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Disposition of Perfluorooctanoic Acid in the Rat after Single and Subchronic Administration

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Perfluorocarboxylic acids are strong acids having excellent heat and chemical resistance . They have various industrial uses because of the surface activity of the perfluoroalkyl group: e.g. in water- and oil-repellent coating compositions, lubricants, foams, and in the polymerization of fluorinated olefins (Guenthner and Vietor 1962, Shinoda and Nomura 1980). In general, perfluorinated hydrocarbons are considered non-toxic and metabolically inert. The toxicity of some perfluorinated carboxylic acids has been more thoroughly studied in recent years, perfluorooctanoic acid (PFOA, $C_7F_{15}COOH$) being one of them. After the report dealing with the human occupational contamination with PFOA (Ubel et al. 1980), studies on the toxic actions of this xenobiotic after oral, dermal, and inhalation exposure in rodents have been carried out (Griffith and Long 1980, Kennedy 1985, Kennedy et al. 1986).

In the rat, a sex-related difference in the urinary excretion of PFOA has been discovered : females have an active renal secretion mechanism for this anion, whereas male rats are lacking in it or it is inactive (Hanhijärvi et al. 1982) . The reduced elimination of PFOA may explain the greater susceptibility of male rats to the toxic actions of PFOA than females (Griffith and Long 1980).

PFOA has been shown to accumulate in the serum and liver in rats (Griffith and Long 1980, Johnson et al. 1984, Kennedy 1985), but a more complete description of its distribution in various organs has not been published Methods based on gas chromatography have been developed for PFOA determinations in biological samples (Belisle and Hagen 1981, Ylinen et al. 1985), because radiolabelled PFOA has not been commercially available for pharmacokinetic studies.

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In this study, the distribution and accumulation of PFOA were assayed in the female and male Wistar rats after a single (50 mg/kg) and subchronic administration (3, 10, and 30 mg/kg/day). The difference between female and male rats in the disposition of PFOA in organs was studied. The xenobiotic was assayed in the samples with gas chromatography and gas chromatography-mass spectrometry in the selected ion monitoring -mode.

MATERIALS AND METHODS

Perfluorooctanoic acid (PFOA) was purchased from Aldrich-Chemie (Steinheim, BRD) and perfluorononanoic acid (PFNA) from Fluorochem Ltd. (Derbyshire, UK). PFOA was dissolved in 0.9% NaCl solution and propylene glycol-water mixture (1:1) for intragavage and intraperitoneal administration, respectively. Tetra-butylammonium hydroxide 1M (Aldrich-Chemie) was further diluted to 0.05M in methanol. Benzyl bromide (E.Merck, Schuchardt, BRD) was diluted in methylene chloride at 0.05% (w/v).

The single dose of PFOA (50 mg/kg) was administered intraperitoneally to 10 weeks old Wistar female (N=20) and male (N=20) rats. The volume of the injected solution was 0.25 ml/100g. PFOA was administered 3, 10, and 30 mg/kg/day by gavage to 36 newly weaned Wistar rats (18 females+18 males) for 28 consecutive days in the subchronic test. The volume of the solution administered was 0.5 ml/100g. The animals were housed in 21±1°C during the tests with dark period from 9 p.m. to 7 a.m. and given tap water and regular rat chow ad libitum. After the single dose, samples were collected for PFOA determination 12, 24-168 (in 24 h intervals), 244 and 336 hours after the administration; in the subchronic test on the 28th day. The serum was collected by cardiac puncture; after decapitation the brain and at necropsy samples from the liver, kidney, lung, spleen, ovary, testis, and adipose tissue were collected and frozen.

PFOA was extracted from tissues with a mixture of diethyl ether:hexane (2:8) after homogenization and making acid with hydrochloric acid (Belisle and Hagen 1981) . PFNA was added to the tissue homogenates prior to the extraction as an internal standard 10 µg or 50 ng/sample for gas chromatographic or mass spectrometric detection, respectively. Benzyl bromide in methylene chloride (1 ml) together with methanolic tetrabutyl-ammonium hydroxide (0.1 ml) were used in the derivatization of the extracted PFOA and PFNA (Ylinen et al. 1985). Lipids were extracted from the liver with chloroform-methanol (2:1). The extract or a sample of the adipose tissue was hydrolysed for 1 hour under reflux with 1 M ethanolic KOH and the procedure

for extraction of PFOA was continued thereafter. The concentration of PFOA in the serum, tissues , and lipid extracts was determined with capillary gas chromatography (Ylinen et al. 1985) equipped with a flame ionization detector (FID) or a mass spectrometer (VG Trio-2 -quadrupole mass spectrometer interfaced with HP 5890 gas chromatograph). The latter was used in the selected ion monitoring (SIM) -mode, when the PFOA concentration in the samples was below the quantitation limit of the FID (1 µg/ml). The intensity of the molecular ion of benzyl-PFOA (m/z 504) and the internal standard, benzyl-PFNA (m/z 554) (Ylinen et al. 1985) , was monitored in the electron impact ionization at 50 eV electron energy and 300 µA ionization current. The method was calibrated by processing standard samples containing 1-200 ng of PFOA and 50 ng of PFNA. The quantitation limit of PFOA was 2 ng/sample; the coefficient of variation of 6 replicate injections of 50 pg of PFOA was 8.0% in the SIM-method.

The biological half-life ($T_{1/2}$) of PFOA in the serum and other tissues was estimated from the equation (linear regression) of the linear relationship between time and concentration of PFOA in the semilogarithmic plot (Fig. 1.).

RESULTS AND DISCUSSION

The time courses of the PFOA concentrations in the serum, liver, kidney, spleen, and brain of the rats after the single dose are shown in Fig. 1. The concentration of PFOA in the serum as well as other tissues assayed was higher in males than females during the whole test. Twelve hours after the administration of PFOA about 10% of the dose was found in the serum of females, whereas about 40% was in the serum of males. After 14 days about 3.5% of the dose was still left in the serum of males. In the females, the fall of the PFOA level in the serum, liver, and kidney occurred in a discontinuous fashion indicating distinct phases. The $T_{1/2}$ of the concentration of PFOA in the serum was 24 and 105 h in the females and males, respectively. In the females, a $T_{1/2}$ of 60 h was estimated in the liver during the first week. In the males, the $T_{1/2}$ was 210 h and a more linear relationship was observed between time and PFOA concentration . Although PFOA was retained by the liver, it was not found in the lipid fraction of it assayed with gas chromatography. In the kidney, the $T_{1/2}$ of the PFOA concentration was 145h and 130h in females and males, respectively. Similar $T_{1/2}$-values of the PFOA concentration were estimated in the spleen as in the liver (females 73h and males 170h) . The unbound fraction of PFOA in the serum was able to penetrate in the brain, as PFOA could be quantitated in the brain tissue. In the samples of

adipose tissue, detectable amounts of PFOA were not found.

Table 1. Concentrations of perfluorooctanoic acid (PFOA) in various tissues of rats after subchronic (28 days) administration with doses 3, 10, and 30 mg/kg/day.

CONCENTRATION OF PFOA, µg/ml, g	Mean (±S.D.;N)		
	Daily PFOA dose (mg/kg)		
	3	10	30
Serum			
Female	2.40 (N=3)	12.47 (4.07;6)	13.92 (6.06;6)
Male	48.60 (10.30;6)	87.27* (20.09;6)	51.65* (11.47;6)
Liver			
F	1.81 (0.49;6)	3.45 (1.36;6)	6.64 (2.64;6)
M	39.90* (7.25;6)	51.71* (11.18;6)	49.77* (10.76;6)
Kidney			
F	0.06 (0.02;6)	7.36 (3.19;6)	12.54 (8.24;6)
M	1.55* (0.71;6)	40.56* (14.94;6)	39.81* (17.67;6)
Spleen			
F	0.15 (0.04;6)	0.38 (0.17;6)	1.59 (0.49;6)
M	4.75* (1.66;6)	7.59* (3.50;6)	4.10* (1.57;6)
Lung			
F	0.24 (N=3)	0.22 (0.15;6)	0.75 (0.26;6)
M	2.95 (0.54;6)	22.58* (4.59;6)	23.71* (5.42;6)
Brain			
F	<L_Q	0.029 (0.019;6)	0.044 (0.018;6)
M	0.398 (0.144;6)	1.464* (0.211;6)	0.710* (0.320;6)
Ovary	<L_Q	0.41 (0.27;6)	1.16 (0.58;6)
Testis	6.24 (2.04;6)	9.35 (4.02;6)	7.22 (3.17;6)

* different from females, $p<0.05$ (Student's t-test)
<L_Q concentration below the quantitation limit of the SIM method (2 ng/sample)

Results of the PFOA assays of the samples taken at the 28th day of the subchronic test are shown in Table 1. The concentration of PFOA in the serum as well as other tissues analysed was higher in the males than females ($p<0.05$) in all the three dose levels. After the single as well as subchronic administration, PFOA was mainly distributed in the serum of rats. Also in the liver, kidney, and lung, organs highly perfused by blood, males and females had high concentrations of PFOA. A significant ($p<0.05$) positive correlation existed between the administered dose and the concentration of PFOA in the liver ($r^2=0.996$), kidney ($r^2=0.933$), spleen ($r^2=0.995$), and lung ($r^2=0.959$) of females. On the contrary, no significant correlation between the administered dose and the concentration of PFOA was observed in the males, as 10 mg/kg/day produced higher PFOA concentrations in serum and organs than 30 mg/kg/day. However, in males, the concentration in the spleen, testis, and brain correlated positively with the concentration in the serum ($r^2=0.969$, 0.971, and 0.976, respectively).

In general, perfluorinated hydrocarbon derivatives are lipophilic compounds (Sargent and Seffl 1970). However, perfluorinated carboxylic acids being strong acids are totally ionized in water solutions: the pK_A-value of PFOA is 2.5 and the $P_{heptane/water}$ in pH 7.4 is <0.01 . Because of the hydrophilic and ionic character of PFOA, it is distributed mainly in the serum and excreted principally in the urine in rats. PFOA is excreted also in the bile, but due to the enterohepatic circulation, the rate of the fecal elimination is slow (Johnson et al. 1984). Metabolism of PFOA in rats is not evident (Ophaug and Singer 1980). Thus, the significant difference in the elimination rate of PFOA in urine between sexes in the rat after a single dose (2-12 mg/animal) explains the sex dependent difference observed also in the tissue concentrations (Hanhijärvi et al. 1982, *Ylinen* et al. 1989, manuscript).

The urinary excretion of PFOA in the subchronic administration with the present doses in female and male rats has been reported elsewhere (Hanhijärvi et al. 1987). The rate of the urinary PFOA excretion was faster in females than males assayed on the 7. and 28. day. However, the difference between females and males was significant only in the group receiving 3 mg/kg/day. PFOA is almost totally bound to serum proteins in rats and any sex difference in the binding is not obvious (Hanhijärvi et al. 1982, *Ylinen* et al. 1989, manuscript). Saturation of the binding sites of PFOA in the serum resulting in an increased urinary elimination is obvious in the group of males receiving the highest dose in the subchronic administration (30

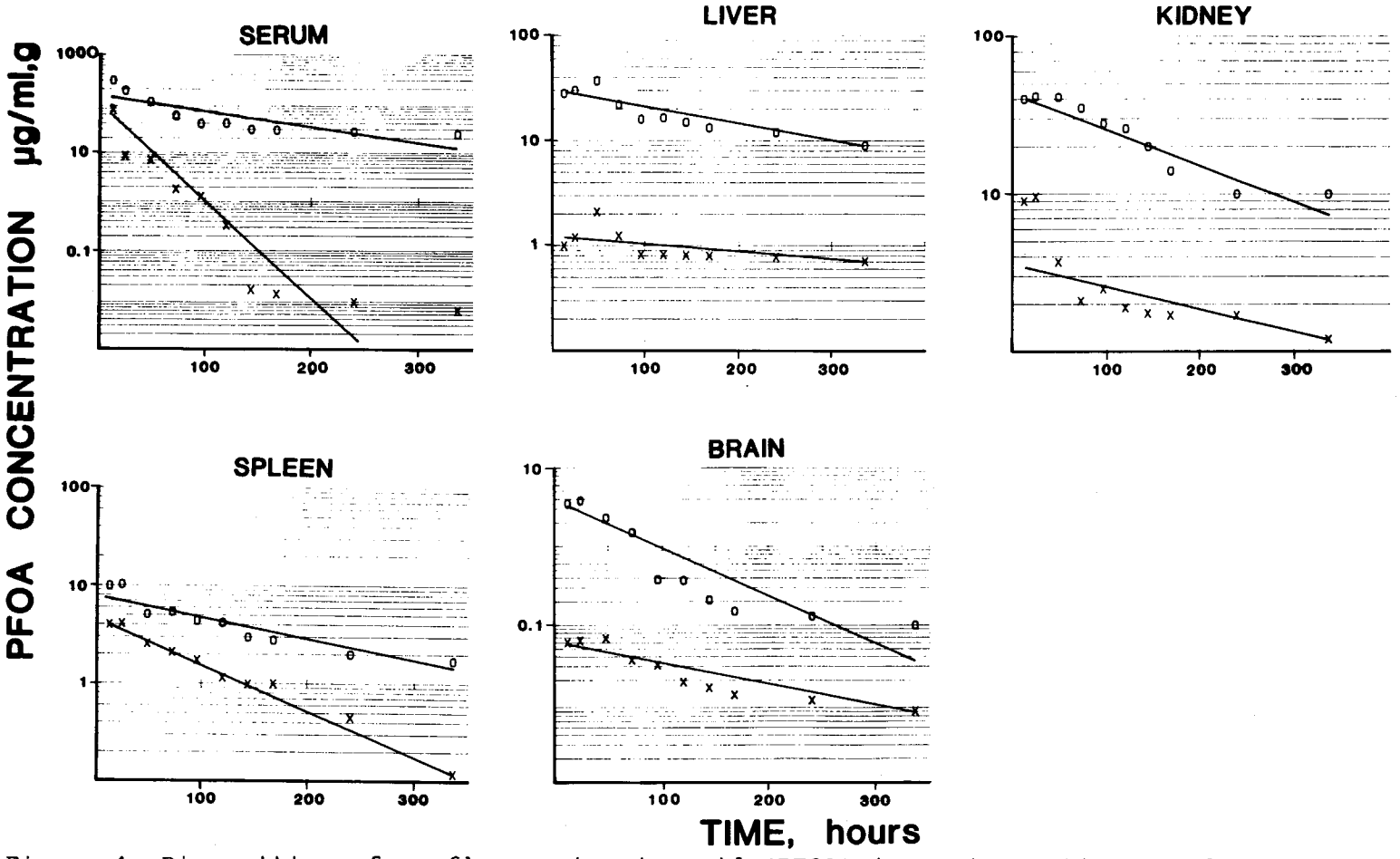

Figure 1. Disposition of perfluorooctanoic acid (PFOA) in various tissues of female and male rats after a single dose (50 mg/kg). Each point represents a mean of the results of two animals.
Female —X— Male —O—

mg/kg/day) . Males receiving 10 mg/kg/day excreted PFOA in urine less than the daily dose on the 28th day (Hanhijärvi et al. 1987), which also explains the higher concentration of PFOA in the serum as well as other tissues of this group than the males receiving 30 mg/kg/day.

The relatively long $T_{1/2}$ of PFOA in the serum of the male rats after a single administration is consistent with the estimate of Kennedy (1985) after dermal application. Strong binding to proteins together with the reduced urinary excretion have an influence on the accumulation of PFOA in the serum and other tissues of male rats. High organic fluorine levels in the serum and urine of workers exposed to perfluorochemicals (mainly PFOA) even 14 months after the removal from exposure indicate a very slow elimination of this xenobiotic also in humans (Ubel et al. 1980).

According to several studies, the liver is the main target organ of the toxic actions of PFOA in rats (e.g. Griffith and Long 1980, Kennedy 1985, Pastoor et al. 1987). Signs of the toxic actions of PFOA in the liver include histopathologic (Griffith and Long 1980) as well as enzymatic changes , and changes in the lipid metabolism (Pastoor et al. 1987). Comparable treatment levels of PFOA have been shown to produce more pronounced histopathologic effects in male rats than in females (Griffith and Long 1980), which is pertinent with the difference between sexes in the disposition of PFOA in the liver. However, PFOA is not expected to be hepatocarcinogenic in rats, although it accumulates in the liver and induces hepatomegaly. Based on the hepatic DNA content, the hepatomegaly was defined as hypertrophy rather than hyperplasia (Pastoor et al. 1987).

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REFERENCES

Belisle J, Hagen DF (1980) A method for the determination of perfluorooctanoic acid in blood and other biological samples. Anal Biochem 101: 369-376

Griffith FD, Long JE (1980) Animal toxicity studies with ammonium perfluorooctanoate. Am Ind Hyg Assoc J 41:576-583

Guenthner RA, Vietor ML (1962) Surface active materials from perfluorocarboxylic and -sulphonic acids. I&EC Prod Res Develop 3:165-169

Hanhijärvi H, Ophaug RH, Singer L (1982) The sex-related difference in perfluorooctanoate excretion

in the rat. Proc Soc Exp Biol Med 171: 50-55
Hanhijärvi H, Ylinen M, Kojo A, Kosma VM (1987) Elimination and toxicity of perfluorooctanoic acid during subchronic administration in the Wistar rat. Pharmacol Toxicol 61: 66-68
Johnson JD, Gibson SJ, Ober RE (1984) Cholestyramine-enhanced fecal elimination of ^{14}C in rats after administration of ammonium ^{14}C-perfluorooctanoate or potassium ^{14}C-perfluorooctanesulphonate. Fundam Appl Toxicol 4: 972-976
Kennedy GL (1985) Dermal toxicity of ammoniumperfluoro-octanoate. Toxicol Appl Pharmacol 81: 348-355
Kennedy GL, Hall GT, Brittelli MR, Barnes JR, Chen HC (1986) Inhalation toxicity of ammonium perfluorooctanoate. Fd Chem Toxic 24: 1325-1329
Ophaug RH, Singer L (1980) Metabolic handling of perfluorooctanoic acid. Proc Soc Exp Biol Med 163: 19-23
Pastoor TP, Lee KP, Perri MA, Gillies PJ (1987) Biochemical and morphological studies of ammonium perfluorooctanoate-induced hepatomegaly and peroxisome proliferation. Exp Mol Pathol 47: 98-109
Sargent JW, Seffl RJ (1970) Properties of perfluorinated liquids. Fed Proc 29: 1699-1703
Shinoda K, Nomura T (1980) Miscibility of fluorocarbon and hydrocarbon surfactants in micelles and liquid mixtures. J Phys Chem 84: 365-369
Ubel FA, Sorensen SD, Roach DE (1980) Health status of plant workers exposed to perfluorochemicals - a preliminary report. Am Ind Hyg Assoc J 41: 584-589
Ylinen M, Hanhijärvi H, Peura P, Rämö O (1985) Quantitative gas chromatographic determination of perfluorooctanoic acid as the benzyl ester in plasma and urine. Arch Environ Contam Toxicol 14: 713-717

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