USE OF ARCHIVED DUPLICATE-DIET SAMPLES TO MEASURE PAST DIETARY EXPOSURE TO DIOXINS IN JAPAN

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Introduction

In this study, past dietary dioxin exposures were measured by means of archived duplicate-diet samples collected in 6 households in 2 Japanese cities in 1990, and the exposures were compared with the results from other studies. Knowledge of past dioxin exposure levels is essential for evaluating current exposure levels. The majority of human dioxin exposure in the general population occurs through dietary intake[1]. The duplicate-diet method can be used to evaluate inter-household differences, and market-basket studies can be used to determine dietary dioxin intake from an average diet. A study using archived market-basket samples showed that the decline in dietary dioxin exposure in Japan leveled off after around 1990[2]. The objective of the present study was to measure the variability of past dietary dioxin exposure among households and to verify the trend of dietary dioxin exposure in Japan over time by means of the complementary sampling method. In this paper, 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), and several non-ortho- and mono-ortho-substituted polychlorinated biphenyls (co-PCBs, those with toxic equivalency factors defined by WHO in 1998[3]) are collectively referred to as dioxins.

Materials and Methods

The samples were collected in 1990 from 3 households in each of 2 Japanese cities Nagano city (area 404 km^2, population 0.36 million) is located inland in the north-central part of Japan's main island, and Kobe city (area 550 km^2, population 1.5 million) is located in the western part of Japan's main island, facing the Seto-naikai Inland Sea. Duplicate-diet samples were prepared and collected daily at each of the 6 test households for 3 consecutive days. The samples included snacks and drinking water and other drinks. Daily samples were homogenized, packed into glass bottles, frozen, shipped to the storage site, and kept frozen until analysis. A list of food items was recorded for each household. At the analytical laboratory, the samples from each household were thawed and mixed to make up a 3-day composite sample on the basis of the daily recorded weight of the diet.

The detailed analytical method will be described elsewhere[4]. Briefly, the composite sample was spiked with ^{13}C-labeled cleanup spikes and centrifuged. The supernatant was alkaline-digested and extracted with *n*-hexane. The precipitate was freeze-dried, mixed with anhydrous sodium sulfate, and Soxhlet-extracted with toluene. The toluene was then solvent-exchanged to *n*-hexane. The *n*-hexane extracts of the precipitate and the supernatant were mixed, treated with concentrated sulfuric acid, and purified by column chromatography on a multilayer silica-gel/modified silica-gel

column and an activated-carbon column. The amount of dioxins in the final concentrate was quantitated by high-resolution gas chromatography/high-resolution mass spectrometry (GC/MS). The 29 dioxin compounds were quantitated by the isotope dilution method. Non-2,3,7,8-subsutituted tetra- to hepta-CDDs and CDFs were quantitated by the internal standard method. The recovery of cleanup spikes was calculated against syringe spikes added just prior to GC/MS injection. A blank test was conducted using a pure water sample. Values below the detection limits (DLs) were treated as half the DL in the calculation of 2,3,7,8-tetraCDD toxic equivalents (TEQs[3]). Fat content was determined by Soxhlet extraction of a portion of the composite sample with (2:1) chloroform/methanol[5].

Results

The blank samples showed negligible amounts of dioxins. The DLs for the dioxin compounds varied among samples and among compounds from 0.3 to 3 fg/g wet weight. The contribution from 1,2,3,4,7,8- and 2,3,4,6,7,8-hexaCDFs may have been overestimated (by up to 12% for PCDF-TEQ and 4% for total TEQ) because GC separation of these compounds was not perfect. Most of the dioxin compounds (except 1,2,3,7,8,9-hexaCDF [0/6] and 1,2,3,4,7,8,9-heptaCDF [1/6]) were detected in all 6 diet samples.

The total amount of the daily diet (a 3-day average), the fat content of the diet, the dioxin TEQ concentration in the diet, and the calculated daily dietary TEQ intake of dioxins are presented in Table 1. The amount of the daily diet ranged from 1.45 kg to 2.15 kg, and fat intake ranged from 26 g to 67 g. The dioxin TEQ concentrations in the diet ranged from 0.048 to 0.26 pg TEQ/g wet weight or from 2.0 to 15 pg TEQ/g fat. The dietary dioxin intake per person per day ranged from 72 to 3.8×10^2 pg TEQ. The major (>10%) contributors to the TEQ values were 3,3',4,4',5-pentaCB (46%), 2,3,4,7,8-pentaCDF (12%), and 1,2,3,7,8-pentaCDD (10%) (values in parentheses are averages for the 6 samples). It is interesting to note that non-2,3,7,8-subsutituted compounds comprised 28% to 73% of tetra- to octa-CDDs and CDFs (in actual, not TEQ, concentration).

Discussion

Past dietary dioxin exposures were directly measured by means of archived duplicate-diet samples collected in 6 households in 2 Japanese cities in 1990. The results revealed the variability of the daily dietary dioxin intake among the households tested. Notably, 1 household from Kobe city showed relatively high co-PCB dietary exposure compared to the other 5 households. The sample size was not sufficient to allow us to discuss the differences between the two sampled cities.

The results from the present study are in accordance with the dietary dioxin intake levels and their time trend expected from 2 other studies conducted in Japan (Figure 1). Japan's Ministry of Health and Welfare (MHW) analyzed archived market-basket samples collected in the Kansai region (in the western part of Japan's main island) in 1977, 1982, 1988, 1992, 1995, and 1998 to measure dietary dioxin exposure[2]. The Japan Environment Agency (JEA) conducted a duplicate-diet study in 9 areas using a collection method similar to that used in the present study, and Nagano and Kobe cities were included in the sampled areas[6]. The difference in sampling areas may partly account for the dietary dioxin exposure being lower in the 2 duplicate-diet studies than in the market-basket study (Figure 1), because the Kansai region showed higher dietary dioxin intake than the other parts of Japan in a nationwide market-basket study in 1998[2].

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Table 1. Diet statistics and daily dietary dioxin intake in 6 households in 2 Japanese cities in 1990[a].

	diet [kg/day]	fat	dioxin conc.[b] [pg TEQ/g diet wet weight]	intake per person [pg TEQ/day]			
				PCDDs	PCDFs	co-PCBs	total dioxins
Nagano-A	1.94	2.4%	4.8E–02	3.7E+01	2.0E+01	3.7E+01	9.4E+01
Nagano-B	2.15	3.1%	8.8E–02	6.5E+01	4.5E+01	8.0E+01	1.9E+02
Nagano-C	1.61	1.9%	6.6E–02	2.4E+01	2.5E+01	5.7E+01	1.1E+02
Kobe-D	1.46	1.8%	2.6E–01	4.0E+01	4.4E+01	3.0E+02	3.8E+02
Kobe-E	1.49	2.0%	1.2E–01	2.5E+01	3.3E+01	1.2E+02	1.8E+02
Kobe-F	1.45	2.3%	5.0E–02	1.1E+01	1.3E+01	4.9E+01	7.2E+01
Nagano mean	1.90	2.5%	6.7E–02	4.2E+01	3.0E+01	5.8E+01	1.3E+02
Kobe mean	1.47	2.0%	1.4E–01	2.5E+01	3.0E+01	1.6E+02	2.1E+02
overall mean	1.69	2.3%	1.1E–01	3.3E+01	3.0E+01	1.1E+02	1.7E+02

a) Measured from 3-day composite samples of archived duplicate diet. b) The sums of PCDDs, PCDFs and co-PCBs.

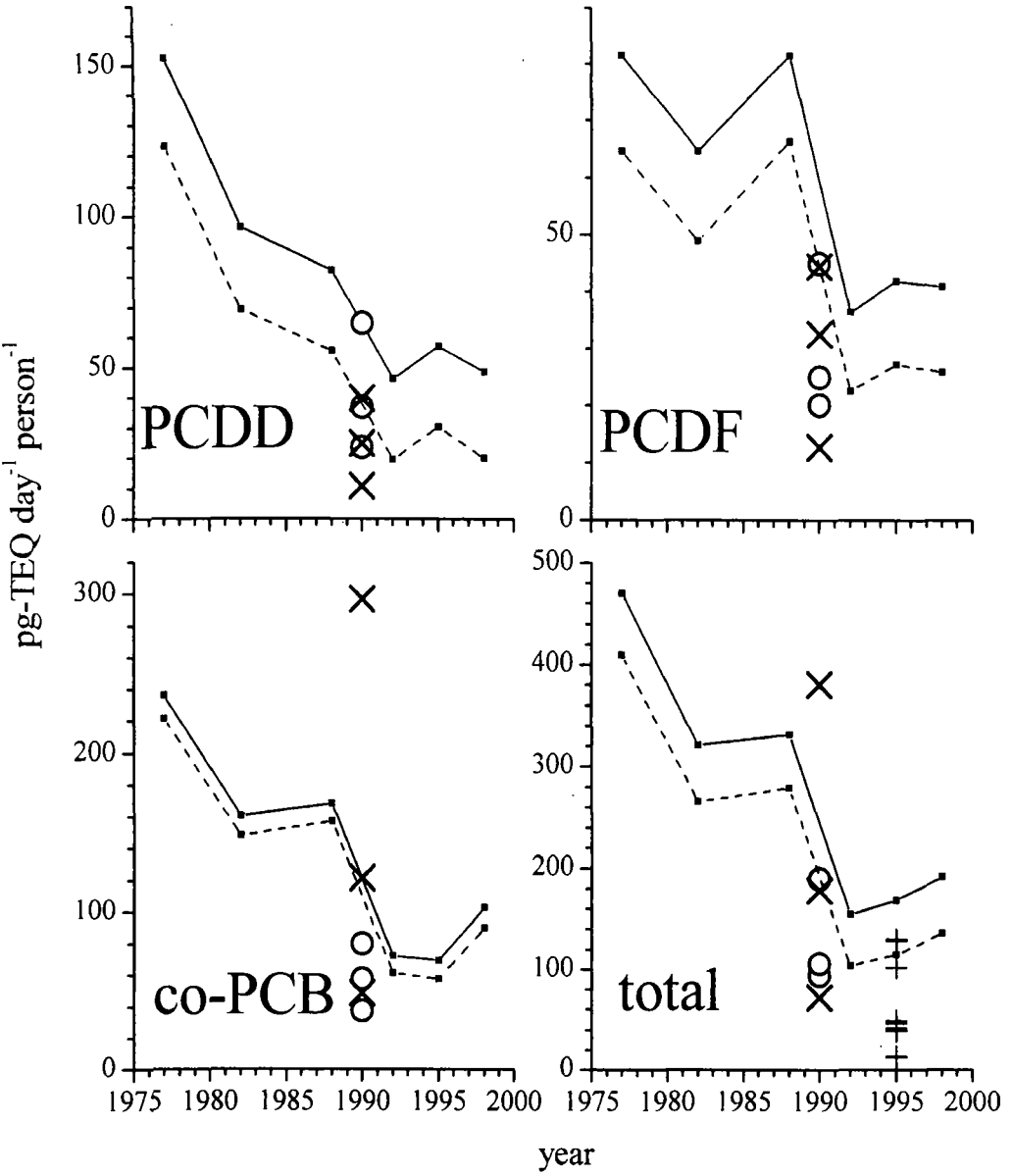

Figure 1. Comparison of daily dietary intake of dioxins. O: Nagano (this study); X: Kobe (this study); solid square: MHW 1977–1998 study (the results from 2 methods for TEQ calculation are shown because of the higher incidence of ND in the MHW study than in the JEA study and the present study; solid line: ND is treated as half the quantitation limit; broken line: ND is treated as zero)[2]; +: JEA study (only the total values were available; the sampling year was around 1995)[6].