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# Distributions of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) in Japan and Their Toxicities

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Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are end products of many fluorochemical compounds in the natural environment. The aim of this review is to summarize several studies in Japan and characterize the toxicities of these compounds. We also compared the levels of contamination with those reported from various countries to illustrate the unique situation of the toxicological issues within Japan.

PFOA and PFOS concentrations in surface water in Japan are in the ranges of 0.1–67,000 ng/L and 0.1–526 ng/L, respectively. While the origin of PFOS in surface water remains unknown, PFOA present in surface water is very likely to have been released from a few industries. The levels of PFOA and PFOS in the atmosphere are 71.8–919 pg/m$^3$ and 2.3–21.8 pg/m$^3$, respectively. The concentrations of PFOA and PFOS in Japanese serum range from an undetectable level to 52.2 ng/ml and from 0.2 to 57.7 ng/ml, respectively. The levels of PFOA and PFOS present in the serum of the inhabitants of Kyoto are higher than those of other cities. One epidemiological study conducted by 3M revealed an increase in prostate cancer mortality [3.3-fold increase (95% CI, 1.02–10.6)] among workers exposed to PFOA. Another study conducted by 3M revealed an increase in bladder cancer mortality (SMR 12.77, 95% CI 2.63–37.35) among workers exposed to PFOS.

PFOA and PFOS had a low order of toxicity in an acute toxicity study in rodents; however, they exhibited versatile toxicities in primates. Both chemicals are carcinogenic in rodents, causing reproductive toxicity, neurotoxicity, and hepatotoxicity. Additionally, peroxisome proliferation and calcium channel modulation are demonstrated effects. There are large interspecies differences in toxicokinetics.

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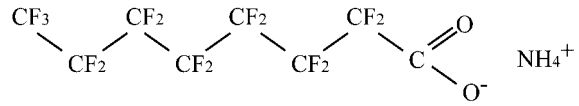
## 1. Introduction

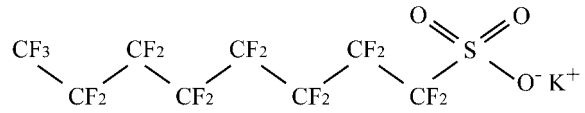
Perfluorinated alkyl compounds (PFCs), of which representative chemicals include perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) (Fig. 1), are a class of special chemicals used in a variety of applications, such as lubricants, paints, cosmetics, and fire-fighting foams.[1,2] PFCs are produced because of their advantageous physical and chemical properties, which include chemical stability, thermal inertness, low surface energy, and their intrinsic amphiphilic nature. These favorable characteristics have resulted in the production of a wide variety of perfluoroalkylated chemicals for application in fire-fighting foams, carpet surface repellent, textile protection, leather protection, paper and wood board protection, special surfactants, and as a polymerization aid for polytetrafluoroethylene (Teflon®).[3,4] According to the OECD's report,[5] approximately 4000–4500 metric tons of PFOA and PFOS were produced in 2002, however, this information is quite limited and these values are not certain. PFOA and PFOS (Fig. 1) are assumed to be the final biodegradation or environmental degradation products of fluorotelomer compounds or perfluorooctanyl sulfonamides.[6,7]

PFOS and PFOA have been important perfluorinated surfactants; however, in 2002, after 50 years of production, 3M Company, one of the largest company that had been producing these compounds, phased out their manufacture.[8]

PFOA and PFOS have been found globally in a variety of living organisms, including humans[9] and wildlife.[10] The worldwide distribution of these compounds has been attributed to their resistance to degradation in ecological systems[2] and their bioaccumulative characteristics.[11] These chemicals have been regulated by various countries including Japan, USA, Canada, Sweden and Denmark.




perfluorooctanoic acid ammonium salt




perfluorooctane sulfonate potassium salt

Fig. 1. Structures of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS).

There is a large difference between the distributions of PFOA and PFOS. PFOA is only detected in selected areas, whereas PFOS is found worldwide.[12,13] There are many studies revealing that these compounds have various toxicities on living organisms including human beings.[14–18]

With increasing worldwide attention to these compounds and the increasing number of studies of these compounds, several reviews are available that describe the occurrence of these chemicals within the global environment.[14–19]

Recent studies revealed a unique situation in Japan in that PFOA contamination more profoundly progressed than PFOS contamination environmentally.[20–22] In agreement with this observation, the serum concentrations of PFOA among Japanese, although representing a limited population in Japan, are assumed to be higher than those among Americans whereas vise versa in the case of PFOS.[36,37]

Our primary aim in this review is twofold. First, we will review the distributions of PFOS and PFOA in the environment in Japan. Second, we will characterize the toxicities of PFOS and PFOA. We have compared data collated in Japan with those from other countries as was necessary to present the unique situation in Japan.

## 2. Distributions of PFOA and PFOS in Environment in Japan

### 2.1 *Surface water*

Several studies have shown the concentrations of PFOA and PFOS in surface water in Japan.[20–23] We summarized the data in the order of the nine districts of Japan from Hokkaido to Okinawa (Table 1).

#### 2.1.1 *PFOA*

The geometric mean concentrations of PFOA in eight districts were within the range of 1 to 3 ng/L except for the Kinki district. In the Kinki district, PFOA concentrations in surface waters were much higher than those in other districts.[21] The systematic investigation of the Kanzaki River system by our group has revealed that there is a single source of PFOA within the Ai River, which is located upstream of the Kanzaki River. The levels of PFOA were 447.74 ng/L in Koshienhama, ranged from 2.14 to 3,750 ng/L in the Kanzaki River and were at a maximum of 67,000 ng/L in the Ai River.[21] The highest concentration was recorded in wastewater from the Ai River Wastewater Treatment Plant. A high concentration PFOA of 87,000 ng/L was also recorded in released sewage water from the same site in a separate survey.[24]

The concentrations of PFOA in other countries are also shown in Table 1. These values are relatively higher than the values in Japan excluding the Kanzaki River system. For example, the PFOA concentrations in Lake Erie and Lake Ontario, Canada/USA, are 30–40 ng/L[25] and the PFOA concentration is 366 ng/L in the Tennessee River.[26] In the Etobicoke Creek in Canada, which has been contaminated by a spill of aqueous fire fighting foam into the groundwater in a fire-training area at a U.S. Air Force Base, PFOA concentration is extremely high, as much as 1000-fold higher than the general water concentrations in Japan even long after (5 or more years) the spill had occurred.[27,28] It should be noted, however,

Table 1
Observed levels of PFOA and PFOS in surface water in Japan and in other countries.

| | | PFOA (ng/L) | | PFOS (ng/L) | | | |
|---|---|---|---|---|---|---|---|
| District | n | GM (GSD) | Range | GM (GSD) | Range | Ref. | Comment |
| River | | | | | | | |
| Hokkaido | 2 | | | 1.20 (1.13) | 1.10–1.30 | (20) | |
| Hokkaido | 1 | | 0.40 | | 1.90 | (21) | |
| Tohoku | 18 | | | 1.07 (2.34) | 0.34–5.47 | (20) | |
| Tohoku | 15 | 1.11 (2.74) | 0.1–4.22 | 1.15 (2.49) | 0.25–4.62 | (21) | |
| Kanto | 26 | | | 6.48 (4.42) | 0.44–157.0 | (20) | |
| Kanto | 14 | 2.84 (3.56) | 0.33–15.08 | 3.66 (3.93) | 0.33–31.42 | (21) | |
| Kanto (Tama river) | 25 | | | | 0.7–440 | (20) | |
| Chubu | 20 | | | 1.40 (3.74) | 0.36–135.0 | (20) | |
| Chubu | 17 | 2.50 (2.23) | 0.28–16.28 | 1.07 (2.36) | 0.24–6.04 | (21) | |
| Kinki | 34 | | | 4.44 (3.47) | 0.30–32.33 | (20) | |
| Kinki | 8 | 21.2 (6.16) | 2.14–456.41 | 5.72 (3.61) | 0.78–37.32 | (21) | |
| Kinki (Kanzaki River) | 52 | | 4.5–67,000 | | 1.5–526 | (21) | |
| Chugoku | 9 | | | 1.14 (3.04) | 0.48–19.58 | (20) | |
| Chugoku | 9 | 1.51 (2.28) | 0.51–8.11 | 1.00 (3.42) | 0.42–25.10 | (21) | |
| Shikoku | 7 | | | 1.44 (3.79) | 0.44–15.78 | (20) | |
| Shikoku | 7 | 3.02 (2.05) | 1.35–13.82 | 1.11 (4.65) | 0.24–14.86 | (21) | |
| Kyushu | 10 | | | 0.78 (1.82) | 0.30–1.68 | (20) | |
| Kyushu | 8 | 1.30 (2.35) | 0.20–3.28 | 0.72 (1.91) | 0.29–1.72 | (21) | |
| Sea | | | | | | | |
| Hokkaido | 1 | | 1.90 | | 2.12 | (21) | |
| Tohoku | 2 | 2.04 (1.06) | 2.09–2.13 | 1.04 (1.90) | 0.61–0.87 | (21) | |
| Kanto | 1 | | 32.21 | | 2.58 | (21) | |
| Kanto | 3 | 166 (1.13) | 154.3–192.0 | 20.1 (1.49) | 12.7–25.4 | (23) | |
| Chubu | 1 | | 11.5 | | 0.65 | (21) | |
| Kinki | 1 | | 448 | | 27.69 | (21) | |
| Hokkaido | 1 | | | | <2.5 | (22) | |
| Kanto | 4 | | | 26 | 8–59 | (22) | d |
| Kinki | 3 | | | 8.7 | <4–21 | (22) | d |
| Kinki (Koshien hama) | 1 | | 447.74 | | 27.69 | (21) | |
| Chugoku | 4 | | | | <4 | (21) | |
| Kyushu | 5 | | | 4.8 | <9–11 | (22) | d |
| Okinawa | 4 | | | | <2.5 | (22) | |
| Lake | | | | | | | |
| Hokkaido | 1 | | | | <2.5 | (22) | |
| Kinki | 3 | | | 3.8 | <4–7.4 | (22) | d |
| Tap Water | | | | | | | |
| Tohoku | 15 | 0.25 (2.26) | 0.00–1.00 | 0.21 (1.72) | 0.10–0.45 | (21) | |
| Kinki | 15 | 15.3 (2.29) | 4.91–42.19 | 3.84 (3.58) | 0.27–12.69 | (21) | |
| River | | | | | | | |
| Tennessee, USA | 40 | 366 (1.50) | 140–498 | 55.1 (2.00) | 16.8–144 | (26) | a |
| Etobicoke Creek, Canada | 13 | | 11–1.1×104 | | nd to 2.2×106 | (29) | b |
| Sea | | | | | | | |
| Hong Kong | | | 0.73–5.5 | | 0.09–3.1 | (30) | |
| South Chi Sea | | | 0.14–16 | | 0.02–12 | (30) | |
| Korea | | | 0.24–320 | | 0.04–730 | (30) | |
| Lake | | | | | | | |
| Erie, Canada/USA | 8 | 34.8 (1.28) | 21–47 | 29.5 (1.50) | 11–39 | (25) | |
| Ontario, Canada/USA | 8 | 41.6 (1.57) | 15–70 | 46.2 (2.00) | 15–121 | (25) | |
| Groundwater | | | | | | | |
| Air Force Base, USA | | | nd–105×103 | | (4–110)×103 | (31) | c |

a: Concentrations following spill of aqueous fire fighting foam into Etobicoke Creek. b: River directly influenced by fluorochemical production facility. c: Fire-training area at Air Force Base. d: Average was taken as arithmetic mean.
GM: geometric mean, GSD: geometric standard deviation, nd: not detected

that the concentration of PFOA is significantly higher in the Kanzaki River system including the Ai River than within the general surface waters in USA. It is therefore very likely that this may be the highest PFOA concentration ever reported excluding an accidental spill. Taken together, the surface water PFOA contamination in districts other than the Kinki district may not be deleterious; however, for those within the Kinki district, preventive action to halt the release of PFOA into the environment may be urgently required.

### 2.1.2 *PFOS*

The current surface water PFOS contamination level is shown in Table 1. PFOS concentrations in Japan were lower in six districts than in the Kanto and Kinki districts. The concentrations were high in the Tama River in the Kanto and Kanzaki River in the Kinki region. A systematic sampling of surface water by our group revealed the highest PFOS concentrations of 440 ng/L in the Tama River and 526 ng/L at Osaka airport.[21] It is interesting to note that the highest recorded water concentration in the Tama River contained wastewater discharged from the Yokota Air Force Base. High concentrations of PFOS were also found in wastewater from Osaka airport. It is confirmed that Light Water that contains PFOS had been used in Osaka airport (Personal communication). However, detail information regarding the type of fire fighting foams used at these airports is not available, aqueous fire fighting foams (AFFFs) have been widely used in the control of oil-related fires at the airport and at other sites.[29,31] Recently, a large amount of AFFF was released from Tomakomai oil refinery fire followed on the heels of the offshore earthquake caused obvious contaminations of PFOA and PFOS.[32] Taking these reports into consideration, the contamination of PFOS around the airport seems to be derived from activities using fire-fighting foams.

PFOS concentration in general surface water in Japan are much lower than those in the USA and Canada.[22] The contamination of the Etobicoke Creek continued for more than 5 years after the occurrence of the spill as shown by a study conducted at the U.S. Air Force Base (Table 1).[28]

## 2.2 *Airborne*

There are limited literatures all over the world that report airborne PFOA and PFOS. This work has been pioneered by Sasaki *et al.*,[33] who demonstrated discernible amounts of PFOS in urban dust (Table 2). Moriwaki *et al.*[34] has confirmed discernible amounts of PFOA and PFOS in indoor dust. Boulanger *et al.* reported that the mean concentration of PFOS in particulate-phase air samples is 6.4 pg/m$^3$ (standard deviation, 3.3) in the U.S.[35] According to the study of Sasaki *et al.*,[33] the concentrations ranged from 69 to 3,700 ng/g-dust for PFOA and from 11 to 2,500 ng/g-dust for PFOS. Harada *et al.*[36] has recently shown that the concentrations of PFOA detected within urban airborne samples were 50-fold higher than those of PFOS. PFOS was found to be significantly higher in the urban atmosphere of Oyamazaki than in the suburban atmosphere of Morioka for both PFOA and PFOS ($p < 0.01$). The authors estimated the inhaled PFOA and PFOS levels as follows; assuming that adult humans inspire 15 m$^3$ of air per day, all particles are respirable, and that PFOA and PFOS on the particles are completely absorbed into the body; the daily intakes of PFOA and

PFOS at the Oyamazaki station, Kyoto are 4 ng/day and 0.1 ng/day, respectively. On the other hand, the estimated daily intakes of PFOA and PFOS from city water were calculated as follows; the mean concentrations of PFOA and PFOS in the city water of Kyoto[21] are multiplied by a daily water intake of 2 L/day, that is, 10.8 ng/day and 9.8 ng/day, respectively. The estimated amount of inhaled PFOA is approximately 40% of the amount ingested from drinking water in Kyoto, whereas the amount of inhaled PFOS is quite lower than that of the ingested PFOS. Even if these estimations have uncertainties, these estimates on the airborne concentrations of PFOA and PFOS have important roles for speculating the origin of contamination and the impact of these compounds on human beings (Table 2).

## 2.3 *Human exposure*

Table 3 shows the serum concentrations of PFOA and PFOS in Japanese people.[22,37–40] This table shows that the concentrations of PFOA and PFOS are higher in males than in females. A time course measurement for the Miyagi and Akita prefectures was performed by Harada *et al.*[37] The study revealed that serum concentrations were increased by a factor of 3 for PFOS and by a factor of 14 for PFOA between 1977 and 2003 in the Miyagi prefecture. The serum concentrations of PFOA increased significantly between 1995 and 2003 in the Akita region, whereas those of PFOS did not increase between 1991 and 2003 (ANOVA, 0.05 as the level of significance).

The city water concentrations of both PFOA and PFOS are higher in the Kinki district than in the Tohoku district.[21] Serum concentration seems to correlate with the city water concentration, although it remains unknown whether the higher PFOA concentrations in city water can explain the higher levels present in the serum of the residents of the Kinki district

Table 2
Indoor and outdoor airborne PFOA and PFOS levels in Japan and in other countries.

| | | | PFOA | | | PFOS | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sampling site | *N* | Unit | GM | GSD | Range | GM | GSD | Range | Ref. |
| Oyamazaki Town | 12 | pg/m$^3$-air | | | | 5.3 | 1.2 | 2.3–21.8 | (33) |
| (on a highway) | | ng/g-dust | | | | 97.4 | 1.2 | 38.0–427.4 | |
| 2001/04–2002/03 | 12 | pg/m$^3$-air | 262.7 | 2.4 | 71.8–919.4 | 5.2 | 1.4 | 2.5–9.8 | (35) |
| | | ng/g-dust | 3413.0 | 2.4 | 469–9049 | 72.2 | 1.8 | 19.7–168.0 | |
| Fukuchiyama city | 12 | pg/m$^3$-air | | | | 0.6 | 1.3 | nd'–2.1 | (33) |
| (on a local road) | | ng/g-dust | | | | 19.2 | 1.2 | nd–60.6 | |
| 2001/04–2002/03 | | | | | | | | | |
| Morioka city | 8 | pg/m$^3$-air | 2.0 | 1.2 | 1.6–2.6 | 0.7 | 1.4 | 0.5–1.2 | (35) |
| (on a local road) | | | | | | | | | |
| 2003/07 | | | | | | | | | |
| Indoor dust | 16 | ng/g-dust | 177.7 | 2.6 | 69.0–3700 | 39.5 | 3.9 | 11.0–2500 | (34) |
| (general home) | | | | | | | | | |
| Lake Ontario | 8 | pg/m$^3$ | | | | 6.4 | 3.3 | | (36) |
| (particulate-phase) | | | | | | | | | |

GM: geometric mean, GSD: geometric standard deviation, nd: not detected

Table 3
Serum or whole blood concentrations of perfluorochemicals in Japanese people.

| | | | | PFOA (ng/mL) | | | PFOS (ng/mL) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sampling site | Year | Sex | *N* | GM | GSD | Range | GM | GSD | Range | Ref. |
| Taiwa (Miyagi) | 2003 | M | 32 | 3.3 | 2.0 | 0.8–52.2 | 5.7 | 2.0 | 0.4–16.9 | (37) |
| (Serum) | | F | 23 | 2.8 | 1.5 | 1.1–8.4 | 3.5 | 2.9 | 0.2–11.4 | |
| | 1977 | M | | | | | | | | |
| | | F | 39 | 0.2 | 2.0 | nd–1.1 | 1.1 | 1.8 | 0.2–3.3 | |
| Yokote city (Akita) | 2003 | M | 66 | 3.4 | 1.5 | 1.6–14.6 | 12.9 | 1.5 | 4.1–36.2 | (37) |
| (Serum) | | F | 50 | 2.5 | 1.6 | 0.4–9.2 | 6.9 | 1.4 | 0.9–14.9 | |
| | 1995 | M | | | | | | | | |
| | | F | 40 | 1.9 | 1.4 | 1.0–4.8 | 8.7 | 1.3 | 5.8–16.1 | |
| | 1991 | M | 16 | 2.2 | 1.4 | 0.9–3.5 | 10.2 | 1.5 | 6.0–29.2 | |
| | | F | 60 | 1.8 | 1.5 | 0.7–4.1 | 7.9 | 1.4 | 3.7–20.2 | |
| Tsukuba city | 2002 | M | 11 | | | | 8.1 | 1.8 | 2.4–20.5 | (22) |
| (Whole blood) | | F | 2 | | | | | | 9.1–11.0 | |
| Yokohama city | 2001 | M | 12 | | | | 6.2 | 1.6 | 2.3–15.2 | (39) |
| (Whole blood) | | F | 10 | | | | 8.7 | 1.7 | 3.1–20.2 | |
| Kyoto city | 2003 | M | 14 | 12.4 | 1.4 | 7.1–19.8 | 28.1 | 1.5 | 16.3–57.7 | (37) |
| (Serum) | | F | 20 | 7.1 | 1.4 | 3.3–11.7 | 13.8 | 1.5 | 6.1–37.2 | |
| | 2004 | M | 5 | 7.5 | 1.4 | 5.2–13.2 | 12.1 | 1.4 | 9.0–19.4 | (38) |
| | | F | 5 | 10.7 | 1.5 | 7.6–20.6 | 10.8 | 1.3 | 8.6–16.4 | |
| | | M(>60 yr) | 5 | 11.6 | 1.3 | 7.6–15.8 | 23.3 | 1.7 | 11.8–49.2 | |
| | | F(>60 yr) | 5 | 14.0 | 1.1 | 12.6–15 | 22.9 | 1.3 | 16.2–33.0 | |
| Hokkaido | 2003 | F | 15 | 0.93 | 2.23 | nd–2.3 | 8.4 | 1.4 | 4.9–17.6 | (40) |
| (Serum, UC) | | fetus | 15 | | | nd | 2.7 | 1.5 | 1.6–5.3 | |

GM: geometric mean, GSD: geometric standard deviation, UC: umbilical cord serum, nd: not detected

(Harada *et al.* Submitted for publication). We suspect that there is an industrial emission source of these compounds. To solve these questions, further studies are required to reveal the origin of contamination and the ability of water treatment facilities to remove PFOA and PFOS from drinking water.

Inoue *et al.* reported the concentrations of PFOA and PFOS in maternal and fetal cord serum samples in Hokkaido.[40] The geometric mean PFOS concentrations (GM) in maternal and fetal cord serum samples were 8.4 ng/ml (range, 4.9–17.6 ng/ml) and 2.7 ng/ml (1.6–5.3 ng/ml), respectively. The concentration of PFOA in maternal serum is relatively lower than that of PFOS, 0.93 ng/ml for GM (range, nd to 2.3 ng/ml), whereas these compounds were undetectable in fetal cord serum. Although the serum PFOS concentrations in Hokkaido[40] are in agreement with those in other places in the study of Harada *et al.*,[37] those of PFOA are not. These results suggest geographical differences in the exposure intensities of PFOA as predicted in the study.[37]

It should be noted that there is a discrepancy between the USA and Japan in terms of the long-term exposure trends of PFOA. From our observations,[37] the serum concentrations of PFOA are still increasing in Japan, whereas those in the USA reached a plateau during the 1980s.[41] Studies in the USA and in Japan both showed that serum PFOS concentrations reached a plateau in the 1990s (Table 3).[37,41] In conclusion, PFOA exposure levels are likely to be heterogeneous even within Japan; thus, a large geographical area should be covered to ensure that higher exposure populations are not overlooked.

## 3. Toxicology of PFOA and PFOS

### 3.1 *Epidemiological study*

#### 3.1.1 *Mortality of PFOA*

A retrospective cohort mortality study, which included 2,788 men and 749 women and thus a total of 3,537 people, was performed on employees at a PFOA-treating fluorochemical plant, wherein PFOA production was limited to the Chemical Division.[42] The cohort was followed up from 1947 to 1989. Standardized mortality ratios (SMRs) adjusted for age, sex, and race were calculated.

The all-causes standardized mortality rates were 0.75 [95% confidence interval (CI) 0.56–0.99] for women and 0.77 (95% CI, 0.69–0.86) for men. Among the men, the cardiovascular standardized mortality rate was 0.68 (95% CI, 0.58–0.80) and that for all-gastrointestinal diseases was 0.57 (95% CI, 0.29–0.99). Ten years of exposure during employment, however, was associated with a 3.3-fold increase (95% CI, 1.02–10.6) in prostate cancer mortality. Human mortality profile and animal studies[42] suggest that PFOA may increase prostate cancer mortality by altering reproductive hormones in male workers. Olsen *et al.* conducted cross-sectional studies that measured PFOA level in serum in relation to several reproductive hormones to determine whether PFOA is associated with changes in reproductive hormones.[43] However, significant hormonal changes that were associated with serum PFOA concentrations were not found.

In conclusion, the absence of both hormonal effects of PFOA and having only a small number of prostate cancer deaths in these workers suggest that this observed increase in mortality from prostate cancer may be due to chance rather than by a cause and effect relationship.

#### 3.1.2 *Mortality of PFOS*

Alexander *et al.* conducted a retrospective cohort mortality study for PFOS in which they followed up all workers with a minimum of one year of cumulative employment at their place of work.[45] By biomonitoring the exposure intensities were classified into three discrete groups, namely, the high exposure group, the low exposure group, and the non-exposed group. A total of 145 deaths occurred among the 2,083 members of the cohort. Sixty-five deaths had occurred among workers in the high exposure employment group. The overall mortality rates for the cohort and exposure groups were lower than expected. The risk of death from bladder cancer was increased for the entire cohort. Three cases of bladder cancer occurred among the workers who held high-exposure jobs (SMR 12.77, 95% CI

2.63–37.35). The authors argued whether these three cases could be attributed to fluorochemical exposure because the possibility by chance alone could not be ruled out. One confounding factor is that although the workers were mainly exposed to PFOS, they were also exposed to other perfluoroalkyl compounds including PFOA.

#### 3.1.3 *Other risks of PFOA and PFOS*

There are several studies that show the effects of PFOA and PFOS on biomarkers such as hepatic enzymes, cholesterol, or lipoprotein of 3M company employees[46,47] and reproductive hormones.[44] No increase in the levels of these biomarkers has been attributed to exposures to PFOA and PFOS.

Two independent studies targeting workers handling PFOA and PFOS in Japan have suggested the probable association of PFOA and PFOS exposures with cancer risk. Such studies might have strong statistical power by applying meta-analyses.

### 3.2 *Animal studies*

#### 3.2.1 *Acute toxicity*

##### 3.2.1.1 *PFOA*

The oral $LD_{50}$ values of PFOA for CD rats were greater than 500 mg/kg for males and 250–500 mg/kg for females,[48] and < 1000 mg/kg for male and female Wistar rats.[49] There was no mortality following an inhalation exposure of 18.6 mg/L PFOA for 1 h in rats.[50] The dermal $LD_{50}$ in rabbits was determined to be greater than 2000 mg/kg.[51]

##### 3.2.1.2 *PFOS*

The oral $LD_{50}$ values of PFOS were 233 mg/kg (95% CI, 160–339) for male rats, 271 mg/kg (95% CI, 200–369) for female rats, and 251 mg/kg (95% CI, 199–318) for the combined values of both sexes.[52] A 1-h $LC_{50}$ of 5.2 mg/L (95% CI, 4.4–6.4) in rats has been reported.[53] PFOS was found to be mildly irritating to the eyes and not an irritant to the skin of rabbits.[54]

#### 3.2.2 *Subchronic toxicity*

##### 3.2.2.1 *PFOA*

Dietary exposure to PFOA for 90 days resulted in a significant increase in liver weight and hepatocellular hypertrophy in male rats at doses as low as 100 ppm (5 mg/kg/day) and in female rats at 1000 ppm (76.5 mg/kg/day).[55]

In a 90-day study of rhesus monkeys, exposure to PFOA at 30 mg/kg/day and above resulted in death, lipid depletion of the adrenal glands, hypocellularity of the bone marrow, moderate follicular atrophy of the lymphoid tissues in the spleen and lymph nodes (Table 4).[55]

In a 6-month study of male cynomolgus monkeys,[56] mortalities were observed in monkeys treated with PFOA at 3 mg/kg/day and at 30/20 mg/kg/day (the dose was decreased to 20 mg/kg/day during the experiment while it was initially 30 mg/kg/day because of severe toxicity). There were no consistent effects on hormone levels. Increased absolute and

Table 4
Subcronic toxicities manifested in monkeys treated with PFOA and PFOS.

| | Dose (mg/kg/day) | Elevated/ Appeared | Reduced/ Decreased |
|---|---|---|---|
| PFOA | | | |
| Resus Monkey[a] | 100 | Death by week 5 (4/4). Anorexia; emesis; pale face and gums; swollen face and eyes; prostration; and body trembling (4/4) | Activity, body weight and body weight gain (4/4) |
| | 30 | Death by week 13 (3/4). Emesis; ataxia; swollen face, eyes, and vulva; pale face and gums; black stools; dehydration; and eyelids ptosis. Plt, PT, APTT, AST | Activity, body weight and body weight gain. RBC, Hb, Hct, ALP, and ALT |
| | 10 | Anorexia; pale and swollen face; and black stools (1/4) | ALP and ALT |
| | 3 | Occasional soft stools; diarrhoea; and emesis | ALP |
| Cynomolgus Monkey[b] | 20 followed by 30[*] | Death (1/6). Oedema and inflammation of oesophagus and stomach; necrosis of heart tissue; and histopathological liver injury (and rhabdomyolysis) (1/6). TG. | Food consumption, body weight and body weight gain, little or no feces (6/6) |
| | 10 | Body weight loss | Body weight and body weight gain |
| | 3 | Death (not related to treatment, 1/4) | Food consumption, body weight and body weight gain, little or no feces (1/4) |
| PFOS | | | |
| Resus Monkey[c] | 4.5 | Death between weeks 5–7 (4/4). Anorexia; emesis; black stool; and dehydration. Diffuse lipid depletion of adrenals; diffuse atrophy of pancreatic acinar cells; and diffuse atrophy of serous alveolar cells | serum cholesterol (significant) |
| | 1.5 | Soft stools; diarrhoea | Body weight, ALP, K (female, significant), cholesterol (1/2 females), P (1/2 females) |
| | 0.5 | Soft stools; diarrhoea; anorexia; emesis; occasional decreases in activity | ALP (slight) |
| Cynomolgus Monkey[d] | 0.75 | Death (pulmonary necrosis with severe inflammation, 1/6). Absolute liver weight; liver to body weight percentages; liver to brain weight ratios (females) Excessive salivation; laboured respiration; hypoactivity and ataxia; constricted pupils; pale gums; liquid and black-coloured feces; dehydration; and recumbent position. | Food consumption and mucoid in mouth. Total cholesterol and HDL-cholesterol (significant), triiodothyronine. |
| | 0.15 | | Triiodothyronine and cholesterol |
| | 0.03 | Death (not related to treatment, 1/4) | Food consumption, body weight and body weight gain, little or no feces (1/4) |

[a]Goldenthal, 90-day test;[55] [b]Buttenhoff *et al.*, 6-month test;[56] [c]Goldenthal, 90-day test;[58] [d]Seacat *et al.*, 6-month test[59]
[*]Initial dose (30 mg/kg/day) was changed to 20 mg/kg/day because PFOA showed severe toxicity at the initial dose.
RBC: erythrocyte, Hb: haemoglobin, Hct: haematocrit, Plt: platelet, PT: prothrombin time, APTT: activated partial thromboplastin time, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, TG: triglyceride, K: potassium, P: phosphorus.

relative liver weights were observed at 3, 10 and 30/20 mg/kg/day. The observed clinical signs included low food consumption, body weight loss, little or no feces, elevation of serum triglycerides, oedema, and inflammation of the oesophagus and stomach in the 30/20 mg/kg/day group. Body weight loss occurred in the 10 mg/kg/day group. Necrosis of the heart tissue and liver injury occurred in one monkey, which died prematurely. The correlation between the doses administered and serum PFOA levels was nonlinear. An amendment of the original protocol was an obligatory decision because of the high mortality exhibited at the highest dose. One animal died during examination even after the protocol had been changed. Although the authors argued that these deaths were not considered to be treatment related, multiple deaths in the highest dose group suggest PFOA toxicity, which is in contrast to the conclusion presented.

#### 3.2.2.2 *PFOS*

The adverse signs of PFOS toxicity observed during the 90-day rat study included increases in liver enzyme levels, hepatic vacuolation, hepatocellular hypertrophy, gastrointestinal effects, haematological abnormalities, weight loss, convulsions, and death. These effects were reported at PFOS doses of 2 mg/kg/day and above.[57]

Goldenthal performed a 90-day study of rhesus monkeys (Table 4).[58] All monkeys in the 4.5 mg/kg/day group died between weeks 5 and 7. The clinical signs in this group included anorexia, emesis, black stool, and dehydration. The following abnormalities were also observed in this group: a significant reduction in serum cholesterol level, marked diffuse lipid depletion of the adrenal glands, moderate diffuse atrophy of pancreatic acinar cells, and moderate diffuse atrophy of serous alveolar cells. The 1.5 mg/kg/day group had soft stools, diarrhoea, reduced body weight, significant reductions in serum alkaline phosphatase and potassium levels, and a reduced serum cholesterol level. Digestive organ symptoms including soft stools, diarrhoea, anorexia, and emesis were also observed in the low dose group receiving PFOS at 0.5 mg/kg/day.

In a 6-month study conducted in cynomolgus monkeys, low food consumption, excessive salivation, labored breathing, hypoactivity, ataxia, hepatic vacuolization and hepatocellular hypertrophy, significant reductions in serum cholesterol levels, and death were observed in monkeys receiving PFOS at 0.75 mg/kg/day.[59] No monkeys survived beyond 3 weeks of treatment at 10 mg/kg/day or beyond 7 weeks of treatment at doses as low as 4.5 mg/kg/day. Pulmonary necrosis with severe inflammation was observed in the histopathology of one of the monkeys in the 0.75 mg/kg/day dose group. Reductions in serum cholesterol and HDL-cholesterol levels were significant in the 0.75 and 0.15 mg/kg/day groups. Triiodothyronine levels were reduced in the 0.15 and 0.75 mg/kg/day groups. No abnormal signs were observed in the 0.03 mg/kg/day group, except for a monkey that died prematurely. The serum concentration of PFOS reached a plateau at approximately 100 days in the 0.75 mg/kg/day group, whereas no plateau was observed in the 0.15 and 0.03 mg/kg/day groups. However, the correlation between the doses administered and serum PFOS levels was nonlinear. A dose-response relationship was not clear in this study and was similar to the study of PFOA.

In summary, although dose-response relationships were established for PFOA and PFOS in rodent studies, they were not established in monkey studies. These compounds seemed to affect the central nervous system of monkeys. Such toxicity warrants further studies.

### 3.2.3 *Chronic toxicity*

#### 3.2.3.1 *PFOA*

The chronic dietary exposure of rats to 300 ppm PFOA (14.2 and 16.1 mg/kg/day for males and females, respectively) for 2 years resulted in increased liver and kidney weights, haematological effects and liver lesions in males and females.[60] In addition, testicular masses were observed in males exposed to PFOA at 300 ppm and ovarian tubular hyperplasia was observed in females after exposure to PFOA at the lowest dose of 30 ppm (1.6 mg/kg/day).[60]

Carcinogenicity studies in Sprague-Dawley (CD) rats show that PFOA is carcinogenic, inducing Leydig cell adenomas in male rats and mammary fibroadenomas in female rats, following dietary exposure to 300 ppm for 2 years (equivalent to 14.2 mg/kg/day in male rats and 16.1 mg/kg/day in female rats).[60] PFOA has also been reported to be carcinogenic to the liver and pancreas of male CD rats at 300 ppm.[61,62]

#### 3.2.3.2 *PFOS*

In a dietary 2-year study conducted in Sprague-Dawley rats, hepatotoxicity, characterized by centrilobular hypertrophy, the presence of centrilobular eosinophilic hepatocytic granules, the presence of centrilobular hepatocytic pigment, or centrilobular hepatocytic vacuolation was noted in male and/or female rats given 5 or 20 ppm PFOS. Hepatocellular centrilobular hypertrophy was also observed in male rats receiving a mid-strength dose (2 ppm PFOS).[63] Significant increases in the incidence rate of cystic hepatocellular degeneration were found in all the male treated groups (0.5, 2, 5, or 20 ppm) compared with the control group. From the pathological findings of the liver, the least observed adverse effect level (LOAEL) was 5 ppm and the no observed adverse effect level (NOAEL) was 2 ppm in female rats. In male rats, the LOAEL was 0.5 ppm and the NOAEL was not established.

The potential carcinogenicity of PFOS has been examined in a dietary 2-year study in Sprague-Dawley rats.[63] Compared with the control group, there was a significant increase in the incidence rate of hepatocellular adenomas in male and female rats receiving the highest dose of 20 ppm; female rats receiving 20 ppm also had significant increases in the combined incidence rates of hepatocellular adenomas and carcinomas. In addition, there were significant increases in the incidence rates of thyroid follicular cell adenomas and combined thyroid follicular cell adenomas and carcinomas in the male recovery group at 20 ppm.

### 3.2.4 *Genotoxicity*

PFOA or PFOS is neither mutagenic nor clastogenic in a variety of tests.[64–75]

### 3.2.5 *Reproductive toxicity*

#### 3.2.5.1 *PFOA*

In a two-generation reproductive toxicity study of PFOA in rats administered at 0, 1, 3, 10, and 30 mg/kg/day,[76] PFOA had significant effects on the fetal growth and development of male rats but to a much lesser degree in female rats. The difference in sensitivity was presumed to be a gender-related difference in the elimination of PFOA.

#### 3.2.5.2 *PFOS*

A two-generation reproductive toxicity study of PFOS was conducted in Sprague-Dawley rats.[77] Reductions in body weight gain and food consumption were observed in the $F_0$ and $F_1$ generations receiving 0.1 mg/kg/day (male rats) and 0.4 mg/kg/day (female rats). In the $F_1$ generation, there are significant reductions in the number of implantation sites, litter size, pup viability, pup body weight, and survival at 1.6 mg/kg/day. In the $F_2$ generation, significant reductions in mean pup body weight were observed at 0.4 mg/kg/day.

### 3.2.6 *Neurotoxicity*

There are limited studies on the neurotoxicities of PFOS and PFOA. Austin *et al.* reported that the treatment of rats with PFOS affected estrous cyclicity and increased serum corticosterone levels while decreasing serum leptin concentrations.[78] PFOS treatment also increased norepinephrine concentrations in the paraventricular nucleus of the hypothalamus.

## 3.3 *Toxicology at cellular and molecular levels*

### 3.3.1 *Peroxisome proliferation*

The common carboxylic functional group shared by peroxisome-proliferating substances and their metabolites plays a role in their ability to induce peroxisome proliferation. Most perfluoroalkyl compounds cause peroxisome proliferation in rats and mice.[79–82] Investigations using sulfur-substituted PFOS were also shown to induce peroxisome proliferation.[82,83]

### 3.3.2 *Membrane effects*

The effects of perfluorinated compounds (PFCs) on membrane fluidity, mitochondrial membrane potential and membrane permeability were investigated.[84] In the PFCs tested, only PFOS increased cell membrane permeability to the hydrophobic ligands utilized. PFOS increased membrane fluidity in fish leukocytes in a dose-dependent manner, whereas perfluorohexane sulfonic acid and perfluorobutane sulfonic acid had no effects in the concentration range tested. The lowest effective concentrations for the membrane fluidity effects of PFOS were 5–15 mg/L. Effects on mitochondrial membrane potential occurred in the same concentration range as that in the effects on membrane fluidity. This suggests that PFOS affects membrane properties at relative low concentrations.

### 3.3.3 *Toxic effects on gap junctions*

The inhibition of gap junctional intercellular communication (GJIC) has been linked to the tumor-promoting properties of many carcinogens.[85] Upham *et al.* reported that perfluorinated fatty acids (PFFAs) with carbon lengths of 7 to 10, including PFOA, inhibited GJIC in a dose-dependent manner, whereas both shorter and longer chain PFFAs did not.[86] Hu *et al.* also reported the inhibition of GJIC by PFOA and PFOS.[87] They concluded that PFFAs including PFOA could potentially act as hepatocarcinogens at gap junctions in addition to their ability to induce peroxisome proliferation.

### 3.3.4 *Effects on calcium channels*

We have recently found that both PFOS and PFOA had effects on action potential and L-type $Ca^{2+}$ currents using isolated guinea-pig ventricular myocytes by the whole-cell patch clamp electrophysiology technique.[88] These discernible effects were as low as 3 μM for PFOS at 1.5 ppm, whereas PFOA was less potent. The practical implications of this study are two-fold. First, PFOS and PFOA have pharmacological effects similar to calcium antagonists such as nifedipine in a relatively shallow resting potential.[88] Second, these pharmacological effects may explain rhabdomyolysis that was observed in subacute studies conducted in monkeys.[56,59] Both studies show the associations of PFOS and PFOA with calcium homeostasis. It should also be noted that nifedipine is reported to be linked with a general increased risk of cancer.[89]

## 4. Toxicokinetics in Humans and Animals

A recent study has shown interspecies differences in the pharmacokinetics of PFOA and PFOS.[90] An ongoing 5-year, half-life study of 9 retired workers has suggested mean serum PFOA and PFOS half-lives of 4.37 years (range 1.50–13.49 years, *SD* = 3.53) and 8.67 years (range 2.29–21.3 years, *SD* = 6.12), respectively.[90] These data provide evidence of the potentials of PFOA and PFOS to accumulate in humans. Their serum half-lives in other animals are summarized in Table 5. There are large interspecies differences in the half-lives of PFOA and PFOS. The rapid excretion of PFOA by female rats compared with male rats is due to active renal tubular secretion (organic acid transport system); this renal tubular secretion is believed to be hormonally controlled. This gender-related difference has not been observed in primates; however, this difference in elimination kinetics in humans is known, and may be associated with blood loss caused by menstruation.[38]

Harada *et al.*[38] has recently built a simple one-compartment model. This model can predict the fates of PFOA and PFOS in sera of rats, monkeys and humans when species specific half-lives are selected. The application of such a simple model suggests that interspecies differences can be attributed to differences in clearance kinetics, which are probably mediated by organic anion transporters(OAT1-3) specific to each species (Table 5).[38]

Table 5
Serum half-lives of PFOA and PFOS.

| | | Serum half-life (days) | | |
|---|---|---|---|---|
| Species | Sex | PFOA | PFOS | Ref. |
| Rat | male | 5.63 days | | (91) |
| | female | 0.08 days | | |
| | male | 105 hours | | (92) |
| | female | 60 hours | | |
| | male | | 7.5 days | (93) |
| Japanese macaque | male | 5.6 days | | (94) |
| | female | 2.7 days | | |
| Cynomolgus monkey | male | | 200 days | (59) |
| Human | male | 4.31 years | 8.67 years | (90) |

## 5. Conclusions

Perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and other fluorinated compounds have been considered to be very useful chemicals for many purposes with a low order of toxicity. Perfluorinated compounds or telomers with longer carbon chain lengths than those of PFOA and PFOS will degrade naturally to PFOA and PFOS.

In the Kinki district, the environmental contamination with PFOA and human exposure to it have been occurring insidiously over the last 20 years. The geographic heterogeneity in the exposure intensities of PFOA is very likely to be associated with industrial activities that might discharge PFOA in wastewater. The correlation of the higher-exposure levels of perfluorinated compounds and the densities of major fluorochemical industries in the Kinki district is not considered to simply have occurred by chance but rather is indicative of the emission of fluorochemicals from these industrial facilities. If this is indeed the case, the identification of the sources and the appropriate regulation of PFOA release are urgently required to be discussed.

However, there is uncertainty regarding the toxicities caused by PFOA and PFOS. There are two major reasons for this uncertainty. First, there are large interspecies differences in toxicities. Primate studies for PFOA and PFOS have revealed new aspects of the manifested toxicity, which had not been detected when carried out in rodents. Thus, subchronic and chronic studies using monkeys will be much more informative and worthwhile for extrapolation to humans to determine the associated effects. Second, the adverse effects of PFOA and PFOS have been reported in epidemiological studies, although they remain inconclusive because of low statistical power. There are many workers in Japan who are being or have been exposed to PFOA and other perfluorochemicals. Epidemiological studies are required for workers in Japanese industries. If conducted appropriately, meta-analysis can be established to present conclusive evidence of these toxicities such as the carcinogenicities of PFOA and PFOS by increasing the statistical power.

For proper risk assessment, dose-response relationship on the toxicities of PFOA and PFOS are still not sound enough at present, while various toxicities are identified. In addition, few data are available for the exposure assessments of PFOA and PFOS. Further studies are indispensable to make risk assessments of PFOA and PFOS. These studies should be directed to include the source of exposure and the pharmacokinetics of these compounds in general population.

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