CONGENER SPECIFIC ANALYSIS OF POLYCHLORINATED BIPHENYLS IN THE ENVIRONMENT AND HUMAN SAMPLES

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Introduction
The production of polychlorinated biphenyls (PCBs) at the industrial scale was started in the USA and Japan in 1929 and 1954, respectively. The estimated quantities of PCBs produced in the Europe and America by 1970 are 450,000 tons, and one million tons in the world. In comparison, the quantities of PCBs produced in Japan by 1972, at the time when the production and use of the PCBs in the open-system were banned, was estimated to be approximately 58,000 tons. In Japan these PCBs were utilized as dielectric fluid for electric appliances (69 %), as heat transfer fluid (16 %), or in carbonless copy (NCR) paper (10 %) and in various other open-ended applications. Each commercial PCB product was a complex mixture of differently chlorinated congeners and used for different purposes. For example, low-chlorinated PCB product (KC300) and high-chlorinated PCB product (KC600) were used in NCR paper and in ship bottom coat, respectively.

In Japan, approximately 5,500 tons of PCBs (no larger than 10 % of the total production) was collected and disposed of by thermal decomposition (1400 °C). Most of the remaining PCBs (approximately 90 %) was handled and stored strictly based upon the law, however, up to this time, substantial amounts of PCBs had been released into the environment and distributed in the air, water, soil and organisms including humans.

In the present study, we analysed all the congeners and isomers of PCBs as well as coplanar PCBs by GC-MS in human samples, mainly milk, in Japan and studied their origin, behaviour and fate in the environment by comparing the specific congenic and isomeric patterns among these samples.

Materials and Methods

Standards
All 209 congeners of PCBs and all commercially available labelled congeners were obtained from CIL and Wellington as authentic standard/mixtures for the assignment and determination. Kanechlor products (KC300, 400, 500 and 600) were used as congener mixtures for the determination of each congener in the industrial product.

GC/MS Analysis
The analysis was carried out using an HP 5890 II gas chromatograph connected to a JMS-700 mass spectrometer (JEOL Ltd. Japan) (operating at a resolution >10 000). Temperature program used for
Assignment

In order to separately analyze all the 209 PCB isomers by GC/MS in terms of resolution, we prepared 15 standard mixtures by classifying these congeners from the point of retention time. For the identification of isomers, we used the standard mixtures, DB-Window, KC mixtures as well as different capillary columns for GC.

Environmental and Human Samples

Environmental samples such as air, water, sediment, rainwater and deposition were collected in Hyogo Prefecture, Japan.

Human breast milk samples and replies to questionnaires on dietary habit were kindly provided from volunteers around Hyogo Prefecture on the basis of informed consent for this study. As a preliminary reference purpose, we obtained adipose tissue from the neck of a middle-aged man after having an informed consent.

Cleanup

Environmental samples were subjected to analysis after n-hexane extraction and sulfuric acid treatments. Milk samples were analyzed by high-resolution GC/MS after extraction of lipid by n-hexane, alkaline treatments.

Results and Discussions

Environmental samples

The chromatograms of congeners in the environmental samples from air, river water and sediment usually are well comparable to those of commercial PCB products (KC300, 400, 500 or 600) reflecting their discharge into particular environmental sites/media from which the samples were collected. In contrast the analysis of PCB isomers indicated some characteristic patterns: From the analysis of di- and tri-chlorinated PCB isomers, the environment samples, such as sediment, water, rain water, and air, showed an increase in the amounts of non-ortho-chlorinated PCB isomers, 3,3’- (#11), 3,4- (#12), 3,4’- (#13), 3,3’,4- (#35) and 3,4,4’- (#37) relatively compared with the products. There found some tendency that the ratio of #180/#118, and of #189/#118 in the sediment and fish samples collected in the estuary and sea were relatively high compare to those of the river sediment. Among so-called coplanar PCB isomers, which are also the target of dioxin monitoring processes, the ratio of #180/#118 and of #170/#118 could be used as a marker for the detection of the past use of KC600 in the vicinity of the sampling area.

Isomers such as 3,3’-dichlorobiphenyl (#11) and 3,3’,4-trichlorobiphenyl (#35), that are not present in KC products, was found in the environmental samples. The possible mechanism of the formation of these isomers may include biodegradation in the sediment, and photo-degradation in the air, however, further studies are needed.

Human samples

We determined PCB congeners in human breast milk samples from 18 volunteer women with no known excessive exposure to PCBs. In the milk samples, total PCB concentrations ranged 81 to 227 ng/g-lipid (Mean ± SD=123±45 ng/g-lipid, n=18). These values are consistent with the ones recently reported by Konishi and co-workers. In their report, they studied chronological trends of chlorinated
organic compounds including PCBs in human breast milk of Osaka prefecture from 1972 to 1998. The average PCB level increased from 1972 (1,302 ng/g-lipid), peaked in 1974 (1,514 ng/g-lipid), and then decreased to about 13 % of the peak level (200 ng/g-lipid) in 1998.

Neither mono-CBs nor di-CBs were detected in the samples. Generally, tetra- to hepta- CBs were predominant among the congeners. The PCB congener pattern in the milk samples appears to be rather similar to those of the environmental media and fish. It should be pointed out that in the case of non-dioxin like PCBs, a more detailed analysis of isomers in each congener is mandatory. For this purpose, isomer patterns of PCBs in human breast milk were determined by GC-MS. From the chromatograms, isomers which are substituted by chlorine at 2-, 4- and 5- positions of phenyl ring, i.e., #74 (2,4,4',5-), #99 (2,2',4,4',5-), #118 (2,3',4,4',5-), #153 (2,2',4,4',5,5'-), and #138 (2,2',3,4,4',5'-), #180 (2,2',3,4,4',5,5'-) and # 187 (2,2',3,4',5,5',6-) were found to be predominant components. In particular, the concentration of #153, the both phenyl-ring of which are substituted at 2-, 4-, and 5- positions, was highest among the isomers, which may reflect the fact that the CYP2B activity was not as high in the human, rat and hamster as in the dog and guinea pig3,4.

In human breast milk samples minor but non-insignificant amounts of isomers were found to be #31 and #28 in Tri-CB, #199 and #196, #203 in Octa-CB: it is thought that #31 was larger in amounts than #28. It was found that these isomers gave a rise to a single or two peaks depending upon a ratio of their concentrations or analytical conditions.

We obtained a preliminary observation on PCB congeners in the adipose tissue obtained from a middle-aged man. Di-CBs were detected in the neck adipose tissue although at a level close to a lower detection limit, and the concentrations of tri-, tetra, and penta-CBs were similar to those of the milk samples. In contrast, higher chlorinated CBs, hexa- to deca-CBs were more prominent in the adipose tissue, which reflects the tendency of accumulation of these PCB congeners. The overall congeneric pattern was similar to those of the milk samples. Due to a very limited number of samples, further studies are needed to conclude this observation.

Perspectives in analysis of PCBs

PCB isomers predominantly found in humans are shown to be #138, #153 and #180. Since identification of all isomers is so money and time-consuming, it is thus impractical for regular monitoring purposes. Selecting appropriate number of PCB isomers may well fulfill the monitoring purposes, although the amounts of selected PCB isomers are needed to truly reflect PCB levels of a given sample. In view of highly different isomeric patterns in the environmental and human samples as shown in the present study, it may be practical to prepare target PCB isomer sets, consisting of different isomers, which are appropriate for the purposes of monitoring of environmental media and humans tissues. We suggest that indispensable isomers for monitoring include #138, #153 and #180, major components in human sample. We also propose that four of the remaining ICES7 congeners, thus #28, #52, #101, #118, should be analyzed for the purpose of total PCB level so that comparison with the existing data can be performed when necessary.

The analysis of other isomers such as #11, #35, #37, #77, #78, #79 is also useful to identify the origin of 3,3'-dichlorobenzidine. Atropisomer PCBs (#84, # 91, #95, #132, #135, #136, #149, #174, #176) plays an important role since they will provide the information upon metabolic status of the organisms. Thus how important which congener is may differ as where the aim of the study is laid. At the same time retrieving and compiling all the isomeric data available are of the great importance especially when the toxicities of each congener are yet to be revealed.

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References