GSTA1 gene was discovered, and the variant allele significantly lowers enzyme expression (Coles et al. 2001, Morel et al. 2002). *GSTM1* and *GSTT1* genes are polymorphic in humans, and the phenotypic absence of enzyme activity is due to the absence of a homozygous and inherited gene (Seidegard et al. 1998, Pemple et al. 1994). GSTM1, a mu class enzyme, detoxifies the reactive metabolites of benzo[a]pyrene and other polycyclic aromatic hydrocarbons (Ketterer et al. 1992). GSTT1 metabolizes various potential carcinogens, such as monohalomethanes, which are widely used as methylating agents, pesticides, and solvents (Guengerich et al. 1995). A polymorphic site at nucleotide 313 (an A-to-G substitution replacing Ile with Val) in the *GSTP1* gene was detected and found to modify the enzyme's specific activity and affinity for electrophilic substrates—for example, benzo[a]pyrene and diol epoxide (Ali-Osman et al. 1997, Watson et al. 1998).

This case control study was carried out to examine whether the genetic polymorphisms of major phase II enzymes *GSTA1*, *GSTT1*, *GSTM1*, and *GSTP1* are associated with the risk of prostate cancer.

Materials and methods

Subjects

The demographic data of both case and control groups are presented in Table 1. The case groups comprised 190 prostate cancer patients (age 70.6 ± 5.9 years) from Kitakyushu City and Miyazaki Prefecture, Japan. The patients were consecutive cases presenting at the University of Occupational and Environmental Health Hospital and Miyazaki University Hospital and had been histologically diagnosed during the period of September 1992 to January 2002. None of the patients refused to participate.

The control group comprised 294 individuals who had visited local medical clinics in Kitakyushu City and Miyazaki City between September 1993 and September 2001 for regular medical health check-ups, including collection of blood and urine specimens (age 67.0 ± 10.4 years). Although no specific age-matching was carried out, the mean ages of the case individuals were similar to the control individuals. The control individuals had no current or previous diagnosis of cancer. All participants completed a questionnaire administered by a trained interviewer that covered medical, residential, occupational, and smoking status. Smoking status was summarized as smoker or never-smoker until the time of the interview. Data for prostate cancer risk factors, such as body mass index, cooking preferences, drug use, and physical activity, were not available. All participants were given an explanation of the nature of the study, and informed consent was obtained. This study was approved by the ethics committees of the University of Occupational and Environmental Health and the University of Miyazaki.

Table 1 Distribution of demographic variables for patients and controls

Variables Age (years)	Controls ($n = 294$)	Patients ($n = 190$)
Mean age (± SD)	67.0 ± 10.4	70.6 ± 5.9
Range	45–94	52–80
Smoking status		
Nonsmoker (%)	91 (31.0%)	57 (30.0%)
Smoker (%)	203 (69.0%)	133 (70.0%)

Genotype analysis

Genomic DNA was isolated from peripheral leukocytes by proteinase K digestion and phenol-chloroform extraction (Sambrook et al. 1989). The genotype of *GSTA1* (*GSTA1*A*-69C and *GSTA1*B*-69T) was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) according to Coles et al. (2001). Briefly, the primers used in the PCR were sense primer (5'-TGT TGA TTG TTT GCC TGA AAT T-3') and antisense primer (5'-GTT AAA CGC TGT CAC CCG TCC T-3'). The amplification was performed by denaturing at 94°C for 5 min, followed by 35 cycles at 94°C for 60 s, annealing at 64°C for 60 s, and extending at 72°C for 60 s in a Perkin-Elmer 9700 (Norwalk, CT, USA). The amplification products (20 µl) were digested by 10 U of restriction endonuclease EarI at 37°C for 12 h (Fig. 1).

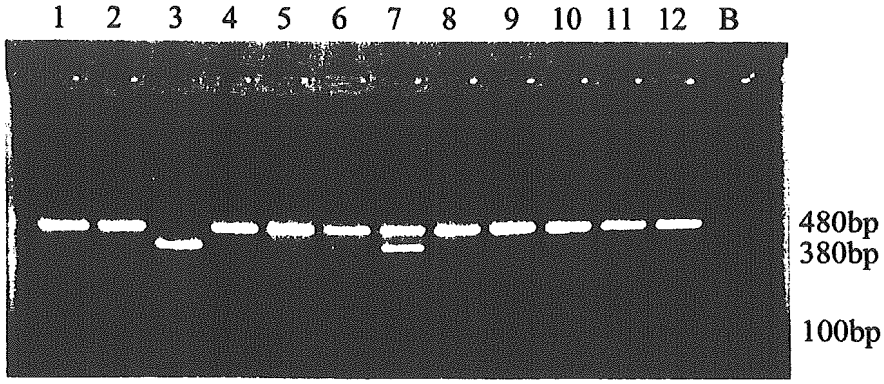

Fig. 1 Examples of restriction fragment length polymorphism (RFLP) of *GSTA1*-specific polymerase chain reaction (PCR) products. The gel shows lanes 1, 2, 4, 5, 6, 8, 9, 10, 11, and 12 homozygous *GSTA1*A* genotype samples; lane 7 a heterozygous genotype sample; lane 3 a homozygous *GSTA1*B* genotype sample; and lane B PCR reagent blank.

A multiple PCR method was used to detect the presence or absence of the *GSTT1* and *GSTM1* genes (Katoh et al. 1996). Briefly, this PCR method had both *GSTT1*- and *GSTM1*-specific primer pairs in the same amplification mixture, and included a third primer pair for β-globin.

The genotype of *GSTP1* exon5 (Ile105Val) was determined by the PCR-RFLP method according to Watson et al. (1998). Briefly, the primers used in the PCR were sense primer (5'-GTA GTT TGC CCA AGG TCA AG-3') and antisense primer (5'-AGC CAC CTG AGG GGT AAG-3'). The amplification products were digested by the restriction endonuclease Alw26I. All digest patterns were determined by resolution on a 2% agarose gel.

Statistical analysis

We used a chi-square test to compare the *GSTA1*, *GSTT1*, *GSTM1*, and *GSTP1* gene polymorphisms in the prostate cancer patients with the expected gene distribution from the healthy control individuals. Crude odds ratios and 95% confidence intervals (CI) were calculated for *GATA1*, *GSTT1*, *GSTM1*, and *GSTP1* genotypes. Odds ratios (OR) were adjusted for age and smoking status by using multiple logistic regression analysis. All statistical analyses were based on two-tailed probabilities. Values of $p < 0.05$ were considered statistically significant. SPSS II for Windows software (version 11.0 J, SPSS Japan, Tokyo, Japan) was used for statistical analysis.

Results

The frequencies of *GSTA1*, *GSTT1*, *GSTM1*, and *GSTP1* genotypes are shown in Table 2. The distribution of *GSTA1*A/*B* genotypes were in good agreement with those expected in a Hardy-Weinberg equilibrium. The frequency of *GSTA1*A/*B* or **B/*B* genotype individuals among prostate cancer cases increased to 26.3% compared with the control groups (19.0%); however, this difference did not reach statistical significance (OR = 1.49; 95% CI, 0.96–2.32) after adjustment for age and smoking status. The *GSTT1* nondeletion genotype was weakly associated with increased incidence of prostate cancer (OR = 1.39; 95% CI, 0.95–2.03). There was no association of the *GSTM1* or *GSTP1 I105V* variant with the risk of prostate cancer.

Based on a hypothesized role for GSTs in modulating the effects of carcinogens present in tobacco smoke, we investigated the combined role of smoking and GSTs. Table 3 outlines the relationship between the *GSTA1*, *GSTT1*, *GSTM1*, and *GSTP1* genotypes and prostate cancer by stratifying by smoking status. Among smokers, the frequency of *GSTA1*A/*B* or **B/*B* genotype was significantly higher in prostate cancer cases (27.8%) compared with the controls (18.2%). The OR of the individuals with *GSTA1*A/*B* or **B/*B* genotype to develop prostate cancer was 1.72 (95% CI, 1.01–2.94). Similarly, the frequency of *GSTT1* nondeletion genotype was significantly higher in prostate cancer cases (63.6%) compared with the controls (51.2%) among smokers (OR = 1.68; 95% CI, 1.06–2.68). No significant associations were observed for genotypes of *GSTM1* and *GSTP1 I105 V* variant with the risk of prostate cancer for either never-smokers or smokers.

To evaluate the interaction between the genotypes, we similarly analyzed the combined genotypes in subgroups (Table 4). The adjusted OR of carrying the combined genotyping of *GSTA1*A/*B* or **B/*B* and *GSTT1* nondeletion was 2.08 (95% CI, 1.14–3.80), with the combined genotyping of *GSTA1*A/*A* and *GSTT1* null as a reference.

Discussion

This study presents the first data on the frequency of the *GSTA1* polymorphism at *GSTA1*A* (-567T, -69C, -52G) and *GSTA1*B* (-567G, -69T, -52A) in a Japanese population. The prevalence of the *GSTA1*A/*A*, **A/*B*, and **B/*B* genotypes in the control population ($n = 294$)

Table 2 Relationship between the *GSTA1*, *GSTT1*, *GSTM1*, and *GSTP1* genotypes and prostate cancer (*OR* odds ratio, *CI* confidence interval)

		Controls % (*n*)	Prostate cancer % (*n*)	OR[a] (95% CI)
GSTA1	**A/*A*	81.0% (238)	73.7% (140)	1
	**A/*B*	17.0% (50)	23.7% (45)	1.48 (0.94–2.35)
	**B/*B*	2.0% (6)	2.6% (5)	1.33(0.39–4.51)
	**A/*B* or **B/*B*	19.0% (56)	26.3% (50)	1.49 (0.96–2.32)
GSTT1	Null genotype	48.3% (139)	39.8% (74)	1
	Nondeletion genotype	51.7% (149)	60.2% (112)	1.39 (0.95–2.03)
GSTM1	Nondeletion genotype	45.5% (131)	50.0% (93)	1
	Null genotype	54.5% (157)	50.0% (93)	0.76 (0.52–1.12)
GSTP1	105 Ile/Ile	72.9% (212)	76.5% (143)	1
	105 Ile/Val	23.7% (69)	20.9% (39)	0.86 (0.55–1.36)
	105 Val/Val	5.4% (10)	2.7% (5)	1.01 (0.32–3.12)
	105 Ile/Val or *105 Val/Val*	27.1% (79)	23.5% (44)	0.87 (0.57–1.35)

[a]ORs were adjusted for age and smoking status; $p < 0.05$

Table 3 Relationship between the *GSTA1*, *GSTT1*, *GSTM1*, and *GSTP1* genotypes and prostate cancer (OR odds ratio, CI confidence interval)

		Controls % (*n*)	Prostate cancer % (*n*)	OR[a] (95% CI)	
	GSTA1	**A/*A*	79.1% (72)	77.2% (44)	1
		**A/*B* or **B/*B*	20.9% (19)	22.8% (13)	1.10 (0.49–2.46)
Never smokers	GSTT1	Null genotype	46.6% (41)	47.4% (27)	1
		Nondeletion genotype	53.4% (47)	52.6% (30)	0.95 (0.49–1.86)
	GSTM1	Nondeletion genotype	37.5% (33)	56.1% (32)	1
		Null genotype	62.5% (55)	43.9% (25)	0.46 (0.23–1.06)
	GSTP1	105 Ile/Ile	71.9% (64)	71.9% (41)	1
		105 Ile/Val or *105 Val/Val*	28.1% (25)	28.1% (16)	1.00 (0.47–2.09)
	GSTA1	**A/*A*	81.8% (166)	72.2% (96)	1
		**A/*B* or **B/*B*	18.2% (37)	27.8% (37)	1.72 (1.01–2.94)[b]
Smokers	GSTT1	Null genotype	48.8% (104)	36.4% (47)	1
		Nondeletion genotype	51.2% (109)	63.6% (82)	1.68 (1.06–2.68)[b]
	GSTM1	Nondeletion genotype	49.3% (105)	47.3% (61)	1
		Null genotype	50.7% (108)	52.7% (68)	0.96 (0.61–1.51)
	GSTP1	105 Ile/Ile	73.3% (148)	78.5% (102)	1
		105 Ile/Val or *105 Val/Val*	26.7% (54)	21.5% (28)	0.84 (0.49–1.44)

[a]ORs were adjusted for age
[b]$p < 0.05$

was 81.0% ($n=238$), 17.0% ($n=50$), and 2.0% ($n=6$), respectively. The distribution of the *GSTA1* polymorphism among different ethnic groups in the literature is as follows: African-American ($n=70$) **A/*A* 61%, **A/*B* 26%, **B/*B* 13%, and Caucasian ($n=278$) **A/*A* 38%, **A/*B* 48%, **B/*B* 14% (Coles et al. 2001). Japanese male genotype frequencies were significantly different from each of these other populations. The comparative genotype frequencies suggest that there may be racial differences in the metabolism of chemicals detoxified by GSTA1, such as activated heterocyclic aromatic amine carcinogen N-acetoxy-PhIP.

In this study, we present the first evidence of an association between *GSTA1*B* (-567G, -69T, -52A) and smoking status among prostate cancer patients. Some reports have shown an association between the incidence of prostate cancer and tobacco smoking (Hickey et al. 2001). We analyzed the prostate cancer risk in relation to *GSTA1* and *GSTT1* genotype and smoking status. Our results showed that *GSTA1*A/*B* or **B/*B* genotypes were associated with a 49% higher but nonstatistically significant increased risk of prostate cancer (OR = 1.49; 95% CI, 0.96–2.32). However, among smokers, the OR of the individuals with these genotypes to develop prostate cancer was 1.72 (95% CI, 1.01–2.72). GSTA1 has been reported to be most efficient in detoxifying N-acetoxy-PhIP, and its presence in tobacco smoke is 22.9 ng/cig (Smith et al. 2001). Therefore, we considered that GSTA1 might play an important role in protecting DNA from tobacco-derived PhIP. Although this observation needs further study, the effect of smoking may be more important for susceptible populations such as those with *GSTA1*A/*B* or **B/*B* genotypes.

Table 4 Combined effects of *GSTA1* and *GSTT1* genotypes among Japanese prostate cancer patients and control individuals (*OR* odds ratio,*CI* confidence interval)

GSTA1	GSTT1	Controls	Cases	OR[a] (95% CI)
**A/*A*	Null	112	56	1
	Nondeletion	120	80	1.36 (0.88–2.10)
**A/*B* or **B/*B*	Null	27	18	1.45 (0.73–2.89)
	Nondeletion	29	32	2.08 (1.14–3.80)[b]

[a]Odds ratios were calculated by comparing control individuals and prostate cancer groups, adjusted for age and smoking status
[b]$p=0.018$

Rebbeck's group reported the *GSTT1* nondeletion genotype to be associated with prostate cancer risk (OR = 1.83; 95% CI, 1.19–2.80) (Rebbeck et al. 1999). Murata's group also reported similar results without statistical significance (OR = 1.6; 95% CI, 0.99–2.51) (Murata et al. 2001). Furthermore, Kelada's group reported a significant interaction between *GSTT1* nondeletion genotype and smoking that elevates the risk of prostate cancer (Kelada et al. 2000). Our results are similar to theirs (OR = 1.39; 95% CI, 0.95-2.03, and for smokers OR = 1.68, 95% CI, 1.06–2.68). These findings are consistent with the knowledge that GSTT1 produces genotoxic metabolites in response to specific exposure such as methyl chloride in cigarette smoke and dichloromethanes (Hallier et al. 1994). GSTT1 is expressed at high levels in the prostate, suggesting that GSTT1 may play a role in prostate carcinogenesis, especially among smokers.

To evaluate the interaction between the genotypes, we analyzed combined genotypes of *GSTA1* and *GSTT1*. The OR of carrying the combined genotyping of *GSTA1*A/*B* or **B/*B* and *GSTT1* nondeletion was 1.36, 1.45, and 2.08 with the combined genotyping of *GSTA1*A/*A* and *GSTT1* nondeletion, *GSTA1*A/*B* or **B/*B* and *GSTT1* null, *GSTA1*A/*A* and *GSTT1* null as a reference. These results suggest that the combined genotyping of *GSTA1*A/*B* or **B/*B* and *GSTT1* nondeletion may be strongly linked to prostate cancer. We considered that this interaction may be caused by

different chemical carcinogens, such as PhIP and methyl chloride, but that the most important and common origin of the chemicals associated with this interaction is tobacco smoke.

On the other hand, no significant association was observed for genotypes of *GSTM1* and *GSTP1 I105V*. Rebbeck's group and Jeronimo's groups reported similar results (Rebbeck et al. 1999, Jeronimo et al. 2002). GSTM1 and GSTP1 metabolize a variety of potential carcinogens, including cigarette smoke-derived chemicals such as benzo[a]pyrene. Nelson et al. reported that GSTP1 has been shown to inhibit the adduction of activated PhIP metabolites to DNA in cell-free systems (Nelson et al. 2001); however, GSTP1 did not play an important role in prostate carcinogenesis in our study.

In conclusion, our data show a significant relationship between prostate cancer and genetic polymorphism of *GSTA1* and *GSTT1*, especially among smokers. These findings may be helpful for researching the risk for, and identifying individuals susceptible to, prostate cancer.

Acknowledgements This work was supported in part by a grant-in-aid for risk analysis research on food and pharmaceuticals and cancer research from the Ministry of Health, Labour and Welfare of Japan.

References

Ali-Osman F, Akanda O, Antoun G, Maso JX, Buolamwini J (1997) Molecular cloning, characterization, and expression in *Escherichia coli* of full-length cDNA of three human glutathione S-transferase Pi gene variants. J Biol Chem 272:10004–10021

Coles BF, Morel F, Rauch C, Huber WW, Yang M, Teitel CH, Green B, Lang NP, Kadlubar FF (2001) Effect of polymorphism in the human glutathione S-transferase A1 promotor on hepatic GSTA1 and GSTA2 expression. Pharmacogenetics 11:663–669

Gronberg H (2003) Prostate cancer epidemiology. Lancet 361:859–864

Guengerich FP, Thier R, Persmark M, Taylor JB, Pemple SE, Ketterer B (1995) Conjugation of carcinogens by theta class glutathione S-transferase: mechanisms and relevance to variations in human risk. Pharmacogenetics 5:S103–S107

Hallier E, Schroeder KR, Asmuth K, Donnermuth A, Aust B, Goergens HW (1994) Metabolism of dichloromethane (methylene chloride) to formaldehyde in human erythrocytes: influences of polymorphism of glutathione transferase theta (GSTT1-1). Arch Toxicol 68:423–427

Hayes RB, Ziegler RG, Gridley G (1999) Dietary factors and risks for prostate cancer among blacks and whites in the United States. Cancer Epidemiol Biomarkers Prev 8:25–34

Hickey K, Do K-A, Green A (2001) Smoking and prostate cancer. Epidemiol Rev 23:115–125

Jeronimo C, Varzim G, Henrique R, Oliveira J, Bento MJ, Silva C, Lopes C, Sidransky D (2002) I105 V polymorphism and promotor methylation of the GSTP1 gene in prostate adenocarcinoma. Cancer Epidemiol Biomarkers Prev 11:445–450

Katoh T, Nagata N, Kuroda Y, Itoh H, Kawahara A, Kuroki N, Ookuma R, Bell DA (1996) Glutathione S-transferaseM1(GSTM1) and T1(GSTT1) genetic polymorphism and susceptibility to gastric and colorectal adenocarcinoma. Carcinogenesis 17:1855–1859

Kelada SR, Kardia SLR, Walker AH, Wein AJ, Malkowicz SB, Rebbeck TR (2000) The glutathione S-transferase-μ and -θ genotypes in the etiology of prostate cancer: genotype-environment interactions with smoking. Cancer Epidemiol Biomarkers Prev 9:1329–1334

Ketterer B, Harris JM, Talaska G, Meyer DJ, Pemble SE, Taylor JB, Lang NP, Kadlubar FF (1992) The human glutathione S-transferase supergene family, its polymorphism, and its effects on susceptibility to lung cancer. Environ Health Perspect 98:87–94

Lin D, Meyer DJ, Ketterer B, Lang NP, Kadlubar FF (1994) Effect of human and rat glutathione S-transferase on the covalent DNA binding of the N-acetoxy derivatives of heterocyclic amine carcinogens in vitro: a possible mechanism of organ specificity in their carcinogenesis. Cancer Res 54:4920–4926

Morel F, Rauch C, Coles B, Ferrec EL, Guillouzo A (2002) The human glutathione transferase alpha locus: genomic organization of the gene cluster and functional characterization of the genetic polymorphism in the hGSTA1 promotor. Pharmacogenetics 12:277–286

Murata M, Wtanabe M, Yamanaka M, Kubota Y, Itoh H, Nagao M, Katoh T, Kamataki T, Kawanura J, Yatani R, Shiraishi T (2001) Genetic polymorphisms in cytochrome P450 (CYP)1A1, CYP1A2, CYP2E1, glutathione S-transferase (GST) M1 and GSTT1 and susceptibility to prostate cancer in the Japanese population. Cancer Lett 165:171–177

Nelson CP, Kidd CR, Sauvageot J, Isaacs WB, De Marzo AM, Groopman JD, Nelson WG, Kensler TW (2001) Protection against 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b]pyridine cytotoxicity and DNA adduct formation in human prostate by glutathione S-transferase P1. Cancer Res 61:103–109

Pemple S, Schroeder KR, Spencer SR, Meyer DJ, Hallier E, Blot HM, Kettler B, Taylor JB (1994) Human glutathione S-transferase cθ (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J 300:271–276

Rebbeck TR, Walker AH, Jaffe JM, White DL, Wein AJ, Malkowicz SB (1999) Glutathione S-transferase-μ (GSTM1) and -θ (GSTT1) genotypes in the etiology of prostate cancer. Cancer Epidemiol Biomarkers Prev 8:283–287

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York, pp 9.1–9.62

Seidegard J, Vorachek WR, Pero RW (1998) Hereditary differences in the expression of the human glutathione transferase active on trans-stilbene oxide are due to gene deletion. Proc Natl Acad Sci USA 85:7293–7297

Shirai T, Sano M, Tamano S, Takahashi S, Hirose M, Futakuchi M, Hasegawa R, Imaida K, Matsumoto K, Wakabayashi K, Sugimura T, Ito N (1997) The prostate: a target for carcinogenicity of 2-amino-1-metyl-6-phenylimidazo[4,5-b]pyridine(PhIP) derived from cooked foods. Cancer Res 57:195–198

Smith CJ, Perfetti TA, Rumple MA, Rodgman A, Doolittle DJ (2001) "IARC Group 2B carcinogen" reported in cigarette mainstream smoke. Food Chem Toxicol 39:183–205

Stuart GR, Holcroft J, Boer JG, Glickman BW (2000) Prostate mutations in rats induced by the suspected human carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. Cancer Res 60:266–268

Veierod MB, Laake P, Thelle DS (1997) Dietary fat intake and risk of prostate cancer:a prospective study of 25708 Norwegian men. Int J Cancer 73:634–638

Watson MA, Stewart RK, Smith GBJ, Massey TE, Bell DA (1998) Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. Carcinogenesis 19:275–280

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Science of the Total Environment xx (2005) xxx–xxx

Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Humankind

ELSEVIER

www.elsevier.com/locate/scitotenv

Fish intake and serum levels of organochlorines among Japanese women

H. Tsukino[a], T. Hanaoka[a,*], H. Sasaki[b], H. Motoyama[b], M. Hiroshima[b], T. Tanaka[b], M. Kabuto[c], W. Turner[d], D.G. Patterson Jr.[d], L. Needham[d], S. Tsugane[a]

[a]*Epidemiology and Prevention Division, Research Center for Cancer prevention and Screening, National Cancer Center 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan*
[b]*Department of Obstetrics and Gynecology, Jikei University School of Medicine, 3-25-8 Nishishimbashi, Minato-ku, Tokyo 105-8461, Japan*
[c]*National Institute for Environmental Studies, 16-2 Onogawa, Ibaragi, Tsukuba, 305-0053, Japan*
[d]*Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy, F-17, Atlanta, Georgia 30341-3724, USA*

Received 24 August 2004; accepted 20 April 2005

Abstract

This study evaluates background serum levels of selected organochlorine compounds among Japanese women of reproductive age and investigates whether lifestyle factors, especially dietary factors, may be associated with these levels. A cross-sectional study was performed on 80 Japanese women, aged 26–43 years, who complained of infertility and were confirmed not to have endometriosis. The serum levels of total toxic equivalency (TEQ), 18 polychlorinated dibenzo-*p*-dioxins (PCDDs)/polychlorinated dibenzofurans (PCDFs), 4 coplanar polychlorinated biphenyls (cPCBs), 36 *ortho*-substituted polychlorinated biphenyls (PCBs), and 13 chlorinated pesticides or their metabolites were measured and data were collected on the women's age, residence, occupation, body mass index (BMI), smoking and alcohol habit and 6 dietary intakes (fish, meats, rice, vegetables, fruits and dairy products). The serum median level of total TEQ was 25.1 pg TEQ/g lipid, that of PCDDs/PCDFs/cPCBs was 11.5 pmol/g lipid, that of PCBs was 0.46 nmol/g lipid, and that of total pesticides was 1.32 nmol/g lipid. The serum levels of total TEQ, PCDDs/PCDFs/cPCBs, PCBs and pesticides were positively associated with age (P for trend=0.003, 0.01, 0.005 and 0.01, respectively) and frequent fish consumption (P for trend=0.002, 0.003, 0.0003 and 0.006, respectively). Other lifestyle factors were not associated with serum organochlorine levels. The present study suggests that Japanese women who consume fish frequently in their reproductive period tend to accumulate organochlorines in their bodies.

Keywords: Fish intake; Organochlorines; Dioxins; Polychlorinated biphenyls; Pesticides; DDT

* Corresponding author. Tel.: +81 3 3547 5201x3391; fax: +81 3 3547 5878.
E-mail address: thanaoka@gan2.res.ncc.go.jp (T. Hanaoka).

0048-9697/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.scitotenv.2005.04.014

1. Introduction

Many environmental organochlorine pollutants including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), coplanar polychlorinated biphenyls (cPCBs), *ortho*-substituted polychlorinated biphenyls (PCBs) and chlorinated pesticides have the potential to mimic or antagonize naturally occurring hormones and might affect wildlife and humans adversely (Kavlock et al., 1996; Wolff et al., 1993). PCDDs and PCDFs were unintended byproducts of several industries and processes: the herbicide industry, the chlorine and paper industry, melting processes and incineration of waste. More than 80% of total PCDDs and PCDFs released into the environment in Japan have been estimated to be derived from incinerators (Watanabe et al., 1999). PCBs and pesticides were used in industry and agriculture until the early 1970s. These organochlorines are resistant to metabolism and are lipid soluble; they bioaccumulate in the food chain, and are found in human adipose tissue, blood, and breast milk (Safe, 2000). Human exposure to organochlorines occurs almost exclusively through food consumption. Various kinds of fish from several supermarkets in Japan were reported to contain high levels of PCDDs, PCDFs and PCBs, and the mean daily intake of total toxic equivalency (TEQ) values of PCDDs, PCDFs and PCBs from fish and shellfish were higher than that from other foods (Tsutsumi et al., 2001). The main foods contributing to dietary intake of chlorinated pesticides including total hexachlorocyclohexane (HCH) and total bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT) have been also reported to be fish and meat among the Japanese population (Nakagawa et al., 1995). These findings suggest that people who often consume fish or meat would accumulate higher levels of PCDDs, PCDFs, PCBs and pesticides in their body.

In the present study, we measured serum levels of organochlorines including 8 PCDDs, 10 PCDFs, 4 cPCBs, 36 PCBs, and 13 chlorinated pesticides or their metabolites in 80 Japanese infertile women in a hospital-based cross-sectional study. The object of the present study was to evaluate background levels of exposure to organochlorines in Japanese women of reproductive age, and to estimate the effect of lifestyle factors, especially dietary factors, on serum organochlorine levels.

2. Subjects and methods

2.1. Subjects and sample collection

Eligible subjects were women aged 20 to 45 years who complained of infertility and consulted doctors in the Department of Obstetrics and Gynecology, the Jikei University School of Medicine, from 1999 to 2000. A total of 139 women were diagnosed laparoscopically according to the revised classification of the American Fertility Society (1985). Fifty-eight women with stage II or greater endometriosis were designated 'cases.' Eighty-one women who were laparoscopically confirmed not to have endometriosis (stage 0 or I) were designated 'controls.' Because accumulation of organochlorines in the body has been proposed as a risk factor for endometriosis (Rier et al., 1993), endometriosis cases might present higher serum organochlorine levels. Of eighty-one control subjects, we excluded one subject whose serum PCB levels could not be measured because of small blood sample volume. Consequently, eighty subjects, aged 26–43 (mean age, 32.9 years), were included in this study. All subjects gave their written informed consent. The study protocol was approved by the Institutional Review Board of the Jikei University School of Medicine, National Cancer Center, National Institute for Environmental Studies, and U.S. Centers for Disease Control and Prevention (CDC).

A fasting blood sample was obtained before the laparoscopic examination. Serum was immediately collected by centrifugation, transferred into a stock tube and stored at −80 °C until analyzed.

2.2. Questionnaire survey

Subjects were interviewed by a single trained interviewer using a structured questionnaire before the laparoscopic examination. The questionnaire included demographic and anthropometric information, occupation, and use of alcohol and tobacco. Regarding dietary habits, subjects were asked how often they consumed 6 food items (fish, meat, rice, vegetable, fruit and dairy products) over the previous year. The frequency of dietary intake was classified into nine categories, i.e., rare, 1–3 times/month, 1–2 times/week, 3–4 times/week, 5–6 times/week, 1 time/day, 2–3 times/day, 4–6 times/day, and more than 7 times/day.

t1.1 Table 1
t1.2 Lipid-adjusted serum median levels of organochlorines and total TEQs among Japanese women

t1.3		Subjects with detectable values[a]	Median level (25th, 75th)[b]	Mean LOD (SD/Maximum)
t1.4	PCDDs/PCDFs/cPCBs (pg/g lipid)			
t1.5	2,3,7,8-TetraCDD[c]	7/80	<LOD (<LOD,<LOD)	2.6 (1.8/12.2)
t1.6	1,2,3,7,8-PentaCDD[c]	30/80	<LOD (<LOD, 5.5)	3.1 (2.2/15.9)
t1.7	1,2,3,4,7,8-HexaCDD[c]	5/80	<LOD (<LOD, <LOD)	5.7 (4.2/25.1)
t1.8	1,2,3,6,7,8-HexaCDD[c]	76/80	26.1 (20.7, 37.1)	5.0 (4.0/26.1)
t1.9	1,2,3,7,8,9-HexaCDD[c]	34/80	<LOD (<LOD, 4.6)	5.2 (4.0/27.1)
t1.10	1,2,3,4,6,7,8-HeptaCDD[c]	77/80	16.8 (13.2, 23.7)	5.6 (4.3/31.8)
t1.11	1,2,3,4,6,7,8,9-OctaCDD[c]	80/80	265.5 (196.0, 389.0)	102.7 (87.2/637.0)
t1.12	2,3,7,8-TetraCDF[c]	1/80	<LOD (<LOD, <LOD)	2.58 (1.6/12.7)
t1.13	1,2,3,7,8-PentaCDF[c]	1/80	<LOD (<LOD, <LOD)	2.9 (2.0/14.3)
t1.14	2,3,4,7,8-PentaCDF[c]	75/80	11.3 (8.7, 13.8)	3.0 (2.1/14.9)
t1.15	1,2,3,4,7,8-HexaCDF[c]	78/80	6.2 (4.3, 8.4)	3.4 (2.5/16.4)
t1.16	1,2,3,6,7,8-HexaCDF[c]	76/80	6.1 (5.0, 7.9)	3.3 (2.5/17.2)
t1.17	1,2,3,7,8,9-HexaCDF[c]	1/80	<LOD (<LOD, <LOD)	3.5 (2.7/18.6)
t1.18	2,3,4,6,7,8-HexaCDF[c]	47/80	2.0 (<LOD, 3.6)	3.5 (2.6/17.3)
t1.19	1,2,3,4,6,7,8-HeptaCDF[c]	39/80	<LOD (<LOD, 5.4)	4.4 (3.4/24.3)
t1.20	1,2,3,4,7,8,9-HeptaCDF[c]	1/80	<LOD (<LOD, <LOD)	4.8 (3.6/24.0)
t1.21	3,4,4′,5-TetraCB[c]	79/80	8.2 (6.3, 11.6)	5.7 (3.8/26.8)
t1.22	3,3′,4,4′,5-PentaCB[c]	77/80	47.6 (31.9, 70.2)	5.4 (3.7/22.7)
t1.23	3,3′,4,4′,5,5′-HexaCB[c]	80/80	34.0 (26.5, 43.7)	6.4 (4.8/31.7)
t1.24	PCBs (IUPAC nos.) (ng/g lipid)			
t1.25	PCB44	76/80	4.4 (3.2, 5.7)	4.6 (4.8/18.2)
t1.26	PCB49	63/80	3.0 (1.8, 4.0)	3.7 (3.0/11.9)
t1.27	PCB52	71/80	5.5 (3.3, 7.7)	3.9 (3.5/13.8)
t1.28	PCB66	79/80	1.8 (1.5, 2.6)	4.4 (2.5/15.4)
t1.29	PCB74	80/80	6.4 (4.9, 9.2)	2.8 (1.6/9.9)
t1.30	PCB87	79/80	1.1 (0.8, 1.9)	2.6 (1.5/9.3)
t1.31	PCB99	80/80	6.8 (4.5, 9.5)	2.5 (1.5/8.5)
t1.32	PCB101	79/80	2.6 (1.9, 4.6)	3.8 (2.2/14.8)
t1.33	PCB105[c]	80/80	2.0 (1.4, 2.9)	4.0 (2.5/19.8)
t1.34	PCB110	79/80	1.8 (1.1, 2.9)	3.6 (2.1/13.9)
t1.35	PCB118[c]	79/79	10.5 (7.2, 15.0)	6.5 (3.6/25.2)
t1.36	PCB128	67/80	0.3 (0.2, 0.5)	2.4 (1.5/8.3)
t1.37	PCB138+158[d]	80/80	16.8 (11.6, 26.2)	2.9 (1.6/10.3)
t1.38	PCB146	80/80	5.9 (3.3, 7.8)	2.5 (1.4/8.6)
t1.39	PCB151	73/73	0.6 (0.4, 0.8)	2.5 (1.5/9.3)
t1.40	PCB153	80/80	36.6 (23.3, 51.0)	2.7 (1.6/10.1)
t1.41	PCB156[c]	79/79	3.4 (2.3, 4.9)	5.5 (3.3/22.9)
t1.42	PCB157[c]	75/76	0.9 (0.6, 1.3)	6.4 (3.8/26.6)
t1.43	PCB167[c]	71/72	1.6 (0.9, 2.2)	7.8 (5.1/36.0)
t1.44	PCB170	80/80	8.2 (5.1, 11.4)	2.6 (1.4/9.29)
t1.45	PCB172	80/80	1.4 (0.9, 2.0)	2.7 (1.6/11.0)
t1.46	PCB178	78/80	2.0 (1.2, 2.7)	2.6 (1.5/9.7)
t1.47	PCB180	80/80	21.6 (12.9, 28.9)	2.7 (1.6/11.0)
t1.48	PCB183	80/80	2.4 (1.5, 3.2)	2.6 (1.5/9.7)
t1.49	PCB187	80/80	8.7 (5.0, 12.5)	2.6 (1.4/9.2)
t1.50	PCB189[c]	60/60	0.4 (0.2, 0.6)	8.4 (5.3/40.9)
t1.51	PCB194	80/80	2.4 (1.5, 3.7)	3.9 (2.8/23.9)
t1.52	PCB195	79/80	0.9 (0.6, 1.4)	7.9 (5.7/47.9)
t1.53	PCB196+203[d]	80/80	1.9 (1.3, 2.9)	2.6 (1.6/9.7)
t1.54	PCB201	79/80	2.4 (1.6, 3.7)	2.6 (1.6/9.7)
t1.55	PCB206	78/80	0.8 (0.5, 1.1)	7.4 (4.8/40.3)
t1.56	PCB209	80/80	0.7 (0.5, 0.9)	9.6 (6.5/56.6)

(continued on next page)

Table 1 (*continued*)

	Subjects with detectable values[a]	Median level (25th, 75th)[b]	Mean LOD (SD/Maximum)
Pesticides (ng/g lipid)			
HCB	2/80	<LOD (<LOD, <LOD)	16.1 (2.7/25.7)
b-HCCH	80/80	93.2 (60.8, 171.0)	8.1 (1.4/13.0)
g-HCCH	2/80	<LOD (<LOD, <LOD)	8.1 (1.4/13.0)
H.EPOX	8/80	<LOD (<LOD, <LOD)	8.1 (1.4/13.0)
Oxychlordane	54/80	9.0 (<LOD, 12.2)	8.1 (1.4/13.0)
trans-NONA	78/80	20.9 (16.0, 29.3)	8.1 (1.4/13.0)
pp-DDE	80/80	221.0 (146.0, 358.5)	8.1 (1.4/13.0)
Dieldrin	4/80	<LOD (<LOD, <LOD)	6.3 (1.1/10.1)
op-DDT	0/80	<LOD (<LOD, <LOD)	16.2 (2.8/25.9)
pp-DDT	7/80	<LOD (<LOD, <LOD)	16.2 (2.8/25.9)
Mirex	1/80	<LOD (<LOD, <LOD)	8.1 (1.4/13.0)
Total TEQ values (pg TEQ/g lipid)			
PCDDs	80/80	8.6 (6.4, 10.8)	
PCDFs	80/80	7.5 (6.3, 9.0)	
cPCBs	80/80	5.1 (3.5, 7.4)	
PCBs	80/80	3.6 (2.4, 5.0)	
PCDDs/PCDFs	80/80	16.1 (13.0, 19.6)	
PCDDs/PCDFs/cPCBs	80/80	21.6 (17.4, 26.9)	
Sum	80/80	25.1 (20.3, 31.8)	

Abbreviations: LOD—limit of detection; SD—standard deviation; CDD—chlorodibenzo-*p*-dioxin; CDF—chlorodibenzofuran; CB—chlorobiphenyl; HCB—hexachlorobenzene; HCCH—hexachlorocyclohexane; H.EPOC—heptachlor epoxide; NONA—nonachlor; DDE—bis(4-chlorophenyl)-1,1-dichloroethene; DDT—bis(4-chlorophenyl)-1,1,1-trichloroethene; TEQ—toxic equivalency.

[a] Number of subjects with values above LOD/number of measured subjects.

[b] 25th—25th percentile; 75th—75th percentile.

[c] WHO-TEF values were assigned.

[d] Combined levels for PCB138,158 and PCB196,203 were analyzed.

2.3. Analytical methods

Serum analyses for a total of 71 compounds, 8 PCDDs, 10 PCDFs, 4 cPCBs, 36 PCBs and 13 selected persistent chlorinated pesticides or their metabolites, were performed at the U.S. Centers for Disease Control and Prevention (CDC) by gas chromatography/high-resolution isotope dilution mass spectrometry. The analytical methods and quality control procedures have been described previously (DiPietro et al., 1997; Patterson et al., 1987; Turner et al., 1994). Because the PCDDs, PCDFs, cPCBs, PCBs and pesticides are lipophilic and concentrate in the body's lipid stores including the lipid in serum, the serum levels for these compounds were adjusted for serum lipid levels. Triglycerides and total cholesterol were used in calculating the total lipid level (2.27 × total cholesterol+triglycerides+62.3). The mean volume of blood used for the analyses was 7.99 g (range: 0.89–13.2 g). Limits of detection (LOD) on a lipid-adjusted basis were calculated for each sample. Because we could not measure PCB138 and 158, or PCB196 and 203 separately, combined values for PCB138/158 and PCB196/203 were reported. The list of organochlorines measured and their mean LOD values are shown in Table 1. TEQ was assessed using the "toxic equivalency factor" (TEF) based upon the relative potency of each congener compared to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as the most potent of the compounds (Van den Berg et al., 2000). The World Health Organization (WHO)-TEF values were assigned 7 PCDDs, 10 PCDFs, 4 cPCBs and 6 PCBs to calculate TEQ values of PCDDs, PCDFs, cPCBs and PCBs in this study (Table 1) (Van den Berg et al., 1998). For values below LOD ('<LOD'), a value of one half the LOD was assigned (Hornung and Reed, 1990). The results were essentially similar when zeros were assigned to values <LOD.

2.4. Statistical analysis

Total levels of PCDDs/PCDFs/cPCBs, PCBs and pesticides were calculated for the sum of serum molar concentration of PCDDs/PCDFs/cPCBs, PCBs and

pesticides, respectively. Differences in log-transformed levels of total TEQ, PCDDs/PCDFs/cPCBs, PCBs and pesticides between subgroups were tested by the analysis of variance (PROC GLM, SAS, SAS Institute Inc., Cary, NC). Tests for trend were assessed by using serum organochlorine levels as continuous variables. P values less than 0.05 (two-tail) were considered to be statistically significant. All analyses were conducted using the SAS (version 8.2) program.

Table 2
Comparisons of serum organochlorine levels according to age, residence, occupation, BMI, smoking and alcohol habit among Japanese women

Variable	No.[a]	Total TEQ (pg TEQ/g lipid)	Total PCDDs/PCDFs/ cPCBs (pmol/g lipid)	Total PCBs (nmol/g lipid)	Total pesticides (nmol/g lipid)	p,p′-DDE (ng/g lipid)
		Median (25th, 75th)[b]	Median (25th, 75th)[b]	Median (25th, 75th)[b]	Median (25th, 75th)[b]	Median (25th, 75th)[b]
Total	80	25.1 (20.3, 31.8)	1.15 (0.84, 1.50)	0.46 (0.35, 0.66)	1.32 (0.92, 1.93)	221.0 (146.0, 358.5)
Age (years)						
24–29	15	23.7 (19.5, 25.7)	1.13 (0.78, 1.35)	0.41 (0.37, 0.54)	1.04 (0.83, 1.38)	191.0 (146.0, 271.0)
30–35	45	23.5 (19.6, 28.7)	1.13 (0.86, 1.41)	0.41 (0.31, 0.63)	1.18 (0.84, 1.70)	201.0 (119.0, 346.0)
36–43	20	31.9 (24.6, 39.0)	1.27 (0.91, 2.25)	0.61 (0.52, 0.83)	1.89 (1.42, 2.62)	297.5 (212.0, 537.0)
P for difference[c]		0.005	0.01	0.003	0.002	0.014
P for trend		0.003	0.01	0.005	0.01	0.02
Residence						
Residential area	61	24.6 (20.5, 32.0)	1.14 (0.84, 1.46)	0.45 (0.34, 0.63)	1.32 (0.89, 1.91)	221.0 (143.0, 323.0)
Shopping or office area	6	29.3 (28.6, 29.6)	1.15 (0.81, 1.27)	0.64 (0.45, 0.72)	1.64 (1.33, 1.70)	287.0 (215.0, 358.0)
Agricultural or fishing area	7	26.0 (17.8, 42.9)	1.41 (0.85, 1.86)	0.53 (0.38, 0.88)	1.26 (0.93, 3.24)	244.0 (146.0, 519.0)
Industrial area	2	26.7 (12.2, 41.2)	1.42 (0.62, 2.23)	0.42 (0.25, 0.60)	2.03 (0.95, 3.11)	441.5 (179.0, 704.0)
P for difference[c]		0.79	0.90	0.47	0.29	0.23
Occupation						
Office worker	43	24.6 (20.1, 32.0)	1.07 (0.83, 1.36)	0.47 (0.32, 0.63)	1.23 (0.85, 1.78)	215.0 (126.0, 318.0)
Specialist	18	25.3 (19.8, 32.2)	1.20 (0.86, 1.61)	0.48 (0.40, 0.71)	1.61 (1.01, 2.54)	250.0 (179.0, 592.0)
Others[d]	13	29.2 (20.7, 32.5)	1.35 (0.78, 1.86)	0.46 (0.38, 0.67)	1.70 (0.89, 1.97)	225.0 (179.0, 359.0)
P for difference[c]		0.86	0.09	0.54	0.29	0.44
BMI						
<19.4	25	27.4 (21.1, 32.5)	1.18 (0.83, 1.46)	0.54 (0.46, 0.67)	1.38 (0.93, 1.83)	221.0 (143.0, 346.0)
19.4–21.0	26	25.8 (20.7, 33.5)	1.18 (0.97, 1.53)	0.44 (0.32, 0.63)	1.32 (0.89, 1.95)	202.0 (150.0, 386.0)
>21.0	25	24.0 (17.8, 26.2)	1.00 (0.84, 1.29)	0.41 (0.31, 0.66)	1.33 (1.07, 2.33)	230.0 (146.0, 323.0)
P for difference[c]		0.22	0.27	0.02	0.82	0.66
P for trend		0.10	0.18	0.06	0.91	0.82
Smoking						
Never	50	25.7 (20.9, 32.2)	1.13 (0.84, 1.46)	0.48 (0.36, 0.66)	1.44 (1.01, 1.95)	228.5 (161.0, 359.0)
Past	10	24.4 (17.7, 31.7)	1.16 (0.83, 1.86)	0.45 (0.36, 0.60)	0.89 (0.62, 2.39)	157.5 (109.0, 453.0)
Current	16	24.6 (18.3, 31.2)	1.19 (0.82, 1.41)	0.41 (0.30, 0.67)	1.12 (0.84, 1.68)	195.5 (132.5, 282.5)
P for difference[c]		0.51	0.27	0.21	0.23	0.24
P for trend		0.36	0.24	0.24	0.89	0.54
Alcohol drinking						
≦3 times a month	37	23.3 (20.0, 26.2)	1.00 (0.83, 1.27)	0.41 (0.33, 0.60)	1.26 (0.92, 1.63)	203.0 (146.0, 281.0)
1–4 times a week	24	26.6 (22.3, 34.3)	1.26 (1.03, 1.94)	0.52 (0.36, 0.77)	1.65 (0.88, 2.55)	226.5 (134.0, 409.0)
≧5 times a week	15	30.1 (17.0, 34.0)	1.21 (0.99, 1.36)	0.60 (0.37, 0.72)	1.45 (0.68, 2.34)	256.0 (105.0, 618.0)
P for difference[c]		0.06	0.20	0.20	0.37	0.33
P for trend		0.06	0.40	0.20	0.11	0.16

[a] Total number of subjects for each item varied due to missing information.
[b] 25th—25th percentile; 75th—75th percentile.
[c] Differences in log-transformed levels between subgroups were tested by the analysis of variance.
[d] Others include merchant, housewife and so on.

3. Results

In this study, three PCDDs/PCDFs/cPCBs (1,2,3,4,6,7,9-heptachlorodibenzo-*p*-dioxin, octachlorodibenzofuran and 3,3′,4,4′-tetrachlorobiphenyl), four PCBs (International Union of Pure and Applied Chemistry nos. 18, 28, 149 and 177), and two pesticides (aldrin and endrin) could not be measured because of analytical conditions. Serum median levels of total TEQ of 7 PCDDs, 9 PCDFs, 3 cPCBs and 6 PCBs were 8.6, 7.5, 5.1 and 3.6 pg TEQ/g lipid, respectively (Table 1). The serum median level of the total TEQ of PCDDs/PCDFs/cPCBs/PCBs was 25.1 pg TEQ/g lipid. Serum median levels of total PCDDs/PCDFs/cPCBs, PCBs and pesticides were 11.5 pmol/g lipid, 0.46 nmol/g lipid and 1.32 nmol/g lipid, respectively (Table 2).

Table 2 shows the serum median levels of total TEQ, 19 PCDDs/PCDFs/cPCBs, 32 PCBs and 11

t3.1 Table 3
t3.2 Relationships between serum organochlorine levels and frequency of food intake among Japanese women

t3.3 / t3.4 Frequency of food intake (n=76)	No.	Total TEQ (pg TEQ/g lipid) Median (25th, 75th)[a]	Total PCDDs/PCDFs/cPCBs (pmol/g lipid) Median (25th, 75th)[a]	Total PCBs (nmol/g lipid) Median (25th, 75th)[a]	Total pesticides (nmol/g lipid) Median (25th, 75th)[a]	p,p′-DDE (ng/g lipid) Median (25th, 75th)[a]
t3.5 Fish						
t3.6 ≦3 times a month	7	17.9 (12.2, 27.7)	0.75 (0.62, 0.99)	0.36 (0.26, 0.61)	0.92 (0.63, 1.38)	146.0 (104.0, 215.0)
t3.7 1–4 times a week	58	24.8 (20.5, 32.0)	1.16 (0.86, 1.46)	0.43 (0.34, 0.61)	1.25 (0.89, 1.83)	221.0 (143.0, 323.0)
t3.8 ≧5 times a week	11	30.7 (23.3, 42.9)	1.41 (0.81, 2.34)	0.73 (0.53, 0.88)	2.27 (1.72, 2.70)	367.0 (277.0, 578.0)
t3.9 *P* for difference[b]		0.001	0.002	0.0002	0.001	0.001
t3.10 *P* for trend		0.002	0.003	0.0003	0.006	0.002
t3.11 Meat						
t3.12 ≦2 times a week	12	24.6 (21.0, 30.9)	1.05 (0.98, 1.29)	0.57 (0.35, 0.64)	1.42 (1.11, 1.84)	235.5 (206.0, 358.0)
t3.13 3–4 times a week	42	25.7 (19.8, 32.0)	1.18 (0.84, 1.44)	0.44 (0.33, 0.61)	1.33 (0.85, 1.96)	205.0 (126.0, 323.0)
t3.14 ≧5 times a week	22	24.9 (21.5, 36.5)	1.16 (0.83, 2.10)	0.48 (0.37, 0.77)	1.32 (0.92, 2.70)	373.5 (146.0, 570.0)
t3.15 *P* for difference[b]		0.80	0.39	0.69	0.57	0.73
t3.16 *P* for trend		0.63	0.35	0.46	0.13	0.16
t3.17 Rice						
t3.18 ≦6 times a week	9	29.2 (20.1, 31.7)	1.12 (0.71, 1.29)	0.47 (0.34, 0.66)	1.23 (0.93, 1.64)	221.0 (183.0, 256.0)
t3.19 Once a day	24	25.8 (21.1, 36.7)	1.23 (0.91, 1.55)	0.46 (0.35, 0.73)	1.32 (0.82, 2.16)	214.0 (128.0, 444.0)
t3.20 ≧2 times a day	43	24.6 (19.8, 29.6)	1.13 (0.84, 1.46)	0.46 (0.36, 0.63)	1.42 (0.92, 1.95)	221.0 (146.0, 367.0)
t3.21 *P* for difference[b]		0.38	0.81	0.94	0.87	0.86
t3.22 *P* for trend		0.41	0.55	0.99	0.85	0.74
t3.23 Vegetable						
t3.24 ≦6 times a week	9	25.9 (19.6, 29.6)	1.18 (1.07, 1.27)	0.44 (0.27, 0.63)	1.52 (1.07, 1.70)	203.0 (195.0, 359.0)
t3.25 Once a day	18	23.6 (20.5, 32.3)	1.15 (0.83, 1.36)	0.46 (0.35, 0.66)	0.96 (0.78, 1.72)	149.0 (124.0, 225.0)
t3.26 ≧2 times a day	49	25.7 (20.7, 32.2)	1.13 (0.85, 1.46)	0.49 (0.37, 0.66)	1.38 (0.98, 2.27)	244.0 (179.0, 386.0)
t3.27 *P* for difference[b]		0.56	0.59	0.35	0.73	0.57
t3.28 *P* for trend		0.64	0.55	0.53	0.78	0.83
t3.29 Fruit						
t3.30 ≦3 times a month	17	26.0 (20.5, 30.1)	1.04 (0.83, 1.44)	0.43 (0.35, 0.57)	1.07 (0.78, 1.64)	190.0 (124.0, 309.0)
t3.31 1–4 times a week	34	23.9 (18.8, 32.2)	1.13 (0.84, 1.27)	0.47 (0.29, 0.66)	1.44 (0.93, 1.95)	222.0 (161.0, 359.0)
t3.32 ≧5 times a week	25	25.7 (23.3, 32.3)	1.24 (0.86, 1.61)	0.47 (0.41, 0.71)	1.38 (0.91, 2.27)	244.0 (150.0, 435.0)
t3.33 *P* for difference[b]		0.31	0.31	0.18	0.37	0.35
t3.34 *P* for trend		0.27	0.49	0.19	0.54	0.50
t3.35 Dairy products						
t3.36 ≦6 times a week	23	26.2 (20.0, 31.7)	1.18 (0.99, 1.63)	0.61 (0.34, 0.72)	1.42 (0.93, 2.51)	227.0 (183.0, 555.0)
t3.37 Once a day	40	25.8 (20.6, 32.8)	1.13 (0.84, 1.32)	0.45 (0.36, 0.64)	1.41 (0.87, 1.90)	211.0 (129.0, 363.0)
t3.38 ≧2 times a day	13	24.6 (20.6, 25.8)	1.06 (0.82, 1.46)	0.43 (0.33, 0.52)	1.18 (1.01, 1.61)	230.0 (179.0, 318.0)
t3.39 *P* for difference[b]		0.76	0.31	0.09	0.31	0.34
t3.40 *P* for trend		0.70	0.46	0.04	0.33	0.24

t3.41 [a] 25th—25th percentile; 75th—75th percentile.
t3.42 [b] Differences in log-transformed levels between subgroups were tested by the analysis of variance.

pesticides according to age, residence, occupation, body mass index (BMI), smoking and alcohol drinking habit among all subjects. Significantly higher levels of total TEQ, PCDDs/PCDFs/cPCBs, PCBs and pesticides were observed in older women (P for trend=0.003, 0.01, 0.005, 0.01, respectively). Serum total PCB levels were inversely related to BMI (P for difference=0.02). No significant differences in the levels of total TEQ, PCDDs/PCDFs/cPCBs, PCBs and pesticides were found with regard to residence, occupation, smoking or alcohol drinking habit.

Table 3 shows the association between serum organochlorine levels and frequency of food intake among all subjects. Levels of total TEQ, PCDDs/PCDFs/cPCBs, PCBs and pesticides were significantly increased with increasing frequency of fish intake (P for trend=0.002, 0.003, 0.0003 and 0.006, respectively). The median levels of total TEQ, PCDDs/PCDFs/cPCBs, PCBs and pesticides with subjects who consumed fish more than five times a week was about 1.7-, 1.9-, 2.0-, 2.5-fold significantly higher than in subjects who did so less than three times a month. Inverse association was observed between dairy product intakes and total PCBs levels (P for trend=0.04). Significant differences in levels of total TEQ, PCDDs/PCDFs/cPCBs, PCBs and pesticides were not found in terms of meat, rice, vegetable and fruit intakes. Furthermore, we analyzed the associations between the frequency of food intake and levels of PCDDs, PCDFs and cPCBs separately (data not shown). Statistically significant positive associations were found between the frequency of fish intake and TEQ levels of PCDFs and cPCBs, and the total levels of PCDDs, PCDFs and cPCBs, respectively. No significant differences in the TEQ levels of PCDDs, PCDFs and cPCBs, and the total levels of PCDDs, PCDFs and cPCBs were found with regard to frequency of meats, rice, vegetables, fruits and dairy products intakes.

4. Discussion

In the present study, we identified the serum levels of total TEQ, PCDDs/PCDFs/cPCBs, PCBs and pesticides among Japanese women of reproductive age, and the possible contributions of age and fish intake to such levels.

The mean or median total TEQ levels of PCDDs/PCDFs previously reported among Japanese with no occupational exposure were 9.8 to 24.9 pg TEQ/g lipid (Arisawa et al., 2003; Kumagai et al., 2002, 2000). The mean or median total TEQ levels of PCDDs/PCDFs/cPCB/PCBs previously reported among Japanese with no occupational exposure were 16 to 61 pg TEQ/g lipid (Arisawa et al., 2003; Tsuchiya et al., 2003). The median level of total TEQ of PCDDs/PCDFs and PCDDs/PCDFs/cPCBs/PCBs in the present study was 16.1 and 25.1 pg TEQ/g lipid. The serum TEQ levels in our study were consistent with those previously reported for other Japanese populations. The mean levels of PCBs (e.g., PCB105, 118, 156, 157, 167, 189) were also similar to the mean levels previously reported for Japanese populations (Arisawa et al., 2003; Tsuchiya et al., 2003). To our knowledge, the serum p,p′-DDE levels of Japanese have not been reported previously. The median level of serum p,p′-DDE was 221.0 ng/g lipid in the present study, and the serum p,p′-DDE level of the present subjects was lower than the serum p,p′-DDE levels of American or Swedish subjects (Laden et al., 2001; Weiderpass et al., 2000). In this study, the contribution of individual organochlorine compounds to the total TEQ was highest from 2,3,4,7,8-PentaCDF (21.8%), followed by 3,3′,4,4′,5-PentaCB (20.1%), 1,2,3,7,8-PentaCDD (13.7%) and 1,2,3,6,7,8-HexaCDD (11.4%) (data not shown).

Because organochlorines are lipophilic, slowly metabolized, and tend to bioaccumulate in the food chain, higher organochlorine levels should be found in the human body as people get older. In fact, some previous studies as well as the present investigation reported that total serum TEQ levels significantly increased with age (Arisawa et al., 2003; Chen et al., 2003; Kumagai et al., 2000; Wittsiepe et al., 2000a). In the present study, serum organochlorine levels tended to be lower as BMI was higher, and a inverse association was found between the BMI and level of total PCBs (P for difference=0.02). A few reports investigated the association between BMI and serum organochlorine levels, but no significant association was found in almost all of these investigations (Arisawa et al., 2003; Kumagai et al., 2002, 2000). One study revealed a positive association between BMI and serum DDE levels (Schildkraut et al., 1999). Further study will be needed to explore in

more detail the possible association between BMI and serum organochlorine levels in human.

Concerning the association between dietary intake and serum organochlorine levels in Japanese, higher serum TEQ levels in frequent fish and meat consumers are plausible, because the estimated mean daily intakes of total TEQ levels of PCDDs, PCDFs and PCBs contaminating foods were highest from fish and shellfish (76.9%), followed by meat and eggs (15.5%) in Japanese (Tsutsumi et al., 2001). In our study, we found that Japanese women of reproductive age who consumed fish frequently tended to accumulate TEQ levels of PCDFs, cPCBs, PCBs in their body. Similar to TEQ levels of PCDFs, cPCBs and PCBs, a positive association was found between fish intake and TEQ levels of PCDDs, although not significant. Because age might be a confounding factor of fish intake, we divided all subjects into three groups according to age (24–29, 30–35 and 36–43 years) and investigated the association between fish intake and serum levels of total TEQ, PCDDs/PCDFs/cPCBs, PCBs and pesticides. In each group, positive associations were also found between fish intake and serum levels of total TEQ, PCDDs/PCDFs/cPCBs, PCBs and pesticides (data not shown).

To our knowledge, five studies have reported on the associations between serum organochlorine levels and dietary intake in Japan (Table 4). Arisawa et al. (2003) measured serum total TEQ levels of 7 PCDDs, 10 PCDFs and 12 PCBs in relation to 11 food items consumed by randomly selected persons who resided in five prefectures of Japan and had no known occupational exposure to dioxins. They reported that frequent coastal fish intake was associated with higher serum TEQ levels of PCDFs ($P=0.03$), and the raw fish intake was positively related to TEQ levels of PCBs ($P=0.03$). Tsuchiya et al. (2003) measured serum total TEQ levels of 7 PCDDs, 10 PCDFs, 4 cPCBs and 8 PCBs among 10 fishermen, 10 farmers and 8 office workers. They reported that in frequent fish eaters, mean TEQ levels of PCDFs, cPCBs, PCBs and total sum TEQ levels were significantly higher than in the infrequent fish eaters. Kitamura et al. (2000) investigated the association between 9 factors of food intake and serum total TEQ levels of 7 TCDDs, 10 TCDFs and 3 PCBs among employees in waste incineration plants. Their study revealed that butter/cheese/lard intake was positively associated with TEQ levels of PCDDs, PCDFs and the total TEQ level, while ordinary daily food including fish, clam, egg, squid and vegetable was positively associated with serum TEQ levels of PCBs. They also analyzed the association between 5 preferable meals' intake and total serum TEQ levels. The observed higher fish intake was significantly associated with higher TEQ levels of cPCBs (77+126+169) in blood, but not with TEQ levels of PCDDs or PCDFs.

Contrary to these results, Kumagai et al. (2000) reported no association between the frequency of fish, meat and milk intake and serum TEQ levels of PCDDs and PCDFs among workers employed at waste-incineration plants. However, in their study, the total TEQ levels of PCDDs and PCDFs were compared between only two categories (<7 times/week and ≧7 times/week) of the frequency of fish, meat and milk intake. They indicated that more detailed information was necessary to clarify the relation between fish consumption and the serum PCDDs and PCDFs levels. Only one study investigated the association between dietary intake and serum levels of pesticides including β-HCH, hexachlorobenzene (HCB), pp′-dichlorodiphenyldichloroethane (pp′-DDD), bis(4-chlorophenyl)-1,1-dichloroethene (DDE) and DDT among Japanese farmers (Hanaoka et al., 2002). The authors reported that fish intake showed a positive but no significant relationship with HCB and DDT serum levels. In the present study, the serum pesticide level significantly increased with the increasing frequency of fish intake (P for trend=0.006), and this result was consistent with a previous report (Hanaoka et al., 2002).

Daily dietary intake of PCDDs, PCDFs and PCBs has been estimated using PCDDs, PCDFs and PCBs levels in foods. Because the PCDD, PCDF and PCB levels in foods and the consumption of foods vary from country to country, the kinds of foods contributing to daily dietary intake of PCDDs, PCDFs and PCBs obviously differ with the country. The main foods contributing to the daily dietary intake of total TEQ levels of PCDDs and PCDFs have been reported to be fish for Japanese (Tsutsumi et al., 2001), Spanish (Llobet et al., 2003b), and Belgian people (Focant et al., 2002), meat and meat products for Americans (Guo et al., 2001; Schecter et al., 2001), and British people (Harrison et al., 1998),

Table 4
Previous reports on the association between food and beverage intake and serum organochlorine level among Japanese populations

Subjects	Kinds of food and beverage	Analyzed organochlorines	Significant associations	Reference
Randomly selected people ($n=253$)	11 Items (beef, pork, milk, eggs, butter, cheese, grilled fish, boiled fish, raw fish, coastal fish, other fish)	TEQ of 7 PCDDs, 10 PCDFs, 12 PCBs	Positive association TEQ of PCDFs—coastal fish TEQ of PCBs—raw fish	Arisawa et al., 2003
Fishermen, farmers, office workers ($n=28$)	Fish	TEQ of 7 PCDDs, 10 PCDFs, 4cPCBs and 8PCBs	Positive association TEQ of PCDFf—fish TEQ of cPCBs—fish TEQ of PCBs—fish Total TEQ—fish	Tsuchiya et al., 2003
Incinerator workers ($n=94$)	9 Factors (ordinary daily food[a], clam/shrimp/bacon, fatty food, rice/egg, mushroom/ham, meat, butter/cheese/lard, dairy product, crab)	TEQ of 7 TCDDs, 10 TCDFs, 3 PCBs	Positive association TEQ of PCDDs—fatty food, mushroom/ham, butter/cheese/lard TEQ of PCDFs—butter/cheese/lard TEQ of PCBs—ordinary daily food, mushroom/ham Total TEQ—butter/cheese/lard	Kitamura et al., 2000
	5 Preferred meals (fatty meals, fish meals, noodles, broiled meat/tempura, grilled eel/fried dumpling)		Positive association TEQ of PCDDs—meat/tempra, eel/dumpling TEQ of PCDFs—eel/dumpling TEQ of PCBs—fish meals Total TEQ—eel/dumpling Inverse association TEQ of PCBs—fatty meals	
Incinerator workers ($n=60$)	3 Items (fish, meat, cow's milk)	TEQ of 5 PCDDs and 5 PCDFs 5 PCDDs, 5 PCDFs	Significant association was not found	Kumagai et al., 2000
Farmers ($n=41$)	7 Foods (meats, fish, vegetables, fruits, rice, green tea, milk)	5 Pesticides (b-hexachlorocyclohexane, b-hexachlorobenzene, pp′-DDE, pp′-DDT, pp′-dichlorodiphenyldichloroethane)	Significant association was not found	Hanaoka et al., 2002
Women of reproductive age ($n=80$)	6 Items (fish, meats, rice, vegetables, fruits, milk)	TEQ of 7 PCDDs, 9 PCDFs, 3 cPCBs, 6 PCBs 7 PCDDs, 9 PCDFs, 3 cPCBs, 32 PCBs, 11Pesticides	Positive association TEQ of PCDFs—fish TEQ of cPCBs—fish TEQ of PCBs—fish Total TEQ—fish PCDDs—fish PCDFs—fish cPCBs—fish PCBs—fish Pesticides—fish Inverse association PCBs—dairy products	This study

[a] Ordinary daily food contains fish, clam, eggs, squid, vegetables etc.

and milk and dairy products for Germans (Malisch, 1998). The main food contributing to daily dietary intake of TEQ levels of PCBs is fish in various countries including Japan (Tsutsumi et al., 2001), Spain (Llobet et al., 2003a), the USA (Schecter et al., 2001), England (Harrison et al., 1998), meat and meat product in England (Harrison et al., 1998), and the dairy product in Canada (Wittsiepe et al., 2000b). The present study revealed that the frequency of fish consumption was the most significant contributor to serum total TEQ levels of PCDDs, PCDFs, cPCBs and PCBs among Japanese women of reproductive age, and these results were consistent with the results of the above studies estimating daily dietary intake for Japanese.

The human health effects associated with low exposure to organochlorine compounds which levels we found in the present study have not yet been fully characterized. However, cancer mortality has been reported to be unaffected by such low levels of exposure to organochlorines (Bertazzi et al., 2001). We will examine the effects on endometriosis of exposure to organochlorine compounds, and the results will be reported in a separate paper in the near future.

In conclusion, we found that among various lifestyle factors, fish consumption was positively associated with serum levels of total TEQ, PCDDs/PCDFs/cPCBs, PCBs and pesticides in Japanese women of reproductive age.

Acknowledgements

This study was a part of a collaborative study with the U.S. Centers for Disease Control and Prevention. We wish to thank Dr. Amanda Niskar and Dr. Carol Rubin for their collaboration in making the study protocol. This work was supported in part by a Grant-in-Aid for Cancer Research and the Second Term Comprehensive 10-year Strategy for Cancer Control from the Ministry of Health, Labour, and Welfare of Japan, and the Grant-in-Aid for Risk Analysis Research on Food and Pharmaceuticals and Cancer Research from the Ministry of Health, Labour, and Welfare of Japan. Hiromasa Tsukino is an awardee of a Research Resident Fellowship from the Foundation for the Promotion of Cancer Research (Japan) for the 2nd Term Comprehensive 10-Year Strategy for Cancer Control.

References

Arisawa K, Matsumura T, Tohyama C, Saito H, Satoh H, Nagai M, et al. Fish intake, plasma omega-3 polyunsaturated fatty acids, and polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzo-furans and co-planar polychlorinated biphenyls in the blood of the Japanese population. Int Arch Occup Environ Health 2003;76:205–15.

Bertazzi PA, Consonni D, Bachetti S, Rubagotti M, Baccarelli A, Zocchetti C, et al. Health effects of dioxin exposure: a 20-year mortality study. Am J Epidemiol 2001;153:1031–44.

Chen HL, Lee CC, Liao PC, Guo YL, Chen CH, Su HJ. Associations between dietary intake and serum polychlorinated dibenzo-*p*-dioxin and dibenzofuran (PCDD/F) levels in Taiwanese. Environ Res 2003;91:172–8.

DiPietro ES, Lapenza Jr CR, Cash TP, Turner WE, Green VE, Gill JB. A fast universal automated cleanup system for the isotope-dilution high resolution mass spectrometric analysis of PCDDs, PCDFs, coplanar-PCBs, PCB congeners, and persistent pesticides from the same serum sample. Organohalog Compd 1997; 31:26–31.

Focant JF, Eppe G, Pirard C, Massart AC, Andre JE, De Pauw E. Levels and congener distributions of PCDDs, PCDFs and non-ortho PCBs in Belgian foodstuffs—assessment of dietary intake. Chemosphere 2002;48:167–79.

Guo X, Longnecker MP, Michalek JE. Relation of serum tetrachlorodibenzo-*p*-dioxin concentration to diet among veterans in the Air Force Health Study with background-level exposure. J Toxicol Environ Health A 2001;63:159–72.

Hanaoka T, Takahashi Y, Kobayashi M, Sasaki S, Usuda M, Okubo S, et al. Residuals of beta-hexachlorocyclohexane, dichlorodiphenyltrichloroethane, and hexachlorobenzene in serum, and relations with consumption of dietary components in rural residents in Japan. Sci Total Environ 2002;286:119–27.

Harrison N, Wearne S, Gem MG, Gleadle A, Startin J, Thorpe S, et al. Time trends in human dietary exposure to PCDDs, PCDFs and PCBs in the UK. Chemosphere 1998;37:1657–70.

Hornung R, Reed L. Estimation of average concentration in the presence of non-detectable values. Appl Occup Environ Hyg 1990;5:48–51.

Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE, Kaattari S, et al. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the US, EPA-sponsored workshop. Environ Health Perspect 1996;104(Suppl. 4):715–40.

Kitamura K, Kikuchi Y, Watanabe S, Waechter G, Sakurai H, Takada T. Health effects of chronic exposure to polychlorinated dibenzo-*p*-dioxins (PCDD), dibenzofurans (PCDF) and coplanar PCB (Co-PCB) of municipal waste incinerator workers. J Epidemiol 2000;10:262–70.

Kumagai S, Koda S, Miyakita T, Yamaguchi H, Katagi K, Yasuda N. Polychlorinated dibenzo-*p*-dioxin and dibenzofuran concen-

trations in the serum samples of workers at continuously burning municipal waste incinerators in Japan. Occup Environ Med 2000;57:204–10.
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;59:362–8.
Laden F, Collman G, Iwamoto K, Alberg AJ, Berkowitz GS, Freudenheim JL, et al. 1,1-Dichloro-2,2-bis(*p*-chlorophenyl) ethylene and polychlorinated biphenyls and breast cancer: combined analysis of five US studies. J Natl Cancer Inst 2001;93:768–76.
Llobet JM, Bocio A, Domingo JL, Teixido A, Casas C, Muller L. Levels of polychlorinated biphenyls in foods from Catalonia, Spain: estimated dietary intake. J Food Prot 2003a;66:479–84.
Llobet JM, Domingo JL, Bocio A, Casas C, Teixido A, Muller L. Human exposure to dioxins through the diet in Catalonia, Spain: carcinogenic and non-carcinogenic risk. Chemosphere 2003b; 50:1193–200.
Malisch R. Update of PCDD/PCDF-intake from food in Germany. Chemosphere 1998;37:1687–98.
Nakagawa R, Hirakawa H, Hori T. Estimation of 1992–1993 dietary intake of organochlorine and organophosphorus pesticides in Fukuoka, Japan. J AOAC Int 1995;78:921–9.
Patterson Jr DG, Hampton L, Lapeza Jr CR, Belser WT, Green V, Alexander L, et al. High-resolution gas chromatographic/high-resolution mass spectrometric analysis of human serum on a whole-weight and lipid basis for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Anal Chem 1987;59:2000–5.
Revised American Fertility Society classification of endometriosis: 1985. Fertil Steril 1985;43:351–2.
Rier SE, Martin DC, Bowman RE, Dmowski WP, Becker JL. Endometriosis in rhesus monkeys (*Macaca mulatta*) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Fundam Appl Toxicol 1993;21:433–41.
Safe SH. Endocrine disruptors and human health—is there a problem? An update. Environ Health Perspect 2000;108:487–93.
Schecter A, Cramer P, Boggess K, Stanley J, Papke O, Olson J, et al. Intake of dioxins and related compounds from food in the US population. J Toxicol Environ Health A 2001;63: 1–18.
Schildkraut JM, Demark-Wahnefried W, DeVoto E, Hughes C, Laseter JL, Newman B. Environmental contaminants and body fat distribution. Cancer Epidemiol Biomarkers Prev 1999;8:179–83.
Tsuchiya Y, Nakai S, Nakamura K, Hayashi K, Nakanishi J, Yamamoto M. Effects of dietary habits and CYP1A1 polymorphisms on blood dioxin concentrations in Japanese men. Chemosphere 2003;52:213–9.
Tsutsumi T, Yanagi T, Nakamura M, Kono Y, Uchibe H, Iida T, et al. Update of daily intake of PCDDs, PCDFs, and dioxin-like PCBs from food in Japan. Chemosphere 2001; 45:1129–37.
Turner WE, DiPietro ES, Cash TP, McClure PC, Patterson Jr DG, Shirkhan H. An improved SPE extraction and automated sample cleanup method for serum PCDDs, PCDFs, and coplanar PCBs. Organohalog Compd 1994;19:31–5.
Van den Berg M, Birnbaum L, Bosveld AT, Brunstrom B, Cook P, Feeley M, et al. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 1998;106:775–92.
Van den Berg M, Peterson RE, Schrenk D. Human risk assessment and TEFs. Food Addit Contam 2000;17:347–58.
Watanabe S, Kitamura K, Nagahashi M. Effects of dioxins on human health: a review. J Epidemiol 1999;9:1–13.
Weiderpass E, Adami HO, Baron JA, Wicklund-Glynn A, Aune M, Atuma S, et al. Organochlorines and endometrial cancer risk. Cancer Epidemiol Biomarkers Prev 2000;9:487–93.
Wittsiepe J, Schrey P, Ewers U, Selenka F, Wilhelm M. Decrease of PCDD/F levels in human blood from Germany over the past ten years (1989–1998). Chemosphere 2000a;40:1103–9.
Wittsiepe J, Schrey P, Ewers U, Wilhelm M, Selenka F. Decrease of PCDD/F levels in human blood—trend analysis for the German population, 1991–1996. Environ Res 2000b;83:46–53.
Wolff MS, Toniolo PG, Lee EW, Rivera M, Dubin N. Blood levels of organochlorine residues and risk of breast cancer. J Natl Cancer Inst 1993;85:648–52.