Organohalogen Compounds, Volume 60, Pages 387-390 (2003)

FAST, SENSITIVE, AND COMPREHENSIVE ANALYTICAL METHODS INCLUDING THREE-DIMENSIONAL ANALYSIS (GCxGC-TOFMS) FOR MEASURING HALOGENATED PERSISTENT ORGANIC POLLUTANTS

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Introduction

At the Centers for Disease Control and Prevention (CDC), the demand for high quality laboratory measurements for environmental contaminants in human populations has steadily increased during the past two decades. This increase in demand for human measurements is due to several factors: 1) recent reports that certain kinds of chemicals that are in the environment are capable of disrupting animal hormone systems; and 2) recent epidemiology studies showing a poor correlation between epidemiological exposure indices and actual human body-burden laboratory measurements. These measurements in human matrices (internal-dose) reduce misclassification and greatly increase the probability of finding an association (if any really exists) between exposure to a chemical and any potential human health effects. In addition to an increased demand for laboratory measurements, there has been a very large increase in the number of analytes that must be measured for epidemiological studies. The number and types of compound classes that epidemiologists, toxicologists, and risk assessors are interested in studying have been increasing steadily over the past 20 years[1]. In the past, we have developed analytical methods that are specific for each compound class[2-6]. These methods very often required a separate serum sample for each of the various compound classes. The results of the various analyses would then be combined together for a comprehensive report on the levels within an individual. Because of the limited amount of matrix that is usually available from epidemiological studies, we have been concentrating on developing a comprehensive analytical method for measuring many chemical compound classes in a single human sample. This paper will outline our current approach to a Universal Human Exposure Assessment Scheme.

Results and Discussion

The outline for the Universal Human Exposure Assessment Scheme is shown in Figure 1. The Scheme shows that a minimum of 180 compounds will be measured in serum/plasma with an additional 38 metabolites measured in urine. We plan to add other compound classes to this Scheme over time.

Analytical methods in use at CDC for measuring environmentally significant chemicals in various human matrices are given in Table 1. Various methods can be used depending on what analytes are required and how much matrix is available for the study. We will present the details and validation for the Semi-Automated Comprehensive Extraction and Multiple Fractionation (SACEMF) method shown in Table 1. This method measures more than 180 chemicals from a single serum/plasma sample. An additional 38 or more chemicals can be added to the exposure assessment by analyzing urinary metabolites (Table 1).

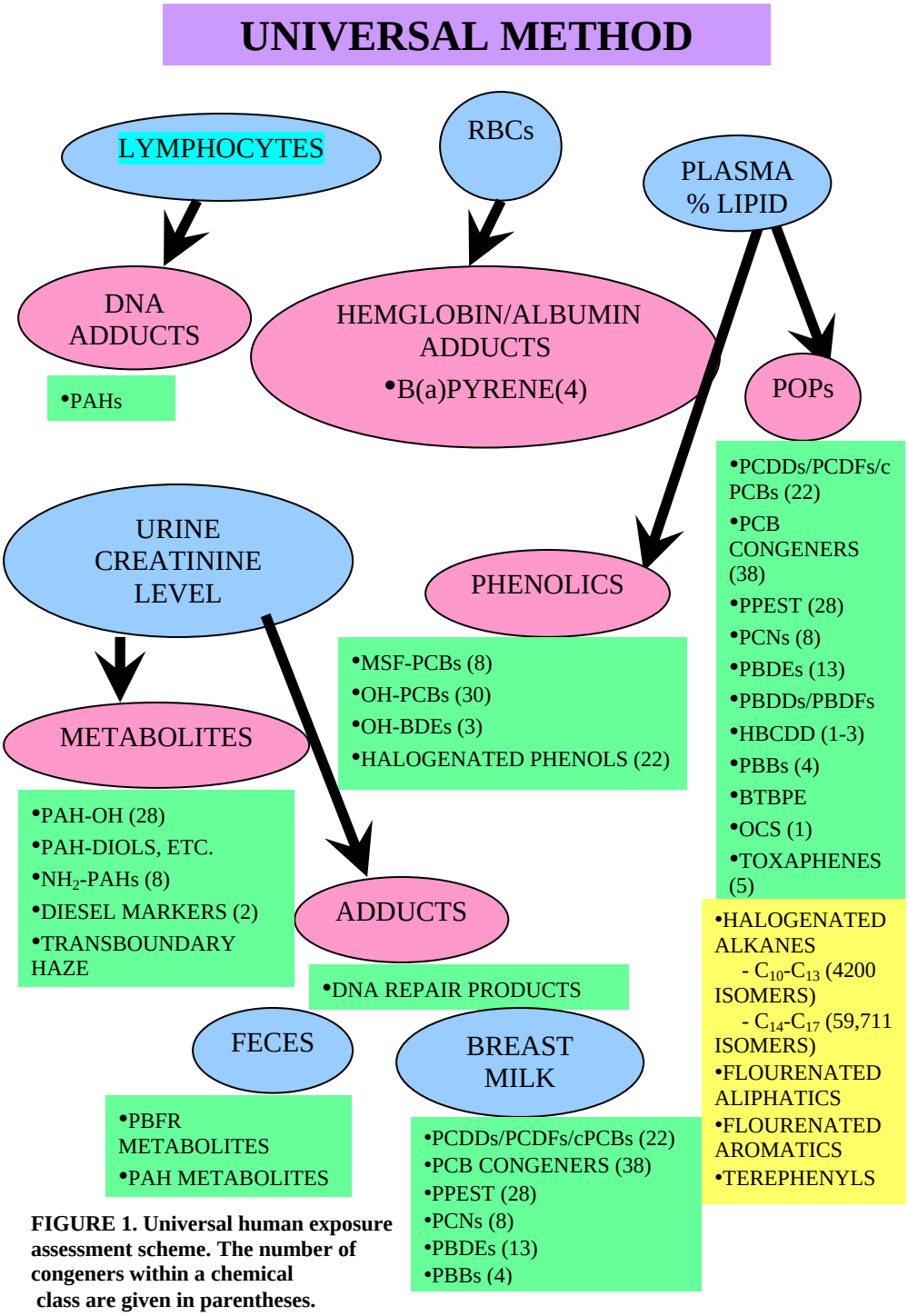

FIGURE 1. Universal human exposure assessment scheme. The number of congeners within a chemical class are given in parentheses.

Organohalogen Compounds, Volume 60, Pages 387-390 (2003)

Organohalogen Compounds, Volumes 60-65, Dioxin 2003 Boston, MA

Table 1. Various Analytical Methods in Use at CDC for Measuring Chemicals in Human Samples.

	MANUAL SOLID-PHASE METHOD	FMS METHOD	BROMINATED FLAME RETARDANTS AND PCBs	PAH METABOLITE METHOD	SACEMF METHOD	HEMOGLOBIN ADDUCT METHOD
MATRIX	Serum/Plasma	Serum/Plasma	Serum/Plasma	Urine	Serum/Plasma	Hemoglobin
AMOUNT OF MATRIX	1 - 2 g	0 – 100 g	4 g	3 mL	1 – 13 g	10 mL Whole Blood
METHOD	Serum/Plasma Formic Acid Water (M) C18 Sorbent Hexane Eluant (M) Silica (Neutral) Florisil (M) Hexane : DCM (1:1) ID-HRGC/HRMS	Serum/Plasma Formic Acid Water (M) C18 Sorbent Hexane Eluant (M) Acid/Basic/Neutral Silica Alumina Carbon (A) ---Forward F1 ---Reverse F2 ID-HRGC/HRMS	Serum/Plasma Formic Acid Water (M) Zymark Rapid Trace SPE workstation (A) Rapid Trace Silica (Neutral) Silica (Acidic) (A) ID-HRGC/HRMS	Urine Buffer Deconjugation (M) Envirolute SPE (M) Derivitization (M) ID - HRGC/HRMS	Serum/Plasma Oasis HLB SPE (A) Activated Silica SPE (A) F1- Hexane/DCM (19:1) Nonpolar Cmpds F2-Hexane/Methanol (9:1) Polar Cmpds F1: Activated Silica Silica (Acidic) Pyrene HPLC Column (A) F1-Non-Polar Cmpds F3- Planar Cmpds F2- Diazomethane Deriviative Silica (Acidic) F1/3 ID-HRGC/HRMS F2 ID-NICI/GCMS	Blood Centrifugation Wash RBC's Lyse Cells (M) Acid Hydrolysis C18 SPE (M) Derivatization of Tetrols NICI/GCMS
ANALYTES	PCBs (38) PP (13 – 28) Toxaphenes (5)	Fraction 1 : PCB (38) NPP (13) Fraction 2: Ds/Fs/cPCBs (22) PCNs (8)	PBDEs (10) PBBs (4) PCBs (38) NPP (11) HBCDD (3)	PAH-OH (28) PAH-NH2 (8) Diesel Markers (2)	F1: PCBs (38) NPP (11) PBDEs (10) PBBs (4) HBCDD (3) Toxaphenes (5) F2: PCBs-OH (30) PBDEs-OH (3) Phenols (10-15) PCBs-MSF (8) F3: PCDDs/F/cPCBs(22) PCNs (8) PBDDs/PBDFs (10)	(+/-) Benzo(a) pyrene -r-7,t-8,t-9,c-10 tetrol -r-7,t-8,t-9,t-10 tetrol -r-7,t-8,c-9,t-10 tetrol r-7,t-8,c-9,c-10 tetrol
SAMPLES PER DAY PER PERSON	24	12	30	35	15	10

PP = Persistent Pesticides. NPP= Nonoxygenated Persistent Pesticides
SACEMF = Semi-Automated Comprehensive Extraction and Multiple Fractionation
M = Manual Step A = Automated Step

Currently, the various fractions from these methods must be analyzed separately on different mass spectrometers. We have been working on a comprehensive GCxGC mass spectrometric method first discussed in 1992[7]. This method has the potential to measure all of the chemicals in a single analytical run. The potential for achieving this goal is shown in Figure 2. This GCxGC-TOFMS chromatogram[8] shows the simultaneous separation and measurement of 58 chemicals (PCBs, PBDEs, and persistent pesticides).

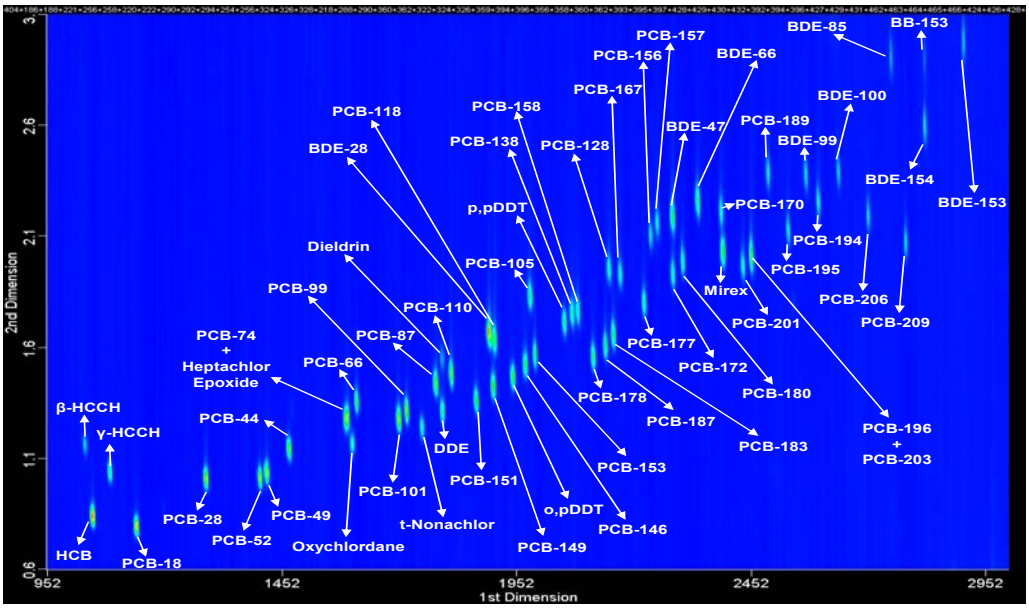

Figure 2. GCxGC/TOFMS chromatogram (contour plot) of the 58 compounds based on analytes characteristic ions**.**

Conclusion: Important advances have been made toward development of a comprehensive method to measure in human samples a large number of chemicals of environmental concern.

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