Organohalogen Compounds, Volume 60, Pages 472-475 (2003)

ANALYSIS OF PERSISTENT ORGANOCHLORINE PESTICIDES IN HUMAN BLOOD BY GC/TOF-MS

Ayumi Mochizuki, Tetsuya Hirai and Yoshinori Fujimine

Analysis Section of EDC Analysis Center, Otsuka Life Science Initiative, Otsuka Pharmaceutical Co., Ltd. 224-18, Ebisuno Hiraishi, Kawauchi-cho,Tokusima 771-0195, Japan

Introduction

Various persistent organic pollutants (POPs) exist in global environment and human is exposed to these pollutants through mainly dietary route and environment. POPs such as organochlorine pesticides persist in human tissue for the relatively long period due to their high lipophilic properties, and subsequent endocrine disrupting effects such as estrogen-related cancer are suspected [1]. Therefore, it is important to evaluate the human exposure to POPs by determining the level of these chemicals in human blood as a typical sample for extensive investigations.

Generally, POPs are analyzed using high-resolution gas chromatography (HRGC) / high resolution double focusing mass spectrometry (HRMS) in the selected ion monitoring mode (SIM). In this method, the each peak can be assigned to the specific compound by the retention time based on mass chromatograms and abundant ratio between two monitoring ions peculiar to chlorine compounds. It can measure with high sensitivity and high resolution (>10,000), however, mass spectrum of each compound can not be obtained. We employed time of flight mass spectrometer (TOF-MS) for the analysis of POPs, which is superior to HRMS in the scanning time, giving each mass spectrum in milli Dalton (mDa) level. Thus we can obtain more positive identification data. Furthermore, unknown compounds can be identified by their mass spectrum while doing quantitative analysis of known compounds.

In this study, we report the analysis of persistent organochlorine pesticides in human blood using HRGC/TOF-MS.

Methods and Materials

Chemicals

Chemicals analyzed in this study were HCB, β-HCH, γ-HCH, Heptachlor epoxide, Oxychlordane, t-nonachlor, Dieldrin, 2,4'-DDT, 4,4'-DDT, Mirex and 4,4'-DDE. Unlabeled compounds and $^{13}C_{12}$-labeled compounds were purchased from Cambridge Isotope Laboratories, Inc. (USA).

Used solvents and reagents were dioxin-analysis grade or persistent pesticide and PCB-analysis grade.

Samples

Blood samples were obtained from 6 healthy volunteers. Donor ages ranged from 25 to 41-years old. The samples were stored at –20□ until analysis.

Preparation of samples for analysis

Extraction

About 30 g of blood was transferred to 200 mL tube, and 2 ng of $^{13}C_{12}$-labeled compounds were added. Lipid fraction containing persistent organochlorine pesticides were obtained by extracting with saturated ammonium sulfate (12 mL) and 25% ethanol/hexane (24 mL), and repeated extraction by hexane (24 mL) two times.

The extracts were washed with ultra-pure water and the organic layer was dried over sodium sulfate and evaporated to dryness. The amount of remaining lipid was determined by weight.

Clean-up

GPC clean-up were carried out on CLNpak EV-G column and CLNpak EV-2000 column (Shodex®, Japan) using 30% acetone/cyclohexane as soluvent and at a flow rate of 4 mL/min. Extracted lipid was dissolved in 5 mL of 30% acetone/cyclohexane and purified by GPC. The fraction with target compounds (18 to 26 minutes after injection) was evaporated and dissolved in 2 mL of 25% toluene/acetonitrile.

The clean-up sample was loaded in ENVI-CARB/LC-NH_2/LC-SI cartridge (20 mL tube, SUPELCO, USA) and target compounds were eluted with 25% toluene/acetonitrile (20 mL). The eluent was evaporated and transferred to a GC autosampler vial tube. This solution was spiked with recovery standards, and concentrated to 20 μL in nonane.

HRGC/TOF-MS analysis

Determination was performed using Hewlett Packard 6890 Series high-resolution gas chromatography interfaced with a Micromass GCT mass spectrometer. Chromatografic separation was achieved by splitless injection on HT8-PCB capillary column (60 m, 0.25 mm i.d.). The column temperature was set at 120□; heated to 180□ at 20□/min; and heated to 260□ at 2□/min and held at 260□ for 11 min. The injector was set at 280□ and transfer line was set at 260□. The TOF was operated in the EI mode with the electron energy of 50 eV, and scan range was 100-450 m/z with a resolution of 6500. Scanning rate was 1 scan/sec.

Results and Discussion

The results of the analysis by GC/TOF-MS are summarized in Table 1 and graphically displayed in Figure 1. All target compounds were detected except γ-HCH. In all subjects, p,p'-DDE was the highest levels residue, ranged from 93 to 680 ng/g-lipid. β-HCH was the secondary highest levels residue, ranged from 41 to 550 ng/g-lipid. In other reports, high level pollution of these compounds had been similarly observed in human blood and other biological samples as particularly highly persistent organochlorine pesticides [2-3]. The range of detection limits on GCT analysis using 30 mL whole blood were 0.3-1.9 ng/g-lipid on each compounds. Although the sensitivity of GCT was inferior to that of HRMS, these detection limits was more than enough to detect almost all target compounds.

During GC/TOF-MS analyses, full scan spectrums are obtained. The examples of TOF mass spectrum obtained from analysis in human blood and corresponding data on NIST mass spectral library are given in Figure 2 and 3. Figure 2 shows β-HCH and Figure 3 shows p,p'-DDE respectively. On GC/TOF-MS analysis, we could give each mass spectrum in mDa level, and more positive identification was possible.

There are some advantages on GC/TOF-MS analysis. First, it can scans quickly (maximum 10 scan/sec) compared with magnetic sector-type mass spectrometry (approximately 0.3 scan/sec). Second, the analysis isn't influenced by the interference matrix because GC/TOF-MS can measure with higher resolution than quadrupole-type mass spectrometry. Consequently we can decide individual pollutants with high accuracy by mDa levels of mass spectrum. Therefore, GC/TOF-MS analysis can provide an useful method to determine the occasional contamination by POPs and unknown pollutants as screening method.

Acknowledgments

The authors wish to acknowledge the advice by Shaw Watanabe from Tokyo University of Agriculture.

References

(1) Francine L, Susan EH, Mary SW, Graham AC, Walter CW, Frank ES, David JH, Int. J. Cancer **2001**, 91, 568-574

(2) Corinnr J. Charlier and Guy J. Plomteux, Clin Chem Lab Med **2002**, 40(4), 361-364

(3) Erika van Wyk, Henk Bouwman, Herman van der Bank, gerhard H. Verdoorn, Dieter Hofmann, Comparative Biochemistry and Physiology Part C **2001**, 129, 243-364

Table 1 Persistent organochlorine pesticides levels in human @@@@@ blood of general population in Japan (n=6)

Compounds	Mean (ng/g-lipid)	SD	Range (ng/g-lipid)
o,p'-DDT	1.7	1.4	0.58-3.7
p,p'-DDT	7.4	4.4	3.1-14
p,p'-DDE	270	220	93-680
á -HCH	140	200	41-550
â -HCH	N.D.*	***	***
HCB	16	4.4	10-23
Dieldrin	6.2	4.7	1.9-14
t-nonachlor	27	25	6.5-72
Oxychlordan	8.1	6.3	3.4-20
Heptachlor epoxide	3.3	3.2	0.80-9.4
Mirex	0.94	0.57	0.36-1.7

* : N.D. - Not detected (á -HCH: limits of detection ranged from 1.2 to 1.9 ng/g-lipid)

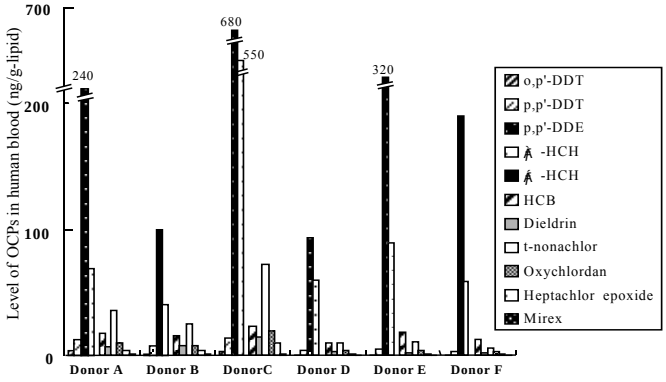

Figure 1 Persistent organochlorine pesticides levels in human blood of general population in Japan

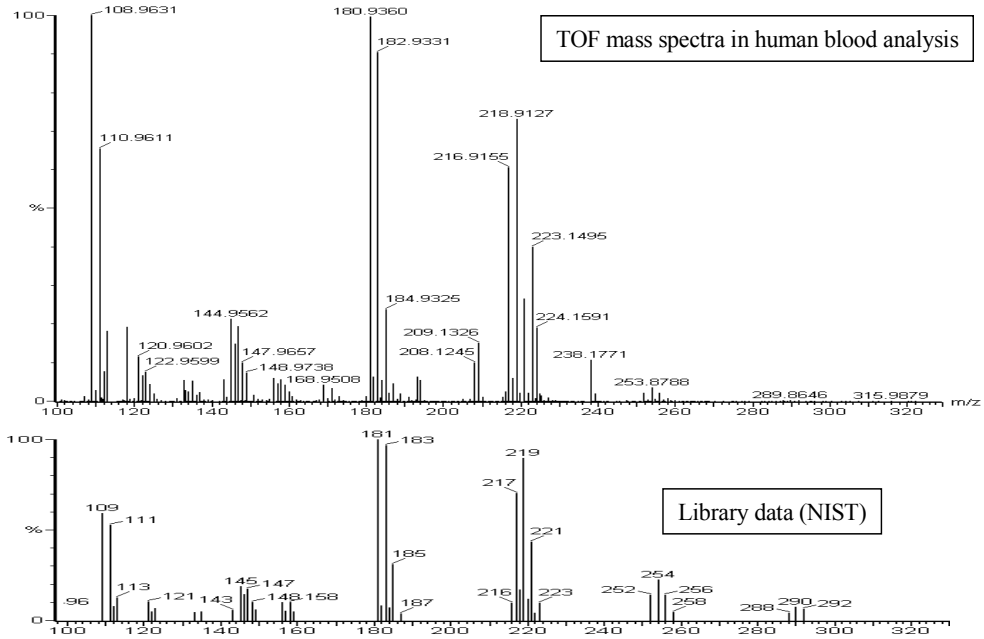

Figure 2 TOF mass spectra obtained from analysis in human blood and library data (Â -HCH)

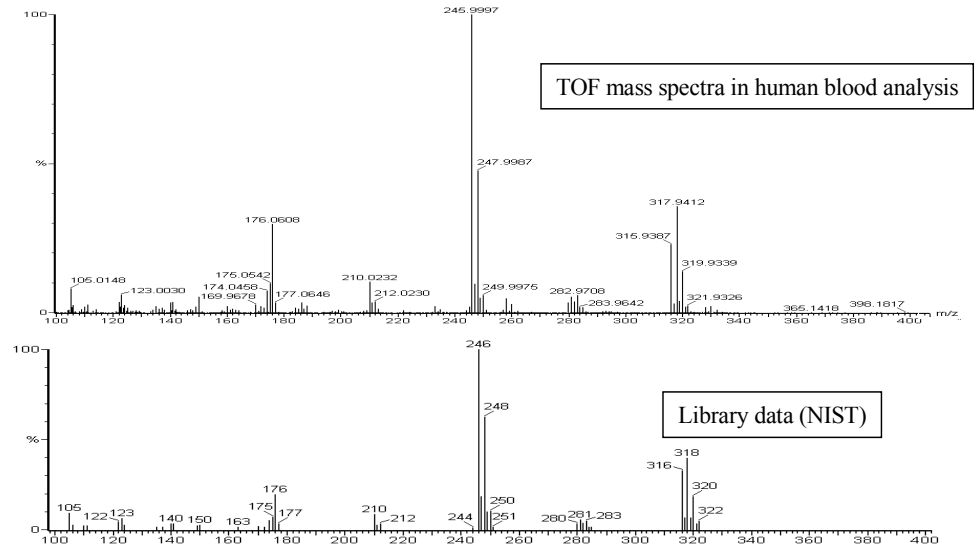

Figure 3 TOF mass spectra obtained from analysis in human blood and library data (p,p'-DDE)