Tohoku J. Exp. Med., 2007, **212**, 63-70

Historical Trends in Human Serum Levels of Perfluorooctanoate and Perfluorooctane Sulfonate in Shenyang, China

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Jin, Y., Saito, N., Harada, K.H., Inoue, K. and Koizumi, A. *Historical Trends in Human Serum Levels of Perfluorooctanoate and Perfluorooctane Sulfonate in Shenyang, China*. Tohoku J. Exp. Med., 2007, **212** (1), 63-70 —— Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are widespread contaminants in the environment, as well as in wildlife and in humans. The PFOS and PFOA concentrations were determined in historical human serum samples collected in Shenyang, China, in 1987 (n = 15), 1990 (n = 33), 1999 (n = 68) and 2002 (n = 119). The serum donors were students, faculty members and university workers. Since the serum PFOA and PFOS levels did not follow a normal or log-normal distribution, a nonparametric method was applied to analyze the historical trends. For the total male and female subjects, the median level of serum PFOA increased significantly from 0.08 μg/l in 1987 to 4.3 μg/l in 2002 ($p < 0.05$), while the median level of serum PFOS also increased significantly from 0.03 μg/l in 1987 to 22.4 μg/l in 2002 ($p < 0.05$). Both the serum PFOA and PFOS levels continued to increase from 1999 to 2002, with remarkable increases observed in females: 6.3-fold increase for PFOA and 13-fold increase for PFOS. In 2002, serum PFOA and PFOS concentrations of female subjects have increased to 4.9 μg/l and 22.4 μg/l in median, respectively, which are comparable to those in U.S.A. and Japan. For male subjects, serum PFOA and PFOS concentrations (1.6 μg/l and 8.3 μg/l in median, respectively) are comparable to those in Italy. The data from this study indicate that females are likely to experience higher exposure to these chemicals. ———— perfluorooctane sulfonate; perfluorooctanoate; serum; long-term trend; China

Received January 22, 2007; revision accepted for publication March 29, 2007.

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Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) have recently received attention due to their widespread occurrence in the environment, in the wildlife and in humans (Kannan et al. 2002; Jin et al. 2004; Nakayama et al. 2005; Dai et al. 2006). In 2000, after 50 years of production, 3M announced phase out of perfluorooctanyl-based chemistry by 2002, because of the persistency in the environment (Renner 2001). However, several manufacturers have continued to use PFOA for the manufacture of fluoropolymers.

The worldwide distribution of PFOS and PFOA contamination has been attributed to their resistance to degradation in ecological systems (Organisation for Economic Co-operation and Development [OECD] 2002; Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency [OPPT] 2003). Their presence in these systems is of great concern because their persistence may have a great impact on the PFOA and PFOS levels found in human serum.

PFOA and PFOS are carcinogens for rodents (OECD 2002; OPPT 2003). At the cellular and molecular levels, these chemicals are classified as peroxisomal proliferators (Vanden Heuvel et al. 2006). The mechanisms of their toxicities, other than in fatty acid metabolism, are not fully understood. PFOS might have an effect on the neuroendocrine system in rodents (Austin et al. 2003; Asakawa et al. 2007). PFOS and PFOA have electrophysiological effects on action potentials and currents (Harada et al. 2005b, 2006; Matsubara et al. 2006).

There have been several reports regarding PFOA and PFOS concentrations in human serum (Olsen et al. 2003; Harada et al. 2004; Calafat et al. 2006; Jin et al. 2006; Kärrman et al. 2006). A recent study revealed that PFOS and other serum fluorochemical concentrations increased between 1974 and 1989, before reaching a plateau in 1989 in the USA (Olsen et al. 2005). However, our research has demonstrated that the serum levels of PFOS and PFOA increased by 3-fold and 14-fold, respectively, between 1977 and 2003 in Japan (Harada et al. 2004). These differing historical trends in the USA and Japan suggest that exposure to PFOA and PFOS may be unique to specific geographic area.

In China, rapid industrial progress has been made in the past 30 years since the Chinese government decided to adopt their "Open the door policy" in the 1980s. Jin et al. (2006) recently reported that the current serum PFOA and PFOS levels were significantly higher in residents of Shenyang than in residents of Chongqing, suggesting geographical differences in China. Furthermore, the current serum levels of PFOS and PFOA were comparable to those in Japan (Harada et al. 2007). It is of particular interest to investigate the historical trends in the levels of human exposure to PFOA and PFOS in Shenyang over the past 30 years, in order to establish whether the serum PFOA and PFOS levels have already reached a plateau as they did in the USA or whether they are still increasing.

The aim of the present study was to investigate the historical trends in human exposure to PFOA and PFOS in Shenyang, China from the mid-1980s to the early 2000s. We demonstrate that the human serum levels of PFOA and PFOS increased significantly in 2002.

Materials and Methods

Experimental design and study population

To evaluate the long-term trends in the PFOA and PFOS concentrations in human serum, historical serum samples were obtained from archived samples collected in 1987, 1990, 1999 and 2002 during annual health checkups. The blood samples were collected from students, faculty members and China Medical University workers, when they underwent annual health checkups at the University Hospital (Shenyang, China). Serum was separated from the red blood cells and other cellular components and stored in polypropylene tubes at –20°C until analysis.

The research protocol of the present study was reviewed and approved by the China Medical University Research Ethics Board on Jan 13, 2003. Informed consent was obtained from all participants.

Reagents

Heptadecafluorooctane sulfonic acid potassium salt (FW. 538.22) and pentadecafluorooctanoic acid ammonium salt (FW. 431.10) were purchased from Fluka

(Milwaukee, WI, USA) as authentic standards for PFOS and PFOA, respectively. The purities of these standards were greater than 98%, therefore the reported concentrations were not corrected for purity. The internal standard [1,2-$^{13}C_2$] perfluorooctanoate was donated by the Environmental Protection Agency of the USA (purity > 99%, originally synthesized by Perkin Elmer, Boston, MA, USA). Tetrabutylammonium hydrogen sulfate (high-performance liquid chromatography [HPLC] grade) was obtained from Acros Organics (Geel, Belgium). Sodium carbonate (> 99.5%) was obtained from Kanto Chemical (Tokyo). Nitrogen gas (G3 grade; > 99.9995%) was obtained from Japan Fine Products Corporation (Kawasaki). Methyl tertiary-butyl ether (MTBE; HPLC grade), methanol (HPLC grade), acetonitrile (HPLC grade) and ammonium acetate (> 97%) were obtained from Wako Pure Chemical Industries (Osaka).

Determination of PFOS and PFOA in serum

The internal standard [1,2-$^{13}C_2$] perfluorooctanoate was added to each serum sample prior to extraction. The serum samples were extracted using a previously described method (Hansen et al. 2001). Briefly, 0.5 ml of serum, 1 ml of 0.5 M tetrabutylammonium hydrogen sulfate solution and 2 ml of 0.25 M sodium carbonate buffer were added to a 15-ml polypropylene tube and mixed. Following addition of 5 ml of MTBE to the solution, the organic and aqueous layers were separated by centrifugation, and the organic layer was removed. The aqueous mixture was rinsed with MTBE and separated twice. The solvent was evaporated at room temperature under a nitrogen gas flow. The sample was then reconstituted in 0.5 ml of methanol. The sample was then passed through a nylon filter (Autovial R5 PUNYL; 0.45-μm pore size; Whatman Japan, Tokyo) to remove any suspended materials and insoluble particles.

Each extracted solution was analyzed by liquid chromatography-mass spectrometry (LC/MS) as previously described (Saito et al. 2003, 2004). Briefly, each methanol extract (injection volume, 10 μl) was chromatographed using HPLC (Agilent 1100 with Zorbax XDB C-18 [3.5 μm, 2.1 × 150 mm]) at a flow rate of 0.2 ml/min. Gradient conditions were used in the mobile phase. Initial mobile phase conditions were 35 : 65 CH_3CN/10 mM CH_3COONH_4 buffer, followed by a 5 min ramp to 45 : 55, hold until 20 min. The chromatographic column was kept at 40°C. Mass spectra were taken using an LC/MS system equipped with an orthogonal spray interface (Agilent 1100MSD SL), employing electrospray ionization in the negative mode. The electrospray probe and ion source were operated at following conditions: capillary voltage: 4 kV, fragmentor voltages: 100 V for PFOA and 200 V for PFOS, nebulizer pressure: 50 psi, desolvation temperature: 350°C, and desolvation gas flow rate: 10 l/min.

The selected ion monitoring was used for quantification of the analytes. The quantification ions were m/z 413 ($C_7F_{15}CO_2^-$) for PFOA and m/z 499 ($C_8F_{17}SO_3^-$) for PFOS. The fragment of PFOA m/z 369 (fragment $C_7F_{15}^-$) and fragment of PFOS m/z 99 (fragment, FSO_3^-) were also monitored as qualifying ions.

Matrix spike studies in serum were performed to evaluate the recovery. The spike concentrations were prepared to contain 10 μg/l of the target analytes in the samples. The mean extraction recoveries from the spiked serum samples ranged from 93% to 101% and from 98% to 109% for PFOS and PFOA, respectively (n = 10 for each compound). The limit of detection (LOD) and limit of quantification (LOQ) were considered to be 3- and 10-fold larger than the signal-to-noise ratio, respectively. The LOD and LOQ were 0.01 μg/l and 0.03 μg/l, respectively, for both analytes.

Statistics

All statistical analyses were carried out using SAS software (Version 8.2; SAS Institute Inc., Cary, NC, USA). Values of $p < 0.05$ were considered to indicate statistical significance. All samples below the LOD were treated as 0.01 in the calculations for arithmetic means and standard deviations (SD), and for geometric means (GM) and geometric standard deviations (GSD). The serum levels of PFOA and PFOS were tested for their normal or log-normal distribution by the Kolmogorov-Smirnov test. Nonparametric analysis of difference in mean of rank sum (Kruskal-Wallis test) was conducted when the distribution was neither normal nor log-normal.

Results

The data for the serum levels of PFOS and PFOA are presented in Table 1. For the samples collected in 1987 and 1990, no information regarding the age and sex was available. For the samples collected in 1999 and 2002, information regarding the age and sex was available, but other pieces of information, such as residential histories, past medical histories, reproductive histories and lifestyles, were not. Tests of associations between age and serum levels of PFOA or PFOS

TABLE 1. Summary of serum PFOA and PFOS levels in the study population from 1987 to 2002.

		1987	1990	1999			2002		
		Total	Total	Total	Male	Female	Total	Male	Female
	n	15	33	68	36	32	119	36	83
Age	Mean ± S.D.	-	-	32.9 ± 15.1	34.5 ± 15.2	31.0 ± 15.0	28.9 ± 8.4	28.5 ± 8.8	29.2 ± 8.3
PFOA	GM (GSD)	0.07 (2.6)	0.06 (3.9)	0.83 (2.0)	0.82 (2.2)	0.85 (1.9)	3.0 (4.1)	2.5 (4.1)	3.3 (4.1)
(μg/l)	Mean ± S.D.	0.11 ± 0.11	0.21 ± 0.60	1.1 ± 0.86	1.0 ± 0.60	1.1 ± 1.1	7.1 ± 9.7	6.2 ± 8.8	7.4 ± 10.1
	Min	0.01	0.01	0.04	0.04	0.19	0.24	0.25	0.20
	25%	0.05	0.03	0.56	0.58	0.56	0.80	0.78	0.80
	Median	0.08	0.05	0.86	0.88	0.78	4.3	1.6	4.9
	75%	0.15	0.14	1.2	1.4	1.1	9.3	9.3	9.3
	Max	0.43	3.4	6.5	2.4	6.5	59.8	40.4	59.8
	KW test between males and females				$p = 0.2198$	-		$p = 0.1245$	-
	KW test between 1999 and 2002 samples				$p = 0.0047$	$p < 0.001$		-	-
PFOS	GM (GSD)	0.03 (2.9)	0.02 (3.3)	1.8 (6.6)	1.6 (8.8)	2.0 (1.9)	12.9 (5.4)	6.4 (5.9)	17.5 (4.7)
(μg/l)	Mean ± S.D.	0.05 ± 0.08	0.08 ± 0.23	5.0 ± 5.7	5.7 ± 6.7	4.2 ± 1.1	31.4 ± 31.8	19.6 ± 25.3	36.5 ± 33.1
	Min	0.01	0.01	0.01	0.01	0.04	0.24	0.24	0.48
	25%	0.01	0.01	0.79	0.33	0.92	3.6	1.7	6.4
	Median	0.03	0.03	3.0	3.2	2.4	22.4	8.3	31.4
	75%	0.04	0.04	7.5	8.7	7.4	50.2	34.5	57.4
	Max	0.26	1.0	27.2	27.2	18.4	145	105	145
	KW test between males and females				$p = 1.000$	-		$p = 0.0196$	-
	KW test between 1999 and 2002 samples				$p = 0.3458$	$p < 0.001$		-	-

KW test, Kruskal-Wallis test.

in 1999 and 2002 demonstrated a lack of significant associations ($p > 0.05$) ($r = 0.04$ for PFOA and $r = –0.18$ for PFOS in 1999; $r = 0.01$ for PFOA and $r = 0.05$ for PFOS in 2002). Therefore, we did not analyze the effects of age on the serum levels of PFOA and PFOS.

The serum levels of PFOA and PFOS did not show log-normal or normal distributions for the total pooled male and female subjects (Kolmogorov-Smirnov test: $p < 0.001$) (Fig. 1). Thus, comparisons for the total pooled male and female subjects were made by the Kruskal-Wallis test. The median serum level of PFOA increased significantly from 0.08 μg/l in 1987 to 4.32 μg/l in 2002, a 54-fold increase ($p < 0.001$, $n = 15$ in 1987 and $n = 119$ in 2002). Moreover, the median serum level of PFOS increased significantly from 0.03 μg/l in 1987 to 22.40 μg/l, a 747-fold increase ($p < 0.001$, $n = 15$ in 1987 and $n = 119$ in 2002).

The serum levels for PFOA and PFOS for the male and female subjects did not show a log-normal or normal distribution in 2002 (Kolmogorov-Smirnov test: $p < 0.001$ for both sexes) (Fig. 2). First, we compared the serum levels collected in 2002 with those collected in 1999. For males, the PFOA levels increased significantly from 1999 to 2002 ($p = 0.0047$, $n = 36$

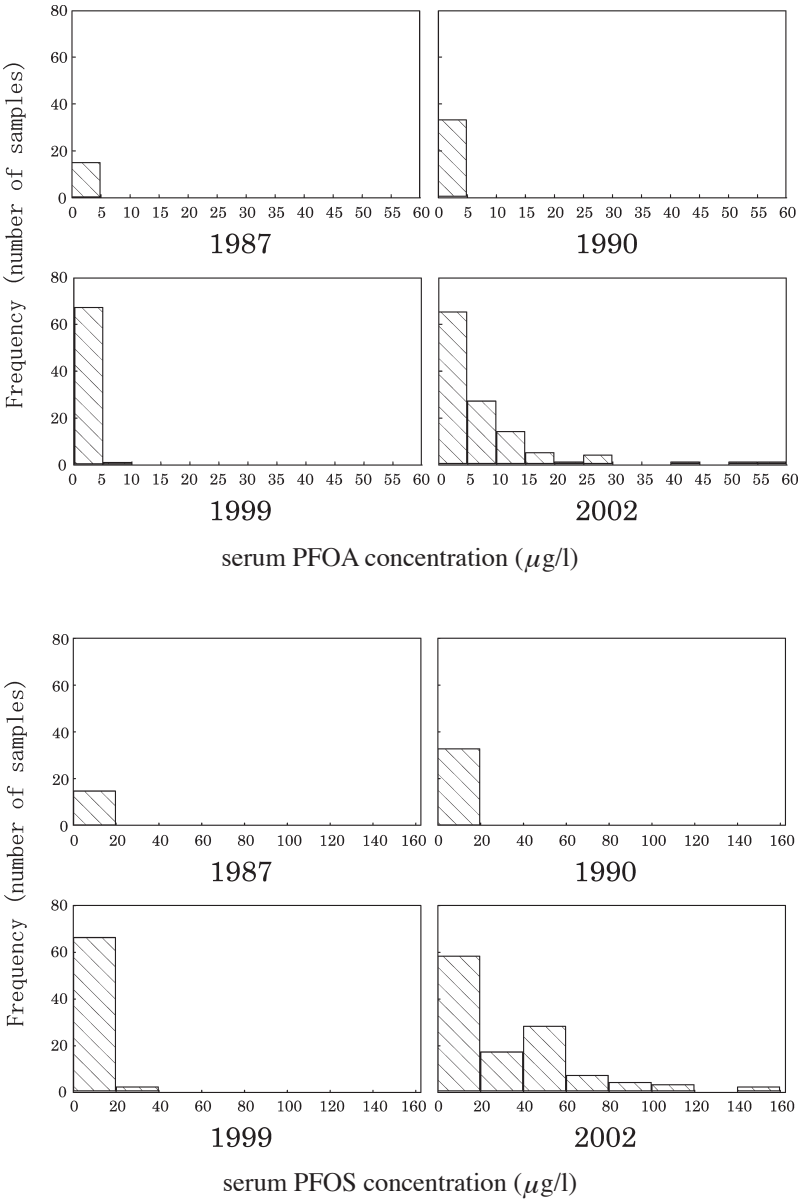

Fig. 1. Serum levels of PFOA and PFOS in the total pooled male and female populations from 1987 to 2002 in Shenyang.

for both 1999 and 2002), while the serum levels of PFOS did not ($p = 0.3458$, $n = 36$ for both 1999 and 2002). In contrast, for females, the serum levels of both PFOA ($p < 0.001$, $n = 32$ in 1999 and $n = 83$ in 2002) and PFOS ($p < 0.001$, $n = 32$ in 1999 and $n = 83$ in 2002) increased significantly from 1999 to 2002 (Fig. 2).

We compared the serum PFOA and PFOS levels between males and females within samples collected in 1999 or 2002. The serum levels in males ($n = 36$) did not differ from those in females ($n = 32$) for either PFOA ($p = 0.2198$) or PFOS ($p = 1.000$) in 1999. However, the PFOA levels in females ($n = 83$) and males ($n = 36$) did not differ ($p = 0.1245$), while the serum PFOS levels were significantly higher in females ($n = 83$) than in males ($n = 36$) in 2002 ($p = 0.0196$).

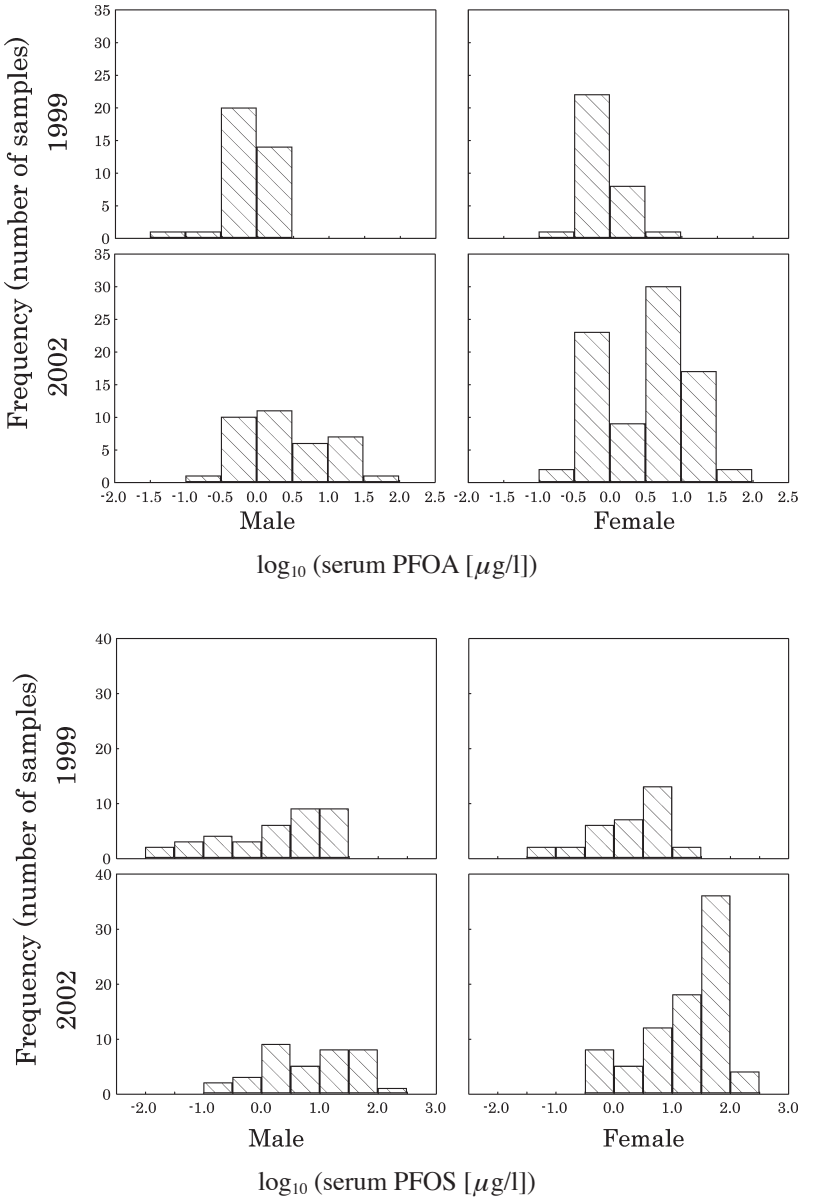

Fig. 2. Serum levels of PFOA and PFOS in males and females in 1999 and 2002.

Discussion

This study represents the first report on the historical trends in the serum PFOA and PFOS levels in Shenyang, China from late 1980s to the early 2000s. As expected, the serum levels of both PFOA and PFOS have increased significantly over recent years. Although the median PFOA levels in the total pooled male and female subjects have increased by 54-fold, the corresponding serum PFOS levels have increased by more than 747-fold. It should also be pointed out that the median serum levels of PFOA and PFOS still continued to increase by 1.8-fold and 2.6-fold in males, respectively, from 1999 to 2002, whereas the corresponding levels in females showed a significant increase of 6.3-fold and 13-fold.

In 2002, serum PFOA and PFOS concentrations of female have increased to 4.9 μg/l and 22.4 μg/l in median, respectively, which are com-

parable to those in USA. (4.7 and 35.8) and Japan (4.4 and 11.8) (Olsen et al. 2003; Harada et al. 2007). For male, serum PFOA and PFOS concentrations (1.6 μg/l and 8.3 μg/l in median, respectively) are comparable to those in Italy (< 3 [LOQ]/< 3 and 4.2/3.5 [male/female]) (Kannan et al. 2004). The rapid increases in the serum levels of both PFOA and PFOS in females are worthy of attention.

A reasonable question to consider would be the sources of exposure to explain such rapid increases in females. Previously several exposure routes have been suggested to play an important role in the exposure to PFOA and PFOS; drinking water (Harada et al. 2003; Saito et al. 2004), airborne dust (Harada et al. 2005a), and house dust (Moriwaki et al. 2003). One report also showed that a high fish consumption group had 2.6-fold higher serum levels of PFOS to the reference population (Falandysz et al. 2006). However these routes are not likely to be specific for female population in Shenyang. Although identification of such sources is beyond the scope of this study due to deficiencies in relevant critical information, the distribution of the serum PFOA and PFOS levels indicates the presence of a female subpopulation with higher exposure levels to PFOA and PFOS. These observations suggest that residential histories may be a determinant for PFOA and PFOS, as previously observed in Japan (Harada et al. 2004, 2007), the USA (Olsen et al. 2003) and China (Jin et al. 2006).

The present study has a major limitation, since the historical samples lacked important pieces of information regarding residential histories, lifestyles, food habits and so on. Thus, no analyses to identify the sources of exposure on an individual basis can be made. In other words, the present historical evaluation of samples between 1987 and 2002 cannot be generalized to the general Chinese population. However, our observation of enormous increases in the serum PFOA and PFOS levels cannot be overlooked, since the exposure levels are rapidly increasing and exceeding the PFOS levels in the USA.

The intense levels of exposure to PFOS raise an alarming signal for Chinese females in Shenyang. Further investigations will be required to specifically characterize the sources of the exposure for the female subpopulation.

Acknowledgments

We are grateful to Drs. Ding Mei, Wang Lie and Zhang Yinghua (China Medical University) for their help and support and to Drs. Mark J. Strynar and Andrew B. Lindstrom (NERL, USEPA) for donating the internal standard. This study was mainly supported by a Grant-in-Aid from National Natural Science Foundation of China (No. 30471435), a Grant-in-Aid from the Ministry of Health, Labor and Welfare of Japan (H15-Chemistry-004) and partly by the Nippon Life Insurance Foundation (Environment-04-08).

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