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Is Fish a Major Source of Fluorinated Surfactants and Repellents in Humans Living on the Baltic Coast?

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Concentrations of 19 perfluorochemicals have been quantified in human blood and in some marine food resources from the region of the Gulf of Gdañsk at the Baltic Sea south coast in Poland. We indicate that in addition to PFOS and PFOA, a further 8 perfluorochemicals bioaccumulate in the human body. Food chain is an important route of exposure for all 10 perfluoroalkyl compounds detected in nonoccupationally exposed humans. Individuals who declared to have a high fish intake in their diet (mainly Baltic fish) on average contained the highest load of all 10 fluorochemicals when compared with the other human subpopulations. Baltic seafood has been found to highly influence human body burden of PFHxS, PFOS, PFOSA, PFHxA, PFHpA, PFNA, PFDA, PFUnDA, and PFDoDA, and to a lesser extent PFOA.

Introduction

Environmental contaminants impacting the quality of food for man is a continuous challenge, and examples of such problematic chemicals include methylmercury and many persistent organohalogenated chemicals, notably the popular insecticides, DDT and its analogues, dieldrin, polychlorinated biphenyls (PCBs), -naphthalenes (PCNs), -dibenzo-*p*-dioxins (PCDDs), -furans (PCDFs), and more recently polybrominated diphenyl ethers (PBDEs). We present evidence here that the food chain is also an important route of intake of several fluorinated chemicals used as surfactants and repellents and they concentrate in human body fluids and tissues.

Perfluorinated chemicals (PFCs) represent a broad group of compounds and have been used by humans for over fifty years. These man-made substances are used for many applications and some have become popular as ionic and neutral surfactants and repellents, which are in consumer products in daily use, such as upholstery, paper, carpets, and leather and textile coatings. These compounds are also used in commercial and industrial applications in fire-fighting film-forming foams, metal plating, or as polymerization aids in the manufacture of fluoropolymers.

Long-chain perfluorinated (sulfonic, carboxylic) acids and alcohols (fluorotelomer alcohols) are PFCs which have attracted considerable attention in recent years among environmental scientists, toxicologists, epidemiologists, and policy makers. This is because at least two perfluoroalkyl compounds, i.e., perfluorooctane sulfonate (PFOS; $C_8F_{17}SO_3^-$) and perfluorooctanooate (PFOA; $C_7F_{15}COO^-$) have been found as persistent contaminants in human body and wildlife worldwide (*1*−*4*). PFOS and PFOA, and also long-chain perfluorochemicals, are purely synthetic and fully fluorinated; they are analogous to naturally occurring fatty acid. One of the mysteries of the complex phenomenon of environmental sources and fate of PFCs is pathways leading to their presence in human body. Due to the potential health problems associated with PFCs, more data on their sources and effects for human are urgently required (*5*).

In this study we indicate that, in addition to PFOS and PFOA, a further eight perfluorochemicals bioaccumulate in the human body. Some of these PFCs detected in human blood samples examined have remarkably different sources and also different pharmacokinetics (*5*−*7*). We show that the food chain is an important route of exposure for all 10 perfluoroalkyl compounds detected in nonoccupationally exposed humans. Perfluochemicals are remarkably stable and perhaps should be included with a broader class of persistent organohalogenated environmental and food-chain contaminants. Nevertheless, most of PFCs are markedly different in their properties from that of DDT and its analogues, dieldrin, PCBs, PCNs, and similar compounds, which are similarly highly lipophilic food-chain contaminants.

A majority of PFCs have an amphiphilic character, and with their thermodynamically strong covalent C−F bonds were initially considered as nonmetabolizeable and nontoxic. Most of them were also considered as having little if any volatility. An example of PFCs with low volatility are fluorotelomer alcohols (FTOHs), which together with some sulfonamide-based PFCs may undergo transformation in the environment to form more persistent PFOA or similar perfluoroalkyl carboxylic acids. Potassium, sodium, ammonium, or some other salts of PFCs are water soluble. Perfluorochemicals with their unique surface modification properties readily bind to surfaces including blood globulins. There is an array of toxic responses reported for laboratory animals exposed to PFCs and in other bioassays, but also a few reports of toxicity for occupationally exposed humans (*4*−*8*). The PFCs surface binding capacity and nearly perfect analogy to natural fatty acids seem to be their special features.

Materials and Methods

Nineteen perfluorochemicals were determined in human blood and in some marine food resources from the region of the Gulf of Gdañsk at the Baltic Sea south coast in Poland (Table 1). The human blood donors were citizens of Gdañsk or were from nearby small towns and villages. They were selected in a way to represent various subpopulations: specifically dockers (all males) and farmers (2 females and 13 males), individuals with declared high intake of Baltic Sea fish (4 females and 11 males), and a general sector of Gdañsk citizens (5 females and 10 males). All contributors were adult (age 19−62) and provided blood donations during July 2003 in the framework of the UE-project COMPRENDO (www.comprendo-project.org) which has a strong focus on

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TABLE 1. Concentrations (pg mL^{-1}; Mean ± SD, Median, and Range) of Perfluorochemicals in Adult Human and Animal Whole Blood Samples

	human blood[a]				animal blood[b]	
compound[c]	dockers	farmers	fish-dish fanciers	reference group	cod	eider duck
PFBuS	<2	<2	<2	<2	<2	<2
PFHxS	510 ± 290 390 (170−1000)	570 ± 340 460 (220−1400)	1400 ± 860 1200 (490−3700)	510 ± 290 390 (170−1000)	100 ± 170 100 (50−800)	1100 ± 600 1100 (400−2900)
PFOS	12000 ± 5700[a] 9700 (5200−24000)	13000 ± 5200 12000 (6600−25000)	41000 ± 23000 34000 (14000−84000)	16000 ± 12000 12000 (6700−46000)	17000 ± 12000 15000 (6100−52000)	22000 ± 14000 22000 (12000−38000)
PFOSA	370 ± 330 220 (56−1100)	300 ± 250 210 (110−1000)	990 ± 650 1100 (280−2600)	440 ± 270 370 (53−900)	5700 ± 2800 5200 (1200−13000)	500 ± 210 490 (100−800)
PFHxA	4.1 ± 4.5 <2 (<2−16)	27 ± 20 21 (5−63)	59 ± 57 33 (14−240)	30 ± 15 29 (4−65)	170 ± 140 160 (<50−690)	<50
PFHpA	67 ± 150 12 (5−570)	56 ± 38 47 (7−150)	190 ± 230 69 (33−790)	170 ± 170 86 (17−470)	120 ± 160 90 (<50−740)	<50
PFOA	2700 ± 1500 2300 (1200−5800)	3400 ± 1700 3600 (1200−6200)	4100 ± 2100 3700 (1700−8700)	3000 ± 1200 2800 (1300−5200)	200 ± 160 90 (50−700)	100 ± 60 60 (60−100)
PFNA	400 ± 190 360 (160−820)	560 ± 150 590 (300−810)	1700 ± 930 1600 (410−3800)	610 ± 360 490 (300−1500)	1200 ± 600 1100 (100−2100)	400 ± 170 320 (300−900)
PFDA	150 ± 84 120 (62−340)	190 ± 50 190 (110−260)	540 ± 350 480 (150−1400)	200 ± 120 170 (90−510)	950 ± 550 1200 (50−1800)	110 ± 50 100 (70−220)
PFUnDA	85 ± 47 74 (29−200)	92 ± 29 87 (41−160)	360 ± 270 340 (73−1100)	110 ± 82 78 (40−300)	1600 ± 1000 1500 (100−3800)	170 ± 70 190 (60−300)
PFDoDA	15 ± 5 10 (4−23)	17 ± 7 15 (6−33)	58 ± 92 30 (11−380)	16 ± 10 12 (6−38)	260 ± 200 240 (50−910)	30 ± 10 30 (10−40)

[a] Each group consisted of 15 donors. [b] Cod and eider duck represented by 18 and 16 individuals, respectively. [c] PFBuS (perfluorobutanesulfonate, $C_4F_9SO_3^-$) (low recovery rate for that compound), PFHxS (perfluorohexanesulfonate ($C_6F_{13}SO_3^-$), PFOSA perfluorooctane-sulfonamide, $C_8F_{17}SO_2NH_2$), PFNA (perfluorononanoic acid, $C_8F_{17}COOH$), PFDA perfluorodecanoic acid, $C_9F_{19}COOH$), PFUnDA (perfluoroundecanoic acid, $C_{10}F_{21}COOH$), PFDoDA (perfluorododecanoic acid, $C_{11}F_{23}COOH$); and perfluorochemicals not detected (<40 to <2000 pg/mL^{-1}) in selected human blood samples: PFTeDA (perfluorotetradecanoic acid, $C_{13}F_{27}COOH$), PFHxDA (perfluorohexadecanoic acid, $C_{15}F_{31}COOH$), PFOcDA (perfluorooctadecanoic acid, $C_{17}F_{35}COOH$), Net-FOSA (*N*-ethylperfluorooctanesulfonamide; $C_8F_{16}SO_2NHC_2H_5$), 7:1FTOH (7:1 fluorotelomer alcohol, $CF_3(CF_2)_6CH_2OH$), 10:1FTOH (10:1 fluorotelomer alcohol, $CF_3(CF_2)_9CH_2OH$), 8:2FTCA (8:2 fluorotelomer acid, $CF_3(CF_2)_7CH_2COOH$), 8:2FTUCA (8:2 fluorotelomer unsaturated acid, $CF_3(CF_2)_6CF{=}CHCOOH$).

endocrine disrupter research. The donors were sampled by professionals at the Blood Bank in Gdañsk. Blood (10−14 mL) sampled for PFCs determinations was collected in precleaned fresh polyethylene tubes with a few crystals of EDTA added, further sealed, and delivered deep-frozen to the analytical laboratory. The donors had no known specific contact with PFCs, and these chemicals are not manufactured in or imported in bulk to Poland (*10*). Cod (*Gadus morhua*) and eider duck (*Sommateria mollisima*) samples were analyzed additionally as they represent an integral part of the Baltic Sea marine foodchain. Cod is a popular fish comestible at the Gdañsk region and together with eider duck these animal samples were collected from the Gulf of Gdañsk in February 2003.

Chemicals and Standards. Methanol (pesticide grade), distilled water (HPLC-grade), tetrabutylammonium hydrogensulfate (TBA), sodium carbonate and sodium hydrogen carbonate, and methyl-*tert*-butyl ether (MTBE) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Nylon syringe filters (13 mm, i.d. 0.1 μm) were purchased from Iwaki (Tokyo, Japan). Milli-Q water was obtained from Milli-Q Gradient-A10 (Millipore).

PFOS (86.9%), PFHxS (98.6%), PFBS (>98%), PFOSA (>95%), and THPFOS were provided by the 3M Company (St. Paul, MN). PFOA (98%) was purchased from Stream Chemicals, Inc. PFNA (97%) and PFBA (99%) were purchased from Avocado Research Chemicals Ltd. (Heysham, Lancashire, U.K.). PFPeA (97%), PFHpA (98%), PFDA (98%), PFUnDA (97%), and PFDoDA (96%) were purchased from Fluorochem Limited, and PFHxA (97%) was purchased from Wako Pure Chemical Industries, Ltd.

Extraction. One mL of whole blood, 10 μL of internal standard, 1 mL of 0.5 M tertrabutylammonium hydrogen sulfate (TBA) solution, and 2 mL of 0.25 M sodium carbonate buffer (adjusted to pH 10) were added to a 15-mL polypropylene tube for extraction. After through mixing, 5 mL of methyl-*tert*-butyl ether (MTBE) was added, and the mixture was shaken for 20 min (rpm 250). The organic and aqueous layers were separated by centrifugation (15 min, rpm 3000), and exact volume of MTBE (4 mL) was removed from the solution. The aqueous mixture was rinsed with MTBE and separated twice; all rinses were combined in a second polypropylene tube. The solvent was allowed to evaporate under a gentle stream of nitrogen gas after adding 1 mL of methanol. The sample was vortexed for 30 s and filtered using a 0.2-μm nylon mesh filter to remove particles that appeared in the final solution of a few of blood samples. Each sample was extracted and analyzed in duplicate.

Instrumental Analysis and Quantification. A 10 μL aliquot of the sample extract was injected into a guard column (XDB-C8, 2.1 mm i.d. × 12.5 mm length, 5 μL; Agilent Technologies, Palo Alto, CA) connected sequentially to a Betasil C18 column (2.1 mm i.d. × 50 mm length, 5 μL; Thermo Hypersil-Keystone, Bellefonte, PA) with 2 mM ammonium acetate aqueous solution (solvent A) and methanol (solvent B) as mobile phases, starting at 10% methanol and increased linearly. At a flow rate of 300 μL/min, the gradient was increased to 30% methanol at 0.1 min, 75% methanol at 7 min, and 100% methanol at 10 min, before reverting to original conditions at 12 min, at the 20-min time point. Column temperature was maintained at 20 °C.

For quantative determination, the HPLC system was interfaced with a Micromass (Beverly, MA) Quattro Ultima Pt mass spectrometer operated in the electrospray negative ionization mode. Instrumental parameters were optimized to transmit the $[M - K]^-$ or $[M - H]^-$ ion for analyte. Cone-gas and desolvation-gas flows were kept at 60 and 750 L/hr, respectively. Source and desolvation temperatures were kept at 120 and 400 °C, respectively. Cone voltage and collision energies were optimized for each analyte (Table 1). In all cases the capillary column was held at 1.0 kV.

13C-PFOA was used as an internal standard and was spiked into blood samples prior to the addition of reagents for extraction. Recoveries of ^{13}C-PFOA ranged from 42 to 129% and the quantification was not based on internal standard recoveries. Quantification was based on the re-

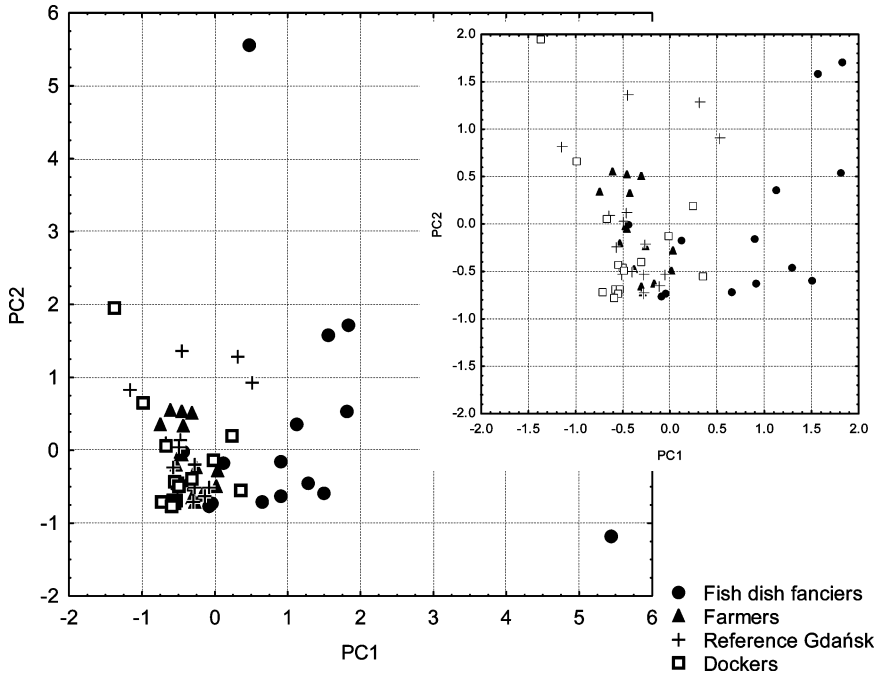

FIGURE 1. Data plot of PFCs in human blood for four subpopulations examined in space of principal components (PC) 1 and 2 after PC analysis. Most of the fish-dish fancier blood donors cluster separately due to significantly higher load of PFCs. PC1 (associated with PFOS, PFOSA, PFUnDA, PFDA, and PFNA) and PC2 (associated with PFDoDA, PFOA, and PFHpA) explained 65.9 and 11.8% of the total variance in the data set, respectively.

sponse of the external standards that bracketed the concentrations found in samples. The method limit of quantification (MLQ) was determined on the linear range of the calibration curve. For human blood samples, a calibration curve containing 2, 10, 50, 200, 1000, and 2000 ng/mL standard, injected at 10 μL, was used. Concentrations in samples that were at least 3-fold greater than the lowest acceptable standard concentration were considered to be valid. A curve point was deemed acceptable if (1) it was back-calculated to be within 30% of the theoretical value when evaluated versus the $^1/_x$ weighted curve, and (2) the peak area of the standard was at least 3 times greater than that in the blank (*3, 11*). The analytical laboratory involved (no. 16; AIST, Tsukuba) participated in a recent PFCs intercalibration round with very good result (*12*).

Statistical Analysis. All statistical analyses were performed with computer software Statistica version 5.0, and associations among PFCs and blood donor groups were displayed on principal components 1 and 2 with unrotated, Varimax-rotated, and Quartimax-rotated matrixes.

Results and Discussion

A cocktail consisting of between 7 and 10 PFCs has been found in blood from humans living on the south coast of the Baltic Sea. There are no earlier reports showing an accumulation in humans for 10 of the 19 PFCs examined in this study. Individuals who declared a high fish intake in their diet (mainly Baltic fish) on average contained the highest load of all 10 fluorochemicals when compared with the other human subpopulations (Figure 1; Table 1). On the basis of absolute concentrations, the concentration of fluorochemicals in this subgroup was roughly 2- to 3-fold greater when compared with the three other subpopulations, with just a few individuals that were the exception. The group of individuals in this study consumed their preferred dish 2−7 times weekly with declared weekly fish intake rate from 800 to 1500 g (1100 ± 300 g), whereas for the other 45 blood donors, fish intake was between 0 and 1600 g weekly.

After evaluation of a data frequency distribution diagram, fish in the diet was the only factor of the parameters assessed (age, gender, residence period, education, occupation, health status, addictions (smoking and alcohol), and access or exposure to pesticides) positively correlated with total PFCs concentration in blood samples. The docker subpopulation had the lowest frequency (three positive findings) and blood load of perfluorohaxanoic acid (PFHxA; $C_5F_{11}COOH$) ($p < 0.01$; U Mann−Whitney test) and perfluoroheptanoic acid (PFHpA; $C_6F_{13}COOH$) among the human donors. No occupational exposure factors become evident (Table 1).

The origin and sources to human and wildlife of PFOS and PFOA, the most often studied and detected in biota among PFCs (*2*−*4*, *7*, *9*, *13*, *14*), are of great interest. The blood concentration quotient of PFOS to PFOA for subpopulations studied here was 9.2 for subpopulation with a high fish intake in their diet and substantially less, i.e., from 3.3 to 4.3, for the other human subpopulations. This finding again implies that Baltic Sea fish are an important source of PFOS and for PFOA, albeit less so than for PFOS, for the Polish people.

PFOA blood concentrations are the most uniform among all subpopulations when compared with all other fluorochemicals. Nevertheless, five individuals (with a high fish intake in their diet) contained significantly higher loads of PFOA in their blood when compared with other donors in this group ($p < 0.05$; U Mann−Whitney test). For these five individuals their PFOA blood load, when compared to any other group or to all 45 donors together, or to the other 55 persons jointly, differed with a high level of significance ($0.008 < p < 0.002$; U Mann−Whitney test). These five individuals declared a weekly fish intake rate of 1500 g per capita. Two of them were near-shore fisherman, and two, father and son fisherman, were known to also consume by-catch of eider duck and other marine diving duck meat. Four individuals in this group were in the age class of 51−62, and the fifth individual was 19. For this subgroup the concentration quotient of PFOS to PFOA showed a clear age-related decrease (probability value) with values spanning 23 to 6.6, for the oldest and youngest individual, respectively. This finding supports the findings of an increasingly widespread environmental contamination with PFOA-based compounds in

the region, and is consistent with the global time-trend in human exposure to PFCs as observed recently in Japan and the United States (*7*, *13*).

In addition to Baltic fish, which have been found to highly influence human body burden of PFHxS, PFOS, PFOSA, PFHxA, PFHpA, PFNA, PFDA, PFUnDA, and PFDoDA, and to a less extent PFOA, other sources of intake may exist, notably for PFOA, including beef (*15*). Since fluorotelomer alcohols could be biodegraded in part to PFOA by microbes via enzymatic route utilizing alcohol dehydrogenase no data are available on the possible relevance to human of enzymatic pathway utilizing human alcohol dehydrogenase.

Literature Cited

(1) Kissa, E. *Fluorinated Surfactants and Repellents*, 2nd ed.; Marcel Dekker: New York 2005.

(2) Giesy, J. P.; Kannan, K. Perfluorochemical surfactants in the environment. *Environ. Sci. Technol.* **2001**, *35*, 1339−1345.

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

(4) Kannan, K.; Corsolini, S.; Falandysz, J.; Fillmann, G.; Kumar, K. S.; Loganathan, B. G.; Mohd, M. A.; Olivero, J.; Van Wouwe, N.; Yang, J. H.; Aldous, K. M. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environ. Sci. Technol.* **2004**, *38*, 4489−4495.

(5) U. S. EPA. *Preliminary Risk Assessment of the Developmental Toxicity Associated with Exposure to Perfluoroctane Acid and its Salts*; Office of Pollution Prevention and Toxics, Risk Assessment Division: Washington, DC, 2003.

(6) Kennedy, G. L.; Butenhoff, J. L.; Olsen, G. W.; O'Connor, J. C.; Seacat, A.; Perkins, R.; Biegel, L.; Murphy, S.; Farrar, D. The toxicology of perfluorooctanoate. *Crit. Rev. Toxicol.* **2004**, *34*, 351−384.

(7) Olsen, G. W.; Huang, H.-Y.; Heizisouer, K. J.; Hansen, K. J.; Butenhof, J. L.; Mandel, J. H. Historical comparison of perfluorooctanesulfonate, perfluorooctane and other fluorochemicals in human blood. *Environ. Health Perspect.* **2005**, *113*, 539−545.

(8) Inoue, K.; Okada, F.; Ito, R.; Kato, S.; Sasaki, S.; Nakajima, S.; Uno, Y., Sata, F.; Yoshimura, Y.; Kisi, R.; Nakazawa, H. Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: Assessment of PFOS exposure in a susceptible population during pregnancy. *Environ. Health Perspect.* **2004**, *112*, 1204−1207.

(9) Hu, W.; Jones, P. D.; Celius, T.; Giesy, J. P. Identification of genes responsive to PFOS using gene expression profiling. *Environ. Toxicol. Pharmacol.* **2005**, *19*, 57−70.

(10) OECD. *Environment, health and safety publications series on risk management No. 19. Results of survey on production and use of PFOS, PFAS and PFOA, related substances and products/ mixtures containing these substances*; ENV/JM/MONO(2005)1; OECD: Paris, 2004.

(11) Taniyasu, S.; Kannan, K.; So, M. K.; Gulkowska, A.; Sinclair, E.; Okazawa, T.; Yamashita, N. Analysis of fluorotelomer alcohols, fluorotelomer acids, and short and long-chain perfluorinated acids in water and biota. *J. Chromatogr. A.* **2005**, *1093*, 89−97.

(12) Kärrman, A.; van Bavel, B.; van Leeuwen, S.; de Boer, J.; Lindström, G. Perfluorinated compounds in human blood and plasma. 1st Worldwide interlaboratory study. *Organohalogen Compd.* **2005**, *67*, 805−808.

(13) Harada, K.; Saito, N.; Yoshinaga, T.; Watanabe, T.; Sasaki, S.; Kamiyama, S.; Koizumi, A. The influence of time, sex and geographic factors on levels of perfluorooctane sulfonate and perfluorooctanoate in human serum over the last 25 years. *J. Occup. Health* **2004**, *46*, 141−147.

(14) Kannan, K.; Corsolini, S.; Falandysz, J.; Oehme, G.; Focardi, S.; Giesy, J. P. Perfluorooctanesulfonate and related fluorinated hydrocarbons in marine mammals, fishes, and birds from coast of the Baltic Sea and the Mediterranean Sea. *Environ. Sci. Technol.* **2002**, *36*, 3210−3216.

(15) Guruge, K. S.; Taniyasu, S.; Miyazaki, S.; Yamanaka, N.; Yamashita, N. Age dependent accumulation of perfluorinated chemicals in beef cattles. *Organohalogen Compd.* **2004**, *66*, 4029−4034.

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