CONCENTRATIONS OF DIOXINS AND RELATED COMPOUNDS AND THEIR EFFECTS TO BIOCHEMICALS IN FUKUOKA RESIDENTS

Yoshito Masuda[1], Koichi Haraguchi[1], Suminori Kono[2], Hiroshi Tsuji[3], Olaf Päpke[4]

[1]Daiichi College of Pharmaceutical Sciences, Fukuoka 815-8155, Japan
[2]Kyushu University, School of Medicine, Fukuoka 812-8582, Japan
[3]Fukuoka University, School of Medicine, Fukuoka 814-0180, Japan
[4]ERGO Laboratory, Geierstrasse 1, 22305 Hamburg, Germany

Introduction

World Health Organization (WHO) renewed the Tolerable Daily Intake (TDI) to 1-4 pg/kg/day of dioxin toxicity equivalents (TEQ) consisting of polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), non-ortho chlorinated coplanar biphenyls (noPCB) and mono-ortho chlorinated biphenyls (moPCB) in 1998[1]. Japanese government also renewed TDI to 4 pg/kg/day of TEQ consisting of the same polychlorinated compounds as WHO in 1999. For understanding the background levels of these chemicals in Japanese, blood samples of Fukuoka residents were collected and analyzed for PCDDs, PCDFs, PCBs and some chlorinated pesticides. The blood samples were also examined for 20 biochemical levels for investigating the health effects that may be caused by TEQ concentrations and aging.

Materials and Methods

Blood sample

About equal numbers in male and female and in ages of 20s, 30s, 40s and 50s were randomly selected from the residents in the Ward of Fukuoka City, and requested for blood donation. The blood samples, 100 ml, from the consented 152 donors were collected at a doctor's office and their health conditions were inquired in October and November of 1999.

Analysis of PCDDs, PCDFs, noPCBs and moPCBs

Blood samples, 70 ml, was added with the internal standards of 7 ^{13}C-PCDDs, 10 ^{13}C-PCDFs, 4 ^{13}C-noPCBs and 8 ^{13}C-moPCBs and mixed with water/ethanol, eluted through silica phase column and extracted with hexane. The hexane solution was evaporated to dryness and remaining lipid was gravimetrically determined. The lipid material was redissolved in hexane and passed through multi layer column of sulfuric acid/silica gel, cesium silicate and potassium silicate. The eluate was concentrated and fractionated on a column of carbon on glass fiber. The column was eluted with hexane/dichloromethane for separating moPCBs, and later reversely eluted with toluene for the fractions of PCDDs, PCDFs and noPCBs. Each fraction was determined for individual congeners by high-resolution gas chromatography/high-resolution mass spectrometer (HRGC/HRMS) in ERGO Laboratory.

Analysis of PCBs and chlorinated pesticides

Blood sample, 10 ml, was extracted with acetone/hexane (2:1) and the extract was concentrated to dryness to determine the lipid weight. The lipid was dissolved in hexane/dichloromethane (1:1), added

with internal standards of 12 ^{13}C-PCBs, and fractionated on a gel permeation column of Bio-Beads S-X3. The fraction for PCBs and pesticides was analyzed for 18 PCB congeners, hexachlorobenzene (HCB), beta-hexachlorocyclohexane (ß-HCH), bis(chlorophenyl)-dichloroethylene (DDE), trans-nonachlor (t-Nonachlor) and others by HRGC/HRMS.

Measurement of biochemicals

Blood sample, 20 ml, was sent to the clinical laboratory, to measure the level of biochemicals, such as thyroxin binding globulin (TBG), triiodothyronine (T3), free-T3, thyroxine (T4), free-T4, thyroid-stimulating hormone (TSH), total cholesterol (TC), triglyceride (TG), creatinine, uric acid (UA), amylase, gamma-glutamyl transpeptidase (γ-GPT), cholinesterase (ChoE), glutamine-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), albumin, free testosterone (F-Testosterone), testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH).

Results and Discussion

Concentrations of PCDDs, PCDFs, PCBs and Pesticides

TEQ concentrations in the blood are calculated by the use of TEQ factors established by WHO in 1998[1], and shows in Table 1.

Table 1. Concentrations of TEQ (ppt in Lipid) in the blood of Fukuoka residents

	N of positive	Mean	S.D.	Median	Min	Max
Age of residents	152	36.7	11.8	35.1	20.0	60.0
PCDDs	152	10.28	4.21	9.50	3.49	26.34
PCDFs	152	5.56	2.77	5.10	1.82	20.66
noPCBs	152	7.75	7.93	5.00	0.28	43.98
moPCBs	152	4.57	3.46	3.61	0.75	18.70
Total TEQ	152	28.15	16.98	23.08	9.13	101.96

Blood donors of 152 were divided into male and female and into ages of 20-29, 30-39, 40-49, 50-60, and cumulative TEQ concentrations in the blood of each group shows in Figure 1.

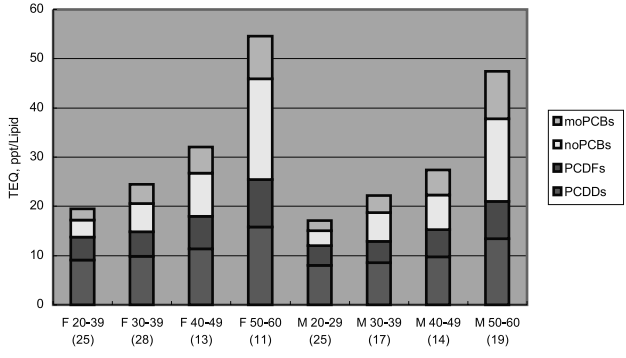

Figure 1. TEQ Concentrations from PCDDs, PCDFs, noPCBs, moPCBs. In the blood of different Age and Sex

Percent of the TEQ contributed from noPCBs to total TEQ increased from 18.1% to 35.3 % in male and from 17.5 % to 37.5 % in female with aging from 20s to 50s, while the TEQ contributed from PCDDs decreased from 46.7 % to 28.3 % in male and from 46.7 % to 28.9 % in female with aging. Concentrations of PCBs, HCB, ß-HCH, DDE and t-Nonachlor in the blood of 151 residents are shown in Table 2. These concentrations also increased with ages from 20s to 50s. Predominant high concentrations of ß-HCH were observed in both sexes, ages 50-60. Japanese environment was presumed to be heavily polluted with PCBs and pesticides in 1970s. These pollutants exposed to human at that time still remain in the aged persons for 30 years.

Table 2. Concentrations of PCBs and Pesticides (ppb in Lipid) in the blood of Fukuoka residents

	N of positive	Mean	S.D.	Median	Min	Max
Total PCBs	151	341	254	278	55	1286
HCB	151	23	16	22	1	85
ß-HCH	138	525	728	244	24	4830
DDE	148	357	250	311	14	1598
t-Nonachlor	151	73	74	50	9	566

Correlation between TEQ, PCBs and Biochemicals

Table 3 lists the correlation coefficients between the levels of TEQ, PCBs, DDE and 20 biochemicals in the blood of Fukuoka residents of male 75 and female 77. Very high correlation coefficients ($p<0.01$) were observed between Age, TEQ and PCBs in both sexes. Correlations between DDE and Age, TEQ or PCBs were weaker than those between TEQ, PCBs and Age, but their significant levels were more than 5 %.

Significantly positive correlations are observed between TC, TG, γ-GTP, GOT or FSH and Age, TEQ or PCBs, and significantly negative correlation between T4 or Free-T4 and Age, TEQ or PCBs in female and between F-Testosterone or Testosterone and Age, TEQ or PCBs in male.

As shown in Figure 2, significantly positive correlations are observed between TEQ and TG in males of age 20-39 and in females of age 40-60, and weak negative correlation between TEQ and T4 in females of age 40-60.

Aging and increasing levels of TEQ and PCBs might cooperatively induce enzyme activities for TG, ?-GTP and GOT and cooperatively change the hormonal levels of thyroxins and sex hormones.

Acknowledgments

This investigation was supported by the grant from the Ministry of Health and Welfare.

References

1. A Brouwer, UG Ahlborg, FXR van Leeuwen, MM Feeley (1998), Chemosphere 37, 1627-43.

Table 3. Correlation coefficients between the levels of TEQ, PCBs, DDE and Biochemicals in the blood of Fukuoka residents, Male 75 and Female 77

	Male				Female			
	Age	TEQ	PCBs	DDE	Age	TEQ	PCBs	DDE
Number	75	75	75	75	77	77	76	76
Age	1,0000				1,0000		**0,6919**	***0,2367***
TEQ	**0,6832**	1,0000			**0,6498**	1,0000	**0,8181**	**0,3891**
PCBs	**0,7057**	**0,8828**	1,0000				1,0000	**0,3810**
DDE	**0,6043**	**0,5590**	**0,5790**	1,0000				1,0000
TBG	0,1542	0,0642	0,0848	0,1220	-0,0165	-0,0729	-0,0439	0,1433
T3	-0,1410	-0,0854	-0,1113	-0,0015	-0,0045	-0,1323	-0,1735	-0,1207
Free-T3	0,1457	0,0096	-0,0443	0,0275	0,0547	-0,1506	-0,1499	-0,0225
T4	-0,0139	-0,0868	-0,0136	-0,0402	**-0,3019**	**-0,3014**	**-0,3431**	-0,0821
Free-T4	0,0283	-0,0595	-0,0350	-0,0242	**-0,3361**	***-0,2901***	**-0,3945**	***-0,2454***
TSH	0,0656	0,0365	0,1298	0,0045	**0,2967**	***0,2499***	**0,3256**	0,0787
TC	***0,2564***	0,2160	***0,2882***	0,1230	**0,4706**	**0,3569**	**0,5151**	-0,0097
TG	***0,2844***	***0,2863***	**0,4644**	**0,3657**	**0,4331**	**0,4270**	**0,5713**	0,0845
Creatinin	-0,1667	-0,0857	-0,1202	-0,1111	**-0,3208**	-0,1241	***-0,2563***	**-0,2164**
UA	0,1034	0,1675	0,1825	-0,0453	0,1026	**0,4208**	**0,3110**	0,0299
Amylase	**-0,2136**	-0,1295	-0,1388	***-0,2371***	-0,0548	-0,1310	-0,0911	-0,0851
?GTP	**0,4353**	**0,5314**	**0,5240**	**0,2972**	**0,3464**	**0,3468**	**0,3868**	0,0846
CheE	-0,0324	-0,0340	-0,0606	0,0043	***0,2686***	0,1047	**0,2244**	0,1853
GOT	***0,2944***	***0,2314***	**0,3415**	***0,2876***	**0,4000**	**0,3977**	**0,3456**	0,0634
GPT	0,1509	0,1900	***0,2428***	***0,2715***	**0,3180**	**0,4186**	**0,3157**	0,0805
Albumin	**-0,4297**	***-0,2372***	**-0,2107**	-0,1036	**-0,3454**	-0,2106	-0,1461	-0,1821
F-Testosteron	**-0,5647**	**-0,3663**	**-0,3749**	**-0,3378**	?	?	?	?
Testosterone	**-0,2248**	***-0,2835***	***-0,2484***	-0,1230	?	?	?	?
LH	***0,2885***	0,1479	0,1433	0,1912	?	?	?	?
FSH	**0,5967**	**0,4650**	**0,4382**	**0,3725**	?	?	?	?

p < 0.01 : Bold 10 point | ***p < 0.05 : Bold 10 point, i*** | **p < 0.1 : Bold 9 point**

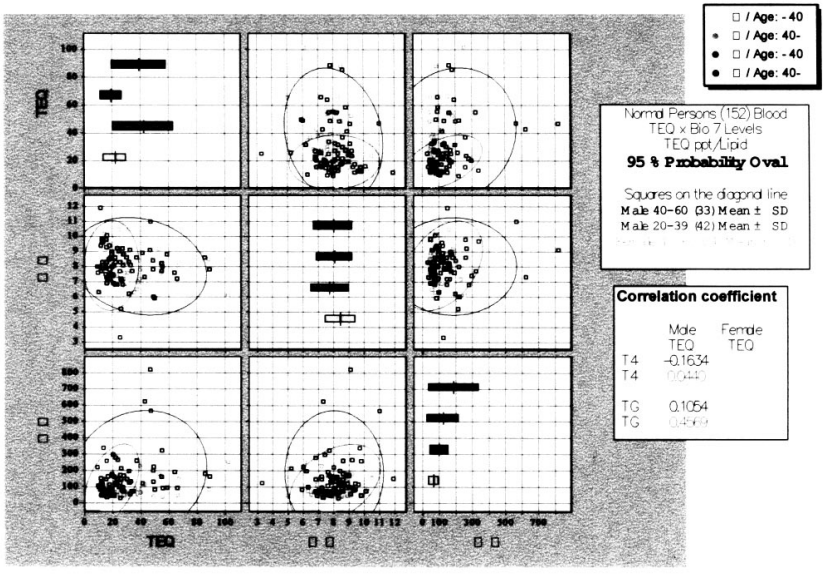

Figure 2. Diagrams of correlations between the levels of TEQ, T4 and TG in different Age and Sex