POLYCHLORINATED NAPHTHALENES, POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS AND COPLANAR POLYCHLORINATED BIPHENYLS IN PLASMA FROM NEW YORK RESIDENTS

Qinting Jiang[1], Kurunthachalam Kannan[2], Nobuyasu Hanari[3], Paul Kwan Sing Lam[1], Barbara Wyrzykowska[3], Nobuyoshi Yamashita[3]

[1]Department of Biology and Chemistry, City University of Hong Kong
[2]Wadsworth Center, New York State Department of Health
[3]National Institute of Advanced Industrial Science and Technology

Introduction

Polychlorinated naphthalenes (PCNs), polychlorinated biphenyls (PCBs), and polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are ubiquitous environmental pollutants. Humans are exposed to these compounds through several pathways and diet being the predominant route. A common mechanism of toxic action of these structurally related compounds is through the activation of aryl hydrocarbon receptor (AhR). Based on the concentrations and toxic potentials of individual congeners of PCBs, PCDDs/Fs and PCNs, it is possible to evaluate the relative contribution by each compound class to the AhR mediated toxicity. This will enable the determination of the most critical contaminant of concern to humans, in order to develop strategies and policies to eliminate the sources of exposures. In this study, we report concentrations of PCNs, PCDDs/Fs, and PCBs in human blood plasma collected from individuals in New York City, USA. Relative contribution of each contaminant group to dioxin-like toxicity is examined to identify the critical contaminant of concern.

Materials and Methods

Blood samples were obtained from volunteers from a local hospital in New York City during 2002. Institutional Review Board (IRB) approvals were obtained to analyze human blood plasma for trace organic contaminants. No personal identifiers were available except age, sex, ethnicity, and body weight.

PCNs, PCBs and PCDDs/Fs were analyzed in blood plasma following the method described elsewhere with some modifications.[1] Approximately, 4 mL of plasma samples were mixed with 30 g of anhydrous sodium sulfate and extracted with a mixture of acetone and hexane (1:1, v/v) for 5 min at 100 °C and then with toluene (2nd step) for 10 min at 175°C in an accelerated solvent extractor (ASE-200, Dionex Co., Sunnyvale, CA, USA). Extraction cells were kept at 2000 psi. Samples were spiked with ^{13}C-labeled coplanar PCBs and PCDDs/DFs as internal standards. The extracts were then concentrated and cleaned by use of a multi-layer silica gel packed into a glass column (30 cm long × 20 mm id) in the following order: silica gel (0.8 g), 2% KOH-silica gel (3 g), silica gel (0.8 g), 44% H_2SO_4-silica gel (4 g), 22% H_2SO_4-silica gel (4 g), silica gel (0.8 g), 10% $AgNO_3$-silica gel (8 g) and anhydrous sodium sulfate (5 g) at the top. The column was pre-washed with *n*-hexane (200 mL) and target analytes were eluted with *n*-hexane (200 mL). The eluant was concentrated to 1 mL, purified and fractionated using activated, basic alumina column chromatography. Alumina (10 g) was activated at 130 ^{o}C for 12 h and packed into a glass column (30 mm length × 12 id) with anhydrous sodium sulfate layer (2 g) on the top. The analytes were eluted with *n*-hexane (10 mL, fraction 1), 0.5% DCM in *n*-hexane (40 mL) and 50% DCM in *n*-hexane (100 mL, fraction 3). All fractions were micro-concentrated to 200 µl under a gentle stream of nitrogen. The target analytes were further fractionated by an HPLC by using a porous graphitic carbon column (100 mm length × 4.6 mm id, 7 µm grain size) (Hypercarb, Hypersil, Thermoelectron Corp., San Jose, CA, USA). An aliquot of 150 µl of the fractions 2 and 3 were injected. The Hypercarb-HPLC column was forward-eluted using 50% DCM in *n*-hexane (15 mL; fractions 2-1 or 3-1) and back-flushed using toluene (45 mL; fractions 2-2 or 3-2). All fractions were spiked with isooctane (30 µl), and micro-concentrated to 30 µl.

The HRGC-HRMS analysis was performed using an HP 6890 GC interfaced with a JEOL JMS-700D HRMS (Japan)

at a resolution R> 10 000 MU (10% valley) in the selected ion monitoring (SIM) mode. Each sample was analyzed using two different GC capillary columns. Tetra- to hexa-CDDs/DFs were separated and quantified using an SP2331 capillary column (0.25 mm i.d.×60 m length and 0.20 µm file thickness; Supelco, Bellefonte, USA), while hepta- and octa-CDDs/DFs, tetra- to hepta-coplanar PCBs and tri- to octa-CNs were separated and quantified using a DB-17 column (0.25 mm i.d.×30 m length and 0.25 µm file thickness; J&W Scientific). The column oven temperature was programmed from 100°C (1 min) to 200°C at a rate of 20°C /min and then to 260°C at 2°C /min, with a final hold time of 35 min for tetra- to hexa-CDDs/Fs; 70 °C (1 min) to 200 °C at a rate of 15 °C /min and then to 270°C at 4 °C /min, with a final hold time of 15 min for coPCBs and hepta-, octa-CDDs/Fs; 70 °C (1 min) to 180 °C at a rate of 15 ° C /min and then to 270°C at 2 °C /min, with a final hold time of 10 min for PCNs. Carrier gas (helium) flow-rate was 15 mL/min. Two µl of the extract was injected in the splitless mode. Data acquisition was controlled by an HP work station. Peaks were identified by retention times compared to standards if signal to noise (S/N) ratio was >3 and were quantified if target/qualifier ion ratios were within 15% of the theoretical values. Seventeen 2,3,7,8-substituted PCDD/F congeners were analyzed in this study. Coplanar PCB congeners analyzed include IUPAC# 77, 123, 118, 114, 105, 167, 156, 157, 180, 170 and 189. Total coPCBs represent sum of all 11 non- and mono-*ortho* PCBs analyzed. Total PCNs represent sum of all tri- through octa-CN congeners analyzed.

Results and Discussion

Concentrations of coplanar PCBs, PCDD/Fs and PCNs in fifteen of the samples analyzed so far are shown in Table 1. Concentrations of sum of non- and mono-*ortho* PCBs were greater than those of total PCNs and PCDDs/Fs. Among 11 non- and mono-*ortho* PCBs analyzed, PCB 180 was the most abundant congener found in plasma samples. Concentrations of PCNs in plasma ranged from <900 to 19,000 pg/g, lipid wt. Although most of the plasma samples did not contain detectable concentrations of PCN congeners, a few samples contained elevated concentrations of PCNs. Octa-CN was the predominant chloronaphthalene congener in the plasma of the individual with the greatest concentration (Fig. 1). This pattern is different from those observed in environmental and biological matrices reported to date. Halowax 1051, a technical PCN preparation contains greater proportions of octa-CN. This suggests that some individuals are exposed to specific sources of PCNs. Further studies are needed to elucidate the sources of PCNs to humans. This is the first report of PCNs in human blood plasma from New York residents.

Table 1. Concentrations (pg/g, lipid wt) of total PCNs, coplanar PCBs and PCDDs/Fs in plasma from New York residents. (non-detects=1/2 limit of detection)

	Age (yr)	Fat %	Total PCNs	Total co-PCBs	Total PCDDs	Total PCDFs
Female Mean	37.7	0.48	1360	31900	205	173
Range	32-42	0.30-0.64	1100-1580	20700-45300	<100-389	136-193
Male Mean	37.9	0.60	3121	21500	348	534
Range	26-52	0.29-0.94	<900-19000	4520-45500	<100-1300	145-3420

Female samples include 2 whites and 1 black and male samples include 10 whites and 2 blacks

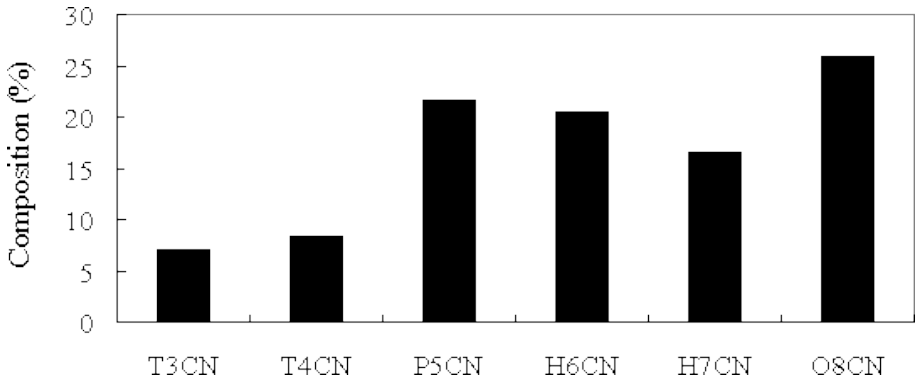

Fig. 1. Profile of PCN congener composition in plasma of an individual with the highest PCN concentration. (non-detects=1/2 limit of detection)

Concentrations of PCDDs in plasma samples were <100-1300 pg/g, lipid wt, which is comparable to that reported for the blood of New York residents collected in 1995-1996[2].Concentrations of PCDFs found in this study (Table 1) were much greater than those reported earlier[2]. Furthermore, in our study, concentrations of PCDFs were similar to those of PCDDs in most of the samples analyzed. The ratio of concentrations of PCDDs to PCDFs, on average, was 1. Earlier studies have reported greater concentrations of PCDDs than of PCDFs in blood samples from New York residents.[2] Greater concentrations of PCDFs in plasma samples in this study suggest an increase in recent exposure to PCDFs.

The dioxin-like toxic equivalency (TEQ) was calculated for coplanar-PCBs, and PCDDs/DFs based on the TEFs suggested by the World Health Organization.[3] To assess the dioxin-like toxicity of toxic PCN congeners, relative potency (REP) values reported in earlier studies were used [4,5] REPs were rounded to a value of either 1 or 5, irrespective of the order of magnitude difference with TCDD. The profile of TEQs contributed by coplanar PCBs, PCNs and PCDDs/Fs is shown in Fig.2. The total TEQs for coplanar PCBs in plasma samples, on average, was 2.03 pg/g, lipid wt. This is lower than the background level of 5.4 pg/g reported for the U.S. general population[6]. PCDDs and PCDFs contributed to more than 90% of total TEQs in all 15 individuals analyzed in this study. The total mean TEQ concentrations for PCDDs/DFs in this study (36.7 pg WHO-TEQ /g lipid wt.) were comparable to the average TEQ serum levels for New York Firefighters (39.0 pg/g)[7]. An average TEQ concentration of 34 pg/g in plasma of males analyzed in this study was higher than 24.2 pg/g reported for the general U.S. male population [6] Furthermore, a TEQ concentration of 47.3 pg/g in females was significantly higher than the reported value of 9.8 pg/g for postpartum blood[2]. This indicates that the exposure to PCDDs/DFs among New York residents is of continuing concern. PCNs concentration less than 5% of the total TEQs.

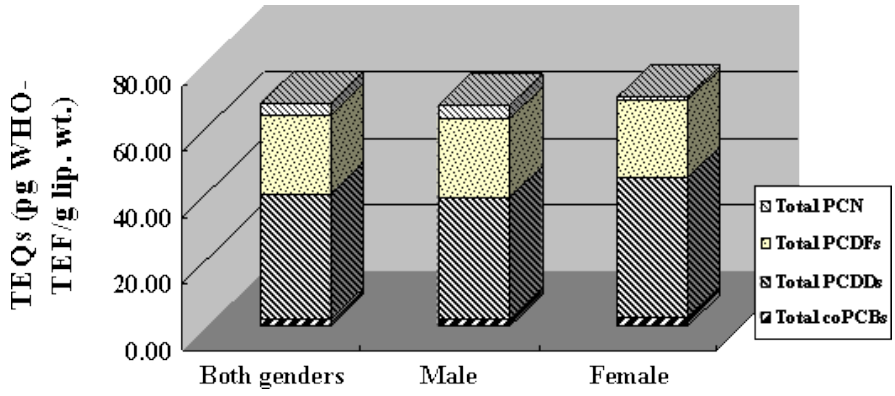

Fig. 2. Mean TEQ concentrations of coplanar PCBs, PCDDs/PCDFs, and PCNs in plasma (pg WHO-TEQ /g lipid wt.) of New York residents. (non-detects=1/2 limit of detection)

References

1. Hanari N., Horii Y., Okazawa T., Falandysz J., Bochentin I., Orlikowska A., Puzyn T., Wyrzykowska B., Yamashita N. (2004) J. Environ. Monit., 06, 305-312.

2. Schecter, A., Kassis, I. and Papke, O. (1998). Chemosphere, 37, 1817-1823.

3. Van den Berg, M., Birnbaum, L., Bosveld, A.T.C., Brunstrom, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., van Leeuwen F.X.R., Liem, A.K.D., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T. (1998). Environ. Health Perspect., 106, 775-792.

4. Villeneuve, D.L., Kannan, K., Khim, J.S., Falandysz, J., Nikiforov, V.A., Blankenship, A.L., Giesy, J.P. (2000). Arch. Environ. Contam. Toxicol., 39, 273-281.

5. Blankenship A. L., Kannan K., Villalobos S. A., Villeneuve D. L., Falandysz J., Imagawa T., Jakobsson E., Giesy J. P. (2000) *Environ. Sci. Technol*. 34, 3153-3158.

6. Schecter, A., Papke, O.(1998). Organohalogen Compounds, 38, 179-181.

7. Kelly, K.J., Connelly, E., Reinhold, G.A., Byren, M., Prezant, D.J. (2002). Arch. Environ. Health, 57, 282-293.