

13 individual samples were extracted as described above and were kept at -20°C until cleanup. For comparison of PBDE congener profiles with a popular seafood, a lipid sample from pooled fillet of seven horse mackerels at the average length of 27 cm used in a previous study (Akutsu et al., 2001) was also analyzed in the manner described below.

In brief, extracted lipid (0.5 g) was spiked with cleanup standard mixtures (^{13}C -labeled PBDEs, #28, #47, #99, #153, #154; 0.5 ng each). The lipid was saponified with alcoholic potassium hydroxide solution under room temperature and PBDEs were extracted with *n*-hexane. Further cleanup was performed on a pre-washed multilayer silica column packed vertically from bottom to top with 2% KOH-silica (1 g), neutral silica (0.6 g), 44% and 22% H_2SO_4 -silica (2 and 1 g), neutral silica (0.6 g), 10% AgNO_3 -silica (1 g), neutral silica (0.6 g), and Na_2SO_4 (3–4 g), using 200 ml of hexane as an eluting solvent. The eluate was evaporated to near dryness and spiked with ^{13}C -labeled BDE-77 in *n*-nonane as a recovery standard. Each PBDE congener in the sample was identified by comparing the GC retention time and ion-abundance ratio of two exact *m/z*'s with the corresponding retention time of an authentic standard and the theoretical ion-abundance ratio of the two exact *m/z*'s, and was measured based on relative response factors from the corresponding cleanup standards listed in Table 1.

3. Results and discussion

In our previous study, we applied a fast cleanup method (automated gel permeation chromatography and mini-column chromatography) and GC/low resolution MS with negative chemical ionization for screening analysis of PBDEs in marine fish (Akutsu et al., 2001). The method showed acceptable performance in the first international interlaboratory study concerning the analysis of PBDEs in eel, porpoise, cormorant, mussel, and sediment samples (de Boer, 2000, 2002). However, in the present study, for the purpose of performing more accurate PBDE identification/quantification, a conventional destructive cleanup method and GC/high resolution MS with electron ionization were applied in the PBDE analysis of the human milk samples. In the present study, the recoveries of the respective cleanup standards for the 21 milk and one fish samples were almost within the acceptable range (60–120%).

Table 3 shows the concentrations of PBDE congeners in the 21 milk samples (8 pooled and 13 individual samples). Firstly, as shown in Fig. 1, the sum of all PBDE congener concentration ($\sum\text{PBDEs}$) in the pooled samples continuously increased during the period between 1973 (all of the individual PBDE concentrations were below the detection limit of 0.01 ng/g lipid) and 1988 (1.6 ng/g lipid). After a decrease at the beginning of the 1990s, the concentration of $\sum\text{PBDEs}$ seemed to

Table 3
Concentrations of PBDEs in human milk samples (ng/g lipid)

Sample ID	Congener														$\sum\text{PBDEs}$
	#28	#37	#75	#47	#66	#77	#100	#99	#85	#154	#153	#138	#183		
OS1973	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	–
OS1978	0.07	ND	ND	0.03	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.10
OS1983	0.15	0.01	ND	0.26	0.02	ND	0.02	0.04	ND	0.01	0.07	ND	0.01	0.01	0.59
OS1988	0.47	0.09	ND	0.67	0.06	ND	0.05	0.08	ND	0.02	0.18	ND	0.02	0.02	1.64
OS1993	0.17	0.02	ND	0.32	0.02	ND	0.07	0.06	ND	0.03	0.21	ND	0.06	0.06	0.96
OS1998	0.10	0.01	ND	1.03	0.02	ND	0.22	0.53	0.04	0.05	0.29	ND	0.02	0.02	2.31
OS1999	0.11	0.01	ND	0.62	0.02	ND	0.18	0.16	0.01	0.03	0.29	ND	0.02	0.02	1.45
OS2000	0.09	0.01	ND	0.53	0.02	ND	0.17	0.15	0.01	0.03	0.34	ND	0.04	0.04	1.39
KA99-1	0.09	ND	ND	0.57	0.02	ND	0.17	0.16	0.01	0.03	0.43	ND	0.09	0.09	1.57
KA99-2	0.30	0.04	ND	0.37	ND	ND	0.11	0.12	ND	0.02	0.31	ND	0.09	0.09	1.36
KA99-3	0.28	0.02	ND	2.15	0.02	ND	0.28	0.39	0.03	0.03	0.27	ND	0.04	0.04	3.51
KA99-4	0.03	ND	ND	0.30	ND	ND	0.08	0.14	ND	0.02	0.17	ND	0.04	0.04	0.78
KA99-5	0.14	0.02	ND	0.52	0.01	ND	0.10	0.11	ND	0.02	0.35	ND	0.06	0.06	1.33
KA99-6	0.18	0.02	ND	0.82	0.06	ND	0.21	0.23	ND	0.06	0.26	ND	0.08	0.08	1.92
KA99-7	0.05	ND	ND	0.20	ND	ND	0.05	0.06	ND	0.01	0.16	ND	0.03	0.03	0.56
KA99-8	37.7	1.10	0.49	187	3.24	0.11	27.2	21.1	2.05	1.33	10.2	0.22	0.08	0.08	291
KA99-9	0.23	0.03	ND	2.25	0.04	ND	0.38	0.45	0.05	0.06	0.40	ND	0.08	0.08	3.97
KA99-10	0.12	ND	ND	0.58	ND	ND	0.10	0.11	ND	0.02	0.14	ND	0.03	0.03	1.10
OK99-1	0.22	ND	ND	1.11	0.02	ND	0.12	0.14	ND	0.03	0.28	ND	0.03	0.03	1.95
OK99-2	0.14	0.01	ND	1.06	0.05	ND	0.21	0.55	0.04	0.07	0.33	0.02	0.05	0.05	2.53
OK99-3	0.03	ND	ND	0.27	ND	ND	0.07	0.08	ND	0.02	0.24	ND	0.02	0.02	0.73

ND: not detected (<0.01 ng/g lipid).

Not shown congeners (BDE-71, 119, and 190) were each below 0.01 ng/g lipid in all samples.

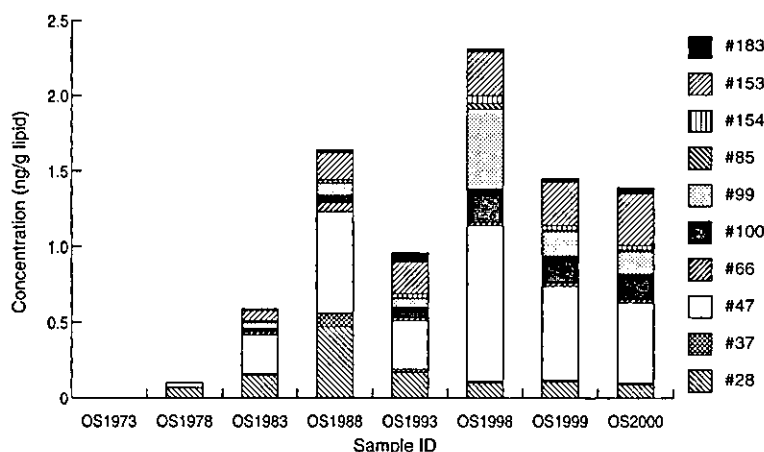


Fig. 1. Time-trend of PBDE concentrations in pooled human milk samples collected from mothers living in Osaka between 1973 and 2000.

increase again and began leveling off. The levels and time-trend of \sum PBDEs in Japanese mothers' milk were not remarkably different from those in Swedish mothers' milk (Meironyté et al., 1999). However, unlike the Swedish study, our study revealed that the contribution of BDE-28 and 153 during the 1983–1993 period was obviously higher than that of BDE-99 (a main component of penta-BDE): 18–29% for BDE-28, 11–22% for BDE-153, and 5–7% for BDE-99. These characteristic congener profiles would partly be explained by past use of tetra-BDE and hexa-BDE in Japan. Both tetra-BDE and hexa-BDE are peculiar products used in Japan (The Chemical Daily Co., 1987–2001; IPCS, 1994; Nishizawa, 1987), and to our knowledge, there is no report on production or use of these products in other countries. Tetra-BDE is reported to be used in Japan until 1990, and the annual demand of it is 1000 metric tons/year for the period between 1986 and 1990 (The Chemical Daily Co., 1987–2001, data before 1985 are not reported). Unfortunately, the chemical composition of tetra-BDE used in Japan is unknown. Tetra-BDE is supposed to be mainly composed by Tr-TeBDEs, especially para- (4- and/or 4'-) and ortho- (2- and/or 2'-) substituted congeners such as BDE-47 (2,2',4,4'-) and BDE-28 (2,4,4'-), based on the known composition of a commercial penta-BDE (Sjödén et al., 1998). Obviously, further retrospective researches are necessary to clarify the details of tetra-BDE (manufacturer, trade name, composition, etc.) used in Japan, and it is important to investigate whether the classification of tetra-BDE reported in the past research is scientifically correct or not. On the other hand, there is a clear evidence for past use of hexa-BDE because "hexa-bromodiphenyl ether (Ref. No. 3-2845)" has been registered in "The Existing Chemical List for the Law Concerning the Examination and Regulation of Manufacture, etc. of Chemical Substances" (The Japa-

nese Government, 1973). The chemical substances on this list were confirmed to be industrially manufactured in, or imported to, Japan at the time of 1973. It has been suggested that a commercial hexa-BDE product (Planelon HB-60P by Mitsui Toatsu Fine Chemicals) consists of a single major hexa-brominated congener and other minor congeners (Ogino et al., 1987). We obtained a bulk substance of technical hexa-BDE (bromine content, 73%; mp, 155–157 °C; trade name, uncertain) manufactured by a Japanese company before 1991. In the preliminary capillary GC/MS analysis, one major peak (>90% contribution) and three minor peaks were found. The major peak was identified as BDE-153, and the three minor peaks as BDE-183, 154, and 99, respectively. Unfortunately, statistical data on past demands for hexa-BDE or penta-BDE in Japan are not available. There is the only limited evidence that some commercial hexa-BDE and penta-BDE were produced/distributed from Japanese companies in 1980s (Nishizawa, 1987). According to the voluntary commitment by nine Japanese companies with global operation (Asahi Glass Co., Ltd., et al., 1995), any PBDE products besides octa-BDE and deca-BDE are supposed to have not been used as flame retardants in Japan since 1995. The commercial production, and use of hexa-BDE and penta-BDE have been probably discontinued in Japan since early 1990s as in the case of tetra-BDE which was cancelled in 1991. In fact, Ohta et al. report that the concentrations of PBDEs (Tr-HxBDEs) in the stocked fish samples obtained from Osaka Bay remarkably decreased between 1989 and 1999; i.e., from 449 to 23.2 ng/g fat in Japanese sea bass (Ohta et al., 2001).

In the Swedish study, PBDE congener profiles in human milk samples are relatively constant through the period between 1972 and 1997 (Meironyté et al., 1999). On the other hand, the congener profiles in the milk

samples collected from mothers living in Osaka changed dramatically from 1988 to 1998; i.e., the concentrations and contributions of BDE-28, 37, and 66 decreased (contributions; 29–4%, 5–0.4%, and 4–0.9%, respectively), while those of BDE-99 and 100 increased (contributions; 5–23% and 3–10%, respectively). Additionally, the concentrations and contributions of BDE-47 once decreased from 1988 to 1993, and then increased (Fig. 1). The decrease of the concentrations of Tr-TeBDEs (BDE-28, 37, 47, 66) from 1988 to 1993 could be explained by the abandonment of the use of tetra-BDE in Japan in 1991. However, the increase of the concentrations of BDE-47, 99, and 100, which were predominant components of penta-BDE, from 1993 to 1998 seemed difficult to be explained. The increase might be related to delayed leakage/disperse of these congeners from the flame-retarded materials in Japanese environment. The upward trend of the intake of foreign foods among Japanese is considerable. The calculated import dependence ratios of several food items (e.g., fishery products, meats, and oils) gradually increase in the last quarter century in Japan (The Ministry of Agriculture, Forestry and Fisheries of Japan, 2001). For example, calculated import dependence ratios of fishery products except feeds and manures in 1975, 1980, 1985, 1990, 1995, and 2000 were 0%, 3%, 14%, 28%, 41%, and 47%, respectively. There is no report about PBDE levels of imported foods, but a number of reports strongly suggest that the environmental penta-BDE contamination expands through 1990s in North American region (Ikonomou et al., 2000, 2001; Stern and Ikonomou, 2000; Lebeuf et al., 2001; Luross et al., 2001; Moisey et al., 2001; Betts, 2002; She et al., 2002). North American countries are large food suppliers for Japan (The Ministry of Economy, Trade and Industry of Japan, 2002). Consequently, the Japanese intakes of penta-BDE components from foreign

foods might have synergistically increased through 1990s. Moreover, according to recent reports, serious penta-BDE pollutions are still occurring in North American countries (Hale et al., 2001a,b, 2002; Betts, 2002; She et al., 2002). Therefore, imported foods should also be monitored.

The recent levels of Σ PBDEs in 12 out of 13 individual milk samples were low (0.56–3.97 ng/g lipid), whereas a value of about two order of magnitudes higher (291 ng/g lipid) was found in 1 sample (KA99-8) collected from a mother living in Kanagawa (Table 3). The concentration of PBDEs found in KA99-8 was comparable to the highest concentrations in human samples reported previously (She et al., 2002); e.g., breast adipose tissue samples of the California women was 86 ± 114 ng/g fat (mean \pm SD, $n = 23$). The levels of the dominant PBDE concentration and congener profiles excluding the outlier value (KA99-8) were similar among mothers living in three regions of Kanagawa, Okayama, and Osaka (Fig. 2). As Ohta et al. have reported (Ohta et al., 2002), the ratios of BDE-153 and 154 are remarkably different between the human milk and marine fish (Fig. 2), and the number of detected congener peaks in human milk samples is clearly less than that in the marine fish (Fig. 3). These results suggest the difference of the uptake/excretion capacity for PBDEs between human and marine fish. Interestingly, a characteristic congener profile was found in KA99-8; the contribution of Hx-HpBDEs was significantly lower than that of other milk samples (Fig. 2). A high level of PBDEs with the similar congener profiles to above was also observed in a serum sample collected from a KA99-8 donor in 1999 (data not shown). The result indicated for certain that body burden of PBDEs in the KA99-8 donor was higher than that in other donors. On the other hand, the levels of organochlorine contaminants

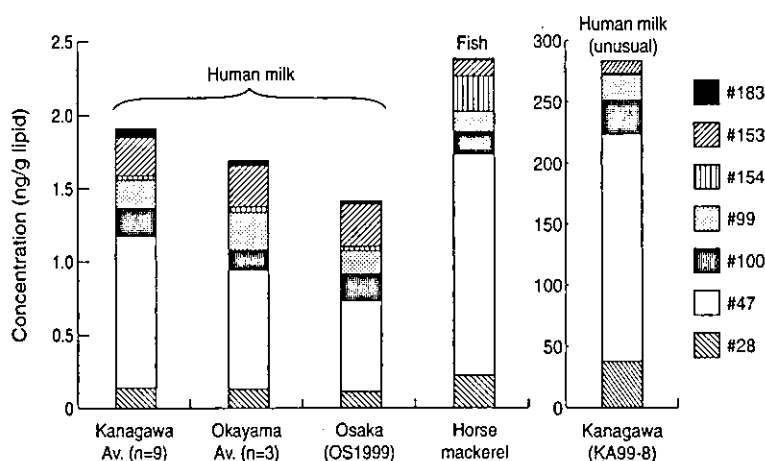


Fig. 2. Levels of PBDEs in Japanese mothers' milk samples collected from mothers living in three different prefectures in 1999, and pooled fish sample from the Seto Inland Sea in 1998.

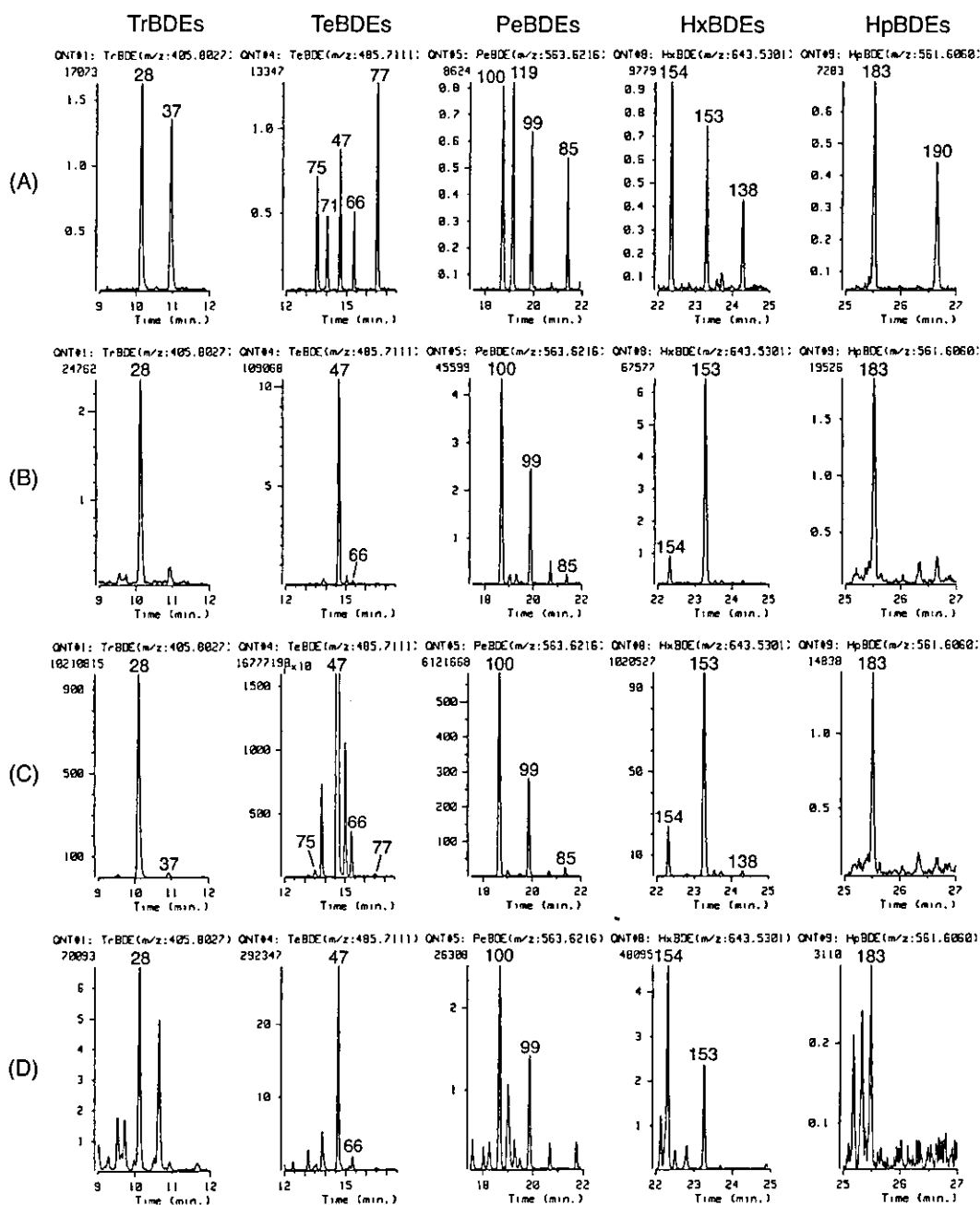


Fig. 3. Representative chromatograms from (A) a standard 16 PBDE mixtures, 0.5 ng/ml each in *n*-nonane, (B) a human milk (KA99-1), (C) a human milk highly contaminated by PBDEs (KA99-8), and (D) a pooled horse mackerel.

(e.g., PCBs, DDT, and HCBs) in the KA99-8 milk sample in our previous report were not higher than those in other nine individual milk samples collected in Kanagawa; i.e., total PCB concentrations in the KA99-8 donor and other nine individuals are 49 and 42–192 ng/g lipid, respectively (Hori et al., 2001). Therefore, intake of fatty marine fish, which was a major source of organochlorine contaminants for Japanese (Konishi et al., 2001), would

not be a main cause of the high PBDE concentration found in KA99-8. The survey was conducted by a brief questionnaire to the KA99-8 donor in 2001, but decisive exposure source was not ascertained. As the cause of extraordinary PBDE concentration found in KA99-8, the intake of accidentally PBDE-contaminated foods as with Yusho incident and respiratory exposure of PBDE-treated materials (e.g., furniture, electrical appliances,

textiles) in her living/working environment were considered, but not confirmed. Long-term follow-up survey may be desirable, although no serious health effects were observed in either the KA99-8 donor or her child at the moment. Additionally, it was uncertain whether the most widely used PBDE product deca-BDE (BDE-209) degraded to highly accumulative/toxic congeners (Tr-HxBDEs) in the actual environment. Obviously, there are many unidentified homologue peaks on the chromatogram of the marine fish sample, although they are of relatively small amount (Fig. 3). A number of them might be decomposition products from technical PBDEs by comparison with the known simple composition of penta-BDE (Sjödín et al., 1998). Therefore, it was also important to continue and develop the environment/human monitoring studies, though the recent levels of Σ PBDEs seemed to be stable or decrease in both Japanese and Swedish mothers' milk samples (Meironyté and Norén, 2001b).

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References

- Akutsu, K., Obana, H., Okihashi, M., Kitagawa, M., Nakazawa, H., Matsuki, Y., Makino, T., Oda, H., Hori, S., 2001. GC/MS analysis of polybrominated diphenyl ethers in fish collected from the Inland Sea of Seto, Japan. *Chemosphere* 44, 1325–1333.
- Andersson, Ö., Blomkvist, G., 1981. Polybrominated aromatic pollutants found in fish in Sweden. *Chemosphere* 10, 1051–1060.
- Asahi Glass Co., Ltd., Albemarle Asano Co., Teijin Chemicals Ltd., Tosoh Co., Nippon Chemicals Co., Ltd., Bromoken (Far East) Ltd., Manac Inc., Miki and Co., Ltd., Mitsui Toatsu Fine Chemicals, Inc., 1995. Voluntary plan in Japan concerning the risk management of selected brominated flame retardants, July 14. Available from <<http://www.oecd.org/pdf/M00032000/M00032868.pdf>>.
- Asplund, L., Athanasiadou, M., Sjödín, A., Bergman, Å., Börjeson, H., 1999. Organohalogen substances in muscle, egg and blood from healthy Baltic salmon (*Salmo salar*) and Baltic salmon that produced offspring with the M74 syndrome. *Royal Swedish Acad. Sci.* 28, 67–76.
- Betts, K.S., 2002. Rapidly rising PBDE levels in North America. *Environ. Sci. Technol.* 36, 50A–52A.
- BSEF (Bromine Science and Environmental Forum), 2000. BSEF factsheets: major brominated flame retardants volume estimates, July 27. Available from <<http://www.bsef.com>>.
- Burreau, S., Zebühr, Y., Ishaq, R., Broman, D., 2000a. Comparison of biomagnification of PBDEs in food chains from the Baltic Sea and the Northern Atlantic Sea. *Organohalogen Compd.* 47, 253–255.
- Burreau, S., Broman, D., Örn, U., 2000b. Tissue distribution of 2,2',4,4'-tetrabromof[14C]diphenyl ether ([14C]-PBDE47) in pike (*Esox lucius*) after dietary exposure—a time series study using whole body autoradiography. *Chemosphere* 40, 977–985.
- Carlson, G.P., 1980a. Induction of xenobiotic metabolism in rats by short-term administration of brominated diphenyl ethers. *Toxicol. Lett.* 5, 19–25.
- Carlson, G.P., 1980b. Induction of xenobiotic metabolism in rats by brominated diphenyl ethers administered for 90 days. *Toxicol. Lett.* 6, 207–212.
- Chen, G., Konstantinov, A.D., Chittim, B.G., Joyce, E.M., Bols, N.C., Bunce, N.J., 2001. Synthesis of polybrominated diphenyl ethers and their capacity to induce CYP1A by the Ah receptor mediated pathway. *Environ. Sci. Technol.* 35, 3749–3756.
- de Boer, J., 1989. Organochlorine compounds and bromodiphenylethers in livers of Atlantic cod (*Gadus morhua*) from the North Sea, 1977–1987. *Chemosphere* 18, 2131–2140.
- de Boer, J., 2000. Draft Report on the BSEF PBDE Interlaboratory Study. The Netherlands Institute for Fisheries Research (RIVO).
- de Boer, J., Cofino, W.P., 2002. First world-wide interlaboratory study on polybrominated diphenylethers (PBDEs). *Chemosphere* 46, 625–633.
- de Boer, J., Wester, P.G., Klamer, H.J., Lewis, W.E., Boon, J.P., 1998. Do flame retardants threaten ocean life? *Nature* 394, 28–29.
- Eriksson, P., Viberg, H., Jakobsson, E., Örn, U., Fredriksson, A., 1999. PBDE, 2,2',4,4',5-pentabromodiphenyl ether, causes permanent neurotoxic effects during a defined period of neonatal brain development. *Organohalogen Compd.* 40, 333–336.
- Eriksson, P., Jakobsson, E., Fredriksson, A., 2001. Brominated flame retardants: a novel class of developmental neurotoxins in our environment? *Environ. Health Perspect.* 109, 903–908.
- European Chemicals Bureau, 2000. European Union risk assessment report; diphenyl ether, pentabromo derivative (pentabromodiphenyl ether).

- Fowles, J.R., Fairbrother, A., B.-Steppan, L., Kerkvliet, N.I., 1994. Immunologic and endocrine effects of the flame-retardant pentabromodiphenyl ether (DE-71) in C57BL/6J mice. *Toxicology* 86, 49–61.
- Gustafsson, K., Björk, M., Burreau, S., Gilek, M., 1999. Bioaccumulation kinetics of brominated flame retardants (polybrominated diphenyl ethers) in blue mussels (*Mytilus edulis*). *Environ. Toxicol. Chem.* 18, 1218–1224.
- Haglund, P.S., Zook, D.R., Buser, H.-R., Hu, J., 1997. Identification and quantification of polybrominated diphenyl ethers and methoxy-polybrominated diphenyl ethers in Baltic biota. *Environ. Sci. Technol.* 31, 3281–3287.
- Hale, R.C., Guardia, M.J.L., Harvey, E., Mainor, T.M., Duff, W.H., Gaylor, M.O., 2001a. Polybrominated diphenyl ether flame retardants in Virginia freshwater fishes (USA). *Environ. Sci. Technol.* 35, 4585–4591.
- Hale, R.C., Guardia, M.J.L., Harvey, E., Gaylor, M.O., Mainor, T.M., Duff, W.H., 2001b. Persistent pollutants in land-applied sludges. *Nature* 412, 140–141.
- Hale, R.C., Guardia, M.J.L., Harvey, E., Mainor, T.M., 2002. Potential role of fire retardant-treated polyurethane foam as a source of brominated diphenyl ethers to the US environment. *Chemosphere* 46, 729–735.
- Hallgren, S., Sinjari, T., Håkansson, H., Darnerud, P.O., 2001. Effects of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) on thyroid hormone and vitamin A levels in rats and mice. *Arch. Toxicol.* 75, 200–208.
- Helleday, T., Tuominen, K.L., Bergman, A., Jenssen, D., 1999. Brominated flame retardants induce intragenic recombination in mammalian cells. *Mutat. Res.* 439, 137–147.
- Holm, G., Norrgren, L., Andersson, T., Thurén, A., 1993. Effects of exposure to food contaminated with PBDE, PCN or PCB on reproduction, liver morphology and cytochrome P450 activity in the three-spined stickleback, *Gasterosteus aculeatus*. *Aquatic Toxicol.* 27, 33–50.
- Hori, S., Kitagawa, M., Oda, H., Nakazawa, H., Shinoda, A., Makino, T., 2001. Analysis of endocrine disruptors in mother's milk and serum of mother's body/cord blood using an on-line GPC-GC/MS system. *Organohalogen Compd.* 52, 301–304.
- Ikonomou, M.G., Fischer, M., He, T., Addison, R.F., Smith, T., 2000. Congener patterns, spatial and temporal trends of polybrominated diphenyl ethers in biota samples from the Canadian west coast and the Northwest territories. *Organohalogen Compd.* 47, 77–80.
- Ikonomou, M.G., Fischer, M., Antcliffe, B., Addison, R.F., 2001. PBDEs on the rise: as reflected by aquatic species from British Columbia and the Arctic. In: BFR 2001 Proceedings, May 14–16, Stockholm, Sweden, pp. 325–328.
- International Programme on Chemical Safety (IPCS), 1994. Environmental Health Criteria 162, Brominated diphenyl ethers. World Health Organization, Geneva. Available from <<http://www.inchem.org/documents/ehc/ehc/ehc162.htm>>.
- Konishi, Y., Kuwabara, K., Hori, S., 2001. Continuous surveillance of organochlorine compounds in human breast milk from 1972 to 1998 in Osaka, Japan. *Arch. Environ. Contam. Toxicol.* 40, 571–578.
- Kuehl, D.W., Haebler, R., 1995. Organochlorine, organobromine, metal, and selenium residues in bottlenose dolphins (*Tursiops truncatus*) collected during an unusual mortality event in the Gulf of Mexico, 1990. *Arch. Environ. Contam. Toxicol.* 28, 494–499.
- Law, R.J., Allchin, C.R., Bennett, M.E., Morris, S., Rogan, E., 2002. Polybrominated diphenyl ethers in two species of marine top predators from England and Wales. *Chemosphere* 46, 673–681.
- Lebeuf, M., Love, K., Trottier, S., 2001. Polybrominated diphenyl ethers in beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary, Canada: levels and temporal trends. In: BFR 2001 Proceedings, May 14–16, Stockholm, Sweden, pp. 333–336.
- Lindström, G., van Bavel, B., Hardell, L., Liljegren, G., 1997. Identification of the flame retardants polybrominated diphenyl ethers in adipose tissue from patients with non-Hodgkin's lymphoma in Sweden. *Oncol. Rep.* 4, 999–1000.
- Lindström, G., Wingfors, H., Dam, M., van Bavel, B., 1999. Identification of 19 polybrominated diphenyl ethers (PBDEs) in long-finned pilot whale (*Globicephala melas*) from the Atlantic. *Arch. Environ. Contam. Toxicol.* 36, 355–363.
- Loganathan, B., Kannan, K., Watanabe, I., Kawano, M., Irvine, K., Kumar, S., Sikka, H.C., 1995. Isomer-specific determination and toxic evaluation of polychlorinated biphenyls, polychlorinated/brominated dibenzo-*p*-dioxins and dibenzofurans, polybrominated biphenyl ethers, and extractable organic halogen in carp from the Buffalo River, New York. *Environ. Sci. Technol.* 29, 1832–1838.
- Luross, J.M., Alace, M., Cannon, C.M., Sergeant, D.B., Whittle, D.M., Solomon, K.R., 2001. Spatial and temporal distribution of polybrominated diphenyl ethers and polybrominated biphenyls in lake trout from the Great Lakes. In: BFR 2001 Proceedings, May 14–16, Stockholm, Sweden, pp. 401–404.
- Manchester-Neesvig, J.B., Valters, K., Sonzogni, W., 2001. Comparison of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in Lake Michigan salmonids. *Environ. Sci. Technol.* 35, 1072–1077.
- Meerts, I.A.T.M., van Zanden, J.J., Luijckx, E.A.C., van Leeuwen-Bol, I., Marsh, G., Jakobsson, E., Bergman, Å., Brouwer, A., 2000. Potent competitive interaction of some brominated flame retardants and related compounds with human transthyretin in vitro. *Toxicol. Sci.* 56, 95–104.
- Meerts, I.A.T.M., Letchere, R.J., Hoving, S., Marsh, G., Bergman, A., Lemmen, J.G., van der Burg, B., Brouwer, A., 2001. In Vitro estragenicity of polybrominated diphenyl ethers, hydroxylated PBDEs, and polybrominated bisphenol A compounds. *Environ. Health Perspect.* 109, 399–407.
- Meironyté, D., Norén, K., Bergman, Å., 1999. Analysis of polybrominated diphenyl ethers in Swedish human milk. A time-related trend study, 1972–1997. *J. Toxicol. Environ. Health Part A* 58, 329–341.
- Meironyté, D., Bergman, Å., Norén, K., 2001a. Polybrominated diphenyl ethers in Swedish human liver and adipose tissue. *Arch. Environ. Contam. Toxicol.* 40, 564–570.
- Meironyté, D., Norén, K., 2001b. Polybrominated diphenyl ethers in Swedish human milk. The follow-up study. In: BFR 2001 Proceedings, May 14–16, Stockholm, Sweden, pp. 303–305.
- Meneses, M., Wingfors, H., Schuhmacher, M., Domingo, J.L., Lindström, G., van Bavel, B., 1999. Polybrominated diphenyl ethers detected in human adipose tissue from Spain. *Chemosphere* 39, 2271–2278.

- Moisey, J., Simon, M., Wakeford, B., Weseloh, D.V., Norstrom, R.J., 2001. Spatial and temporal trends of polybrominated diphenyl ethers detected in Great Lakes herring gulls, 1981 to 2000. In: BFR 2001 Proceedings, May 14–16, Stockholm, Sweden, pp. 153–157.
- Nagayama, J., Tsuji, H., Takasuga, T., 2000. Comparison between brominated flame retardants and dioxins or organochlorine compounds in blood levels of Japanese adults. *Organohalogen Compd.* 48, 27–30.
- Nishizawa, H., 1987. In: Handbook of the Flame Retardation of Polymer, the Chemistry and Practical Technology (an enlarged edition). Taiseisya Co., Ltd., Tokyo (in Japanese).
- Ogino, Y., Okamoto, Y., Yoshimura, H., 1987. On environmental survey of chemicals (XXVI); determination of organobrominated compounds. In: Annual Report of Okayama Prefectural Institute for Environmental Science and Public Health, No. 11, pp. 83–88 (in Japanese).
- Ohta, S., Ishizuka, D., Nishimura, H., Nakao, T., Aozasa, O., Shimadzu, Y., Ochiai, F., Kida, T., Miyata, H., 2000. Real situation of contamination by polybrominated diphenyl ethers as flame retardant in market fish and mother milk of Japan. *Organohalogen Compd.* 48, 218–221.
- Ohta, S., Ishizuka, D., Nishimura, H., Nakao, T., Aozasa, O., Miyata, H., 2001. Time-dependent alteration of PBDEs contamination observed in the long-term stock-fish samples (Japanese sea bass and grey mullet: 1986–1999). In: The Proceedings of the 10th Annual Meeting of Japan Society for Environmental Chemistry, May 23–25, Matsuyama, Japan, pp. 74–75 (in Japanese).
- Ohta, S., Ishizuka, D., Nishimura, H., Nakao, T., Aozasa, O., Shimadzu, Y., Ochiai, F., Kida, T., Nishi, M., Miyata, H., 2002. Comparison of polybrominated diphenyl ethers in fish, vegetables, and meats and levels in human milk of nursing women in Japan. *Chemosphere* 46, 689–696.
- Sellström, U., Jansson, B., Kierkegaard, A., de Wit, C., 1993. Polybrominated diphenyl ethers (PBDE) in Biological samples from the Swedish environment. *Chemosphere* 26, 1703–1718.
- She, J., Petreas, M., Winkler, J., Visita, P., McKinney, M., Kopec, D., 2002. PBDEs in the San Francisco bay area: measurements in harbor seal blubber and human breast adipose tissue. *Chemosphere* 46, 697–707.
- Sjödin, A., Jakobsson, E., Kierkegaard, A., Marsh, G., Sellström, U., 1998. Gas chromatographic identification and quantification of polybrominated diphenyl ethers in a commercial product, Bromkal 70-5DE. *J. Chromatogr. A* 822, 83–89.
- Sjödin, A., Hagmar, L., K.-Wehler, L., K.-Diab, K., Jakobsson, E., Bergman, Å., 1999. Flame retardant exposure: polybrominated diphenyl ethers in blood from Swedish workers. *Environ. Health Perspect.* 107, 643–648.
- Stern, G.A., Ikonomou, M.G., 2000. Temporal trends of polybrominated diphenyl ethers in SE Baffin beluga: increasing evidence of long range atmospheric transport. *Organohalogen Compd.* 47, 81–84.
- Strandman, T., Koistinen, J., Vartiainen, T., 2000. Polybrominated diphenyl ethers (PBDEs) in placenta and human milk. *Organohalogen Compd.* 47, 61–64.
- The Chemical Daily Co., Ltd., 1987–2001. Yearly features about annual demand of flame retardants. In: The Chemical Daily (Japanese-language daily newspaper).
- The Japanese Government, 1973. Hexabromodiphenyl ether (Ref. No. 3-2845). In: The Existing Chemical List for the Law Concerning the Examination and Regulation of Manufacture, etc. of Chemical Substances (in Japanese).
- The Ministry of Agriculture, Forestry and Fisheries of Japan, 2001. Food Balance Sheet 1960–2000 (in Japanese).
- The Ministry of Economy, Trade and Industry of Japan, 2002. White Paper on International Trade 2001 (in Japanese).
- The Ministry of Health and Welfare of Japan, 1972. Analysis Method for PCB in Mother's Milk (in Japanese).
- von Meyerinck, L., Hufnagel, B., Schmoldt, A., Benthe, H.F., 1990. Induction of rat liver microsomal cytochrome P-450 by the pentabromo diphenyl ether Bromkal 70 and half-lives of its components in the adipose tissue. *Toxicology* 61, 259–274.
- Watanabe, I., Kashimoto, T., Tatsukawa, R., 1987. Polybrominated biphenyl ethers in marine fish, shellfish and river and marine sediment in Japan. *Chemosphere* 16, 2389–2396.
- Zhou, T., Ross, D.G., DeVito, M.J., Crofton, K.M., 2001. Effects of short-term in vivo exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weanling rats. *Toxicol. Sci.* 61, 76–82.

(9)

自治体焼却施設における堆積粉塵中ダイオキシン類濃度と労働者の血清中ダイオキシン類濃度との関係

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抄録：自治体焼却施設における堆積粉塵中ダイオキシン類濃度と労働者の血清中ダイオキシン類濃度との関係：熊谷信二ほか。大阪府立公衆衛生研究所一焼却施設 13 ヶ所において、労働者の血清中ダイオキシン類濃度と堆積粉塵中ダイオキシン類濃度を測定し、両者の関係を検討した。その結果、以下のことがわかった。1) 堆積粉塵の分析により、ダイオキシンの中では八塩化ダイオキシンおよび七塩化ダイオキシンが、またジベンゾフランの中では、八塩化ジベンゾフランおよび七塩化ジベンゾフランが主成分であることがわかった。ダイオキシンとジベンゾフランを合わせた毒性等量は 0.54～33 ngTEQ/g と一般土壌の 1,000～10,000 倍のレベルであった。2) 全焼却施設労働者の血清中のダイオキシン毒性等量、ジベンゾフラン毒性等量、およびダイオキシンとジベンゾフランを合わせた毒性等量の平均値はそれぞれ 16, 12 および 28 pgTEQ/g 脂肪であった。血清中の六塩化および七塩化ジベンゾフラン濃度が一般人よりも有意に高くなっており、勤務中にこれらの物質の取り込みがあると考えられた。3) 堆積粉塵中ダイオキシン類濃度と血清中ダイオキシン類濃度の関係の検討では、過去 7 年以内に施設内の全面的な改造工事を行った 3 施設を除けば、血清中の七塩化ジベンゾフランは堆積粉塵中の濃度と正に相関していた。したがって、焼却施設の堆積粉塵中ダイオキシン類濃度を測定すれば、労働者の血清中ダイオキシン類濃度測定を含む健康調査を実施することが必要か、否かの判断が可能かもしれない。

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1. 目的

1999 年に大阪府能勢町の焼却施設における労働者のダイオキシン類への高濃度の曝露が報告されて以来¹⁾、焼却施設で働く労働者の間に健康影響への不安が広がっている。このため、厚生労働省では、1999～2001 年に計 26 ヶ所の焼却施設において、労働者の健康調査を実施した²⁻⁴⁾。血中ダイオキシン類濃度の各施設ごとの平均値は 11～40 pg 毒性等量 (TEQ)/g 脂質と、ほぼ一般人のレベルであったが、一部の焼却施設の労働者では 100 pgTEQ/g 脂質を超える者も見られた。また、私どもは、1998～2000 年に計 6 ヶ所の焼却施設において、労働者の血清中ダイオキシン類濃度測定を実施した^{5, 6)}。血清中ダイオキシン類濃度の各施設ごとの平均値は 23～35 pgTEQ/g 脂質 (コプラナーポリ塩化ビフェニルを除く) と、ほぼ一般人のレベルであった。

このように、大阪府能勢町以外の焼却施設の場合、血中ダイオキシン類濃度の平均値は一般人のレベルであり、一応、安心できる状況と言える。ただし、一部の労働者で一般人のレベルを超える者が見られたことから、血中ダイオキシン類濃度測定を含む健康調査の実施を求める声が広がっている。そこで、協力の得られた自治体の焼却施設 7 ヶ所において、労働者の血清中ダイオキシン類濃度を測定した。本報では、これら 7 ヶ所のデータと既報の 6 ヶ所のデータと合わせて、焼却施設労働者の血清中ダイオキシン類濃度の特徴を検討した。

もうひとつの目的は、焼却施設の堆積粉塵中ダイオキシン類濃度が労働者の曝露指標として使用できるかを明らかにすることである。焼却施設は全国で約 22,000 ヶ所 (うち、一般廃棄物焼却施設 1,900 ヶ所、産業廃棄物焼却施設 3,000 ヶ所、未規制小型廃棄物焼却施設 17,000 ヶ所) あり^{7, 8)}、これら施設の労働者を合わせれば、数万人に上ると考えられる。しかし、血中ダイオキシン類濃度の測定を実施している機関は少なく、1 年間に測定できる能力は全機関合わせても 2,000 検体以下と推定さ

Table 1. Characteristic of incineration plants and subjects

	A	B	C	D	E	F	G
Incinerator							
Year of beginning of operation	1988	1980	1975, 95	1981	1986	1979	1974, 88
Number of incinerators	3	3	4	1	2	2	2
Type of incinerator	Stoker	Stoker	Stoker	Fluid bed	Fluid bed	Stoker	Stoker
Instrument for removing dust *	EP	EP	EP, BF	BF	EP	EP	EP, BF
Operating hours (h/d)	24	24	24	8	16	16	8, 24
Incinerated volume (ton/d)	160	300	600	20	40	60	170
Number of operation workers	28	38	74	7	7	13	29
Dioxin concentration							
Exhaust gas (ng TEQ/Nm ³)**	8.9-42	0.82-1.4	0.072-12	0.22-590	11-260	11-53	0.021-40
Fly ash (ng TEQ/g)**	42-43	2.9-3.5	1.1-20	1.7	10-34	22-200	1.4-55
Subjects							
Number	10	10	10	7	7	6	10
Age (yr, mean (SD))	50.4 (5.4)	45.4 (3.4)	47.8 (4.4)	44.1 (5.5)	40.6 (11.1)	38.7 (8.3)	55.6 (4.6)
Employment duration (yr, mean (SD))	22.0 (5.3)	9.4 (3.0)	23.6 (3.3)	11.0 (5.1)	10.3 (3.4)	15.7 (7.3)	31.0 (2.7)
	H	I	J	K	L	M	
Incinerator							
Year of beginning of operation	1973, 91	1983, 93	1992	1978	1993	1992	
Number of incinerators	3	2	3	2	3	2	
Type of incinerator	Stoker	Stoker	Stoker	Stoker	Stoker	Fluid bed	
Instrument for removing dust *	EP, BF	EP	BF	EP	BF	BF	
Operating hours (h/d)	24	24	24	24	24	24	
Incinerated volume (ton/d)	450	80	450	180	120	270	
Number of operation workers	44	26	76	33	26	36	
Dioxin concentration							
Exhaust gas (ng TEQ/Nm ³)**	0.002-27	1.0-4.6	0.042-5.0	0.01-14	0.003-3.3	0.0003-18	
Fly ash (ng TEQ/g)**	0.27-8.8	7.1	3.0	7.3-120	0.84-3.5	5.7-8.7	
Subjects							
Number	10	3	10	9	5	20	
Age (yr, mean (SD))	48.1 (3.8)	43.0 (11.3)	44.4 (6.9)	36.3 (7.0)	43.0 (6.1)	40.8 (5.6)	
Employment duration (yr, mean (SD))	25.4 (2.5)	14.7 (16.8)	20.1 (5.8)	9.3 (3.6)	18.4 (4.2)	13.3 (6.2)	

*: EP=Electrostatic precipitator, BF=Bag filter,

** : The minimum and maximum values are shown for incineration plants with two or more regular measurements.

れる。このため、全焼却施設の労働者の血中濃度測定を実施するには、10年以上の期間が必要になる。したがって、今後、血中濃度を測定できる機関を増やすことが必要であるが、現状では、むしろ測定の必要な焼却施設、つまり曝露の高い可能性のある焼却施設を絞り、そこに資源（血中ダイオキシン類濃度の測定能力）を集中する方が有効であると考えられる。

このような観点に立ち、焼却施設の堆積粉塵中ダイオキシン類濃度が労働者の曝露指標として使用できるかを検討した。堆積粉塵の測定であれば、測定コストも比較的安く、また、測定を実施できる機関も多い。したがっ

て、短期間で全国の焼却施設での測定が可能となると考えられる。

2. 方法

2-1. 施設の概要

Table 1に焼却施設の概要を示す。焼却施設A～Fについては、既に血清中ダイオキシン類濃度測定を実施し報告している^{5, 6)}。今回、新たに焼却施設G～Mについて調査を行ったため、全部で13カ所となった。

焼却施設の稼働開始時期は1973～95年であり、古いものから比較的新しいものまでである。各施設の焼却炉数

Table 2. Characteristics of incinerator workers and controls

	Incinerator workers	Controls	Significance
Workers (n)	117	50	
Age (yr, mean \pm SD)	44.7 \pm 7.8	45.8 \pm 7.2	n.s.*
Age distribution			
20-29 yr (n (%))	3 (2.7)	1 (2.0)	n.s.**
30-39 yr (n (%))	28 (23.9)	7 (14.0)	
40-49 yr (n (%))	56 (47.9)	26 (52.0)	
50-60 yr (n (%))	30 (25.6)	16 (32.0)	
Employment duration at incineration plant (yr, mean \pm SD)	17.5 \pm 8.7	0	
Dietary habit (times/wk, mean \pm SD):			
Fish	6.3 \pm 3.3	7.4 \pm 3.2	$p < 0.05$ *
Meal	6.1 \pm 3.2	6.3 \pm 3.0	n.s.*
Milk	3.6 \pm 4.0	4.2 \pm 4.8	n.s.*
Current smoking habit:			
Smokers (workers, n (%))	63 (53.8)	23 (46.0)	n.s.**
Ex-smokers (workers, n (%))	29 (24.8)	13 (26.0)	
Non-smokers (workers, n (%))	25 (21.4)	14 (28.0)	
Smoking habit index (cigarclcs/d \times yr, mean \pm SD)	470 \pm 443	378 \pm 369	n.s.*
Body mass index (kg/m ² , mean \pm SD)	23.4 \pm 3.2	24.3 \pm 3.1	n.s.*

*: t-test. **: χ^2 -test

は1~4基であった。焼却炉のタイプは、ストーカ方式および流動床方式がそれぞれ10カ所および3カ所であった。煤塵除去装置は、電気集塵器およびバッグフィルターがそれぞれ6カ所および4カ所、電気集塵器およびバッグフィルターの両方を採用している所が3カ所であった。稼働時間は、全連続式(24時間稼働)、准連続式(16時間稼働)およびバッチ式(8時間稼働)がそれぞれ9カ所、2カ所および1カ所であり、全連続式とバッチ式の両方ある所が1カ所であった。実処理量は20~600トン/日であり、焼却炉の運転に携わっている労働者数は7~76名であった。

排ガス中ダイオキシン類濃度は0.0003~590 ngTEQ/Nm³、飛灰中ダイオキシン類濃度は0.27~200 ngTEQ/gであり、低いケースから高いケースまで含まれている。

2-2. 調査対象者

各施設から勤続年数の長い男性労働者3~20名、計117名を選び調査対象者とした。Table 1に各施設の調査対象者の人数、平均年齢および平均勤続年数を示す。平均年齢は36.3~55.6歳と施設による差が見られる。また、焼却施設での平均勤続年数は9.3~31.0年とやはり施設による差が見られる。Table 2に調査対象者全体の年齢、勤続年数、食事習慣(魚、肉、牛乳)、喫煙習

慣、肥満度(Body Mass Index)を示す。

2-3. 血清中ダイオキシン類濃度測定

1) 試薬

ヘキサンは関東化学製(ダイオキシン分析用)を、ジクロロメタンおよびエタノールは和光純薬製(ダイオキシン分析用)を、無水硫酸ナトリウムは和光純薬製(PCB・フタル酸分析用)を、硫酸アンモニウム、水酸化カリウムおよびn-ノナンは和光純薬製(特級)を使用した。また、積層カラムの充填剤は和光純薬製(ダイオキシン分析用)を、アルミナ(塩基性、活性度I)はICN製(ダイオキシン分析用)を使用した。ダイオキシン類の標準液はWellington Laboratories製を使用した。

2) 採血

血中脂質量の食事前後の変動による、血清中ダイオキシン類濃度(1gの脂質中濃度として表す)の見かけ上の変動を少なくするため朝食前に採血した。採血量は約100 mlとし、10 mlの真空採血管(テルモ製ベノジェクトII VP-P100)10本を使用した。血液が凝固した後、3,000 rpmで10分間の遠沈を行い、上層の血清をガラス容器に移し、分析時まで-30℃で凍結保存した。

3) 前処理

血清40 mlに内部標準物質(¹³C-2,3,7,8-ダイオキシン,¹³C-2,3,7,8-ジベンゾフランおよび¹³C-コブラナポリ塩化

ビフェニルのアセトン溶液)を添加し混合した。次に、飽和硫酸アンモニウム 27 ml, エタノール 27 ml, ヘキサン 80 ml を加えて 15 分間振とうした。2 層が分離した後、水層を別の分液ロートに移し、ヘキサン 80 ml を加えて、15 分間振とうした。2 層が分離した後、水層を捨て、ヘキサン層を 1 回目のヘキサン層と合わせ、その後、精製水 (ヘキサン洗浄水) 16 ml で 1 回, 40 ml で 2 回洗浄した。次いで、無水硫酸ナトリウムで水分を除去し、ロータリーエバポレータで濃縮した。重量を測定したビーカーに濃縮液を移し、室温で蒸発乾固させて重量を測定し、脂質重量を算出した。

脂質重量を測定後、1N 水酸化カリウム-エタノール溶液 10 ml を加え、スターラで 2 時間攪拌して脂質を分解した。次いで、試料を分液ロートに移し、精製水 10 ml およびヘキサン 25 ml を加え、15 分間振とうした。2 層が分離した後、水層を別の分液ロートに移し、ヘキサン 25 ml を加えて、15 分間振とうした。2 層が分離した後、水層を捨て、ヘキサン層を 1 回目のヘキサン層と合わせ、その後、精製水 20 ml で 2 回洗浄した。次いで、無水硫酸ナトリウムで水分を除去し、ろ過 (Advantec 製 5A) 後、濃縮した。

次いで、試料を多層シリカゲルカラムに添加し、ヘキサン 75 ml で溶出させた。濃縮後、アルミナカラム (アルミナ 3 g) に添加し、0.5% ジクロロメタン-ヘキサン 10 ml を通して 1st フラクションとした。次いで、1% ジクロロメタン-ヘキサン 75 ml を通して 2nd フラクションとした。さらに、50% ジクロロメタン-ヘキサン 30 ml を通して 3rd フラクションとした。2nd フラクションを濃縮し先細試験管に移し、さらに濃縮後、n-ノナン 500 μ l (回収率算出用の内部標準物質 ^{13}C -1,2,3,4-四塩化ダイオキシンを含む) を添加してモノオルト-ポリ塩化ビフェニルの分析試料とした。3rd フラクションを濃縮し先細試験管に移し、さらに濃縮後、n-ノナン 10 μ l (回収率算出用の内部標準物質 ^{13}C -1,2,3,4-四塩化ダイオキシンを含む) を添加してダイオキシン、ジベンゾフランおよびノンオルト-ポリ塩化ビフェニルの分析試料とした。

なお、施設 A~C については、調査を行った 1998 年当時は、ダイオキシン類の中にコプラナーポリ塩化ビフェニル (コプラナー PCB) は含まれていなかったため、測定していない。また、焼却過程から発生するダイオキシン類の中でコプラナー PCB の占める割合は小さい⁹⁾。これらの事情から、以下の記述は、ダイオキシンおよびジベンゾフランに絞ることとする。

4) 高分解能ガスクロマトグラフ-高分解能質量分析計による測定

分析試料 2 μ l を高分解能ガスクロマトグラフ-高分解能質量分析計 (日本電子製 Mstation JMS700) に注入

し、ダイオキシン類を定量した。定量下限は、四塩化、五塩化および六塩化のダイオキシンおよびジベンゾフランでは 1 pg/g 脂質、七塩化および八塩化のダイオキシンおよびジベンゾフランでは 2 pg/g 脂質である。

5) ブランク値および定量下限の取り扱い

ブランク試験では、八塩化ダイオキシンが検出されたため、サンプル値からブランク値を差し引いたものを測定値とした。その他の物質については、ブランク試験からは検出されなかった。

毒性等量 (TEQ, WHO1998) の算出において、ある物質の濃度が定量下限未満の場合は、その半分の値を使用した。また、各焼却施設ごとの平均値および標準偏差、あるいは調査対象者全体の平均値および標準偏差の算出においても同様に取り扱った。

2-4. 堆積粉塵中ダイオキシン類濃度測定

1) 試薬

トルエンは和光純薬製 (ダイオキシン分析用) を塩酸は和光純薬製 (特級) を使用した。他の試薬は血清中ダイオキシン類分析の場合と同様である。

2) サンプリング

焼却炉および集塵器周辺の梁など、あまり清掃されていない場所 5~6 ヶ所を選び、堆積している粉塵を採取し、混合して 1 サンプルとした。粒径の大きい粉塵は空気中での沈降速度が速いため、作業現場では粒径の小さい粉塵が浮遊していると考えられる。そこで、堆積粉塵を 32 μ m のメッシュに通し、通過したものを分析試料とした。

3) 前処理

分析試料 0.5~1 g を秤量し、2N 塩酸 10 ml で 1 時間処理後、プフナロートでろ過した。ろ液は分液ロートに移し洗浄水と合わせて 40 ml とし、トルエン 30 ml を加え 15 分間振とうした。2 層が分離した後、水層を別の分液ロートに移し、トルエン 30 ml を加えて、15 分間振とうした。2 層が分離した後、水層を捨て、トルエン層を 1 回目のトルエン層と合わせた。プフナロートのろ過残渣は風乾した後、トルエンによりソックスレ抽出を 16 時間行った。次に、ろ液からのトルエン抽出液およびソックスレ抽出によるトルエン抽出液を合わせ、水 40 ml で 2 回洗浄し、無水硫酸ナトリウムで水分を除去後、濃縮・乾固した。

乾固した試料を少量のヘキサンで溶解し内部標準液を添加した後、多層シリカゲルカラムおよびアルミナカラムを通して分析試料とした。測定は、高分解能ガスクロマトグラフ-高分解能質量分析計を用いて行なった。これらの操作および分析条件は血清中ダイオキシン類測定の場合と同じである。

Table 3. Concentrations of PCDDs and PCDFs in the deposited dust on beams in the incineration plants (unit ng/g)

	A	B	C	D	E	F	G	H	I	J	K	L	M
2, 3, 7, 8-TCDD	0.25	0.03	0.41	0.02	0.01	0.38	1.0	0.34	0.20	0.46	0.16	0.03	0.01
1, 2, 3, 7, 8-PeCDD	1.6	0.30	2.0	0.11	1.8	3.2	8.0	2.0	1.3	2.6	1.5	0.17	0.05
1, 2, 3, 4, 7, 8-HxCDD	2.1	0.65	2.9	0.14	5.3	4.2	13	3.2	1.9	4.0	2.6	0.24	0.10
1, 2, 3, 6, 7, 8-HxCDD	4.8	1.3	5.3	0.26	5.3	11	20	5.6	3.6	7.5	5.7	0.56	0.27
1, 2, 3, 7, 8, 9-HxCDD	3.3	0.87	3.8	0.17	3.1	8.5	18	4.6	2.9	6.0	3.8	0.34	0.22
1, 2, 3, 4, 6, 7, 8-HpCDD	41	11	51	1.9	790	73	170	50	30	86	54	2.7	2.0
OCDD	97	29	130	3.4	1800	150	390	170	72	360	150	4.6	7.9
2, 3, 7, 8-TCDF	0.41	0.07	0.59	0.14	0.72	0.65	1.8	0.76	0.52	0.88	0.44	0.04	0.04
1, 2, 3, 7, 8-PeCDF	1.3	0.34	2.3	0.58	5.0	2.0	6.4	2.4	1.6	3.2	2.1	0.19	0.39
2, 3, 4, 7, 8-PeCDF	1.7	0.43	2.7	0.57	7.4	2.5	8.5	2.6	1.8	3.2	2.7	0.20	0.56
1, 2, 3, 4, 7, 8-HxCDF	3.0	0.81	4.2	0.87	13	4.4	14	4.9	2.9	6.9	4.6	0.20	0.64
1, 2, 3, 6, 7, 8-HxCDF	2.9	0.78	4.1	0.80	17	4.9	15	4.6	2.9	6.7	5.0	0.20	0.66
1, 2, 3, 7, 8, 9-HxCDF	0.39	0.14	0.37	0.11	2.4	1.8	6.2	1.8	1.4	2.9	2.3	0.06	0.65
2, 3, 4, 6, 7, 8-HxCDF	3.9	1.3	4.8	1.6	5.4	9.2	29	9.8	6.0	13	10	0.30	1.7
1, 2, 3, 4, 6, 7, 8-HpCDF	15	6.1	24	3.4	120	32	91	38	16	59	35	1.10	5.3
1, 2, 3, 4, 7, 8, 9-HpCDF	3.0	0.82	2.7	0.85	4.5	4.4	17	4.9	3.4	8.9	6.8	0.09	1.7
OCDF	17	4.5	14	3.0	210	25	79	29	15	61	37	0.40	9.2
TEQ-PCDD	3.3	0.72	4.1	0.21	19	6.7	16	4.2	2.6	5.7	3.4	0.34	0.14
TEQ-PCDF	2.2	0.61	3.1	0.71	14	3.8	12	4.0	2.5	5.5	4.1	0.20	0.73
TEQ-PCDD/PCDF	5.5	1.3	7.3	0.92	33	11	28	8.2	5.1	11	7.5	0.54	0.87

The dust samples were analyzed after being passed through a 32 μ m sieve.

2-5. 統計検定

先に述べたように、焼却施設 A～F の調査結果については既に報告しているが^{5, 6)}、その調査の中で、職業的にダイオキシン類曝露のない一般人（男性）50名を選びコントロールとしていた。Table 2 にこの集団の年齢、食事習慣（魚、肉、牛乳）、喫煙習慣、肥満度を示す。平均年齢は45.8歳と本報の焼却施設労働者とはほぼ同じであり、年齢構成にも有意差は見られない。また、魚の摂取回数を除く食事習慣、喫煙習慣、肥満度にも有意差は見られない。魚の摂取回数については、一般人の方が有意に多いが、大きな差ではないため、この集団をコントロールとして、血清中ダイオキシン類濃度の中央値の有意差検定を行った（Mann-Whitney *U* 検定）。

堆積粉塵中ダイオキシン類濃度と血清中ダイオキシン類濃度（各施設の平均値）の関係については、いずれも対数変換を行った後、ピアソンの相関係数を算出し有意性の検定を行った。なお、血清中ダイオキシン類濃度の各施設における代表値として平均値を使用したのは、労働者総体への累積曝露を表すためである。また、対数変換したのは、堆積粉塵中ダイオキシン類濃度および血清中ダイオキシン類濃度（各施設の平均値）の施設間分布がいずれも正規分布よりも対数正規分布に近かったためである。

3. 結果

3-1. 堆積粉塵中ダイオキシン類濃度

Table 3 に堆積粉塵中のダイオキシン類濃度を示す。ダイオキシンとジベンゾフランを合わせた毒性等量（TEQ-PCDD/PCDF）は0.54～33 ngTEQ/gであった。同族体パターンを見ると、ダイオキシンの中では、いずれの施設においても八塩化ダイオキシンがもっとも高く、次いで1,2,3,4,6,7,8-七塩化ダイオキシンが高かった。ジベンゾフランの中では、8カ所の施設においては1,2,3,4,6,7,8-七塩化ジベンゾフランがもっとも高く、5カ所の施設においては八塩化ジベンゾフランがもっとも高かった。

3-2. 血清中ダイオキシン類濃度

Table 4 に各焼却施設の労働者の血清中ダイオキシン類濃度の平均値を示す。ダイオキシンとジベンゾフランを合わせた毒性等量（TEQ-PCDD/PCDF）の各施設における平均値は19～41 pgTEQ/g 脂質であった。

Table 5 に全焼却施設労働者および一般人の血清中ダイオキシン類濃度の平均値、標準偏差、中央値および範囲を示す。焼却施設労働者の血清中ダイオキシン毒性等量（TEQ-PCDD）、ジベンゾフラン毒性等量（TEQ-PCDF）、およびダイオキシンとジベンゾフランを合わせた毒性等量（TEQ-PCDD/PCDF）の平均値はそれぞれ

Table 4. Mean concentrations of PCDDs and PCDFs in the serum of the incinerator workers in each incineration plant (unit: pg/g lipid)

	A	B	C	D	E	F	G	H	I	J	K	L	M
2, 3, 7, 8-TCDD	2	2	3	2	1	2	3	2	2	1	1	1	1
1, 2, 3, 7, 8-PeCDD	9	10	13	8	8	8	13	8	8	8	6	7	6
1, 2, 3, 4, 7, 8-HxCDD	3	3	5	3	5	4	7	3	4	3	3	3	3
1, 2, 3, 6, 7, 8-HxCDD	24	48	62	19	25	24	54	42	36	38	38	35	29
1, 2, 3, 7, 8, 9-HxCDD	5	8	12	4	7	7	11	5	5	6	4	5	5
1, 2, 3, 4, 6, 7, 8-HpCDD	24	29	41	31	46	24	55	22	28	28	19	14	24
OCDD	260	499	521	356	249	253	679	416	368	554	141	233	170
2, 3, 7, 8-TCDF	3	1	1	1	2	2	2	1	1	1	1	1	2
1, 2, 3, 7, 8-PeCDF	2	2	1	1	3	1	2	1	1	1	1	1	3
2, 3, 4, 7, 8-PeCDF	13	15	18	16	21	14	24	15	16	12	11	11	24
1, 2, 3, 4, 7, 8-HxCDF	6	6	6	6	13	5	11	6	5	5	6	4	19
1, 2, 3, 6, 7, 8-HxCDF	6	8	6	7	23	8	19	8	7	7	8	5	33
1, 2, 3, 7, 8, 9-HxCDF	1	1	1	1	2	1	1	1	1	1	1	1	3
2, 3, 4, 6, 7, 8-HxCDF	4	6	4	3	22	5	11	3	4	3	4	1	24
1, 2, 3, 4, 6, 7, 8-HpCDF	15	19	9	8	50	12	39	9	7	13	14	3	82
1, 2, 3, 4, 7, 8, 9-HpCDF	1	1	1	1	5	1	2	1	1	1	1	1	9
OCDF	2	2	2	1	3	1	1	1	1	1	1	1	3
TEQ-PCDD	15	18	24	13	14	13	24	15	15	14	12	13	12
TEQ-PCDF	9	10	11	10	17	9	17	10	10	8	7	7	21
TEQ-PCDD/PCDF	23	28	35	23	31	23	41	24	25	22	19	20	33
	(14-32)	(16-37)	(21-69)	(13-39)	(15-58)	(11-36)	(22-82)	(16-47)	(13-36)	(15-29)	(14-24)	(11-31)	(14-60)

(): Range of TEQ-PCDD/PCDF

Table 5. Concentrations of PCDDs and PCDFs in the serum of the incinerator workers and controls (unit: pg/g lipid)

	Mean	SD	Median	Range	Mean	SD	Median	Range
2, 3, 7, 8-TCDD	2	1	2	(ND-6)	2	1	2	(ND-4)
1, 2, 3, 7, 8-PeCDD	9	4	8	(3-32)	9	5	8	(3-34)
1, 2, 3, 4, 7, 8-HxCDD	4	2	3	(ND-13)	3	2	3	(ND-13)
1, 2, 3, 6, 7, 8-HxCDD	37	19	34	(9-100)	36	20	32	(11-78)
1, 2, 3, 7, 8, 9-HxCDD	7	4	5	(ND-29)	7	5	6	(ND-24)
1, 2, 3, 4, 6, 7, 8-HpCDD	30	24	23	(7-130)	31	37	23	(7-230)
OCDD	350	450	180	(49-2100)	410	570	230	(73-3100)
2, 3, 7, 8-TCDF	1	1	1	(ND-5)	2	1	1	(ND-8)
1, 2, 3, 7, 8-PeCDF	2	2	1	(ND-10)	1	1	ND	(ND-7)
2, 3, 4, 7, 8-PeCDF	17	9	15	(5-52)	15	6	14	(6-34)
1, 2, 3, 4, 7, 8-HxCDF	9	7	6**	(2-41)	6	3	5	(ND-12)
1, 2, 3, 6, 7, 8-HxCDF	13	13	9***	(2-75)	6	3	6	(2-15)
1, 2, 3, 7, 8, 9-HxCDF	1	1	ND	(ND-7)	1	0	ND	(ND-ND)
2, 3, 4, 6, 7, 8-HxCDF	9	10	4***	(ND-59)	3	2	3	(ND-11)
1, 2, 3, 4, 6, 7, 8-HpCDF	28	34	14***	(3-170)	6	3	6	(3-16)
1, 2, 3, 4, 7, 8, 9-HpCDF	3	4	ND	(ND-21)	1	0	ND	(ND-ND)
OCDF	2	2	ND	(ND-10)	1	1	ND	(ND-3)
TEQ-PCDD	16	7	14	(5-52)	16	7	15	(6-41)
TEQ-PCDF	12	8	10**	(4-43)	9	4	9	(4-21)
TEQ-PCDD/PCDF	28	12	25	(11-82)	25	10	23	(11-56)

Incinerator workers: n=117. Controls: n=50. **: $p < 0.01$, ***: $p < 0.001$ by the Mann-Whitney U test

れ16, 12および28 pgTEQ/g脂質であった。一般人との比較(中央値)では、ジベンゾフラン毒性等量は焼却施設労働者で有意に高かったが、他の2つの毒性等量には有意差は見られなかった。

各物質ごとに見ると、ダイオキシンでは焼却施設労働者と一般人で有意な差は見られなかったが、ジベンゾフランでは、3種類の六塩化ジベンゾフランおよび1種類の七塩化ジベンゾフランが焼却施設労働者で有意に高かった。特に1,2,3,4,6,7,8-七塩化ジベンゾフランは中央値で一般人の約2倍であり、平均値で約4倍であった。

3-3. 堆積粉塵中ダイオキシン類濃度と血清中ダイオキシン類濃度の関係

Figure 1に、堆積粉塵中に多く含まれていた1,2,3,4,6,7,8-七塩化ダイオキシン、八塩化ダイオキシン、1,2,3,4,6,7,8-七塩化ジベンゾフラン、および八塩化ジベンゾフランについて、各施設の堆積粉塵中濃度と血清中濃度の関係を示すが、いずれの物質も有意な相関は認められなかった。しかし、調査時点より過去7年以内のうちに、焼却施設の全面的な改造工事が行われた施設(J, L, M)を除くと、血清中1,2,3,4,6,7,8-七塩化ジベンゾフランについては有意な正の相関が見られた。

Figure 1には、ダイオキシン毒性等量およびジベンゾフラン毒性等量についても、堆積粉塵中濃度と血清中濃度の関係を示しているが、有意な相関は見られなかった。また、施設J, LおよびMを除外しても有意な相関は見られなかった。

4. 考 察

本報で調査した焼却施設の堆積粉塵中ダイオキシン類濃度は0.54~33 ngTEQ/gであり、これは一般土壌(中央値0.76 pgTEQ/g)¹⁰⁾の1,000~10,000倍のレベルに相当する。これらの粉塵は焼却炉や電気集塵器などの設備内部のものではなく、焼却炉や電気集塵器周辺の梁などの上に堆積していたものである。したがって、労働者が日常的に勤務している時に吸入している粉塵とほぼ同質と考えられ、勤務中のダイオキシン類曝露が懸念される。

焼却施設A~Fの労働者の血清中七塩化ジベンゾフランが一般人よりも有意に高かったことは既に報告している^{5, 6)}。今回、さらに7つの焼却施設で追加調査を行い、全体として1,2,3,4,6,7,8-七塩化ジベンゾフランが一般人よりも有意に高く、中央値で約2倍に、平均値で約4倍になっていることが確認された。堆積粉塵中の同族体パターンでも七塩化ジベンゾフランは高く、観察された血清中濃度の上昇は勤務中の取り込みに起因するものと考えられる。また、3種類の血清中六塩化ジベンゾフランが一般人よりも有意に高くなっていることも確認さ

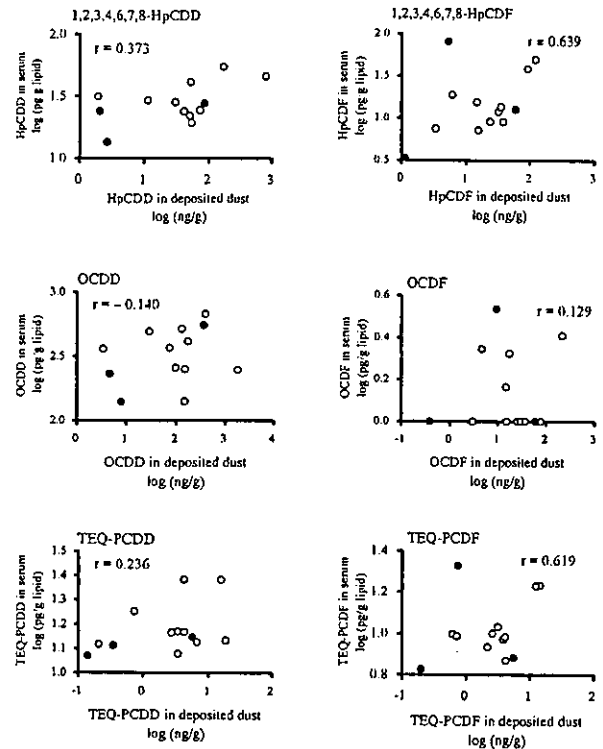


Fig. 1. Relationship between dioxin concentration in deposited dust and that in serum. Both x-axis and y-axis are the logarithm of the dioxin concentration. Black circles are plants J, L and M, where the deposited dust may be different from dust inhaled by workers. The correlation coefficients were calculated using the ten data except for the data obtained from plants J, L and M.

れた。堆積粉塵中の同族体パターンでは六塩化ジベンゾフランはそれほど高いわけではないが、一般人の血清中濃度が低いため(Table 5)、勤務中の取り込みによる上昇が明確になったものと思われる。

堆積粉塵中濃度が高かった八塩化ジベンゾフランについては、労働者の血清中濃度の上昇は明確ではなかった。この物質の血清中濃度は一般人で1 pg/g脂質と低く、かつ個人差も小さい。にもかかわらず、血清中濃度の上昇が明確に見られないのは、この物質の体内での代謝速度が大きい可能性、あるいは排泄速度が大きい可能性が考えられる。

また、堆積粉塵中濃度が高かった七塩化および八塩化ダイオキシンは、労働者の血清中濃度の上昇は見られなかった。これら物質の血清中濃度の平均値は一般人でそれぞれ31および411 pg/g脂質と比較的高く、かつ個人差も大きい。このため勤務中の取り込みによる血清中の上昇が明確にならなかったものと考えられる。

大阪府能勢町の焼却施設労働者の調査¹⁾では、コントロールが設定されていないが、本報のコントロールと比較すると、八塩化ダイオキシンを除くすべての物質の

平均値が1.5倍以上であり、特に、六塩化ジベンゾフランおよび七塩化ジベンゾフランの上昇が著しい。また、Schecterら¹¹⁾は、ドイツの焼却場労働者の調査を行っており、ダイオキシンとジベンゾフランを合わせた毒性等量 (TEQ-PCDD/PCDF) はコントロールと同程度であったと報告している。物質ごとに比較すると、古い焼却場の労働者では、八塩化ダイオキシン、六塩化ジベンゾフランおよび七塩化ジベンゾフランが高くなっていた。本報でも、六塩化ジベンゾフランおよび七塩化ジベンゾフランが上昇しており、これが焼却場労働者の特徴と言える。

堆積粉塵中ダイオキシン類濃度と血清中ダイオキシン類濃度の関係を、七塩化および八塩化ダイオキシン、七塩化および八塩化ジベンゾフラン、そして毒性等量について検討したが、いずれも有意な相関は見られなかった。原因として、比較的近い過去に焼却施設の全面的な工事が行われた場合には、調査時点で堆積していた粉塵が労働者が長期にわたり吸入してきた粉塵と質的に異なっている可能性が考えられる。例えば、施設 M の労働者の血清中七塩化ジベンゾフラン濃度は、平均値で一般人の14倍になっており (Table 4)、明らかに勤務中の取り込みの影響と考えられるが、堆積粉塵中七塩化ジベンゾフラン濃度は1.7 ng/gと調査した施設の中では低い方であった (Table 3)。この施設では調査の前年に全面的な設備の改造工事を行っており、堆積粉塵と労働者が長期にわたり吸入してきた粉塵の質とが大きく異なっていたと考えられる。

ヒトにおけるダイオキシン類の半減期は約7年であり¹²⁾、この期間に施設内の全面的な工事がなければ、調査時に堆積していた粉塵中のダイオキシン類濃度は、労働者が体内に蓄積したダイオキシン類を反映している可能性がある。このため、過去7年以内に全面的な工事を行った3施設を除外して解析を行った。その結果、七塩化ジベンゾフランで有意な正の相関が認められた。したがって、労働者の血清中濃度は堆積粉塵中濃度のある程度反映していると考えられることができる。このことから、焼却施設の堆積粉塵中ダイオキシン類濃度を測定すれば、労働者の血中ダイオキシン類濃度測定を含む健康調査を実施することが必要か、否かの判断がある程度可能と言える。ただし、施設 M のように、施設内の全面的な改造工事などにより、堆積粉塵の質が変化した場合には、このような方法は誤った方針を示すことになる。

5. まとめ

焼却施設 13カ所において、労働者の血清中ダイオキシン類濃度と堆積粉塵中ダイオキシン類濃度を測定し、両者の関係を検討した。その結果、以下のことがわかった。

- 1) 堆積粉塵中ダイオキシン類濃度は0.54～33 ngTEQ/gと一般土壌の1,000～10,000倍のレベルであり、労働者の勤務中のダイオキシン類曝露が懸念された。
- 2) 焼却施設労働者において、3種類の六塩化ジベンゾフランおよび1種類の七塩化ジベンゾフランの血清中濃度、および血清中ジベンゾフラン毒性等量が一般人よりも有意に高くなっており、勤務中の取り込みに起因すると考えられた。
- 3) 堆積粉塵中ダイオキシン類濃度と血清中ダイオキシン類濃度の関係の検討では、過去7年以内に施設の全面的な工事を行った3施設を除けば、七塩化ジベンゾフランで有意な正の相関が見られた。したがって、焼却施設の堆積粉塵中ダイオキシン類濃度を測定すれば、労働者の血中ダイオキシン類濃度測定を含む健康調査を実施することが必要か、否かの判断がある程度可能と言える。

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参考文献

- 1) 豊能郡美化センターダイオキシン問題に係る調査研究委員会。豊能郡美化センターダイオキシン問題に係る調査研究報告書。東京：中央労働災害防止協会、1999。
- 2) ダイオキシン類による健康影響調査研究委員会。清掃業等におけるダイオキシン類等の労働者へのばく露実態の把握に関する調査研究 (平成11年度)。東京：中央労働災害防止協会、2000。
- 3) ダイオキシン類による健康影響調査研究委員会。清掃業等におけるダイオキシン類等の労働者へのばく露実態の把握に関する調査研究 (平成12年度)。東京：中央労働災害防止協会、2001。
- 4) ダイオキシン類による健康影響調査研究委員会。清掃業等におけるダイオキシン類等の労働者へのばく露実態の把握に関する調査研究 (平成13年度)。東京：中央労働災害防止協会、2002。
- 5) Kumagai S, Koda S, Miyakita T, Yamaguchi H, Katagi K, Yasuda N. Polychlorinated dibenzo-p-dioxin and dibenzofuran concentrations in the serum samples of workers at continuously burning municipal waste incinerators in Japan. *Occup Environ Med* 2000; 57: 204-210.
- 6) Kumagai S, Koda S, Miyakita T, Ueno M. Polychlorinated dibenzo-p-dioxin and dibenzofuran concentrations in serum samples of workers at intermittently burning municipal waste incinerators in Japan. *Occup Environ Med* 2002; 59: 362-368.

- 7) 環境庁. 一般廃棄物焼却施設の排ガス中のダイオキシン類濃度等について. (<http://www.env.go.jp/recycle/dioxin/ippai/ippai-nod.pdf>) 東京：環境庁, 2001.
- 8) 環境庁. 産業廃棄物焼却施設の排ガス中のダイオキシン類濃度等について. (<http://www.env.go.jp/recycle/dioxin/sanpai/sanpai-noudo.pdf>) 東京：環境庁, 2001.
- 9) ダイオキシン類による健康影響調査研究委員会. 清掃業等におけるダイオキシン類等の労働者へのばく露実態の把握に関する調査研究委員会報告書 (廃棄物処理施設の作業環境の実態調査). 東京：中央労働災害防止協会, 2000.
- 10) 環境省. ダイオキシン類による人の曝露実態調査について. 東京：環境省, 2002.
- 11) Schecter A, Pöpke O, Ball M, et al. Dioxin concentrations in the blood of workers at municipal waste incinerators. *Occup Environ Med* 1995; 52: 385-387.
- 12) 中央環境審議会環境保健部会, 生活環境審議会, 食品衛生調査会. ダイオキシンの耐容一日摂取量 (TDI) について. 東京：厚生省, 1999.

Relationships between Dioxin Concentrations in Deposited Dust and those in Serum of Workers at Municipal Waste Incineration Plants

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Abstract: Relationships between concentrations of polychlorodibenzo-*p*-dioxins (PCDDs) and polychlorodibenzofurans (PCDFs) in deposited dust and those in the serum of incinerator workers were examined at 13 municipal incineration plants. The following results were found. 1) Dust analysis showed that dominant constituents were octachlorodibenzo-*p*-dioxin (OCDD) and 1, 2, 3, 4, 6, 7, 8-heptachlorodibenzo-*p*-dioxin (HpCDD) among PCDDs, and 1, 2, 3, 4, 6, 7, 8-heptachlorodibenzofuran (HpCDF) and octachlorodibenzofuran (OCDF) among PCDFs. The toxicity equivalents (TEQs) of total PCDDs and PCDFs in the deposited dust were 0.54 to 33 ngTEQ/g, which were about 1,000 to 10,000 times as high as those in general soil. 2) The mean of serum TEQ of PCDDs, that of PCDFs and that of total PCDDs and PCDFs in the incin-

erator workers were 16, 12 and 28 pg TEQ/g lipid, respectively, for all the incinerator workers. Concentrations of hexachlorodibenzofurans (HxCDFs) and HpCDFs in the serum were significantly higher in the incinerator workers than the general population, which suggests that these workers had inhaled these chemicals during their work. 3) The concentration of 1, 2, 3, 4, 6, 7, 8-HpCDF and the TEQ value of PCDFs and in the serum were positively correlated with those in dust except for three plants where large-scale remodeling of the equipment was conducted within the past seven years. Consequently, deposited dust analysis may be useful for judging whether health examination containing blood dioxin measurement is necessary or not.

(*San Ei Shi* 2004; 46: 1-9)

The Proficiency Testing of Determination of Dioxins in Food

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Introduction

Food intake is the main route of human dioxin exposure, making the determination of dioxins in food indispensable for risk assessment and risk management of dioxins. The uncertainty of analytical results, however, can be very great because of the low concentration of the analytes and complicated cleanup procedures. The risk assessment of dioxins based on analytical results also suffers from a similar degree of uncertainty. The Ministry of Health, Labor and Welfare of Japan has published "Guideline for the Determination of Dioxins in Food" to standardize the analytical procedures. The guideline contains the quality assurance procedures to obtain reliable analytical results and recommends participation in the relevant proficiency testing scheme. The proficiency testing provides the fair evaluation of the analytical results. The central science laboratory in England and the food and drug safety center in Japan offer the proficiency testing on food. The National Institute of Health Sciences of Japan (NIHS) also has carried out proficiency testing of dioxins in food since 1998 to assure the quality of analytical results for dioxins. In this presentation we will show the results of 5 rounds of proficiency testing.

Methods and Materials

Samples The samples used in the proficiency testing are listed in Table 1. Table 1 also shows the number of participants and the TEQ of each sample.

BCR CRM607 and BCR RM 534 were prepared by the European Commission's Institute for Reference Materials and Measurements. Eleven certified value for PCDDs and PCDFs were given to CRM607. Eleven values were assigned for PCDDs and PCDFs in RM534 although not certified. CARP-1 was prepared by the National Research Council of Canada. Eighteen concentrations are certified, including PCBs. The custom-prepared standard solutions containing native PCDDs,

PCDFs and PCBs were prepared by Wellington Laboratories (Canada).

Other samples (freeze-dried fish and freeze-dried spinach) were prepared by the Japan Food Research Laboratories. The homogeneity of the samples was verified by the Japan Food Research Laboratories and the NIHS.

Analytical methods All participants determined dioxins by HRGC/HRMS as stipulated in the "Guideline for the Determination of Dioxins in Food".

Statistical analysis The mean and the standard deviation (SD) of the concentrations reported for each compound from the participants were calculated. There was the possibility of outliers, but the application of tests for outliers such as the Grubbs test was not advisable due to the small number of participants. The robust mean and the robust SD were then calculated using algorithm A¹. The RSDs of TEQ in Table 1 were calculated from the robust mean and the robust SD. Examples of the statistical results are shown in Table 2. One participant reported a very high concentration of OCDD. This outlying high value led to the high mean (2.85 pg/g) and the large SD (6.33 pg/g). The robust mean and SD of the same data were 0.67 pg/g and 0.25 pg/g, respectively, after the effect of the outlier was eliminated. The z-Score of each participant was calculated using the robust mean and robust SD. The techniques of participants who gave a z-score of more than 3 or less than -3 were regarded as unsatisfactory, and review of their analytical procedures was recommended.

Results and Discussion

Year 1998 A CRM was used to verify the trueness of the results. The participants used the same standard solution, provided by the NIHS. The mean values of the results reported for two isomers were out of the confidence intervals of the certified values. All the results reported by two participants fell within the 95% confidence interval of the certified value. The other 4 participants reported results outside the 95% confidence interval but *the number of the outlying results was only 1-3. Reproducibility calculated from the 6 participants was 2.8-48 % RSD for each isomer and 6.6 % RSD for total TEQ.

Year 1999 The same CRM was used to compare the results with those in 1998. Many reports suggested that fish is the main route of dioxin intake, making the reliability of analysis of dioxins in fish crucial². CARP-1 was then included in the proficiency testing. One plausible reason for poor reproducibility was the difference

among the standard solutions used by the participants. Mixed standard solutions of PCDDs, PCDFs and PCBs were used to estimate the variation in standard solutions among the participants.

For 6 isomers, the mean of participants was outside the confidence intervals of the certified values. The reproducibility for CRM607 (TEQ) was 11% RSD and larger than that in 1998. The decline in analytical performance probably arose from the difference between standard solutions. In 1998, all participants performed the determinations using the same standard solution. In 1999, each participant used their own standard solution. The number of participants increased to 15 in 1999, and inexperienced laboratories were included. This explains the increase in RSD.

The difference in the mean of the reported value for the mixed standard solution sample and the stated concentration was below 10%. The reproducibility of the standard solution sample was 8-15 RSD %. Bavel reported the RSDs of reported values of participants in proficiency testing in which a standard solution was used³. The RSDs after removing the outliers were, with one exception, 10-17%. These results are similar to ours. The analysis of the solution required no cleanup procedure and the results were expected to represent the variability of the standard solutions of participants. According to the manufacturer's statement, the range of standard solution concentration is $\pm 5\%$, corresponding to an RSD of 2.9%. The higher reproducibility suggested other causes, such as the change in the concentration of the internal standards due to unsuitable storage conditions.

The mean of the reported values for CARP-1 was within the confidence interval of the certified value. The reproducibility of TEQ was 8.0% RSD. The TEQ of CARP-1 was 79 pg/g and was fairly large compared with the CRM607 (3.3). The large TEQ of CARP-1 led to its small reproducibility RSD.

Year 2000 Another RM and a standard solution with different isomer concentrations were used. The mean of the reported value for the RM was lower than the reference value for all compounds with reference values. The reproducibility of RM534 (TEQ) was 18% RSD. The reason for this poor reproducibility was not clear. The bias and reproducibility of the mixed standard solution sample were comparable to those in 1999. Differences in the standard solution used by the participants could not explain the large negative bias or large RSD.

Year 2001 As mentioned above, dioxin intake from marine fish is of great concern, and the use was requested of samples from wild polluted marine fish. The TEQ of CARP-1 is higher than that of wild fish, so it did not seem appropriate for proficiency testing aiming at the assurance of quality for analysis of common foods. Because no appropriate samples made of marine fish were available, we attempted the preparation of our own samples. Since 1998, no vegetable samples had been used in the proficiency testing, in spite of public concern about the contamination of leaf vegetables by dioxins.⁴ For assurance of the performance of the vegetable analysis, a sample made of spinach was also prepared. Both samples were confirmed to be homogeneous and were thus suitable for proficiency testing. The reproducibilities of TEQ for the fish sample and spinach sample were 10% and 30%, respectively. The TEQ of the spinach sample was quite low (0.34 pg/g) at 1/20 of that of the fish sample. The large RSD was not extraordinary taking the low TEQ into consideration.

Year 2002 Another marine fish sample was prepared from grey mullet. Grey mullet contain more fat than sea bass and require further cleanup procedures. The results are likely to represent the actual analytical performance. The reproducibility was 7.1% RSD and comparable to the result of CARP-1.

The results of 5 rounds of proficiency testing revealed several problems with the determination of dioxins in foods. The variability of the standard solution is of major importance. Periodical confirmation of the validity of the standard by the use of CRM or by participation in proficiency testing is strongly recommended.

Although the TEQ of sea bass or grey mullet samples was about 1/10 of that of CARP-1, the reproducibility RSDs were comparable. These results show that repeated participation in proficiency testing improves the analytical skills of the laboratories. It is clear that for proficiency testing, the use of samples representing actual foods is preferable. Our attempted production of samples led to sufficiently homogeneous samples of fish and vegetables that could be prepared by freeze-drying. This technique opens the possibility of preparing samples from a variety of foods, leading to enhanced the effectiveness of proficiency testing.

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