant toxicity data, and calculation of a final plant value (FPV), if sufficient data are available. The FPV represents the least concentration from a toxicity test with an important aquatic plant species, in which the concentrations of test material have been measured; the end point monitored in the study is biologically important. For the derivation of a freshwater FPV for PFOS, a chronic study with milfoil, *Myriophyllum sp,* was selected to comply with data acceptability requirements outlined in the GLI. This study

fulfilled all necessary requirements for data acceptability (i.e., measured PFOS water concentrations, biological, and ecologically important end points) and had the least genus mean chronic value (GMAV). Thus, based on the results from the *Myriophyllum* toxicity test, the FPV was determined to be 2.3 mg PFOS/L.

Using the methods outlined in the GLI, a CMC was calculated for PFOS by dividing the FAV (42 µg PFOS/L) by 2, this resulted in a value of 21 µg PFOS/L. The CCC is determined as the lower value between the FCV (5.1 µg PFOS/L) and the FPV (2,300 µg PFOS/L), thus the CCC is 5.1 µg PFOS/L.

3.4.2 Critical Body Burden of PFOS in Fish

The critical body residue (CBR) hypothesis provides a framework for analyzing aquatic toxicity in terms of mode of action and tissue residue concentrations (McCarty and Mackay 1993; Di Toro et al. 2000). The key assumption of the hypothesis is that adverse effects are elicited when the molar concentration of a chemical in an organism's tissues exceeds a critical threshold. Under steady state conditions, the CBR can be expressed mathematically as the end point of the effect concentration (in water) determined in an aquatic test and the BCF:

$$CBR = BE \times BCF$$

where CBR is the critical body residue (mmol/kg), BE is the biological effect level (LC_{50}, EC_{50}, or some chronic level), and BCF is the bioconcentration factor.

The BCF used in this type of analysis can be derived either experimentally or empirically through the use of QSARs. Implicit in this hypothesis is the assumption that a chemical is accumulated in tissues via a partitioning process, and it has reached a steady state within the test period. Thus, the CBR is a time-independent measure of effect for organisms exposed to the chemical. One limitation of this assumption is that, in many cases, organisms may not have achieved a steady state concentration such that using the BCF would over-estimate the actual whole body concentration one would expect during a standard aquatic acute toxicity test. In addition, this model does not take into account accumulation of chemicals into target tissues that occurs in a manner different from that observed on a whole body basis (Barron et al. 2002). Thus, these factors may result in an overestimate of the CBR that would under-estimate the risk an aquatic organism would be exposed to in a natural setting. For this analysis, kinetic parameters of accumulation have been used instead of the BCF to evaluate tissue concentrations associated with toxicity in bluegill.

To estimate a critical body residue level for PFOS in fish, we used data from a bluegill bioconcentration study in which significant mortality occurred at the greatest dose (Drottar et al. 2001). In this study, bluegill sunfish were exposed to 0.086 or 0.87 mg PFOS/L for up to 62 d, followed by a depuration period. However, at 0.87 mg PFOS/L, mortality was noted by day 12, with 100% mortality being observed by day 35. At this high dose, no fish survived to the

Table 10 Cumulative mortality and whole body PFOS concentrations in bluegill exposed to 1.0 mg/L in a bioconcentration study[a]

Time (d)	PFOS (µg/kg, wt/wt)	Number exposed	Cumulative mortality
0.2	1,577	55	0
1.0	2,519	55	0
3.0	33,703	55	0
7.0	81,690	55	0
14	158,743	55	16
21	177,969	55	35
28	241,799	55	52

[a] PFOS concentration values are means for fish sampled on the indicated dates. Concentrations are expressed on a wet weight basis

end of the uptake phase of the study. Mortality and whole body PFOS concentration data, collected during the study at the 0.87 mg PFOS/L exposure concentration, are given in Table 10. Probit analysis was used to estimate a critical body residue concentration; tissue PFOS concentration was used as the independent variable and mortality as the dependent variable. The use of probit analysis allowed for the calculation of point estimates along the dose–response curve. The 28-d LD_{50}, calculated from whole body concentrations, was 172 mg PFOS/kg, wt/wt. The 95% lower and upper confidence limits for the LD_{50} were 163 and 179 mg PFOS/kg, respectively. As an estimate of a no observable adverse effect level (NOAEL) for PFOS-induced mortality in bluegill, we extrapolated down to the LD_{01}. The LD_{01} was 109 mg PFOS/kg, wt/wt. The 95% lower and upper confidence limits for the LD_{01} were 87 and 123 mg PFOS/kg, wt/wt, respectively. From this statistical evaluation of the data, the tissue concentration that would not be expected to cause adverse effects in fish is 109 mg PFOS/kg. However, due to potential differences in species sensitivity, the lower 95% confidence limit of the LD_{01} was used as a conservative estimate of a NOAEL. Based on this analysis, tissue concentrations less than 87 mg PFOS/kg would not be expected to cause acute effects in fish. However, in this bluegill study no evaluation of other non-lethal end points, including development or reproduction, was made. In addition, there are not sufficient data to critically evaluate differences in accumulation, tissue distribution, or target organ toxicity across fish species. Therefore, it may be necessary to incorporate uncertainty factors in the estimated CBR to take into account interspecies differences.

3.4.3 PFBS

Because acute toxicity data are too limited to calculate a water quality criterion, a Tier II water quality criterion is derived as directed by GLI guidance. In this methodology, a secondary acute value (SAV) is calculated by dividing the least acute toxicity value (LC_{50}) by an application factor or secondary acute factor (SAF). The SAF is a factor used to compensate for the lack of sufficient acute toxicity data that is normally required for calculating Tier I water quality

criteria. The magnitude of the SAF corresponds to the number of satisfied minimum data requirements given in the Tier I methodology and can range from 4.3 up to 21.9 for chemicals that, for example, only meet 7 or 1 of the data requirements, respectively. For PFBS, the lowest acute toxicity value was for the fathead minnow (*P. promelas*) that had a 96-hr LC_{50} of 1,938 mg PFBS/L (Table 11). The SAF, based on the three acceptable acute toxicity data values that meet the Tier I requirements, is 8.0 as prescribed by GLI guidance. Thus, the SAV for PFBS was calculated as shown in Eqs. 5 and 6 below:

Table 11 Summary of genus mean acute values (GMAVs) and genus mean chronic values (GMCVs) for aquatic organisms exposed to PFBS[a]

Organism	Genus/species	Test duration	Media	GMAV (mg PFBS/L)
Acute				
Bluegill	*Lepomis macrochirus*	96 hr	FW	6,452
Water flea	*Daphnia magna*	48 hr	FW	2,183
Fathead minnow	*Pimephales promelas*	96 hr	FW	1,938
Chronic				
Water flea	*Daphnia magna*	21 d	FW	707

[a] Genus mean acute values were based on geometric means of acute toxicity values by genus, whereas genus mean chronic values were based on geometric means of NOAEC values from chronic studies
FW = Fresh Water

$$\text{secondary acute value (SAV)} = \frac{\text{lowest acute value}}{\text{secondary acute factor (SAF)}} \quad (5)$$

$$\text{SAV} = \frac{1938 \text{ mg/L}}{8.0} = 242 \text{ mg PFBS/L} \quad (6)$$

To calculate a secondary chronic value (SCV) the SAV is divided by a final acute–chronic ratio (FACR). However, to date, only one chronic toxicity study, on *D. magna,* could be used to calculate a species-specific ACR, which is needed in the derivation of a FACR. For this species, the SMAV was 1,938 mg PFBS/ L, and the chronic value (CV) was 707 mg PFBS/L. The ACR, calculated from these data, was 3.1. However, to calculate a FACR, a minimum of three species-specific ACR values are needed, and as a result, default ACR values are used to replace the missing data as prescribed in the GLI Tier II methodology. The default ACR value of 18 is substituted for each of the two missing ACR values; this resulted in a FACR of 10. The SCV is then calculated by dividing the SAV by the FACR. The SCV for PFBS was calculated as follows in Eqs. 7 and 8:

$$\text{secondary chronic value (SCV)} = \frac{\text{secondary acute value (SAV)}}{\text{final acute to chronic ratio (FACR)}} \quad (7)$$

$$SCV = \frac{242 \text{ mg/L}}{10} = 24 \text{ mg PFBS/L} \qquad (8)$$

Using Tier II methodology, the SCV for PFBS was determined to be 24 mg PFBS/L.

As required by the GLI guidance, a FPV needs to be determined to evaluate the potential hazard a chemical may pose to aquatic plant communities. This value can be based on 96-hr tests conducted with an alga, or a chronic test conducted with an aquatic vascular plant. To date, only a single toxicity study has been conducted with the green alga, *S. capricornutum*, on PFBS that meets the data requirements as outlined in the GLI guidance. In this test, the 96-hr EC_{50}, based on cell count, was 2,347 mg PFBS/L, and the NOEC and LOEC values were 1,077 and 2,216 mg PFBS/L, respectively. The chronic value from these data was 1,545 mg PFBS/L. Thus, the FPV for PFBS was determined to be 1,500 mg PFBS/L.

From methods outlined in the GLI, a CMC for PFBS was calculated by dividing the FAV by 2 and produced a value of 120 mg PFBS/L. The secondary continuous criterion (SCC) is determined as the lower value between the SCV (24 mg PFBS/L) and the FPV (1,500 mg PFBS/L); thus, the SCC for PFBS is 24 mg/L.

3.4.4 PFOA

A review of available acute toxicity data for PFOA with freshwater organisms indicates that there is insufficient data to calculate a Tier I water quality criterion, because only five of the data requirements were met. Consequently, a Tier II water quality criterion was derived as provided for in the GLI guidance. For PFOA, the least GMAV was for the water flea (*D. magna*) that had a 48-hr EC_{50} of 297 mg PFOA/L (Table 12). The SAF, based on five Tier I data requirements being met, was 6.1 as prescribed by GLI guidance. Thus, the SAV for PFOA was calculated as shown in Eq. 9:

$$SAV = \frac{297 \text{ mg/L}}{6.1} = 49 \text{ mg PFOA/L} \qquad (9)$$

To calculate a SCV, the SAV was divided by a FACR. To date, only two acceptable chronic toxicity studies are available that can be used to calculate the species-specific ACR needed to derive a FACR. For *D. magna*, if a SMAV of 297 mg PFOA/L and a CV of 22 mg PFOA/L are used, the ACR was 10. For *O. mykiss*, the SMAV was 752 mg PFOA/L, and the chronic value was 40 mg PFOA/L. From these two values the ACR was determined to be 19. However, a minimum of three species-specific ACR values are needed to calculate a FACR and as a result, a default ACR value of 18 was used to replace the missing data,

Table 12 Summary of genus GMAVs and GMCVs for aquatic organisms exposed to perflurooctanoate (PFOA)[a]

Test species	Genus/species	Test duration	Media	GMAV (mg PFOA/L)
Acute				
Midge	*Chironomus tentans*	96 hr	FW	1,090
Rainbow Trout	*Oncorhynchus mykiss*	96 hr	FW	752
Bluegill	*Lepomis macrochirus*	96 hr	FW	601
Fathead minnow	*Pimephales promelas*	96 hr	FW	511
Water flea	*Daphnia magna*	48 hr	FW	297
Chronic				
Rainbow trout	*Oncorhynchus mykiss*	85 d	FW	40[b]
Water flea	*Daphnia magna*	21 d	FW	21[b]

[a]Genus mean acute values were based on geometric means of acute toxicity values by genus
[b]Values represent GMCV and are based on geometric means of NOAEC values from chronic studies
FW = Fresh Water

as provided for in the GLI Tier II methodology. Thus, the FACR, calculated from these three ACR values, was 17. The SCV for PFOA was calculated as shown in Eq. 10:

$$\text{SCV} = \frac{49 \text{ mg/L}}{17} = 2.9 \text{ mg PFOA/L} \tag{10}$$

By using Tier II methodology, the SCV for PFOA was determined to be 2.9 mg PFOA/L.

Sufficient data were available to calculate a FPV for PFOA that would be protective of aquatic plant communities. To date, acceptable toxicity tests have been conducted with the algae (*S. capricornutum*), and with two milfoil species, *M. spicatum* and *M. sibiricum*. *Myriophyllum* was determined from these studies to be the most sensitive aquatic plant genus, with a 42-d EC_{50} of 34 mg PFOA/L, and a NOAEC of 23.9 mg PFOA/L, using reductions of biomass (dry wt) as the end point. Based on the NOAEC for *Myriophyllum* sp., the FPV for PFOA was determined to be 23.9 mg/L.

Using GLI methodology, a CMC for PFOA was calculated by dividing the FAV by 2, this produced a value of 25 mg PFOA/L. The SCC is determined as the lower value between the SCV (2.9 mg PFOA/L) and the FPV (23.9 mg PFOA/L), therefore, the SCC for PFOA is 2.9 mg/L.

3.5 Water Quality Criteria for the Protection of Wildlife

3.5.1 PFOS

Toxicity reference values (TRVs) have been derived from chronic effects on reproduction in which mallards or quail were chronically exposed via the diet (Newsted et al. 2007). The TRVs were based on quail because treatment-related reproductive effects were observed at 10 mg PFOS/kg, wt/wt feed; in contrast, toxicological and ecological effects were not noted in mallards at this dietary concentration. TRVs were derived from dietary exposure and tissue PFOS concentrations with the intention of protecting fish-eating water birds. These species were selected because they harbor some of the greatest liver and serum PFOS concentrations, when compared to lower trophic level avian species; thus, avian TRVs, protective of all avian species, were derived from the characteristics of trophic level IV fish-eating birds such as eagles and ospreys. Many of these bird species are sensitive to other classes of organic compounds and may provide an early warning for the presence and effects of contaminants within contaminated aquatic ecosystems (Ankley et al. 1993; Bowerman et al. 1998). In addition, by factoring in characteristics of predatory birds, such as weights, daily food consumptions, and species-specific transfer coefficients, contributions of PFOS from both aquatic and terrestrial exposure pathways can be incorporated into the derivation of avian TRVs (Giesy et al. 1994; Giesy and Kannan 1998).

3.6 Derivation of PFOS TRVs for a Level IV Avian Predator

TRVs for level IV birds were developed by using the uncertainty factor (UF) approach, as described in the US EPA GLI methodology (US EPA 1995). In this approach, three categorical uncertainties were delineated. These included the following: (1) uncertainty with LOAEL to NOAEL extrapolation (UF_L), (2) uncertainty related to duration of exposure (UF_S), and (3) uncertainty related to inter-taxon extrapolations (UF_A). In this approach, UFs for each category are assigned values between 1 and 10 that are based on available scientific findings and best professional judgment (Chapman et al. 1998). Using the data from the quail reproduction study, and the characteristics of a level IV avian predator, a final UF of 24 was assigned; this UF accounted for data gaps and extrapolations in the analysis (Table 13). TRVs, that were based on dietary concentrations, average daily intake (ADI), and egg PFOS concentrations, were 0.42 mg PFOS/kg feed, 0.032 mg PFOS/kg bwt/d, and 2.6 μg PFOS/ml egg yolk, respectively (Table 14). Because sex-specific differences in adult serum and liver PFOS concentrations were observed in the toxicity studies, TRVs based on these end points represent a range of values that encompass all avian reproductive conditions. The sex-specific differences in serum and liver PFOS concentrations, at study termination, were probably a

Table 13 Assignment of uncertainty factors for the calculation of a generic trophic level IV avian predator toxicity reference value (TRV) for PFOS[a]

Uncertainty factors	Notes
Inter-taxon extrapolation (UF_A)	The laboratory study used to determine a threshold dose was from northern bobwhite quail; this species belongs to the same taxonomic class but is in a different order, $UF_A = 6$
Toxicological end point (UF_L)	An LOAEL, but not a NOAEL, was determined in the quail study, based on multiple end points that included reproduction. Furthermore, the difference between the LOAEL and the control was less than 20% for the affected reproductive end points. Taken together with other study data, the $UF_L = 2$
Exposure duration (UF_S)	The quail reproductive study was conducted for 20 wk; several important life stages were evaluated including embryonic development and offspring growth and survival, so $UF_s = 2$
Overall UF for TRV	$UF = 6 \times 2 \times 2 = 24$

[a]Selection of uncertainty factors based on the Great Lake Initiative (US EPA 1995)

Table 14 PFOS toxicity reference values (TRVs) for a generic trophic level IV avian predator based on dietary, liver, and serum toxic doses[a]

	Male LOAEL	TRV[b]	Female LOAEL	TRV[b]
ADI (mg PFOS/kg bwt/d)[c]	0.77	0.032	0.77	0.032
Liver (µg PFOS/g, wt/wt)	88	3.7	4.9	0.20
Serum (µg PFOS/ml)	141	5.9	8.7	0.36
Egg Yolk (µg PFOS/ml)			62	2.6

[a]LOAEL values based on bobwhite quail definitive study
[b] TRV estimated with total uncertainty factor of 24 derived using the US EPA GLI protocol
[c]ADI= Average daily intake (mg PFOS/kg bwt/d); estimates were based on pen averages

result of PFOS being transferred to eggs from adult females during egg-laying. This is substantiated by the fact that during the pre-reproductive phase of the study, serum concentrations in females were similar to those observed in males (Newsted et al. 2007). Therefore, the reproductive condition of the bird affects the relevant serum and liver values.

Water quality criteria for the protection of avian species can be calculated using modified procedures that are explained in GLI guidance (US EPA 1995). Modifications to the GLI procedures were focused primarily on the derivation of BAFs that are used to model the accumulation of residues from water in trophic level III and IV fish. The procedures outlined in the GLI for deriving these BAFs are based on chemical-specific K_{ow} values. However, because PFOS has surfactant qualities, a K_{ow} value has not been directly measured in the laboratory. Consequently, literature values were used to estimate the potential biomagnification of PFOS into upper trophic level fish. The site-specific nature of most field-derived BAF values resulted in a baseline BAF being calculated from laboratory BCF values. Several laboratory BCF values have been determined for fish exposed to waterborne PFOS (Drottar et al. 2001; Martin et al.

2003b). For bluegill (*L. macrochirus*), a BCF of 3,614, based on whole body PFOS concentrations, was derived by kinetic analysis (Drottar et al. 2001). In rainbow trout (*O. mykiss*), a BCF of 1,100 was derived from carcass PFOS concentrations (Martin et al. 2003b). The baseline BAF for PFOS was calculated as the geometric mean of these BCF values (BAF=1,994). To address potential biomagnifications rates into trophic level IV fish, literature values from a Laurentian Great Lakes food chain study were used (Kannan et al. 2005). In this study, PFOS concentrations in predatory fish, including whitefish and Chinook salmon, were approximately 10–20 times greater than that measured in prey fish. However, PFOS concentrations in the predatory fish were based on liver concentrations, a tissue for which PFOS is preferentially accumulated compared to whole body concentrations. As a result, the biomagnification factor (BMF) from this study could have been approximately four to five times greater if whole-body PFOS concentrations had been used in these analyses (Martin et al. 2003b). A BMF value of 5 was used to predict the concentration of PFOS in trophic level IV. Finally, for the consumption of piscivorous birds by upper trophic level avian predators (e.g., herring gull by eagles), the BAF is derived by multiplying the baseline BAF for fish by a BMF to account for the biomagnification from fish into birds. Currently, there are few reliable data from field studies that can be used to derive a BMF. As a result, the data from the mallard and northern bobwhite definitive reproduction studies were used to calculate a BMF (Newsted et al. 2007). Based on the results of the definitive reproduction study with bobwhite quail and mallards, BMFs were calculated by dividing the mean concentration of PFOS in liver by the concentration of PFOS in the diet (Leonards et al. 1997). Liver concentrations were used to calculate the BMF, because liver PFOS concentrations were less variable than serum concentrations and would be a better measure of accumulation. Finally, only the male liver PFOS concentrations were used in the calculation of the BMF, because their liver concentrations were unaffected by laying of eggs, as was observed in females from these treatment groups. The effect of laying eggs and the resulting loss of PFOS from the hens would introduce a bias and underestimate the actual BMF. In northern bobwhites males from the 10 ppm PFOS treatment, an average liver concentration of 88 µg PFOS/g resulted in a BMF of 8.8. For mallards from the 10 ppm treatment group, an average liver concentration of 61 µg PFOS/g resulted in a BMF of 6.1. Using the geometric mean of the northern bobwhite and mallard BMF values, an overall BMF of 7.3 was calculated, and this value was used in the water quality criterion calculations. This value is similar to that observed for bald eagles from the Great Lakes, where PFOS concentrations in the livers of bald eagles were 10–20 times greater than that measured in the livers of salmon (Kannan et al. 2005).

According to the GLI guidelines, three avian species are selected to represent avian wildlife in the WQC calculations. The use of these representative species is meant to be protective of all avian wildlife. The three species are the bald eagle, the herring gull, and the belted kingfisher. These birds are all residents of the

Great Lakes basin and are likely to experience the greatest exposures to contaminants through the food web. Exposure parameters, including body weights (BW), feeding rates (F_{TLi}), drinking rates (W), and trophic level dietary composition (as food ingestion rate and food item percent in the diet), for each representative avian species are listed in Table 15. Calculation of a water quality criteria for the protection of avian species were based on these species-specific parameters, as shown in Eq. 11:

$$WV = \frac{\frac{TD}{UF_A \; xUF_S \; xUF_L} x \; BW}{W + \sum \left(F_{TLi} \; xBAF^{WL}_{TLi} \right)} \tag{11}$$

where WV = wildlife value in milligrams of PFOS per liter (mg PFOS/L); TD = test dose or threshold dose in mg of PFOS per kg per day (mg PFOS/kg, bwt/d) for the test species; UF = uncertainty factor for extrapolating toxicity across species (UF_A), for sub-chronic to chronic exposures (UF_S), and for LOAEL to NOAEL extrapolations (UF_L). All UF values are unit-less; BW = average body weight in kilograms (kg) for the representative species; F_{TLi} = species-specific average daily amount of food consumed (kg/d) for trophic level (i); W = species-specific average daily amount of water consumed (L/d); BAF^{WL}_{TLi} = bioaccumulation factor for wildlife food in trophic level (i). For consumption of piscivorous birds by other birds, the BAF is derived by multiplying the trophic level III BAF by the biomagnification factor (L/kg).

WVs for the three avian species were calculated from exposure values given in the GLI guidance (Table 15), and using the following fate and toxicological properties for PFOS.

Fate properties:		Toxicological properties	
BAF_3	1,994	$ADI_{(LOAEL)}$	0.77 mg PFOS/kg
bwt/d			
BAF_4	9,970	Total UF	24
BMF	7.3		
BMF_{other}	0		

Using these data, the calculated wildlife values for the individual species were the following:

Herring gull:	41 ng PFOS/L
Bald eagle:	71 ng PFOS/L
Kingfisher:	36 ng PFOS/L

The final avian wildlife value was calculated as the geometric mean of all three avian wildlife values, thus

Avian wildlife value:	47 ng PFOS/L

Table 15 Exposure parameters for three avian surrogate species identified for protection

Species	Adult body wt (kg)	Water ingestion rate (L/d)	Food ingestion rate of each prey in each trophic level (kg, wt/wt/d)	Trophic level of prey (% diet)
Herring gull	1.1	0.063	TL3: 0.192; TL4: 0.0480; Other: 0.0267	Fish: 90 (TL3: 80; TL4: 20). Other: 10
Bald eagle	4.6	0.160	TL3: 0.371; TL4: 0.0929 PB: 0.0283; Other: 0.0121	Fish: 92 (TL3: 80; TL4: 20). Birds: 8: (PB: 70; other: 30)
Belted Lingfisher	0.15	0.017	TL3: 0.0672	TL3: 100

TL3 or TL4 = trophic level III or IV fish; PB = piscivorous birds; Other = non-aquatic birds and mammals

3.7 Derivation of PFBS TRVs for a Level IV Avian Predator

As with PFOS, the TRV was derived by using the UF methodology described in the US EPA GLI methodology (US EPA 1995). Using the data from the quail reproduction study (Newsted et al. 2008), and the characteristics of a level IV avian predator, a final UF of 12 was assigned to account for data gaps and extrapolations in the analysis (Table 16). TRVs, based on dietary, ADI, and egg PFOS concentrations, were 50 mg PFBS/kg feed, 7.3 mg PFBS/kg bwt/d, and 5.7 mg PFBS/ml whole egg, respectively (Table 17). Unlike PFOS, sex-specific differences in accumulation of PFBS from the diet into blood serum and liver of quail were not great and were generally less than 1.6-fold. Thus, the reproductive condition of the bird may not be important in determining concentrations of PFBS in serum and liver; however, because sample size was small, the toxicological or ecological significance of these differences is unknown.

Table 16 Assignment of uncertainty factors for the calculation of a generic trophic level IV avian predator toxicity reference value (TRV) for PFBS[a]

Uncertainty factors	Notes
Inter-taxon extrapolation (UF$_A$)	The laboratory study used to determine a threshold dose was from northern bobwhite quail; this species belongs to the same taxonomic class but is in a different order, UF$_A$ = 6
Toxicological end point (UF$_L$)	A NOAEL was determined from a quail study and was based on multiple end points that included reproduction. Taken together with other study data, the UF$_L$ = 1
Exposure duration (UF$_S$)	The quail reproductive study was conducted for 20 wk and evaluated several important life stages including embryonic development and offspring growth and survival, so UF$_s$ = 2
Overall UF for TRV	UF = 6×1×2 = 12

[a]Selection of uncertainty factors based on the Great Lake Initiative (US EPA 1995)

Table 17 PFBS TRVs for a generic trophic level IV predator based on dietary, liver, and serum toxic doses[a]

	Male		Female	
	NOAEL	TRV[b]	NOAEL	TRV[b]
ADI (mg PFBS/kg bwt/d)[c]	87.7	7.3	87.8	7.3
Liver (mg PFBS/g, ww)	16	1.3	30	2.5
Serum (mg PFBS/ml)	68	5.7	104	8.7
Egg yolk (mg PFBS/ml)			68	5.7

[a]NOAEL values based on bobwhite quail definitive study
[b]TRV estimated with total uncertainty factor of 12 derived using the US EPA GLI protocol
[c]ADI = average daily intake (mg PFBS/kg bwt/d); estimates were based on pen averages

3.8 PFBS Water Quality Criteria for Protection of Aquatic Predatory Birds

A baseline BAF for PFBS was calculated from laboratory BCF values (Wildlife International 2001a–g; Martin et al. 2003b). For bluegill (*L. macrochirus*), a steady state BCF of less than 1, based on whole body PFOS concentrations, was derived by kinetic analysis (WLI 2001d). This result was similar to that found in rainbow trout (*O. mykiss*), where a BCF was also determined to be less than 1 (Martin et al. 2003b). Thus, as a conservative measure, the baseline BAF was estimated to be 1 for PFBS. Unlike PFOS, no biomagnification of PFBS into upper trophic level fish was assumed, since laboratory studies have not indicated that PFBS is bioaccumulated via the diet (Martin et al. 2003b); the BAF for upper trophic level was also assumed to be 1.0.

The biomagnification of PFBS into avian species from consumption of fish was determined from the quail dietary reproduction study (Newsted et al. 2008). In this study, liver concentrations in both adult male and female quail were approximately 30-fold less than that measured in the feed, indicating that biodiminution may have occurred. In addition, no significant sex-related differences were observed in the study; this indicated that reproductive condition may not be important in predicting bird tissue concentrations. A BMF of 1.0 was used as conservative value to account for potential food web accumulation of PFBS into upper trophic level birds.

WV values for the three avian species were calculated from exposure values given in the GLI guidance (Table 15) and from the following fate and toxicological properties for PFBS.

Fate properties:		Toxicological properties	
BAF_3	1	$ADI_{(LOAEL)}$	87.8 mg PFBS/kg bwt/d
BAF_4	1	Total UF	12
BMF	1		
BMF_{other}	0		

Using these data, the calculated wildlife values for the individual species were the following:

Herring gull:	24 mg PFBS/L
Bald eagle:	16 mg PFBS/L
Kingfisher:	13 mg PFBS/L

The final avian wildlife value was calculated as the geometric mean of all three avian wildlife values, thus

Avian Wildlife Value:	17 mg PFBS/L

3.9 QSAR Analyses

Little toxicity information exists for many PFCs, especially in vivo toxicity data. Therefore, QSARs were developed to estimate the toxicity of PFCs for which no measured information is available. The results of in vitro and in vivo toxicity studies with PFCs have shown that the two principle determinants of biological activity and bioaccumulation are (1) the length of the fluorinated carbon chain and (2) the functionality of the head group (Goecke-Flora and Reo 1996; Hu et al. 2002; Lau et al. 2007). Specifically, the results of these studies have shown that the bioaccumulation potential and toxicity of PFCs increase with increasing fluorinated carbon chain length and that, in general, compounds that have sulfonic acid moieties tend to be more toxic than their carboxylic acid counterparts. In addition, the presences of primary and secondary amides have a significant effect on the toxicity of these compounds (Starkov and Wallace 2003). These findings can also be extended to aquatic organisms, where chain length, head group functionality, as well as the presence of amide groups, can also influence the toxicity and bioaccumulation potential of fluorochemicals.

Although few bioconcentration studies with PFCs in aquatic organisms exist, laboratory studies with fish have shown that the bioaccumulation potential of perfluorinated carboxylates (PFCAs) and perfluorinated sulfonates (PFAS) is related to chain length, with the greatest accumulation being observed for those compounds with the longest fluorinated carbon chains (Martin et al. 2003a, 2003b; Condor et al. 2008). In rainbow trout, PFCA and PFAS compounds, with fluorinated carbon chains shorter than seven and six, respectively, do not bioaccumulate and typically have BCFs less than 1.0 (Fig. 3). Bioconcentration factors were found to increase by a factor of approximately 8 for each additional fluorinated carbon for PFCAs with chain lengths of 8–12 (Martin et al 2003b). However, PFCAs with fluorinated carbon chains greater than 12 accumulated in rainbow trout to a lesser degree than did PFCAs with shorter carbon chains; this suggests that bioaccumulation potential may be limited by molecular size. Although only limited laboratory data are available for PFAS compounds, the general relationship
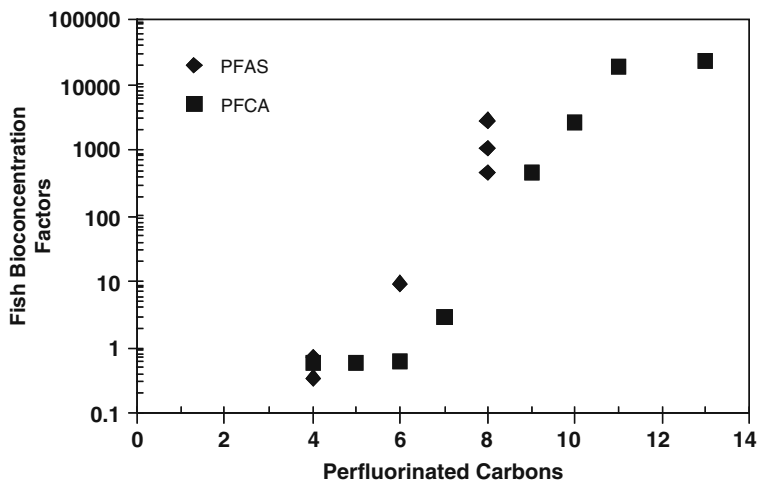
Fig. 3 Relationship between fluorinated carbon chain length of perfluorinated sulfonates (PFAS) and perfluorinated carboxylates (PFCA), and bioconcentration in several fish species including rainbow trout, fathead minnow, and bluegill

between fluorinated carbon chain length and bioaccumulation potential for these compounds is similar to that of the PFCAs, wherein both BCF and BAF values increase with chain length. For rainbow trout, a comparison of BCFs for PFBS (4 carbons), perfluorohexane sulfonate (PFHxS; 6 carbons), and PFOS (8 carbons) indicated that the BCF for PFHS was approximately 100-fold less than PFOS (8 carbons), whereas the BCF value for PFBS was at least 1,500-fold less than the PFOS BCF value. However, because a measurable BCF for PFBS may result from tissue concentrations being less than the method detection limit (MDL) (Martin et al. 2003b), a definitive evaluation could not be made. However, in studies with bluegill, the BCF value for PFOS was shown to be approximately 11,000-fold greater than the value determined for PFBS (3M 2003; NICNAS 2005). Collectively, these data indicate that, for PFAS compounds, an approximately 100-fold increase in bioaccumulation potential occurs when two fluorinated carbons are added to the chain length (for PFAS with 4–8 carbons). However, bioconcentration studies have not yet been conducted with PFAS with greater than eight fluorinated carbons, and it is, therefore, not known if this relationship will be borne out in future studies. Moreover, molecular size limitations may affect the bioaccumulation potential of PFAS compounds as it does with PFCAs. Finally, a comparison of bioconcentration and bioaccumulation factors of PFCA and PFAS compounds, with carbon chain lengths of five or greater, indicates that PFAS compounds tend to have greater bioaccumulation potentials than do PFCA compounds of the same carbon chain lengths, in a manner that is similar to that observed for mammalian species (Ohmori et al. 2006; Lau et al. 2007).

Studies conducted in vitro with several different mammalian cell lines have shown that the length of the carbon chain of fluorinated compounds is related to their potency as measured by cytotoxicity or inhibition of gap junctional intercellular communication (GJIC) (Fig. 4). GJIC is a process by which cells exchange small molecules (ions, second messengers, low molecular weight metabolites, etc.) and is involved in normal growth, development, and differentiation of tissues (Trosko and Rush 1998). In a study with rat liver epithelial cells (WB-F344), PFCAs with carbon chain lengths of 7–10 rapidly and reversibly inhibited GJIC in a dose-dependent manner. In contrast, PFCAs with carbon chain lengths of 2–5, 16, or 18 did not appreciably inhibit GJIC (Upham et al. 1998). In addition, PFOS also inhibited GJIC in a dose-dependent manner and was more potent than PFOA, a C7 carboxylate. In a subsequent study, PFOS, perfluorooctane sulfonamide (PFOSA), and PFHS all inhibited GJIC in a dose-dependent manner, whereas exposure to PFBS, a 4-carbon PFAS, did not affect GJIC (Hu et al. 2002). The toxicity of PFCs to mammalian cells is also directly proportional to the length of the fluorinated carbon chain, and such chain length is the primary determinant of toxicity (Fig. 4). In the study by Hu et al. perfluorinated carboxylates with carbon chain lengths less than five were not cytotoxic; toxicity increased with increasing carbon chain lengths for those compounds with chain lengths of 5–13 (Kleszczynski et al. 2007; Mulkiewicz et al. 2007). However, for PFCAs with greater than 13 fluorinated carbons, the relationship between chain length and toxicity deviated from linearity, with potency actually being less than that observed for
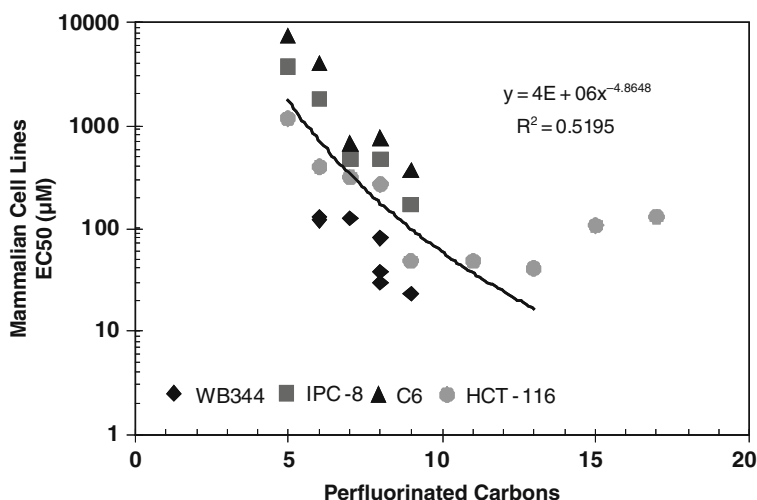

Fig. 4 Relationship between cytotoxicity and gap junctional intercellular communication (GJIC) inhibition and fluorinated carbon chain length of PFAS and PFCA in several mammalian cell lines. Regression model includes all perfluorinated compounds except for those with carbon chain lengths greater than 13

perfluorotetradecanoic acid (PFTeDA: C13). In summary, the results from both the GJIC and the cytotoxicity assays show that the primary determinant of perfluorinated compound potency is the length of the fluorinated carbon chain, and although the functionality of the head group may influence potency, the significance of this influence can only be ascertained when additional in vitro toxicity data are available.

The length of the fluorinated carbon chain and functionality of the head group are also related to the aquatic toxicity of perfluorinated chemicals, as affirmed in several freshwater species, including rainbow trout, bluegill, and fathead minnow (Fig. 5A), and the cladoceran *D. magna* (Fig. 5B). In fish, the toxicity of both PFAS and PFCA compounds was directly related to carbon chain length; PFCA compounds were less toxic than PFAS compounds with equivalent carbon chain lengths.

Results of acute toxicity studies on *D. magna*, with PFAS and PFCA compounds, have shown increased toxicity as carbon chain length increases; the PFAS compounds tend to be more toxic than PFCA compounds with equivalent chain lengths. Interestingly, the toxicity of saturated (FTCA) and unsaturated (FTUCA) fluorotelomer carboxylic acid was related to the length of the carbon chain, with toxicological potency increasing with increasing chain length (Boudreau et al. 2002; Phillips et al. 2007). When all groups of PFCs were compared as to chain length of their fluorinated carbons, FTCA and FTUCA compounds were generally more toxic than were PFAS or PFCA compounds. Although the basis for the greater toxicity of these fluorotelomers to *D. magna* has not yet been fully examined, insight may be gained from results of a metabolic study with rat hepatocytes, in which the metabolism and disposition of fluorotelomer alcohols were evaluated (FTOH; Martin et al. 2005). In this study, 8:2 FTOH was first oxidized to a transient fluorotelomer aldehyde or and then was further oxidized to either an unsaturated aldehyde, 8:2 FTCA that could then be converted to its unsaturated form, 8:2 FTUCA. These unsaturated fluorotelomer metabolites eventually react with glutathione. Because these compounds may also react with other cellular nucleophiles, such reactivity could result in toxicity, as was observed in studies of the effects of PFCs on *D. magna*. However, more detailed, mechanistic studies are needed before the underlying basis for the toxicity of these compounds to aquatic organisms can be understood.

The presence of amide functional groups also increased the toxicity of PFAS compounds to fish (Fig. 5A), and to *D. magna* (Fig. 5B), when compared to PFAS compounds with equivalent carbon chain lengths. The effect of amides on the toxicity of PFAS compounds is not unexpected, because such effects were observed when the impact of such amides on mitochondrial energetics of mammals was studied (Langely 1990; Schnellmann and Manning 1990; Starkov and Wallace 2002). In these studies, PFCs such as PFOA and PFOS, as well as a fully saturated amide, *n*-ethyl perfluorooctane sulfonamidoethanol (*n*-EtFOSE), were found to be relatively weak inhibitors of mitochondrial oxidative phosphorylation and appeared to act in a non-selective manner on
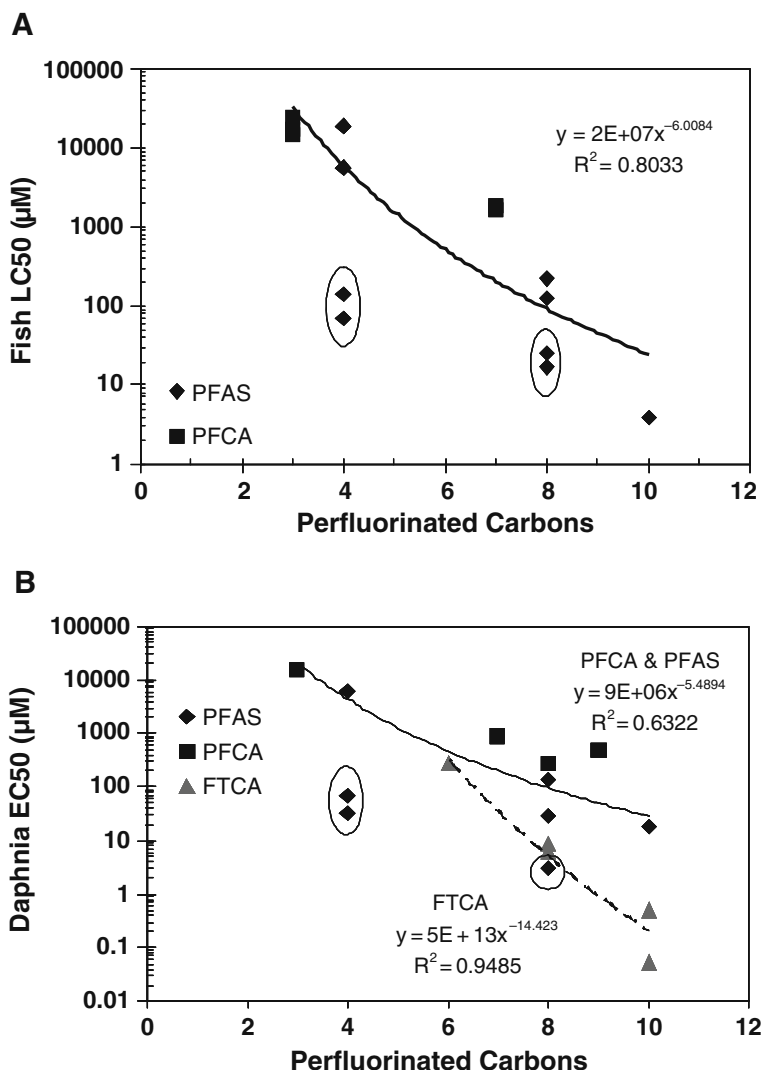
Fig. 5 Acute toxicity of PFAS, PFCA, and fluorotelomer carboxylic acids (FTCA and FTUCA) to aquatic organisms. (**A**) LC_{50} values for bluegill, fathead minnow, and rainbow trout. (**B**). EC_{50} mortality value for the cladoceran, *Daphnia magna*. Fish regression models include both PFAS and PFCA data, while models based on daphnids represent PFAS/PFCA and fluorotelomer data, respectively. Circles identify alcohol and amide sulfonic acids and were not included in the regression analyses

mitochondrial membrane permeability at relatively high concentrations (>100 μM). In contrast, perfluorinated chemicals with secondary amide groups, such as PFOSA and n-EtFOSA, were potent inhibitors (at 5–50 μM levels) of mitochondrial oxidative phosphorylation. The effects of perfluorinated

chemicals on mitochondrial bioenergetics are similar to the protonophoric mechanism of action that is observed in the liver of rats exposed to dinitrophenol. Under this hypothesis, compounds that have ionizable amide groups with favorable pKa values can shuttle protons back into the mitochondrial matrix and dissipate the proton motive force generated by the electron transport, thus disrupting oxidative phosphorylation. Therefore, it is not simply the presence of an amide group that impacts the potency of a fluorinated compound to inhibit mitochondrial function. It is also important that the amide group become protonated under prevailing physiological conditions. As such, the substituted amides such as PFOSA, *n*-ethyl perfluorooctane sulfonamidoacetic acid (PFO-SAA), *n*-methyl perfluorooctane sulfonamidoethanol (*n*-MeFOSE), and *n*-ethyl perfluorooctane sulfonamide (n-EtFOSA) are potent inhibitors of mitochondrial oxidative phosphorylation, whereas other compounds, such as *n*-EtFOSE and *n*-EtFOSAA that lack the protonated amide, are not effective inhibitors of mitochondrial phosphorylation.

In studies conducted with freshwater algae and macrophytes, the toxicological potency of PFCA was found not to be related to the length of the fluorinated carbon chain, as was observed for aquatic organisms (Fig. 6). For example, in toxicity studies conducted with the green alga *S. capricornutum*, the toxicity of PFCA compounds was not related to carbon chain length, but rather the EC_{50} values were found to vary between 100 and 400 µM for PFCs with chain lengths of 2–9 carbons (Fig. 6A). Because toxicity data for PFAS compounds is limited, the nature of the relationship between chain length and toxicity is difficult to quantify. Notwithstanding, chain length appears to an important determinant of toxicity in that PFBS (C4) was approximately 50-fold less toxic than was PFOS (C8). However, additional study results will be required before the relationship between carbon chain length and toxicity can elucidated. The effect of amide groups on toxicity is also observed for *S. capricornutum,* in that PFBS was significantly less toxic than amide-containing PFBS compounds such as methyl perfluorobutane sulfonamidoethanol and methyl perfluorobutane sulfonamide. The absence of a relationship between PFAS and PFCA chain length and toxicity in aquatic plants was observed in toxicity studies conducted with the aquatic macrophytes, *Lemna* sp. (Fig. 6B; Boudreau et al. 2002; 3M 2003). In these studies, the toxicity of PFCAs and PFASs ranged from approximately 100 to 300 µM, but there was no linear relationship between either carbon chain length or functional head group and toxicity. In contrast, the toxicity of saturated and unsaturated fluorotelomer carboxylic acids was related to carbon chain length for compounds with 4–8 fluorinated carbons (Fig. 6B). The relationship between carbon chain length and toxicity deviated from linearity for fluorotelomers with greater than eight carbons. For 10:2 FTCA and 10:2 FTUCA compounds, toxicity actually decreased from that observed for 8:2 fluorotelomers, in a manner similar to that observed for the mammalian cell line HCT-116, in which cytotoxicity of PFCA compounds with greater than 13 carbons were less toxic than the most toxic PFCA evaluated, PFTeDA (Fig. 4).
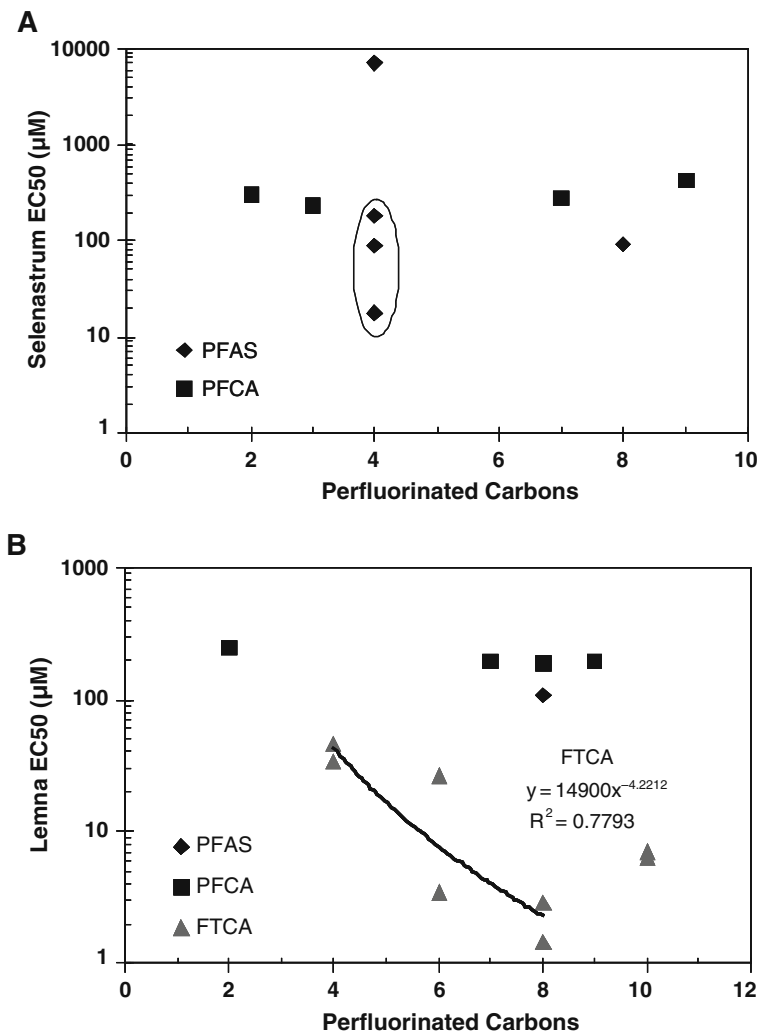
Fig. 6 Inhibition of growth and/or biomass of aquatic plants exposed to fluorinated chemicals. (**A**) Data from studies with *Selenastrum capricornutum* exposed to PFAS acid and PFCA acids. (**B**) Data from studies conducted with duckweed, *Lemna* sp exposed to PFAS, PFCA, FTCA, or FTUCA. Circles identify alcohol and amide sulfonic acids and were not included in the regression analyses

Results available in the literature on the toxicity of PFCs to aquatic organisms indicate that toxicity of fluorinated chemicals is related to length of the fluorinated carbon chain; the nature of functional groups has relatively little effect on the toxic potency of these compounds. Moreover, the addition of amide groups that can be protonated, under environmental conditions,

tends to increase toxic potency of PFCs relative to PFCs of similar chain length. However, additional studies are needed with other fluorinated compounds, as well as with other aquatic species, before we can accept as correct this finding on the potency of fluorinated chemicals to aquatic organisms. Notwithstanding, no such linear relationship between carbon chain length and toxicity has yet been verified for PFCA and PFAS compounds and aquatic plants. The exception to this finding is for saturated and unsaturated fluorotelomer carboxylic acids, wherein chain length was the primary determinant of toxic potency. Additional studies are needed to more fully understand this relationship and also to evaluate the ecological significance of this finding within the context of current environmental concentrations of these compounds. Although the analysis given above indicates that structure–activity relationships can be derived from existing data, there are still numerous data gaps that need to be addressed to quantify the toxicity of different classes of perfluorinated compounds and the relative susceptibility of aquatic organisms and plants. When such data are available it will be feasible to develop more sophisticated models to predict the toxicity of fluorinated compounds to aquatic organisms.

4 Conclusions

From available aquatic organism toxicity data, PFOS concentrations that were protective to selected aquatic species were calculated for surface waters and fish tissues. Using the Great Lakes Initiative, water concentrations of PFOS were calculated to protect aquatic plants and animals. The final plant value was calculated as 8.2 mg PFOS/L; the secondary chronic value for aquatic animals was calculated as 0.46 mg PFOS/L. Based on these calculations, chronic water concentrations less than or equal to 0.46 mg PFOS/L should not pose a significant adverse risk to aquatic organisms, and concentrations of 0.78 mg PFOS/L should be protective of aquatic organisms under acute exposure scenarios. A critical body residue level for PFOS in fish tissues was calculated from a bluegill bioaccumulation study with PFOS. Based on the lower 95% confidence interval of the LC_{01}, a tissue PFOS concentration of 87 mg/kg, wt/wt was calculated as the threshold value below which PFOS is not expected to pose a risk to fish populations. However, results from a toxicity study, wherein PFOS concentrations were measured in freshwater mussels, indicate that significant mussel mortality is associated with tissue concentrations of 88.8 mg PFOS/kg, wt/wt. Thus, it is possible that freshwater mussels are more sensitive to PFOS exposure than are fish. It is also probable that additional studies are needed to more accurately address the tissue residue concentrations of PFOS associated with toxicity in fish.

5 Summary

PFCs are released into the environment via their uses as wetting agents, lubricants, stain resistant treatments, and foam in fire extinguishers. PFOS is the terminal breakdown product of many commercially used perfluorinated compounds and is often the predominant PFC found in the environment. PFOS is resistant to chemical and biological changes and does not significantly degrade under environmental conditions. As a result of its low volatility and strong soil adsorption PFOS has little mobility in the environment. In laboratory and field tests, PFOS has been shown to bioconcentrate in fishes. More information is available about PFOS than for any other PFC. Toxicity studies with plants, invertebrates, and vertebrates from both terrestrial and aquatic habitats have been conducted with PFOS. Therefore, PFOS is used as an example compound in this chapter. Based on available toxicity studies, concentrations of PFOS were calculated that are protective of aquatic plants and organisms in surface waters. A critical body concentration of PFOS was calculated for fish that would be protective of top predators.

References

3M (2003) Environmental and health assessment of perfluorooctane sulfonic acid and its salts. Prepared by 3M Company with J. Moore (Holly house Inc., J. Rodricks and D. Turnbull (Environ Corp) and W. Warren Hicks (Cadmus Group). August 2003.

Ankley GT, Niemi, GJ, Lodge KB, Harris HJ, Beaver DL, Tillitt DE, Schwartz T, Giesy JP, Jones PD, Hagley C (1993) Uptake of planar polychlorinated biphenyls and 2,3,7, 8-substituted polychlorinated dibenzofurans and dibenzo-p-dioxins by birds nesting in the lower Fox River and Green Bay, Wisconsin, USA. Arch Environ Contam Toxicol 24:332–344.

Ankley GT, Kuehl DW, Kahl MD, Jensen KM, Butterworth BC, Nichols JW (2004) Partial life-cycle toxicity and bioconcentration modeling of perfluorooctanesulfonate in the northern leopard frog (*Rana pipiens*). Environ Toxicol Chem 23:2745–2755.

Barron MG, Hansen JA, Lipton J (2002) Association between contaminant tissue residues and effects in aquatic organisms. Rev Environ Contam Toxicol 173:1–37.

Boudreau T, Sibley P, Mabury SA, Muir DCG, Solomon KR (2002) Toxicity of perfluoroalkyl carboxylic acids of different chain lengths to selected freshwater organisms. Department of Environmental Biology, Masters Thesis, University of Guelph, ON, p. 134.

Boudreau TM, Sibley PK, Mabury SA, Muir DCG, Solomon KR (2003a) Laboratory evaluation of the toxicity of perfluorooctane sulfonate (PFOS) on *Selenastrum*

capricornutum, Chlorella vulgaris, Lemna gibba, Daphnia magna, and *Daphnia pulicaria.* Arch Environ Contam Toxicol 44:307–313.

Boudreau TM, Wilson CJ, Cheong WJ, Sibley PK, Mabury SA, Muir DCG, Solomon KR (2003b) Response of the zooplankton community and environmental fate of perfluorooctane sulfonic acid in aquatic microcosms. Environ Toxicol Chem 22:2739–2745.

Bowerman WW, Best DA, Grubb TG, Zimmerman M, Giesy JP (1998) Trends of contaminants and effects in bald eagles of the Great Lakes Basin. Environ Monitor Assess 53:197–212.

CCME (1999) A protocol for the derivation of water quality guidelines for the protection of aquatic life. Canadian Environmental Quality Guideline. Canadian Council of Ministers of the Environment, Ottawa.

Chapman PM, Fairbrother A, Brown D (1998) Critical evaluation of safety (uncertainty) factors for ecological risk assessment. Environ Toxicol Chem 17:99–108.

Condor JM, Hoke RA, De Wolf W, Russell MH, Buck RC (2008) Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. Environ Sci Technol 42:995–1003.

Desjardins D, Sutherland CA, VanHoven RL, Krueger HO (2001a) PFOS: A 96-hr toxicity test with the freshwater alga (*Anabaena flos-aquae*). Wildlife International, Ltd., Project Number 454A-110B, EPA Docket AR226-0186.

Desjardins D, Sutherland CA, VanHoven RL, Krueger HO (2001b) PFOS: A 96-hr toxicity test with the marine diatom (*Skeletonema costatum*). Wildlife International, Ltd., Project Number 454A-113A, EPA Docket AR226-1030a056.

Desjardins D, Sutherland C, VanHoven RL, Krueger HO (2001c) PFOS: A 7-d toxicity test with duckweed (*Lemna gibba* G3). Wildlife International, Ltd. Project Number 454-111, EPA Docket AR226-1030a054.

Di Toro DM, McGarth JA, Hansen DJ (2000) Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. I. Water and tissue. Environ Toxicol Chem 19:1951–1970.

Dixon DA (2001) Fluorochemical decomposition process. Theory, modeling, and simulation. WR Wiley Environmental Molecular Sciences Lab., Pacific Northwest National Lab., Richmond, WA.

Drottar KR, Krueger HO (2000a) PFOS: A 96-hr toxicity test with the freshwater alga (*Selenastrum capricornutum*). Wildlife International, Ltd., Project Number 454A-103A, EPA Docket AR226-0085.

Drottar KR, Krueger HO (2000b) PFOS: A 48-hr static acute toxicity test with the cladoceran (*Daphnia magna*). Wildlife International, Ltd., Project No. 454A-104, EPA Docket AR226-0087.

Drottar KR, Krueger HO (2000c) PFOS: A 96-hr static acute toxicity test with the freshwater mussel (*Unio complanatus*). Wildlife International, Ltd., Project No. 454A-105, EPA Docket AR226-0091.

Drottar KR, Krueger HO (2000d) PFOS: A 96-hr static acute toxicity test with the saltwater mysid (*Mysidopsis bahia*). Wildlife International, Ltd. Project No. 454A-101, EPA Docket AR226-0095.

Drottar KR, Krueger HO (2000e) PFOS: A 96-hr shell deposition test with the eastern oyster (*Crassostrea virginica*). Wildlife International, Ltd., Project No. 454A-106, EPA Docket AR226-0089.

Drottar KR, Krueger HO (2000f) PFOS: A semi-static life-cycle toxicity test with the cladoceran (*Daphnia magna*). Wildlife International Ltd., Project No. 454A-109, EPA Docket AR226-0099.

Drottar KR, Krueger HO (2000 g) PFOS: A flow through life-cycle toxicity test with the saltwater mysid (*Mysidopsis bahia*). Wildlife International, Ltd., Project No. 454A-107, EPA Docket AR226-0101.

Drottar KR, Krueger HO (2000 h) PFOS: A 96-hr static acute toxicity test with the fathead minnow (*Pimephales promelas*). Wildlife International, Ltd., Project No. 454-102, EPA Docket AR226-0083.

Drottar KR, Krueger HO (2000i) PFOS: An early life-stage toxicity test with the fathead minnow (*Pimephales promelas*). Wildlife International, Ltd., Project No. 454-108, EPA Docket AR226-0097.

Drottar KR, VanHoven RL, Krueger HO (2001) Perfluorooctanesulfonate, potassium salt (PFOS): A flow-through bioconcentration test with the bluegill (*Lepomis macrochirus*). Wildlife International, Ltd., Project No. 454A-134, EPA Docket AR226-1030a042.

Ellefson M (2001a) Solubility of PFOS in natural seawater and an aqueous solution of 3.5% of sodium chloride. 3M Company, 3M Environmental Laboratory, Laboratory Project No. E00-1716, EPA Docket AR226-1030a026.

Ellefson M (2001b) Solubility of PFOS in octanol. 3M Company, 3M Environmental Laboratory, Laboratory Project No. E00-1716, EPA Docket AR226-1030a027.

Ellefson M (2001c) Solubility of PFOS in water. 3M Company, 3M Environmental Laboratory, Laboratory Project No. E00-1716, EPA Docket AR226-1030a025.

Ellefson M (2001d) Soil adsorption/desorption study of potassium perfluorooctanesulfonate (PFOS). 3M Company, 3M Environmental Laboratory, Laboratory Project No. E00-1311, EPA Docket AR226-1030a030.

Fisk AT, Norstrom RJ, Cymbalisty CD, Muir DCG (1998) Dietary accumulation and depuration of hydrophobic organochlorines: Bioaccumulation parameters and their relationship with the octanol/water partition coefficient. Environ Toxicol Chem 17:951–961.

Giesy JP, Ludwig JP, Tillitt DE (1994) Dioxins, dibenzofurans, PCBs and colonial fish-eating water birds. In: Schecter A (ed) Dioxin and health. New York, NY, Plenum Press, pp. 249–307.

Giesy JP, Kannan K (1998) Dioxin-like and non-dioxin-like toxic effects of polychlorinated biphenyls (PCBs): Implications for risk assessment. Crit Rev Toxicol 28:511–569.

Giesy JP, Kannan K (2001) Global distribution of perfluorooctane sulfonate in wildlife. Environ Sci Technol 35:1339–1342.

Giesy JP, Kannan K (2002) Perfluorochemical surfactants in the environment. Environ Sci Technol 36:146A–152A.

Giesy JP, Newsted JL (2001) Selected fluorochemicals in the Decatur Alabama Area. 3M Report, Project No. 178041. EPA Docket AR226-1030a161.

Gledhill WE, Markley BJ (2000a) Microbial metabolism (biodegradation) studies of perfluorooctane sulfonate (PFOS). I. Activated sludge/sediment. Springborn Laboratories, Inc., Wareham, MA. Lab ID Number 290.6120, EPA Docket AR226-1030a034.

Gledhill WE, Markley BJ (2000b) Microbial metabolism (biodegradation) studies of perfluorooctane sulfonate (PFOS). II. Aerobic soil biodegradation. Springborn Laboratories, Inc., Wareham, MA. Lab ID Number 290.6120, EPA Docket AR226-1030a035.

Gledhill WE, Markley BJ (2000c) Microbial metabolism (biodegradation) studies of perfluorooctane sulfonate (PFOS). III. Anaerobic sludge biodegradation. Springborn Laboratories, Inc., Wareham, MA. Lab ID Number 290.6120, EPA Docket AR226-1030a036.

Goecke-Flora CM, Reo NV (1996) Influence of carbon chain length on the hepatic effects of perfluorinated fatty acids. Chem Res Toxicol 9:689–695.

Hanson ML, Sibley PK, Brain RA, Mabury SA, Solomon R (2005) Microcosm evaluation of the toxicity and risk to aquatic macrophytes from perfluorooctane sulfonic acid. Arch Environ Contam Toxicol 48:329–337.

Hatfield T (2001a) Screening studies on the aqueous photolytic degradation of potassium perfluorooctane sulfonate (PFOS). 3M Environmental Laboratory, Lab Request No. W2775, St. Paul, MN, EPA Docket AR226-1030a041.

Hatfield T (2001b) Hydrolysis reactions of perfluorooctane SULFONATE (PFOS). 3M Environmental Laboratory, Lab Request No. W1878. St. Paul, MN, EPA Docket AR226-1030a039.

Hekster FM, Laane RWPM, de Voogt P (2003) Environmental and toxicity effects of perfluoroalkylated substances. Rev Environ Contam Toxicol 179:99–121.

Hu W, Jones PD, Upham BL, Trosko JE, Lau C, Giesy JP (2002) Inhibition of gap junctional intercellular communication by perfluorinated compounds in rat liver and dolphin kidney epithelial cell lines in vitro and Sprague-Dawley rats *in vivo*. Toxicol Sci 68:429–439.

Jacobs RL, Nixon WB (1999) Determination of the melting point/melting range of PFOS. Wildlife International, Ltd., Project No. 454C-106. EPA Docket AR226-0045.

Johnson JD, Gibson SJ, Ober RE (1984) Cholestyramine-enhanced fecal elimination of carbon-14 in rats after administration of ammonium [14-C] perfluorooctanoate and potassium [14-C] perfluorooctane sulfonate. Fund Applied Toxicol 4:972–976.

Jones PD, Hu W, DeCoen W, Newsted JL, Giesy JP (2003) Binding of perfluorinated fatty acids to serum protein. Environ Toxicol Chem 22:2639–2649.

Kannan K, Tao L, Sinclair E, Paskva S, Jude DL, Giesy JP (2005) Perfluorinated compounds in aquatic organisms at various levels in a Great Lakes food chain. Arch Environ Contam Toxicol 48:559–566.

Kannan K, Franson JC, Bowerman WW, Hansen KJ, Jones PD, Giesy JP (2001b) Perfluorooctane sulfonate in fish-eating water birds including bald eagles and albatrosses. Environ Sci Technol 35: 3065–3070.

Kannan K, Koistinen J, Beckmen K, Evans T, Gorzelany JF, Hansen KJ, Jones PD, Helle E, Nyman M, Giesy JP (2001a) Accumulation of perfluorooctane sulfonate in marine mammals. Environ Sci Technol 35:1593–1598.

Kleszczynski K, Gardzielewski P, Mulkiewicz E, Stepnowski P, Skladanowski AC (2007) Analysis of structure-cytotoxicity in vitro relationships (SAR) for perfluorinated carboxylic acids. Toxicol In Vitro 21:1206–1211.

Kurume Laboratory (2001) Final report, Bioconcentration test of 2-perfluoroalkyl (C = 4-14) ethanol [This test was performed using 2-(perfluorooctyl)-ethanol (Test substance number K-1518)] in carp. Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan. EPA Docket AR226-1276.

Kurume Laboratory (2002) Final report, Biodegradation test of salt (Na, K, Li) of perfluoroalkyl (C = 4-12) sulfonic acid, test substance number K-1520 (test number 21520). Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan.

Lange CC (2001) The 35-d aerobic biodegradation study of PFOS. Pace Analytical Services, Minneapolis, MN. 3M Project Number E01-0444. EPA Docket AR226-1030a040.

Langely AE (1990) Effects of perfluoro-n-decanoic acid on respiratory activity of isolated rat liver mitochondria. J Toxicol Environ Health 29:329–336.

Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J (2007) Perfluoroalkyl acids: A review of monitoring and toxicological findings. Toxicol Sci 99:366–394.

Leonards PEG, Zierikzee Y, Brinkman UATh, Cofino WP, van Straalen NM, van Hattum B (1997) The selective dietary accumulation of planar polychlorinated biphenyls in the otter (*Lutra lutra*). Environ Toxicol Chem 16:1807–1815.

MacDonald MM, Warne AL, Stock NL, Mabury SA, Solomon KR, Sibley PK (2004) Toxicity of perfluorooctane sulfonic acid and perfluorooctanoate to *Chironomus tentans*. Environ Toxicol Chem 23:2116–2123.

Martin JW, Mabury SA, O'Brian PJ (2005) Metabolic products and pathways of fluorotelomer alcohols in isolated rat hepatocytes. Chem Biol Interact 155:165–180.

Martin JW, Mabury SA, Solomon KR, Muir DCG (2003a) Dietary accumulation of perfluorinated acids in juvenile rainbow trout (*Oncorhynchus mykiss*). Environ Toxicol Chem 22:189–195.

Martin JW, Mabury SA, Solomon K, Muir DCG (2003b) Bioconcentration and tissue distribution of perfluorinated acids in rainbow (*Oncorhynchus mykiss*). Environ Toxicol Chem 22:196–204.

McCarty LS, Mackay D (1993) Enhancing ecotoxicological modeling and assessment. Environ Sci Technol 27:1719–1728.

Moody CA, Martin JW, Kwan WC, Muir DCG, Mabury SA (2001) Monitoring perfluorinated surfactants in biota and surface water samples following an accidental release of firefighting foam into Etobicoke Creek. Environ Sci Technol 36:545–551.

Mulkiewicz E, Jastorff B, Skladanowski AC, Kleszczynski K, Stepnowski P (2007) Evaluation of the acute toxicity of perfluorinated carboxylic acids using eukaryotic cell lines, bacteria and enzymatic assays. Environ Toxicol Pharmacol 23:279–285.

Newsted JL, Beach SA, Gallagher SP, Giesy JP (2008) Acute and chronic effects of perfluorobutane sulfonate (PFBS) on the mallard and northern bobwhite quail. Arch Environ Contam Toxicol 54:535–545.

Newsted JL, Coady KK, Beach SA, Butenhoff JL, Gallagher S, Giesy JP (2007) Effects of perfluorooctane sulfonate on mallard and northern bobwhite quail exposed chronically via the diet. Environ Toxicol Pharmacol 23:1–9.

NICNAS (2005) Potassium perfluorobutane sulfonate. Report to the National Industrial Chemicals Notification and Assessment Scheme, Sydney Australia.

Oakes KD, Sibley PK, Martin JW, MacLean DD, Solomon KR, Mabury SA, Van Der Kraak GJ (2005) Short-term exposures of fish to perfluorooctane sulfonate: Acute effects on fatty acyl-CoA oxidase activity, oxidative stress, and circulating sex steroids. Environ Contam Toxicol 24:1172–1181.

Obourn J, Frame SR, Bell RH, Longnecker DS, Elliott GS, Cook J (1997) Mechanisms for the pancreatic oncogenic effects of the peroxisome proliferator wyeth-14,643.Toxicol Appl Pharmacol 145:425–436.

OECD (Organization for Economic Cooperation and Development) (2002) Hazard assessment of perfluorooctane sulfonate (PFOS) and its salts. ENV/JM/RD 17/Final. 21 November, 2002. Paris, p. 362.

Ohmori K, Kudo N, Katayama K, Kawashima Y (2006) Comparison of the toxicokinetics between perfluorocarboxylic acids with different carbon chain length. Toxicol 184:135–140.

Olson CT, Anderson ME (1983) The acute toxicity of perfluorooctanoic and perfluorodecanoic acids in male rats and effects on tissue fatty acids. Toxicol Appl Pharmacol 70:362–372.

Palmer SJ, Krueger HO (2001) PFOS: A frog embryo teratogenesis assay-*Xenopus*(FETAX). Wildlife International, Ltd., Project No. 454A-116. EPA Docket AR226-1030a057.

Palmer SJ, Van Hoven RL, Krueger HO (2002a) Perfluorooctanesulfonate, potassium salt (PFOS): A 96-hr static acute toxicity test with the rainbow trout (*Oncorhynchus mykiss*). Wildlife International Ltd. Report No. 454A-145. EPA Docket AR226-1030a044.

Palmer SJ, van Hoven RL, Krueger HO (2002b) Perfluorooctanesulfonate, potassium salt (PFOS): A 96-hr static renewal acute toxicity test with the sheepshead minnow (*Cyprinodon variegatus*). Wildlife International Ltd. Report No. 454A-146A.

Phillips MM, Dinglasan-Panlilio MJA, Mabury SA, Solomon KR, Sibley PK (2007) Fluorotelomer acids are more toxic than perfluorinated acids. Environ Sci Technol 41:7159–7163.

RIVM (2001) Guidance document on deriving environmental risk limits in the Netherlands. In: Traas TP (ed) Report No. 601501 012. National Institute of Public Health and the Environment, Bilthoven, The Netherlands.

Robertson JC (1986) Potential for environmental impact of AFA-6 surfactant. Beak Consultants Ltd. Missassauga, Ontario, Canada. EPA Docket AR226-1030a043.

Sargent J, Seffl R (1970) Properties of perfluorinated liquids. Fed Proc 29:1699–1703.

Schaefer EL, Flaggs RS (2000) PFOS: An activated sludge respiration inhibition test. Wildlife International, Ltd., Project No. 454E-101, EPA Docket AR226-0093.

Schnellmann RG, Manning RO (1990) Perfluorooctane sulfonamide: A structurally novel uncoupler of oxidative phosphorylation. Biochem Biophys Acta 1016:344–348.

Sohlenius AK, Andersson K, Bergstrand A, Spydevold O, De Pierre JW (1994) Effects of perfluorooctanoate, a potent peroxisome proliferator in rat, on morris hepatoma 7800C1 cells, a rat cell line. Biochem Biophys 1213:63–74.

Starkov A, Wallace KB (2002) Structural determinants of fluorochemicals-induced mitochondrial dysfunction. Toxicol Sci 66:244–252.

Sutherland CA, Krueger HO (2001) PFOS: A 96-hr toxicity test with the freshwater diatom (*Navicula pelliculosa*). Wildlife International, Ltd., Project Number 454A-112. EPA Docket AR226-1030a055.

Taniyasu S, Kannan K, Horii Y, Hanari N, Yamashita N (2003) A survey of perfluorooctane sulfonate and related perfluorinated organic compounds in water, fish, birds, and humans from Japan. Environ Sci Technol 37:2634–2639.

Tolls J, Haller M, Graaf ID, Thijssen MATC, Sijm DTHM (1997) Bioconcentration of LAS: Experimental determination and extrapolation to environmental mixtures. Environ Sci Technol 31:3426–3431.

Tolls J, Sijm DJHM (1995) A preliminary evaluation of the relationship between bioconcentration and hydrophobicity for surfactants. Environ Toxicol Chem 14:1675–1685.

Trosko JE, Rush RJ (1998) Epigenetic toxicology as toxicant-induced changes in signaling leading to altered gap junctional intercellular communication. Toxicol Lett 102/103:71–78.

Upham BL, Deocampo ND, Wurl B,Trosko JE (1998) Inhibition of gap junctional intercellular communication by perfluorinated fatty acids is dependent on the chain length of the fluorinated tail. Int J Cancer 78:491–495.

US EPA (1985) Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. PB-85-227049. US Environmental Protection Agency, NTIS, Springfield, VA, USA.

US EPA (1995) Final Water Quality Guidance for the Great Lakes System: Final Rule. Federal Register 60: 15366-15425. US EPA, Washington, DC, USA.

US EPA (2001) Perfluorooctyl sulfonates; proposed significant new use rule. Federal Register 65:62319–62333.

Van Hoven RL, Stenzel JI, Nixon WB (1999) Determination of the vapor pressure of PFOS using the spinning rotor gauge method. Wildlife International, Ltd., Project Number 454C-105. EPA Docket AR226-0048.

Wildlife International Ltd (2001a) PFBS: An activated sludge respiration test. Wildlife International Ltd., Project No. 454E-102A.

Wildlife International Ltd. (2001b) Perfluorobutane sulfonate, Potassium salt (PFBS): A 48-hour static acute toxicity test with the cladoceran (*Daphnia magna*). Wildlife International Ltd., Project No. 454A-118A.

Wildlife International Ltd (2001c) Perfluorobutane sulfonate, potassium salt (PFBS): A 96-hour static acute toxicity test with the fathead minnow (*Pimephales promelas*). Wildlife International Ltd., Project No. 454A-115.

Wildlife International Ltd. (2001d) Perfluorobutane sulfonate, Potassium salt (PFBS): A 96-hour static acute toxicity test with bluegill (*Lepomis macrochirus*). Wildlife International Ltd., Project No. 454A-114.

Wildlife International Ltd (2001e) PFBS: A 96-hour static acute toxicity test with the freshwater alga (*Selenastrum capricornutum*). Wildlife International Ltd., Project No. 454A-129.

Wildlife International Ltd (2001f) PFBS: A 96-hour toxicity test with the saltwater mysid (*Mysiodopsis bahia*). Wildlife International Ltd., Project No. 454A-128.

Wildlife International Ltd (2001 g) PFBS: A semi-static life-cycle toxicity test with the cladoceran (*Daphnia magna*). Wildlife International Ltd., Project No. 454A-130.

Yamada T, Taylor PH (2003) Laboratory-scale thermal degradation of perfluoro-octanyl sulfonate and related substances. Environmental Sciences and Engineering Group, University of Dayton, Research Institute, Dayton, OH.