[Chem. Pharm. Bull. 26(3) 956—966 (1978)]

UDC 615.456.3.011.4.014.23.076.9

Fate of Perfluorochemicals in Animals after Intravenous Injection or Hemodilution with Their Emulsions

Kazumasa Yokoyama,[1a] Kouichi Yamanouchi,[1b] Harumasa Ohyanagi, and Takao Mitsuno[1a]

Kobe University, School of Medicine, First Department of Surgery[1a] *and The Green Cross Corporation*[1b]

(Received August 29, 1977)

The tissue distribution and excretion of perfluorochemicals (PFCs) in the mixed PFC emulsion, which were a mixture of 7 parts Perfluorodecalin (FDC) and 3 parts Perfluorotripropylamine (FTPA), were studied in rats and dogs hemodiluted to a hematocrit of 5% and monkeys to 1%. The half life of both PFCs in rats given 4 g/kg body weight were calculated from their expiratory excretion rate to be about 7 days for FDC and 63 days for FTPA. Both PFCs in the mixed PFC emulsion were excreted according to respective excretion rate, indicating no mutual effect of combined use on respective excretion rate. Both PFCs were taken up into the liver, spleen and bone marrow in the majority and eliminated through expiration as the main route and bile as the minor route, the maximum deposition occurring in 4 days after infusion. FDC localized in the tissues was rapidly eliminated and no longer detected both in dogs at 6 months after hemodilution and in monkeys at 4 months. On the other hand, FTPA was also fairly rapidly eliminated from the tissues, but at a slower rate and it was retained only in the liver and spleen in both of dogs at 6 months and monkeys at 4 months in a very small amount. The absence of excess fluoride ion in urine and the coincidence of mass spectra of FDC and FTPA retained in the liver with those of the standard suggested that both of them were not catabolized *in vivo*.

Keywords——perfluorochemical emulsion; perfluorodecalin; perfluorotripropylamine; elimination; distribution; metabolism

One of the major impediments of perfluorochemical (PFC) emulsion as an artificial blood substitute has been that the compounds are retained in the tissues of animals for a long period of time. In order to solve this problem, 20 or more kinds of PFCs were examined by many workers.[2] From these enormous efforts, perfluorodecalin (FDC) has been chosen as the most suitable compound, due to its rapid elimination from the body without emphysema,[2b,d,f] but it has been difficult to prepare the fine and stable emulsion with a conventional emulsifying agent as phospholipids or Pluronic polymers.[2d,3]

The particle size of the emulsion is apparently very important because the toxic effect is closely related to it; the larger, the more toxic.[4] Furthermore, larger particles are removed from the circulation more rapidly than smaller ones when the emulsion is intravenously injected.[4,5] Therefore, it is essential to prepare the fine and stable emulsion for developing the PFC emulsion as an artificial blood substitute.

1) Location: *a*) *13/7, Kusunoki-cho, Ikuta-ku, Kobe*; *b*) *44, 5/3, Miyakojima-nakadori, Miyakojima-ku, Osaka.*

2) *a*) L.C. Clark, Jr. and F. Gollan, *Science*, **152**, 1755 (1966); *b*) L.C. Clark, Jr., E.P. Wessler, M.L. Miller, and S. Kaplan, *Microvascular Res.*, **8**, 320 (1974); *c*) R.P. Geyer, *New Engl. J. Med.*, **289**, 1077 (1973); *d*) K. Yokoyama, K. Yamanouchi, M. Watanabe, T. Matsumoto, H. Okamoto, T. Suyama, and R. Naito, *Fed. Proc.*, **34**, 1478 (1975); *e*) K. Yokoyama, K. Yamanouchi, and R. Murashima, *Chem. Pharm. Bull.* (Tokyo), **23**, 1368 (1975); *f*) W.I. Rosenblum, M.G. Hadfield, A.J. Martinez, and P. Schatzki, *Arch. Pathol. Lab. Med.*, **100**, 213 (1976).

3) L.C. Clark, Jr., E.P. Wessler, S. Kaplan, M.L. Miller, C. Becker, F. Becattini, and V. Obrock, *Fed. Proc.*,, **34**, 1468 (1975).

4) R.P. Geyer, *Fed. Proc.*, **34**, 1499 (1975).

5) H. Okamoto, K. Yamanouchi, and K. Yokoyama, *Chem. Pharm. Bull.* (Tokyo), **23**, 1452 (1975).

For this purpose, a comprehensive screening test was performed to select the suitable FDC emulsion as an artificial blood substitute, which is stable both *in vitro* and *in vivo*. As the result of various trials, the combined use of FDC and perfluorotripropylamine (FTPA) was found to produce a fine and stable emulsion.[6] FTPA used is one of the acceptable compounds as the material of artificial blood because of its low toxicity and fairly rapid elimination from the body. Its half life in the body of rats is around 65 days, which is shorter than that of other perfluoroalkylamine compounds but longer than that of FDC. PFCs in the PFC emulsion used in this study, which were selected by the screening test, were a mixture of 7 parts FDC and 3 parts FTPA. The mixture was emulsified with Pluronic F68 with addition of a small amount of yolk phospholipids. The preparation was low toxic (LD_{50}: about 35 g/kg body weight as PFC) and had high activity as an oxygen carrying agent *in vivo*. The rats exchange-transfused with this preparation unitl hematocrit reached 4% survived for around 60 hrs, while those with Ringer lactate solution died within 30 min.[7]

The purpose of this study is to determine the distribution of such different types of PFCs in the PFC emulsion throughout the body and to clarify whether long-term retention occurs in various organs following intravenous injection of mixed PFCs. In addition, it is also studied whether or not the combined use of both PFCs in mixed form, which have different excretion rate, influences mutually their excretion rate.

Materials and Methods

1. Materials——PFCs utilized in this study were FDC (Imperial Smelting Company, U.K.) and FTPA (Dainippon Ink. Co., Japan). The chemical formulae and important physical constants are listed in Table I.

Table I. Chemical Formulae and Physical Properties of PFCs

	Perfluorodecalin[a] (FDC)	Perfluorotri-propylamine[a] (FTPA)
Chemical formulae	$C_{10}F_{18}$	$C_9F_{21}N$
(mol wt.)	(462.11)	(521.07)
Bp (°C)	142	129
Density (g/cc)	1.945 (20°)	1.823 (25°)
Vapor Pressure (Torr, at 37°)	12.7	20.0
Refractive index (n_D^{25})	1.313	1.279
Proton content[b] (g atom/l)	$<0.3\times10^{-4}$	$<1.01\times10^{-4}$

a) Purity (by GC): FDC, 98.6%; FTPA, 96.8%.
b) By NMR.

Benzotrifluoride (BTF) was used as an internal standard for gas chromatography. 1,1,2-Trichlorotrifluoroethane (FC-113) and fluorinated polyether (Freon E_4; Dupont Chemical, U.S.A.) were used for extracting the PFCs retained in blood and for capturing them in expiration, respectively. These reagents were purified by the method of distillating fractionation before use.

2. Preparation of PFC Emulsions[8]——Six kinds of PFC emulsions were prepared in this experiment; FDC emulsified with yolk phospholipids (PL), FTPA with Pluronic F68 (Asahi Denka Co., Ltd., Japan) and 4 sorts of mixtures of FDC and FTPA, of which mixing ratio was 8: 2, 7: 3, 6: 4 and 5:5 by volume, emulsified with both Pluronic F68 and a small amount of PL (Vitrum AB, Sweden). These emulsions were prepared with a Manton Gaulin homogenizer (Manton Gaulin Co., U.S.A.) at the 2nd pressure of 50 kg/cm² and the total pressure of 150 kg/cm² under the nitrogen gas stream. The resultant emulsions were filtered with a Millipore membrane (Nippon Millipore Ltd., Japan) of 0.45 micron pore size, filled in a 100 ml glass vial and

6) R. Naito (ed.), "Fluosol-DA® as a Candidate for Erythrocyte Substitution," Tech. Inform. Ser. No. 4, The Green Cross Corp., Osaka, 1976, pp. 41—52.
7) T. Matsumoto, M. Watanabe, T. Hamano, S. Hanada, T. Suyama, and R. Naito, *Chem. Pharm. Bull.* (Tokyo), **25**, 2163 (1977).
8) K. Yokoyama, A. Suzuki, I. Utsumi, and R. Naito, *Chem. Pharm. Bull.* (Tokyo), **22**, 2966 (1974).

then, sterilized at 115° for 30 min. In animal experiments, these emulsions were made isotonic and isooncotic with Krebs Ringer bicarbonate balanced salt and low molecular hydroxyethyl starch (M.W. 110000, Ajinomoto Co., Ltd., Japan), respectively, just prior to injection.

3. Determination of PFCs in Tissues and Blood——The method of determination herein used complied with our report[9] as outlined below. One ml of blood, tissue homogenates (0.2 to 0.3 g wet tissues), or bile was placed in a test tube and then, 3 ml of ethanol was added. By centrifugation for 10 min at 3000 rpm, the PFCs were settled down with tissue fragments and supernatant was discarded. Five ml of FC-113 was added to residual precipitation in the tube for extracting them. After centrifugation again, the lower FC-113 layer was washed twice with water and was dried over anhydrous sodium sulfate. One ml aliquot was transferred into another test tube and 1 to 2 ml of 1.0% (v/v) benzotrifluoride solution in FC-113 was added as the internal standard. Then, this sample was analyzed by gas chromatography.

4. Determination of PFCs in Expiration——The determination of PFCs excreted through expiration was carried out by our previous reported method.[2e] Three glass absorbance tubes, 30 cm tall and 3 cm i.d., each containing 20 ml of Freon E_4 as the absorbent and 30 ml of water as covering layer were used to capture the PFCs in expiration.

Two rats given the PFC emulsion were placed in a glass vessel (about 10 l capacity) for 2 or 3 hrs at several intervals after the injection and the air was sucked with a suction pump through the exhaust pipe. The air inspired through the inlet pipe was led into the absorbance tubes through the vessel and the vaporized compounds in the expiration were captured in Freon E_4. The air flow was kept between 450 and 500 ml per minute during the experiment. One ml of Freon E_4 was collected into a test tube and accurately 1 ml of 1.0% (v/v) BTF solution in Freon E_4 was added as the internal standard. Then the sample was analyzed by gas chromatography. The capturing efficiencies of FDC and FTPA were 93.2 and 91.4%, respectively.

5. Gas Chromatography——The gas chromatography was done by the method of our previous report.[9] A Shimadzu gas chromatograph model GC-4BPTF equipped with flame ionization detectors was used in this experiment. A column used for determining PFCs in FC-113 was a coiled glass tube, 2 m long and 4 mm i.d. packed with 20% Silicone OV-17 on Chromosorb W AW (DMCS), 60 to 80 mesh, and for determining PFCs in Freon E_4 was a same size tube packed with 15% Carbowax-1540. Two types of analysis were carried out under the same operating condition. The temperature of column oven, injection port and detector were 60, 175 and 180°, respectively. Nitrogen gas flow rate was 40 ml per minute. Under these conditions, FDC and FTPA were able to be determined simultaneously.

6. Determination of Fluoride Ion in the Urine——Fluoride contents in the urine were determined with an Orion specific fluoride ion electrode model 69—09 (Orion Research Corp., U.S.A.) by the method of Venkateswarlu as outlined below.[10] One ml of urine was diluted with 5 ml of TISAB (Ammonium acetate, 384 g/l, 11.6 M HCl 213.5 ml/l, 1,2-cyclohexane diamine tetraacetic acid 19.8 g/l and cresol red 0.07 g/l, pH 5.2) and then, the fluoride contents were directly measured with the specific ion electrode.

7. Gas Chromatography-Mass Spectrometry (GC-MS)——Preparation of analytical samples: PFCs in the liver and spleen of rats intravenously infused with the PFC emulsion were extracted with trichlorotrifluoroethane as reported previously.[11] The extracts were dried over anhydrous sodium sulfate and aliquot was injected into the GC–MS.

GC–MS: A Shimadzu LKB GC–MS Model 9000 instrument was employed. The chromatographic column was 6 m × 3 mm glass coiled tube with 15% DOP on acid washed Chromosorb W 60/80 mesh (Nihon Chromato Works, Ltd., Japan). Analyses were carried out at an oven temperature of 50° isothermally, while the molecular separator and ion source were held at 180 and 210°, respectively. The flow rate of carrier gas (helium) was 30 ml/min and the trap current was 60 μA. The mass spectra were recorded at electron energies of 20 and 70 eV.

Mass Chromatography: The GC–MS–Computer System, an LKB 9000 operated on-line with a Shimadzu PAC-300DG Computer was employed. Following injection of the sample, magnet scans converting the mass range (*m/e* 195—540) were initiated automatically after a selected delay and with 5 seconds intervals. After completion of the run, characteristic ions plotted on the display terminal were compared with those of reference PFCs.

8. Animal Experiments——Rats: A total of 100 Wistar strain male rats weighing 150—180 g was used in this study. The rats were intravenously injected with 16—80 ml/kg body weight of the mixed PFC, FDC, or FTPA emulsion, corresponding to 4—20 g/kg as PFC and the concentration of PFCs in the tissues was determined at various intervals through 8 weeks after injection by the method described above. In order to determine the fluoride content in the urine, 10 rats injected with 32 ml/kg of the mixed PFC emulsion, of which PFCs consisted of 7 parts FDC and 3 parts FTPA, were housed in a metabolic cage throughout the 8 weeks and urine was collected with a polyethylene beaker daily.

9) K. Yamanouchi, R. Murashima, and K. Yokoyama, *Chem. Pharm. Bull.* (Tokyo), **23**, 1363 (1975).
10) P. Venkateswarlu, *Anal. Chem.*, **46**, 878 (1974).
11) K. Yamanouchi and K. Yokoyama, Proc. Xth Intern. Cong. Nutrition-Symposium on PFC Artificial Blood, Kyoto, 1975, pp. 91—102.

Biliary excretion of PFCs in rats: At various intervals during 4 weeks after injection of 32 ml/kg of the mixed PFC emulsion the rats were anesthetized with urethane (250 mg/kg, *i.m.*) and a catheter was cannulated into the hepatic bile duct. Then, the concentration of PFCs in the bile collected during 8 hrs was determined by the gas chromatographic method described above and the total amount of PFCs excreted through the bile each day was calculated.

Exchange transfusion of PFC emulsion in dogs and monkeys:[12] Six beagle dogs weighing 8 to 10 kg and 2 green monkeys weighing 3.8 and 4.2 kg were used in this experiment. The animals fasted overnight were anesthetized with intermittent halothane administration under pure oxygen respiration. A catheter was inserted into the femoral artery and vein. The animals were bled at 10 ml/kg for 10 min from the femoral artery and simultaneously infused with HES solution (lactated Ringer solution containing 6% hydroxyethyl starch) 1.5 times the shed blood volume through the femoral vein to 20% hematocrit value. Thereafter, the mixed PFC emulsion, of which PFCs consisted of 7 parts FDC and 3 parts FTPA, was infused into the femoral vein instead of the HES solution until hematocrit reached 5% in dogs and 1% in monkeys. Eight hours after the end of exchange transfusion, the autologous erythrocyte suspension was reinfused, whereby the hematocrit reached 20%. Then, the animals housed in a common cage and allowed to take commercial food and water *ad. libitum* in an air conditioned room. All the animals survived well without ill effect throughout the experimental period. The dogs exchange-transfused were sacrificed at 2, 4 and 6 months after operation and the monkeys at 2 and 4 months, and the concentration of PFCs in the tissues was determined.

Results and Discussion

1. Excretion of FDC and FTPA in Rats following Intravenous Injection of Their Emulsions

Table II shows the comparative data on the retention of FDC and FTPA in main organs of rats following intravenous injection of their emulsions.

Table II. Comparison of Tissue Retention of FDC and FTPA in Rats following Intravenous Injection of Their Emulsions

Tissues	Time after injection	% of given dose	
		FDC	FTPA
Liver	1 week	8.50±2.38	10.40±3.33
	2 weeks	0.50±0.12	4.60±1.03
	4 weeks	0.09±0.02	2.30±0.73[a]
Spleen	1 week	4.00±1.25	6.90±1.14
	2 weeks	0.40±0.19	5.80±2.01
	4 weeks	0.20±0.16	1.20±0.50[a]
Kidney	1 week	0.04±0.01	0.03±0.01
	2 weeks	N.D.	tr.
	4 weeks	N.D.	N.D.
Lung	1 week	tr.	0.01±0.00
	2 weeks	N.D.	tr.
	4 weeks	N.D.	N.D.

Dose: 4 g/kg body weight as PFC.
tr.: trace, less than 10 μg/g tissue.
N.D.: not detected, less than 1 μg/g tissue.
a) significantly different from the FDC group, $p < 0.01$.

Both PFCs were emulsified with yolk phospholipids for FDC and with Pluronic F68 for FTPA to a similar particle size distribution, about 0.1 μ in average diameter, and the concentration of PFCs in organs was determined through 4 weeks after injection of 4 g/kg body weight as PFCs. Both PFCs were taken up into the liver and spleen in the majority, the maximum deposition in these organs occurring in 4 days after injection, but significant difference was found in the elimination rate between both PFCs. The concentration of

12) *a*) H. Ohyanagi, M. Sekita, T. Matsuda, S. Okumura, and T. Mitsuno, *Resp. and Cir.* (in Jap.), **22**, 380 (1974); *b*) M. Sekita, *Jap. J. Surg.*, **3**, 184 (1973).

FDC retained in the liver at 4 weeks after injection was less than 0.1% of the given dose, while that of FTPA was about 3%.

Our previous report[2e] has clarified that PFCs were mainly excreted through expiration and the elimination curves of those from the body were found to be exponential as shown in Fig. 1. Since the primary excretion route of PFCs was expiration, the retained amounts of the substances in the body were calculated by summing the total amounts of expiratory PFCs excreted. The half life of FDC and FTPA in the body were calculated to be 7 days and 65 days, respectively, from the elimination curves shown in Fig. 1.

The mixed PFC emulsion selected as an artificial blood substitute consists of FDC and FTPA having a different elimination rate. This made us conceive that the combined use of different types of these substances might accelerate their elimination each other as found in an azeotropic mixture. In order to clarify whether the combined use of FDC and FTPA mutually affecting their elimination rate or not, rats were injected with 4 g of mixture of FDC and FTPA in emulsified form at the ratio of 8: 2, 7: 3, 6: 4 and 5: 5 and the elimina-

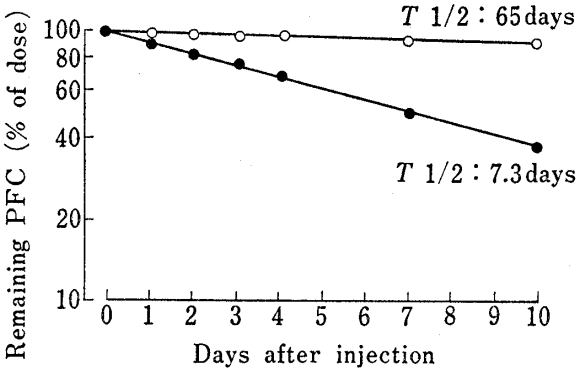

Fig. 1. Elimination Curves of FDC and FTPA in Rats through Expiration

Dose: 4 g/kg body weight as PFC.
—○—, FTPA; —●—, FDC.

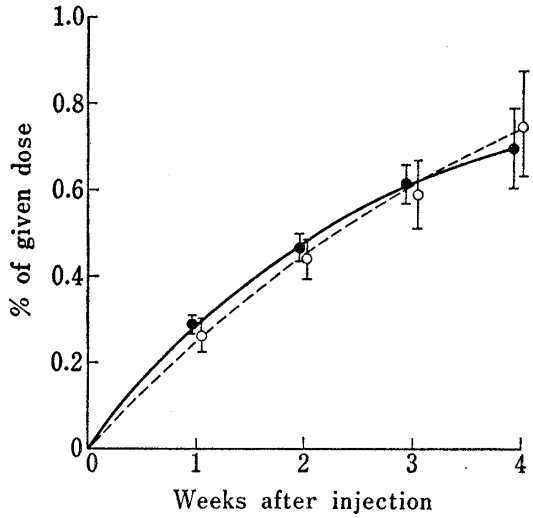

Fig. 2. Cumulative Curves of Biliary Excretion of FDC and FTPA in Rats following Intravenous Injection of Mixed PFC Emulsion

Dose: 8 g/kg body weight as PFC.
—●—, FDC excreted; ---○---, FTPA excreted.

Table III. Expiratory Elimination Rate Constant (K) and consequently Calculated Half Life of FDC and FTPA in Rats

PFC in emulsion	Ratio of FDC: FTPA	Elimination rate constant PFC	K ($\times 10^3$)	Half life
FDC alone	—	FDC	3.95 hr^{-1}	7.3 days
FDC/FTPA	8:2	FDC	3.70	7.8
		FTPA	0.444	64.5
FDC/FTPA	7:3	FDC	3.99	7.2
		FTPA	0.420	68.8
FDC/FTPA	6:4	FDC	4.01	7.0
		FTPA	0.453	63.8
FDC/FTPA	5:5	FDC	3.66	7.9
		FTPA	0.459	64.2
FTPA alone	—	FTPA	0.446	64.7

Dose: 4 g/kg body weight as PFC.

tion rate of FDC and FTPA from the body in the mixed PFC emulsion was compared to that of FDC and FTPA injected alone. The results are shown in Table III. The half life in all groups, which was calculated from the elimination rate constant through expiration, ranged from 7 to 8 days in FDC and from 63 to 70 days in FTPA, and no significant difference was found in the elimination rate in all groups, indicating no mutual effect of the combined use of FDC and FTPA on their elimination rate.

As mentioned above, PFCs were mainly excreted through expiration, but the biliary excretion was also noted. Fig. 2 shows the cumulative excretion curves of FDC and FTPA through bile during 1 month after injection of the mixed PFC emulsion. The excreted amounts of PFCs through bile was far little as compared to that through expiration and only 0.7% of the given dose in FDC and 0.75% in FTPA were found, indicating that the biliary excretion is a minor route. No significant difference was observed in the biliary excretion between FDC and FTPA.

2. Distribution and Elimination of FDC and FTPA in and from Tissues following Intravenous Injection of the Mixed PFC Emulsion in Animals

Rats were injected with 80 ml/kg body weight of the mixed PFC emulsion corresponding to 20 g/kg as PFCs and the concentration of FDC and FTPA in tissues were determined separately. The results are shown in Table IV.

Table IV. Distribution and Retention of PFCs in Rats after Injection of Mixed PFC Emulsion

Organs	Time after infusion	mg/g wet tissue		
		FDC	FTPA	Total PFC
Liver	4d	73.3 ±13.3	29.6 ± 4.2	102.0 ± 13.7
	1w	64.9 ± 7.5	28.8 ± 2.5	93.7 ± 7.9
	2w	38.2 ± 6.4	21.4 ± 2.9	59.6 ± 9.2
	4w	7.3 ± 2.9	9.2 ± 2.6	16.5 ± 5.1
	8w	0.59± 0.11	2.7 ± 0.47	3.3 ± 0.60
Spleen	4d	153.8 ± 9.5	68.1 ± 4.9	221.9 ± 1.44
	1w	199.1 ±71.8	91.9 ± 3.9	291.1 ±110.9
	2w	61.3 ±16.9	30.2 ±10.6	91.5 ± 27.4
	4w	38.5 ± 3.4	15.1 ± 2.8	53.6 ± 6.1
	8w	0.97± 0.20	3.1 ± 0.59	4.1 ± 0.75
Lung	4d	3.3 ± 1.6	2.1 ± 1.0	5.4 ± 2.6
	1w	2.9 ± 0.4	2.3 ± 0.2	5.2 ± 0.45
	2w	tr.	tr.	tr.
	4w	tr.	tr.	tr.
	8w	N.D.	N.D.	N.D.
Kidney	4d	11.5 ± 4.4	6.1 ± 2.7	17.6 ± 7.2
	1w	6.0 ± 1.2	3.6 ± 0.8	9.6 ± 1.9
	2w	1.98± 0.38	1.76± 0.34	3.74± 0.71
	4w	0.73± 0.60	1.25± 1.0	1.97± 1.51
	8w	N.D.	N.D.	N.D.
Femoral marrow	4d	32.7 ±11.0	14.3 ± 4.2	47.0 ± 15.2
	1w	38.5 ± 5.7	18.7 ± 3.6	60.6 ± 3.9
	2w	24.3 ± 2.6	12.9 ± 2.4	37.2 ± 4.9
	4w	14.2 ± 5.2	12.3 ± 4.5	26.6 ± 9.6
	8w	0.09± 0.02	0.8 ± 0.05	0.90± 0.10

Dose: 20 g/kg body weight as PFC.
The values represent the mean ± standard deviation for 8 to 10 rats.
tr.: trace, less than 10 μg/g tissue. d: days. w: weeks. N.D.: not detected, less than 1 μg/g tissue.

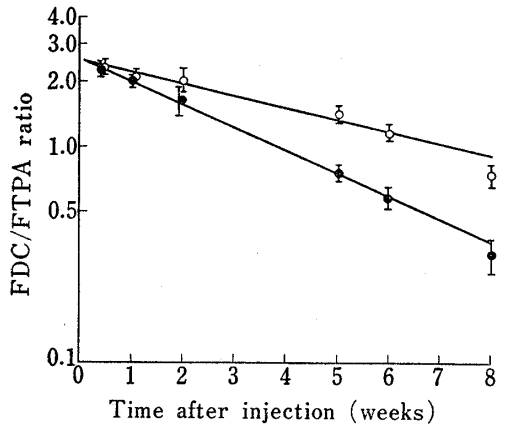

Fig. 3. Change of FDC/FTPA Ratio in Liver and Spleen of Rats following Injection of Mixed PFC Emulsion

Dose: 20 g/kg body weight as PFC.
—●—, in liver; —○—, in spleen.

Both PFCs in the mixed PFC emulsion were taken up into the liver, spleen and bone marrow in the majority at an early stage after injection and the maximum concentration of both PFCs in the liver reached at 4 days after injection, being 65 mg/g wet tissues in FDC and 29 mg/g in FTPA, respectively. The concentration of both PFCs so localized in the tissues decreased fairly rapidly with time, and was found only less than 1 mg/g in FDC in both liver and spleen and about 3 mg/g in FTPA in those organs 8 weeks after the injection. As indicated, FDC was eliminated from the tissues at a rate far faster than FTPA.

Fig. 3 shows the time course of FDC/FTPA ratio of PFCs retained in the liver and spleen following injection of the mixed PFC emulsion. The ratio of FDC to FTPA in both organs decreased with time and its declining curve seemed to be exponential, suggesting that the elimination of both PFCs from the organs also followed a first order process.

Table V shows the distribution and elimination of FDC and FTPA in dogs exchange-transfused with the mixed PFC emulsion until hematocrit reached below 5%. The net dose given as PFCs was about 15 g/kg body weight, which was calculated by subtracting the amount of PFCs in the shed blood from the total amounts infused. The concentrations of FDC and FTPA in tissues of dogs hemodiluted 4 weeks previously were the highest in the spleen, being at levels of 50 to 60 mg/g wet tissues. The concentrations of both PFCs in the

Table V. Distribution of FDC and FTPA in Tissues of Dogs Exchange-transfused with Mixed PFC Emulsion

Tissues	PFC concentration (mg/g wet tissue)					
	1 month		2 months		6 months	
	FDC	FTPA	FDC	FTPA	FDC	FTPA
Brain	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Heart	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Lung	0.11	0.63	tr.	tr.	N.D.	N.D.
Liver	18.97	29.43	tr.	7.67	N.D.	tr.
Spleen	48.07	60.65	tr.	5.38	N.D.	0.99
Kidneys	0.51	1.03	tr.	tr.	N.D.	N.D.
Adrenals	4.30	5.62	tr.	tr.	N.D.	N.D.
Pancreas	6.88	8.81	tr.	tr.	N.D.	N.D.
Small intestine	0.63	0.79	N.D.	tr.	N.D.	N.D.
Large intestine	tr.	0.70	N.D.	N.D.	N.D.	N.D.
Stomach	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Testicle	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Muscle	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Femoral marrow	4.05	4.92	tr.	1.33	N.D.	N.D.
Adipose tissue	0.74	2.11	N.D.	N.D.	N.D.	N.D.
Bile (μg/ml)	55.80	102.90	21.90	38.70	N.D.	13.20

The dogs were exchange-transfused with mixed PFC emulsion until hematocrit reached below 5%.
The values represent the mean for 2 dogs.
tr.: trace, less than 10 μg/g tissue.
N.D.: not detected, less than 1 μg/g tissue.

liver were about a half of that in the spleen. Small amounts of both PFCs were also found in the lung, kidneys, adrenal, pancreas, intestine, bone marrow, adipose tissues and bile at 4 weeks, but PFCs were not detected in the brain, heart and testicle in any amount. The elimination rate of FDC from these tissues were faster than that of FTPA and at 2 months after hemodilution none or only trace amount of FDC was found, while about 1 mg/g wet tissue of FTPA in the spleen and a trace in the liver and bone marrow still remained even at 6 months.

Table VI. Distribution of FDC and FTPA in Tissues of Monkeys Exchange-transfused with Mixed PFC Emulsion ($n=2$)

Tissues	PFC concentration (mg/g wet tissue)			
	2 months		4 months	
	FDC	FTPA	FDC	FTPA
Brain	N.D.	N.D.	N.D.	N.D.
Heart	N.D.	N.D.	N.D.	N.D.
Lung	N.D.	N.D.	N.D.	N.D.
Liver	11.00	9.62	N.D.	2.20
Spleen	5.37	4.64	N.D.	2.16
Kidneys	N.D.	N.D.	N.D.	N.D.
Adrenals	0.88	0.22	N.D.	N.D.
Pancreas	1.84	0.39	N.D.	N.D.
Small intestine	N.D.	tr.	N.D.	N.D.
Large intestine	N.D.	N.D.	N.D.	N.D.
Stomach	N.D.	N.D.	N.D.	N.D.
Testicle	N.D.	N.D.	N.D.	N.D.
Muscle	N.D.	N.D.	N.D.	N.D.
Femoral marrow	9.84	6.47	N.D.	N.D.
Adipose tissue	0.65	N.D.	N.D.	N.D.
Blood	N.D.	N.D.	N.D.	N.D.
Bile	N.D.	N.D.	N.D.	N.D.

The monkeys were exchange-transfused with mixed PFC emulsion until hematocrit reached below 1%.
tr.: trace, less than 10 μg/g tissue.
N.D.: not detected, less than 1 μg/g tissue.

Similar results were also found in the monkeys hemodiluted with the mixed PFC emulsion until hematocrit reached 1% as shown in Table VI. The net dose given as PFCs was calculated to be about 25 g/kg body weight. At 2 and 4 months after hemodilution the monkeys were sacrificed and the concentration of PFCs in the tissues was determined. At 2 months the concentration of both PFCs were found to be higher in the liver, spleen and bone marrow than in the other tissues and no difference of the concentration in those tissues was observed between FDC and FTPA. However, FDC so localized rapidly decreased thereafter and FDC was not detected in any organs at 4 months after hemodilution, while approximately 2 mg/g of FTPA were still found only in the liver and spleen.

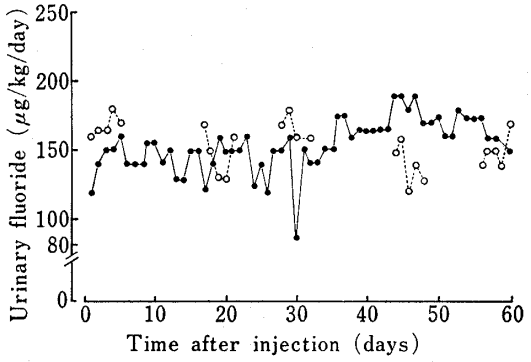

Fig. 4. Urinary Fluoride Ion Content in Rats following Intravenous Injection of Mixed PFC Emulsion

---○---: control (saline 80 ml/kg).
—●—: mixed PFC emulsion (80 ml/kg).

3. Biological Inertness of FDC and FTPA

Generally, the PFCs are biologically inert and probably not metabolized in the body.

In order to confirm the biological inertness of FDC and FTPA, the determination of fluoride contents in urine and the GC–MS analyses of PFCs in the liver of rats were made following intravenous injection of the mixed PFC emulsion, FDC, or FTPA emulsion.

Fig. 4 shows the fluoride content in urine through 2 months after injection of the mixed PFC emulsion. The concentration of urinary fluoride ion in the mixed PFC emulsion group

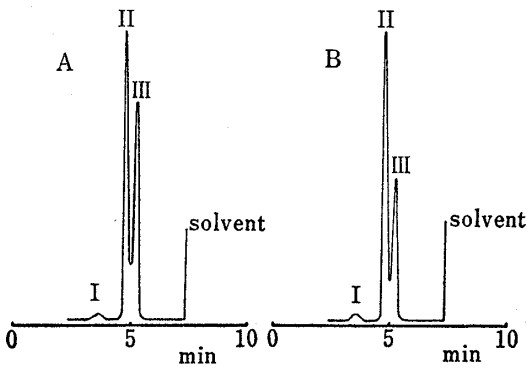

Fig. 5. Comparison of Gas Chromatograms of Standard FDC and Retained FDC in Liver of Rats following Intravenous Injection of Mixed PFC Emulsion 4 Weeks previously

Conditions: column, 6 m × 4 mm glass column packed with 15% DOP on Chromosorb W; column temp., 50°.
A: standard FDC, B: FDC extracted from liver,
I: impurities, II: *trans* FDC, III: *cis* FDC.

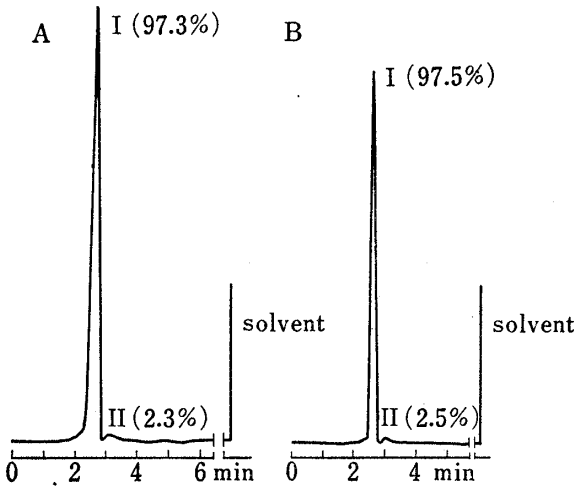

Fig. 6. Comparison of Gas Chromatograms of Standard FTPA and Retained FTPA in Liver of Rats following Intravenous Injection of Mixed PFC Emulsion 4 Weeks previously

Conditions: same as described in Fig. 5.
A: standard FTPA, B: FTPA extracted from liver,
I: FTPA, II: impurities.

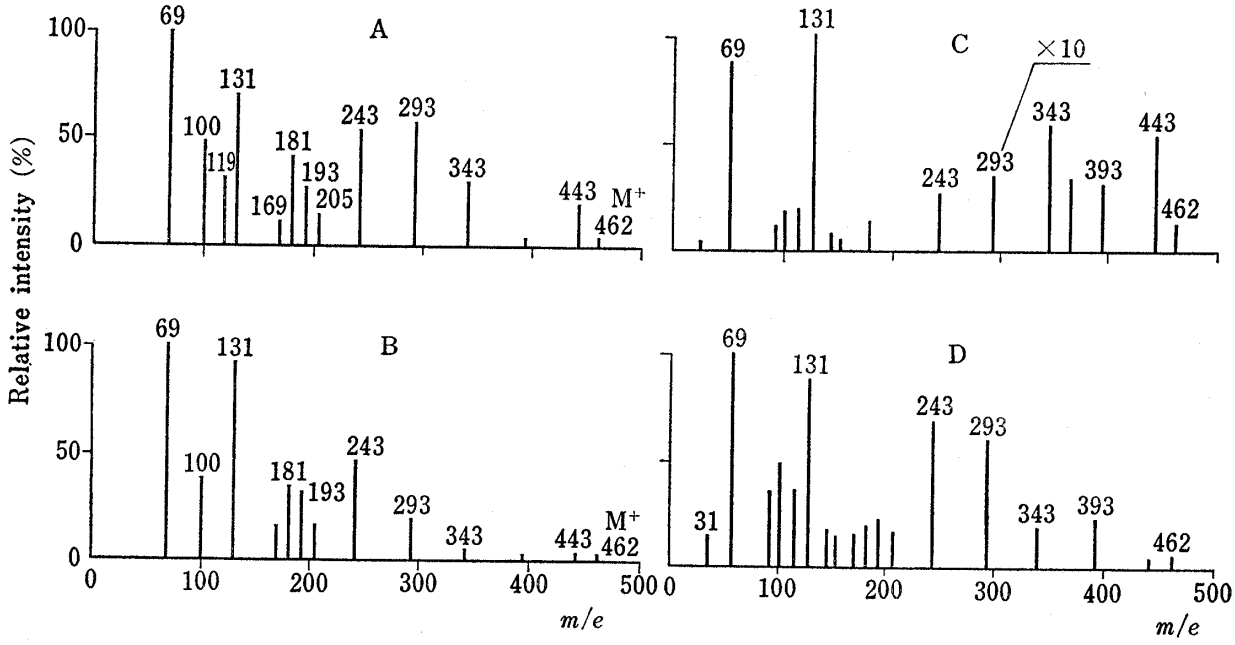

Fig. 7. Mass Spectra of FDC

A: standard FDC, B: FDC retained in liver, C: *trans* FDC (purity, 98.8%), D: *cis* FDC (purity, 88.2%).

did not exceed 200 μg/kg/day and no difference was found between the mixed PFC emulsion and control groups.

Fig. 5 shows gas chromatograms of FDC extracted with trichlorotrifluoroethane from the liver of rats at 4 weeks after injection of FDC emulsion. The gas chromatogram of the liver sample had profiles different from that of standard. The enhancement of the peaks of *trans* isomer and minor impurities and the reduction of that of *cis* isomer were observed, but no new peaks appeared. The relative changes of peak height on gas chromatograms were due to the difference of their excretion rate. Actually, the *cis* isomer of FDC was eliminated at a rate somewhat faster than the *trans* one and the half-retention of *cis*

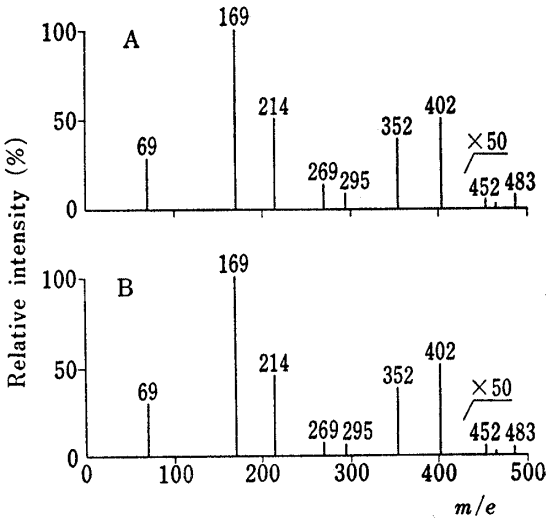

Fig. 8. Mass Spectra of FTPA Retained in Liver and Standard FTPA

A: standard FTPA, B: FTPA retained in liver.

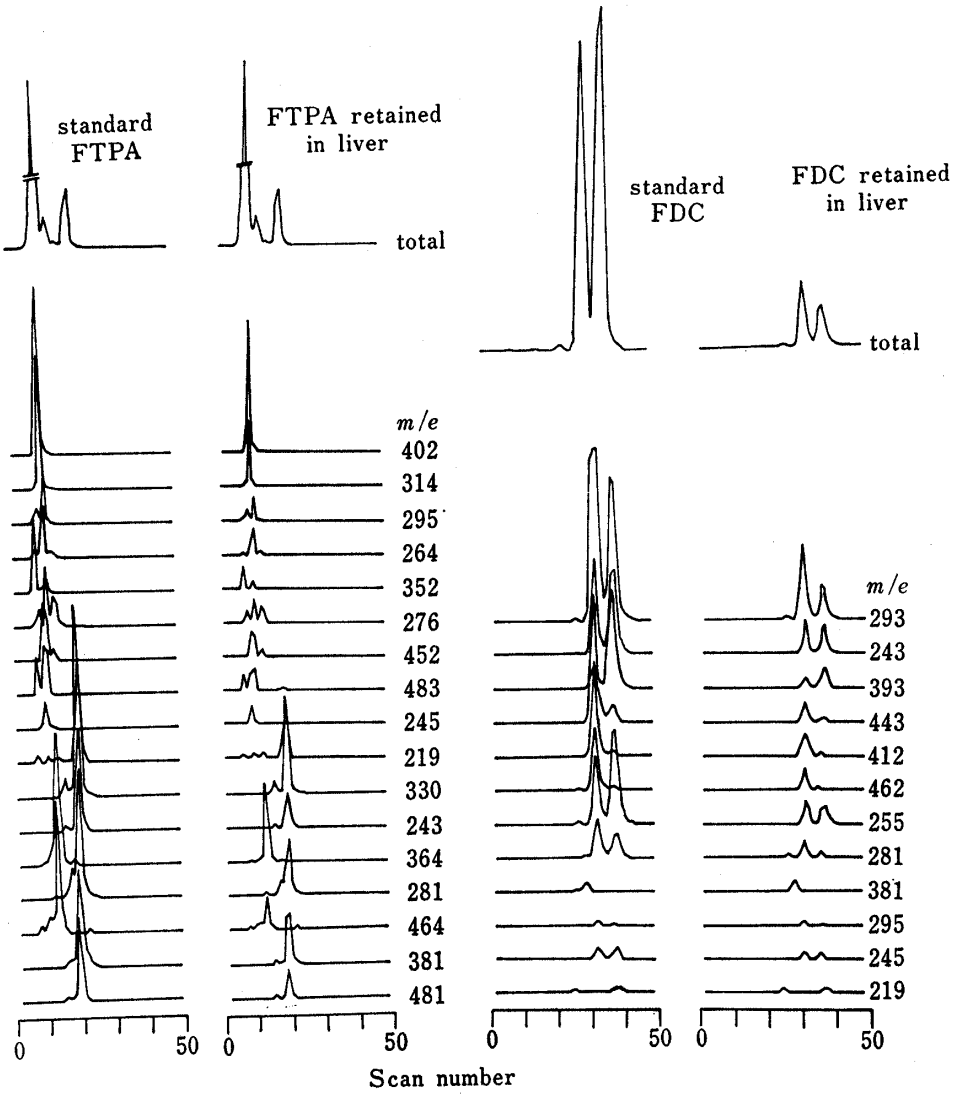

Fig. 9. Mass Chromatograms of FDC and FTPA Retained in Liver of Rats and of Standard Ones

and *trans* isomers in the body of rats given 4 g/kg body weight were 6.0 and 7.5 days, respectively.[13]

On the other hand, no change of gas chromatographic pattern of FTPA was observed between the standard and the liver sample as shown in Fig. 6.

Further studies on the catabolism of FDC and FTPA were carried out by mass spectrometric and mass chromatographic analyses for both PFCs in the liver of rats. Fig. 7 shows the mass spectrum of FDC which was extracted from the liver of rats intravenously given 20 g/kg of FDC in emulsified form 4 weeks previously. A slight difference of fragment ions in relative intensity at *m/e* 131, 293 and 343 was observed between the standard and the liver sample, but no new fragment ions on the mass spectra of the liver samples were detected. The fragmentation pattern of the liver sample resembled that of *trans* FDC more than *cis* one.[11] Therefore, the enhancement of relative intensity of fragment ions at *m/e* 131 and the reduction of those at *m/e* 293 and 343 were due to the increase of relative concentration of *trans* isomer in the liver.

Fig. 8 shows the mass spectrum of the FTPA retained in the liver and standard FTPA. The pattern of both spectra agreed precisely, indicating absence of degradation products in the liver sample.

The introduction of unsaturation and/or hydrogenation into the FDC and FTPA molecules in the body resulted in the difference of the fragmentation pathway and of the fragment ion intensity on the mass spectra as compared to their standard PFCs. Thus, the samples of FDC and FTPA retained in the liver of rats injected with corresponding emulsion 4 weeks previously were analysed by mass chromatography. The results are illustrated in Fig. 9.

As can be seen in Fig. 9, no mass chromatographical difference between the liver sample and standard in both PFCs was found at the scanning range of *m/e* 195 to 490 in FDC and of *m/e* 195 to 540 in FTPA, indicating the absence of catabolites of both PFCs in the liver. The findings obtained from mass spectrometric analyses suggested that both FDC and FTPA were not decomposed *in vivo*.

Acknowledgement We wish to thank Drs. R. Naito and T. Suyama, The Green Cross Corporation, for helpful discussion and gifts of PFC emulsions; to Messrs. M. Watanabe and R. Murashima for valuable technical assistance.

13) K. Yokoyama, K. Yamanouchi, and R. Murashima, Proc. Xth Intern. Cong. Nutrition-Symposium on PFC Artificial Blood, Kyoto, 1975, pp. 103—112.