CONTAMINATION PROFILES OF SHORT-CHAIN POLYCHLORINATED n-ALKANES IN FOODSTUFF SAMPLES FROM JAPAN

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Introduction

Polychlorinated n-alkanes (PCAs) are group of chemicals manufactured by chlorination of liquid *n*-paraffin or paraffin wax that contain 30 to 70% chlorine by weight. Large amounts of PCAs are widely used as plasticizers for vinyl chloride, lubricants, paints, and flame retardants and number of other industrial applications. Annual global production of PCAs is approximately 300 kilo tones, with a majority having medium-carbon-chain (C_{14}-C_{19}) length. According to the investigation made by Kagaku Kogyo Nippon-Sha, the annual consumption of PCAs in Japan was about 83,000 tons in between 1986-2001. Short-carbon-chain (C_{10}-C_{13}) has been placed on the Priority Substance List under Canadian Environmental Protection Act and on the Environmental Protection Agency Toxic Release Inventory in the USA due to its potential to act as tumor promoters in mammals. Data on environment levels of PCAs is meager, nevertheless, PCAs have been measured at relatively high concentrations in biota from Sweden[1], biota, sediment from Canada[2,3] and marine biota and human milk from the Canadian Arctic[4].

In our earlier study, we reported concentrations of short-chain PCAs from sewage treatment plant (STP) collected from Tama River, Tokyo and river water and sediment from Tokyo and Osaka[5]. STP influent water contained greater short-chain PCAs concentrations than STP effluent. In addition, some river water and sediment samples contained detectable concentrations of short-chain PCAs, which was similar to other industrial countries. However, there is no study conducted to explore the contamination profiles of short-chain PCAs in human foodstuff samples. In the present study, we analyzed eleven foodstuff samples that were purchased from various supermarkets in order to know the short-chain PCAs concentrations in the foodstuff and possible human total daily intake (TDI) amounts.

Materials and Methods

Eleven foodstuff sample composites was purchased in supermarkets as follows; 1) Grain and grain products including boiled rice, rice processed food, barley, wheat/corn flour, bread, sweet bread, noodle, macaroni, instant noodle, rice vermicelli, corn starch etc., 2) Nuts and nut products which encompass a mixture of peanut, almond, walnut, giant corn, cashew nut, sweet potato, potato etc., 3) Sugar and sugar products which encompass a composition of sugar, honey, strawberry jam, rice cracker, candy, cake, biscuit, chocolate, soy sauce, salt, food seasoning, beer, wine, cola etc., 4) Oil which encompasses a mixture of margarine, butter, vegetable oil, beef tallow, mayonnaise etc., 5) Beans or vegetable products which encompass a mixture of soybean, carrot, spinach, pimento, tomato, leek, corn marigold, broccoli, radish, onion, cabbage, cucumber, spring onion, parsley, ginger, garlic, pickle, mushroom, seaweed etc., 6) Fruits and fruit products which encompass citrus fruit, apple, banana, strawberry, grapes, cherry, tomato juice etc., 7) Fish and fish products samples composited from salmon, tuna, bream, flat fish, horse mackerel, sardine, cattle fish, octopus, crab, salted fish, half-dried fish, canned fish, boiled & seasoned fish, fish products, fish ham etc., 8) Shell and shell products which include short-necked clam, scallop, clam, canned scallop, boiled & seasoned shell, ark shell etc., 9) Meat and meat products which include pork, beef, lamb, ham sausage, chicken, roasted ham etc., 10) Egg samples which include chicken and quail egg and 11) Milk and milk products which consist of milk, cheese, fresh cream, yogurt, kamanbeera cheese, and other products., All the samples were homogenated with laboratory mixture to smooth paste, stored at $-20^{o}C$ until analyzed.

The known amount of sample homogenate was mixed and powdered with Na_2SO_4 and Soxhlet extracted for 16-h with dichloromethane. The extracts were concentrated up to 20 mL using rotary evaporator; from this, 1 mL was used for fat determination and 5 mL was used for short-chain PCAs analysis. The cleanup of extracts was performed with florisil, while lipid rich samples were pre-cleaned with sulfuric acid-silicagel column chromatography. $^{13}C_{12}$-2,3,3',5,5'-PeCB-111 and $^{13}C_{12}$-2,2',3,3',4,4',5-HpCB-170 were used as internal standards. The Promochem PCA-technical standard mixtures (PCA-C_{10}, C_{11}, C_{12}, and C_{13}) were analyzed by high resolution gas chromatography / high resolution mass spectrometry with electron capture negative ionization (HRGC/HRMS-ECNI), Thermoelectron Finningan MAT95XL and used as reference standard for calibration purpose. In general, for standardizing the HRGC/HRMS-ECNI methods, we conducted the short-chain PCAs analysis using DB-5 MS column (J&W, 15m x 0.25mm [0.1µm]) with 200 ng of technical PCAs standard (e.g., Promochem). The result of SCAN analysis by ECNI in low-resolution showed the specific mass spectrum, and the monitor ion $[M-Cl]^-$ for SIM analysis in high-resolution was proposed[5]. The chromatograms in SIM analysis showed typical broad peaks and the calibration curves of standard materials were prepared at 0.5, 1, 5, 20, 50 ng/µL standard solutions. The detection limits for all congeners were in between <0.1 ng/g wet wt (grains), <0.2 ng/g wet wt (nuts, sugar, beans/vegetables, fruit, fish, shell fish and egg), <0.3 ng/g wet wt (milk), <0.5 ng/g wet wt (meat) and <2.0 ng/g wet wt (oil), less than this level is consider as ND (not detected). The concentrations of foodstuff samples were expressed as ng/g wet weight basis unless specified otherwise. The complete analytical methodology that we adopted in present study was reported in our earlier study[5].

Results and Discussion

Concentrations of short-chain PCAs with chlorine molecule of 4 to 11 were shown in Table 1. Blank samples showed not detected of any of short chain PCA congeners (Table 1). In foodstuff samples, PCA congeners with 4 chlorines were not detected in any of 11 analyzed sample homogenates. Carbon homologue 10 was the most prevalent contaminant followed by PCA congeners with increasing carbon number. These observations are slightly different than observations made on sewage treatment plant and water samples that show greater accumulation of carbon homologues 11, 13 and 12. PCA congeners with 5, 6, and 7 chlorine molecules accumulated comparatively greater than those with higher chlorine numbers. Oil contained maximum the PCA concentration (140 ng/g wet wt), followed by shellfish or crustaceans (18 ng/g wet wt), fish (16 ng/g wet wt), while milk, nuts, fruit, beans or vegetables, egg, sugar and grains, which had the minimum concentration (<3.0 ng/g wet wt) as shown in Table 1.

Table 1. Concentrations (ng/g wet wt) of short-chain PCAs in foodstuff samples collected from Japan

Sample Item	Grains	Nuts	Sugar	Oil	Beans	Fruit	Fish	Shell	Meat	Egg	Milk
Fat (%)	0.54	0.90	1.9	94	0.92	0.04	9.4	0.72	25	11	7.0
QL*	0.10	0.2	0.2	2.0	0.2	0.2	0.2	0.2	0.5	0.2	0.3
C10H18Cl4	<0.1	<0.2	<0.2	<2.0	<0.2	<0.2	<0.2	<0.2	<0.5	<0.2	<0.3
C10H17Cl5	0.14	<0.2	<0.2	<2.0	<0.2	<0.2	0.28	0.46	0.22	<0.2	<0.3
C10H16Cl6	0.57	0.47	0.42	8.6	0.50	0.39	0.87	2.0	1.5	0.49	<0.3
C10H15Cl7	0.64	0.61	0.75	18	0.47	0.53	2.4	3.9	2.0	0.45	0.38
C10H14Cl8	0.44	0.34	0.50	18	0.31	0.28	4.3	3.6	1.3	0.36	0.37
C10H13Cl9	0.13	<0.2	<0.2	9.3	<0.2	<0.2	2.6	1.1	<0.5	<0.2	<0.3
C10H12Cl10	<0.1	<0.2	<0.2	4.9	<0.2	<0.2	0.88	<0.2	<0.5	<0.2	<0.3
C10H11Cl11	<0.1	<0.2	<0.2	<2.0	<0.2	<0.2	<0.2	<0.2	<0.5	<0.2	<0.3
Total C10 PCA	1.9	1.4	1.7	59	1.3	1.2	11	11	5.1	1.3	0.75
C11H20Cl4	<0.1	<0.2	<0.2	<2.0	<0.2	<0.2	<0.2	<0.2	<0.5	<0.2	<0.3
C11H19Cl5	0.17	<0.2	0.26	<2.0	0.24	0.28	0.34	0.28	0.65	0.28	<0.3
C11H18Cl6	0.16	<0.2	0.22	3.5	<0.2	<0.2	0.32	0.63	0.53	0.20	<0.3
C11H17Cl7	0.24	<0.2	0.21	14	0.21	<0.2	0.94	1.7	0.75	0.20	<0.3
C11H16Cl8	<0.1	<0.2	<0.2	8.3	<0.2	<0.2	1.1	1.1	<0.5	<0.2	<0.3
C11H15Cl9	<0.1	<0.2	<0.2	4.6	<0.2	<0.2	0.83	0.65	<0.5	<0.2	<0.3
C11H14Cl10	<0.1	<0.2	<0.2	<2.0	<0.2	<0.2	0.36	<0.2	<0.5	<0.2	<0.3
C11H13Cl11	<0.1	<0.2	<0.2	<2.0	<0.2	<0.2	<0.2	<0.2	<0.5	<0.2	<0.3
Total C11 PCA	0.57	<0.2	0.69	30	0.45	0.28	3.9	4.4	1.9	0.69	<0.3
C12H22Cl4	<0.1	<0.2	<0.2	<2.0	<0.2	<0.2	<0.2	<0.2	<0.5	<0.2	<0.3
C12H21Cl5	<0.1	<0.2	<0.2	<2.0	<0.2	<0.2	<0.2	<0.2	<0.5	<0.2	<0.3
C12H20Cl6	<0.1	<0.2	<0.2	<2.0	<0.2	<0.2	<0.2	0.23	<0.5	<0.2	<0.3
C12H19Cl7	<0.1	<0.2	<0.2	12	<0.2	<0.2	0.30	0.65	<0.5	<0.2	<0.3
C12H18Cl8	<0.1	<0.2	<0.2	16	<0.2	<0.2	0.34	0.53	<0.5	<0.2	<0.3
C12H17Cl9	<0.1	<0.2	<0.2	8.1	<0.2	<0.2	0.25	0.23	<0.5	<0.2	<0.3
C12H16Cl10	<0.1	<0.2	<0.2	5.0	<0.2	<0.2	<0.2	<0.2	<0.5	<0.2	<0.3
C12H15Cl11	<0.1	<0.2	<0.2	3.8	<0.2	<0.2	<0.2	<0.2	<0.5	<0.2	<0.3
Total C12 PCA	<0.1	<0.2	<0.2	45	<0.2	<0.2	0.89	1.6	<0.5	<0.2	<0.3
C13H24Cl4	<0.1	<0.2	<0.2	<2.0	<0.2	<0.2	<0.2	<0.2	<0.5	<0.2	<0.3
C13H23Cl5	<0.1	<0.2	<0.2	<2.0	<0.2	<0.2	<0.2	<0.2	<0.5	<0.2	<0.3
C13H22Cl6	<0.1	<0.2	<0.2	<2.0	<0.2	<0.2	<0.2	0.29	<0.5	<0.2	<0.3
C13H21Cl7	<0.1	<0.2	<0.2	2.8	<0.2	<0.2	<0.2	0.55	<0.5	<0.2	<0.3
C13H20Cl8	<0.1	<0.2	<0.2	4.4	<0.2	<0.2	<0.2	0.36	<0.5	<0.2	<0.3
C13H19Cl9	<0.1	<0.2	<0.2	2.9	<0.2	<0.2	<0.2	<0.2	<0.5	<0.2	<0.3
C13H18Cl10	<0.1	<0.2	<0.2	<2.0	<0.2	<0.2	<0.2	<0.2	<0.5	<0.2	<0.3
C13H17Cl11	<0.1	<0.2	<0.2	<2.0	<0.2	<0.2	<0.2	<0.2	<0.5	<0.2	<0.3
Total C13 PCA	<0.1	<0.2	<0.2	10	<0.2	<0.2	<0.2	1.2	<0.5	<0.2	<0.3
Total short-chain (C10-13) PCAs	2.5	1.4	2.4	140	1.8	1.5	16	18	7.0	2.0	0.75

*QL indicates quantitation limit;
Blank analysis showed less than detection limit of any short-chain PCAs congeners

The concentration in the category 4 "Oil" (140 ng/g wet wt) was remarkably higher than those of any other category as reported using a thin layer chromatograph analysis by a previous study conducted at UK[6]. The category 4 included vegetable oil (542.7g), mayonnaise (292.7g), margarine (15g), butter (61g), and beef tallow (12.2g). The butter sampled originated in northern Japan, and the origins of other products are unknown. The oil food products were not measured to determine which food product contributed the most to the highest concentration. Concentrations in fish (16 ng/g wet wt) and shellfish (18 ng/g wet wt) categories were lower than expected, but showed the second highest concentrations in this study. Most fish and shellfish were from Japanese fresh water systems or Japanese fishery harbors except tuna from Italy, Spain, and Japan, salted salmon from the USA, salted mackerel from Norway, dried smelt from Canada, and clams and other fish/shell products from China. The third highest concentration (7.0 ng/g wet wt) was reported from the meat category including pork, beef (USA/Japan), chicken (Japan), ham, and lamb (New Zealand). It was surprising, however, that the milk category showed the lowest concentration among the eleven food categories. The category comprised of whole milk (832 g), cream (76 g), and yogurt (76 g), which originated in Japan. The fat content of the whole milk was about 7.0% (Table 1).

Table 2. Total daily intake of short-chain PCAs for humans from Japan (ng/kg/day)

Food Item	Ages								
	1 to 6	7 to 14	15 to 19	20 to 29	30 to 39	40 to 49	50 to 59	60 to 69	>70
Grains	30.8	23.6	17.4	15.1	14.7	14.6	14.7	14.8	14.7
Nuts	7.6	5.6	2.9	2.3	2.3	2.4	2.7	2.8	3.3
Sugar	25.0	12.7	10.7	13.2	15.3	14.9	15.8	13.4	9.0
Oil	112.9	84.8	63.2	54.0	50.8	47.8	45.1	36.3	31.5
Beans	33.2	25.4	16.7	15.0	16.0	16.8	20.4	22.0	21.9
Fruit	12.6	7.0	4.1	2.9	2.8	3.5	5.4	6.6	6.8
Fish	38.7	29.2	22.6	19.8	21.3	26.7	31.7	30.8	30.8
Shell	5.2	3.9	3.0	2.6	2.8	3.6	4.2	4.1	4.1
Meat	32.1	26.4	22.8	17.6	16.1	13.5	12.7	10.7	9.1
Egg	5.7	3.7	3.1	2.2	2.2	2.1	2.1	2.0	1.9
Milk	22.8	18.9	6.8	3.4	3.1	2.9	3.4	3.8	4.3
Total	326.6	241.2	173.3	148.6	147.4	148.8	158.2	147.3	137.4

There is very limited data available on PCAs (particularly short-chain PCAs) in foodstuff and thus the observed fish concentrations in this study are comparable to the reported studies on fish. Comparatively lower concentrations (170 ng/g fat weight basis) were noticed in this study compared to fish samples from Sweden[1] with 1,400-1,600 ng/g fat weight basis. However, the lipid concentrations of shellfish in our study (2500 ng/g fat weight basis) were similar to industrialized countries. Short-chain PCAs are also detected in beluga whales (0.41 to 166), carp (0.9) and rainbow trout (2.7) from Canada on µg/g wet weight basis[7]. Short-chain PCAs were detected in mussel, pike, plaice, pouting (<0.03-3.25) from UK on µg/g wet weight basis[6, 8]. The yellow perch and zebra mussel from Detroit River, USA contained greater amount of PCAs (1,150-1,210 ng/g dry weight).

The water short-chain PCAs concentrations were 0.008 to 0.22 ng/mL[5], which were several orders of magnitude lower than fish, shell, and other foodstuff collected from Japan. These trends suggest possible bio-accumulation of short-chain PCAs compounds in aquatic animals. Elevated contamination profiles of short-chain PCAs in oil, fish and shellfish can result in significant ingestion exposure by humans. Total daily intakes of short-chain PCAs for people in Japan were calculated for different age groups of people based on the food consumption survey data (Table 2). The results show a relatively uniform total intake between 15 and 69 years old, mainly because young generations prefer food with the fat and meat categories and the preference of older generations shifts to fish in addition to vegetables and potatoes. The highest total intake is 7.9 μg/day, equivalent to 158 ng/kg/day for a 50 kg human, while total daily intake for 6 year olds is 6.8 μg/day, equivalent to 324 ng/kg/day for a 21-kg first-year elementary school student. A conclusion of the recent review on mammalian toxicity test results led to a NOAEL of 100 mg/kg/day. Our risk assessment for human health concludes that the food intake is the main exposure pathway, and that the total food intake of short-chain PCAs poses no risk at present.

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