with the GC/MS measurement were also sufficiently removed.

Methylation using diazomethane, which has been widely used, was adopted for the GC/MS measurement of the phenolic compounds such as HO-PCBs, PCP and TBBP-A. These compounds were sufficiently esterified within 3 h at ambient temperature.

3.7. Recovery study

The optimized sample extraction and cleanup method was evaluated by measuring the absolute recovery of the 59 compounds. Swine liver, heart, kidney, and cattle adipose tissues spiked with each standard compound at 1–5 ng/g were used for the recovery study, according to the scheme shown in Fig. 4. Table 2 summarizes the recovery data, which were considered satisfactory, and there were no problems encountered during the evaluation. TBBP-A, pentachlorobenzene and endrine aldehyde, however, appeared to have relatively lower recoveries Table 3.

Swine kidney occasionally gave interferences for the determination of 4-$MeSO_2$-heptaCB174 in Fraction-B, although no interferences were detected from the other matrices. However, the interferences could be removed by passing Fraction-B from the kidney sample through a 22% sulfuric acid silica-gel column (0.3 g of 22% sulfuric acid silica-gel packed in a disposable SPE cartridge) with 5 ml DCM as the eluent.

In this research, lipid extraction by ASE, GPC cleanup, and silica-gel SPE fractionation were automated. Manual work was still needed for parts of the liquid–liquid distribution extraction, the eluate concentration procedure, and the centrifugation.

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Bulletin of Environmental Contamination and Toxicology

Cleanup Method Using Disposable Tandem Cartridge System for the Determination of Dioxins in Human Milk by Enzyme-Linked Immunosorbent Assay

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For dioxin analysis, the establishment of the appropriate analytical method and the quality control procedure are indispensable for reliable measurements. On the other hand, a method that is simple, rapid, inexpensive, and highly sensitive for the screening of dioxins has been highly demanded by the public and the government. One of the methods that may satisfy these requirements is an enzyme-linked immunosorbent assay (ELISA), and there have been some reports on the measurement of dioxins using ELISA. We have already reported the development of a dioxin toxicity evaluation method for human milk by ELISA (Sugawara et al. 1998; Ishizuka et al. 2001; Sugawara et al. 2002; Saito et al. 2003a). However, the dioxin analysis by ELISA usually requires a sufficient cleanup similar to the GC/MS method due to the extremely low content of the dioxins, though ELISA is generally said to have the ability of high specificity.

A cleanup method for the analysis of human milk, in which an alkaline digestion step and a three-layer H_2SO_4/silica-gel cartridge were used, was reported in a previous paper (Saito et al. 2003a). Although an excellent cleanup effect could be obtained by employing this method, it required an overnight alkaline digestion treatment (about 12 hours) and a liquid-liquid distribution extraction using hexane. Accordingly, a more effective cleanup method was developed using the tandem column combined with a commercially available multi-layer silica-gel cartridge and an alumina cartridge. Furthermore, in order to assess the usefulness of the proposed cleanup method, the assay validation was carried out by comparing a conventional GC/MS method and the proposed ELISA method for the determination of dioxins in human milk samples.

MATERIALS AND METHODS

All of the dioxin standards were from Wellington Laboratories (USA) and were diluted with decane to the appropriate concentrations. Most of the organic solvents such as hexane, acetone, dichloromethane, toluene, diethylether, ethanol and methanol (MeOH) were of dioxin analysis quality from Kanto Kagaku (Tokyo, Japan) or Wako Pure Chemicals (Osaka, Japan). Decane was of special quality

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grade and was redistilled prior to use.

The multi-layer silica-gel column packed in a disposable cartridge tube was from GL Sciences, Inc. (Tokyo, Japan). It is made of 0.9g silica-gel, 3g 2% KOH/silica-gel, 0.9g silica-gel, 4.5g 44% H_2SO_4/silica-gel, 6g 22% H_2SO_4/silica-gel, 0.9g silica-gel, 3g 10% $AgNO_3$/silica-gel and 6g sodium sulfate. The cartridge was washed with 100 mL of hexane prior to use. The Sep-pak Plus Alumina (Basic type) cartridge was from Waters (Japan), and was washed with 10 mL of hexane prior to use. The tandem cartridge column of a multi-layer silica-gel cartridge and a Sep-pak Plus Alumina cartridge was prepared as follows; the column outlet part of the multi-layer silica-gel cartridge was connected in series to the inlet part of the Sep-pak Plus Alumina cartridge.

The surrogate standard for ELISA, 2,3,7-trichloro-8-methyldibenzo-*p*-dioxin (TMDD) was synthesized by Sanborn et al. (Sanborn et al.1998). Goat anti-rabbit antibody coupled to horseradish peroxydase and 3,3', 5,5'-tetra-methylbenzidine (TMB) were purchased from Sigma-Aldrich (USA). All other immunoreagents including coating hapten III for 2,3,7,8-TCDD and the antiserum for this ELISA were described in a previous report (Sugawara et al. 2002). All the other chemicals were of PCB analysis quality grade or special quality grade, and used without further purification.

The fat was extracted from human milk according to a previously described procedure (Saito et al. 2003a), and then dissolved with ca.2 mL of hexane. The hexane solution was applied to the tandem cartridge. After washing the cartridge with 160 mL of hexane, the multi-layer silica-gel cartridge, the upper cartridge of the tandem cartridge, was removed, and subsequently the dioxin fraction was obtained by eluting the Sep-pak Plus Alumina cartridge with 5 mL of 60% DCM/hexane. To the eluate was added 60 μL of a 100 ppm Triton X-100 methanol solution as a keeper, the solution was nearly dried out by a nitrogen stream, and the residue was re-dissolved into 60 μL of MeOH-DMSO (1:1).

The ELISA assay was carried out using a dry plate, which was prepared according to the reported method (Ishizuka et al. 2001). An outline of the method is shown below. Briefly, microtiter plates (Sumitomo Bakelite, Tokyo, Japan) were coated with the optimized concentration (0.5 μg/mL, 100 μL/well) of coating antigens (hapten III) (Sugawara et al. 1998) in carbonate-bicarbonate coating buffer (pH 9.6). They were incubated overnight at 4 °C. On the following day, the coated plates were washed 5 times with 0.05% (v/v) Tween 20 in phosphate buffered saline (PBS, pH 7.5) and were incubated for 30 min at room temperature with 300 μL of a 0.5% (w/v) bovine serum albumin (BSA) with sucrose in PBS (blocking solution) per well. After the removal of the blocking solution, the plates were dried *in vacuo* for 4 hr at 25 °C. They were then put into separate aluminum bags, and were packed *in vacuo*. Standards were prepared in 1:1:2 (v:v:v) DMSO:MeOH with 100 ppm Triton X-100 : PBS containing 2 mg/mL BSA

(PBSB). After an initial blocking step with BSA-PBS, and a wash step, 50 μL of the standards were added to the standard wells in a microtiter plate. The sample wells contained 25 μL of PBSB, then 25 μL of a human milk sample in DMSO-MeOH was added. Next, 50 μL of the antiserum diluted in PBSB was added to each well. The final ratio of DMSO-MeOH to PBSB was 1:3. The plates were incubated for 90 min. Following a wash step, 100 μL of goat anti-rabbit antibody coupled to horseradish peroxydase was added (diluted in PBS + 0.05% Tween 20). After a 60-min incubation period, the plates were washed with wash buffer, and 100 μL of an enzyme substrate containing TMB was added to each well. After 20 min, the color reaction was stopped by the addition of 50 μL of 2 M sulfuric acid. The resultant color was measured at 450 nm with a Model 550 Microplate Reader (Bio-Rad Laboratories Inc., Hercules, CA) in the single wavelength mode, and the dioxin levels in the human milk samples were calculated on the basis of a standard curve derived from a fit of absorbance versus the logarithm of the concentration.

The analysis of human milk by the GC/MS method was carried in accordance with a previous report (Saito et al. 2003b). The toxic equivalent quantity (TEQ) was calculated using WHO-TEF1998.

RESULTS AND DISCUSSION

The multi-layer silica-gel cartridge was made by modifying the multi-layer silica-gel column (Lamparski et al. 1980; Smith et al. 1984), which has been widely used for dioxin cleanup in various kinds of samples. A cleanup method for the GC/MS analysis of house dust, in which the usefulness of a multi-layer silica-gel cartridge method compared to an alkaline digestion method, was already reported in a previous paper (Saito et al. 2003b). In our preliminary experiment, we tried to develop a simpler cleanup method that allows the extracted fat to be directly treated using a multi-layer silica-gel cartridge without subjecting it to the alkaline digestion step. The multi-layer silica-gel cartridge was a useful method that was able to effectively remove the coexisting material of matrix origin such as the lipids and pigments in the human milk fat. However, the percentage of the fat content in the human milk depends on the subject. An excess amount of fat was often observed to spoil the cleanup performance of the multi-layer silica-gel cartridge. Moreover, the ELISA occasionally showed a false negative value or a low measurement value due to insufficient purification, even if the level of the fat content was in the range of the processing performance of the multi-layer silica-gel cartridge for the purification. We then tried further purification using alumina column chromatography, which has been also widely used for the dioxin cleanup. We have adopted the idea and created a tandem cartridge column system, i.e., a disposable alumina cartridge connected in series to the output of the multi-layer silica-gel cartridge was prepared in order to improve the cleanup efficiency. Furthermore, the maximum loading level of the human milk fat onto the multi-layer silica-gel cartridge was examined.

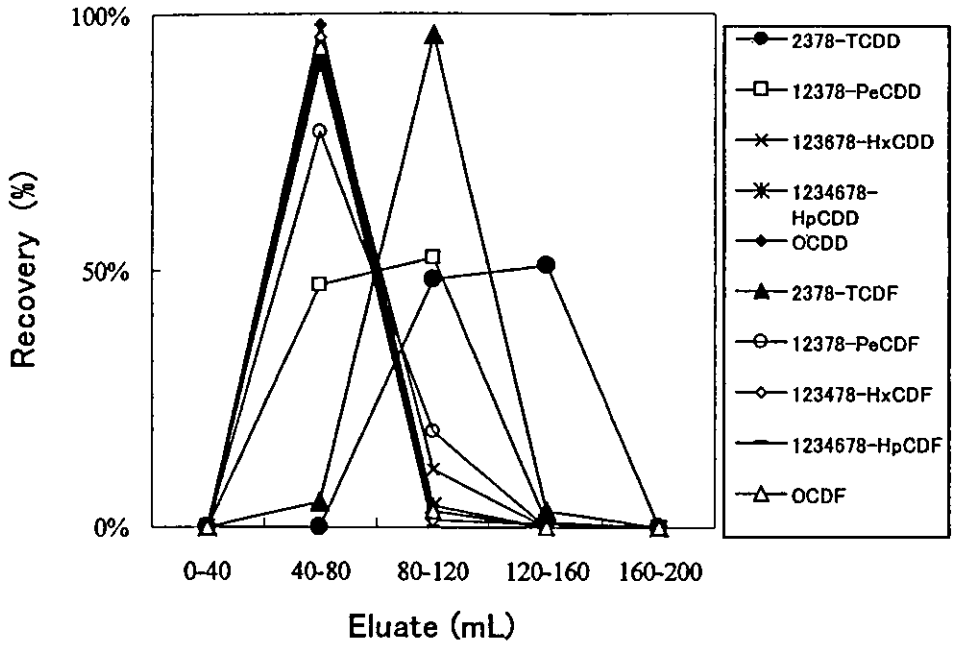

Figure 1. Elution profile of PCDD/Fs from a multi-layer silica-gel cartridge. Forty mL each of hexane eluate was collected as a fraction.

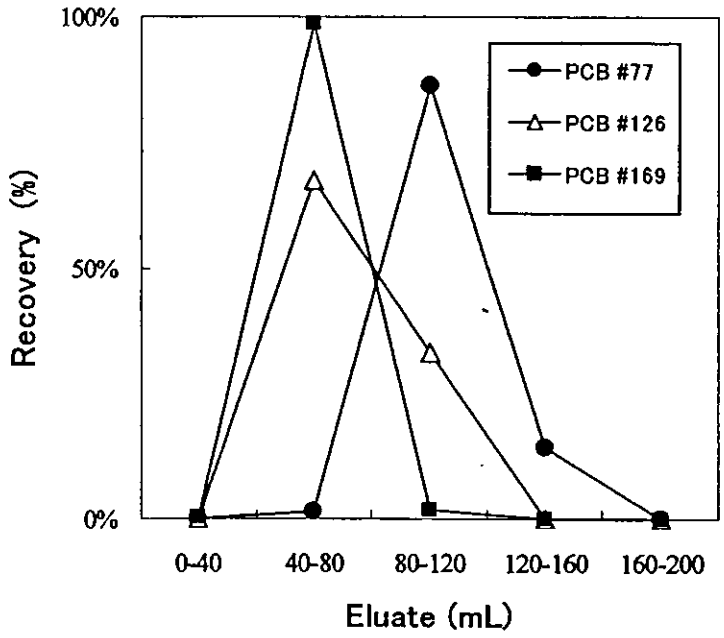

Figure 2. Elution profile of non-ortho PCBs from a multi-layer silica-gel cartridge. Forty mL each of hexane eluate was collected as a fraction.

The maximum loading level of the human milk fat onto the multi-layer silica-gel cartridge was estimated. Each fat (1,1g, 1,2g, 1.3g, 1.5g, 1.8g, 2.3g) extracted from actual human milk (six specimens) was applied to the multi-layer silica-gel cartridge, and the maximum loading level of the fat was judged by observing the carbonization reaction in the 44% H_2SO_4/silica-gel layer of the cartridge. That is, the limit state was that the brown color layer formed along with the reaction in the H_2SO_4/silica-gel layer does not reach the KOH/silica-gel layer. As a result, the fat level of 1.5 g or less was found to be suitable. Accordingly, the fat maximum loading level in the multi-layer silica-gel cartridge was judged to be 1.5g.

The eluting fraction profile of each congener of the PCDD/Fs and Co-PCBs was examined using a multi-layer silica-gel cartridge with hexane as the mobile phase. The dioxin standards, 10 kinds of PCDD/Fs (200 pg each), and 3 kinds of non-ortho-PCBs (1000 pg each) were added to the multi-layer silica-gel cartridge, and subsequently the cartridge was washed with hexane. Each 40 mL of effluent was collected as a separate fraction. Figure 1 and Figure 2 revealed that all of the target chemical substances were eluted with 160 mL of hexane.

For the ELISA, it was preferable to remove the non-planar PCBs and mono-ortho PCBs eluted from the multi-layer silica-gel cartridge as much as possible. There were a lot of PCBs residues in the eluate although the cross-reactivity of these PCBs in the ELISA is extremely low compared to the PCDD/Fs. We then paid attention to the alumina column chromatography already used in the analysis of dioxin, and examined a simple cleanup method with a commercially available pre-packed alumina cartridge. In that case, in order to simplify the operation as much as possible, the alumina cartridge was connected under the multi-layer silica-gel cartridge in series. Such an operation was based on the following idea: dioxins seemed not to be easily eluted from the alumina cartridge when using a non-polar solvent such as hexane, because the basic alumina has the ability to maintain dioxins stronger than silica-gel. The following method was then examined: After the dioxins eluted from the multi-layer silica-gel cartridge were trapped in the alumina cartridge, the multi-layer silica-gel cartridge was removed. Subsequently, the dioxins were eluted from the alumina cartridge with an appropriate solvent. The elution behavior of the dioxins from the alumina cartridge was then examined. As a result, it turned out that both the PCDD/Fs and non-ortho Co-PCBs were not eluted in the first 160mL of hexane, and were eluted in a subsequent 5mL of 60% DCM/hexane.

Figure 3 shows the relationship between the TMDD equivalents by ELISA and GC/MS values (Total-TEQ) determined in the actual human milk samples. Fairly good agreements between the GC/MS values and ELISA values were obtained from a linear regression analysis ($r = 0.947$, $n = 7$). The strong correlation between ELISA and TEQ suggests that the ELISA using the proposed cleanup method indicated its usefulness as a toxicity evaluation method for dioxins in human milk.

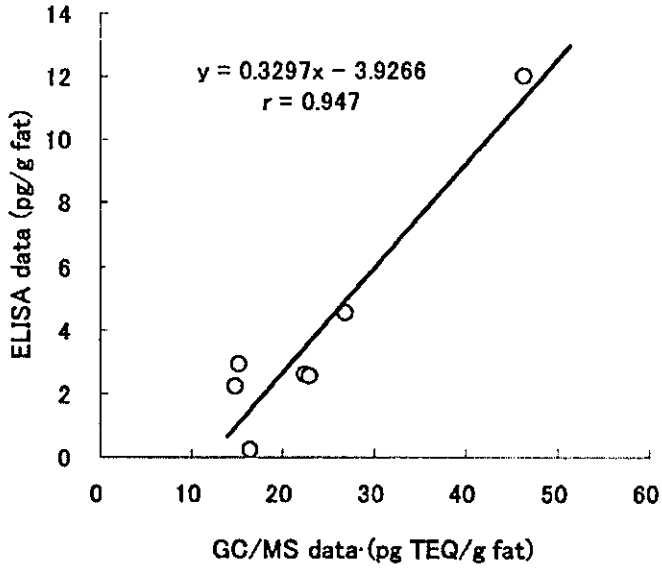

Figure 3. Correlation between GC/MS data and ELISA data.

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Industrial Health 2003, 41, 167–174

Review Article (7)

Exposure Evaluation of Dioxins in Municipal Waste Incinerator Workers

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Abstract: In Japan, the largest source of dioxin is solid waste incineration plants. Because workers employed at these plants handle fly ash and slag contaminated by dioxins, they can take dioxins into the body during work and their health may be adversely effected. This paper describes the dioxin exposure concentration, daily dioxin intake and blood dioxin level in workers employed at municipal incineration plants. The estimated dioxin exposure concentrations were 0.5 to 7.2 pg TEQ/m³ in the daily operation and 0.2 to 92,000 pg TEQ/m³ in the periodic maintenance. It was also expected that the daily dioxin intake can exceed the tolerable daily intake (TDI) in incineration plants with fly ash of high dioxin concentration. The mean of blood dioxin concentration was 346 pg TEQ/g lipid in the highest exposed worker group of the Toyono-gun incineration plant and those were 11 to 40 pg TEQ/g lipid in the other incineration plants.

Key words: Dioxins, Dioxin exposure, Daily dioxin intake, Blood dioxin level, Municipal waste incineration plant

Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs) are chemically and biologically similar compounds and are also highly toxic chemicals. "Dioxins" is the general term for these chemicals. They have already contaminate the environment such as air, water and soil, and foods such as fish, meat and vegetables[1-3]. Dioxins are also found in human adipose tissue, blood and milk[1, 4-6]. The main sources of dioxins are from the production of organic chlorinated herbicides, bleaching of paper/pulp and incineration of waste[7-9]. In Japan, incinerators are the major source[10] because most domestic solid waste (kitchen refuse, paper, wood, cloth, plastic etc.) is incinerated in municipal waste incinerators without sufficient measures to prevent the generation of these chemicals. Indeed, the grounds surrounding some incineration plants are contaminated with high levels of dioxins, causing concern among the residents about the adverse health effects of dioxins. Workers employed at incineration plants are also concerned about adverse effects on their health. In Japan, about 1,500 municipal waste incineration plants for domestic waste and 3,700 private incineration plants for industrial waste are in operation[11, 12] and tens of thousands of workers are estimated to be employed at them. Because the workers handle fly ash and slag contaminated by dioxins, they may intake dioxins during their work. The present paper reviews the exposure evaluation of dioxins in municipal waste incinerator workers in Japan.

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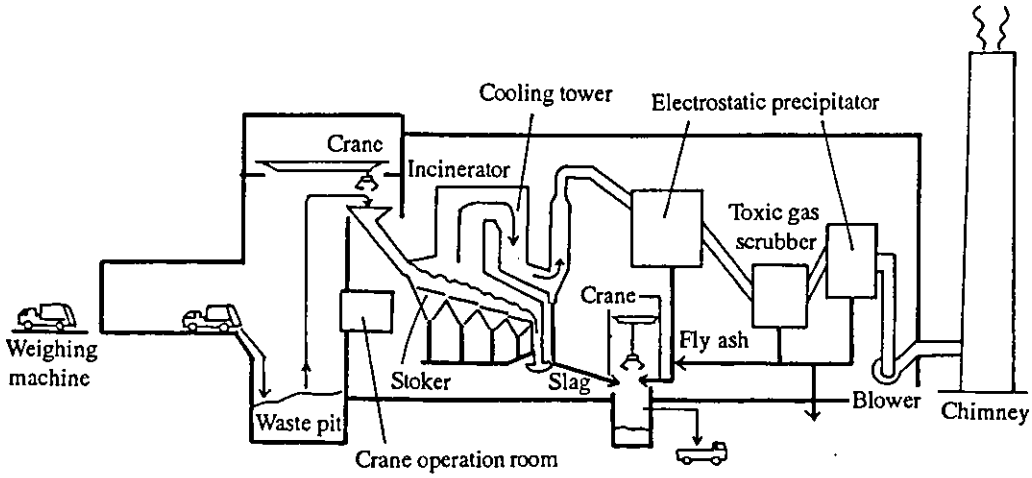

Fig. 1. Incineration process in a municipal waste incineration plant[13].

Incineration Process and Tasks of Incineration Workers

Incinerators are classified according to the incineration system, e.g., stoker, fluid bed and other types, and also according to their operation hours, e.g., full continuously burning type (24 hr), semi continuously burning type (16 hr) and batch type (8 hr).

Figure 1 shows an incinerator of the stoker type[13]. First, the collected waste is weighed and put into the waste pit. Next, the waste is transported into the incinerator by crane. In the incinerator, the waste is dried and burned while being conveyed to the bottom by the stoker. The combustion gas is passed through the cooling tower, electrostatic precipitator (EP) and toxic gas scrubber, and emitted from the chimney. Slag remaining at the bottom of the incinerator and fly ash collected by EP are transported into the ash pit. The slag and fly ash accumulated in the ash pit are loaded onto a truck and carried to reclaimed land. In this process, if the conditions of combustion and cooling are not appropriate, dioxins are synthesized in the incinerator, cooling tower and EP, and contaminate the slag, fly ash and exhaust gas. Recently, a bag filter (BF) has been substituted for EP at many incineration plants, because EP generates high levels of dioxins.

For the fluid bed type, hot air is blown into the incinerator from many holes at the bottom, and sand in the incinerator is heated and flung upward. Next, waste is transported into the incinerator and the waste is burned by contact with the hot sand. The combustion gas is passed through the cooling tower, EP and toxic gas scrubber, and emitted from the chimney. Because control of the burning condition is more difficult for the fluid bed type than for the stoker type, dioxins are more easily synthesized.

The median of dioxin concentrations in the exhaust gas of municipal waste incinerators is 1.0 ng TEQ/m^3 (= 1,000 pg TEQ/m^3) in 2000[11], which is about 8,000 times that in general air (0.13 pg TEQ/m^3) in 2001[14]. Medians of dioxin concentrations in the slag and fly ash are 0.02 and 3 ng TEQ/g (= 20 pg and 3,000 pg TEQ/g) in 1998–99[15], respectively, which are about 6 and 900 times that in general soil (3.2 pg TEQ/g, 2001)[14].

The tasks of incinerator workers are classified into "daily operation" and "periodic maintenance". The daily operation consists of weighing of waste, crane operation, incinerator operation, carrying slag and fly ash, and daily inspection and maintenance. The periodic maintenance is conducted several times in a year. First, the insides of the incinerator, cooling tower, EP and BF are cleaned by the incinerator workers or outside workers employed at maintenance companies. Next, these apparatuses are inspected and repaired mainly by the outside workers.

Estimated Dioxin Exposure Concentrations during Work Activities

It is difficult to directly measure dioxin exposure concentrations during work activities, because flow rate of personal sampler is too low to collect enough airborne dust to quantitatively determine dioxins. Thus, total dust concentrations in the breathing zone of incinerator workers were measured and the dioxin exposure concentrations were estimated by multiplying the total dust exposure concentrations by the dioxin concentrations in deposited dust,

Table 1. Total dust exposure concentrations and estimated dioxin exposure concentrations of incinerator workers (from Ref. 13)

	Plant	Sample number	Total dust exposure concentration (mg/m^3)		Estimated dioxin exposure concentration ($pgTEQ/m^3$)		Work duration (hr)
			Mean	Range	Mean	Range	
Daily operation	A, C	8	0.39	0.11–1.50	2.0	0.5–7.2	8
Periodic maintenance (cleaning the inside of equipment)							
1. Incinerator							
I. Removing clinker	A, C	6	55	30–97	29	9.2–48	1*
II. Removing clinker and slag	B	3	420	130–780	1.7	0.5–3.1	4*
2. Duct under stoker	A, C	6	82	12–170	36	13–70	1*
3. Cooling tower	A, B	5	210	53–420	48	0.2–110	1*
4. Electrostatic precipitator							
I. Removing fly ash deposited at discharge position	A	3	120	51–200	880	370–1500	0.5*
II. Removing fly ash by compressed air	B	2	1800	1500–2000	81000	71000–92000	1*

*: Work duration during one periodic maintenance.

fly ash and slag[13].

Table 1 shows the total dust exposure concentrations and the estimated dioxin exposure concentrations. In daily operations, the total dust exposure concentrations were 0.11 to 1.50 mg/m^3 with a mean of 0.39 mg/m^3. Using the dioxin concentrations in dust deposited in the workplace, the dioxin exposure concentrations were estimated to be 0.5 to 7.2 pg TEQ/m^3 with a mean of 2.0 pg TEQ/m^3 (Table 1). The Ministry of Health, Labor and Welfare, Japan, recommends an administrative level of 2.5 pg TEQ/m^3 for airborne dioxins in workplace[16]. The above estimation of dioxin exposure concentration suggests that though the mean value is less than the administrative level, the maximum value exceeded the administrative level even in the daily operation. Consequently, in such cases, dust emission must be reduced in the workplace and/or protective respirators and clothing should be worn.

For the periodic maintenance, two methods of cleaning the inside of the incinerator and EP were found in the above study. At plants A and C, the workers removed glassy lumps (clinker) adhering to the wall with a stick in the incinerator and removed fly ash adhering to the discharge hole in the EP (method I). At plant B, the workers removed clinker and all slag remaining on the stoker by shovel in the incinerator and removed all fly ash adhering to the wall using compressed air in the EP (method II). The inside of incinerator and EP was extremely dusty with slag and fly ash during the cleaning process by method II, and the mean values of total dust exposure concentrations were 420 mg/m^3 in the incinerator and 1800 mg/m^3 in the EP. On the other hand, with method I, the mean values of total dust exposure concentrations were 55 mg/m^3 in the incinerator and 120 mg/m^3 in the EP, which were still high but about 1/10 those of method II.

By multiplying the total dust exposure concentrations by the dioxin concentrations in slag (0.004 to 1.1 ng TEQ/g), the dioxin exposure concentrations during cleaning of the inside of the incinerator were estimated to be 0.5 to 48 pg TEQ/m^3. By multiplying the total dust exposure concentrations by the dioxin concentrations in fly ash (7.3 to 64 ng TEQ/g), the dioxin exposure concentrations during cleaning of the inside of the EP were estimated to be 370 to 92,000 pg TEQ/m^3, which were 150 to 37,000 times the administrative level. Consequently, highly efficient protective respirators and clothing should be worn during equipment cleaning procedures.

Estimated Daily Dioxin Intake

How much dioxin does an incinerator worker take into the body? Daily dioxin intake was estimated for the cases of fly ash of 1, 10, 100 and 1000 ng TEQ/g based on the following assumptions[13]. It is assumed that the body weight is 60 kg and the respiratory ventilation is 1 m^3/hr. In one year, there are 250 working days and the periodic maintenance is done 4 times. Total dust exposure concentration during each work is assumed to be the same as the mean value shown in Table 1. The worker inhales the same dust as the deposited dust during the daily operation, the slag while cleaning the inside of the incinerator and cooling tower, and the fly ash while cleaning the inside of the EP. Dioxin concentrations in the slag and in the deposited dust are 1/100 and 1/10 times, respectively, that in the fly ash[13]. Environmental intake, such as through meals and the air, is

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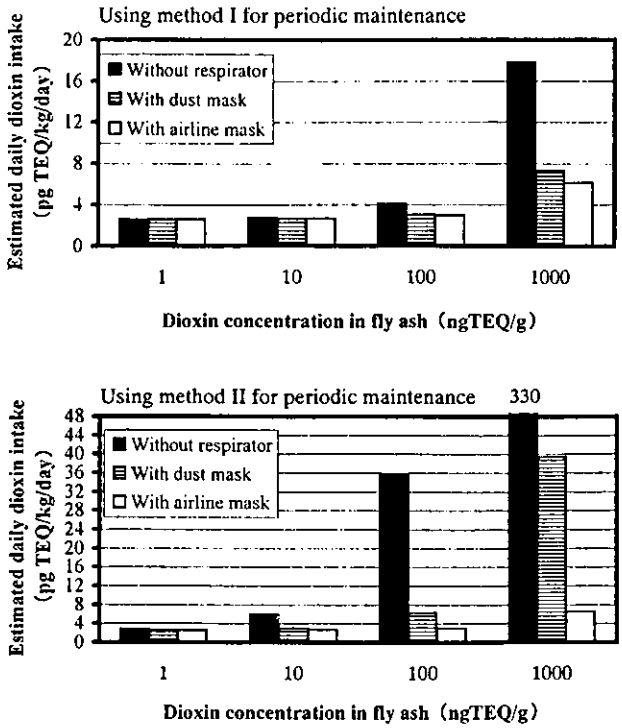

Fig. 2. Estimated daily dioxin intake in workers employed at continuously burning incineration plants.
The graphs are based on data in Reference 13.

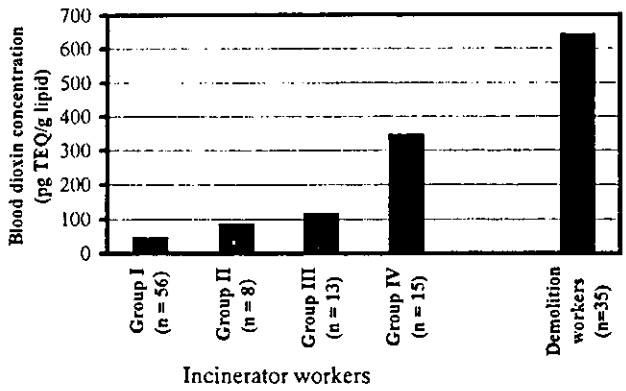

Fig. 3. Blood dioxin concentrations in incinerator workers and demolition workers at the Toyono-gun incineration plant.
Blood dioxin concentration = PCDDs + PCDFs + CoPCBs. Group I: workers who had not work in the incineration building and not handled slug and fly ash, Group II: workers who had not worked in the incineration building but handled the solidified ash, Group III: workers who had worked in the incineration building, Group IV: workers who had worked in the incineration building as well as engaged in maintenance of the incinerator. This graph is based on References 21 and 22.

2.60 pg TEQ/kg/day, as reported by the National Environmental Council[17]. The protection factors of dust masks and airline masks are 10 and 1000, respectively[18].

Figure 2 shows results of the estimation[13]. With method I, the estimated dioxin intakes for the fly ash of 1 and 10 ng TEQ/g are less than the tolerable daily intake (TDI: 4 pg TEQ/kg/day) recommended by the National Environmental Council[19], but the intake for the fly ash of 100 ng TEQ/g exceeds TDI if protective respirator is not worn during the cleaning of the inside of incinerator, and the intake for the fly ash of 1000 ng TEQ/g exceeds TDI even if an airline mask is worn. With method II, the estimated dioxin intake for the fly ash of 1 ng TEQ/g is less than TDI, but the intake for the fly ash of 10 ng TEQ/g exceeds TDI if protective respirator is not worn, the intake for the fly ash of 100 ng TEQ/g exceeds TDI even if a dust mask is worn, and the intake for the fly ash of 1000 ng TEQ/g exceeds TDI even if an airline mask is worn. In the case of the fly ash of 1000 ng TEQ/g, because the airborne dust in the incineration plants must be highly contaminated, dioxin intake during daily operation can be high even if an airline mask is worn only during the cleaning of the inside of incinerator. In a report of the former Ministry of Welfare[20], dioxin concentrations in fly ash ranged from 0.01 to 240 ng TEQ/g for continuously burning incineration plants to which the old dioxin guideline was not applicable, and ranged from 0.00 to 24 ng TEQ/g for the plants to which the guideline was applicable. Thus, in incineration plants with fly ash of high dioxin concentration, the daily dioxin intake of the workers can exceed TDI.

Blood Dioxin Level in Incinerator Workers

Figure 3 shows blood dioxin concentrations in workers employed at the Toyono-gun incineration plant[21]. The blood dioxin concentration in workers who had not work in the incineration building and not handled slug and fly ash (group I) was 46 pg TEQ/g lipid on the average, which was almost the same level as that of the general population. The blood dioxin concentration in workers who had not worked in the incineration building but handled the solidified ash (group II) and that in workers who had worked in the incineration building (group III) were 85 and 115 pg TEQ/g lipid, respectively, which were higher than the general level. The blood dioxin concentration in workers who had worked in the incineration building as well as engaged in maintenance of the incinerator (group IV) was 346 pg TEQ/g lipid, which was about ten times the general level.

In the plant, dioxin concentration in the fly ash remaining in the EP was 320 ng TEQ/g and that in the ash solidifying machine was 1,500 ng TEQ/g. This finding suggests that

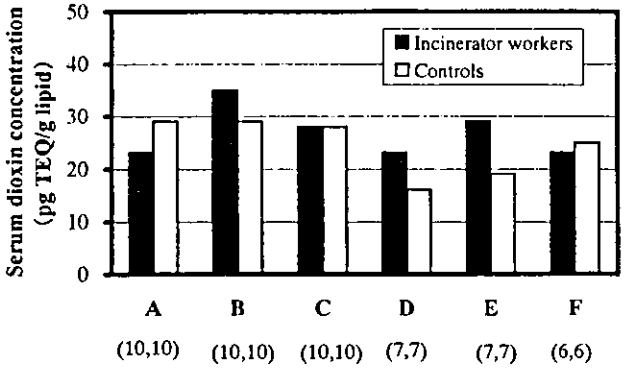

Fig. 4. Serum dioxin concentrations in incinerator workers and controls in six cities.

Serum dioxin concentration = PCDDs + PCDFs. ( ): Number of subjects. This graph is based on References 24 and 25.

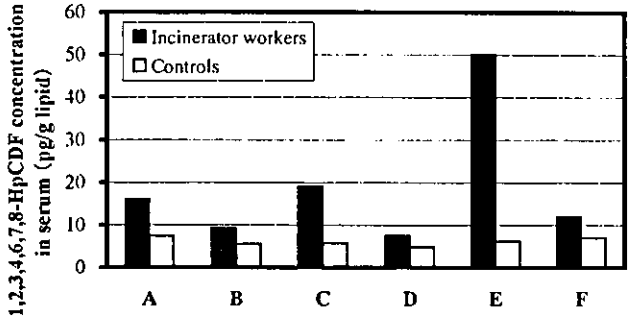

Fig. 5. 1,2,3,4,6,7,8-Heptachlorodibenzofuran concentrations in serum in incinerator workers and controls.

This graph is based on References 24 and 25.

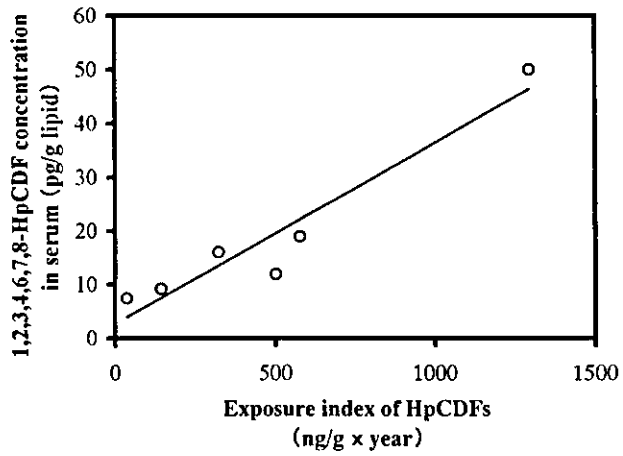

Fig. 6. Relationship between exposure index and serum concentration.

the daily dioxin intake in the workers can exceed TDI based on the above estimation. Dioxin concentration in the dust deposited at the bottom of the toxic gas scrubber and that of the chimney were 96,000 and 120,000 ng TEQ/g, respectively, which were much higher than that in the fly ash. These extremely high levels were due to accumulation of dioxins in alkaline liquid circulating in the toxic gas scrubber. This finding suggests that attention should be paid to dust other than fly ash.

The Toyono-gun incineration plant was demolished in 2000. The workers wore airline masks inside the equipment and dust masks outside it. They also wore protective clothing. However, the blood dioxin concentrations increased to 680 pg TEQ/g lipid on the average at the end of the work (Fig. 3)[22]. In order to clarify why the blood dioxin level increased, heating tests of the dust remaining inside the equipment was conducted[23]. The results showed that when the workers cut the parts of the equipment with a gas burner, dioxins in the dust adhering to the parts were vaporized and the workers inhaled the vapor passing through the dust mask. This means that adhering dust should be removed before heating the parts and workers should wear respirators that shut out vapor.

In 1998–2000, we measured serum dioxin concentrations of 50 workers at six municipal waste incineration plants[24,25]. For these plants, the dioxin concentrations in the exhaust gas were 0.072 to 590 ng TEQ/m^3, which were representative of Japanese municipal waste incineration plants. For comparison, 50 controls matched for age (± 5 years) were selected from the general population. The mean serum dioxin concentrations in the incinerator workers at each plant ranged from 23 to 35 pg TEQ/g lipid (PCDDs + PCDFs), while that in the controls ranged from 16 to 29 (Fig. 4). This finding suggests that the dioxin level in the incinerator workers was almost the same as that in the controls.

However, significant increases in the serum 1,2,3,4,6,7,8-HpCDF concentration were found at all six plants (Fig. 5). Figure 6 shows the relationship between the occupational exposure index and serum concentration, where the occupational exposure index was defined as the product of the employment duration at the incineration plant and the 1,2,3,4,6,7,8-HpCDF level in the deposited dust. As the occupational exposure index increases, the serum concentration also increases. This finding suggests that the incinerator workers took dioxins into their bodies while they were working.

The Ministry of Health, Labor and Welfare also carried out blood dioxin measurements of 298 workers at 26 municipal waste incineration plants in 1999–2001[26–28]. For these plants, dioxin concentrations in the exhaust gas were

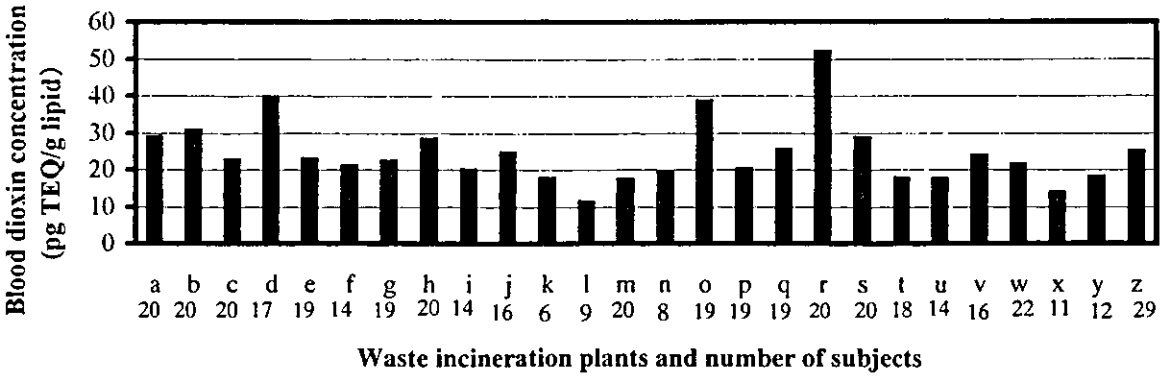

Fig. 7. Mean blood dioxin concentrations in workers employed at 26 waste incineration plants.
Blood dioxin concentration = PCDDs + PCDFs + Co-PCBs. This figure graph is based on References 26, 27 and 28.

0.035 to 450 ng TEQ/m^3. The mean blood dioxin concentration at each plant ranged from 11 to 40 pg TEQ/g lipid (Fig. 7, PCDDs + PCDFs + Co-PCBs). The mean values were similar to the general level, but the individual values were higher than 100 pg TEQ/g lipid in some cases, where dioxins were taken into the workers' bodies while they were working.

Strategy for Future Research

The number of municipal waste incineration plants where blood dioxin measurements have been carried out is probably less than 100. Except for the Toyono-gun plant, there has been no observation of very high blood dioxin levels. However, as there are about 1,500 municipal waste incineration plants in operation in Japan, this number is too small to conclude that the dioxin body burden of incinerator workers is not high, so that further research is necessary. But the cost of blood dioxin measurement is very high and the number of laboratories that can do blood measurements is small.

Under such circumstances, let us consider what can be done. As stated above, because the serum HpCDF level is correlated to the occupational exposure index, the serum level can be estimated from the HpCDF concentration in deposited dust and employment duration. There are many laboratories that can conduct dust measurements and the cost is lower than for blood measurements. Consequently, measurement of dioxin concentration in the deposited dust can be conducted in all municipal waste incineration plants of Japan in the first year. Based on the dust measurement results, plants needing further inspection can be identified, and the blood dioxin concentrations in the workers can be measured in the next year.

For our study at the six plants, there was no correlation of TEQ values between the serum and the exposure index, probably because the exposure index was low. If a similar study can be carried out for plants with high TEQ value and a correlation is found for TEQ values between the serum and the exposure index, our proposed strategy will be more efficient.

Guidelines for Control of Dioxin Exposure

In April 2001, the Ministry of Health, Labor and Welfare issued guidelines for controlling dioxin exposure at waste incineration plants[16]. The guidelines prescribe the election of work leader, education of workers on occupational health, establishment of measures against dioxin generation, direction of workers to use respiratory and skin protection, and control of workers' health. The workplace should be classified as control area I, II or III based on the airborne dioxin level measured every six months, and the working environment and work practices should be controlled to decrease dioxin exposure of the workers. Periodic general health examinations and special care of workers with anxiety about their health are also necessary. If there is the possibility of a high dioxin intake by the workers, such as in an accident, specific medical examinations should be done and the blood dioxin level should be measured if necessary.

The guidelines also include prescriptions for demolishing an incineration plant. The dioxin concentration in the dust adhering to the inside of the equipment and the airborne dioxin concentration in the workplace should be measured before the demolition work begins. Based on these measurements, the workplace will be classified as control area I, II or III, and the demolition method and grade of respiratory and skin protection are determined. For example,

if the dioxin concentration in the adhering dust is 4.5 ng TEQ/g or more, the workplace is classified as control area III, and portable electric tools and hydraulic cutters can be used, but not gas burners. When workers always handle highly contaminated dust (> 3.0 ng TEQ/g), they should wear air-supply respirators, airtight clothing and gloves for handling chemicals. If the work environment management and work practice are kept in good control according to the guidelines, the dioxin exposure of workers can be decreased considerably.

Exposure Evaluation of Dioxins for Outside Workers

This paper has focused on the dioxin exposure of workers employed at municipal waste incineration plants. Because for many municipal waste incineration plants, work with high dioxin exposure, such as cleaning and repairing the inside of equipment, is performed by the outside workers employed at maintenance companies, evaluation of their dioxin exposure is necessary. In the future, demolition will increase at old incinerators to which the Law Concerning Special Measures against Dioxins[29] is not applicable. Consequently, the evaluation of dioxin exposure for the demolition workers will also be necessary.

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Time-trend (1973–2000) of polybrominated diphenyl ethers in Japanese mother's milk

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Abstract

The time-trend and recent concentrations of polybrominated diphenyl ethers (PBDEs) in Japanese mother's milk were investigated. The time-trend of 16 PBDEs (BDE-28, 37, 47, 66, 71, 75, 77, 85, 99, 100, 119, 153, 154, 138, 183, and 190) in pooled milk samples from mothers living in Osaka between 1973 and 2000 was analyzed. Additionally, PBDE concentrations in individual milk samples collected from 13 mothers living in Kanagawa and Okayama in 1999 were measured. The total concentration of all PBDE congeners ($\sum$PBDEs) measured in the pooled samples increased during the period between 1973 (<0.01 ng/g lipid) and 1988 (1.64 ng/g lipid), and remained low afterwards while showing remarkable changes in PBDE congener profiles. The $\sum$PBDEs in the 1999 individual milk samples were also low (0.56–3.97 ng/g lipid), except for a single sample (291 ng/g lipid). The source of this exposure could not be identified.

Keywords: Flame retardant; GC/MS; Human milk

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are flame retardants used in polymeric materials for fire safety of furniture, textiles and electronics (IPCS, 1994). The total production of the three major commercial PBDE products (i.e., deca-BDE, octa-BDE, and penta-BDE) in 1999 is reported to be 54 800, 3825, and 8500 metric tons, respectively (BSEF, 2000). Several penta-BDE components (BDE-47, 99, 153, etc.) have high potential for bioaccumulation/biomagnification (Holm et al., 1993; Gustafsson et al., 1999; Burreau et al., 2000a,b; Hale et al., 2002), and these components have been detected in various biological samples obtained from freshwater/marine fishes (Andersson and Blomkvist, 1981; Watanabe et al., 1987; de Boer, 1989; Loganathan et al., 1995; Asplund et al., 1999; Akutsu et al., 2001; Manchester-Neesvig et al., 2001; Hale et al., 2001a), from aquatic/terrestrial animals (Sellström et al., 1993; Haglund et al., 1997; de Boer et al., 1998; Lindström et al., 1999; Law et al., 2002; She et al., 2002), and even from humans (Lindström et al., 1997; Meironyté et al., 1999, 2001a,b; Meneses et al., 1999; Sjödin et al., 1999; Ohta et al., 2000, 2002; She et al., 2002). In recent years, the flame retardant penta-BDE has been phased out in the European Community following the results of the EU scientific risk assessment for penta-BDE (European Chemicals Bureau, 2000). In addition, the voluntary discontinuance of production and import of PBDEs other than octa-BDE and deca-BDE were pledged by Japanese companies in 1995 (Asahi Glass Co., Ltd., et al.,

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1995). However, serious penta-BDE problems are now arising in North America where 98% of the global use (8290 tons) of penta-BDE in 1999 is reported (BSEF, 2000). Surprisingly high concentrations of penta-BDE components have recently been found in North American biota; e.g., salmonids in Lake Michigan (Manchester-Neesvig et al., 2001), freshwater fishes in Virginia (Hale et al., 2001a), and harbor seal blubber and also human breast adipose tissue samples of residents in the San Francisco Bay area (She et al., 2002). In addition, Hale et al. report that relatively high levels of penta-BDE (1100–2290 μg/kg dry weight) in "biosolid" fertilizers (recycled sewage sludges) are land-applied on US farms (Hale et al., 2001b). It has been suggested that the possible sources of these penta-BDE contaminations are discarded penta-BDE treated materials (e.g., flame-retarded polyurethane form) as well as direct vapor emission/wastewater from the related industries (Hale et al., 2001a,b, 2002).

PBDEs are suspected to be transferred via placenta and breast milk from the mother to the offspring in mammals (Kuehl and Haebler, 1995; Strandman et al., 2000; Law et al., 2002; She et al., 2002), and the available data suggest that some of these congeners are potential thyroid disruptors and developmental neurotoxicants (Fowles et al., 1994; Eriksson et al., 1999, 2001; Meerts et al., 2000; Hallgren et al., 2001; Zhou et al., 2001). Other endocrine and genetic effects have also been observed (Carlson, 1980a,b; von Meyerinck et al., 1990; Fowles et al., 1994; Helleday et al., 1999; Chen et al., 2001; Meerts et al., 2001). Therefore PBDE levels in mothers are of great concern relating to the healthy growth of fetuses and infants. Meironyté et al. report that the levels of PBDEs found in Swedish mothers' milk increased continuously during the period between 1972 and 1997, while other organochlorine contaminants decrease (Meironyté et al., 1999). In Japan, it has been reported that approximately a total of 0.1 million metric tons of technical PBDE products (mainly deca-BDE) were used during the 1986–2000 period (The Chemical Daily Co., 1987–2001). However, there are only a few studies on the levels of PBDEs in Japanese human samples (Nagayama et al., 2000; Ohta et al., 2000, 2002), and virtually no time-trend data are available. The purpose of this pilot study was to investigate the time-trend of PBDE concentrations in Japanese mothers' milk.

2. Materials and methods

Table 1 shows the PBDE congeners monitored in this study. The non-labeled and ^{13}C-labeled PBDE standards were purchased from Cambridge Isotope Laboratories (MA, USA) or Wellington Laboratories (Ontario, Canada).

Table 1
PBDE congeners and their monitor ions used in this study

Class	Congener	Substitution	Abbreviation	Monitor ions (*m/z*)		Ion ratio[a]	Reference CS[b]
Native	TrBDE	2,4,4'-	BDE-28	405.8027	407.8006	1:0.97	^{13}C-BDE-28
		3,4,4'-	BDE-37				
	TeBDE	2,4,4',6-	BDE-75	485.7111	483.7132	1:0.69	^{13}C-BDE-47
		2,3',4',6-	BDE-71				
		2,2',4,4'-	BDE-47				
		2,3',4,4'-	BDE-66				
		3,3',4,4'-	BDE-77				
	PeBDE	2,2',4,4',6-	BDE-100	563.6216	565.6196	1:0.97	^{13}C-BDE-99
		2,3',4,4',6-	BDE-119				
		2,2',4,4',5-	BDE-99				
		2,2',3,4,4'-	BDE-85				
	HxBDE	2,2',4,4',5,6'-	BDE-154	643.5301	641.5321	1:0.77	^{13}C-BDE-154
		2,2',4,4',5,5'-	BDE-153				^{13}C-BDE-153
		2,2',3,4,4',5-	BDE-138				^{13}C-BDE-154
	HpBDE	2,2',3,4,4',5',6-	BDE-183	561.6060	563.6039	1:0.97	^{13}C-BDE-154
		2,3,3',4,4',5,6-	BDE-190				
^{13}C-labeled	TrBDE	2,4,4'-	^{13}C-BDE-28	417.8429	419.8409	1:0.97	–
	TeBDE	2,2',4,4'-	^{13}C-BDE-47	497.7514	495.7534	1:0.69	–
		3,3',4,4'-	^{13}C-BDE-77				–
	PeBDE	2,2',4,4',5-	^{13}C-BDE-99	575.6619	577.6598	1:0.97	–
	HxBDE	2,2',4,4',5,6'-	^{13}C-BDE-154	655.5704	653.5724	1:0.77	–
		2,2',4,4',5,5'-	^{13}C-BDE-153				

[a] Calculated theoretical ratio from the relative natural abundance of bromine isotopes (^{79}Br, 50.69%; ^{81}Br, 49.31%).
[b] Cleanup standard referenced for recovery correct of each congener groups. Listed PBDEs are arranged in elution order from a GC column (J&W DB-1).

Table 2
Milk sample information

Sample ID	Location	Year	Type[a] P/I (n)	Age range (average)	% Lipid range (average)	Frequency of parturition
OS1973	Osaka	1973	P (21)	25–29 (26.3)	NA[b]	1
OS1978	Osaka	1978	P (21)	25–29 (26.4)	NA	1
OS1983	Osaka	1983	P (19)	25–29 (26.7)	NA	1
OS1988	Osaka	1988	P (24)	25–29 (26.5)	NA	1
OS1993	Osaka	1993	P (30)	25–29 (27.2)	1.57–6.20 (3.86)	1
OS1998	Osaka	1998	P (35)	25–29 (27.2)	1.70–7.78 (3.94)	1
OS1999	Osaka	1999	P (30)	25–29 (27.1)	1.32–6.01 (3.68)	1
OS2000	Osaka	2000	P (27)	25–29 (27.2)	2.48–6.22 (3.89)	1
KA99-1	Kanagawa	1999	I	24	2.37	1
KA99-2	Kanagawa	1999	I	39	1.98	2
KA99-3	Kanagawa	1999	I	38	4.02	3
KA99-4	Kanagawa	1999	I	31	3.50	2
KA99-5	Kanagawa	1999	I	31	2.44	2
KA99-6	Kanagawa	1999	I	33	3.59	3
KA99-7	Kanagawa	1999	I	37	2.63	2
KA99-8	Kanagawa	1999	I	23	3.30	1
KA99-9	Kanagawa	1999	I	35	3.02	4
KA99-10	Kanagawa	1999	I	32	2.43	2
OK99-1	Okayama	1999	I	NA	2.24	1
OK99-2	Okayama	1999	I	NA	3.07	1
OK99-3	Okayama	1999	I	NA	3.83	1

[a] Pooled (P) or individual (I), number of sample donars in each pool is shown in parenthesis.
[b] NA: not available.

Organic solvents used for extraction and cleanup of samples were of pesticide analysis grade and were purchased from Wako Pure Chemicals (Osaka, Japan). Silica (Kieselgel 60 Art. 7734) of column chromatography grade was purchased from Merck (Darmstadt, Germany), and chemical-impregnated silica (10% $AgNO_3$, 22% H_2SO_4, 44% H_2SO_4, and 2% KOH) of dioxin analysis grade from Wako Pure Chemicals. Water was deionized and purified using a Milli-Q cartridge system (Millipore, MA, USA) and then washed with *n*-hexane.

GC/MS analyses were performed on a HP5890 series II gas chromatograph (Hewlett-Packard, CA, USA) coupled to a JMS-700 (JOEL, Tokyo, Japan). The GC conditions were as follows: column, DB-1 (J&W Scientific, CA, USA) 15 m, 0.25 mm i.d., 0.25 μm film thickness; column temperature program, 140 °C (held for 2 min) to 180 °C at 10 °C/min, 180–220 °C at 3 °C/min, and then 220–325 °C (held for 5 min) at 10 °C/min; carrier gas, helium; column head pressure, 10 psi; injection temperature, 275 °C; injection volume, 2 μl using the splitless injection mode (splitless time, 1.5 min). All target PBDEs were eluted from the column within 30 min under the above-mentioned conditions. The MS conditions were as follows: detection mode, electron ionization mode (selected ion monitoring mode, monitoring ions of each target congener are shown in Table 1); electron energy, 38 eV; filament current, 600 μA; ion source temperature, 270 °C; resolution, 10000 (10% valley definition).

Information about the samples is shown in Table 2. All the sample donors were healthy women and informed consent was obtained from each of them. For the time-trend of PBDE concentrations the pooled milk lipid samples collected in the years of 1973, 1978, 1983, 1988, 1993, 1998, 1999, and 2000 from mothers living in Osaka were analyzed. The volunteers (19–35 mothers per sampling year) were between 25 and 29 years old, and the breast milk was collected within the period of 30 and 90 days from their first parturition. Lipid was quantitatively extracted from each milk sample with a mixture of potassium oxalate, ethanol, diethyl ether, and hexane according to the official method for analysis of PCB in mother's milk (The Ministry of Health and Welfare of Japan, 1972). The milk lipid samples were pooled per each sampling year by mixing the individual lipids equally by weight, and were kept at −20 °C until cleanup. Additionally, 10 individual milk samples were collected at Tokai University Hospital, Kanagawa, within 30 days from parturition in 1999. A part of these individual 10 milk samples had previously been analyzed for several organochlorine contaminants. The concentrations of these organochlorine contaminants in each sample were close to the average levels reported in Japan (Konishi et al., 2001): total PCB concentrations in these samples ranged from 42 to 192 ng/g lipid (Hori et al., 2001). Additional three milk samples were collected from mothers living in Okayama within the period of 30–90 days from parturition in 1999. Lipids from these