PERSISTENT ORGANIC POLLUTANTS IN THE INHABITANTS OF SINGAPORE

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Abstract

POPs in the blood of inhabitants of Singapore were investigated. Organochlorine pesticides (OCPs) were detected in all samples with *p,p'*-DDE (median 141 ng g^{-1} lipid) most abundant. Total PCBs (41 congeners) were in a range of 0- 124 ng g^{-1} lipid, and concentrations of the sum of 7 BDE congeners varied from 0 to 30.1 ng g^{-1} lipid - generally lower than that of PCBs and OCPs.

Pesticides such as HCB were positively correlated with PCB congeners such as CB 47, CB 118. The common source and similar environmental behavior of pesticides and PCBs is a possible explanation. Age was found to be positively correlated with some pesticides and PCBs, but negatively correlated with BDEs. This may be a result of only recent exposure to PBDEs, or because half-lives of PBDE are shorter than that of PCBs and pesticides.

A model was established using principal components regression. *p,p'*-DDE was regressed against variables (i.e. age, BMI, dietary patterns, gender) to interpret the inter-correlationships. The negative coefficients of seafood, fish and chicken consumption were unexpected, and most likely due to a limited sample number. However, these regression coefficients were not statistically significant except that of intercept (*p*-value =0.048<0.05) and age (*p*-value =0.006<0.05).

Introduction

The ubiquity of persistent organic pollutants (POPs) in various environmental media and human tissues has become a matter of significant concern within the global scientific community. It has been reported that POPs can induce a variety of toxic responses, including immunologic, teratogenic, reproductive, carcinogenic and neurological effects[1]. Although detailed studies have now been undertaken in many other countries on the concentrations of POPs in human blood, there is a paucity of data available from Singapore. This study focuses on determining the residual levels of POPs, namely organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) in inhabitants of Singapore. Moreover, the correlations of donors' demographic characteristics and dietary patterns with their POPs levels will be evaluated. A model has been established to identify the factors that affect levels of POPs in human blood.

Materials and Methods

Our primary investigation objects were students and staff in the National University of Singapore who have lived

in Singapore for at least 5 years. Donors were recruited via advertisement on a voluntary basis. 20ml of blood were drawn from each volunteer, and was centrifuged at 3000rpm for 10 minutes. The top layer consisting of the serum was removed and placed in a n-hexane wash vial, and kept frozen until analysis. Donors were asked to complete a questionnaire which details their weight and age, eating habits, place of residence, and lifestyle.

Glassware used for analysis was baked at 450 °C overnight and then rinsed with acetone, n-hexane and dichloromethane (DCM) before use. All chemicals used were of high purity. Blood extraction and cleanup method followed the method developed by Hovander et al.[2], and is only briefly outlined here. The serum spiked with recovery standards was first denatured by HCl and iso-propanol, and then extracted with hexane: methyl tert-butyl ether (MTBE) (1:1, v:v), followed by concentrated sulfuric acid and gel permeation chromatogram cleanup. Lipid content was determined gravimetrically. Sample analysis and quantification was performed using a Shimadzu QP-2010 (Shimadzu Asia-Pacific, Singapore) gas chromatograph coupled with a mass spectrometer (GC-MS) on a DB-5 ms (J&W Scientific, USA) capillary column. A procedural blank was also run with every batch of 5 samples to assess potential sample contamination. Internal standards were also used for quantification. Statistical data analyses were performed using xlstat2007 software (Addinsoft, USA). Target compounds included 7 pesticides (alpha-, beta-, gamma- and delta-HCH; cis- and trans- chlordane; p,p'-DDT, p,p'-DDD and p,p'-DDE), 41 PCB congeners (CB 17, 18, 28&31, 33, 44, 49, 52, 70, 74, 82, 87, 95, 99, 101, 105, 110, 118, 128, 138, 149, 151, 153&132, 156, 158, 169, 170, 171, 177, 180, 183, 187, 191, 194, 195, 201, 205, 206, 208 and 209) and 7 PBDE congeners (BDE 28, 47, 99, 100, 153, 154, 183).

Results and Discussion

A total of 49 volunteers were recruited, but 5 blood samples were spoilt and only 44 from 27 females and 17 males were successfully determined with POPs. Donors were between 12 to 81 years of age. Excluding two special samples (one aged 12 and another one 81), the average age of donors was 27.5. The Body Mass Index (BMI) ranged between 15.8 and 30.1.

OCPs were detected in all blood samples in this study (Table 1). Total OCP concentrations were derived from the sum of HCB, HCHs, Chlordanes and DDT and its metabolites. The sum of OCPs varied in the samples ranging from a minimum of 46.0 - 1360 ng g^{-1} lipid. The most abundant OCPs found in this study were DDT and its metabolites. They were detected in all samples and had a median concentration of 141 ng g^{-1} lipid. Total PCBs were in the range of 0- 124 ng g^{-1} lipid, with the hexa and hepta substituted PCBs the most abundant (median of 5.60 and 5.39 ng g^{-1} lipid respectively). PBDEs were detected in 95.2% samples for this study. Concentrations of sum of 7 BDE congeners (BDE 28, 47, 99, 100, 153, 154 and 183) in the samples varied from 0 to 30.1 ng g^{-1} lipid, which is generally lower than that of PCBs and OCPs. BDE 153 was the most abundant congener (median 1.03 ng g^{-1} lipid) and was the most frequently detected congener with 39 samples reporting values above LOD.

Table 1 Summary of POPs in human blood

Statistic	Minimum	Maximum	Median	Mean	Standard deviation	Percentage detected
HCB	0.00	396	18.3	43.3	77.9	92.9%
HCHs	0.00	791	87.2	153	204	90.5%
Chlordanes	0.00	56.4	0.00	6.30	11.9	42.9%
DDTs	10.7	1140	141	250	244	100%
Total pesticides	46.0	1360	352	452	361	100 %
tri CBs	0.00	13.7	0.00	1.64	3.79	26.2%
tetra CBs	0.00	19.1	0.00	1.93	4.57	42.9%
penta CBs	0.00	29.2	1.08	3.61	6.12	71.4%
hexa CBs	0.00	58.0	2.37	5.60	9.63	83.3%
hepta CBs	0.00	46.1	3.00	5.39	9.31	66.7%
octa CBs	0.00	4.24	0.00	0.36	0.96	21.4%
nona CBs	0.00	2.85	0.00	0.09	0.45	9.52%
Total CBs	0.00	124	10.9	18.8	25.1	85.7%
BDE 28	0.00	1.87	0.00	0.32	0.51	42.9%
BDE 47	0.00	16.4	0.00	3.27	4.90	45.2%
BDE 100	0.00	7.65	0.91	1.67	2.07	73.8%
BDE 99	0.00	14.3	0.00	1.42	2.60	40.5%
BDE 154	0.00	2.85	0.20	0.39	0.58	61.9%
BDE 153	0.00	7.90	1.03	1.47	1.66	92.9%
BDE 183	0.00	1.63	0.00	0.13	0.32	21.4%
Total BDEs	0.00	30.1	6.14	8.67	8.39	95.2%

Linear correlations between POPs detected in this study were evaluated using Spearman's rank correlation coefficient (r_s). Compounds/congeners were considered to be correlated if the absolute value of the correlation coefficient was larger than 0.5 with a *p*-value smaller than 0.05. p,p'-DDE were positively related with HCB and some PCB congeners such as CB 47, CB 118 (r_s= 0.3-0.5, p <0.5), meaning that higher pesticide levels in blood are indicative of higher amounts of PCBs. The common source and similar environmental behavior of pesticides and PCBs is a possible explanation for this phenomenon. Many PCB and PBDE congeners were significantly correlated with each other, especially tetra CBs with other CBs, BDE 47 with other BDEs, which implies that these congeners are possible indicators for detecting others in human blood. Age was found to be positively correlated with some pesticides and PCBs, which suggests an age dependent accumulation of these contaminants. However, negative correlations were found between age and BDEs, even though they were insignificant. This may be because young people have more chances to contact electronic products which are the main source of PBDEs. Recent application of PBDEs in industry, and relatively shorter half-lives of PBDE probably make the age dependence accumulation less obvious. Other factors such as BMI, food consumption, gender, alcohol and

cigarette consumption were also investigated to determine any effects on the levels of POPs, however, no significant findings were observed.

In order to investigate how variables (i.e. age, BMI, dietary habit, gender) affect the level of POPs, a model was built using regression analysis. Principal components regression was applied to avoid erroneous inferences caused by multi-collinearity. As *p,p'*-DDE was the most frequent and abundant POP detected in this study, *p,p'*-DDE was taken as an example to interpret the inter-correlationships among level of POPs and the variables mentioned above. The multiple regression equation derived was as follows:

log DDE =
1.15+3.14E-02*age+2.41E-02*BMI-5.81E-02*seafood[a]-1.40E-02*fish[a]+3.27E-02*meat[a]-8.54E-02*chicken[a]+1.64*milk[b] -0.183*gender[c]

[a]: data recorded in gram consumed per week

[b]: data recorded in liter consumed per week

[c]: designating 0 for male, 1 for female

The multiple correlation coefficient R^2 was 0.434, which means 43.4% of variability can be explained by the equation. The negative values from the coefficient realtionships of seafood, fish and chicken consumption were unexpected, as it is known that the major route of POPs in human body is ingestion of food[3]. This equation also shows that males have higher levels of POPs than females. However, these regression coefficients were not statistically significant except that of intercept (*p*-value =0.048<0.05) and age (*p*-value =0.006<0.05). This is probably due to the limited sample number of the study (44), meaning that data was insufficient to clearly indicate relationships.

Future work will focus on collecting more blood samples, and mining the factors related to the levels of POPs in the blood, such occupation, place of living, number of electric appliance at home, and number of children if the donor is a female. These factors will then be incorporated in to the model which established using an advanced regression method -- support vector machines. This model will more accurately interpret the functional relations between levels of POPs and their affecting factors. And thus, we may be able to predict one's POPs content if those factors are known.

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