DIOXIN-LIKE PCB CONGENER LEVELS IN ADIPOSE TISSUE SAMPLES FROM TURKISH MEN

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Introduction

It is clear that humans are exposed to a complex mixture of dioxin-like compounds[1,2] and human health risk posed by exposure to these chemicals. Dioxin-like compounds are extremely persistent in the environment, and their high lipophilicity results in their bioconcentration into biota and biomagnification through the food chain[3]. Consequently, dioxin-like compounds released to the atmosphere can travel long distances before they are deposited onto water, soil and vegetation causing a widespread occurrence of such compounds[4]. There are numerous compounds that are considered of "dioxin-like" toxicity. The three most general types of dioxin are PCDDs, PCDFs and PCBs. Among these, tetrachlorodibenzo-*p*-dioxin or 2,3,7,8-TCDD represents the most common and toxic of all the congeners. A number of PCB congeners show 'dioxin-like' toxicity. These PCBs have no or only one chlorine atom in the ortho-position. The phenyl rings of these molecules can rotate and can adopt a coplanar structure, which leads to the same toxicity as the PCDDs and the PCDFs. The WHO identified 12 PCBs to be dioxin-like (non-ortho: #77, #81, #126, #169 and mono-ortho: #105, #114, #118, #123, #156, #157, #167 and #189)[5]. The toxic effects of these substances include immuno- and reproductive toxicity, they negatively influence the organism's growth and the development of the central nervous system, and in addition they have carcinogenic potency. The effects, however, become only manifest after a certain critical body burden has been exceeded[6].The toxic equivalency factor (TEF) approach[7] is the most common method used to characterize the toxicity of human exposure to dioxin-like compounds.

Materials and Methods

23 human adipose tissue samples were taken during surgical operations from the Ankara University, School of Medicine from different donors which had been living in the Ankara area for at least 5 years between June 2004 and September 2004. All subjects participated in the study voluntarily and all were male. All subjects acknowledged their participation by signing an informed consent form. The age of the subjects ranged from 28 to 44 years (mean age 36.1±4.6). Each subject completed a questionnaire to provide personal information such as smoking, occupation, dietary habits, weight, and height. All the subjects were mixed food consumers

Extraction and Cleanup

1.5 to 2 g of adipose tissue were ground with Isolute HM-N, spiked with ^{13}C labelled PCDD/F and PCB standards and extracted by use of pressurized liquid extraction (ASE 200, Dionex GmbH, Idstein, Germany) with n-hexane:acetone 3:1 as extraction solvent. The extract was evaporated to dryness for gravimetrical determination of the lipid content. The residue was resolved in 2 to 3 ml of n hexane.

Instrumentation

The PCB analysis was performed with a high resolution mass spectrometer Finnigan MAT 95S (Thermo Electron GmbH, Bremen, Germany) coupled with an Agilent GC 6890 (Agilent Technologies, Palo Alto, CA, USA). Chromatographic separation was achieved by splitless injection (cold injection system CIS4, Gerstel GmbH, Mülheim, Germany) of 1 µl on a Restek Rtx-2330 column with a length of 60 m, ID 0.25 mm, ft 0.1 µm (Restek GmbH, Sulzbach, Germany). The GC oven was programmed as follows: 90°C initial hold for 1.5 min, increase

at a rate of 20°C min^{-1} to 170°C, hold for 7 min., followed by an increase of 3°C min^{-1} to 260°C and a final hold at 260°C for 10 min. The MS was operated in SIM mode at a resolution of 10000 and the two most intense ions of the molecular ion cluster were monitored for the unlabelled and labelled isomers.

Statistical evaluation
Concentrations dioxin-like PCBs were expressed as WHO-TEQ values. A non-parametric test (Kruskal-Wallis analysis of variance and the Mann-Whitney *U* test) were used for data analysis. We determined the relationships among biological and chemical data by correlation analysis and multivariate principal component analysis (PCA).

Results and Discussion
In this study Dioxin-like PCBs (non-ortho PCBs #77, #81, #126, #169 and mono-ortho PCBs #105, #114, #118, #123, #156, #157, #167, #189) which had not been measured in Turkey yet, have been studied in the male adipose tissue. The levels of PCB congeners found in adipose tissue samples are presented in Table 1. The measured 12 dioxin-like PCBs were present in almost all of the samples, except PCB #81 and #123 (91.3 %). The mean level of the sum of all dioxin-like PCBs found in the samples was 13959 pg g^{-1} lipid. Mono-ortho PCB congeners accounted for greater than 99% of the concentrations of dioxin-like PCBs in men. The compound found at the highest concentration was PCB #118, followed by the congeners #156 and #105. These congeners were responsible for about 81.6% of the total concentration of the non-ortho and mono-ortho PCBs in the adipose tissue. On the other hand, non-ortho PCB congeners #81, #77, #126 and #169 were the congeners found at the lowest levels, being responsible for only 0.8% of the total dioxin-like PCB levels found in the tissue but, contribution of these PCB congeners to the TEQ had been reported as 53.9%.
3,3',4,4',5-Pentachlorobiphenyl (PCB #126) is the most important one of the 12 dioxin-like PCBs because of its high WHO-TEF of 0.1 and its concentration level is found as 32.3±24.4 in this study. TEQs were estimated using WHO-TEFs[6]. Concentrations of TEQs in males were found between 1.6-24.5 pg g^{-1} lipid in this study.

Table 1: Levels of dioxin-like PCBs in Turkish male adipose tissue (pg g^{-1} lipid)

	IUPAC No.	n	Concentration (pg/g lipid) Mean±SD
Non-ortho PCB:			
3,3',4,4'-Tetrachlorobiphenyl	PCB #77	23	33.2±19.2
3,4,4',5-Tetrachlorobiphenyl	PCB #81	23	7.9±9.6
3,3',4,4',5-Pentachlorobiphenyl	PCB #126	23	32.3±24.4
3,3',4,4',5,5'-Hexachlorobiphenyl	PCB #169	23	36.0±21.5
Mono-ortho PCB:			
2,3,3',4,4'-Pentachlorobiphenyl	PCB #105	23	1605±1497
2,3,4,4',5-Pentachlorobiphenyl	PCB #114	23	372±296
2,3',4,4',5-Pentachlorobiphenyl	PCB #118	23	6359±5745
2',3,4,4',5-Pentachlorobiphenyl	PCB #123	23	66.7±77
2,3,3',4,4',5-Hexachlorobiphenyl	PCB #156	23	3423±2697
2,3,3',4,4',5'-Hexachlorobiphenyl	PCB #157	23	623±534
2,3',4,4',5,5'-Hexachlorobiphenyl	PCB #167	23	924±901
2,3,3',4,4',5,5'-Heptachlorobiphenyl	PCB #189	23	477±396
TEQ			**6.67**

As the aim of this study is to determine dioxin-like PCBs, the amounts of other PCB congeners were not analyzed. Thus, the associations between dioxin-like PCBs and other PCB congeners are not evaluated in this study. On the other hand, no correlations were found between dioxin-like PCB concentrations and age, body mass index (BMI) and smoking status.

In previous studies that were conducted on women living in Ankara 7 indicator PCBs (#28, #52, #101, #118, #138, #153, #180) were detected. When the results of this study are also considered, it is once more argued that PCB exposure to people living in Ankara is inevitable. Although limited studies conducted in Turkey has reported the presence of PCB compounds in human, environment and food, Çok et al.[8,9] reported in the first study aiming the investigation of PCBs in humans that the indicator PCBs were present in both milk and adipose tissues of women .In this study human adipose tissue was collected from men living in Ankara which is an industrialized city in the central Turkey, quite far away from the sea and big lakes. Food intake is the main route of exposure to dioxin-like compounds for humans and for wild life. Nevertheless, all subjects reported having a mixed diet including meat and fish. As PCB resources for people living in Ankara careless disposal practices, accidents, leakage from various industrial facilities and from chemical waste disposal sites could be considered. To our knowledge, PCBs have been found only in closed applications in Turkey. There is no information about any source or any use of semi-closed applications of PCBs. Moreover, there are no strict regulations addressing the disposal of PCBs and controlling PCBs still in use in Turkey. On the other hand, main sources of dioxin-like PCBs could be spelt out as releases from commercial PCB formulation, emissions from combustion processes and solar photolysis of higher chlorinated PCBs.

According to some researchers[10] one of the major sources of dioxin-like PCBs is municipal solid waste incineration rather than PCB formulations. De nevo synthesis of dioxin-like PCBs was observed in the combustion of municipal solid wastes and resulted from the dimerization of chlorobenzenes[11].

As this study is the first in Turkey presenting values about dioxin-like PCBs in adipose tissue, it is not possible to compare it with previous values. The results suggest that human background contamination by dioxin-like PCBs is lower than that generally found in industrialized countries[12-14] .

In Turkey, organochlorine pesticide residues have been monitored in the Turkish population by carrying out regional surveys at given time intervals since 1976[15-18]. But there are very few data of PCB levels in human. Moreover there have been no national document for PCDDs/PCDDFs and dioxin-like PCB contamination for human. Because of this reason, the results of the present study are very important to provide baseline data on the concentrations of dioxin-like PCB contaminants in Turkey.

This study put forward the exposure to PCBs of Turkish population. Even not in detailed manner, Turkish population's exposure to PCB had been reported previously. The support of the results of this study with PCDDs and PCDFs results will help to evaluate the toxicological implications (for instance, the effects on fertility) on humans and wild life.

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