EVALUATION OF HUMAN TOENAIL AS A NON-INVASIVE BIOMONITORING MATRIX FOR
ASSESSING HUMAN EXPOSURE TO ENVIRONMENTAL ORGANIC POLLUTANTS BY
OPTIMIZED SAMPLE PREP AND GC/HRMS ANALYSIS

A Dissertation
presented to
the Faculty of the Graduate School
at the University of Missouri

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

By
PAUL HERBERT PETERMAN
Dr. J. David Robertson, Dissertation Supervisor

MAY 2013

© Copyright by Paul Herbert Peterman 2013

All Rights Reserved

The undersigned, appointed by the Dean of the Graduate School, have examined the
dissertation entitled

EVALUATION OF HUMAN TOENAIL AS A NON-INVASIVE BIOMONITORING MATRIX FOR
ASSESSING HUMAN EXPOSURE TO ENVIRONMENTAL ORGANIC POLLUTANTS BY
OPTIMIZED SAMPLE PREP AND GC/HRMS ANALYSIS

Presented by Paul Herbert Peterman

A candidate for the degree of Doctor of Philosophy

And hereby certify that, in their opinion, it is worthy of acceptance.

J. David Robertson, Ph. D, Advisor and Committee Chair

Susan Z. Lever, Ph. D

Kent S. Gates, Ph. D

C. Michael Greenlief, Ph. D

Frederick S. vom Saal, Ph. D

DEDICATION

In loving memory of my mother, Leona Ernestine Peterman, who loved to learn and care for those in God's creation.

This is dedicated to my wife, Tina M. Lunsford Peterman, Ph. D., Phi Beta Kappa, fellow scholar, lover, best friend, and inspiring humble person always yearning to learn more. Thank you for both sharing the path and blazing the end of the journey of graduate school at Mizzou with your unwavering encouragement.

This is also dedicated to my children, Jessica, Elizabeth, and Macie, grandson Jayden, son-in-law Joe, and others yet to be. I was very fortunate to be your adopted dad. May your lives be fulfilled using your God-given talents and abilities.

Finally, I dedicate this to my older siblings, John Peterman and Pauline Strehlow, and my siblings in-law, Gary, Joel, Chris, Debra, Joann, and Susan for their caring and encouragement.

ACKNOWLEDGEMENTS

With profound gratitude, I acknowledge the help, inspiration, and moral support of a number of people who have helped my journey through graduate school, research, and this dissertation:

Dave Robertson, my advisor, who convinced me that some of the best academic research has occurred despite difficult challenges, lack of ideal laboratory conditions, and some failures along the way. Thank you for understanding the pitfalls of sophisticated analytical instruments failing. Thank you for your enthusiasm, trust in my ability, and reassuring me at times that I did not have to be completely successful but to assess and learn from whatever results occurred.

Adrian Covaci, friend, mentor, and Belgian researcher with hair biomonitoring, thank you so much for your valuable, encouraging, insightful advice. Though far away, your email responses helped bolster my confidence to try toenail biomonitoring and that my best efforts could succeed and make a difference to society.

Fred vom Saal, committee member, friend, and collaborator on previous MU research, thank you again for whetting my academic research appetite in 1998. Further research with Julia Taylor on BPA led me to return to graduate school for my own academic research effort.

Carl Orazio, my former supervisor at the US Geological Survey, who also pursued his Ph. D later in life and personally understood the rigors of graduate school while working at an environmental laboratory. Thank you for buoying my career by

professional trips for Belgium EU Workshop and presenting several papers at Dioxin 2006 in Oslo. Thanks for approving my flex-time work schedule at USGS that allowed me the opportunity to attend MU graduate school. Also, thank you for your enthusiasm, support, artful cartoons, and trust in my ability.

Robert Gale, friend, colleague at USGS, and effective scientific devil's advocate. Despite your growing up in California, you embody the best characteristics of someone from the Show-Me State. Thank you especially for your insights and patience to work around or to solve anomalies in software data processing and chromatography/mass spectrometry. Thank you also for tackling last minute issues with MS WORD.

James Zajicek, friend, colleague at USGS, and enthusiastic supporter for my academic research. Thank you so much for sharing your insights and knowledge about biochemistry information pertinent to my research.

Kevin Feltz, friend and colleague at USGS, who appreciated and often shared with my various attempts to improve sample preparation techniques. Thank you.

Vickie Schroeder, friend and colleague at USGS, who really mastered my flash GPC column preparation technique and demonstrated its potential by another person.

Kathy Echols, friend, immediate supervisor and co-author at USGS, for your support in my buying custom columns and my optimizing analytical techniques.

Don Tillitt, David Alvarez, and Chris Schmitt at USGS, thank you for your support.

Julia Towns-Campbell and Kathy Richter at USGS, thanks for help with Endnote.

Tom Crabtree, friend, and experienced high resolution MS expert at his best with helpful advice especially whenever the analyst or instruments are not working well.

Dan Difeo of SGE for promoting my capillary column separations by donating a high performance narrow bore column and keeping me informed about new capillary chromatography accessories, Stefano Galli of MEGA in Legnano Italy for supplying excellent deactivated retention gaps, Scott Hallstrom and Brock Chittim of Wellington Labs for their generous help with isotopically-labeled and trace-level calibration standards, Ben Priest of Cambridge Isotope Labs for help with labeled compounds, Sean Crook at Waters Corporation for insightful troubleshooting help with high resolution MS, Paolo Magni of Thermo Italy and Konrad Grob, Jr. in Switzerland for extremely valuable chromatographic advice.

John Brockman and James Guthrie, fellow beginning graduate students who now work with Dr. Robertson at MURR. Thank you for your friendship and support.

Dave Meier, fellow graduate student in our Robertson Research Group at MU, who graduated ahead of me. Thanks for often blazing the trail ahead of me.

Jerry Brightwell and recently Ryan Coffin, able and competent staff secretaries in the MU Chemistry Dept, thank you for your consistent professionalism and great job of shepherding us MU Chemistry graduate students through our long journey.

Various members of MU IT Department who expertly answered or at least tried their best to provide help at the HELP Desk phone line or on campus. Thank you.

Finally, MU Chemistry professors, who taught me during the past years, including Gates, Sharp, Harmata, Loepky, Greenlief and Leigh. Plus, encouraging advice from Rainer Glaser about my computations of HOMO-LUMO energy levels of PBDEs. Thank you again. I learned a lot and yet learned how much I still don't know.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	ii
LIST OF FIGURES	vii
LIST OF TABLES	x
ABBREVIATIONS AND ACRONYMNS	xiii
ABSTRACT	xv
Chapter 1. INTRODUCTION	1
Human Health Biomonitoring	1
Test Hypothesis by Measuring DDT/DDE, PBDEs, Triclosan, BPA, and Phthalate Metabolites in Toenail Samples	19
CHAPTER 2: METHODS	22
Sample Collection	22
Sample Cleaning	23
Sample Sets and Surrogates Spiked	23
Dissolution and Extraction of Toenail	26
Lipid removal using Miniature Flash Gel Permeation Chromatography (GPC)	30
Methylation of (Polar) Phenols and Acids with Iodomethane	35
Cleanup with 0.5-g Basic Alumina SPE	35
Fractionation of Non-Polar Compounds with 4-g Basic Alumina Flash Chromatography Column	37
GC/Full-scan Mass Spectrometry to Identify Unsuspected Compounds	39
GC/High Resolution Mass Spectrometry (GC/HRMS) Selected ion monitoring	40
CHAPTER 3. ANALYTICAL RESULTS and DISCUSSION	53
Dissolution of Nail	53

Lipid removal by GPC flash column.....	54
Cleanup with SPE Basic Alumina Glass Cartridge.....	56
Final Cleanup and Separation with Basic Alumina Flash Chromatography	57
GC/full-scan MS to identify unsuspected compounds in Set 1 samples	58
GC/full-scan MS to identify unsuspected compounds in Set 3 samples	66
GC/HRMS Analysis to detect and quantitate target polar compounds.....	85
GC/HRMS analysis of non-polar organic pollutants in Sets 1, 2, and 4	89
Replicate Analysis through Time of Multiple Toenail Samples of Individuals A and C	105
Summary	109
CHAPTER 4. RESULTS and DISCUSSION – TOENAILS AS BIOMONITORING MATRIX.....	112
Biomonitoring PCB congeners	118
Biomonitoring of moderately-persistent PBDEs.....	125
Biomonitoring Synthetic Musk Fragrance Compounds After Their Identification	129
Biomonitoring of a Transient Polar Compound, Triclosan.....	131
House cats as possible “sentinels” of home-related pollutants for humans?.....	133
Summary	137
CHAPTER 5. LIMITATIONS AND FUTURE RESEARCH	141
Improve Toenail Digestion and Extraction.....	141
Target Compounds with Shorter-Half Lives whose Exposures Might be Integrated in Nails.....	142
Continue to Search for and Identify Unsuspected Compounds	143
Future Toenail Biomonitoring Research – Validate with a Diverse Population, Target Compounds with Known or Suspected Exposures, and Other Matrices.....	144
BIBLIOGRAPHY	145
VITA.....	154

LIST OF FIGURES

Figure	Page
1. Three routes of chemical exposure and fate within the human body.	4
2. Structures and properties of DDT, DDE, and DDD isomers.	12
3. Structures and properties of prominent polybrominated-diphenyl ethers	15
4. Structures and properties of a di-alkyl Phthalate and a mono-alkyl-ester phthalate metabolite.....	17
5. Structure and properties of Bisphenol A and Triclosan.....	19
6. Overall Method Flow Chart for Toenail Samples.....	24
7. Structure of dithiothreitol used to break down keratin	28
8. Sample Set 1 Culture tubes.....	29
9. Top reservoir and adapter-cap for nitrogen-pressurized flash GPC.....	31
10. Flash GPC elution was collected in appropriate-sized vials.....	33
11. Orange-colored beta-carotene, eluted before 4.5 mL, as did lipid.	33
12. Lipid can be seen to coat the walls of evaporated GPC collection vials.....	34
13. Cleanup with 0.5-g Basic Alumina (and 2-g Na ₂ SO ₄) SPE Cartridge.....	36
14. Non-polar organic pollutants were chromatographed by a 4-g basic alumina flash column	38
15. GC/HRMS analysis with a 30 m x 0.15 mm i.d. BPX5 column of a low calibration mixture of 80 PCBs (6 pg each injected)	48
16. Four selected ions from Group 3 of the GC/HRMS analysis of PCBs.....	49
17. GC/HRMS analysis with a 13 m x 0.15 mm i.d. BPX5 column.....	50
18. The last portion of the same composite chromatogram from GC/HRMS analysis of basic alumina Fraction 2	51

19.	Culture tubes of all human toenail extracts of Set 4 (Table 2).....	54
20.	Total ion chromatogram of Fraction 2 of Set 1 blank (1B-1) compared with that of Toenail (3T-1)	59
21.	Pairs of chromatograms from 33.4-36.8 min of blank and nail samples.....	61
22.	Mass spectrum at 34.29 min in nail sample confirmed as chlordane.....	63
23.	Mass spectrum at 35.09 min in nail sample confirmed as trans-nonachlor	64
24.	Pairs of chromatograms of m/z 498 (¹³ C-PBDE 47; 79 Internal Standard) and 486 (PBDE 47) in blank and nail samples.....	65
25.	Mass spectrum at 9.75 min was tentatively identified as 2,4-dimethyl-quinazoline	67
26.	Mass spectrum at 15.25 min was tentatively identified as 6-methoxy-2-methyl-benzothiazole.....	68
27.	Mass spectrum at 17.44 min from Nail 11T-3 was confirmed as benzophenone...	70
28.	Mass (m/z 243) chromatograms from 22.9-26.5 min of Set 3 samples	71
29.	Mass chromatograms (m/z 241 and 253 for native- and ¹³ C-BPA-dimethyl ether)	72
30.	Mass chromatograms (m/z 302 and 314 for native- and ¹³ C-triclosan-dimethyl ether)	74
31.	Mass spectrum at 31.93 min from Nail 11T-3 was tentatively identified as 2-anilino-4-methylquinoline	76
32.	Mass spectrum at 46.87 min from Nail 11T-3 was tentatively identified as octocrylene	77
33.	Mass spectrum at 18.52 min was tentatively identified as 4-(4-methoxyphenyl)-3-butene-2-one	78
34.	Mass spectrum at 16.20 min of nail 11T-3 sample could be of a C ₁₃ H ₁₆ N ₂ related to Figure 25	79
35.	Mass spectrum at 30.23 min of nail 11T-3 with apparent molecular mass of 261. 80	
36.	Mass spectrum at 34.30 min of nail 10T-3 showed an apparent molecular mass of 244	81
37.	Summed ion chromatograms (m/z 130-750) from 8.0-17.3 min of Set 3 blank	83

38.	Tentative identification of methyl anthranilate or methyl 2-amino-benzoate	83
39.	Tentative identification of 4-methoxy-benzoate	84
40.	Tentative identification of methyl 2-(dimethylamino)-benzoate	84
41.	Tentative identification of methyl 2-(methylamino)-benzoate	84
42.	Tentative identification of 2-(acetylamino)-benzoate	85
43.	From Set 3 samples, GC/HRMS selected ion chromatograms of m/z 241.1229 for dimethyl ether-BPA and m/z 253.1631 for ¹³ C-BPA surrogate	87

LIST OF TABLES

Table	Page
1. Analytical Challenge to Measure Contaminants below 1 ng in Toenail Samples....	20
2. Toenail Clippings and QC Samples with Method Steps and Analytes Targeted.....	25
3. GC/HRMS Selected Ion Monitoring of Fraction 1 (PCBs, Pesticides)	42
4. PCB Congeners and Pesticides in Fraction 1 by GC Retention Time	43
5. GC/HRMS Selected Ion Monitoring of PBDEs, Pesticides, and ¹³ C-Surrogates in Basic Alumina - Fraction 2	44
6. GC/HRMS Selected Ion Monitoring for Triclosan, Bisphenol A, and Other Polar Compounds	46
7. Concentrations (ng/g) with Surrogate Recoveries of DDE and Several Polar Compounds in Set 3 Toenail Samples.....	88
8. Concentrations (ng/g) of p,p'-DDE and Several PBDEs in Set 1 Samples	89
9. Concentrations (ng/g) of Organochlorine Pesticides in Set 2 Samples	91
10. Background-Subtracted Concentrations (ng/g) of PBDEs in Set 2 Blank, Toenails, and Soap Washes.....	91
11. Concentrations (ng/g) of Organochlorine Pesticides in Set 4 Blank Samples	93
12. Blank-Subtracted Concentrations (ng/g-nail) of POPs Pesticides in Set 4 Toenail Samples	93
13. Blank-Subtracted Concentrations (ng/g-wet wgt) of Organochlorine Pesticides in Soap Washes	95
14. Blank-Subtracted Concentrations (ng/g) of Synthetic Musk Fragrances in Set 4 Toenail Samples	96
15. Concentrations (ng/g) of PBDEs in Set 4 Blanks	97
16. Blank-subtracted Concentrations (ng/g) of PBDEs in Set 4 Toenail Samples.....	98

17.	Blank-Subtracted Concentrations (ng/g) of PBDEs in Set 4 Soap Washes of Toenails	99
18.	Percent Recoveries of ¹³ C-PBDEs and DDE in Set 4 Toenail Samples and Blanks .	100
19.	Amount of PCBs (ng) in Set 4 Blanks	101
20.	Blank-Subtracted Concentrations (ng/g) of PCBs in Set 4 Toenail Samples.....	103
21.	Blank-Subtracted Concentrations of PCBs in Set 4 Toenail Soap Washes	104
22.	Percent Recoveries of ¹³ C-PCB Surrogates in Set 4 Toenail Samples	105
23.	Blank-Subtracted Concentrations (ng/g-nail) of Organochlorine Pesticides in Multiple Toenail Samples from Two Individuals	106
24.	Blank-Subtracted Concentrations (ng/g-nail) of PBDEs in Multiple Toenail Samples from Two Individuals	106
25.	Blank-Subtracted Concentrations (ng/g-nail) of Selected PCBs in Multiple Toenail Samples from Two Individuals.....	108
26.	Lipid-Based Concentrations (ng/g lipid) of Organochlorine Pesticides in Nails	113
27.	Concentrations (ng/g lipid) of Organochlorine Pesticides in Serum, Tissue, and Hair Samples from Extant Data	113
28.	Lipid-Based Concentrations (ng/g-lipid) of PCBs in Toenails, Extant Serum, Tissue, and Hair Samples	120
29.	Relative Potencies of Neurological and Dioxin-like Toxicity Reported for PCBs ...	124
30.	Blank-Subtracted Lipid-Based Concentrations (ng/g-lipid) of PBDEs in Toenails..	127
31.	Lipid-Based Concentrations (ng/g-lipid) of PBDEs in Extant Samples	127
32.	Blank-Subtracted Lipid-Based Concentrations (ng/g-lipid) of Musk Fragrances in Toenails and Extant Data	130
33.	Concentration (ng/g-wet weight) of Triclosan in Set 3 Toenails and Extant Data	132
34.	Tentatively-Identified Unsuspected Compounds by Full-scan Mass Spectra in Toenail Samples from Set 3	133
35.	Lipid-Based Concentrations (ng/g-lipid) of PBDEs in Cat Toenails and Extant Cat Serum	135

36.	Lipid-Based Concentrations (ng/g lipid) of Pesticides in Cat Toenails and Extant Cat Serum	136
37.	Lipid-Based Concentrations (ng/g-lipid) of PCBs in Cat Toenails and Extant Cat Serum	138

ABBREVIATIONS AND ACRONYMS

2,4,5-T	2,4,5,-Trichlorophenoxyacetic acid
ABS	Acrylonitrile Butadiene Styrene
Bis-TriBr-PE	1,2-bis(2,4,6-tribromophenoxy)ethane
BPA	Bisphenol A
CDC	Centers for Disease Control and Prevention
DCM	Dichloromethane
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenylethylene
DDT	Dichlorodiphenyltrichloroethane
DEHP	Di(ethyl-hexyl)-phthalate
Dioxin	2,3,7,8-Tetrachlorodibenzo- <i>p</i> -dioxin
EPA	Environmental Protection Agency
GC	Gas Chromatography
GC/MS	Gas Chromatography/Mass Spectrometry
GC/HRMS	Gas Chromatography/High Resolution Mass Spectrometry
GPC	Gel Permeation Chromatography
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane (equivalent to BHC called benzene-hexachloride)
INAA	Instrumental Neutron Activation Analysis
ICP/MS	Inductively Coupled Plasma/Mass Spectrometry

LC/MS/MS	Liquid Chromatography/Mass Spectrometry/Mass Spectrometry
LDLP	Lipid-Depleted Lipoprotein
Log K	Log K (Partition Coefficient) usually based on octanol/water
NHANES	National Health and Nutrition Examination Survey
NHATS	National Human Adipose Tissue Survey
NIST	National Institute of Standards and Technology
PBDEs	Polybrominated Diphenyl ethers
PCA	Pentachloroanisole
PCBs	Polychlorinated Biphenyls
PCNs	Polychlorinated Naphthalenes
PFOA	Perfluorooctanoic Acid
PFOS	Perfluorooctanesulfonate
POPs	Persistent Organic Pollutants
ppb	parts per billion
ppm	parts per million
sd	Standard deviation
SPE	Solid Phase Extraction (Cartridge)
S-X3	Styrene-divinylbenzene (3% crosslinked)
TBBPA	Tetrabromobisphenol A
USGS	United States Geological Survey

ABSTRACT

Human biomonitoring is an analytical challenge to find environmental organic chemicals of varying polarity, persistency, and potential toxicity in a suitable, ideally non-invasive matrix at ppb levels that are significantly above method blanks. Compared with more traditional matrices of adipose tissue, serum, and urine, toenail clippings samples are non-invasive, compact, can be shipped without refrigeration, stored indefinitely at room temperature, and processed without concerns for biohazards. With both hydrophilic and hydrophobic layers, toenails contain 1-2% lipid, which is several times higher than serum. Toenails grow slowly and are trimmed every 2-3 months, which offers the potential to integrate both chronic and pulsed episodic exposures. Using toenail samples (65 to 340 mg) donated from four individuals and an indoor house cat, the hypothesis that toenails are a suitable biomonitoring matrix was tested by analyzing for persistent pesticides, over 50 PCB congeners, moderately persistent PBDEs, and transient compounds of triclosan and bisphenol A by using GC/High Resolution MS (GC/HRMS) analysis and for unsuspected compounds using GC/full scan MS. Although not fully digested and dissolved, toenails averaged 1.22% lipid (sd 0.20%, n=10). Lipid was separated and determined using a new small single-use 2-g S-X3 gel permeation chromatography flash column with high purity nitrogen. Multiple toenail samples from one individual were collected for over a year for replicate analysis, p,p'-DDE averaged 0.82 ng/g-nail, sd 0.28, n=5 and 65.2 ng/g-lipid, sd 15.3, n=5 on lipid-adjusted basis. *Trans*-nonachlor averaged 3.08 ng/g-nail, sd 1.03, n=5; mean 254 ng/g-

lipid, sd 97, n=5. PBDE 28 averaged 0.29 ng/g-nail, sd 0.10, n=5; mean 24.8 ng/g-lipid, sd 13.3, n=5. PBDE 85 averaged 0.25 ng/g-nail, sd 0.06, n=5; mean 20.8 ng/g-lipid, sd 6.2, n=5. PBDE 153 averaged 1.82 ng/g-nail, sd 0.51, n=6; mean 150 ng/g-lipid, sd 49.3, n=6. Most effectively biomonitored in toenails were normally transient triclosan (mean 58.3 ng/g-nail, sd 6.6, n=2), chlordanes, DDT, PBDEs, and PCBs including congeners with 2,5- or 2,3,6-chlorine substitution (PCBs 52, 49, 44, 70, 95, 101, 87, and 110), which are suspect neurotoxins, but are rarely found in extant serum biomonitoring data. Toenail soap wash samples indicated little (< 4%) or no exogenous contamination, except for the musks galaxolide and tonalide in most samples, which ranged up to 30%, likely from topical application. The one cat toenail sample had elevated concentrations of PBDEs and especially chlordanes. Unsuspected tentatively identified compounds included a UV Filter compound, octocrylene, a hydroxyl-methyl benzothiazole, and several compounds used in flavors or fragrances.

CHAPTER 1. INTRODUCTION

Human Health Biomonitoring

The Centers for Disease Control and Prevention (CDC) defines “biomonitoring” as:

“the assessment of human exposure to an environmental chemical via the measurement of that chemical, its metabolite(s), or reaction product(s) in human blood, urine, milk, saliva, adipose, or other tissue in individuals taken separately but generally taken together to constitute a population.¹”

“Blood, serum, and urine levels reflect the amount of a chemical that actually gets into the body by all routes of exposure, including ingestion, inhalation, and dermal absorption. The measurement of an environmental chemical in a person’s blood or urine is an indication of exposure; it does not by itself mean that the chemical causes disease or an adverse effect.²”

Human biomonitoring began gradually in the 1930s and 1940s when investigations of people dying from toxic substances implicated exposure to toxic metals lead and mercury. With the invention of gas chromatography (GC) in 1952 and an electron capture detector in 1958, organochlorine pesticides, e.g. dichlorodiphenyltrichloroethane or DDT, became detectable in various sample extracts. In 1962, the ecologist Rachel Carson in her book, *Silent Spring*³ warned about toxicity and long-term reproduction failures in animals (and likely humans) from widespread use of pesticides like DDT. Thus, federal agencies like the CDC and some state agencies began human health monitoring for not only toxic metals but also pesticides.

By directly measuring the elements or compounds in human samples, human biomonitoring inherently considers all sources and exposure routes instead of

estimating exposure by separately measuring concentrations in air, water, food, house dust, and other possible sources. Human biomonitoring can identify new chemical exposures, trends and changes in exposure, and vulnerable populations linked to certain contaminated sites.⁴ For example in the US, the CDC's National Health and Nutrition Examination Survey (NHANES) effectively targeted lead in human blood samples and showed a concomitant drop of lead in blood concentrations during 1976-1980, when the US reduced lead in gasoline and other products.¹

For organic compound analyses of human samples, early investigations for DDT and other pesticides led to measurements of polychlorinated biphenyls (PCBs), which as industrial chemicals and not pesticides were initially unknown to analysts targeting pesticides. With similar chromatographic properties, PCBs were initially unsuspected analytical interferences to DDT on a GC with electron capture detector which is highly sensitive to compounds containing multiple halogens. An early environmental application of GC coupled to a mass spectrometer (GC/MS) was to identify some of the PCBs, which range from mono- to deca-chlorobiphenyl.⁵ By the mid-1970s, PCBs were banned for commercial use except for closed electrical transformers and these were phased out as replacement compounds were found. Subsequently, CDC researchers gradually added other target organic pollutants including "dioxin" (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), which was a trace-level but highly toxic impurity in the herbicide 2,4,5-T and the Vietnam War defoliant, Agent Orange. Generally, the earliest organic pollutants targeted were all widely used persistent organic pollutants (POPs) with biological half-lives of years. Some less persistent compounds were later targeted

if they were incidentally detected in human specimens or were suspected toxicants. As analytical equipment became more sensitive and selective (e.g. GC with high resolution mass spectrometer, GC/HRMS), methods and techniques were developed first to respond to illnesses from unsuspected toxicants and toxic occurrences (e.g. dioxin contamination of Love Canal, NY or Times Beach, MO) and later to measure concentrations in the general population. For measuring chronic environmental exposure to POPs, analysts originally needed tens of grams of invasive adipose tissue biopsies to provide a sufficient sample mass and lipophilic matrix to measure pollutants, especially those dioxin-like, at concentrations far below one part per billion.⁶ The National Human Adipose Tissue Survey began in 1967 and continued into the 1980s under the oversight of the US Environmental Protection Agency (EPA).⁷ Tissues were taken from both autopsied cadavers and surgical patients to create a representative national sample.

Alternate, less invasive biomonitoring matrices such as blood, urine, breast milk, umbilical cord, hair, nails, and saliva were typically first employed in forensic investigations and later evaluated for biomonitoring as the pharmacokinetics of the compounds was better understood and as newer, more sensitive analytical instruments with enhanced trace-level techniques were developed.⁸ By 1990, CDC researchers had switched to blood samples (< 10 mL) as it is a less invasive sample matrix for measuring POPs including dioxin, DDE (persistent metabolite of the pesticide DDT), and PCBs.⁶

When a persistent compound was detected in blood, which typically contains only 0.5% lipid by mass, the concentration of the POP was made comparable to adipose

tissue values by normalizing each value to a per gram-lipid basis, assuming equilibrium with bodily lipids (Figure 1). Lipid-based concentrations of various persistent lipophilic organic compounds in matrices like blood or breast milk (3-5% lipid by mass) generally compared closely to those in adipose tissue as shown for example in pesticides and PCBs in 293 women from Long Island, NY.⁹

Figure 1. Three routes of chemical exposure and fate within the human body. Adapted from Needham et al. (2005).⁶ Matrices colored green have been routinely analyzed in biomonitoring; matrices colored pink have only recently and rarely been analyzed.

Subsequent work has demonstrated that even long-lived POPs concentrations differ in various human matrices and even among fractions of human blood characterized by differences in lipoprotein density (High Density Lipoprotein, Low Density L, Very Low Density L, and Lipoprotein-Depleted consisting primarily of albumin). In blood from five fasting Swedish volunteers, the majority of POPs, including 73% of DDE, were found in the LDLP or lipoprotein-depleted fraction.¹⁰ The authors conceded that the POPs distribution may have changed had they eaten instead of fasted. In major human biomonitoring studies including those from the CDC, human

volunteers do not necessarily fast before donating their blood for specimen biomonitoring. Presumably POPs concentrations balance out with the analysis of many specimens adjusted to lipid-based concentrations.

As with the variations with lipid type in blood, concentrations of organic compounds can differ by more than expected in human fatty tissues.¹¹ Blood and multiple tissues samples from different fat compartments (subcutaneous, visceral, retroperitoneal, and pelvic) were collected from seven donors who had all fasted and were analyzed for POPs consisting of 10 PCB congeners and 22 chlorinated pesticides.¹¹ Only two subjects showed similar patterns and relative concentrations in all fat compartments. In the majority of subjects there were major differences in concentrations in subcutaneous as compared with other fat compartments. In some subjects, patterns of PCB concentrations (high or low) matched high or low pesticide concentrations but in other subjects the PCB patterns were opposite. Hypotheses proposed to explain the differences included the ideas that lipid composition may have varied between subjects and even between different adipose tissue sites within the same subject, individual metabolism was different, site-specific functions in certain tissues favored solubility or binding of certain compounds, and different transport proteins favored certain compounds in different fatty tissues.¹¹ Rönn et al. (2011)¹² reported that some PCBs (congeners 74, 99, 105, and 118) were positively correlated with fat mass and others (PCB 156, 157, 169, 170, 180, 189, 194, 206, and 209) were negatively correlated, especially in women. Both gender and age factors were thought to involve differences in hormonal activity that affected distribution of compounds in

human matrices. Some cancer researchers have deliberately analyzed cancerous tissue including breast tissue and breast fluids for suspected carcinogens, but generally without strong correlation if they lacked an accompanying comprehensive cytogenic cell study.¹³ Studies of pollutant transfer from pregnant and postpartum mothers to their children have been forced to consider various matrices (maternal blood, breast milk, placenta, umbilical cord tissue, and umbilical cord blood) to determine the best matrices for biological monitoring. For example, umbilical cord tissue was found to be the best sample matrix to assess fetal contamination by persistent chemicals by Fukata et al. (2005).¹⁴

In general for all compounds, possible human exposure and fate pathways exist (Figure 1). Optimum human biomonitoring often requires a biological “speciation” approach by measuring the most appropriate compound or metabolite in the most appropriate or accessible matrix.⁶ Nonpersistent compounds with biological half-lives shorter than a few days within humans are usually measured by targeting metabolites in hydrolyzed extracts of urine whereas persistent compounds with longer biological half-lives on the order of months or years are now measured in blood extracts because adipose tissue is usually too invasive. Unfortunately, urine samples in particular provide only a short exposure time frame, as demonstrated by phthalate metabolite concentrations that are directly proportional to 48-h personal care product use.¹⁵ Measuring several phthalates directly in blood extracts by GC/MS is rarely possible because they are ubiquitous and variable in lab blanks.

Human biomonitoring studies for inorganic exposure have successfully used the human nail for a number of trace nutrients and potentially toxic trace elements. Because of prevalent disulfide bonds in hair and nail keratin, the metals lead and mercury and elements like arsenic preferentially bind in the keratin and are typically higher in hair and nail than in blood, urine, or other tissues except bone for lead.¹⁶ Similar to some organics noted earlier, concentrations of elements vary throughout all human matrices. Some essential elements like iron have specific biochemical functions in certain matrices as iron is in blood hemoglobin. Other elements are essential at low levels and still others are non-essential or toxic. For elemental analysis, a standard nail sample is either a clipping from the big toe or thumb and is typically analyzed using instrumental neutron activation analysis (INAA) or inductively coupled plasma-mass spectrometry (ICP-MS).¹⁶

Two elements that have been effectively biomonitoring in human nail are arsenic and selenium.¹⁷⁻²⁰ Selenium is an essential trace nutrient that, among other benefits, helps to prevent toxicity from compounds such as methyl-mercury. In a rat model using INAA, Brockman et al. (2011)²¹ found that nail was an effective noninvasive biomonitor for determining the Hg/Se molar ratio. Arsenic is comparable to environmental organic pollutants because it is a known toxin, a suspected carcinogen, and humans can be exposed to it from environmental sources. Arsenic biomonitoring is challenging because it is cleared through the blood stream within hours and through the body within a few days.¹⁹ The nail matrix has become the preferred biomonitor for arsenic in humans because of the preferential retention in the keratin and the nail's integration of periodic

exposures during the nail's slow consistent growth.¹⁹ In 1996, Karagas et al.²² reported in a pilot study that toenail was an effective biomonitor of people in New Hampshire being exposed to trace levels of arsenic in their drinking water. In another biomonitoring study in India using both hair and fingernails, Mandal et al. (2003)¹⁹ not only measured total arsenic but different arsenic species and metabolites aided by separation with a high performance liquid chromatograph coupled to ICP-MS. Exogenous contamination of arsenic was a concern for hair samples, so the researchers found the two dimethyl metabolites, dimethyl-arsenous acid and dimethyl-arsenic acid, in nail to be ideal biomarkers for arsenic exposure in humans.¹⁹

From the literature on biomonitoring exposure to inorganics with human nails, there are additional advantages of using a human nail matrix. Researchers have been able to successfully conduct human biomonitoring with nails even in remote areas of the world like Kenya because sampling the human nail is noninvasive making it possible for many more people to participate. In addition, the nail is much easier to transport long distances without needing special handling such as refrigeration, and nails are not biohazards like blood containing AIDS that is prevalent especially in Africa.²³

The toenail could also offer similar advantages as a noninvasive biomonitoring sampling matrix for measuring chronic environmental organic pollutant exposure. Collecting toenail clippings for toenail samples in any human biomonitoring program is noninvasive compared with biopsies of human adipose or breast tissue, collecting blood or breast milk samples at a medical lab or clinic, or collecting multiple urine samples during an entire 24-hour period for certain compounds with short half-lives or

metabolites. For nail samples, an analytical laboratory and personnel do not have to worry about viruses and biohazards that a medical lab and personnel do with handling human blood. Human hair and nails are both keratin-containing soft tissue storage sites, which can be sampled with minimal invasion for possible use in biomonitoring (Figure 1).

Nail keratin is more complex than hair.²⁴ A nail's strong keratin structure helps shield it from external contamination and its slow growth integrates chronic internal exposure from the blood stream. Alternating hydrophilic/lipophilic layers within the nail plate (Inset, Figure 1) may enable incorporation of both lipophilic and hydrophilic organic compounds. Hair has slightly higher lipids (3%) than nails (1-2%) whereas nails are more hydrated (15% water). Hair only grows lengthwise, so researchers can time exposures by monthly degree of, for example, drug use.²⁵ In contrast, accurate timing of (drug) exposure with nails is nearly impossible because nails grow in both length and thickness and consist of three layers with alternating lipophilic/hydrophilic properties.²⁶ The primary middle nail plate layer with more water content resembles a hydrophilic gel membrane; the thinner top and bottom nail plate layers are slightly lipophilic as they contain most of the lipids in nails.^{26, 27}

Nail studies with three anti-fungal drugs with biological half-lives of 21 to 36 h assessed the extent and rates of incorporation into nails during daily dose regimen and pulse dosing of every few days or weeks.^{25, 28} Fluconazole (Diflucan), a hydrophilic drug (log K 0.5), was studied along with two lipophilic drugs (itraconazole and terbinafine or Lamisil, log K 6). Fluconazole at relatively high plasma concentrations penetrated the

nails very rapidly. Within 8 h after the first oral dose, the concentration in the distal (end) of the nail plate was already 50% of the peak nail concentration. Nail concentrations ranged from one to three times higher than the corresponding plasma concentration during treatment and the compounds were detectable in the nail clippings for five months after treatment.²⁵ The two lipophilic drugs penetrated more slowly during the first few weeks because most of the drug was protein bound by albumin and their concentrations kept rising throughout the dose period with similar concentrations observed in nails as in plasma. They also persisted with at least one metabolite in nails being found months after ending treatment.²⁵ Thus, nails appear to be a suitable matrix for storing both lipophilic and hydrophilic compounds and show the possibility of integrating exposures for organic compounds.

Recently, researchers in China and Japan used liquid chromatography-tandem mass spectrometry (LC/MS/MS) to target perfluoroalkyl compounds in two small biomonitoring studies with nail concentrations compared with serum values, and in one study also urine and hair concentrations.^{29, 30} Although such compounds are being phased out in the US because of effects on kidney, liver, and other organs, they are still widely produced and used in China in a wide variety of products including non-stick cooking items, and stain-resistant carpet. Their biological half-lives in humans are on the order of years, however, they are not retained in lipid but in protein.³⁰ Of the two most common perfluoroalkyl organics, perfluorooctane sulfonate or PFOS in nail was correlated with serum values in both studies, but in one study averaged nearly 10 times less in nail than in serum.²⁹ In both studies, perfluorooctanoic acid or PFOA was lower

in nail than in serum.^{29, 30} At least two washes of nail samples before extraction were necessary to remove most of the exogenous nail (and hair) contamination and extreme care had to be taken to minimize lab background from various perfluorinated plastic products.^{29, 30}

For comparison, hair biomonitoring for organic compounds has primarily targeted drugs as reviewed by Srogi.³¹ Several recent studies by Covaci et al. have targeted environmental pollutants DDT, DDE, other pesticides, and some PCBs in hair.³²⁻³⁴ Because hair is much more porous than nails, it is more susceptible to contamination from sweat and external dust/smoke contamination. Pre-rinsing or washing sampled hair two or more times is essential to minimize external contamination, but it also removes some bioincorporated compounds.^{31, 34}

Altshul et al. (2004)³⁴ compared concentrations of PCBs and pesticides (including DDT) in hair with those in blood from 10 volunteers in Boston. Unfortunately, a long interval gap of 10-25 months occurred between the sampling dates for serum and hair. Even with this lapse, they were able to report a strong correlation ($r=0.8$) for concentrations of the highly persistent p,p'-DDE in hair versus blood.³⁴ Hair/serum *ratios* of pesticide and PCB concentrations ranged from about 1 for p,p'-DDE to 9 for o,p'-DDT in males and 48 for o,p'-DDT in females. Preliminary results based on only five men and women suggest that the ratios for estrogenic o,p'-DDT may indicate that o,p'-DDT is preferentially retained in women's hair. This is in contrast to the national CDC study using serum in which no o,p'-DDT was detected even in the 95th percentile.²

The DDT family series of compounds should be included among compounds evaluated in toenail samples for human biomonitoring. One of the first pesticides used extensively around the world from the 1940s until the late 1960s, DDT was banned or phased out by many countries in the 1970s. About 23 tropical countries, including Mexico, have continued to use it especially for malaria control.^{35, 36} Having environmental half-lives in years, the lipophilic DDT isomers (20% o,p'-; 80% p,p'-) and persistent metabolites, DDE and DDD, are so ubiquitous that "no living organism may be considered DDT-free" (Figure 2).³⁵ Including these persistent compounds in any biomonitoring investigation would provide a type of positive control. By 1978, o,p'-DDT was confirmed to be weakly estrogenic.³⁷ More recently, researchers found that o,p'-DDT binds also to other steroid receptors and p,p'-DDE is anti-androgenic.³⁸⁻⁴⁰

DDT-series Compounds

Lipophilic: Log K (octanol/water): 6 to 7
 Biological Half-Life: In Years
 Good "positive control" as environmental analytes
 p,p'-DDE – Most persistent
 Most are weakly estrogenic; p,p'-DDE is anti-androgenic

Figure 2. Structures and properties of DDT, DDE, and DDD isomers.

Reproduction processes in mammals involve several hormones binding to specific proteins and steroid receptors in certain organs and are complicated with elaborate feedback mechanisms to maintain homeostasis. Because hormones are potent at parts per billion (ppb) or less, such processes are vulnerable to

agonistic/antagonistic effects of other chemicals especially if they compete for several trace proteins or receptors.^{41, 42} Collectively, such chemicals are called endocrine disruptors. Certain widely used organic chemicals that people are chronically exposed to collectively have low, acute toxicity but are potential disrupters of estrogen, androgen, thyroid, and other hormone systems by multiple mechanisms at very low (ng/g) concentrations. For DDT-related analytes, the p,p'-DDE metabolite was the most persistent compound detected by GC/HRMS in virtually all CDC human serum extracts, ranging from a detection limit of 0.05 ng/g blood to 12 ng/g (95 percentile) or in blood lipid-basis to 1,860 ng/g-lipid.^{2, 43} For Mexican-Americans, concentrations were, on average, about double these values.

Polybrominated diphenyl ethers (PBDEs) are another group of lipophilic organic compounds that are suspected of endocrine-disruption in at least two different ways (Figure 3). First, PBDEs, and especially hydroxyl-PBDE metabolites, are anti-estrogenic.⁴⁴ Second, the thyroid system may be susceptible, not directly from the PBDEs but from the hydroxyl metabolites that bind strongly to transthyretin, a transport protein of the thyroid hormone system.⁴⁵ During the past 25 years, PBDEs have been used in high volumes commercially as “additive” flame retardants in three commercial mixtures (Penta, Octa, and Deca-BDE) based on the average number of bromine substitution. To be effective, PBDEs have typically constituted from 10 to 25% (by weight) of the commercial product.⁴⁶ By late 2013, the few chemical companies making PBDE flame retardants will begin phasing out the Deca-BDE mixture (97% deca-BDE) because of related PBDE toxicity concerns.⁴⁷ Through sunlight photolysis or metabolism even by

fish, deca-BDE becomes debrominated to lesser brominated but more toxic PBDEs.⁴⁸ It has been used worldwide in high impact plastic casing for computers and electronics, in wires and cables, and minor use for textiles and upholstery. The Penta-BDE mixture (tetra-, penta-, and low percentages of hexa-BDEs) has been used in North America in polyurethane foam for mattresses and upholstery, phenolic and epoxy resins, but it is also being phased out because of a ban in California by 2008 and a ban in Europe for several years.⁴⁹ The Octa-BDE mixture (primarily hepta- and octa-BDEs plus hexa- and nona-BDEs) was used much less, primarily in rigid ABS or acrylonitrile butadiene styrene plastic and computer monitor cases, and, with similar bans in Europe and in California by 2008, was also being phased out.

European researchers first identified PBDEs in the environment in the 1980s and became very concerned in the 1990s when PBDE concentrations in human breast milk rose almost exponentially.⁴⁹ Though lipophilic, PBDEs have much shorter biological half-lives of weeks to months compared to biological half-lives of years for DDT or PCBs. Along with food contamination, indoor dust is suspected to be the most important chronic exposure pathway for PBDEs.⁵⁰

For PBDEs in blood, results from the CDC were reported in 2009 from biomonitoring blood samples collected during 2003-2004.² Based on approximately 2000 human blood samples, CDC reported geometric mean concentrations (normalized to ng/g-lipid) for BDE 28 (1.19), BDE 47 (20.5), BDE 100 (3.93), and BDE 153 (5.69).² They also occasionally measured PBDEs 66, 99, 154, and 183. The tri-BDE 17 was also targeted but was not detected. They did not list deca-BDE 209.

Figure 3. Structures and properties of prominent polybrominated-diphenyl ethers (PBDEs) compared with the thyroid hormone, thyroxine or T4. Like PCBs, mono-through deca-PBDE congeners have been numbered from 1 to 209 for convenience.

Hites (2004)⁵¹ reviewed reported PBDE concentrations in people and environmental samples throughout the world by 2004. Conspicuously missing from human data was BDE 209, the most commonly used PBDE. It was probably not included because it is quickly metabolized, is difficult to chromatograph before breaking down, and is typically found in lab blanks. Before 2004, PBDEs were reported in only a few human samples from the US in units of ng/g-blood. The most prominent PBDE, also subject to lab background, was the 2,2',4,4'-tetrabromo-congener (BDE 47) at an average 0.5 ng/g-blood in women from Indiana and ranging from 0.1 to 5 ng/g-blood in 50 Laotian immigrants who lived in San Francisco.⁵² Although PBDEs were not reported in human biomonitoring with hair, PBDEs were reported in hair and several organs from hedgehogs (similar to ground hogs) from road kills in Belgium.⁵³ Mean PBDE

concentrations were highest in the animals' livers (40 ng/g wet wgt), then adipose fat (10 ng/g), and then hair (2 ng/g). Seven PBDE congeners were measured by GC/negative ion CI MS. In the Belgian animal hair, PBDE 99 (Figure 3) and not PBDE 47 was the highest concentration congener even though PBDE 47 was highest in the other organs. For some animals, penta-BDE 99 is typically more susceptible to metabolism.⁵³

Metabolites of dialkyl phthalates comprise a third group of organic compounds of biomonitoring interest. They present a significant analytical challenge as the parent compounds, i.e. diethyl- dibutyl-, di-(ethylhexyl)- and butylbenzyl-phthalate (Figure 4) are typically too prevalent in blank QC samples to be measured. Phthalates are widely-used commercially as plasticizers in various plastic products including flexible PVC plastics, garden hoses, children's toys, food packaging, blood-storage bags, and as diluent-additives in detergents, fragrances, shampoo, nail polish, and hair spray.¹⁵ Animal exposures during the fetal period have shown lowered testosterone levels and testicular atrophy.¹⁵ Decline in human sperm quality and DNA damage in sperm have also been associated with phthalate concentrations in men.^{15, 54} Phthalates are moderately lipophilic but are generally metabolized to mono-ester organic acids and excreted within 1-2 days.⁵⁵ Mono-ester metabolites of several phthalates, including dibutyl- and di-ethyl-hexyl-, are anti-androgenic.

Although phthalates and phthalate metabolites are of biomonitoring interest, they have been rarely detected in blood or breast milk. Nearly all researchers, including those from the CDC measure seven mono-ester phthalic acid metabolites in urine extracts by LC/MS/MS to avoid lab contamination concerns and to directly measure the

biologically-active compounds.⁶ However, at first, contamination at CDC occurred in their analyses until they found a suitable alternate enzyme. Their original glucuronidase enzyme not only hydrolyzed the Phase II glucuronide metabolites but also with lipase activity converted any dialkyl phthalates present to the mono-ester metabolites.⁵⁶ Phthalate metabolites were measured in many of CDC's 2800 urine samples. Concentrations of mono-ethyl phthalate were highest, ranging up to 5000 µg/L.²

Di(alkyl) Phthalates → Mono-alkyl Metabolites

Moderately Lipophilic: Log K (octanol/water): 4 to 5 Biological Half-Life: In a Few Days Widely used in cosmetics, lotions, nail polish, hair spray, food packaging, children's toys, car interiors, ... Metabolites are anti-androgenic Associated with decline in sperm quality/DNA damage
--

Di-(n-butyl) Phthalate

Mono-(n-butyl) Phthalate (Metabolite)

Figure 4. Structures and properties of a di-alkyl Phthalate and a mono-alkyl-ester phthalate metabolite.

In a human reference population in 2000, CDC studied the relative amounts of phthalate metabolites in both urine and serum and the percentage of the free phthalic acid form as glucuronide.⁵⁷ The most hydrophilic (mono-ethyl-phthalic acid) in serum was only 3% of the urine concentration, but more lipophilic compounds (mono-butyl- and mono-(ethyl-hexyl)-phthalic acid) in serum were 47% and 100%, respectively. The

portion in free phthalic acid form was the same in both urine and blood, i.e. mono-ethyl-phthalic acid, was 71% in free form; others were $\leq 16\%$ in free form.

Subsequently, Swedish and US CDC researchers targeted phthalate diesters and metabolites in 42 Swedish women who had given birth several weeks prior. No diesters or metabolites were detected in breast milk, blood, or serum.⁵⁸ However, most phthalate metabolites were detected in the women's urine samples comparable to those from the general population in US and in Germany.

Finally, the most transient group of compounds of biomonitoring interest includes triclosan, 5-chloro-2-(2,4-dichlorophenoxy) phenol, a common antibacterial agent in many hand soaps and dish detergents and bisphenol A (BPA), an industrial chemical that is moderately lipophilic (log K 4) with a biological half-life of only 6 h (Figure 5). BPA is a monomer used in high volume (thousands of tons annually) chemical production of various polymeric resins, commercial polycarbonate plastics, and other co-polymers. Humans can be exposed to BPA leaching from resins that line food-containing cans, teeth sealants in dentistry, reusable polycarbonate carboys used in commercial bottled water, polycarbonate commercial serving containers at restaurants, beverage glasses, and disposable plastic eating utensils. In 1980, Peterman et al.⁵⁹ reported finding BPA in the environment in the effluent of a paper mill that deinked and recycled wastepaper. In 1993, Krishnan et al.⁶⁰ discovered that BPA leached from plastic polycarbonate flasks into autoclaved distilled water at 3 $\mu\text{g/L}$, which caused positive estrogenic findings in several different bioassays. Recently in reviewing their own research and the overall BPA literature, vom Saal et al.⁶¹ stressed that endocrine-

disrupting effects occurred at very low doses. In mice, sexual differences in neurology and behavior differences between males and females were affected at low doses.^{62, 63}

Bisphenol A is another compound that will require a “speciation” approach.⁶

With a short biological half-life, BPA would typically be measured in a urine sample.

Only the free form of BPA is toxicologically active so measuring that concentration is very important.⁶

Figure 5. Structure and properties of Bisphenol A and Triclosan.

For BPA, a review of about 20 references found that people throughout the world have BPA ranging from 0.1 to 10 ng/mL in both blood and tissue.⁶³ In 400 urine samples, CDC found BPA ranged from 0.1 to 5 ng/mL (or µg/g creatinine).⁶⁴

Test Hypothesis by Measuring DDT/DDE, PBDEs, Triclosan, BPA, and Phthalate Metabolites in Toenail Samples

The hypothesis of this work is that the human toenail is a suitable matrix for human biomonitoring of environmental organic contaminants. Using geometric mean

lipid-adjusted concentrations of representative compounds from CDC for serum samples, ng amounts anticipated in a 0.25 g toenail sample after adjusting for 1.2% lipid is shown in Table 1. It is a daunting analytical challenge to measure compounds below 1 ng in a toenail sample and is likely the reason it has not been done before.

Table 1. Analytical Challenge to Measure Contaminants below 1 ng in Toenail Samples

		CDC Geo. Mean (ng/g-lipid)	Total ng in 2 g serum	Total ng expected in 0.25 g Toenails
Compound Name				
(Persistent)	Hexachlorobenzene	15.5	0.2	0.047
p,p'-DDE	268	3.38	0.82
	p,p'-DDT	---		
	trans-nonachlor	16.9	0.212	0.052
	PCB 52	2.59	0.032	0.008
	PCB 99	4.52	0.056	0.014
	PCB 138/158	17.7	0.222	0.054
	PBDE 47	19.5	~ 0.2	0.060
	PBDE 153	5.41	~ 0.06	0.017
	Bisphenol A	In urine -->	2.41 µg/L	(?)
	Triclosan	In urine -->	13.6 µg/L	(?)
(Transient)				

To test this hypothesis with the implied analytical challenge, an optimized method was developed to carefully analyze for several organic compounds that are likely to be present because of their persistence or current wide use and are important for their potential to disrupt multiple endocrine systems at low concentrations, especially during fetal development. DDE and several POPs are included because of their longtime history in biomonitoring and presence in nearly everyone in the world. Also targeted are emerging pollutants, PBDE flame retardants, which are moderately persistent. And finally, biologically active transient compounds with very short

biological half-lives including BPA, tetrabromobisphenol A (a flame retardant), triclosan and phthalate metabolites are also targeted. The polar phenols and acidic phthalate metabolites will need to be derivatized (e.g. methylation) for trace-level detection by GC/HRMS. Although the phenols in their “free” phenolic form are biologically active, they are usually conjugated as glucuronides in humans, which would require a separate glucuronidase enzymatic step to determine the total concentration in human toenails. To avoid missing an important compound of biomonitoring interest in the toenail sample extracts, GC/full-scan MS analysis is used to detect and identify by the compound’s mass spectrum. When possible, compounds detected will be tentatively identified by matching mass spectra with NIST Mass Spectral Library resident in the GC/MS system.

In summary, our hypothesis is that the toenail offers a minimally-invasive biomonitoring sampling matrix for measuring chronic environmental organic pollutant exposure. A nail’s strong keratin structure helps shield it from external contamination and its slow growth integrates chronic internal exposure from the blood stream. Alternating hydrophilic/lipophilic layers within the nail plate enables incorporation of both lipophilic and hydrophilic organic compounds. Although the small mass of toenail clippings requires an optimized method of sample preparation with minimal lab contamination, no sample carryover, and sensitive analytical instrument(s), its use is justified if the toenail matrix can be used for measuring chronic exposure to elusive compounds.

CHAPTER 2: METHODS

Sample Collection

To evaluate the concept and consistency of analyzing toenail clippings for biomonitoring while optimizing the method, toenail clipping samples were collected from the same male (individual A) for five years. Clipping samples (typically 100 to 300 mg) were collected approximately every two to three months and stored at room temperature away from sunlight and bright fluorescent lights in clean glass culture tubes with Teflon-lined caps (Fisher Scientific Co). Each toenail was clipped while the foot was placed on a large sheet of aluminum foil (solvent pre-rinsed with hexane) to collect the nails. Each sample of clippings was transferred to a culture tube with clean tweezers to avoid any lint or tiny bits of adjoining skin tissue. Because of the limited mass of individual toenail clippings samples and possible differences in clippings from different toes, replicate analyses of subsamples of an individual toenail sample were not done. Alternatively, multiple (i.e. six for non-polar, two for polar) toenail samples from individual A collected during 1.5 years were used for replicate analyses with the assumption that exposures would be similar during that time period. To further evaluate the nail as a biomonitor among other individuals, several additional toenail samples were donated from a 53 year old female (individual B), and from two additional males ages 59 (individual C) and 51 (individual D). Finally for a contrasting non-human sample, a nail clipping sample was taken as part of normal grooming from a family pet (neutered male cat, age 15), who had lived 14 years in the same home. None of the

toenails sampled were ever coated with nail polish or acetone-containing nail polish remover.

Sample Cleaning

Before extraction, each nail clipping sample was precleaned by sonicating 1 min with sodium laurate (supplied in a glass jar, 99% pure, Sigma, St. Louis, MO) dissolved in Fisher Environmental-grade water to a dilute (0.1 mg/mL) low-sudsing solution. Each nail sample was then sonicated with three 2-mL Fisher water rinses. The soap solution and three nail rinses were transferred to a separate glass culture tube for extraction and analysis with each associated toenail clipping sample to assess any superficial contaminants from exogenous sources.

Sample Sets and Surrogates Spiked

In all, four toenail sample sets were extracted and analyzed using a method that targeted either non-polar or more polar compounds, which allowed for a wider range of analytes within each polarity group (Table 2 and Figure 6). Three initial sample sets (Sets 1-3, Table 2) used toenails only from individual A to test and optimize the method before a larger sample set (Set 4) with nails from four individuals and the cat was run. Because of the small mass of a nail clipping sample (100-350 mg), it was extremely important to develop and optimize a method that minimized lab contamination and background. Some method steps were changed slightly from set to set to optimize the extent of sample nail digestion, to reduce lab background, and to measure previously unsuspected compounds. In the initial sample set (Set 1, Table 2), extracts of the

samples (blanks 1B-1 and 2B-1, one toenail sample 3T-1, and a soap and water pre-wash 3W-1) were processed first for polar phenolic compounds and then later after methylation were combined with non-polar organic pollutants. Next, the method was modified to minimize unnecessary steps and reduce lab background. Sample Sets 2 and 3 were analyzed separately for non-polar and polar compounds, respectively (Table 2). Each set consisted of two procedure blanks, two toenail clipping samples, and two soap/water pre-washes. Sample Set 4 (Table 2), run for non-polar compounds, included three procedure blanks (one blank without a digesting enzyme, Proteinase K), a soap/water blank, three nail samples from individual A, two nail clipping samples from individual C, and a single nail clipping sample from individuals B and D and the cat. For

Figure 6. Overall Method Flow Chart for Toenail Samples

Table 2. Toenail Clippings and QC Samples with Method Steps and Analytes Targeted

Sample	Type	Donor	M/F	Age	Date collected	Nail mass mg	% Lipid	GC/MS		GC/HRMS	F1-Least Polar	F2 Basic Alumina	Non-Polar Compounds		Polar Phenols-Methylated		
								30 m	13 m	30 m	HCB,DDE,PCBs	HCH, PCA, DDT, Chlordanes, PBDEs, Musks	F1-SPE	F2,F3-SPE			
1B-1	Blank	Set 1							FS	F1,2	HCB, DDE			PBDEs	Triclosan	BPA, TBBPA (Ph. Met)	
2B-1	Blank									F1,2	HCB, DDE			PBDEs	Triclosan	BPA, TBBPA (Ph. Met)	
3T-1	Toenail	A	M	57	Nov-05	186.6	1.21%	FS		F1,2	HCB, DDE			PBDEs	Triclosan	BPA, TBBPA (Ph. Met)	
3W-1	Wash									F1,2	HCB, DDE			PBDEs	Triclosan	BPA, TBBPA (Ph. Met)	
4B-2	Blank	Set 2								F1,2	HCB, DDE		HCH, PCA, DDT Chlordanes	PBDEs			
5B-2	Blank									F1,2	HCB, DDE		HCH, PCA, DDT Chlordanes	PBDEs			
6T-2	Toenail	A	M	57	Jan-06	192.3	1.31%			F1,2	HCB, DDE		HCH, PCA, DDT Chlordanes	PBDEs			
6W-2	Wash									F1,2	HCB, DDE		HCH, PCA, DDT Chlordanes	PBDEs			
7T-2	Toenail	A	M	57	Mar-06	190.4	0.87%			F1,2	HCB, DDE		HCH, PCA, DDT Chlordanes	PBDEs			
7W-2	Wash									F1,2	HCB, DDE		HCH, PCA, DDT Chlordanes	PBDEs			
8B-3	Blank	Set 3							FS		F1,2					Triclosan	BPA, TBBPA (Ph. Met)
9B-3	Blank								FS		F1,2					Triclosan	BPA, TBBPA (Ph. Met)
10T-3	Toenail	A	M	58	May-06	271.5	Partial	FS		F1,2,3					Triclosan	BPA, TBBPA (Ph. Met)	
10W-3	Wash									F1,2					Triclosan	BPA, TBBPA (Ph. Met)	
11T-3	Toenail	A	M	58	Jul-06	294.3	Partial	FS		F1,2,3					Triclosan	BPA, TBBPA (Ph. Met)	
11W-3	Wash									F1,2					Triclosan	BPA, TBBPA (Ph. Met)	
12B-4	Blank	Set 4								F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
13B-4	Blank									F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
14B-4	Blank									F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
15T-4	Toenail	A	M	58	Oct-06	339.5	1.16%			F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
15W-4	Wash									F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
16T-4	Toenail	A	M	58	Feb-07	295	1.29%			F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
16W-4	Wash									F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
17T-4	Toenail	A	M	59	May-07	159	1.63%			F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
17W-4	Wash									F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
18T-4	Toenail	B	F	53	2010	65.5	1.23%			F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
18W-4	Wash									F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
19T-4	Toenail	M-cat	M	15	Apr-10	120	1.12%			F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
19W-4	Wash									F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
20T-4	Toenail	C	M	59	Jan-09	195.9	1.03%			F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
20W-4	Wash									F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
21T-4	Toenail	C	M	59	Apr-09	321.6	1.22%			F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
21W-4	Wash									F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
22T-4	Toenail	D	M	51	Nov-07	188.7	1.23%			F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	
22W-4	Wash									F2	F1,2	DDE, PCBs	HCH, PCA, DDT Chlordanes	PBDEs	Dieldrin	Musks	

best accuracy and to correct for analyte recovery losses throughout the method, the “isotope dilution technique” was used. For non-polar compounds, several ^{13}C -stable-labelled surrogates were spiked into each sample at the beginning of the method including: ^{13}C -mono- to deca-PBDEs and 35 ^{13}C -PCB congeners from Wellington Labs (Guelph, ON Canada) plus ^{13}C -p,p'-DDE from Cambridge Isotope Labs (Andover, MA). For polar compounds, ^{13}C -surrogates for each sample included ^{13}C -triclosan, ^{13}C -bisphenol A, ^{13}C -tetrabromobisphenol A, and several ^{13}C -mono-phthalate metabolites from the same companies. Surrogate spiking solution volume was kept to a minimum (0.1 mL), especially to avoid denaturing the enzyme, Proteinase K (from Tritirachium album, BioUltra grade, Sigma, St. Louis) when used in Set 4.

Dissolution and Extraction of Toenail

A first important challenge was to break up, digest, and solubilize toenail clippings for effective solvent extraction of trace levels of organic contaminants without introducing any significant lab contamination or destructive reagents. Strong acids were first attempted for digestion, but the toenail material was impervious to hydrochloric or sulfuric acid for at least several weeks at room temperature. Strong bases were not tried for nail digestion because certain pollutants like DDT would break down (to DDE and DDD), as shown by Covaci and Schepens (2001)³² from a dilute NaOH basic extraction. A possible extractor using liquid nitrogen was considered but rejected because of likely BPA contamination from its polycarbonate sample holder. A small powered commercial mill may have been suitable but was unavailable to break up nail clippings to very small fragments. For extracting hair, Covaci and Schepens (2001)³²

tried 16 extraction methods (dilute base, dilute acids, enzyme, and various Soxhlet) to extract hair for environmental pollutants. They chose an initial incubation of 3 M HCl at 40 °C for 12 h and then liquid-liquid extraction with hexane:dichloromethane (4:1), but acid-labile pesticides (dieldrin and heptachlor epoxide) were not targeted.³²

Researchers using nail to monitor for cocaine, a diester (like phthalates) which is susceptible to hydrolysis from a high pH extraction, used a pH 5 potassium phosphate buffer for three days to extract their small nail slivers for cocaine.^{31, 65}

The ultimate goal was to denature the protein matrix sufficiently to allow extraction by organic solvents while not chemically degrading the analytes and not hydrolyzing triglycerol lipids. Biochemical reagents such as dithiothreitol (Figure 7), and enzyme, proteinase K, are known to break down protein.⁶⁶ Therefore, for all sample sets 1-4 (Table 2), nail clippings were pre-washed and hand-ground in a 5-mL glass conical mortar vial with matching glass pestle (Kimble Chase, Vineland, NJ) with at least 5 mg dithiothreitol (DTT, Sigma, St. Louis), a reducing agent commonly used to break disulfide bonds of cystine in proteins. DTT dissolved in dichloromethane (DCM, Optima grade, Fisher) was added to the wet nails in the vial to promote extraction of small organic molecules, but DTT is known to work only on the solvent-accessible areas.⁶⁷ Cystine, common in nail keratin protein, typically occurs from two cysteine amino acids “cross-linking” within the protein. Although a slow process, toenail was more effectively broken down by alternating hand-grinding (to increase surface area) with sonicating the vial with DTT reagent and added water. After sufficient time (hours up to several days) of digesting, a white fluffy suspension from each toenail sample was observed to form.

Figure 7. Structure of dithiothreitol used to break down keratin

This suspension was transferred to a clean (15 mL) glass culture tube for DCM extraction (Figure 8). Typically, some bits of toenail clippings remained in the mortar vial, though noticeably thinner and softer, and required further hand-grinding to dissolve as much nail as possible before transferring to the culture tube. By comparing total lipid and analyte concentrations from multiple nail clippings samples from individual A that were dissolved or digested to varying extents, it was possible to assess the importance of a nearly complete dissolution or digestion of an entire nail clippings sample.

In an effort to improve the extent of nail dissolution in sample set 4, a digesting Proteinase K enzyme (Sigma, St. Louis, MO) at 1 mg/mL Fisher water in a culture tube at 37 °C was added to the toenail samples with slow rocking agitation. After several days of slow progress, it was necessary to then add 5-10 mg DTT in granular form to each sample tube followed by multiple sonication every day or two. After seven days of digesting, the white fluffy suspension from each toenail sample was transferred to a

Figure 8. Sample Set 1 Culture tubes (Left to Right) of two procedure blanks (with DTT), one soap wash, and toenail sample, designated 1B-1, 2B-1, 3W-1, and 3T-1 (Table 2).

clean culture tube for DCM extraction. To the remaining nail material in each sample tube, freshly dissolved Proteinase K and DTT were added for another ten days of digestion and periodic sonication before again transferring the white nail suspension to the original sample culture tube. Remaining toenail clippings were noticeably thinner and softer to hand-grind in a mortar vial before transferring to the culture tube, except for the relatively hard nails clipped from the cat that required an hour of hand-grinding to get most of the nail into a suspension and into the tube for extraction.

For all sample sets, each ground nail sample was extracted three to four times with 3-mL DCM to extract small organic compounds from the (aqueous) nail suspension still containing bits of undissolved nail. Each extract was carefully removed from the bottom layer with a glass transfer pipet to another culture tube and combined in a

15-mL glass culture tube. For Set 4 that had more extensive emulsions in sample extracts, a fourth 3-mL DCM extraction was routinely used. Whenever emulsions complicated extract partitioning, most of the top water layer was decanted to a separate culture tube and extracted separately with DCM three to four times. In Set 3 which only targeted phenolic and acidic compounds, two DCM extracts were made, initially at neutral pH 7 and later at pH 3 after acidification with several drops of dilute HCl. With a pKa of 9-10, bisphenol A, tetrabromobisphenol A, and triclosan were expected to be in the neutral extract, but the mono-phthalate metabolites were expected to be in the acidic extract. For Set 3, only “free” or unconjugated phenols were targeted. No glucuronidase/sulfatase enzyme was added to the aqueous dissolved nail solution before acidification to release any glucuronide metabolites present for a possible third DCM extract. In general, each DCM sample extract was gently concentrated with high purity nitrogen to 0.25 mL to prepare for a miniaturized 2-g flash gel permeation chromatography (GPC) step to remove lipids.

Lipid removal using Miniature Flash Gel Permeation Chromatography (GPC)

Based on size-exclusion chromatography, GPC with BioBeads S-X3 (BioRad, Hercules, CA) is an effective technique to remove lipids from environmental samples extracted for various organic compounds to be analyzed using gas chromatography (GC).⁶⁸ However, any automated GPC system is prone to carryover from one sample to the next, prone to trapped gas bubbles requiring pumping the solvent upwards through the column, and is designed for large samples up to 100 g using a large amount (350 mL)

of solvent. The availability of long narrow (45 cm x 5 mm i.d.) glass flash columns, custom made by Kimble Chase, Vineland, NJ made it possible to devise a miniaturized 2-g flash GPC with similar length but five times narrower i.d. that uses high purity nitrogen (0.5 bar) for downward DCM flow (Figure 9). A single sample use for each column avoided carryover from any previous sample. A 40-mL reservoir with 20-400 GPI glass screw thread on top of the column connects to the nitrogen and the bottom end of the column is tapered to a glass male Luer lock for a small glass fiber plug (0.45 μ m). Miniaturized by a factor of 35, the smaller GPC used only a small eluant (< 10 mL DCM), which minimized solvent and lab background.

Figure 9. Top reservoir and adapter-cap for nitrogen-pressurized flash GPC. A 0.25-mL *beta*-carotene test solution was applied to the column before adding DCM eluant.

All sample sets (1-4) used the same GPC column packing procedure. To wet-pack S-X3 (2.00 g, 3% cross-linked gels, 40-80 μm) in an empty column, S-X3 and DCM were added alternately in partial portions until within 30 min the S-X3 swelled and settled as a contiguous gel without any significant air bubbles. A small plug of cleaned glass wool was then added on top (to keep gel from floating into the reservoir) and DCM flowed under pressure until the bottom of the glass wool.

To carefully collect the drops of eluting DCM solvent, a stainless steel vial rack with new specially-chosen GC glass vials was placed under the column end when the reservoir was opened for a manual injection of a DCM extract (0.25 mL). The column was re-pressurized for a 0.25-mL rinse before 10 mL DCM was added. GC glass vials were chosen for their consistent volumes, lot-tested by manufacturers for minimal contaminants, and suitably packaged to minimize exposure to lab dust. With convenient volumes known to the nearest 0.1 mL, vials were chosen for accurate collection (1.6-mL "Maximum Recovery" vial and 0.3-mL amber conical vial, Waters, Milford, MA or Shamrock Glass, Seaford, DE) and a conical 1.0-mL vial (Sun-SRI, Rockwood, TN). The sequence of vials used began with two 1.6 mL vials, a 1.0 mL vial, two 0.3 mL vials, and two more 1.6 mL vials (Figures 10-11). The two small (0.3 mL) conical vials were strategically placed at the end of the elution of bulkier lipid and at the beginning of the elution of smaller molecule (mass <1000 Da.) organic contaminants/pollutants. For Sets 1, 2, and 4, the bulkier lipid eluted in 0-4.5 mL DCM just ahead of smaller molecule organic pollutants from 4.5-8.0 mL DCM.

Figure 10. Flash GPC elution was collected in appropriate-sized vials (left to right, two 1.6 mL, one 1.0 mL, two 0.3 mL amber, and two 1.6 mL) for beta-carotene check.

A key feature of this miniaturized GPC column was that the separated lipid was collected accurately in the first four tared glass GC vials (Figures 10-11). After evaporation, the lipid mass, left mostly in vials 2-4, was then measured directly with a five-decimal place balance negating the need to use any of the valuable small extract for a separate lipid determination (Figure 12).

Figure 11. Orange-colored beta-carotene, eluted before 4.5 mL, as did lipid.

Figure 12. Lipid can be seen to coat the walls of evaporated GPC collection vials (2-4) of elution from 1.6-4.5 mL for extracts of both toenail samples in Set 2 (vials “A” or “B”).

For Sets 1, 2, and 4, one GPC eluate (4.5-8.0 mL) was collected for all targeted organic compounds. However, for Set 3 targeted for phenolic and acidic compounds, two portions of the GPC eluate were collected ([A] 4.2 – 4.8mL and [B] 4.8-8.0 mL) to ensure at least partial recovery of early eluting, bulky phthalate metabolites in [A] despite also collecting the remainder of the eluting lipid. From each sample in all sets, collected GPC eluate solutions were combined in a glass culture tube and concentrated to < 0.5 mL with a gentle stream of high purity nitrogen. For quality control and initial miniature GPC method development, GPC elutions were checked (a) gravimetrically with cod liver oil or corn oil for lipid recovery; (b) colorimetrically with *beta*-carotene (Figure 11), a bulky C₄₀ unsaturated pigment with intense orange color that elutes just

before the early di-(ethylhexyl) phthalate (DEHP); and (c) radiometrically with ^{14}C -DEHP or ^{14}C -cholesterol. GPC elutions were consistent, provided that the 2.0 g mass of S-X3 media was accurately apportioned and the S-X3 gel swelled effectively with minimal trapped air bubbles.

Methylation of (Polar) Phenols and Acids with Iodomethane

Sets 1 and 3 included a methylation with back-extraction step for converting polar phenolic and acidic compounds to much less polar methyl ethers or methyl esters, which appeared as sharp detectable peaks by GC/MS. After concentrating the eluates to < 0.5 mL, 0.75-mL 5% K_2CO_3 (Sigma and Fisher water) was added to deprotonate the compounds (at pH 12), 10-mL acetone to homogenize the solutions, and 0.25-mL iodomethane to methylate the anions (reaction times of several hours or overnight) at room temperature. A high purity nitrogen stream removed most of the acetone before extraction (three times) with 3-mL hexane, concentration to 0.5 mL, and cleanup using a 3-mL glass solid phase extraction (SPE) cartridge with 0.5-g basic alumina topped with 2-g Na_2SO_4 , a common drying agent (Figure 13).

Cleanup with 0.5-g Basic Alumina SPE

Following GPC, a 3-mL glass SPE cartridge with glass fiber frits pre-packed with 0.5-g basic alumina (Macherey-Nagel, Bethlehem, PA) was used as a clean-up step for all methods. Alumina is a common sorbent for normal phase liquid chromatography, and basic alumina is especially suited to remove (by chemi-sorbing) fatty acids that would have at least partially been collected via GPC. For Set 3, the basic alumina SPE was

Figure 13. Cleanup with 0.5-g Basic Alumina (and 2-g Na_2SO_4) SPE Cartridge

topped with the drying agent, 1 g Na_2SO_4 , as the final cleanup/fractionation step for the methylated derivatives of phenolic/acidic compounds. Several fractions, using eluants each progressively more polar, were collected from the SPE in Set 3 using 6-mL eluants of DCM, 2% acetone in DCM, and finally 10% acetone in DCM, to ensure elution of both methyl ethers and methyl esters. In the other sample sets for non-polar compounds, a basic alumina SPE was used to remove residual fatty acids while eluting targeted compounds with 6-mL DCM. Fractions were collected in culture tubes and concentrated to 0.5 mL with a gentle stream of high purity nitrogen.

Fractionation of Non-Polar Compounds with 4-g Basic Alumina Flash Chromatography Column

Lacking a clean room, a 4-g basic alumina flash chromatography column was used to provide a clean environment (0.5 bar high purity nitrogen) to replace open-air gravity normal phase chromatography columns which are prone to contamination from lab air or dust. With higher performance flash-sized basic alumina available, (40-63 μm , Sorbent Technologies, Atlanta, GA), eluant solvent volume could be reduced. The flash column was used for Sets 1, 2, and 4 to split non-polar compounds into two eluate fractions. Previously, I reported consistent separations with this flash column (irrespective of room humidity) with significantly lower contamination of PBDEs and PCBs in blank samples.⁶⁹ Storing basic alumina in a stoppered glass flask at 130 °C minimized dust but required slightly stronger eluant strength than in Figure 14. Clean, empty glass columns were pre-heated to 130 °C before dry-packing. The first (least polar) fraction (1) now used 30 mL 1% DCM in cyclopentane to isolate DDEs, hexachlorobenzene (HCB), and common *ortho*-substituted PCBs. The eluant, cyclopentane, with slight aromaticity and slightly increased elutropic strength compared with hexane, was key to separating common *ortho*-substituted PCBs from dioxin-like non-*ortho*-PCBs.⁶⁹ Subsequently, Fraction 2 with 15 mL 0.5% acetone in DCM was used to elute many other compounds including PBDEs, chlordanes, and other organochlorine pesticides. An additional more polar fraction was considered, but natural (more polar) sterols and cholesteryl esters could also have eluted to hamper GC/MS detection of possible pollutants. For quality assurance, elution checks were made by measuring

Figure 14. Non-polar organic pollutants were chromatographed by a 4-g basic alumina flash column with 60 mL 0.5% DCM in cyclopentane eluant followed by 4 mL of 2%, 10%, 25%, and 100% DCM. Fraction 1 had DDE (light blue), (most PCBs, not shown), and a few pesticides; Fraction 2 had PBDEs, chlordanes, dieldrin, and a few non-*ortho*-PCBs (final: 15 mL 0.5% acetone in DCM).⁶⁹

¹⁴C-p,p'DDE (typically in Fraction 1, Figure 14) with a Beckman LS 6500 scintillation counter.

GC/Full-scan Mass Spectrometry to Identify Unsuspected Compounds

Trace-level detection and quantitation of targeted compounds were done effectively by using a highly selective GC/High Resolution Mass Spectrometer (GC/HRMS). This research was greatly helped by also using a less sensitive and less selective benchtop GC/quadrupole MS (GC/MS) in full-scan MS analysis for a broader investigation to detect and identify unsuspected compounds and to confirm targeted compounds at higher concentrations. A GC/full scan MS analysis was done for Set 1 after GC/HRMS was run with a short capillary column to target relatively few analytes and for Set 3 to investigate polar compounds following methylation in several fractions before targeted GC/HRMS analysis (Table 2, p.25). Final 0.3 mL eluates (from basic alumina) in nonane were analyzed by injecting 3 μ L into a cool-on-column inlet on a Thermo 8000 Top GC/TRACE MS Plus system (Austin, TX) with a 30 m x 0.15 mm i.d. BPX5 column (SGE, Austin, TX). A deactivated retention gap (0.25 m x 0.25 mm and 2.5 m x 0.25 mm i.d., MEGA Scientific, Legnano, Italy) was connected with glass press-tight connectors (Restek, Bellefonte, PA) to the column to accommodate the syringe needle for on-column injection. The column was temperature programmed from 110 $^{\circ}$ C-330 $^{\circ}$ C at 3 $^{\circ}$ C/min and helium pressure programmed from 300-500 kPa at 3 kPa/min. For maximum sensitivity, the TRACE MS Plus was tuned by the analyst before mass calibration by the computer data system (with perfluoro-*t*-butylamine) with built-in specifications. With the photomultiplier set at 350 V, GC/full scan (m/z 50-750) MS

spectra were acquired at the fastest speed possible (0.7 s/scan) and data processed using Thermo XCalibur (V1.4) software. Identifying compounds by mass spectra was aided by the data system reference MS libraries, totaling nearly 500,000 spectra, NIST 2011 Mass Spectral Library (NIST, Washington, D.C.) and Wiley Registry Seventh Edition MS Library (Wiley, Indianapolis, IN).

GC/High Resolution Mass Spectrometry (GC/HRMS) Selected ion monitoring

The Waters Autospec GC/HRMS system was setup at a mass resolution of at least 10,000 (Resolving Power, 10% valley) in Selected Ion Monitoring (SIM) mode, a highly selective technique for targeted compounds. For example, at m/z 500, the instrument in this mode could selectively detect two different compounds with masses differing by only 0.05 Da if their concentrations were within a factor of 10 of each other. The magnet was held within each group of selected ions during a specified time range at a “lock mass” of known accurate mass. Within a limit of 1.5 times the lowest mass in the group, the accelerating voltage was rapidly switched by the data system (downward within a factor of 1.5) from one ion to another within an ion group for a dwell period (12 to 40 ms) for each ion. Also, an extra delay or settling time (10 ms) for each ion was required to stabilize from the voltage for the previous ion. With GC peak widths of only a few seconds, it was important to minimize the total time to cycle within a group to about 500 ms or less to provide sufficient data points to measure the Gaussian-shaped analyte GC peaks. Each ion mass was specified accurately to four decimal places and multiple ion groups were analyzed tailored to analytes known or suspected in each basic

alumina eluate fraction (Tables 3-6). Given the HRMS mass range limitation within an ion group and various GC retention times of analytes, ions selected often had to be alternate, less abundant, fragment ions of compounds with excessively large mass compared to other compounds eluting within the same GC time frame. GC/HRMS SIM data were acquired and data processed using Waters' Masslynx software, and comprehensive quantitative analysis was performed using Waters' Targetlynx software. After retention times of analyte standards were established for current GC conditions, SIM groups of selected accurate mass ions in electron impact ionization mode were acquired with the Autospec HRMS at 36 eV electron energy, filament trap current of 500 μ A, photomultiplier at 350 V, and source temperature of 250 $^{\circ}$ C.

GC/HRMS SIM methods shown in Tables 3-6 became more comprehensive than in Set 1, when the number of analytes (ions) targeted were fewer. In basic alumina Fraction 1, mainly DDE (metabolite of DDT), 13 C-DDE surrogate, pentachlorobenzene, and hexachlorobenzene were targeted in Set 1 (Table 2). PCBs (tri- to decachloro-) were included in case nail concentrations greatly exceeded method blank levels. Fraction 2 of Set 1 was analyzed for di- to deca-PBDEs, two metabolites, pentachloroanisole (of pentachlorophenol) and oxychlordane (of chlordane), plus methylated ether derivatives of BPA, triclosan, tetrabromobisphenol A, tri- to hexa-PCB hydroxyl metabolites, and methylated esters of phthalate metabolites. Possible metabolites were targeted because of concern that lab background levels of parent compounds may mask actual levels in the nails. Later sample sets 2 and 4 included additional compounds in Fraction 2 that were originally unsuspected (e.g. chlordanes and synthetic musk fragrances).

Table 3. GC/HRMS Selected Ion Monitoring of Fraction 1 (PCBs, Pesticides)

SIM Group 1				SIM Group 2			
(2.8-7.0 min)	(14 ions)	Dwell (ms)	Ion Ratio	(7.0-15.2 min)	(24 ions)	Dwell (ms)	Ion Ratio
Di-PCBs	222.0003	15	1.00	Tri-PCBs	255.9614	15	1.00
Di-PCBs	223.9974	15	0.66	Tri-PCBs	257.9584	15	0.99
¹³ C-Di-PCBs	234.0406	15	1.00	Tetra-PCNs	263.9067	15	0.76
¹³ C-Di-PCBs	236.0376	15	0.66	Tetra-PCNs	265.9038	15	1.00
Lock Mass	242.9856	16	---	¹³ C-Tri-PCBs	268.0016	15	1.00
Lock Mass Check	242.9856	12	---	¹³ C-Tri-PCBs	269.9986	15	0.99
Penta-Cl-benzene	247.8521	15	0.61	Tetra-PCBs	289.9224	15	0.76
Penta-Cl-benzene	249.8491	15	1.00	Tetra-PCBs	291.9194	15	1.00
Tri-PCBs	255.9614	15	1.00	Lock Mass	292.9824	16	---
Tri-PCBs	257.9584	15	0.99	Lock Mass Check	292.9824	12	---
¹³ C-Tri-PCBs	268.0016	15	1.00	Penta-PCNs	299.8648	15	1.00
¹³ C-Tri-PCBs	269.9986	15	0.99	Penta-PCNs	301.8618	15	0.66
Hexa-Cl-benzene	283.8102	15	1.00	¹³ C-Tetra-PCBs	301.9626	15	0.76
Hexa-Cl-benzene	285.8072	15	0.82	¹³ C-Tetra-PCBs	303.9597	15	1.00
				o,p'- & p,p'-DDEs	315.9380	15	0.76
				o,p'- & p,p'-DDEs	317.9351	15	1.00
				Penta-PCBs	323.8834	15	0.61
				Penta-PCBs	325.8804	15	1.00
				¹³ C-p,p'-DDE	327.9783	15	0.76
				¹³ C-p,p'-DDE	329.9753	15	1.00
				¹³ C-Penta-PCBs	335.9236	15	0.61
				¹³ C-Penta-PCBs	337.9207	15	1.00
				Hexa-PCBs	359.8414	15	1.00
				Hexa-PCBs	361.8385	15	0.82
SIM Group 3				SIM Group 4			
(15.2-23.5 min)	(22 ions)	Dwell (ms)	Ion Ratio	(23.5-35.0 min)	(20 ions)	Dwell (ms)	Ion Ratio
Penta-PCBs	323.8834	15	0.61	Hepta-PCBs	393.8025	15	1.00
Penta-PCBs	325.8804	15	1.00	Hepta-PCBs	395.7995	15	0.98
Hexa-PCNs	333.8258	15	1.00	¹³ C-Hepta-PCBs	405.8428	15	1.00
Hexa-PCNs	335.8229	15	0.82	¹³ C-Hepta-PCBs	407.8398	15	0.98
¹³ C-Penta-PCBs	335.9236	15	0.61	Octa-PCBs	427.7635	15	0.87
¹³ C-Penta-PCBs	337.9207	15	1.00	Octa-PCBs	429.7606	15	1.00
o,p'-DDT	351.9147	15	0.64	¹³ C-Octa-PCBs	439.8038	15	0.87
o,p'-DDT	353.9118	15	1.00	¹³ C-Octa-PCBs	441.8008	15	1.00
Hexa-PCBs	359.8414	15	1.00	Lock Mass	454.9729	12	---
Hexa-PCBs	361.8385	15	0.82	Lock Mass Check	454.9729	16	---
¹³ C-Hexa-PCBs	371.8817	15	1.00	Nona-PCBs	461.7245	15	0.76
¹³ C-Hexa-PCBs	373.8788	15	0.82	Nona-PCBs	463.7216	15	1.00
Hepta-PCBs	393.8025	15	1.00	Hexa-Br-Biphenyls	467.7006	15	1.00
Hepta-PCBs	395.7995	15	0.98	Hexa-Br-Biphenyls	469.6986	15	0.66
Lock Mass	404.9761	16	---	¹³ C-Nona-PCBs	473.7648	15	0.76
Lock Mass Check	404.9761	12	---	¹³ C-Nona-PCBs	475.7619	15	1.00
¹³ C-Hepta-PCBs	405.8428	15	1.00	Deca-PCB	497.6826	15	1.00
¹³ C-Hepta-PCBs	407.8398	15	0.98	Deca-PCB	499.6797	15	0.87
Octa-PCBs	427.7635	15	0.87	¹³ C-Deca-PCB	509.7229	15	1.00
Octa-PCBs	429.7606	15	1.00	¹³ C-Deca-PCB	511.7199	15	0.87
¹³ C-Octa-PCBs	439.8038	15	0.87				
¹³ C-Octa-PCBs	441.8008	15	1.00				

KEY: Quality Assurance Tolerance for ratios of ion abundances is within 15%

PCBs = Polychlorinated Biphenyls; PCNs = Polychlorinated Naphthalenes;

DDE = Dichlorodiphenyldichloroethylene; DDT = Dichlorodiphenyltrichloroethane

Table 4. PCB Congeners and Pesticides in Fraction 1 by GC Retention Time

SIM									
Analyte	Min.	Group	Surrogate Std	Internal Std	Analyte	Min.	Group	Surrogate Std	Internal Std
Pentachlorobenzene	3.78	1	¹³ C-p,p'-DDE	None	Hexa-PCB 149	15.21	3	¹³ C-PCB 153	
Di-PCBs 4/10	4.53	1	¹³ C-PCB 4	¹³ C-PCB 9	Hexa-PCB 153/168	16.49	3	¹³ C-PCB 153	
Di-PCB 6	5.25	1	¹³ C-PCB 8	¹³ C-PCB 9	Hexa-PCB 141	17.15	3	¹³ C-PCB 153	
Di-PCB 8	5.41	1	¹³ C-PCB 8		Hexa-PCB 137	17.41	3	¹³ C-PCB 138	¹³ C-PCB 162
Hexachlorobenzene	5.48	1	¹³ C-p,p'-DDE	None	Hexa-PCB 138/158	17.91	3	¹³ C-PCB 138	
Tri-PCB 19	5.82	1	¹³ C-PCB 19	¹³ C-PCB 37	Hexa-PCB 129	18.18	3	¹³ C-PCB 138	
Tri-PCB 18	6.41	1	¹³ C-PCB 19		Hexa-PCB 128	19.23	3	¹³ C-PCB 167	¹³ C-PCB 162
Tri-PCB 16	6.94	2	¹³ C-PCB 28	¹³ C-PCB 37	Hexa-PCB 167	19.41	3	¹³ C-PCB 167	
Tri-PCBs 31/28	7.88	2	¹³ C-PCB 28		Hexa-PCB 156	20.56	3	¹³ C-PCB 156	¹³ C-PCB 162
Tri-PCB 33	8.16	2	¹³ C-PCB 28		Hexa-PCB 157	20.80	3	¹³ C-PCB 157	¹³ C-PCB 162
Tri-PCB 22	8.47	2	¹³ C-PCB 28						
Tetra-PCB 54	7.33	2	¹³ C-PCB 54	¹³ C-PCB 79	Hepta-PCB 188	15.91	3	¹³ C-PCB 188	¹³ C-PCB 162
Tetra-PCB 52	9.04	2	¹³ C-PCB 52	¹³ C-PCB 79	Hepta-PCB 178	18.09	3	¹³ C-PCB 188	
Tetra-PCB 49	9.17	2	¹³ C-PCB 52		Hepta-PCB 187	18.54	3	¹³ C-PCB 188	
Tetra-PCB 44	9.77	2	¹³ C-PCB 52		Hepta-PCB 183	18.82	3	¹³ C-PCB 188	
Tetra-PCB 41	10.21	2	¹³ C-PCB 52		Hepta-PCB 174	19.78	3	¹³ C-PCB 180	¹³ C-PCB 194
Tetra-PCB 40	10.53	2	¹³ C-PCB 70	¹³ C-PCB 79	Hepta-PCB 177	20.04	3	¹³ C-PCB 180	
Tetra-PCB 74	11.28	2	¹³ C-PCB 70		Hepta-PCB 171	20.28	3	¹³ C-PCB 180	
Tetra-PCB 70	11.48	2	¹³ C-PCB 70		Hepta-PCB 180/193	21.34	3	¹³ C-PCB 180	
Tetra-PCB 66	11.58	2	¹³ C-PCB 70		Hepta-PCB 191	21.55	3	¹³ C-PCB 180	
Tetra-PCB 60	12.31	2	¹³ C-PCB 70		Hepta-PCB 170	22.80	3	¹³ C-PCB 170	¹³ C-PCB 194
o,p'-DDE	12.31	2	¹³ C-p,p'-DDE	None	Hepta-PCB 189	24.47	4	¹³ C-PCB 189	¹³ C-PCB 194
					Octa-PCB 202	20.04	3	¹³ C-PCB 202	¹³ C-PCB 194
Penta-PCB 104	9.47	2	¹³ C-PCB 104	¹³ C-PCB 111	Octa-PCB 201	20.48	3	¹³ C-PCB 202	
Penta-PCB 95	11.48	2	¹³ C-PCB 95	¹³ C-PCB 111	Octa-PCB 200	21.76	3	¹³ C-PCB 202	
Penta-PCB 84	12.38	2	¹³ C-PCB 101	¹³ C-PCB 111	Octa-PCB 199	23.10	3	¹³ C-PCB 202	
Penta-PCB 90	12.46	2	¹³ C-PCB 101		Octa-PCB 203	23.44	4	¹³ C-PCB 202	
Penta-PCB 101	12.53	2	¹³ C-PCB 101		Octa-PCB 194	26.18	4	¹³ C-PCB 205	¹³ C-PCB 194
Penta-PCB 99	12.72	2	¹³ C-PCB 101		Octa-PCB 205	26.35	4	¹³ C-PCB 205	
Penta-PCB 119	12.96	2	¹³ C-PCB 101		Nona-PCB 208	24.76	4	¹³ C-PCB 208	¹³ C-PCB 206
Penta-PCB 97	13.42	2	¹³ C-PCB 101		Nona-PCB 207	25.22	4	¹³ C-PCB 208	
Penta-PCB 87	13.75	2	¹³ C-PCB 101		Nona-PCB 206	27.93	4	¹³ C-PCB 208	
p,p'-DDE	13.86	2	¹³ C-p,p'-DDE	None	Deca-PCB 209	29.30	4	¹³ C-PCB 209	¹³ C-PCB 206
Penta-PCB 85	13.89	2	¹³ C-PCB 101						
Penta-PCB 110	14.17	2	¹³ C-PCB 101						
Penta-PCB 123	15.31	3	¹³ C-PCB 123	¹³ C-PCB 111	Internal Standards:				
Penta-PCB 118	15.52	3	¹³ C-PCB 118	¹³ C-PCB 111	¹³ C-Di-PCB 9	5.03	1		
o,p'-DDT	15.95		¹³ C-p,p'-DDE	None	¹³ C-Tri-PCB 37	10.10	2		
Penta-PCB 114	15.99	3	¹³ C-PCB 114	¹³ C-PCB 111	¹³ C-Tetra-PCB 79	13.00	2		
Penta-PCB 105	16.77	3	¹³ C-PCB 105	¹³ C-PCB 111	¹³ C-Penta-PCB 111	13.67	2		
Hexa-PCB 151	14.62	2	¹³ C-PCB 153	¹³ C-PCB 162	¹³ C-Hexa-PCB 162	19.08	3		
Hexa-PCB 135	14.81	2	¹³ C-PCB 153		¹³ C-Octa-PCB 194	26.16	4		
					¹³ C-Nona-PCB 206	27.92	4		

Table 5. GC/HRMS Selected Ion Monitoring of PBDEs, Pesticides, and ¹³C-Surrogates in Basic Alumina - Fraction 2

SIM Group 1	GC Retention 2.15-4.95 min	16 ions m/z	Dwell (ms)	Ion Ratios	SIM GROUP 2	GC Retention 4.95-7.6 min	14 ions m/z	Dwell (ms)	Ion Ratios
Traseolide	4.47	215.1436	12	1.00	Lock mass check ion	---	292.9824	10	---
Hexachlorocyclohexanes α-3.40, γ-3.93,		216.9145	12	0.76	Lock mass ion	---	292.9824	20	---
Hexachlorocyclohexanes β-4.03, δ-4.74		218.9115	12	1.00	Dacthal	6.17	298.8836	20	0.78
Lock mass ion	---	242.9856	15	---	Dacthal	6.17	300.8807	20	1.00
Lock mass check ion	---	242.9856	10	---	Di-PBDEs	5.04 - 6.78	325.8942	20	0.51
Galaxolide; Tonalide	4.52; 4.59	243.1749	12	1.00	Di-PBDEs	5.04 - 6.78	327.8921	20	1.00
Mono-PBDEs	3.16, 3.26, 3.39	249.9816	12	---	Chlordene (s)	Unknown	337.8571	20	1.00
Galaxo-, Tona-, Traseo-lide	4.47, 4.52, 4.59	258.1984	12	Varies	¹³ C-Di-PBDE 15	6.76	337.9344	20	0.51
¹³ C-Mono-PBDE 3	3.39	260.0239	12	---	Chlordene (s)	Unknown	339.8542	20	0.80
Trifluralin; Benfluralin	2.99; 3.04	264.0232	12	Varies	¹³ C-Di-PBDE 15	6.76	339.9324	20	1.00
Pentachloroanisole	3.45	277.8627	12	0.65	Heptachlor Epoxide	7.09	352.8442	20	1.00
Pentachloroanisole	3.45	279.8597	12	1.00	Heptachlor Epoxide	7.09	354.8413	20	0.80
Trifluralin (M+.)	2.99	292.0545	12	1.00	Oxychlordane	7.01	386.8052	20	1.00
Tribromoanisole (s)	2.85	300.7686	12	1.00	Oxychlordane	7.01	388.8023	20	1.00
Tribromoanisole (s)	2.85	302.7666	12	0.97					
Benfluralin (M+.)	3.04	306.0702	12	1.00					

SIM GROUP 3	GC Retention 7.6-11.8 min	23 ions m/z	Dwell (ms)	Ion Ratios	SIM GROUP 4	GC Retention 11.8-17.35 min	22 ions m/z	Dwell (ms)	Ion Ratios
Tetra-PCBs (#81, 77)	9.29; 9.67	289.9224	12	0.77	Tetra-PCDD (dioxin)	< 11.8-12.5	319.8965	12	0.77
Tetra-PCBs (#81, 77)	9.29; 9.67	291.9194	12	1.00	Tetra-PCDD (dioxin)	< 11.8-12.5	321.8936	12	1.00
Methyl Triclosan	8.46	301.9668	12	0.77	Penta-PCB (#126)	12.66	323.8834	12	0.62
Methyl Triclosan	8.46	303.9640	12	1.00	Penta-PCB (#126)	12.66	325.8804	12	1.00
p,p'-DDE (most in F1)	9.19	315.9380	12	0.77	¹³ C-PCB 126	12.64	335.9236	12	0.62
p,p'-DDE (most in F1)	9.19	317.9351	12	1.00	¹³ C-PCB 126	12.64	337.9207	12	1.00
DDD (o,p'- and p,p')	9.45; 10.84	319.9507	12	1.00	Penta-PCDF (furans)	12.5-	339.8597	12	1.00
DDD (o,p'- and p,p')	9.45; 10.84	321.9478	12	0.45	Penta-PCDF (furans)	12.5-	341.8567	12	0.66
¹³ C-p,p'-DDE	9.18	327.9783	12	0.77	p,p'-DDT	12.11	351.9147	12	1.00
¹³ C-p,p'-DDE	9.18	329.9753	12	1.00	p,p'-DDT	12.11	353.9118	12	0.64
Endosulfan I and II	8.18; 10.49	336.8760	12	1.00	Hexa-PCB (#169)	15.66	359.8415	12	1.00
Endosulfan I and II	8.18; 10.49	338.8731	12	0.64	Hexa-PCB (#169)	15.66	361.8385	12	0.80
Dieldrin & Endrin	9.09; 9.81	342.9018	12	0.61	¹³ C-Hexa-PCB 169	15.65	371.8817	12	1.00
Dieldrin & Endrin	9.09; 9.81	344.8989	12	1.00	¹³ C-Hexa-PCB 169	15.65	373.8788	12	0.80
Chlordanes (cis-, trans-)	7.87; 8.19	372.8260	12	1.00	Endosulfan Sulfate	11.94	386.8400	12	1.00
Chlordanes (cis-, trans-)	7.87; 8.19	374.8231	12	0.95	Endosulfan Sulfate	11.94	388.8371	12	0.64
Lock mass ion	---	404.9760	15	---	Lock mass ion	---	404.9760	10	---
Lock mass check ion	---	404.9760	10	---	Lock mass check ion	---	404.9760	15	---
Tri-PBDEs	8.21 - 11.60	405.8027	12	1.00	Tetra-PBDEs	12.52 - 17.00	483.7132	12	0.68
Nonachlors (cis-, trans-)	8.21; 10.37	406.7870	12	0.87	Tetra-PBDEs	12.52 - 17.00	485.7111	12	1.00
Tri-PBDEs	8.21 - 11.60	407.8006	12	0.98	¹³ C-Tetra-PBDEs	Various	495.7534	12	0.69
Nonachlors (cis-, trans-)	8.21; 10.37	408.7840	12	1.00	¹³ C-Tetra-PBDEs	Various	497.7514	12	1.00
¹³ C-Tri-PBDE 28	10.61	417.8429	12	---					

SIM GROUP 5	GC Retention 17.35-23.2 min	20 ions m/z	Dwell (ms)	Ion Ratios	SIM GROUP 6	GC Retention 23.2-31.05 min	20 ions m/z	Dwell (ms)	Ion Ratios
Hexa-PBDEs (M-2Br)	21.26 ->	481.6975	15	0.68	Lock mass check ion	---	554.9665	10	---
Hexa-PBDEs (M-2Br)	21.26 ->	483.6955	15	1.00	Lock mass ion	---	554.9665	16	---
Methoxy-Tetra-PBDEs	Unknown	513.7238	15	0.68	HexaBr-cyclododecane	Undetermined	560.7284	15	0.77
Methoxy-Tetra-PBDEs	Unknown	515.7218	15	1.00	Hepta-PBDEs (M-2Br)	26.32 - 31.28	561.6060	15	1.00
Methoxy-Cl-TetraBr-PBDEs	Unknown	549.6826	15	1.00	HexaBr-cyclododecane	Undetermined	562.7264	15	1.00
Methoxy-Cl-TetraBr-PBDEs	Unknown	551.6805	15	0.81	Hepta-PBDEs (M-2Br)	26.32 - 31.28	563.6040	15	0.97
Lock mass ion	---	554.9665	16	---	Methoxy-Penta-PBDEs	Unknown	593.6323	15	1.00
Lock mass check ion	---	554.9665	10	---	Methoxy-Penta-PBDEs	Unknown	595.6303	15	0.98
Penta-PBDEs (M+.)	16.92 - 22.04	563.6216	15	1.00	Hexa-PBDEs (M+.)	23.2 - 27.52	641.5321	15	0.77
Penta-PBDEs (M+.)	16.92 - 22.04	565.6196	15	0.97	Hexa-PBDEs (M+.)	23.2 - 27.52	643.5301	15	1.00
TetraBr-BPA-dime-ether	Undetermined	569.7864	15	1.00	¹³ C-Hexa-PBDEs	23.70-26.41	653.5724	15	0.77
TetraBr-BPA-dime-ether	Undetermined	571.7844	15	0.69	¹³ C-Hexa-PBDEs	23.70-26.41	655.5704	15	1.00
¹³ C-Penta-PBDEs	Various	575.6619	15	1.00	Bis(TriBrPhenoxyEthane)	28.71	685.5582	15	0.80
¹³ C-Penta-PBDEs	Various	577.6598	15	0.97	Bis(TriBrPhenoxyEthane)	28.71	687.5562	15	1.00
Methoxy-Penta-PBDEs	Unknown	593.6323	15	1.00	¹³ C-Bis(TriBr-PhenoxyEt)	28.69	697.5985	15	0.80
Methoxy-Penta-PBDEs	Unknown	595.6303	15	0.98	¹³ C-Bis(TriBr-PhenoxyEt)	28.69	699.5965	15	1.00
Hexa-PBDEs (M+.)	21.26 ->	641.5322	15	0.77	Hepta-PBDEs (M+.)	26.32 - 31.28	721.4407	15	1.00
Hexa-PBDEs (M+.)	21.26 ->	643.5302	15	1.00	Hepta-PBDEs (M+.)	26.32 - 31.28	723.4386	15	0.97
¹³ C-Hexa-PBDE 154	22.12	653.5724	15	0.77	¹³ C-Hepta-PBDEs	27.56; 29.31	733.4809	15	1.00
¹³ C-Hexa-PBDE 154	22.12	655.5704	15	1.00	¹³ C-Hepta-PBDEs	27.56; 29.31	735.4788	15	0.97
SIM GROUP 7	GC Retention 31.05-36.1 min	14 ions m/z	Dwell (ms)	Ion Ratios	SIM GROUP 8	GC Retention 36.1-39.7 min	12 ions m/z	Dwell (ms)	Ion Ratios
Octa-PBDEs (M-2Br)	31.19 - 35.36	639.5165	25	0.77	Nona-PBDEs (M-2Br)	36.57 - 37.97	719.4250	30	1.00
Octa-PBDEs (M-2Br)	31.19 - 35.36	641.5144	25	1.00	Nona-PBDEs (M-2Br)	36.57 - 37.97	721.4229	30	0.97
Hexa-PBDEs	Unnecessary	641.5321	25	0.77	¹³ C-Nona-PBDEs	36.96; 37.96	731.4652	30	1.00
Hexa-PBDEs	Unnecessary	643.5301	25	1.00	¹³ C-Nona-PBDEs	36.96; 37.96	733.4632	30	0.97
Nona-PBDEs (M-2Br)	QC Check	719.4250	25	1.00	Lock mass check ion	---	770.9598	15	---
Nona-PBDEs (M-2Br)	QC Check	721.4229	25	0.97	Lock mass ion	---	770.9598	29	---
Hepta-PBDEs (M+.)	< - 31.28	721.4406	25	1.00	Octa-PBDEs (M+.)	QC Check	799.3511	30	0.82
Hepta-PBDEs (M+.)	< - 31.28	723.4386	25	0.97	Octa-PBDEs (M+.)	QC Check	801.3491	30	1.00
Lock mass ion	---	770.9598	20	---	Nona-PBDEs (M+.)	36.57 - 37.97	879.2596	30	1.00
Lock mass check ion	---	770.9598	15	---	Nona-PBDEs (M+.)	36.57 - 37.97	881.2575	30	0.97
Octa-PBDEs (M+.)	31.19 - 35.36	799.3512	25	0.82	¹³ C-Nona-PBDEs (M+.)	36.96; 37.96	891.2998	30	1.00
Octa-PBDEs (M+.)	31.19 - 35.36	801.3491	25	1.00	¹³ C-Nona-PBDEs (M+.)	36.96; 37.96	893.2978	30	0.97
¹³ C-Octa-PBDEs (M+.)	31.96; 33.91	811.3912	25	0.82					
¹³ C-Octa-PBDEs (M+.)	31.96; 33.91	813.3892	25	1.00					
SIM GROUP 9	GC Retention 39.7-47 min	15 ions m/z	Dwell (ms)	Ion Ratios	SIM GROUP 9	GC Retention 39.7-47 min	15 ions m/z	Dwell (ms)	Ion Ratios
Deca-PBDE 209 (M-2Br)	41.77	797.3355	30	0.82	Deca-PBDE 209 (M+.)	41.77	957.1701	30	0.87
Deca-PBDE 209 (M-2Br)	41.77	799.3334	30	1.00	Deca-PBDE 209 (M+.)	41.77	959.1680	30	1.00
¹³ C-PBDE 209 (M-2Br)	41.75	811.3737	30	1.00	Deca-DiPh-Ethane	44.09	971.2048	30	1.00
¹³ C-PBDE 209 (M-2Br)	41.75	813.3716	30	0.78	Deca-DiPh-Ethane	44.09	973.2029	30	0.76
Lock mass ion	---	865.9581	25	---	¹³ C-PBDE 209 (M+.)	41.75	973.2063	30	0.81
Lock mass check ion	---	865.9581	15	---	¹³ C-PBDE 209 (M+.)	41.75	975.2042	30	0.45
					¹³ C-PBDE 209 (M+.)	41.75	977.2022	30	0.17
					¹³ C-Deca-DiPh-Ethane	44.08	983.2533	30	0.81
					¹³ C-Deca-DiPh-Ethane	44.08	985.2513	30	1.00

Continued ----->

Table 6. GC/HRMS Selected Ion Monitoring for Triclosan, Bisphenol A, and Other Polar Compounds

SIM GROUP 1	GC Retention 5.0-16.5 min	6 ions m/z (mass)	Dwell (ms)	Ion Ratios
Most Phthalates (< Octyl)	Variety possible	149.0239	40	1.00
¹³ C ₄ -Phthalates (several surrogates)	Several	153.0373	40	1.00
Methyl Phthalates (methylated metabolites)	Variety possible	163.0395	40	0.20-0.50
¹³ C ₄ -Methyl Phthalates (several surrogates)	Several	167.0529	40	0.20-0.50
Lock mass check ion	---	180.9888	25	---
Lock mass ion	---	180.9888	45	---
SIM GROUP 2	GC Retention 16.5-30 min	18 ions m/z (mass)	Dwell (ms)	Ion Ratios
Bisphenol A (as dimethylether)	17.37	241.1229	25	1.00
¹³ C ₁₂ -BPA (as dimethylether)	17.36	253.1631	25	1.00
Bisphenol A (as dimethylether) M+.	17.37	256.1463	25	0.22
¹³ C ₁₂ -PCB 101 (Int. Std)-Fragment ion	18.57	265.9859	25	1.00
¹³ C ₁₂ -BPA (as dimethylether) M+.	17.36	268.1865	25	0.22
¹³ C ₁₂ -PCB 101 (Int. Std)-Fragment ion	18.57	269.9800	25	0.20
Lock mass check ion	---	292.9824	20	---
Lock mass ion	---	292.9824	35	---
Triclosan (as methyl ether)	18.80	301.9668	25	1.00
Triclosan (as methyl ether)	18.80	303.9640	25	0.97
¹³ C-Triclosan (as methyl ether)	18.79	314.0070	25	1.00
p,p'-DDE [detected with polar compds?]	20.17	315.9380	25	0.77
¹³ C-Triclosan (as methyl ether)	18.79	316.0041	25	0.97
p,p'-DDE [detected with polar compds?]	20.17	317.9351	25	1.00
Hydroxy-Tetra-PCBs (as methyl ether)	Undetermined	319.9329	25	0.77
Hydroxy-Tetra-PCBs (as methyl ether)	Undetermined	321.9301	25	1.00
¹³ C-p,p'-DDE [recovered w/polars?]	20.15	327.9783	25	0.77
¹³ C-p,p'-DDE [recovered w/polars?]	20.15	329.9753	25	1.00
SIM GROUP 3	GC Retention 30-45 min	10 ions m/z (mass)	Dwell (ms)	Ion Ratios
Hydroxy-Tetra-PBDEs (as methyl ether)	Undetermined	513.7238	30	0.68
Hydroxy-Tetra-PBDEs (as methyl ether)	Undetermined	515.7218	30	1.00
Tetrabromo-BPA (as dimethylether)	38.44	554.7629	30	0.69
Lock mass check ion	---	554.9665	20	---
Lock mass ion	---	554.9665	35	---
Tetrabromo-BPA (as dimethylether)	38.44	556.7609	30	1.00
¹³ C-Tetrabromo-BPA (as dimethylether)	38.40	566.8032	30	0.68
¹³ C-Tetrabromo-BPA (as dimethylether)	38.40	568.8011	30	1.00
Hydroxy-Penta-PBDEs (as methyl ether)	Undetermined	593.6323	30	1.00
Hydroxy-Penta-PBDEs (as methyl ether)	Undetermined	595.6303	30	0.98

Only a short 13 m capillary column was used in Sets 1-2 with GC/HRMS (Table 2, p.25).

For all samples, final 0.3 mL eluates (Fractions 1 and 2) from basic alumina in nonane were analyzed by injecting 3 μ L into an Agilent 6890N GC/Autospec HRMS. Eluates from Set 4 were injected using a heated (240 $^{\circ}$ C) direct (splitless) inlet for Fraction 1 and cool-on-column inlet for Fraction 2. For Set 4, two narrow (0.15 mm i.d.), highly efficient BPX5 (SGE) capillary columns with 0.1 μ m film thickness were co-resident in the GC/HRMS; a 30-m column for Fraction 1 of PCBs (Figures 15-16) and a few non-polar POPs pesticides and a 13-m column for most POPs and mono- to deca-PBDEs in Fraction 2 (Figures 17-18). For the 30-m BPX5 capillary column, the oven temperature was programmed from 165 $^{\circ}$ C to 250 $^{\circ}$ C at 3 $^{\circ}$ C/min and to 325 $^{\circ}$ C at 12 $^{\circ}$ C/min while helium carrier gas was pressure programmed from 370 kPa to 475 kPa at 3 kPa/min. For the 13-m capillary BPX5 column, the oven was temperature programmed from 150 $^{\circ}$ C to 334 $^{\circ}$ C at 4 $^{\circ}$ C/min and helium carrier gas was pressure-programmed from 245 kPa to 386 kPa at 3 kPa/min.

GC/HRMS chromatograms of PCBs in Figures 15 and 16 show both the chromatographic separations by the longer (30 m) capillary column in the composite chromatogram in Figure 15 and the instrument's mass selectivity and sensitivity with stacked chromatograms of individual (accurate mass) ions in Figure 16. The three lowest levels of analytical calibration mixtures of native PCBs with 13 C-surrogates PCBs were purchased (Wellington Labs) to complement existing 13 C-PCBs standards for spiking into every sample to provide accurate, quantitative trace-level analysis. These PCB mixtures contained sufficient PCB congeners to match the majority of PCBs typically

Figure 15. GC/HRMS analysis with a 30 m x 0.15 mm i.d. BPX5 column of a low calibration mixture of 80 PCBs (6 pg each injected) as a composite of 14 selected ion chromatograms from the 4 ion groups measured (Tables 3-4) for basic alumina Fraction 1 analytes.

Figure 16. Four selected ions from Group 3 of the GC/HRMS analysis of PCBs (Figure 15), from 15.25-23.25 min, are stacked to show the instrument's sensitivity and selectivity. From bottom to top, penta- to octa-PCBs were measured using m/z 325.8804, 359.8414, 393.8025, and 429.7606 (Table 3), respectively.

Figure 17. GC/HRMS analysis with a 13 m x 0.15 mm i.d. BPX5 column is shown as an overlaid composite chromatogram from the first four ion groups measured (Table 5) for basic alumina Fraction 2. Analytes include mono- through tetra-PBDEs (numbered congener peaks in green), pesticides, three synthetic musk fragrances, and four non-*ortho*-PCBs.

Figure 18. The last portion of the same composite chromatogram from GC/HRMS analysis of basic alumina Fraction 2 (Figure 17) from ion groups five through nine includes the rest of the PBDEs (penta- through deca-).

detected, to challenge chromatographic resolution, and to exceed the number of congeners typically targeted by the CDC in their blood biomonitoring. The GC/HRMS analysis in Figures 15 and 16 show the highest of the three mixtures at 2 pg/ μ L (6 pg injected from 3 μ L). GC/HRMS analysis of the lowest concentrated mixture at 0.1 pg/ μ L (0.3 pg injected) showed that all PCB congener peaks were still detectable, demonstrating the potential for measuring PCBs in nails if lab background was sufficiently low.

GC/HRMS chromatograms in Figures 17 and 18 show the complete analysis possible in one 41 minute run with a short 13-m BPX5 column for the compounds eluted into the basic alumina Fraction 2. In Figure 17, mono- through tetra-PBDEs are numbered peaks (ion chromatograms in green) while pesticides, three fragrances, and four non-*ortho*-PCBs are labeled with names alongside the peaks. Calibration standards shown in Figure 17 ranged from 25 to 80 pg injected. GC/HRMS sensitivity was low for DDT, because the 1.5 mass range limitation forced choosing its low abundant molecular ions. Penta- through deca-PBDEs follow in Figure 18 in the latter half of the run from 17-42 min.

CHAPTER 3. ANALYTICAL RESULTS AND DISCUSSION

Various criteria were used to evaluate the nail methods used, including effectiveness in digesting/dissolving nail for solvent extraction, extent of lab contamination of various compounds in method blank samples, recovery of ^{13}C -surrogates, detection limits of analytes, replicate analysis of multiple samples from the same individuals collected over an extended time, and breadth of method to measure a variety of compounds at ppb concentrations, typical of biomonitoring requirements.

Dissolution of Nail

An important first step was to evaluate the effectiveness of the methods to break up the toenail matrix through various means (grinding, DTT reagent, and enzyme digestion) for effective solvent (DCM) extraction of small organic compounds. Of the total of 13 toenail samples processed, all had residual nail material consisting of small thin slivers that was not fully dissolved. After the two Individual A samples of Set 2 (Table 2, p.25) were ground and processed, the residue in the conical mortar vials was allowed to dry passively. In each vial, undissolved nail, measured 96.9 and 124.9 mg, respectively, or approximately half of each initial 190 mg sample. By Set 4 (Table 2, p.25), the attempt to more thoroughly dissolve a nail sample led to adding Proteinase K enzyme digestion for up to two weeks. Solutions of dissolved and digested nail in extracts from Set 4 were clearer, consistent with less suspended (undissolved) nail (Figure 19). However, emulsions in DCM extracts of Set 4 samples were more difficult to resolve than in previous sample sets.

Lipid removal by GPC flash column

During the time of using flash GPC for samples in Set 4, quality assurance elution tests were performed separately with ^{14}C -cholesterol and ^{14}C -palmitic acid using the same two GPC columns after their use for the soap wash blank sample and a soap wash of one of the nail samples, respectively. For ^{14}C -cholesterol, no activity was observed in the first collection vial, 1% in the second vial, 70% in the third vial, 23% in the small fourth vial (from 4.2-4.8 mL), and only 3% in each of the fifth and sixth vials destined for

Figure 19. Culture tubes of all human toenail extracts of Set 4 (Table 2). The top layer in tubes is aqueous, bottom layer is in DCM solvent, and small intermediate density layer suggests denatured keratin protein.

GC/HRMS demonstrating a 94% separation of this bulky steroid. For the 16-chain fatty acid, ^{14}C -palmitic acid eluted in a broader range (0%, 2%, 43%, 39%, 8%, and 8%) with 84% in vials 1-4 before the normal GPC collection range began. However, for the polar compounds targeted in Set 3, both quality assurance tests confirmed that the small fourth vial (collected in an extra early GPC eluate) contained significant amounts of bulkier, polar compounds, including naturally occurring ones.

For eight of the 10 human nail samples in Sets 1, 2, and 4, the percent lipid measured collectively in the first four vials of the flash GPC was consistent between 1.03 and 1.31 weight percent (Table 2, p.25); mean 1.21%, sd 0.09. Including the other two nail samples with lipid values of 0.87% and 1.63% resulted in a mean 1.22%, sd 0.20, $n=10$. The former (0.87%) corresponds to Sample 7T-2, whose dried nail residue weighed about half of the original nail sample, demonstrating incomplete dissolution. The higher value (1.63%) corresponds to Sample 17T-4 (Figure 19), which was a smaller sample than others from Individual A and with the additional Proteinase K and time, may have dissolved more completely. Also it is possible that with such a small nail sample, lipids from bits of skin included with the nail clippings increased the total nail lipid percentage. Such "stratum corneum" lipids that constitute 2% of skin on a human foot⁷⁰ may have solubilized in the soap wash (Sample 17W-4), that results (still to be covered) showed some exogenous contamination. Still another possibility for the higher lipid value is that a residual amount of styrene-divinylbenzene co-polymer from the S-X3 GPC column may have sloughed off from the cleanup column. Set 3 for targeted polar

compounds used just the first three GPC vials for measuring lipid so the measured 0.5% in that set correlates with only half of the total observed in the other sample sets.

Cleanup with SPE Basic Alumina Glass Cartridge

An SPE glass cartridge with 0.5 g basic alumina was used with every sample from all of the methods. Except for Set 3, the SPE cartridge was only a quick intermediate cleanup step following GPC and before final 4-g basic alumina flash chromatography. However, for Set 3 with methylation and more polar analytes, an SPE cartridge was the final cleanup/fractionation step before GC/full scan MS and GC/HRMS. Ordinarily, SPEs made in the US contribute various artifacts and thus are unsuitable for trace-level analyses, but the German-made Macherey-Nagel SPEs performed well to clean up any unreacted polar compounds without leaching any artifacts into the final sample extracts, as evidenced by relatively clean GC/full scan MS analyses for the Set 3 blank and samples run (Table 2, p.25). Because the 0.5 g basic alumina in an SPE cartridge was less active than the heated 4-g basic alumina packed in a flash column and because some acetone used in Set 3 was likely present, most ether and ester derivatives (after methylation) eluted along with cholesterol with DCM from the SPE. Subsequently, smaller amounts of the same compounds were eluted using a slightly more polar (2% acetone in DCM) eluant, but again without any contaminants.

Final Cleanup and Separation with Basic Alumina Flash

Chromatography

Basic alumina flash elutions shifted slightly from expected in Set 1, necessitating a slight adjustment in solvent elution strength in subsequent sample sets as was noted in Chapter 2. Basic alumina became slightly more adsorptive when it was carefully stored in a heated, tightly-covered glass Erlenmeyer flask to minimize exposure to lab dust and moisture. A few analytes expected in Fraction 1, e.g. ^{13}C -DDE, were retained until Fraction 2 and several analytes expected in Fraction 2, e.g. methyl ether derivatives of ^{13}C -BPA and ^{13}C -tetrabromobisphenol A likely remained on the column for Set 1. To compensate, the GC/HRMS was set up to run both fractions for a certain group of analytes to ensure detection and Set 3 for just polar analytes used just an SPE. In Set 4, a third more polar fraction was collected, in case Fraction 2 analytes such as synthetic musk fragrances of ethers and ketones were retained until Fraction 3.

Elutions from basic alumina also occasionally shifted if too much residual DCM was in a sample extract applied to the alumina column. Just before a sample extract was applied, a complete solvent exchange from moderately polar, volatile DCM to non-polar, less volatile isooctane was necessary to prevent analytes expected in Fraction 2 (0.5% acetone in DCM) to shift into Fraction 1 (only 1% DCM in cyclopentane). Solvent exchange was successful by routinely adding slightly more isooctane than the DCM and blowing down to the original volume. With a significantly higher boiling point (100 °C) than DCM (40 °C), isooctane also kept the extract from going dry as high purity nitrogen was blown into each sample tube to purge the DCM.

In general, basic alumina flash chromatography with high purity nitrogen kept background compounds very low and effectively fractionated compounds as described in the Methods chapter. GC/full-scan MS analyses of Fraction 1 of Set 1 nail and blank samples detected various non-polar long-chain hydrocarbons as expected. In Fraction 2 of all Set 1 blanks and sample, slightly more polar polycyclic aromatic hydrocarbons were detected. Also consistently in Fraction 2 were the ^{13}C -PBDEs surrogates. Native PBDEs were higher in the Set 1 nail sample than in the blank samples and several other compounds that were not in the blank samples were identified in the nail sample.

GC/full-scan MS to identify unsuspected compounds in Set 1 samples

Analyses with GC/full-scan MS with unit mass resolution were important to detect and identify unsuspected compounds present in the nail samples and not in blanks amidst background hydrocarbon, siloxane, and phthalate compounds in all samples. These less selective GC/MS analyses were also helpful to assess how well the method cleaned up and fractionated the sample extracts with a minimum of lab background and artifacts from sorbents, reagents, and technique.

For example, a total ion chromatogram of Fraction 2 of Set 1 blank (1B-1) was compared with that of Fraction 2 of individual A Nail (3T-1) in Figure 20. Almost all of the peaks shown were common to both blank and nail sample. Based on ^{13}C -PCB congener 101, internal standard of 5 ng total (~ 25 ppb) comprising most of the small peak at 34.91 min of each chromatogram, each compound peak shown ranges from < 1 ng to about 500 ng, which corresponds to ppb concentrations.

RT: 21.41 - 62.07

Figure 20. Total ion chromatogram of Fraction 2 of Set 1 blank (1B-1) compared with that of Toenail (3T-1).

Common polycyclic aromatic hydrocarbons in both blank and nail samples include phenanthrene (24.79 min), methyl phenanthrenes (28.31, 28.56, 29.09, and 29.29 min), fluoranthene (33.55 min), and pyrene (35.14 min). Beginning at 47.69 min, almost all major late-eluting compounds were long chain hydrocarbons (C_{26} to C_{32}) that were retained in Fraction 2. After comparing both total ion chromatograms, two late-eluting compounds in the nail sample at 52.70 min and 54.37 min were identified that were not in the blank sample (Figure 20). Mass spectra matched those in the NIST MS Library for squalene and cholesta-3,5-diene, respectively, which occur naturally in biological tissue and are not chemical pollutants or contaminants.

Further GC/MS data processing was necessary to uncover all of the ^{13}C -surrogates and other compounds which were in the nail sample but not in the blank sample. For more specificity, low target compounds could be recognized by displaying several mass chromatograms of a compound's abundant ions. For example, the internal standard, ^{13}C -PCB 101, was more easily located (at 34.91 min) in each sample by selecting an abundant ion (m/z 338) of its molecular ion cluster shown in the bottom two traces in Figure 21. Because the areas of each internal standard peak at 34.91 min were nearly identical (within 2%), the GC/MS analysis of the nail sample was directly comparable with that of the blank sample.

Some low unsuspected compounds were recognized by reviewing all mass spectra for detectable ions with masses higher than that from the solvent and background ($> m/z$ 130). In that way and using mass chromatograms to pinpoint a peak, several chlordane-related compounds were recognized and then identified. In Figure

Figure 21. Pairs of chromatograms from 33.4-36.8 min of blank and nail samples were compared for top two total ion chromatograms (TIC) and with peak areas for mass chromatograms m/z 373 (chlordane), 409 (nonachlor), and 338 for ^{13}C -penta-PCB 101 internal standard. Similar peak areas for the internal standard (5 ng total) at 34.91 min confirm data of both sample extracts can be directly compared.

21, mass chromatograms of m/z 373 and 409 are shown from 33.4-36.8 min for both a blank sample and Individual A nail (3T-1) from Set 1. In the nail sample, two isomeric peaks (34.30 and 34.96 min) for (trans- and cis-) chlordane and one trans-nonachlor (35.09 min) were detected with peak responses in the 10^4 range (6×10^4 in Figure 21), but not in the blank sample with only 10^2 (3×10^2) background noise level. Using the data system's built-in "Refine" automatic spectral processing feature, an enhanced background subtracted, full-scan mass spectrum near the apex of the peak (34.29 min) closely matched the NIST MS Library spectrum for chlordane (Figure 22) and later our own spectrum and retention times from an analytical chlordane standard. Similarly, an enhanced background subtracted, full-scan mass spectrum of trans-nonachlor at 35.09 min matched the NIST MS Library reference spectrum (Figure 23) and also later our own spectrum and retention times from a trans-nonachlor standard. Later in the GC/MS chromatograms, the ^{13}C -surrogate of PBDE 47 was detected in both the blank and toenail samples at 45.98 min, shown in the top two m/z 498 mass chromatograms (Figure 24). Peak areas of the surrogate in the samples were nearly identical (230,633 and 220,530, respectively) and, compared to the peak areas at 48.75 min for the internal standard (^{13}C -PBDE 79) at 2 ng total added into the final GC vial, the ^{13}C -PBDE 47 was consistently well recovered (76% and 82%) through the entire Set 1.

2008-Oct27-05 #2014 RT: 34.29 AV: 1 RF: 6.00.3 NL: 4.40E4
T: [0.0] + c EI det=349.00 Full ms [50.00-750.00]

(replib) trans-Chlordane

Figure 22. Mass spectrum at 34.29 min in nail sample confirmed as chlordane. A loss of chlorine corresponds to the base peak cluster.

2008-Oct27-05#2081 RT: 35.09 AV: 1 RF: 6.00.3 NL: 3.10E4
T: (0.0) + c EI det=349.00 Full ms [50.00-750.00]

(mainlib) trans-Nonachlor

Figure 23. Mass spectrum at 35.09 min in nail sample confirmed as trans-nonachlor.

Figure 24. Pairs of chromatograms of m/z 498 (^{13}C -PBDE 47; 79 Internal Standard) and 486 (PBDE 47) in blank and nail samples were compared (44.3-50.2 min). Mass spectrum of nail sample at 45.98 min shows molecular ion clusters and fragment ions for both compounds.

For the native PBDE 47 in the blank and toenail samples, corresponding m/z 486 mass chromatograms showed an extremely small peak in the blank sample at 46.01 min with peak area 1182, but a much larger peak at 45.99 min in the toenail sample with peak area 83,092. Based on the ^{13}C -PBDE 47 (2 ng total or 10.7 ng/g) and assuming a similar response factor for PBDE 47, an approximate concentration for PBDE 47 was 4 ng/g (or ppb). The background-subtracted mass spectrum in the bottom of Figure 24 at 45.98 min in the toenail sample shows both ^{13}C -PBDE 47 and PBDE 47 with

corresponding molecular ion clusters of m/z 494-502 and 482-490 and $(M-2Br)^+$ fragment ions with losses of 158.

GC/full-scan MS to identify unsuspected compounds in Set 3 samples

GC/full-scan MS was also used to analyze for more polar compounds, including phenolic and acidic compounds detectable following methylation (Set 3), in several fractions of two Individual A toenail samples (10T-3 and 11T-3) and a representative blank sample, either of 8B-3 or 9B-3. Using a typical DCM extraction, GPC collection, methylation, and a first (DCM) eluate from basic alumina SPE cartridge, some compounds of possible interest were detected in both toenail samples but not in a blank sample. Although compounds of natural origin, e.g. cholesterol, were also detected in nail samples but not in a blank, this section will focus primarily on other compounds.

Reporting generally in GC elution order, an early eluting compound with molecular mass 158 at 9.8 min was tentatively identified in both nail samples as 2,4-dimethyl quinazoline but was not detected in a blank sample as shown by comparing m/z 158 chromatograms (Figure 25). The mass spectrum closely matched the NIST Library that included several dimethyl quinazoline isomers with formula $C_{10}H_{10}N_2$. The abundant, even mass, molecular ion is consistent with a stable aromatic compound with an even number of nitrogens. An abundant fragment ion (m/z 143) shows an expected loss of a methyl group. This was one of several compounds containing nitrogen and, from a detailed literature search, is not a compound used commercially.

In Figure 26, an early eluting compound at 15.25 min with apparent molecular ion at m/z 179 was tentatively identified as 6-methoxy-2-methyl-benzothiazole by

PHP-2009-Jan-13-03 #229 RT: 9.75 AV: 1 RF: 6.00,3 NL: 8.68E6
T: {0,0} + c EI det=349.00 Full ms [50.00-750.00]

Figure 25. Mass spectrum at 9.75 min was tentatively identified as 2,4-dimethyl-quinazoline by NIST Library match after mass (m/z 158) chromatograms from 8.5-11.4 min of blank and nail samples of Set 3 showed a compound peak at 9.8 min in both nails but not blank.

PHP-2009-Jan-13-03 #685 RT: 15.25 AV: 1 RF: 6.00.3 NL: 1.25E5
T: (0.0) + c EI det=349.00 Full ms [50.00-750.00]

Nail 11T-3 at 15.25 min

(mainlib) Benzothiazole, 6-methoxy-2-methyl-

Figure 26. Mass spectrum at 15.25 min was tentatively identified as 6-methoxy-2-methyl-benzothiazole by NIST Library match after mass (m/z 179) chromatograms from 14.6-21.3 min of blank and nail samples of Set 3 showed a peak at 15.25 min in both nails but not blank.

matching the NIST Library. Because of the methylation step in the method, it is possible that the compound may have been originally 6-hydroxy-2-methyl-benzothiazole. The abundant odd number molecular ion is consistent with an aromatic compound containing an odd number of nitrogen. The main fragment ions are a methyl loss (m/z 164) and a likely additional CO loss (m/z 136).

Benzophenone was identified by the mass spectrum at a retention time of 17.44 min, which is consistent with unpublished USGS data run recently. Mass m/z 182 chromatograms in blank and nail samples were compared in the top of Figure 27. In the blank sample, a very small peak (area 26,517) was detected, but in the nail samples, much larger peaks with respective areas 1.7 million and 2.1 million were detected. In the bottom of Figure 27, a mass spectrum from sample 11T-3 at 17.44 min closely matches a reference mass spectrum of benzophenone from the NIST Library.

Just a few seconds later (at 23.64 min) in each nail sample of Set 3 (Figure 28) was a synthetic musk fragrance compound with molecular mass 258 that matched the mass spectra of at least two $C_{18}H_{26}O$ isomers (common names, Galaxolide and Versalide, bottom and lower bottom matches). By mass spectrometry, these isomeric compounds cannot be distinguished, although a Galaxolide standard did match GC times using GC/HRMS. Subsequently, in Set 4, several synthetic musk fragrance compounds including galaxolide were targeted in the expanded basic alumina Fraction 2 (Table 2, p.25).

PHP-2009-Jan-13-03 #866 RT: 17.44 AV: 1 RF: 6.003 NL: 5.84E5
T: (0.0) + c EI det=349.00 Full ms [50.00-750.00]

Nail 11T-3 at 17.44 min

Figure 27. Mass spectrum at 17.44 min from Nail 11T-3 was confirmed as benzophenone by a standard after mass (m/z 182) chromatograms from 14.6-21.3 min of blank and nail samples of Set 3 showed a compound peak at 17.4 min, primarily in nail samples.

Next-eluting analytes in the Set 3 chromatograms were BPA-dimethyl ether (BPA-DME) and surrogate, ^{13}C -BPA-DME at 31.4 min (Figure 29). In both nail samples, GC/full scan MS detected both BPA-DME and the ^{13}C -surrogate (5 ng total) that was

RT: 22.88 - 26.52

PHP-2009-Jan-13-03 #1380 RT: 23.64 AV: 1 RF: 6.00,3 NL: 2.78E5
T: {0,0} + c EI det=349.00 Full ms [50.00-750.00]

Nail 11T-3

Figure 28. Mass (m/z 243) chromatograms from 22.9-26.5 min of Set 3 samples show a compound peak at 23.64 min in the nail samples only, whose mass spectra (red) match that of two $C_{18}H_{26}O$ isomers (galaxolide and below, versalide) from NIST Library (blue).

RT: 30.87 - 32.14

PHP-2009-Jan-13-03 #2025 RT: 31.42 AV: 1 RF: 6.00,3 NL: 5.86E4
 T: {0,0} + c EI det=349.00 Full ms [50.00-750.00]

Figure 29. Mass chromatograms (m/z 241 and 253 for native- and ^{13}C -BPA-dimethyl ether) from 30.8-32.1 min of Set 3 samples show both were detected at 31.4 min in both nail samples, but neither was detected in a blank sample, due to a bad injection and poor recovery of the ^{13}C -BPA surrogate. Sample mass spectrum (31.42 min) of both compounds co-eluting matched NIST spectrum of BPA-dimethyl ether.

spiked at the beginning of extract. However, neither BPA-DME nor surrogate was detected by GC/full scan MS in a Set 3 blank sample, either due to a poor, partial injection by the GC autosampler and/or because of a poor recovery of the surrogate through the method. Checking the ^{13}C -PCB 101 (5 ng total) internal standard peak areas for the blank and nail samples using m/z 338 at 32.54 min showed the blank sample internal standard peak area (11,108) was about **four times lower** than the nails (45,723 and 51,665), indicating that only a partial injection of the blank had been made. Unfortunately, Blank 9B-3 which was not run by GC/full scan MS had more BPA than in Blank 8B-3. Complete quantitative data for BPA in all Set 3 samples will be reported and discussed in the GC/HRMS section upcoming in this chapter.

Methyl ether derivatives of another polar analyte, triclosan, with ^{13}C -surrogate (10 ng total) eluted at 33.1 min (Figure 30). The surrogate was well recovered in samples, even in the blank sample after allowing for the poor injection. In both nail samples, peak areas for the native triclosan-methyl ether were double that of the surrogate for an estimated 20 ng total in each nail sample and thus a concentration of about 70 ng/g. Although triclosan-methyl ether was not detected in the blank (only partially injected) by GC/full scan MS, more sensitive GC/HRMS analyses were also done and will be discussed.

The non-polar ^{13}C -p,p'-DDE surrogate (5 ng total), eluting at 34.35 min, was among those spiked in Set 3 samples to assess whether it would be recovered through the method. The DDE surrogate was detected in all Set 3 samples in alumina SPE-Fraction 1 by GC/full scan MS, and percent recoveries were determined by GC/HRMS.

RT: 32.52 - 33.79

PHP-2009-Jan-13-03 #2162 RT: 33.07 AV: 1 RF: 6.00,3 NL: 4.82E4
 T: {0,0} + c EI det=349.00 Full ms [50.00-750.00]

Nail 11T-3

Figure 30. Mass chromatograms (m/z 302 and 314 for native- and ^{13}C -triclosan-dimethyl ether) from 32.3-34.1 min of Set 3 samples show both were detected at 33.1 min in both nail samples, but only surrogate was detected in a blank sample. Mass spectrum (33.07 min) of both compounds co-eluting matches NIST spectrum of methoxy-triclosan.

The surrogate, ^{13}C -tetrabromo-BPA (5 ng total) was searched for in all of Set 3 samples and fractions run by GC/full scan MS, but was not detected using the abundant ion chromatograms (m/z 555, 557, and 559), necessitating further GC/HRMS analyses.

Other unsuspected compounds were found in Fraction 1 of Set 3 samples. In both nail samples, a compound peak at 31.95 min with abundant m/z 233 was tentatively identified as 2-anilino-4-methyl quinoline by NIST Library match (Figure 31). At 46.87 min, a compound peak with molecular mass 360 was detected in both nails and less in a blank sample, even after adjusting for poor injection. It matched the NIST Library as octocrylene, a UV filter compound used in sun blocking lotions (Figure 32). Its mass spectrum showed the octyl-alkyl group loss as the base peak cluster at m/z 248-248, with a rare oxygen loss to m/z 232 and CO loss further to m/z 204. Although the molecular ion is m/z 361, which is consistent for an odd number nitrogen compound, it readily loses a hydrogen to m/z 360, a loss more typically seen in aldehyde compounds.

At least one compound was detected in only one (10T-3) of the two nail samples and not in a blank sample at 18.52 min (Figure 33). It was tentatively identified by matching the NIST Library of 4(4-methoxyphenyl)-3-butene-2-one (Figure 33), which is a flavoring agent and more commonly called anisalacetone. In the original nail extract before methylation, it is possible that the methoxy-group was a hydroxyl-group.

Several mass spectra of detected compounds did not match NIST Library but were interpreted. The mass spectrum in Figure 34 from nail sample 11T-3 appears to be related to the $\text{C}_{10}\text{H}_{10}\text{N}_2$ compound in Figure 25 because of similar ions. With an apparent molecular mass of 200, loss of a methyl group (m/z 185) and a base peak of

RT: 31.00 - 33.54

PHP-2009-Jan-13-03 #2067 RT: 31.93 AV: 1 RF: 6.00,3 NL: 4.95E5

T: [0,0] + c EI det=349.00 Full ms [50.00-750.00]

Figure 31. Mass spectrum at 31.93 min from Nail 11T-3 was tentatively identified as 2-anilino-4-methylquinoline by NIST Library match after mass (m/z 233) chromatograms from 31.0-33.6 min of Set 3 samples compared show a compound peak at 31.9 min in the nail samples only.

RT: 46.13 - 47.98

PHP-2009-Jan-13-03 #3306 RT: 46.87 AV: 1 RF: 6.00,3 NL: 1.49E5
 T: [0,0] + c EI det=349.00 Full ms [50.00-750.00]

Figure 32. Mass spectrum at 46.87 min from Nail 11T-3 was tentatively identified as octocrylene by NIST Library match after mass (m/z 360) chromatograms from 46.1-48.1 min of Set 3 samples showed a peak at 46.9 min in all samples, especially in the nails.

m/z 158, the additional 42 mass units correspond to either having an added C₃H₆ or C₂H₂O. Based on the hydrocarbon fragment losses, we theorize an elemental formula of C₁₃H₁₆N₂. Mass m/z 158 chromatograms in Set 3 samples show the compound was not detected in the blank but in both nails. Another unidentified compound that was

PHP-2009-Jan-13-02 #956 RT: 18.52 AV: 1 RF: 6.003 NL: 6.84E5
T: (0.0) + c EI det=349.00 Full ms [50.00-750.00]

Figure 33. Mass spectrum at 18.52 min was tentatively identified as 4-(4-methoxyphenyl)-3-butene-2-one by NIST Library match after mass (m/z 161) chromatograms from 16.6-23.3 min of Set 3 samples showed a peak at 18.52 min in just nail sample 10T-3.

detected at 30.2 min in Fraction 1-Set 3 nail samples was not detected in the blank sample. Based on m/z 261 chromatograms (Figure 35) for the samples, the unknown compound with an apparent molecular mass of 261 was about 100 times higher in nail sample 10T-3 than in nail 11T-3. Its mass spectrum from nail 10T-3 indicates a

RT: 14.53 - 18.32

PHP-2009-Jan-13-03 #763 RT: 16.20 AV: 1 RF: 6.00,3 NL: 1.81E6
 T: {0,0} + c EI det=349.00 Full ms [50.00-750.00]

Figure 34. Mass spectrum at 16.20 min of nail 11T-3 sample could be of a $C_{13}H_{16}N_2$ related to Figure 25 with an apparent molecular mass 200 after mass (m/z 158) chromatograms for Set 3 samples showed a 16.2 min peak for both nail samples but no detected peak in the blank sample.

PHP-2009-Jan-13-02 #1925 RT: 30.21 AV: 1 RF: 6.00,3 NL: 3.35E6
 T: {0,0} + c EI det=349.00 Full ms [50.00-750.00]

Figure 35. Mass spectrum at 30.23 min of nail 11T-3 with apparent molecular mass of 261 for an odd number of nitrogen and (M+2)⁺ possibly a sulfur. Mass (m/z 261) chromatograms for Set 3 samples showed a 30.2 min peak for both nail samples but none in the blank. The peak was about 100 times higher in nail 10T-3 than in 11T-3.

compound with an odd number of nitrogen, the m/z 77 fragment ion strongly hints of a phenyl-substitution, and the loss of M-43 at m/z 218 from M-(CO-CH₃)⁺ (Figure 35).

Slightly later at 34.3 min, an unknown compound was detected in both nail

PHP-2009-Jan-13-02 #2264 RT: 34.30 AV: 1 RF: 6.00,3 NL: 1.06E5
T: {0,0} + c EI det=349.00 Full ms [50.00-750.00]

Figure 36. Mass spectrum at 34.30 min of nail 10T-3 showed an apparent molecular mass of 244 and base peak m/z 215 from an ethyl loss. Mass (m/z 215 and 244) chromatograms for Set 3 samples showed no peak in the blank sample but a 34.3 min peak in both nail samples and higher in nail 10T-3.

samples but not the blank sample with an apparent molecular mass of 244, and base peak of m/z 215 likely from the loss of an ethyl group. Abundant high mass ions suggest at least some aromatic groups in the molecule (Figure 36). This unknown compound of mass 244 may possibly be a fragrance compound related to the molecular mass 258 fragrance compound of galaxolide (Figure 28).

GC/full scan MS data for Set 3 Neutral Extract -- Fraction 2 (2% acetone in DCM from basic alumina SPE) showed some of the same compounds from Fraction 1 were still detected but no new compounds were detected. If more polar compounds were

present but still adsorbed onto the SPE, a stronger eluant, e.g. with a higher percentage of acetone in DCM, would have been necessary to elute those types of compounds. In Set 3 samples, an early GPC eluate with two alumina SPE fractions was collected to target possible phthalate metabolites. However, no target phthalate metabolites were detected. Only high concentrations of fatty acids (as methyl esters), cholesterol, and minor sterols (sitosterol and lanosterol) were detected as would be expected in the early GPC eluate fraction. No anthropogenic compounds were detected in either fraction of the early GPC eluate, which confirmed that the small flash GPC elution performed as expected to isolate small organic molecules after bulkier sterols, fatty acids, and phthalates. Set 3 samples were also extracted a second time at pH< 3 with DCM for stronger acidic compounds before the methylation step and the following were analyzed using GC/full scan MS: Blank 9B-3 and Nail 10T-3 with normal GPC-Fraction 1 SPE, Nail 11T-3 with normal GPC-Fraction 2 SPE, and Nail 10T-3 with early GPC-Fraction 1. Only a relatively few compounds were detected (Figure 37) and primarily included various substituted benzoic acid compounds (detected as methyl ester) (Figures 38-42).

Para-methoxy-benzoate (Figure 39), probably as para-hydroxy-benzoic acid in the pH<3 extract before methylation, also can occur naturally or as a hydroxyl-metabolite of “methyl paraben” (para-hydroxy-benzoate), which is widely used commercially in personal care products of shampoo, lotions, and cosmetics. Methyl anthranilate or methyl 2-(dimethylamino)-benzoate (Figure 40) is both naturally occurring in various fruits and used commercially as a flavoring agent and in perfumes.⁷¹ Several other compounds detected and tentatively identified in the Set 3 pH< 3 samples

Figure 37. Summed ion chromatograms (m/z 130-750) from 8.0-17.3 min of Set 3 blank and nail samples extracted at pH<3 with varying GPC eluates and SPE fractions.

Figure 38. Tentative identification of methyl anthranilate or methyl 2-amino-benzoate. Mass spectrum at 8.56 min of Nail 10T-3 (top in red) matches NIST Library.

Figure 39. Tentative identification of 4-methoxy-benzoate by NIST Library match of mass spectrum at 9.37 min of Nail 10T-3 (top in red).

Figure 40. Tentative identification of methyl 2-(dimethylamino)-benzoate by NIST Library match (bottom, blue) of mass spectrum at 9.95 min of Nail 10T-3 (top, red).

Figure 41. Tentative identification of methyl 2-(methylamino)-benzoate by NIST Library match of mass spectrum at 10.19 min of Nail 10T-3 (top, red).

Figure 42. Tentative identification of 2-(acetylamino)-benzoate by NIST Library match of mass spectrum at 16.45 min of Nail 10T-3 with Early GPC, SPE-1 (top, red).

were all amino-substituted benzoic acid (methyl esters) (Figures 40-42).

GC/HRMS Analysis to detect and quantitate target polar compounds

More sensitive and selective GC/HRMS analyses complemented GC/full scan MS

analyses of Set 3 samples for more polar, transient compounds and will be discussed

followed by GC/HRMS analyses for the less polar, persistent compounds in samples

from Sets 1, 2, and 4. For example, GC/HRMS selected ion chromatograms for BPA and

the surrogate ^{13}C -BPA in Set 3 samples (Figure 43) complemented those by GC/MS

(Figure 29) by detecting all analytes, even the surrogate that was only partially

recovered in blank samples. Peaks of BPA-dimethyl ether and the surrogate at 17.3 min

tailed in most sample analyses, because a slightly earlier flash GPC eluate was taken and

the 0.5-g basic alumina SPE cartridge after methylation may not have been optimal for

GC with 5% of the final extract (3 μL of 60 μL) injected for trace-level analyses. Results

for targeted polar analytes and surrogates in Set 3 samples plus non-polar p,p'-DDE and

surrogate are tabulated in Table 7, albeit with the caveat that any DDT present may

have been converted to DDE from the basic buffer (pH 12) conditions before methylation. Analyses of the first two compounds listed, triclosan and p,p'-DDE, were successful in Set 3. Their surrogates were consistently recovered ($\geq 64\%$). Background levels of triclosan and DDE in both blank samples were either very low or not detected at the GC/HRMS detection limit of 0.1 ng/g, therefore only 0.25 ng/g was blank-subtracted from the toenail sample concentrations (average of blanks plus 3 times standard deviation) (Table 7). Thus, blank-corrected "free" triclosan (without any enzyme deconjugation step) in toenail samples was 53.6 and 62.9 ng/g (mean 58.3, sd 6.6, n=2) and p,p'-DDE 1.00 and 2.02 ng/g. In soap washes of the Set 3 toenail samples, p,p'-DDE was not detected and triclosan was low (1.1 and 2.8 ng/g) compared to concentrations in the toenail samples.

Although BPA analyses in Set 3 had lower surrogate recoveries in the blanks, isotope dilution quantitation still provided accurate concentrations by self-correcting BPA concentrations for the lower recoveries. Unfortunately, relatively high blank concentrations (average 9.09 ng/g) plus three times standard deviation) totaled 12.23 ng/g for the blank BPA subtraction from each toenail sample. After blank subtraction, BPA was 15.0 ng/g in one toenail sample, but meaningless in the other sample (0 ng/g). Set 3 soap washes had BPA concentrations similar to the original toenail sample concentrations, so again, one of the two blank-subtracted soap wash samples would have had a meaningless blank-corrected BPA concentration. Finding BPA in method blanks may require a pooling of several toenail samples to overcome it in future research.

Figure 43. From Set 3 samples, GC/HRMS selected ion chromatograms of m/z 241.1229 for dimethyl ether-BPA and m/z 253.1631 for ^{13}C -BPA surrogate at 5 ng total show integrated peaks labeled with retention time and peak area. Not shown were ^{13}C -BPA in soap washes 10W-3 and 11W-3 (peak areas 235,900 and 196,917, respectively).

Analyses of tetrabromobisphenol A were also problematic with consistently very low surrogate recoveries especially in blanks and soap washes that prevented determining accurate blank-subtracted concentrations in the toenail samples, which originally were measured at 4.9 and 8.9 ng/g (Table 7). Low recoveries may have been caused by a later than expected elution through the flash S-X3 GPC column, because some phenolic compounds can be retained longer than other neutral compounds.⁷³

Not included in Table 7 for Set 3 samples were mono-methyl-phthalic acids, metabolites of various phthalates, and several ¹³C-surrogates spiked for the method's quality assurance. None of these phthalate metabolites or surrogates were detected in any of Set 3 sample extracts, including early GPC eluates and pH < 3 sample extracts, by GC/full scan MS or by GC/HRMS. Lack of methylation efficiency, shown to be a concern even with standards, was likely a major factor.

Table 7. Concentrations (ng/g) with Surrogate Recoveries of DDE and Several Polar Compounds in Set 3 Toenail Samples

ANALYTE NAME	METHOD BLANKS					TOENAIL SAMPLES				SOAP RINSES	
	8B-3	9B-3	AVG (3 * SD)		Subtract from Toenail Samples - [Avg + 3 * SD]]	10T-3	11T-3		10W-3	11W-3	
						A	10T-3	A	11T-3		
	ng/g	ng/g				May-06	NET	Jul-06	NET	ng/g	ng/g
Successful:											
Triclosan (as methyl ether)	0.1	ND< 0.1	0.1	0.15	-0.25	53.8	53.6	63.1	62.9	1.1	2.8
p,p'-DDE (non-polar metab. of DDT)	ND< 0.1	ND< 0.1	0.1	0.15	-0.25	1.25	1.00	2.27	2.02	ND< 0.1	ND< 0.1
Problematic:											
Bisphenol A (as dimethyl ether)	9.83	8.35	9.09	3.14	-12.23	27.2	15.0	10.5	---	30.2	8.37
Tetrabromo-BPA (dimethyl ether)	---	---	---	---	-(Indeterminate)	4.9	< 4.9	8.9	< 8.9	---	---
13C-SURROGATE RECOVERY	8B-3	9B-3				10T-3	11T-3			10W-3	11W-3
	%	%				%	%			%	%
Triclosan (as methyl ether)	70%	66%				80%	64%			66%	68%
p,p'-DDE	72%	75%				80%	65%			78%	78%
Bisphenol A (dimethyl ether)	16%	26%				82%	77%			71%	78%
Tetrabromo-BPA (dimethyl ether)	< 1%	< 1%				3%	6%			1%	1%
		Denotes Data that do not meet common Quality Assurance Criteria									
ND <		Denotes Not Detected at Specified Detection Limit									
---		Denotes Indeterminate because blank exceeded sample or poor recovery shown by surrogate									

GC/HRMS analysis of non-polar organic pollutants in Sets 1, 2, and 4

GC/HRMS analyses will now focus on non-polar persistent organic pollutants beginning with Set 1 samples followed by those in Sets 2 and 4. The sensitive and selective GC/HRMS was an ideal detector after good sample cleanup for concentrations as low as 0.003 ng/g, provided that targeted analytes in blank samples were consistently very low or not detected. The first targeted analyte, p,p'-DDE, the persistent metabolite of p,p'-DDT, was detected at a net value of 0.323 ng/g after subtracting the average of the blanks plus three times the standard deviation between the blanks (Table 8). Despite extreme caution during sample preparation, detecting p,p'-DDE in the blanks at < 0.1 ng/g was not surprising at an environmental lab where samples can often reach 10,000 ng/g p,p'-DDE. In Set 1, p,p'-DDE and the ¹³C-DDE surrogate were partially recovered in both fractions from basic alumina flash chromatography, but the isotope dilution quantitative technique based on the surrogate still resulted in an accurate quantitation. Subsequently in later methods, the eluant was adjusted to 1% DCM

Table 8. Concentrations (ng/g) of p,p'-DDE and Several PBDEs in Set 1 Samples

ANALYTE NAME	METHOD BLANKS					TOENAIL SAMPLE			SOAP WASH
	1B-1	2B-1	AVG (3 * SD)	3 * SD	Subtract from Toenail Sample - [Avg + 3 * SD]	3T-1 A Nov-05	3T-1 Lipid=1.21% NET	3T-1 NET	3W-1 NET
	ng/g	ng/g				ng/g	ng/g	ng/g-lipid	ng/g
p,p'-DDE	0.020	0.009	0.014	0.023	-0.038	0.361	0.323	27	0.008
tri-PBDE 17	0.113	0.051	0.082	0.132	-0.214	0.422	0.208	17	---
tri-PBDE 28	0.124	0.096	0.110	0.060	-0.171	0.505	0.335	28	---
tetra-PBDE 47	1.59	1.14	1.36	0.95	-2.31	10.5	8.19	677	---
penta-PBDE 100	0.27	0.10	0.18	0.37	-0.55	1.57	1.02	84	---
penta-PBDE 99	0.63	0.58	0.60	0.10	-0.70	7.02	6.32	522	0.18
hexa-PBDE 153	0.05	0.14	0.10	0.18	-0.28	1.59	1.31	108	---

in cyclopentane (instead of 0.5%) to match the slightly stronger alumina adsorbency caused by storing the alumina in a tightly closed glass flask in an oven rather than in one vulnerable to moisture in the air. Several compounds including hexachlorobenzene and pentachloroanisole were not listed in Table 8 for the Set 1 toenail sample because the compounds were present in blank samples at or higher than in the toenail sample.

Six PBDEs, ranging from tri- to hexabromo-diphenyl ethers, were detected in all Set 1 samples in the expected second fraction from alumina, but at concentrations higher in the first toenail sample (3T-1) for valid blank-subtracted concentrations (Table 8). Blank-subtracted concentrations in the soap wash sample were almost all less than the blank samples. Relative proportions of PBDE congener concentrations typified environmental samples except that the higher PBDE 153 was more prominent in human samples; this result is discussed in the next chapter about relevance to biomonitoring.

For Set 2 samples, GC/HRMS analyses targeted chlordanes, following their detection and identification by GC/full scan MS in the Set 1 sample along with an expanded list of persistent, non-polar organochlorine pesticides (Table 9) to complement the moderately persistent, PBDE flame retardant compounds (Table 10). By targeting only non-polar compounds with fewer steps than Set 1, blanks had lower background for some compounds like hexachlorobenzene, pentachloroanisole, methyl ether metabolite of triclosan, and beta-hexachlorocyclohexane. Thus, toenail samples had valid blank-subtracted concentrations of more analytes (shaded in blue in Table 9). An exception was that p,p'-DDE was slightly higher in Set 2 blanks, which caused concern for accurately measuring p,p'-DDE in toenail samples after blank subtraction.

Table 9. Concentrations (ng/g) of Organochlorine Pesticides in Set 2 Samples

ANALYTE NAME	METHOD BLANKS					TOENAIL SAMPLES				SOAP WASHES	
	4B-2	5B-2	AVG (3 * SD)		Subtract from Toenail Samples - [Avg + 3 * SD]]	6T-2 A	6T-2	7T-2 A	7T-2	6W-2	7W-2
	ng/g	ng/g				Jan. 2006	NET	Mar. 2006	NET	Jan. 2006	NET
Hexachlorobenzene	0.013	0.017	0.015	0.003	-0.023	0.330	0.31	0.21	0.19	0.024	---
Pentachloroanisole	0.010	0.010	0.010	est. 0.005	est. -0.015	1.34	1.33	0.89	0.88	---	---
Methyl (ether) Triclosan	ND < 0.10	ND < 0.10	< 0.10	est. 0.05	est. -0.15	0.42	0.31	0.37	0.26	---	---
β-Hx-Chloro-Cyclohexane	ND < 0.05	ND < 0.05	< 0.05	est. 0.005	est. -0.055	0.08	0.03	0.11	0.06	---	---
Dieldrin	ND < 0.15	ND < 0.15	< 0.15	est. 0.005	est. -0.155	ND < 0.15	---	ND < 0.15	---	---	---
Chlordanes:											
Heptachlor	ND < 0.04	ND < 0.04	< 0.04	est. 0.005	est. -0.045	0.38	0.34	1.28	1.24	---	---
Heptachlor Epoxide	ND < 0.02	ND < 0.02	< 0.02	est. 0.005	est. -0.025	0.21	0.19	0.16	0.13	---	---
Oxychlordane	ND < 0.04	ND < 0.04	< 0.04	est. 0.005	est. -0.045	0.04	---	ND < 0.04	---	---	---
trans-Chlordane	0.05	< 0.04	0.05	0.008	-0.069	8.52	8.45	5.92	5.85	0.46	0.19
cis-Chlordane	0.08	< 0.04	0.06	0.029	-0.147	5.33	5.18	3.70	3.55	0.34	0.17
trans-Nonachlor	0.06	0.06	0.06	0.004	-0.071	4.60	4.53	3.31	3.23	0.09	---
cis-Nonachlor	ND < 0.04	< 0.04	< 0.04	est. 0.005	est. -0.045	0.25	0.20	0.36	0.31	---	---
DDT Series:											
p,p'-DDE	0.05	0.33	0.19	0.20	-0.78	1.40	0.62	1.27	0.49	---	---
p,p'-DDD	ND < 0.8	ND < 0.8	< 0.8	ND	est. -0.85	ND < 0.8	---	ND < 0.8	---	---	---
p,p'-DDT	ND < 0.8	ND < 0.8	< 0.8	ND	est. -0.85	ND < 0.8	---	ND < 0.8	---	---	---

ND < Not Detected at Specified Detection Limit
est. Estimated for Quality Assurance

Table 10. Background-Subtracted Concentrations (ng/g) of PBDEs in Set 2 Blank, Toenails, and Soap Washes

ANALYTE	METHOD BLANKS					TOENAIL SAMPLES				SOAP WASHES	
	4B-2	5B-2	AVG 3 *SD		Subtract from Toenails and Soap Washes - [Avg+3*SD]]	6T-2 A	6T-2	7T-2 A	7T-2	6W-2	7W-2
	ng/g	ng/g				Jan-06	NET	Mar-06	NET	Jan-06	Mar-06
tri-BDE 17	ND < 0.05	ND < 0.05	< 0.05	ND	est. 0.055	0.15	0.10	0.21	0.16	---	---
tri-BDE 28	< 0.05	ND < 0.05	< 0.05	ND	est. 0.055	0.36	0.31	0.49	0.43	---	---
tetra-BDE 49	ND < 0.06	ND < 0.06	< 0.06	ND	est. 0.065	0.57	0.51	0.82	0.75	---	---
tetra-BDE 47	2.05	3.11	2.58	2.25	4.83	12.8	8.0	13.5	8.7	---	---
tetra-BDE 66	ND < 0.06	ND < 0.06	< 0.06	ND	est. 0.065	0.28	0.21	ND < 0.06	---	---	---
penta-BDE 100	0.10	0.27	0.19	0.36	0.55	1.50	0.95	1.49	0.94	---	---
penta-BDE 99	0.27	0.76	0.51	1.05	1.56	9.19	7.63	9.68	8.12	---	---
penta-BDE 85	ND < 0.10	ND < 0.10	< 0.10	ND	est. 0.15	0.32	0.17	0.41	0.26	---	---
hexa-BDE 154	0.10	ND < 0.10	0.10	---	est. 0.15	0.19	0.04	0.18	0.03	---	---
hexa-BDE 153	ND < 0.10	ND < 0.10	< 0.10	ND	est. 0.15	2.32	2.17	2.04	1.89	---	---
nona-BDE 208	0.86	0.91	0.88	0.11	0.99	ND < 0.20	---	0.76	---	---	---
nona-BDE 207	< 0.20	0.21	0.21	0.03	0.24	ND < 0.20	---	0.40	0.16	---	---
nona-BDE 206	ND < 0.20	< 0.20	< 0.20	---	est. 0.25	ND < 0.20	---	0.30	0.05	---	---

ND < Not Detected at Specified Detection Limit
est. Estimated for Quality Assurance
PBDE Concentrations are self-corrected for recovery by isotope dilution quantitation technique using ¹³C-surrogates

Despite fewer steps in Set 2 than Set 1, PBDEs in blanks and toenail samples were very similar, except now 13 PBDE congeners were detectable (Table 10). Most PBDEs were consistently measured in both toenail samples except for two nona-BDEs in sample 7T-2, which may indicate recent exposure to the common flame retardant, deca-BDE 209 followed by metabolic debromination. Deca-BDE 209 was not detected above the blanks average plus three standard deviations (0.3 ng/g). Resulting blank-subtracted PBDE concentrations in the two toenail samples in Table 10 were shaded blue for easier inspection.

Results of Set 4 sample analyses are presented in the next 12 tables (Tables 11-22) grouped by analyte and sample types. Because toenail sample masses in Set 4 ranged from 65.5 mg to about 300 mg, five different columns (shaded yellow) in Table 11 were used to compute blank-subtracted concentrations of organochlorine pesticides to match the varying masses of toenail samples in Table 12. At low ppb concentrations, achieving consistently low blanks was most important for the smaller toenail sample sizes. Set 4 was the first time that the enzyme Proteinase K was used in toenail sample digestion. Therefore to evaluate whether the enzyme solution contained any targeted compounds, one of Set 4 blanks, 12B-4, did not contain Proteinase K while two blanks, 13B-4 and 14B-4, contained Proteinase K. At least for organochlorine pesticides, Proteinase K solutions did not contain any higher background of these targeted pesticides, so all three blanks were included and averaged in Table 11. Generally, compound background levels in Set 4 blanks (Table 11) were comparable to Set 2 blanks (Table 9), however hexachlorobenzene was both higher and more variable in Set 4

Table 11. Concentrations (ng/g) of Organochlorine Pesticides in Set 4 Blank Samples

ANALYTE NAME	METHOD BLANKS			For 18T-4			SUB.			For 19T-4			SUB.			For 17T-4			SUB.			For 20T-4, 22T-4			SUB.			For 15T-4, 16T-4,			SUB.		
	12B-4	13B-4	14B-4	Blank/0.0655 g			from			Blank/0.12 g			from			Blank/0.159 g			from			Blank/0.190 g			from 20T			and 21T-4			from 15T		
	No			Concentration			18T-4			Concentration			19T-4			Concentration			17T-4			Concentration			or 22T-4			Concentration			16T, 21T		
	Proteinase K			ng/g	ng/g	ng/g	AVG.	3 SD	+ 3 SD	ng/g	ng/g	ng/g	AVG.	3 SD	+ 3 SD	ng/g	ng/g	ng/g	AVG.	3 SD	+ 3 SD	ng/g	ng/g	ng/g	AVG.	3 SD	+ 3 SD	ng/g	ng/g	ng/g	AVG.	3 SD	+ 3 SD
	Total ng	Total ng	Total ng																														
Hexachlorobenzene	0.047	0.114	0.034	0.99	1.97	2.96	0.54	1.08	1.62	0.41	0.81	1.22	0.34	0.68	1.02	0.22	0.43	0.65				0.34	0.68	1.02	0.22	0.43	0.65						
Pentachloroanisole	0.053	0.050	0.048	0.77	0.11	0.88	0.42	0.06	0.48	0.31	0.05	0.36	0.26	0.04	0.30	0.17	0.03	0.19				0.26	0.04	0.30	0.17	0.03	0.19						
Me- (ether) Triclosan	ND < 0.05	ND < 0.05	ND < 0.05	0.76	0.05	0.81	0.42	0.03	0.44	0.31	0.02	0.33	0.26	0.02	0.28	0.17	0.01	0.18				0.26	0.02	0.28	0.17	0.01	0.18						
gamma-HCH	ND < 0.02	ND < 0.02	ND < 0.02	0.31	0.23	0.53	0.17	0.13	0.29	0.13	0.09	0.22	0.11	0.08	0.18	0.07	0.05	0.12				0.11	0.08	0.18	0.07	0.05	0.12						
beta-HCH	ND < 0.02	ND < 0.02	ND < 0.02	0.31	0.23	0.53	0.17	0.13	0.29	0.13	0.09	0.22	0.11	0.08	0.18	0.07	0.05	0.12				0.11	0.08	0.18	0.07	0.05	0.12						
Dieldrin	ND < 0.07	ND < 0.07	ND < 0.07	1.03	0.19	1.22	0.56	0.10	0.67	0.42	0.08	0.50	0.36	0.06	0.42	0.23	0.04	0.27				0.36	0.06	0.42	0.23	0.04	0.27						
Chlordanes:																																	
Oxychlordane	ND < 0.01	ND < 0.01	ND < 0.01	0.15	0.09	0.24	0.08	0.05	0.13	0.06	0.04	0.10	0.05	0.03	0.08	0.03	0.02	0.05				0.05	0.03	0.08	0.03	0.02	0.05						
Heptachlor Epoxide	ND < 0.01	ND < 0.01	ND < 0.02	0.20	0.26	0.47	0.11	0.14	0.26	0.08	0.11	0.19	0.07	0.09	0.16	0.04	0.06	0.10				0.07	0.09	0.16	0.04	0.06	0.10						
trans-Chlordane	0.067	0.068	0.070	1.04	0.07	1.11	0.57	0.04	0.61	0.43	0.03	0.46	0.36	0.02	0.38	0.23	0.01	0.24				0.36	0.02	0.38	0.23	0.01	0.24						
cis-Chlordane	0.059	0.059	0.062	0.92	0.07	0.99	0.50	0.04	0.54	0.38	0.03	0.40	0.32	0.02	0.34	0.20	0.02	0.22				0.32	0.02	0.34	0.20	0.02	0.22						
trans-Nonachlor	0.068	0.068	0.071	1.05	0.09	1.14	0.58	0.05	0.62	0.43	0.04	0.47	0.36	0.03	0.39	0.23	0.02	0.25				0.36	0.03	0.39	0.23	0.02	0.25						
cis-Nonachlor	ND < 0.02	ND < 0.02	ND < 0.02	0.31	0.05	0.35	0.17	0.03	0.19	0.13	0.02	0.14	0.11	0.02	0.12	0.07	0.01	0.08				0.11	0.02	0.12	0.07	0.01	0.08						
DDT Series:																																	
o,p'-DDE	ND < 0.01	ND < 0.01	ND < 0.01	0.15	0.23	0.38	0.08	0.13	0.21	0.06	0.09	0.16	0.05	0.08	0.13	0.03	0.05	0.08				0.05	0.08	0.13	0.03	0.05	0.08						
p,p'-DDE	0.029	0.052	0.027	0.55	0.64	1.19	0.30	0.35	0.65	0.23	0.26	0.49	0.19	0.22	0.41	0.12	0.14	0.26				0.19	0.22	0.41	0.12	0.14	0.26						
o,p'-DDD	ND < 0.05	ND < 0.05	ND < 0.05	0.76	0.14	0.90	0.42	0.08	0.49	0.31	0.06	0.37	0.26	0.05	0.31	0.17	0.03	0.20				0.26	0.05	0.31	0.17	0.03	0.20						
p,p'-DDD	ND < 0.05	ND < 0.05	ND < 0.05	0.76	0.14	0.90	0.42	0.08	0.49	0.31	0.06	0.37	0.26	0.05	0.31	0.17	0.03	0.20				0.26	0.05	0.31	0.17	0.03	0.20						
o,p'-DDT	ND < 0.05	ND < 0.05	ND < 0.05	0.76	0.23	0.99	0.42	0.13	0.54	0.31	0.09	0.41	0.26	0.08	0.34	0.17	0.05	0.22				0.26	0.08	0.34	0.17	0.05	0.22						
p,p'-DDT	ND < 0.05	ND < 0.05	ND < 0.05	0.76	0.14	0.90	0.42	0.08	0.49	0.31	0.06	0.37	0.26	0.05	0.31	0.17	0.03	0.20				0.26	0.05	0.31	0.17	0.03	0.20						
ND < Not Detected at Specified Detection Limit																																	
NOTE: In a Soap Wash sample BLANK (not shown), no analytes were detected above the (average blank + 3 SD) concentrations.																																	

ND < Not Detected at Specified Detection Limit

NOTE: In a Soap Wash sample BLANK (not shown), no analytes were detected above the (average blank + 3 SD) concentrations.

Table 12. Blank-Subtracted Concentrations (ng/g-nail) of POPs Pesticides in Set 4 Toenail Samples

ANALYTE NAME	TOENAIL SAMPLES							
	15T-4	16T-4	17T-4	18T-4	19T-4	20T-4	21T-4	22T-4
	Oct 2006	Feb 2007	May 2007	Jan 2010	Apr 2010	Jan 2009	Apr 2009	Nov 2007
Date Sampled:	Oct 2006	Feb 2007	May 2007	Jan 2010	Apr 2010	Jan 2009	Apr 2009	Nov 2007
Sample Submitter	A	A	A	B	M-cat	C	C	D
Mass (g):	0.340	0.295	0.159	0.0655	0.120	0.196	0.322	0.189
	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
Hexachlorobenzene	---	---	---	---	---	---	---	---
Pentachloroanisole	0.16	0.38	0.34	0.47	0.95	0.16	0.24	---
Methyl (ether) Triclosan	1.78	0.20	0.07	---	---	---	---	---
gamma-HCH (Lindane)	0.05	---	0.13	---	0.14	---	---	0.16
beta-HCH	0.11	0.16	0.24	---	---	0.18	0.12	0.20
Dieldrin	---	---	---	0.69	---	---	0.24	---
Chlordanes:								
Oxychlordane	---	0.09	---	0.38	0.26	0.13	0.09	0.13
Heptachlor Epoxide	0.07	0.09	0.08	---	0.31	0.07	0.09	---
trans-Chlordane	3.68	4.45	6.53	7.11	13.6	4.25	4.41	0.59
cis-Chlordane	2.40	2.81	4.18	4.56	8.22	1.86	1.96	0.33
trans-Nonachlor	1.92	2.27	3.47	4.11	9.48	1.32	1.57	0.32
cis-Nonachlor	0.16	0.19	0.40	0.69	0.80	0.30	0.24	0.22
DDT Series:								
o,p'-DDE	---	---	---	---	---	0.13	0.11	---
p,p'-DDE	0.79	1.14	1.07	0.57	0.67	2.81	2.89	1.31
o,p'-DDD	---	---	---	---	---	---	---	---
p,p'-DDD	---	---	---	---	---	---	---	---
o,p'-DDT	---	---	---	---	---	0.64	0.39	---
p,p'-DDT	---	---	---	---	---	0.89	1.04	1.33

--- Not Detected or not detected above method blank

blanks, resulting in no blank-subtracted concentrations in any Set 4 toenail sample (Table 12). In contrast, p,p'-DDE was consistently lower in Set 4 blanks, which enabled blank-subtracted concentrations in all toenail samples, even the one (18T-4) that was only 65.5 mg. As in all methods, p,p'-DDE was quantitated in all samples based on the ¹³C-p,p'-DDE surrogate recovery, which will be included in the upcoming table with ¹³C-PBDE surrogates. With several of Set 4 toenail samples from other individuals, both isomers of DDT and the second isomer (o,p'-) of DDE were now detected and reported (Table 12). In all Set 4 toenail samples (Table 12), trans-Chlordane had the highest concentration, followed closely by other chlordane components of cis-Chlordane, and trans-Nonachlor.

After blank-subtraction, concentrations in Set 4 soap washes of the toenail samples for organochlorine pesticides primarily contained only the major chlordane components (Table 13). Such compounds are likely from air and dust from homes treated with chlordane for termites before its ban in 1988. It is plausible that some amount would adsorb to the outside nail surface and then be released in an ultrasonic soap bath.

Earlier in this chapter, a synthetic musk fragrance compound, Galaxolide, (or isomer, Versalide) was identified in a Set 3 toenail sample (11T-3) by GC/full scan MS. Being neutral with little polarity, galaxolide and two other related synthetic musk compounds were compatible with and were thus added to Set 4 analyses of the second fraction from basic alumina. Table 14 lists blank-subtracted concentrations of traseolide, galaxolide, and tonalide in Set 4 toenail samples, soap washes, and blank

Table 13. Blank-Subtracted Concentrations (ng/g-wet wgt) of Organochlorine Pesticides in Soap Washes

Sample Submitter Mass (g): ANALYTE NAME	SOAP WASHES of TOENAILS							
	15W-4	16T-4	17T-4	18T-4	19T-4	20T-4	21T-4	22T-4
	A	A	A	B	M-cat	C	C	D
	0.34	0.295	0.159	0.0655	0.120	0.196	0.322	0.189
	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
Hexachlorobenzene	---	---	---	---	---	---	---	---
Pentachloroanisole	---	---	---	---	0.45	---	---	---
Methyl (ether) Triclosan	0.06	---	---	---	---	---	---	---
gamma-HCH (Lindane)	---	---	---	---	---	---	---	---
beta-HCH	---	---	---	---	---	---	---	---
Dieldrin	---	---	---	---	---	---	---	---
<u>Chlordanes:</u>								
Oxychlordane	---	---	---	---	---	---	---	---
Heptachlor Epoxide	---	---	---	---	---	---	---	---
trans-Chlordane	0.19	0.16	0.75	1.04	2.50	0.23	0.06	---
cis-Chlordane	0.13	0.10	0.44	0.68	1.47	0.08	0.04	---
trans-Nonachlor	0.11	0.06	0.43	0.65	1.48	0.04	0.03	---
cis-Nonachlor	0.11	---	0.23	0.53	0.40	0.17	0.12	---
<u>DDT Series:</u>								
o,p'-DDE	---	---	---	---	---	---	---	---
p,p'-DDE	---	---	---	---	---	---	---	---
o,p'-DDD	---	---	---	---	---	---	---	---
p,p'-DDD	---	---	---	---	---	---	---	---
o,p'-DDT	---	---	---	---	---	---	---	---
p,p'-DDT	---	---	---	---	---	---	---	---
--- Not Detected or not detected above method blank								

average plus three standard deviations amount subtracted for each toenail and soap wash sample. Galaxolide was highest of the fragrance compounds in all samples and exceeded 1000 ng/g (one ppm) even in many of the blank-subtracted toenail samples, except for samples 19T-4 (cat) and 22T-4.

Results of Set 4 sample analyses of basic alumina-Fraction 2 continue with PBDEs and a newer brominated flame retardant, 1,2-bis(2,4,6-tribromophenoxy)ethane, abbreviated as Bis-TriBr-PE in Tables 15-18. Set 4 blanks in Table 15 show significantly higher amounts of PBDEs 49, 47, 100, and 99 in the blank samples 13B-4 and 14B-4 that

Table 14. Blank-Subtracted Concentrations (ng/g) of Synthetic Musk Fragrances in Set 4 Toenail Samples

	TOENAIL SAMPLES							
	15T-4	16T-4	17T-4	18T-4	19T-4	20T-4	21T-4	22T-4
Date Sampled:	Oct 2006	Feb 2007	May 2007	Jan 2010	Apr 2010	Jan 2009	Apr 2009	Nov 2007
Sample Submitter	A	A	A	B	M-cat	C	C	D
Mass (g):	0.340	0.295	0.159	0.0655	0.120	0.196	0.322	0.189
ANALYTE NAME	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
Traseolide	0.25	2.24	0.72	---	---	0.40	0.45	---
Galaxolide	700	1,030	2,300	3,440	100	1,860	1,880	41.6
Tonalide	25.0	48.3	30.4	136	11.8	57.1	126	4.43

ANALYTE NAME	SOAP WASHES							
	15W-4	16W-4	17W-4	18W-4	19W-4	20W-4	21W-4	22W-4
	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
Traseolide	---	---	---	---	---	---	---	---
Galaxolide	44.3	46.0	383	572	---	565	141	---
Tonalide	0.61	0.45	10.5	27.3	---	7.9	1.08	---

ANALYTE NAME	METHOD 4 BLANKS [Avg + 3*Std Dev] Subtracted from SAMPLES ABOVE							
	15T-4	16T-4	17T-4	18T-4	19T-4	20T-4	21T-4	22T-4
	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
Traseolide	0.20	0.20	0.38	0.92	0.50	0.32	0.20	0.32
Galaxolide	17.4	17.4	32.7	79.9	43.6	27.5	17.4	27.5
Tonalide	1.02	1.02	1.91	4.66	2.54	1.61	1.02	1.61

had Proteinase K enzyme than in the blank sample 12B-4 without Proteinase K. Those PBDEs are major constituents in the “Penta” formulation used commercially. Deca-BDE 209, all three nona-BDEs and several octa-BDEs were only slightly higher in the two blank samples with Proteinase K. Therefore, for blank-subtraction of PBDEs in Set 4 samples, the enzyme significantly raised the method background of common PBDEs, so method blank 12B-4 was excluded in the averaging process. Blank-subtracted concentrations of PBDEs in Set 4 toenail samples therefore lack most values in Table 16 for PBDEs 17, 49, 47, and 100. The larger blank subtraction for PBDE 99 likely affected its accurate measurement in the nails. Of the remaining PBDEs with values in Table 16

Table 15. Concentrations (ng/g) of PBDEs in Set 4 Blanks

ANALYTE NAME	No Prot. K 12B-4 NOT AVERAGED	METHOD BLANKS 13B-4 14B-4 WITH PROTEINASE K		For 18T-4 Blank/0.0655 g Concentration		SUB. from 18T-4 [Avg + 3 SD]	For 19T-4 Blank/0.12 g Concentration		SUB. from 19T-4 [Avg + 3 SD]	For 17T-4 Blank/0.159 g Concentration		SUB. from 17T-4 [Avg + 3 SD]	For 20T-4, 22T-4 Blank/0.190 g Concentration		SUB. from 20T or 22T-4 [Avg + 3 SD]	For 15T-4, 16T-4 and 21T-4 Blank/0.30 g		SUB. from 15T 16T, 21T [Avg + 3 SD]
	Total ng	Total ng	Total ng	ng/g	ng/g	AVG.	ng/g	ng/g	AVG.	ng/g	ng/g	AVG.	ng/g	ng/g	AVG.	ng/g	ng/g	AVG.
tri-BDE 17	ND < 0.005	0.05	ND < 0.005	0.42	1.46	1.88	0.23	0.80	1.02	0.17	0.60	0.77	0.14	0.50	0.65	0.09	0.32	0.41
tri-BDE 28	ND < 0.005	ND < 0.005	0.01	0.08	0.05	0.12	0.04	0.03	0.07	0.03	0.02	0.05	0.03	0.02	0.04	0.02	0.01	0.03
tetra-BDE 49	ND < 0.005	0.06	0.02	0.57	1.20	1.76	0.31	0.65	0.96	0.23	0.49	0.73	0.20	0.41	0.61	0.12	0.26	0.38
tetra-BDE 47	0.06	1.80	0.71	19.2	35.3	54.5	10.5	19.3	29.7	7.89	14.5	22.4	6.61	12.2	18.8	4.18	7.71	11.9
tetra-BDE 66	ND < 0.005	0.05	ND < 0.005	0.42	1.45	1.87	0.23	0.79	1.02	0.17	0.60	0.77	0.14	0.50	0.64	0.09	0.32	0.41
penta-BDE 100	0.01	0.46	0.22	5.17	7.93	13.1	2.82	4.33	7.15	2.13	3.27	5.40	1.78	2.74	4.52	1.13	1.73	2.86
penta-BDE 99	0.03	0.85	0.64	11.3	6.80	18.1	6.19	3.71	9.90	4.67	2.80	7.47	3.91	2.34	6.26	2.48	1.48	3.96
penta-BDE 85	ND < 0.01	0.01	0.01	0.22	0.01	0.23	0.12	0.004	0.12	0.09	0.003	0.09	0.08	0.002	0.08	0.05	0.00	0.05
hexa-BDE 154	0.01	0.02	0.01	0.22	0.18	0.40	0.12	0.10	0.22	0.09	0.07	0.17	0.08	0.06	0.14	0.05	0.04	0.09
hexa-BDE 153	ND < 0.01	0.03	ND < 0.01	0.28	0.53	0.81	0.15	0.29	0.44	0.11	0.22	0.33	0.10	0.18	0.28	0.06	0.12	0.18
hexa-BDE 139	ND < 0.01	ND < 0.01	ND < 0.01	0.15	0.05	0.20	0.08	0.03	0.11	0.06	0.02	0.08	0.05	0.02	0.07	0.03	0.01	0.04
hexa-BDE 140	ND < 0.01	0.03	ND < 0.01	0.27	0.51	0.79	0.15	0.28	0.43	0.11	0.21	0.33	0.09	0.18	0.27	0.06	0.11	0.17
hexa-BDE 138	ND < 0.01	ND < 0.01	ND < 0.01	0.15	0.05	0.20	0.08	0.03	0.11	0.06	0.02	0.08	0.05	0.02	0.07	0.03	0.01	0.04
hepta-BDE 183	ND < 0.01	0.02	0.02	0.27	0.14	0.41	0.15	0.07	0.22	0.11	0.06	0.17	0.09	0.05	0.14	0.06	0.03	0.09
octa-BDE 201	0.02	0.04	0.03	0.53	0.46	0.99	0.29	0.25	0.54	0.22	0.19	0.41	0.18	0.16	0.34	0.12	0.10	0.22
octa-BDE 204	ND < 0.015	ND < 0.015	0.05	0.47	1.04	1.51	0.26	0.57	0.83	0.20	0.43	0.62	0.16	0.36	0.52	0.10	0.23	0.33
octa-BDE 197	ND < 0.015	0.02	0.02	0.24	0.06	0.30	0.13	0.03	0.16	0.10	0.02	0.12	0.08	0.02	0.10	0.05	0.01	0.07
octa-BDE 203	ND < 0.015	0.04	0.01	0.42	0.84	1.26	0.23	0.46	0.69	0.17	0.35	0.52	0.14	0.29	0.43	0.09	0.18	0.27
octa-BDE 196	ND < 0.015	0.08	0.07	1.17	0.31	1.48	0.64	0.17	0.81	0.48	0.13	0.61	0.40	0.11	0.51	0.26	0.07	0.32
octa-BDE 205	ND < 0.015	0.06	0.04	0.79	0.73	1.53	0.43	0.40	0.83	0.33	0.30	0.63	0.27	0.25	0.53	0.17	0.16	0.33
nona-BDE 208	0.06	0.17	0.09	1.98	2.87	4.85	1.08	1.56	2.65	0.82	1.18	2.00	0.68	0.99	1.67	0.43	0.63	1.06
nona-BDE 207	0.02	0.10	0.06	1.23	1.51	2.74	0.67	0.83	1.50	0.51	0.62	1.13	0.42	0.52	0.95	0.27	0.33	0.60
nona-BDE 206	0.02	0.15	0.08	1.71	2.12	3.83	0.94	1.16	2.09	0.71	0.87	1.58	0.59	0.73	1.32	0.37	0.46	0.84
deca-BDE 209	0.08	0.23	0.13	2.70	3.24	5.94	1.48	1.77	3.24	1.11	1.33	2.45	0.93	1.12	2.05	0.59	0.71	1.30
Bis-TriBr-PE	ND < 0.02	ND < 0.02	ND < 0.02	0.31	0.05	0.35	0.17	0.03	0.19	0.13	0.02	0.15	0.11	0.02	0.12	0.07	0.01	0.08
ND < Not Detected at Specified Detection Limit NOTE: Only Method Blanks 13B-4 and 14B-4 were averaged and used for blank-subtracting concentrations in Method 4 samples Blank for the Soap Wash was not shown because after blank subtraction, all concentrations for soap wash blank were < [avg + 3 SD]																		

for Set 4, the hexa-BDE 153 is likely the best indicator for relative amount of PBDEs in the various toenail samples. In toenail samples from individual A in Set 4, blank-subtracted PBDE 153 concentrations were 1.16, 2.50, and 1.87 ng/g (mean 1.84, sd 0.67, n=3), which closely compare to 1.31 ng/g for Set 1 and 2.17 and 1.89 ng/g for Set 2 (mean 1.79, sd 0.44, n=3). In contrast, toenail samples from individual C in Set 4 contained sevenfold less PBDE 153 (mean 0.25, sd 0.12, n=2). In soap washes of Set 4 toenail samples, there were very few blank-subtracted PBDE concentrations in Table 17, similar to none in Table 10 for Set 2 washes. Therefore, there was no significant exogenous PBDE contamination detected in the soap washes.

Table 16. Blank-subtracted Concentrations (ng/g) of PBDEs in Set 4 Toenail Samples

	TOENAIL SAMPLES							
	15T-4	16T-4	17T-4	18T-4	19T-4	20T-4	21T-4	22T-4
Date Sampled:	Oct 2006	Feb 2007	May 2007	Jan 2010	Apr 2010	Jan 2009	Apr 2009	Nov 2007
Sample Submitter	A	A	A	B	M-cat	C	C	D
Mass (g):	0.340	0.295	0.159	0.0655	0.120	0.196	0.322	0.189
ANALYTE NAME	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
tri-BDE 17	---	---	---	---	---	---	---	---
tri-BDE 28	0.13	0.22	0.33	0.15	0.19	0.36	0.34	0.34
tetra-BDE 49	---	---	---	---	---	---	---	---
tetra-BDE 47	---	9.92	---	---	---	---	5.0	---
tetra-BDE 66	---	---	---	---	---	---	---	---
penta-BDE 100	---	---	---	---	---	---	---	---
penta-BDE 99	2.77	7.31	2.63	14.0	21.4	0.43	3.74	---
penta-BDE 85	0.21	0.29	0.32	0.71	0.79	0.18	0.28	0.16
hexa-BDE 154	0.21	0.39	0.18	1.10	1.95	0.15	0.20	0.17
hexa-BDE 153	1.16	2.50	1.87	2.15	3.48	0.16	0.33	0.45
hexa-BDE 139	0.06	0.09	0.07	0.17	0.46	0.03	0.05	0.10
hexa-BDE 138	---	---	---	0.35	0.45	0.14	0.07	---
hepta-BDE 183	0.04	0.07	---	---	0.19	---	0.03	0.10
octa-BDE 197	---	---	---	---	0.05	---	---	0.11
nona-BDE 208	---	---	---	---	---	---	---	---
nona-BDE 207	---	---	---	---	---	---	---	---
nona-BDE 206	---	---	---	---	---	---	---	---
deca-BDE 209	0.53	2.28	3.51	---	3.17	3.28	2.98	4.82
Bis-TriBr-PE	0.91	1.16	0.11	---	---	---	---	---
--- Not Detected at Specified Detection Limit or < [Blank Avg + 3SD]								

Surrogate ^{13}C -PBDE and ^{13}C -DDE recoveries in Set 4 blanks and toenail samples were tallied in Table 18. For ^{13}C -PBDEs, 167 out of 176 measurements were recovered above 40%, while 10 out of 11 ^{13}C -DDE recoveries exceeded 40%. In the Blank 13B-4, a few volatile surrogates had low recoveries, likely when a small solvent extract nearly went dry. A few computations exceeded 100%, when a few corresponding internal

Table 17. Blank-Subtracted Concentrations (ng/g) of PBDEs in Set 4 Soap Washes of Toenails

	15W-4	16W-4	17W-4	18W-4	19W-4	20W-4	21W-4	22W-4
Date Sampled:	Oct 2006	Feb 2007	May 2007	Jan 2010	Apr 2010	Jan 2009	Apr 2009	Nov 2007
Sample Donated by:	A	A	A	B	M-cat	C	C	D
Mass Rinsed (g):	0.340	0.295	0.159	0.0655	0.120	0.196	0.322	0.189
ANALYTE NAME	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
tri-BDE 17	---	---	---	---	---	---	---	---
tri-BDE 28	---	---	---	---	---	0.07	---	0.07
tetra-BDE 49	---	---	---	---	---	---	---	---
tetra-BDE 47	---	---	---	---	---	---	2.50	---
tetra-BDE 66	---	---	---	---	---	---	---	---
penta-BDE 100	---	---	---	---	---	---	0.19	---
penta-BDE 99	---	---	---	---	---	---	2.82	---
penta-BDE 85	---	---	---	---	---	---	---	---
hexa-BDE 154	---	---	---	0.10	0.22	---	---	---
hexa-BDE 153	0.02	---	---	0.16	0.17	---	---	---
hexa-BDE 139	---	---	---	---	---	---	---	---
hexa-BDE 138	---	---	---	---	---	---	---	---
hepta-BDE 183	---	---	---	---	---	---	---	---
octa-BDE 197	---	---	---	---	---	---	---	---
nona-BDE 208	---	---	---	---	---	---	---	---
nona-BDE 207	---	---	---	---	---	---	---	---
nona-BDE 206	---	---	---	---	---	---	---	---
deca-BDE 209	---	---	---	---	---	---	---	---
Bis-TriBr-PE	---	---	---	---	---	---	---	---

--- Not Detected above Method Blank

standards added in the final vial may have been partially lost from evaporation and were therefore detected with slightly less response than expected from calibration standards.

Because of renewed interest in biomonitoring PCB congeners and, with the newly acquired (Wellington Labs, Ontario, Canada) comprehensive set of low level PCB calibration and ¹³C-PCB surrogate standards, PCBs were targeted in Set 4 samples. PCBs

Table 18. Percent Recoveries of ¹³C-PBDEs and DDE in Set 4 Toenail Samples and Blanks

Sample Submitter SURROGATE	BLANKS			TOENAIL SAMPLES							
	12B-4	13B-4	14B-4	15T-4	16T-4	17T-4	18T-4	19T-4	20T-4	21T-4	22T-4
	%	%	%	A	A	A	B	M-cat	C	C	D
¹³ C-di-BDE 15	49	9	55	83	57	61	53	46	75	77	86
¹³ C-tri-BDE 28	54	17	66	91	64	69	62	50	84	92	94
¹³ C-tetra-BDE 47	70	43	65	98	59	64	63	55	80	87	94
¹³ C-tetra-BDE 77	68	58	64	93	57	65	66	55	79	90	94
¹³ C-penta-BDE 100	68	35	76	108	60	78	72	62	73	97	103
¹³ C-penta-BDE 99	71	58	82	116	66	82	74	62	89	102	108
¹³ C-penta-BDE 126	69	68	80	116	64	84	74	63	93	108	111
¹³ C-hexa-BDE 154	62	42	72	89	50	66	63	51	66	78	98
¹³ C-hexa-BDE 153	69	64	78	101	57	75	72	55	78	91	103
¹³ C-hexa-BDE 169	61	67	68	113	60	70	69	54	85	102	98
¹³ C-hepta-BDE 183	65	71	80	81	60	69	64	58	88	99	114
¹³ C-octa-BDE 197	85	91	92	121	92	61	100	77	101	71	143
¹³ C-octa-BDE 205	83	80	94	117	74	47	87	65	90	60	147
¹³ C-nona-BDE 207	61	68	76	96	63	49	65	52	93	72	109
¹³ C-deca-BDE 209	55	48	47	82	21	11	28	28	38	33	53
¹³ C-bis-triBrPE	68	72	97	120	87	82	85	64	106	108	122
¹³ C-p,p'-DDE	88	11	92	75	84	90	60	73	49	88	91

were accurately measured, both individually as 53 congeners and as homolog totals.

For 20 of the 53 congeners, accuracy was improved using matching ¹³C-PCB surrogates.

An important key to the successful PCB analyses in Set 4 samples was achieving consistently low blanks at or below 1 ng total (Table 19) in a 50-year old building prone to PCB contamination from previous electrical equipment and light fixtures.

Consistently low PCB blanks in the Set 4 sample set were some 20 times lower than nominal blank levels by other analysts using extraction and cleanup procedures that were less isolated to lab air and dust and who used much higher volumes of reagents and chromatographic eluants. All PCBs were measured in basic alumina-Fraction 1

Table 19. Amount of PCBs (ng) in Set 4 Blanks

PCB Congener (Number)	METHOD BLANKS			Amt Blank-Subtracted	
	12B-4	13B-4	14B-4	Avg [Avg + 3SD]	
	No Proteinase K WITH PROTEINASE K			Avg	[Avg + 3SD]
	Total ng	Total ng	Total ng	Total ng	Total ng
Di-PCB 15 *	0.007	0.010	0.008	0.008	0.014
Tri-PCB 18	0.120	0.043	0.046	0.070	0.200
Tri-PCB 31/28 *	0.041	0.024	0.026	0.030	0.058
Tri-PCB 33	0.043	0.023	0.026	0.031	0.064
Tetra-PCB 52 *	0.047	0.037	0.023	0.036	0.071
Tetra-PCB 49	0.017	0.014	0.007	0.013	0.030
Tetra-PCB 44	0.027	0.024	0.012	0.021	0.044
Tetra-PCB 41	0.014	0.016	0.010	0.013	0.023
Tetra-PCB 74	0.011	0.012	0.010	0.011	0.014
Tetra-PCB 70 *	0.032	0.031	0.025	0.029	0.042
Tetra-PCB 66	0.013	0.014	0.013	0.013	0.015
Tetra-PCB 60	0.009	0.008	0.007	0.008	0.010
Tetra-PCB 81 *	ND < 0.003	ND < 0.003	ND < 0.003	0.003	0.005
Tetra-PCB 77 *	0.005	0.005	0.006	0.005	0.007
Penta-PCB 95 *	0.066	0.062	0.051	0.060	0.083
Penta-PCB 84	0.016	0.022	0.015	0.018	0.029
Penta-PCB 101 *	0.063	0.066	0.052	0.060	0.082
Penta-PCB 99	0.023	0.021	0.015	0.020	0.032
Penta-PCB 97	0.021	0.017	0.017	0.019	0.026
Penta-PCB 87	0.037	0.032	0.031	0.033	0.043
Penta-PCB 85	0.012	0.014	0.010	0.012	0.018
Penta-PCB 110	0.056	0.067	0.063	0.062	0.079
Penta-PCB 123 *	0.003	0.003	0.004	0.003	0.005
Penta-PCB 118 *	0.052	0.044	0.042	0.046	0.061
Penta-PCB 105 *	0.042	0.026	0.038	0.035	0.060
Hexa-PCB 135	0.011	0.010	0.011	0.011	0.013
Hexa-PCB 153 */168	0.028	0.029	0.023	0.027	0.036
Hexa-PCB 141	0.016	0.013	0.014	0.014	0.019
Hexa-PCB 137	0.007	0.005	0.007	0.007	0.010
Hexa-PCB 138 */158	0.030	0.031	0.029	0.030	0.032
Hexa-PCB 129	ND < 0.003	ND < 0.003	0.006	0.004	0.008
Hexa-PCB 128	0.007	0.012	0.009	0.009	0.017
Hexa-PCB 167 *	ND < 0.003	0.004	0.004	0.004	0.006
Hexa-PCB 156 *	0.008	0.009	0.009	0.008	0.010
Hexa-PCB 157 *	ND < 0.003	ND < 0.003	ND < 0.003	0.003	0.004
Hepta-PCB 178	0.007	0.007	0.006	0.007	0.008
Hepta-PCB 187	0.003	0.004	0.004	0.004	0.006
Hepta-PCB 183	0.003	0.003	0.003	0.003	0.004
Hepta-PCB 174	0.008	0.009	0.007	0.008	0.011
Hepta-PCB 177	0.003	0.004	0.003	0.003	0.004
Hepta-PCB 171	ND < 0.003	0.003	0.003	0.003	0.006
Hepta-PCB 180 */193	0.004	0.005	0.004	0.004	0.006
Hepta-PCB 170 *	0.003	0.004	0.005	0.004	0.006
Octa-PCB 202 *	0.003	0.003	0.003	0.003	0.006
Octa-PCB 199	0.006	0.007	0.006	0.006	0.008
Octa-PCB 194	0.004	0.004	0.004	0.004	0.007
Nona-PCB 208 *	0.003	0.003	0.003	0.003	0.006
Nona-PCB 206	0.004	ND < 0.003	0.003	0.003	0.005
Deca-PCB 209 *	0.005	0.004	0.006	0.005	0.007

* Denotes Quantitation with same ¹³C-PCB congener

<u>Homolog quantitation:</u>					
SUM of Di-PCBs	0.032	---	---	0.011	0.067
SUM of Tri-PCBs	0.250	0.161	0.117	0.176	0.379
SUM of Tetra-PCBs	0.143	0.131	0.042	0.105	0.271
SUM of Penta-PCBs	0.413	0.440	0.376	0.410	0.506
SUM of Hexa-PCBs	0.169	0.108	0.149	0.142	0.235
SUM of Hepta-PCBs	0.003	0.005	0.009	0.006	0.016
SUM of Octa-PCBs	0.010	0.007	---	0.006	0.021
SUM Di-Octa-PCBs	1.020	0.852	0.693	0.860	1.500

except PCBs 15, 81, and 77 that eluted in Fraction 2. Dioxin-like PCBs 126 and 169 were targeted but not detected. For simplicity, the far right column in Table 19 used to blank subtract from toenail samples and soap washes listed the amount (ng) based on blank average plus three standard deviations. For each toenail and soap wash sample, that amount divided by the mass of each sample gave the appropriate concentration to subtract for each congener and each sample.

Blank-subtracted concentrations of PCBs in Set 4 toenail samples and soap washes were listed in Tables 20 and 21, respectively. Reviewing the toenail samples data for individual A toenails in Table 20, sample 17T-4 at 37.6 ng/g total was significantly higher than 7.0 ng/g and 4.5 ng/g, respectively, for samples 15T-4 and 16T-4. In soap wash data of Table 21, PCBs were highest for the wash sample (17W-4) of that 17T-4 toenail sample, which seems to indicate that much of the elevated PCB concentration for sample 17T-4 was from exogenous contamination.

For quality assurance, percent recoveries of the ^{13}C -PCB surrogates in Set 4 toenail samples are listed in Table 22. Nearly all (150 out of 160 measurements) in the expected basic alumina fraction exceeded 40% recovery except for ^{13}C -PCBs 28 and 105, which were near the cutoff for fraction one to fraction two and they split between the two fractions. For ^{13}C -PCB 28 and 105, both basic alumina fractions were checked and quantitation was made based on the fraction which contained the majority of the surrogate. The percentages slightly above 100% for ^{13}C -PCBs 15, 81, and 77 in fraction two occurred because the internal standards in those samples that are used to calculate the ^{13}C -PCB surrogates had a slightly lower response than in the calibration standards.

Table 20. Blank-Subtracted Concentrations (ng/g) of PCBs in Set 4 Toenail Samples

	TOENAIL SAMPLES							
	15T-4	16T-4	17T-4	18T-4	19T-4	20T-4	21T-4	22T-4
	Oct 2006	Feb 2007	May 2007	Jan 2010	Apr 2010	Jan 2009	Apr 2009	Nov 2007
Date Sampled:	A	A	A	B	M-cat	C	C	D
Sample Submitter	0.340	0.295	0.159	0.0655	0.120	0.196	0.322	0.189
Mass (g):	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
ANALYTE NAME	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
Di-PCB 15 *	0.06	0.05	0.20	---	0.03	---	0.03	0.35
Tri-PCB 18	0.14	---	---	---	1.05	---	0.09	---
Tri-PCB 31/28 *	0.32	0.20	0.45	0.16	0.87	0.24	0.59	0.69
Tri-PCB 33	0.34	0.09	0.23	0.11	0.67	0.11	0.64	0.57
Tetra-PCB 52 *	0.08	0.18	1.41	---	0.73	0.28	0.33	1.47
Tetra-PCB 49	0.08	0.11	0.58	---	0.29	0.13	0.21	0.54
Tetra-PCB 44	0.11	0.13	0.79	---	0.47	0.16	0.28	1.13
Tetra-PCB 41	0.08	0.13	0.65	---	0.29	0.09	0.13	0.92
Tetra-PCB 74	0.13	0.16	0.64	0.17	0.42	0.24	0.26	1.08
Tetra-PCB 70 *	0.29	0.24	2.15	0.58	1.36	0.60	0.70	4.56
Tetra-PCB 66	0.17	0.18	0.87	0.27	0.62	0.37	0.45	1.82
Tetra-PCB 60	0.06	0.08	0.32	0.06	0.20	0.07	0.09	0.65
Tetra-PCB 81 *	0.003	0.005	0.01	0.02	---	0.01	0.01	0.01
Tetra-PCB 77 *	0.01	0.003	0.03	---	0.01	0.02	0.03	0.19
Penta-PCB 95 *	0.14	0.19	3.58	0.65	2.08	0.65	0.83	5.92
Penta-PCB 84	0.02	0.06	1.05	---	0.48	0.12	0.13	1.87
Penta-PCB 101 *	0.39	0.34	4.65	1.19	2.86	1.04	1.36	10.3
Penta-PCB 99	0.15	0.14	1.71	0.30	0.94	0.36	0.47	3.64
Penta-PCB 97	0.17	0.11	1.44	0.39	0.98	0.35	0.50	3.92
Penta-PCB 87	0.29	0.16	2.30	0.68	1.63	0.55	0.89	7.03
Penta-PCB 85	0.09	0.03	0.64	0.11	0.42	0.10	0.25	2.01
Penta-PCB 110	0.39	0.21	3.00	0.80	2.09	0.57	1.25	14.5
Penta-PCB 123 *	0.03	---	0.12	0.07	0.16	0.02	0.06	0.43
Penta-PCB 118 *	0.74	0.23	2.34	1.25	2.33	0.93	1.39	9.98
Penta-PCB 105 *	0.43	---	0.44	0.58	2.11	0.43	1.78	3.76
Hexa-PCB 135	0.07	0.04	0.52	0.30	0.38	0.18	0.28	1.65
Hexa-PCB 153 */168	0.32	0.20	0.93	0.52	0.93	0.50	0.74	3.92
Hexa-PCB 141	0.11	0.03	0.24	0.18	0.41	0.19	0.25	1.42
Hexa-PCB 137	0.03	---	0.10	0.04	0.16	0.06	0.10	0.55
Hexa-PCB 138 */158	0.31	0.13	0.52	0.71	0.98	0.45	0.65	3.28
Hexa-PCB 129	0.04	0.01	0.07	0.08	0.19	0.05	0.09	0.58
Hexa-PCB 128	0.02	---	0.03	---	0.11	0.05	0.04	0.12
Hexa-PCB 167 *	0.09	---	0.02	---	0.06	0.02	0.03	0.18
Hexa-PCB 156 *	0.03	0.05	0.06	0.18	0.19	0.09	0.11	0.46
Hexa-PCB 157 *	0.03	0.01	0.03	0.09	0.06	0.02	0.03	0.11
Hepta-PCB 178	0.03	0.02	0.04	---	---	0.04	0.05	0.10
Hepta-PCB 187	0.13	0.09	0.16	0.21	0.29	0.24	0.30	0.65
Hepta-PCB 183	0.06	0.03	0.06	0.10	0.14	0.11	0.13	0.31
Hepta-PCB 174	0.06	0.02	0.06	0.10	0.18	0.15	0.17	0.49
Hepta-PCB 177	0.04	0.02	0.03	0.06	0.08	0.08	0.10	0.23
Hepta-PCB 171	0.02	0.01	0.01	---	0.04	0.05	0.04	0.14
Hepta-PCB 180 */193	0.12	0.10	0.10	0.07	0.10	0.16	0.20	0.26
Hepta-PCB 170 *	0.12	0.11	0.09	0.14	0.16	0.19	0.19	0.33
Octa-PCB 202 *	0.02	0.02	0.03	---	0.06	0.04	0.05	0.18
Octa-PCB 199	0.11	0.09	0.08	0.11	0.21	0.12	0.13	0.36
Octa-PCB 194	0.06	0.06	0.05	---	---	0.08	0.09	0.07
Nona-PCB 208 *	0.02	0.01	0.01	---	0.08	0.03	0.03	0.12
Nona-PCB 206	0.07	0.05	0.03	0.10	0.09	0.05	0.05	0.09
SUM: 52 PCB congeners	6.6	4.1	32.9	10.4	28.0	10.4	16.6	92.9

* Denotes Quantitation with same ¹³C-PCB congener**HOMOLOG Quantitation:**

SUM of Trichloro-PCBs	0.61	0.27	0.49	0.24	3.17	---	1.80	1.21
SUM of Tetrachloro-PCBs	0.82	1.15	7.29	0.58	4.53	3.61	2.97	13.7
SUM of Pentachloro-PCBs	2.33	2.14	26.7	8.95	21.3	6.82	10.8	76.9
SUM of Hexachloro-PCBs	0.44	0.50	4.03	3.22	5.64	2.71	3.98	22.9
SUM of Heptachloro-PCBs	0.58	0.14	0.38	0.89	1.11	0.74	1.00	3.20
SUM of Octachloro-PCBs	0.89	0.10	---	---	2.56	0.13	0.04	0.40
Homolog SUM Tri-Octa-PCBs	5.7	4.3	38.9	13.9	38.3	14.0	20.6	118.3

Table 21. Blank-Subtracted Concentrations of PCBs in Set 4 Toenail Soap Washes

	15W-4	16W-4	17W-4	18W-4	19W-4	20W-4	21W-4	22W-4
Date Sampled:	Oct 2006	Feb 2007	May 2007	Jan 2010	Apr 2010	Jan 2009	Apr 2009	Nov 2007
Sample Donated by:	A	A	A	B	M-cat	C	C	D
Mass Rinsed (g):	0.340	0.295	0.159	0.0655	0.120	0.196	0.322	0.189
ANALYTE NAME	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
Tri-PCB 18	---	---	---	---	---	---	---	---
Tri-PCB 31/28 *	---	---	---	---	---	---	---	---
Tri-PCB 33	---	---	---	---	---	---	---	---
Tetra-PCB 52 *	---	---	---	---	---	---	---	---
Tetra-PCB 49	---	---	---	---	---	---	---	---
Tetra-PCB 44	---	---	---	---	---	---	---	---
Tetra-PCB 41	---	---	---	---	---	---	---	---
Tetra-PCB 74	---	---	0.06	---	---	---	---	0.03
Tetra-PCB 70 *	---	---	0.25	---	---	---	---	0.09
Tetra-PCB 66	---	---	0.10	---	---	---	---	0.04
Tetra-PCB 60	---	---	0.04	---	---	---	---	---
Penta-PCB 95 *	---	---	0.58	---	---	---	---	0.12
Penta-PCB 84	---	---	0.09	---	---	---	---	---
Penta-PCB 101 *	---	---	0.70	---	---	---	---	0.28
Penta-PCB 99	---	---	0.25	---	---	---	---	0.09
Penta-PCB 97	---	---	0.26	---	---	---	---	0.15
Penta-PCB 87	---	0.01	0.47	---	---	---	---	0.21
Penta-PCB 85	---	---	0.13	---	---	---	---	0.03
Penta-PCB 110	---	---	0.64	---	---	---	---	0.23
Penta-PCB 123 *	---	0.01	0.02	---	---	---	---	0.03
Penta-PCB 118 *	---	0.03	0.54	---	---	---	---	0.30
Penta-PCB 105 *	---	0.01	0.14	0.43	---	---	---	0.04
Hexa-PCB 135	---	0.01	0.12	0.04	---	---	---	0.05
Hexa-PCB 153 */168	---	0.02	0.21	---	---	---	---	0.10
Hexa-PCB 141	---	---	0.07	---	---	---	---	0.05
Hexa-PCB 137	---	---	0.01	---	---	---	---	0.02
Hexa-PCB 138 */158	---	0.06	0.18	0.29	---	---	---	0.16
Hexa-PCB 129	---	0.01	0.03	---	---	---	---	0.02
Hexa-PCB 128	---	---	---	---	---	---	---	---
Hexa-PCB 167 *	---	---	---	---	---	---	---	---
Hexa-PCB 156 *	---	0.02	0.02	0.08	---	0.01	---	0.04
Hexa-PCB 157 *	---	0.01	0.01	0.07	0.01	---	---	0.02
Hepta-PCB 178	---	---	---	---	---	---	---	---
Hepta-PCB 187	---	0.02	0.04	0.03	---	---	---	0.03
Hepta-PCB 183	---	0.01	0.03	0.01	---	---	---	0.02
Hepta-PCB 174	---	0.01	---	---	---	---	---	---
Hepta-PCB 177	---	---	0.01	0.01	---	---	---	0.01
Hepta-PCB 171	---	---	---	---	---	---	---	---
Hepta-PCB 180 */193	---	0.01	---	0.01	---	---	---	0.01
Hepta-PCB 170 *	---	0.02	0.01	0.11	---	---	---	0.02
Octa-PCB 202 *	---	---	---	---	---	---	---	---
Octa-PCB 199	---	0.01	---	---	---	---	---	0.01
Octa-PCB 194	---	---	---	---	---	---	---	---
Nona-PCB 208 *	---	---	---	---	---	---	---	---
Nona-PCB 206	---	---	---	---	---	---	---	---
SUM: 49 PCB congeners	---	0.27	5.01	1.09	0.01	0.01	---	2.20

* Denotes Quantitation with same ¹³C-PCB congener

<u>HOMOLOG Quantitation:</u>								
SUM of Trichloro-PCBs	---	---	---	---	---	---	---	---
SUM of Tetrachloro-PCBs	---	---	---	---	---	---	---	---
SUM of Pentachloro-PCBs	---	---	2.96	---	---	---	---	1.85
SUM of Hexachloro-PCBs	---	0.12	1.12	---	---	---	---	0.51
SUM of Heptachloro-PCBs	---	0.07	0.18	0.16	---	---	---	0.14
SUM of Octachloro-PCBs	---	---	---	---	---	---	---	---
Homolog SUM Tri-Octa-PCBs	---	0.18	4.26	0.16	---	---	---	2.49

Table 22. Percent Recoveries of ^{13}C -PCB Surrogates in Set 4 Toenail Samples

	TOENAIL SAMPLES							
	15T-4	16T-4	17T-4	18T-4	19T-4	20T-4	21T-4	22T-4
	Date Sampled: Oct 2006	Feb 2007	May 2007	Jan 2010	Apr 2010	Jan 2009	Apr 2009	Nov 2007
	Sample Submitter: A	A	A	B	M-cat	C	C	D
Mass (g):	0.340	0.295	0.159	0.0655	0.120	0.196	0.322	0.189
^{13}C -PCB Surrogate	%	%	%	%	%	%	%	%
^{13}C -Di-PCB 15	84	75	102	102	95	78	79	85
^{13}C -Tri-PCB 28	17	82	80	22	28	38	24	55
^{13}C -Tetra-PCB 52	42	83	82	59	78	73	77	83
^{13}C -Tetra-PCB 70	55	82	84	57	79	73	79	82
^{13}C -Tetra-PCB 81	90	92	118	138	125	86	87	107
^{13}C -Tetra-PCB 77	85	84	114	134	118	85	82	104
^{13}C -Penta-PCB 95	50	80	82	52	75	66	72	80
^{13}C -Penta-PCB 101	71	90	94	65	90	85	87	92
^{13}C -Penta-PCB 123	79	94	97	68	95	85	88	93
^{13}C -Penta-PCB 118	76	92	95	66	91	84	87	90
^{13}C -Penta-PCB 105	27	85	79	20	22	38	14	46
^{13}C -Hexa-PCB 153	75	85	89	64	84	76	80	85
^{13}C -Hexa-PCB 138	78	89	92	60	85	78	83	88
^{13}C -Hexa-PCB 167	76	86	88	62	84	76	81	86
^{13}C -Hexa-PCB 156	75	86	88	56	82	72	80	86
^{13}C -Hexa-PCB 157	80	91	92	57	84	70	83	90
^{13}C -Hepta-PCB 180	84	91	94	69	89	80	85	91
^{13}C -Hepta-PCB 170	78	84	88	55	78	66	78	85
^{13}C -Octa-PCB 202	76	80	85	62	79	72	76	80
^{13}C -Nona-PCB 208	99	97	98	96	96	96	96	96

All but PCBs 15, 81, 77 and partially 28 and 105, were in Basic Alumina Fraction 1

Replicate Analysis through Time of Multiple Toenail Samples of Individuals A and C

To reiterate, replicate analysis of the same toenail sample collected on a specific date was not done because sample mass was too small near the detection limits to routinely collect and analyze samples based on an individual's left or right foot and subsampling errors from a larger pool of nails were likely because toenails are not homogenous. Therefore, measurements of precision in the true sense are not available

in this study. However, replicate analyses were done through time for up to five or six multiple toenail samples from individual A over an extended year and from two samples from individual C for part of a year (Tables 23-25).

Table 23. Blank-Subtracted Concentrations (ng/g-nail) of Organochlorine Pesticides in Multiple Toenail Samples from Two Individuals

Date Sampled: Sample Submitter Mass (g): ANALYTE NAME	TOENAIL SAMPLES										
	6T-2	7T-2	15T-4	16T-4	17T-4			20T-4	21T-4		
	Jan 2006	Mar 2006	Oct 2006	Feb 2007	May 2007			Jan 2009	Apr 2009		
	A	A	A	A	A			C	C		
	0.192	0.190	0.340	0.295	0.159	n=5		0.196	0.322	n = 2	
	ng/g	ng/g	ng/g	ng/g	ng/g	MEAN	SD	ng/g	ng/g	MEAN	SD
Pentachloroanisole	1.33	0.88	0.16	0.38	0.34	0.62	0.48	0.16	0.24	0.20	0.05
beta-HCH	0.03	0.06	0.11	0.16	0.24	0.12	0.08	0.18	0.12	0.15	0.05
Chlordanes:											
trans-Chlordane	8.45	5.85	3.68	4.45	6.53	5.79	1.86	4.25	4.41	4.33	0.11
cis-Chlordane	5.18	3.55	2.40	2.81	4.18	3.62	1.10	1.86	1.96	1.91	0.07
trans-Nonachlor	4.53	3.23	1.92	2.27	3.47	3.08	1.03	1.32	1.57	1.44	0.18
DDT Series:											
o,p'-DDE			---	---	---			0.13	0.11	0.12	0.02
p,p'-DDE	0.62	0.49	0.79	1.14	1.07	0.82	0.28	2.81	2.89	2.85	0.06
o,p'-DDT			---	---	---			0.64	0.39	0.51	0.17
p,p'-DDT	---	---	---	---	---			0.89	1.04	0.97	0.11
--- Not Detected or not detected above method blank											

Table 24. Blank-Subtracted Concentrations (ng/g-nail) of PBDEs in Multiple Toenail Samples from Two Individuals

Sample No. - Set: Date Sampled: Sample Submitter Mass (g): ANALYTE NAME	TOENAIL SAMPLES													
	3T-1	6T-2	7T-2			15T-4	16T-4	17T-4			20T-4	21T-4		
	Nov 2005	Jan 2006	Mar 2006			Oct 2006	Feb 2007	May 2007			Jan 2009	Apr 2009		
	A	A	A			A	A	A			C	C		
	0.187	0.192	0.190	n = 3		0.340	0.295	0.159	n = 2, 3, 5 or 6		0.196	0.322	n = 2	
				MEAN	SD	ng/g	ng/g	ng/g	MEAN	SD	ng/g	ng/g	MEAN	SD
tri-BDE 28	0.335	0.31	0.43	0.36	0.06	0.13	0.22	0.33	0.29	0.10	0.36	0.34	0.35	0.01
tetra-BDE 47	8.19	8.0	8.7	8.30	0.36	---	9.92	---			---	5.0		
penta-BDE 100	1.02	0.95	0.94	0.97	0.04	---	---	---			---	---		
penta-BDE 99	6.32	7.63	8.12	7.36	0.93	2.77	7.31	2.63	5.80	2.47	0.43	3.74	2.09	2.34
penta-BDE 85		0.17	0.26	0.22	0.06	0.21	0.29	0.32	0.25	0.06	0.18	0.28	0.23	0.07
hexa-BDE 153	1.31	2.17	1.89	1.79	0.44	1.16	2.50	1.87	1.82	0.51	0.16	0.33	0.25	0.12
hexa-BDE 139						0.06	0.09	0.07	0.07	0.02	0.03	0.05	0.04	0.01
hepta-BDE 183						0.04	0.07	---	0.05	0.02	---	0.03		
deca-BDE 209						0.53	2.28	3.51	2.11	1.50	3.28	2.98	3.13	0.21
--- Not Detected at Specified Detection Limit or < [Blank Avg + 3SD]														

Replicated analyses from individual A were from sets 1, 2, and 4 and the two samples from individual C from set 4. In Table 23, p,p'-DDE was the only compound listed that was quantitated with a ^{13}C -surrogate, resulting in a mean 0.82 ng/g-nail, sd 0.28, n=5 in individual A and 2.85 ng/g, sd 0.06, n=2 for individual C. Using concentrations of *trans*-nonachlor as indicator for chlordanes, the two samples from individual C were very similar (mean 1.44 ng/g-nail, sd 0.18, n=2) but the five samples from individual A varied more (mean 3.08 ng/g, sd 1.03, n=5), which may indicate that exposures for individual A varied more during the extended year than for individual C's partial year.

PBDEs overall were best analyzed in sets 1 and 2 of individual A samples that were not affected by slight contamination in Proteinase K enzyme used in set 4 (Table 24). PBDEs 47, 99, and especially 100 were measured consistently (mean 8.30 ng/g-nail, sd 0.36; mean 7.36 ng/g-nail, sd 0.93; mean 0.97 ng/g-nail, sd 0.04, n=3, respectively). In set 4 for samples from both individuals, elevated blank concentrations of PBDEs 47, 100, and to some extent 99 eliminated those congeners. Several minor PBDEs (28, 85, and especially 153) were unaffected and could be the best biomonitoring indicators. Measurements throughout all sets for individual A samples were PBDE 28, mean 0.29 ng/g-nail, sd 0.10, n=6; PBDE 85, mean 0.25 ng/g, sd 0.06, n=5; and PBDE 153, mean 1.82 ng/g, sd 0.51, n=6.

For PCBs in individuals A and C, concentrations (ng/g-nail) with mean and standard deviation from two samples each are shown for half of the 52 congeners and for homolog quantitation (Table 25). Not included in Table 25 was sample 17T-4 for individual A with relatively high PCBs in the soap wash sample 17W-4 that indicated

Table 25. Blank-Subtracted Concentrations (ng/g-nail) of Selected PCBs in Multiple Toenail Samples from Two Individuals

ANALYTE NAME	TOENAIL SAMPLES							
	15T-4	16T-4			20T-4	21T-4		
	Date Sampled: Oct 2006	Feb 2007			Jan 2009	Apr 2009		
	Sample Submitter: A	A			C	C		
Mass (g):	0.340	0.295			0.196	0.322		
	ng/g	ng/g	MEAN	SD	ng/g	ng/g	MEAN	SD
Tetra-PCB 52 *	0.08	0.18	0.13	0.07	0.28	0.33	0.31	0.04
Tetra-PCB 49	0.08	0.11	0.10	0.02	0.13	0.21	0.17	0.06
Tetra-PCB 44	0.11	0.13	0.12	0.01	0.16	0.28	0.22	0.08
Tetra-PCB 74	0.13	0.16	0.15	0.02	0.24	0.26	0.25	0.02
Tetra-PCB 70 *	0.29	0.24	0.26	0.03	0.60	0.70	0.65	0.07
Tetra-PCB 66	0.17	0.18	0.17	0.01	0.37	0.45	0.41	0.05
Penta-PCB 95 *	0.14	0.19	0.17	0.04	0.65	0.83	0.74	0.13
Penta-PCB 101 *	0.39	0.34	0.37	0.03	1.04	1.36	1.20	0.23
Penta-PCB 99	0.15	0.14	0.14	0.01	0.36	0.47	0.42	0.08
Penta-PCB 97	0.17	0.11	0.14	0.04	0.35	0.50	0.43	0.10
Penta-PCB 87	0.29	0.16	0.22	0.09	0.55	0.89	0.72	0.24
Penta-PCB 110	0.39	0.21	0.30	0.12	0.57	1.25	0.91	0.47
Penta-PCB 118 *	0.74	0.23	0.49	0.36	0.93	1.39	1.16	0.32
Hexa-PCB 135	0.07	0.04	0.05	0.02	0.18	0.28	0.23	0.07
Hexa-PCB 153 */168	0.32	0.20	0.26	0.08	0.50	0.74	0.62	0.17
Hexa-PCB 138 */158	0.31	0.13	0.22	0.12	0.45	0.65	0.55	0.14
Hexa-PCB 156 *	0.03	0.05	0.04	0.02	0.09	0.11	0.10	0.01
Hepta-PCB 187	0.13	0.09	0.11	0.03	0.24	0.30	0.27	0.04
Hepta-PCB 183	0.06	0.03	0.04	0.02	0.11	0.13	0.12	0.01
Hepta-PCB 180 */193	0.12	0.10	0.11	0.01	0.16	0.20	0.18	0.03
Hepta-PCB 170 *	0.12	0.11	0.11	0.01	0.19	0.19	0.19	0.00
Octa-PCB 199	0.11	0.09	0.10	0.01	0.12	0.13	0.13	0.00
SUM: 52 PCB congeners	6.6	4.1	5.4	1.8	10.4	16.6	13.5	4.4
* Denotes Quantitation with same ¹³ C-PCB congener								
HOMOLOG Quantitation:								
SUM of Trichloro-PCBs	0.61	0.27	0.44	0.24	---	1.80	1.80	---
SUM of Tetrachloro-PCBs	0.82	1.15	0.98	0.23	3.61	2.97	3.29	0.46
SUM of Pentachloro-PCBs	2.33	2.14	2.24	0.13	6.82	10.8	8.81	2.83
SUM of Hexachloro-PCBs	0.44	0.50	0.47	0.04	2.71	3.98	3.34	0.89
SUM of Heptachloro-PCBs	0.58	0.14	0.36	0.31	0.74	1.00	0.87	0.19
SUM of Octachloro-PCBs	0.89	0.10	0.50	0.56	0.13	0.04	0.09	0.07
Homolog SUM Tri-Octa-PCBs	5.7	4.3	4.98	0.96	14.0	20.6	17.3	4.65

exogenous or lab contamination of PCBs. Although nearly all concentrations of PCB congeners in samples from individuals A and C were < 1 ng/g-nail, standard deviations were remarkably low (usually within ± 0.1 ng/g). All PCB congeners were quantitated

based on a ^{13}C -PCB surrogate, but only 10 of the 25 congeners shown in Table 25 had the identical congener surrogate.

Summary

Because of the importance of eliminating possible exogenous superficial nail contamination, the analytical results showed the need for a preliminary soap wash before a second soap wash and rinses that would be saved for analysis. Although it seemed tedious at the time, also analyzing a soap wash and rinse blank sample with each nail set provided valuable quality assurance that no apparent contamination arose from the soap wash and Fisher water rinses.

Though an added expense, stable-labeled ^{13}C -surrogates spiked at low ng amounts provided ultimate quality assurance for analyte recoveries, typically above 50%, throughout the method for each and every sample and facilitated accurate and consistent analyses by GC/HRMS. For example, p,p'-DDE was consistently measured in several sets of individual A toenail samples because fluctuations in surrogate recovery inherently corrected for the same fluctuations for native p,p'-DDE. Having sufficient number of ^{13}C -surrogates of PBDE and especially PCB congeners, including those more transient and "episodic," was extremely valuable. Quality assurance, method sensitivity and selectivity were also enhanced by using GC/HRMS combined with high performance narrow bore capillary columns for sharper peaks with higher signal/noise and superior separation of close-eluting compounds and isomers. Detection limits for analytes using GC/HRMS needed to be and were far below 1 ng/g with limits as low as 0.003 ng/g for all dioxin-like PCBs including 77 and 81. In the majority of cases, extremely low

concentrations were consistently measurable in method blanks to compute a 99% confidence interval of 3 standard deviations added to the average method blank for optimum blank-correction concentrations in samples.

Even with the best of GC and HRMS, the method benefitted and relied on the miniature GPC for consistent lipid removal and accurate percent lipid measurements, with single use, no carryover from any other sample. Cleanup with just the 0.5 g basic alumina glass SPE of the methylated polar phenolic extract was relatively good and would have been better with a normal GPC eluate. Methylation with iodomethane did not appear to contribute any significant artifacts to interfere with searching for unknown compounds. Iodomethane may not have been ideal for all targeted compounds; diazomethane could have been tried if artifacts were minor. Overall, lab background was minimized effectively by using high purity, flash-sized basic alumina straight from the jar (heated) into a flash chromatography column that was solvent-rinsed and dried with high purity nitrogen and pressurized high purity nitrogen during use.

Concerning the important early part of toenail sample preparation, hand grinding toenails, digestion, and dissolution were very slow tedious steps in the method. A miniature mill with individual stainless steel vessels that can be thoroughly cleaned for reuse could break down the nails to much smaller pieces with much more surface area accessible for digestion. The reagent, dithiothreitol, a strong reducing agent with sonication was apparently effective to at least partially digest the nail keratin without reacting with too many target compounds, but probably reduced the acidic phthalate

metabolites. Proteinase K enzyme was also somewhat successful but it took a number of days and the enzyme supplied to us had detectable PBDEs that compromised some of the PBDE concentrations in the sample set 4. Unless a different lot or supplier will not have any measurable contamination, use of Proteinase K is not recommended.

Measuring several targeted analytes was challenging and troublesome.

Bisphenol A in method blanks was variable and too high to distinguish BPA in toenails from blanks or soap washes and recoveries of the surrogate of tetrabromobisphenol A were consistently < 10%. Phthalate metabolites would best be analyzed directly with an LC/MS/MS system, possibly after the GPC step, to determine whether they can be found in toenail extracts.

CHAPTER 4. RESULTS AND DISCUSSION – TOENAILS AS BIOMONITORING MATRIX

There is general agreement that collecting toenail clippings for human toenail samples in any human biomonitoring program is non-invasive compared with biopsies of human adipose or breast tissue, collecting blood or breast milk samples at a medical lab or clinic, or collecting multiple urine samples during an entire 24-hour period for certain compounds with short-half lives or metabolites. Therefore, to further evaluate toenail clippings as a suitable biomonitoring matrix to assess human exposure to environmental organic compounds, results of the analyses of toenail samples is compared with extant data from various other human matrices. Comparisons of quantitative biomonitoring data of targeted compounds includes persistent organochlorine pesticides, PCB congeners with varying degrees of persistency in humans, moderately persistent PBDE flame retardants, two synthetic musk fragrances, Galaxolide and Tonalide, and a compound with a very short biological half-life, triclosan.

Biomonitoring of persistent organochlorine pesticides

Blank-subtracted, lipid-based concentrations of persistent organochlorine pesticides in toenail samples from this study (Table 26) are compared to extant literature lipid-based data of the pesticides in serum samples by CDC of those older than age 20,² adipose tissue from the former EPA program, National Human Adipose Tissue Survey,⁷ and in both hair and serum samples from 10 volunteers from Harvard University (Table 27).³⁴ Concentrations in Tables 26 and 27 were all tabulated in units of ng/g-lipid. The percent lipid in each toenail sample was isolated using the flash GPC

Table 26. Lipid-Based Concentrations (ng/g lipid) of Organochlorine Pesticides in Nails

Date Sampled: Sample Submitter Mass (g): Lipid (%): ANALYTE NAME	TOENAIL SAMPLES									
	6T-2	7T-2	15T-4	16T-4	17T-4	18T-4	20T-4	21T-4	22T-4	
	Jan 2006	Mar 2006	Oct 2006	Feb 2007	May 2007	Jan 2010	Jan 2009	Apr 2009	Nov 2007	
	A	A	A	A	A	B	C	C	D	
Mass (g):	0.192	0.190	0.340	0.295	0.159	0.0655	0.196	0.322	0.189	
Lipid (%):	1.31	0.87	1.16	1.29	1.63	1.23	1.03	1.22	1.23	
	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	
Hexachlorobenzene	23	22	---	---	---	---	---	---	---	
Pentachloroanisole	101	101	14	29	21	38	16	19	---	
Methyl (ether) Triclosan	24	30	154	15	4	---	---	---	---	
gamma-HCH (Lindane)	---	---	4	---	8	---	---	---	13	
beta-HCH	2	7	9	12	15	---	18	10	17	
Dieldrin	---	---	---	---	---	56	---	20	---	
Chlordanes:										
Oxychlordane	---	---	---	7	---	31	13	7	11	
Heptachlor Epoxide	14	15	6	7	5	---	7	7	---	
trans-Chlordane	645	673	317	345	401	578	412	361	48	
cis-Chlordane	396	408	207	218	256	371	181	161	27	
trans-Nonachlor	346	372	166	176	213	334	128	129	26	
cis-Nonachlor	15	36	14	14	24	56	29	20	18	
DDE and DDTs:										
o,p'-DDE	---	---	---	---	---	---	13	9	---	
p,p'-DDE	48	56	68	88	66	46	273	237	106	
o,p'-DDT	---	---	---	---	---	---	62	32	---	
p,p'-DDT	---	---	---	---	---	---	86	86	108	

--- Not Detected or not (detected) above method blank average + 3 STD

Table 27. Concentrations (ng/g lipid) of Organochlorine Pesticides in Serum, Tissue, and Hair Samples from Extant Data

Date Sampled: Sample Submitter	SERUM	SERUM	ADIPOSE	HAIR	HAIR:SERUM
	n~1380	> Age 20	n=407	n=10	n=10
	2003-4	2003-4	1983	2003	2003
	CDC	CDC	NHATS	Altshul et al.	Altshul et al.
	Geometric	95th	Geometric	Median	
ANALYTE NAME	Mean	Percentile	Mean	1.8% Lipid	HAIR:SERUM RATIO
	ng/g	ng/g	ng/g	ng/g	
Hexachlorobenzene	15.5	29.0	31	28	1.1
Pentachloroanisole					
Methyl (ether) Triclosan					
gamma-HCH (Lindane)	---	< 8	---		
beta-HCH	7.9	62.2	100		
Dieldrin	---	19.5	60		
Chlordanes:					
Oxychlordane	10.6	39.2	90	NM	<< 1
Heptachlor Epoxide	---	20.6	90		
trans-Chlordane					
cis-Chlordane					
trans-Nonachlor	16.9	74.7	120	36	1.2
cis-Nonachlor					
DDE and DDTs:					
o,p'-DDE				41	6.2
p,p'-DDE	268	1,990	343	229	0.8
o,p'-DDT	---	< 8	14	34	26
p,p'-DDT	---	20.7	294	113	12

--- Not Detected or not (detected) above method blank

BLANK SPACE Denotes Not Targeted for Analysis

NM Not Meaningful, Some Not Detected

cleanup step and a microbalance as described in chapter 2. Most toenail percent lipid values were within a narrow range (1.03-1.31%), except for 0.87% (Sample 7T-2) and 1.63% (Sample 17T-4). The lowest lipid value in Sample 7T-2 was the nail sample that was least digested, based on the amount of residual nail fragments following extraction. Subsequently, extra care was used to grind nails and in Set 4, Proteinase K enzyme was added for more complete digestion.

As expected, the pervasive DDT metabolite, p,p'-DDE, was detected in all toenail samples, even in Set 3 with its emphasis on more polar compounds. Set 3 concentrations of DDE were not included in Table 26 because of a potential for any DDT present to convert to DDE with a strong basic buffer (K_2CO_3). In the five toenail samples collected over an extended year from individual A and analyzed in Sets 2 and 4 for non-polar compounds, p,p'-DDE concentrations were very similar (mean 65 ng/g-lipid; sd 15, n=5). Consistent and accurate p,p'-DDE values were aided by the isotope dilution quantitation technique (with the surrogate, ^{13}C -p,p'-DDE). Samples (20T-4, 21T-4, and 22T-4) from individuals C and D had up to fourfold higher DDE concentrations and detectable DDT, suggestive of more recent DDT exposure. Comparing p,p'-DDE lipid-based concentrations with extant serum, adipose tissue, and hair lipid-based data (Table 27), individual C toenail samples (mean 255 ng/g-lipid) nearly matched the geometric mean of CDC's national serum samples (268 ng/g-lipid), adipose tissue mean from 1983 (343 ng/g-lipid), and the median of 10 hair samples from volunteers at Harvard University (229 ng/g-lipid). In toenails from the other three individuals, DDE was lower, ranging from 46 to 106 ng/g-lipid. The p,p'-DDT, detected in toenail samples from

individuals C and D, concentrations (86, 86, and 108 ng/g-lipid) exceeded the 95th percentile (highest 5%) of the CDC serum samples (20.7 ng/g-lipid), nearly matched the median of the 10 hair samples (113 ng/g-lipid), and were only three times less than the geometric mean of the 1983 adipose tissue survey (Table 27). Both hair and serum lipid-based DDT concentrations were reported in the Harvard study of 10 donors and Altshul et al. (2004)³⁴ reported that both isomers of DDT were much higher in hair than serum, with hair to serum ratios of 26 and 12.

Hexachlorobenzene was found in individual A toenails (23 and 22 ng/g-lipid) in Set 2, but because blanks in Set 4 using Proteinase K enzyme were higher, this compound was only measured in individual A. Individual A toenail concentrations of hexachlorobenzene fell within CDC's geometric mean (15.5 ng/g-lipid) and 95th percentile (29.0 ng/g-lipid) and close to the Harvard hair median (28 ng/g-lipid), and mean of 1983 adipose tissue (31 ng/g-lipid). Toenail concentrations of another persistent compound, beta-hexachlorocyclohexane, like hexachlorobenzene which was phased out of use years ago, again were low and fell within CDC's 95th percentile. We hypothesize that such compounds may arise from a long-term body burden in individuals arising from historic exposure; lipid-adjusted concentrations in toenails were very similar to CDC serum data.

Pentachloroanisole, an environmental metabolite of pentachlorophenol methylated by bacteria, was detected in nearly all of the toenail samples. Pentachloroanisole is not a human metabolite as pentachlorophenol in human liver is either conjugated as a glucuronide or metabolized to tetrachlorohydroquinone.⁷⁴ Like

pentachloroanisole, the methyl ether of the antibacterial, triclosan, can also be converted by bacteria. Variable concentrations of methyl ether triclosan were found solely in toenails from individual A (Table 26). As noted in Chapter 3, “free” triclosan itself was detected and quantitated at concentrations of 53.6 and 62.9 ng/g-wet weight or nearly 100 times higher on a lipid-basis in samples from the same individual (A) in Set 3 analysis for more polar compounds. It is possible that with small, periodic exposures to soaps, approximately 1% of triclosan was converted by skin bacteria to the methyl ether of triclosan, absorbed through the skin, and measured along with other non-polar compounds.

Although not originally targeted in toenail samples and thus not detected in Set 1 until GC/full-scan MS analysis, chlordane was subsequently detected and quantitated in all toenail samples analyzed in Sets 2 and 4 for nonpolar compounds (Table 26). Chlordane provides a good example of the use and analysis of toenail as a biomonitoring matrix to detect and quantify compounds not targeted that are important environmental organic compounds and pollutants indicative of chronic human exposure. Because termites are common in Missouri, many Missouri homes or soil around homes were treated by a professional exterminator with chlordane before its ban in 1989. Comparing concentrations of a major chlordane component, *trans*-nonachlor, in toenail samples (Table 26), individual D (26 ng/g-lipid) was much lower than individual C (mean 129 ng/g-lipid, sd 0.7, n=2), individual B (334 ng/g-lipid), or individual A (254 ng/g-lipid, sd 97, n=5). Comparing with extant serum *trans*-nonachlor data (Table 23), individual D (26 ng/g-lipid) compared closely to CDC’s geometric mean

(16.9 ng/g-lipid) and to hair samples median (36 ng/g-lipid) from Massachusetts. The higher values from Missouri may reflect the fact that termites and termite treatment is less prevalent in the colder northeast. The *trans*-nonachlor concentrations of all toenail samples measured in this study exceeded the CDC 95th percentile serum concentrations, those in hair from Massachusetts, and were slightly above the mean for 1983 adipose tissue samples. In contrast, for the metabolite, oxychlordan, toenail concentrations were much lower when detected (7 to 31 ng/g-lipid) and was detected in individual A in only one of five toenail samples near the detection limit (7 ng/g-lipid). Compared with extant oxychlordan data, toenail sample concentrations, when detected, were all less than the CDC 95th percentile (39.2 ng/g-lipid) and, except for individual B, were very close to the CDC geometric mean for serum (10.6 ng/g-lipid). Our nail results for oxychlordan are consistent with the Harvard hair/serum data where the metabolite was very low or not detected in most hair samples but was consistently measured in serum samples which resulted in a very low ($\ll 1$) but not consistently measurable hair/serum ratio.³⁴

A plausible explanation for hair containing higher amounts of transient compounds like DDT and much lower amounts of metabolites (formed within the body) like oxychlordan is important because of similar tendencies shown for toenail results thus far. Altshul et al. (2004)³⁴ reasoned that hair (like toenails) is able to “sequester”³⁴ or incorporate within its cells some of the less persistent compounds in blood that people are chronically exposed to before a certain amount of a compound is partially metabolized (e.g. chlordan to oxychlordan) in the liver and incorporated into adipose

tissue. With the example of chlordanes in the toenail samples, the majority of oxychlordanes would be expected to form within an individual's liver as a metabolite and is, therefore, less likely to be in a steady state. During the slow growth process of hair and toenails, hair and toenails may integrate frequent exposures and partial sequestering resulting in proportionately higher concentrations of parent compounds and proportionately lower concentrations of metabolites.

Biomonitoring PCB congeners

Lipid-based concentrations (ng/g-lipid) of various PCB congeners in Set 4 toenails are compared with extant data from serum, tissue, and hair samples in Table 28. PCBs were quantitated two ways -- by specific individual congener peaks (or occasional doublets of two co-eluting congeners or isomers not distinguished by mass spectrometry) and by homolog groups (having the same number of chlorines). Results of this tally are given at the bottom of Table 28. Homolog group quantitation is the most complete tally of "total PCBs" and a check to determine whether the number of individual congeners targeted with quantitative calibration standards constituted a similar total for PCBs or whether additional congeners should have been included. In Table 28, PCB totals from both quantitation methods compared well, indicating that there were few other PCB congeners present and detected that were not already among the individuals targeted. In every toenail sample analyzed in Set 4, penta-PCBs comprised the highest concentrations of any homolog group.

Looking further at PCB concentrations in Table 28, the two samples from individual C (20T-4 and 21T-4) are quite consistent even though one nail mass (0.196 g)

was nearly half the mass of the 21T-4 (0.322 g). Sample 22T-4 from individual D had considerably more PCBs than all other toenail samples. Toenail samples from individual A (15T-4 through 17T-4) generally had consistent concentrations for the first two samples (Table 25, previous chapter). However, sample 17T-4 had higher PCBs that were also in the toenail soap wash (17W-4), indicating exogenous PCB contamination, which would have been proportionately more significant because of the smaller mass sample size (0.159 g) of 17T-4. For this reason, results for sample 17T-4 are not listed in Table 28.

Toenail lipid-based (ng/g-lipid) PCB congener concentrations listed in Table 28 are compared with lipid-based extant data of the CDC Fourth National serum-based Biomonitoring Study,² breast tissue from a Canadian women's study of 213 benign and 217 cancerous tissue samples,⁷⁵ and median hair concentrations matched with the hair/serum ratio of the 10 donors from Harvard University from 2003.³⁴ In this study and the CDC study, toenail PCB concentrations were determined by GC/HRMS often using the identical congener ¹³C-PCB surrogate, whose recovery through the entire method self-corrected the value for the native PCB congener. For the higher chlorinated, hepta-nona-PCBs, extant breast tissue geometric mean concentrations generally matched the highest (95th) percentile for CDC serum samples. Toenail concentrations of the higher chlorinated, hepta-nona-PCBs generally fell within the

Table 28. Lipid-Based Concentrations (ng/g-lipid) of PCBs in Toenails, Extant Serum, Tissue, and Hair Samples

	TOENAIL SAMPLES						SERUM		BREAST TISSUE	HAIR	H:S RATIO
	15T-4 Oct 2006	16T-4 Feb 2007	18T-4 Jan 2010	20T-4 Jan 2009	21T-4 Apr 2009	22T-4 Nov 2007	n=1300 > Age 20		Benign n=213 Cancer n=217	n=10 2003	n=10 2003
Date Sampled:											
Sample Submitter	A	A	B	C	C	D	CDC 2003-4		Aronson et al.	Altshul et al.	
Mass (g):	0.340	0.295	0.0655	0.196	0.322	0.189	Geo. Mean	95th % Tile	1995-1997 Geo. Mean	Median	HAIR/ SERUM
Lipid (%):	1.16	1.29	1.23	1.03	1.22	1.23	ng/g	ng/g	Bng/g , Cng/g	1.8% Lipid	RATIO
ANALYTE NAME	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	Bng/g , Cng/g	ng/g	RATIO
Di-PCB 15 *	5	4	---	---	2	28					
< Tri-PCB 18 >	12	---	---	---	7	---				24	
< Tri-PCB 31 > -28 *	27	16	13	24	48	56	5	11		68	9
Tri-PCB 33	29	7	9	11	52	47				32	
< Tetra-PCB 52 * >	7	14	---	27	27	120	3	7		77	51
< Tetra-PCB 49 >	7	8	---	13	17	44	1	3		21	
< Tetra-PCB 44 >	9	10	---	16	23	92	2	5		54	
Tetra-PCB 41	7	10	---	9	11	75				27	
Tetra-PCB 74	12	12	14	23	22	88	5	24		17	2
< Tetra-PCB 70 * >	25	18	47	59	58	371				65	
Tetra-PCB 66	14	14	22	36	37	148	1	4		56	
Tetra-PCB 60	5	6	5	7	7	53				20	
Tetra-PCB 81 *	0.3	0.4	1.3	0.7	0.5	1.0	---	0.01			
Tetra-PCB 77 *	0.8	0.2	---	2	2	16					
<< Penta-PCB 95 * >>	12	15	53	63	68	481					
<< Penta-PCB 84 >>	2	5	---	11	10	152				67	
< Penta-PCB 101 * >	34	27	96	101	111	835	2	6		136	50
[Penta-PCB 99]	13	11	24	35	39	296	5	19	18 , 20	23	3
< Penta-PCB 97 >	14	9	31	34	41	319				35	
< Penta-PCB 87 >	25	12	55	54	73	572	1	3		47	
Penta-PCB 85	7	3	9	10	20	163					
<< Penta-PCB 110 >>	34	17	65	56	102	1,180	1	4		136	
Penta-PCB 123 *	3	---	5	2	5	35					
[Penta-PCB 118 *]	64	18	101	90	114	811	7	34	25 , 30	72	3
[Penta-PCB 105 *]	37	---	47	42	146	306	1	7	6 , 7	25	10
<< Hexa-PCB 135 >>	6	3	25	17	23	134				36	
[Hexa-PCB 153 */168]	27	16	43	48	61	319	24	101	98 , 105	222	2
< Hexa-PCB 141 >	9	3	15	19	21	115				85	
Hexa-PCB 137	3	---	3	6	8	45					
[Hexa-PCB 138 */158]	26	10	57	44	53	267	18	77	67 , 74	198	3
Hexa-PCB 129	3	1	6	5	8	48					
[Hexa-PCB 128]	2	---	---	5	4	10	---	1			
[Hexa-PCB 167 *]	8	---	---	2	3	15	1	4		3.8	
[Hexa-PCB 156 *]	2	4	15	9	9	37	3	17	17 , 19	8.5	
Hexa-PCB 157 *	2	1	7	2	2	9	1	4			
[Hepta-PCB 178]	2	1	---	4	4	8	1	7			
[Hepta-PCB 187]	11	7	17	23	24	53	5	26	24 , 26	88	
[Hepta-PCB 183]	5	2	8	10	10	25	2	8	10 , 10	39	
<< Hepta-PCB 174 >>	5	2	8	15	14	40				66	
Hepta-PCB 177	4	2	5	7	8	19	1	8		31	
Hepta-PCB 171	1	0.4	---	5	3	11				16	
[Hepta-PCB 180 */193]	10	8	6	16	16	21	19	88	66 , 72	71	1
[Hepta-PCB 170 *]	11	8	11	18	15	27	7	30	32 , 34	29	2
[Octa-PCB 202 *]	2	1	---	3	4	14					
[Octa-PCB 199]	9	7	9	12	11	29	4	21		13	
[Octa-PCB 194]	6	5	---	8	7	6	4	20			
[Nona-PCB 208 *]	2	1	---	3	2	10					
[Nona-PCB 206]	6	4	8	5	4	7	3	14			
SUM: 52 PCB congeners	568	321	842	1,011	1,357	7,557				2,640	
* Denotes Quantitation with same ¹³ C-PCB congener											
HOMOLOG Quantitation:							KEY:				
SUM of Tri-PCBs	52	21	19	---	147	99	< >	Less Persistent (2,5-ring Cl substituents)			
SUM of Tetra-PCBs	70	89	47	351	243	1,112					
SUM of Penta-PCBs	201	166	727	662	886	6,255	<< >>	Less Persistent (2,3,6-ring Cl substituents)			
SUM of Hexa-PCBs	38	38	262	263	326	1,859					
SUM of Hepta-PCBs	50	11	72	71	82	260	[]	More Persistent (no vicinal H-substituents)			
SUM of Octa-PCBs	77	8	---	13	3	33					
SUM Tri-Octa-PCBs	488	333	1,128	1,360	1,688	9,617					

geometric mean and highest (95th) percentile of CDC serum and near the geometric mean of Canadian breast tissue, but often far exceeded the CDC serum and the breast tissue data for the less chlorinated, tri-hexa-PCBs. For generally all PCBs, lipid-adjusted toenail concentrations matched extant hair sample data. Consistent with our nail measurements, the hair/serum ratios of some of the PCBs from each of the 10 donors were high.³⁴ Tetrachloro-PCB 52 (2,5- 2',5'-substituted) and pentachloro-PCB 101 (2,4,5- 2',5') were both reported to be less persistent in humans because of the vicinal (two neighboring) hydrogen substituents at the 3 and 4 (meta, para) ring positions of at least one ring that are more easily attacked by the body's Cytochrome P450 enzyme system for metabolism.^{76, 77} Yet, Altshul et al. (2004)³⁴ reported hair/serum lipid-based ratios of 51 and 50 for PCBs 52 and 101, respectively. To further evaluate data of other less persistent PCB congeners of similar structures (chlorines substituted at 2,5- and 2,3,6- both with vicinal hydrogens), those congeners in Table 28 were color and character coded (< > and << >>), respectively. PCB congeners reported to be most persistent in humans (mammals) are categorized by having no vicinal hydrogens (especially not at meta, para positions) and were color and character coded ([]).

Altshul et al. (2004)³⁴ interpreted the higher hair concentrations (than in serum) for less persistent PCBs by again using the term, "sequester." Vascularized hair (and of course nails) keep growing slowly and steadily and are able to "sequester" some portion of various compounds in the blood that the human body has been recently exposed to before some degree of metabolism occurs in the liver. If chronic exposures keep occurring, it is possible that hair and nails can effectively integrate periodic "sequester"

episodes over the long term and ultimately complement biomonitoring data from currently default serum samples.

Based on PCB human exposures of GE workers at their New York facility to three types of PCB commercial mixtures (Aroclors 1016, 1254 and 1260) containing less chlorinated PCBs (Aroclor 1016) to highly chlorinated PCBs (Aroclor 1260), Brown and Lawton (2001)⁷⁶ had access to and studied their workers' serum PCB concentrations periodically enough to compute metabolic clearance rates. Their first-order clearance rates varied from a rapid 10 yr^{-1} for trichloro-PCB 28 to a very slow 0.003 yr^{-1} for very persistent, heptachloro-PCB 180⁷⁶. Even within the so-called 'more consistent' group, there still was a ">100-fold intercongener range" in the first order rates⁷⁶. Further, they remarked, "Because of this, after cessation of exposure the congener distribution patterns will vary continuously, and resemble those listed ... at only one instant in time."⁷⁶

Larry Hansen provided further insight about human exposure to PCBs and risk assessment of PCB congeners:

"... pulses of exposure to more labile mixtures may contribute to developmental effects without leaving a residue record ... humans and other animals are intermittently exposed to profiles of PCBs that leave sparse records of their presence ... pulses of these labile and unexpected congeners would be anticipated following every fish meal, although the intermittent levels would not be as high and persistent as with the accidentally exposed workers. In fact, such pulses of total PCB after fish meals have been recorded with maximum serum levels being reached in about 10 hr and declining rapidly to near ambient by 48 hr."⁷⁸

Hansen advocated an expanded list of 97 PCB congeners that merited monitoring,⁷⁸ of which about 80 congeners were analyzed by GC/HRMS in this toenail study. In a later

book chapter, Hansen settled for 30 major “steady-state” persistent PCB congeners and about 18 PCBs that accumulate in exposure sources including fish but are rapidly metabolized by mammals so they generally are present only transiently even if at rather high levels.⁷⁷ Specific “episodic” PCB congeners of most important concern were PCBs 18, 52, 95, 101, 110, and 149.⁷⁷

In humans, PCB congeners can also be preferentially retained into different organs, which would affect achieving a “steady state” distribution that could be biomonitored in adipose tissue or serum.⁷⁹ For example, PCB congeners with 2,3,6-chlorine substitution including PCBs 84, 110, and 95, and/or their hydroxyl-metabolites preferentially bind to lung cells.⁷⁸ Some of the less-chlorinated PCBs with 2,5- or 2,3,6-chlorine substitution are able to weaken and cross the blood-brain barrier and cause various neurotoxicity effects. Four specific neurotoxicity rankings by relative potency of PCB congeners from Hansen⁷⁸ were tallied in Table 29 corresponding to those PCBs found in toenail samples. Rankings included dopamine depletion, phorbol ester binding, disruption in brain calcium, and ryanodine binding associated with dysregulation of calcium ion (Ca^{2+}) channels signaling. Such effects are most important in neurological development along with disrupting thyroid homeostasis and finally, at least a correlation with a chromosome duplication phenomena associated with genetic disorders like autism spectrum disorder.⁸⁰⁻⁸⁸ In biochemical tests with 35 different PCB congeners, PCB 95 (having both 2,5- and a 2,3,6-substitution) showed the strongest effect on ryanodine-binding that affected calcium channel control at only 145 nM.⁸⁹ Other slightly less potent PCBs with effects at doses still < 1000 nM or 1 μM included

Table 29. Relative Potencies of Neurological and Dioxin-like Toxicity Reported for PCBs

ANALYTE NAME	(Excerpted from Table 2 of Hansen, 1998)				Dioxin-like AHH Enzyme Induction
	Dopamine Depletion	Phorbol Binding	Brain Calcium	Brain Ca++ Channels	
Di-PCB 15 *	0.00	0.00			
< Tri-PCB 18 >	0.78				
< Tri-PCB 31 > -28 *	0.36/0.33	-- / < 0.3	-- / 0.35		
Tri-PCB 33	0.35				
< Tetra-PCB 52 * >	0.74	1.00	0.49	0.33	
< Tetra-PCB 49 >	0.66				
< Tetra-PCB 44 >	0.56				
Tetra-PCB 41					
Tetra-PCB 74					
< Tetra-PCB 70 * >				0.10	
Tetra-PCB 66	0.25			0.00	
Tetra-PCB 60					
Tetra-PCB 81 *					Strong
Tetra-PCB 77 *	0.00	0.00	0.00		Strong
< << Penta-PCB 95 * >> >				1.00	
<< Penta-PCB 84 >>					
< Penta-PCB 101 * >					
[Penta-PCB 99]					
< Penta-PCB 97 >					
< Penta-PCB 87 >					
Penta-PCB 85					
<< Penta-PCB 110 >>					
Penta-PCB 123 *					Moderate
[Penta-PCB 118 *]		< 0.3	0.36		Moderate
[Penta-PCB 105 *]		0.29	0.45		Moderate
<< Hexa-PCB 135 >>					
[Hexa-PCB 153 */168]		< 0.3 / --	0.36 / --	0.10 / --	
< Hexa-PCB 141 >					
Hexa-PCB 137					
[Hexa-PCB 138 */158]					
Hexa-PCB 129					
[Hexa-PCB 128]		< 0.3	0.49		Minor
[Hexa-PCB 167 *]					Minor
[Hexa-PCB 156 *]		< 0.3	0.44		Moderate
Hexa-PCB 157 *					Moderate
[Hepta-PCB 178]					
[Hepta-PCB 187]					
[Hepta-PCB 183]					
<< Hepta-PCB 174 >>					
Hepta-PCB 177					
Hepta-PCB 171					
[Hepta-PCB 180 */193]		0.00 / --	0.50 / --		
[Hepta-PCB 170 *]					
[Octa-PCB 202 *]					
[Octa-PCB 199]					
[Octa-PCB 194]					
[Nona-PCB 208 *]					
[Nona-PCB 206]					

* Denotes Quantitation with same ¹³C-PCB congener

PCBs 84, 52, 183, 187, 170, 101, 180, 18, and 110.⁸⁹ Most important was their finding that multiple congeners generated an additive effect.⁸⁹ Therefore for effective biomonitoring of PCB congeners, it appears to be highly beneficial to use a biological matrix like toenails (or hair) that retains or “sequesters” more transient but more potentially toxic compounds.

Biomonitoring of moderately-persistent PBDEs

Lipid-based concentrations of the moderately-persistent PBDEs in toenail samples are compared with extant sample data in Table 31. In Table 30 in the first three toenail samples (3T-1, 6T-2, and 7T-2) of individual A run in Sets 1 and 2, PBDE 47 (mean 761 ng/g-lipid, sd 208, n=3) and PBDE 99 (mean 679 ng/g-lipid, sd 222, n=3) were highest, followed by PBDEs 153 (164 ng/g-lipid, sd 55, n=3), PBDE 100 (mean 88 ng/g-lipid, sd 18, n=3), PBDE 28 (mean 34 ng/g-lipid, sd 14, n=3) and PBDE 17 (mean 14 ng/g-lipid, sd 5.8, n=3). With Set 2 for just non-polar compounds, PBDEs 49, 85, and 154 were also measured in each sample.

Unfortunately, as mentioned in Chapter 3, concentrations of some PBDEs in Set 4 toenail samples were compromised by PBDE contamination in the Proteinase K digesting enzyme found in both Set 4 blanks with the enzyme but not in the blank without the enzyme (Table 15, p.97). Blank subtraction of Set 4 samples almost completely eliminated most PBDEs associated with the “penta” formulation (PBDEs 17, 49, 47, 100, and to some extent, 99), but did not affect PBDEs 28, 85, 154, 153, 139, 138, 183, and 209.

With those limitations, PBDE 153 appears to be the best PBDE biomarker for comparing concentrations among all toenail samples with extant data. With all six toenail samples of individual A included (Table 30), the mean and standard deviation of PBDE 153 was 150 ± 49 ng/g-lipid. Although only one toenail sample was available for comparison from individual B, concentration of PBDE 153 in individual B (175 ng/g-lipid) was close to the mean value from individual A (150 ng/g-lipid). In contrast, PBDE 153 concentrations were lower in toenails from individuals C (mean 21 ng/g-lipid) and D (37 ng/g-lipid).

Extant lipid-adjusted PBDE data (Table 31) included CDC serum geometric mean and highest (95th) percentile,² the mean and range in adipose tissue samples from New York⁹⁰ and breast tissue samples from California,⁹¹ and median or mean and highest breast milk samples from North Carolina,⁹² 30 breast milk samples from the Philippines,⁹³ and 30 matching hair samples from the same Filipino women.⁹³ Four PBDEs (28, 47, 100, and 153) were quantitated by the CDC in sufficient number of serum samples to compute a geometric mean for each congener. The CDC sera geometric means closely matched the median lipid-based concentrations of breast milk collected in North Carolina.⁹² In a similar comparison for the higher chlorinated PCB congeners earlier, the CDC highest (95th) percentile for PBDEs 28, 47, 100, 99, 85, 154, and 153, generally matched the means of both the New York adipose tissue samples and the California breast tissue samples (Table 31). PBDE concentrations in toenails from this study exceeded the CDC highest (95th) percentile for sera and best matched the ranges observed in the adipose tissue samples from New York. Comparing PBDE

Table 30. Blank-Subtracted Lipid-Based Concentrations (ng/g-lipid) of PBDEs in Toenails

	TOENAIL SAMPLES									
	3T-1	6T-2	7T-2	15T-4	16T-4	17T-4	18T-4	20T-4	21T-4	22T-4
Date Sampled:	Nov 2005	Jan 2006	Mar 2006	Oct 2006	Feb 2007	May 2007	Jan 2010	Jan 2009	Apr 2009	Nov 2007
Sample Submitter	A	A	A	A	A	A	B	C	C	D
Mass (g):	0.187	0.192	0.190	0.340	0.295	0.159	0.0655	0.196	0.322	0.189
Lipid (%):	1.21	1.31	0.870	1.16	1.29	1.63	1.23	1.03	1.22	1.23
ANALYTE NAME	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
Tri-BDE 17	17	7	18	---	---	---	---	---	---	---
Tri-BDE 28	28	23	50	11	17	20	13	35	28	28
Tetra-BDE 49	---	39	86	---	---	---	---	---	---	---
Tetra-BDE 47	677	608	997	---	769	---	---	---	411	---
Tetra-BDE 66	---	16	---	---	---	---	---	---	---	---
Penta-BDE 100	84	72	108	---	---	---	---	---	---	---
Penta-BDE 99	522	582	933	239	567	161	1,140	42	306	---
Penta-BDE 85	---	13	30	18	23	20	58	18	23	13
Hexa-BDE 154	---	3	3	18	30	11	89	15	17	14
Hexa-BDE 153	108	165	218	100	194	115	175	16	27	37
Hexa-BDE 139	---	---	---	5	7	4	14	3	4	8
Hexa-BDE 138	---	---	---	---	---	---	29	13	5	---
Hepta-BDE 183	---	---	---	3	5	---	---	---	2	8
Octa-BDE 197	---	---	---	---	---	---	---	---	---	9
Nona-BDE 207	---	---	18	---	---	---	---	---	---	---
Nona-BDE 206	---	---	6	---	---	---	---	---	---	---
Deca-BDE 209	---	---	---	46	177	215	---	319	244	392
Bis-TriBr-PE	---	---	---	78	90	6	---	---	---	---

--- Not Detected at Specified Detection Limit or < [Blank Avg + 3SD]

Table 31. Lipid-Based Concentrations (ng/g-lipid) of PBDEs in Extant Samples

	SERUM (US)		ADIPOSE TISSUE (NY)			BREAST TISSUE (CA)			BREAST MILK (NC)		BREAST MILK-HAIR (Philippines)			
Date Sampled:	n=1960 2003-4		n = 52 2003-4			n = 152 2004			n=303 2004-6		n=30 MILK 2008		n=30 HAIR 2008	
Sample Submitter:	CDC		Johnson-Restrepo			Petreas et al.			Daniels et al.		Malarvannan		Malarvannan	
Mass (g):	Geo. 95th		et al.						CDC		et al.		et al.	
Lipid (%):	Mean % Tile		Mean Lowest Highest			Mean Lowest Highest			Median Highest		Mean Highest		Mean Highest	
ANALYTE NAME	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
Tri-BDE 17	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Tri-BDE 28	1.2	8.0	3.3	< 0.1	42	---	---	---	2	50	0.1	0.6	0.5	1.5
Tetra-BDE 49	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Tetra-BDE 47	21	163	132	1.3	2,720	86	4.3	3,141	28	1,430	1.3	4.6	8.8	33
Tetra-BDE 66	---	1.3	---	---	---	---	---	---	---	11	---	---	---	---
Penta-BDE 100	3.9	37	68	< 1	1,850	15	0.8	442	5	188	0.2	1.0	1.0	3
Penta-BDE 99	---	42	74	< 1	1,380	35	1.3	470	5	299	0.4	4.4	3.6	19
Penta-BDE 85	---	4.1	6.9	< 1	153	---	---	---	0.5	27	---	---	---	---
Hexa-BDE 154	---	4.2	8.3	< 1	240	3	0.2	82	0.3	13	---	0.4	0.5	2.8
Hexa-BDE 153	5.7	66	92	< 1	3,180	20	0.8	371	6	229	0.3	1.3	1.6	8.1
Hexa-BDE 139	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Hexa-BDE 138	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Hepta-BDE 183	---	---	---	---	---	---	---	---	---	15	---	0.5	1.3	7.5
Octa-BDE 197	---	---	---	---	---	---	---	---	---	---	0.2	0.8	1	3
Nona-BDE 207	---	---	---	---	---	---	---	---	---	---	0.1	0.3	4	11
Nona-BDE 206	---	---	---	---	---	---	---	---	---	---	---	---	5	15
Deca-BDE 209	---	---	---	---	---	---	---	---	---	---	0.5	3.4	44	140

--- Not Detected at Specified Detection Limit or < [Blank Avg + 3SD]

BLANK SPACE Denotes Not Targeted for Analysis

concentrations in breast milk from both North Carolina and the Philippines, Filipino women had concentrations approximately 20 times lower for the same tri- to hepta-PBDEs (Table 31). In matching hair samples from the same Filipino women, PBDE concentrations were generally about five times higher than in their breast milk, possibly demonstrating the hair's ability to integrate chronic exposures of PBDEs.⁹³ The combined matrices study of Filipino women was the only one to also analyze for the highest brominated PBDEs including the transient deca-BDE 209, which was about 50 times higher in hair than in breast milk and only slightly less than deca-BDE 209, when detected, in toenails from this study.

Two minor PBDEs, PBDE 17 (2,4- 2'-substituted) and PBDE 49 (2,4- 2',5'-), that were quantitated in individual A Set 2 toenail samples were not reported in any of the extant data in Table 31. PBDE 17 was targeted by CDC but was rarely if ever found in their sera samples.² PBDE 49 apparently was neither targeted nor reported in the extant data listed. However, recent biomedical neurotoxicity testing,^{81, 82, 94} again with ryanodine binding that affects calcium ion channel regulation, showed that both minor PBDEs 17 and 49 are more potent modulators of the ryanodine receptors than other PBDEs and may add to effects already noted for certain neurotoxic PCBs like PCB 95.⁹⁵ Because toenails may integrate chronic exposures of the moderately persistent PBDEs including potent neurotoxic congeners, it is very important to target the entire range of PBDEs even for minor and more transient compounds. This work also demonstrates that it is important to carefully evaluate and pre-screen any alternative reagents (such as Proteinase K) to avoid compromising any pertinent concentrations of PBDEs or other

analytes. In individual A Set 4 samples from 2007, a relatively new flame retardant, 1,2-bis(2,4,6-tribromophenoxy)ethane or Bis-TriBr-PE was detected and quantitated (Table 30) because it was included in the commercial calibration standard mixture after it was first reported in 2005 as a new alternative brominated flame retardant.⁹⁶

Biomonitoring Synthetic Musk Fragrance Compounds After Their Identification

Two isomeric synthetic musk fragrance compounds, galaxolide and tonalide, were added to the analyte list along with another isomer, traseolide, in Sample Set 4 for biomonitoring of non-polar compounds in toenail samples. After their tentative identification by GC/full-scan MS, they were later confirmed by both GC retention time and mass spectrum of each pure chemical standard. Researchers described them as potential endocrine-disrupting compounds, and found them in adipose tissue and breast milk, thereby providing an impetus in this study to determine whether they could be detected in human toenail samples.⁹⁷⁻¹⁰⁰ Because traseolide was very low in human toenails (Table 14), lipid-adjusted concentrations were shown for only galaxolide and tonalide, which were lowest in individual D (3,380 and 361 ng/g-lipid, respectively), followed by increasingly higher concentrations for individual A, C, and B (Table 32). For these two fragrance compounds, concentrations (8,960 and 1,050 ng/g-lipid, respectively) for the house cat were included to determine if the compounds would be detectable in cat nails above the levels in blanks and to contrast differing exposures to house cats and humans of compounds in human personal care products. Without having a daily shower or personal care products applied to skin like humans in what

Table 32. Blank-Subtracted Lipid-Based Concentrations (ng/g-lipid) of Musk Fragrances in Toenails and Extant Data

	TOENAIL SAMPLES							
	15T-4	16T-4	17T-4	18T-4	19T-4	20T-4	21T-4	22T-4
Date Sampled:	Oct 2006	Feb 2007	May 2007	Jan 2010	Apr 2010	Jan 2009	Apr 2009	Nov 2007
Sample Submitter	A	A	A	B	M-cat	C	C	D
Lipid (%):	1.16	1.29	1.63	1.23	1.12	1.03	1.22	1.23
Mass (g):	0.340	0.295	0.159	0.0655	0.120	0.196	0.322	0.189
ANALYTE NAME	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
Galaxolide (HHCB)	60,300	79,800	141,000	280,000	8,960	181,000	154,000	3,380
Tonalide (AHTN)	2,150	3,740	1,870	11,100	1,050	5,540	10,300	361

EXTANT DATA:	ADIPOSE TISSUE (NY)			BREAST MILK (MA)			BREAST MILK (Switzerland)		
	n = 49			n = 39			n = 54		
	2003-4			2004			2004-2006		
	Kannan et al.			Reiner et al.			Schlumpf et al.		
	Mean	Lowest	Highest	Mean	Lowest	Highest	Mean	Lowest	Highest
ANALYTE NAME	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
Galaxolide (HHCB)	178	12	798	220	< 5	917	55	6.1	310
Tonalide (AHTN)	42	< 8	134	47	< 5	144	14	4.8	29

could be called “therapeutic exposures,” the house cat would be primarily limited to air exposure and minor exposure to residual compounds on human hands when the cat was touched. For nails of both individual D and the house cat that had the lowest concentrations of fragrances, no fragrance compounds (above relatively high blanks) were detected in their soap washes in contrast to their appearance in soap washes of nails from the other three individuals. Soap wash concentrations for individuals A, B, and C ranged up to 30% of that measured in toenails from those individuals, which indicated some adsorption or absorption of the musk compounds likely occurred from direct topical application in a similar way as that reported for measuring selenium after nails were exposed for 5 min to selenium-containing Selsun Blue shampoo.¹⁸ The appearance of these compounds in the soap washes highlights the importance of multiple soap washes whenever targeting compounds associated with personal care products.

Comparing lipid-based toenail concentrations with extant sample concentrations in adipose tissue from New York, breast milk from Massachusetts, and from Switzerland, even the lowest toenail concentrations observed in this work (Individual D) were higher than the highest tissue or human milk sample from the US. Assuming the possibility of toenails integrating periodic exposures during several months of growth before being clipped, it appears plausible that commonly used synthetic musk compounds could be higher in the toenails than in other matrices. If high concentrations are found in soap washes of toenails, it is likely that higher concentrations are due to the nails adsorbing or absorbing topically applied compounds.

Biomonitoring of a Transient Polar Compound, Triclosan

The antibacterial compound, triclosan, was measured only as “free” form in the two toenail samples of Individual A in Set 3. Background-subtracted concentrations in the toenail samples averaged 58.3 ng/g with a standard deviation of 6.6 ng/g. Quantitation by GC/HRMS was aided by having the ¹³C-labelled surrogate of triclosan to correct for any recovery loss or variation through Set 3. Ordinarily, the transient compound triclosan has been biomonitoring in urine samples as the total of free and deconjugated forms by the CDC.^{2, 101} The geometric mean and 95th percentile total concentrations in µg/L (ppb) extant urine data are compared with the toenail data in Table 33. Also included were “total” triclosan concentrations from a post-mortem sampling of adipose tissue, liver, and brain from 11 cadavers from Belgium.¹⁰² Using the ¹³C-triclosan surrogate, Geens et al. (2012)¹⁰² detected triclosan in the highest concentrations and most prevalent (10 of 11) in the liver samples (mean 2.65 ng/g;

Table 33. Concentration (ng/g-wet weight) of Triclosan in Set 3 Toenails and Extant Data

	TOENAIL SAMPLES		ADIPOSE TISSUE -- LIVER (BELGIUM) --		BRAIN		URINE (US)	
	10T-3	11T-3	n = 11		n = 11		n = 11	
Date Sampled:	May 2006	Jul 2006	2002 - Post-mortem		2002 - Post-mortem		2002	
Sample Submitter	A	A	Geens et al.		Geens et al.		Geens et al.	
			Detected (7 of 11)		Detected (10 of 11)		Detected (1 of 11)	
Mass (g):	271.5	294.3	Mean	Highest	Mean	Highest	Mean	95th
ANALYTE NAME	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
Triclosan	53.6	62.9	0.61	3.92	2.65	24.8	0.23	13.0 461

highest 24.8 ng/g), followed by lower concentrations in fewer (7 of 11) adipose tissue samples, and finally a small amount in one brain.¹⁰² Compared with extant data of various other matrices on a “total” basis, the “free” concentrations of triclosan in Individual A toenails are several times higher than the CDC geometric “total” mean and within seven times of the 95th percentile “total” concentration. Triclosan in each soap wash of Individual A toenails (10T-3 and 11T-3) was relatively low (1.1 and 2.8 ng/g or 2-4%, respectively) indicating low exogenous contamination although the possibility of toenails being directly exposed to triclosan for adsorption or absorption through some unlabeled personal care product cannot be ruled out. Although only based on two sample detections without multiple soap washes, it is possible that more toenail research and analyses will show that toenails effectively integrate human periodic exposures to transient compounds such as triclosan.

Several tentatively-identified compounds were also detected by GC/full-scan MS in Set 3 toenail samples from Individual A for polar compounds and are listed in Table 34 with their respective Chem Abstracts numbers. Many of the compounds appear to be either flavor or fragrance agents including some possibly from natural sources. The substituted benzothiazole compound listed, likely originating as 6-hydroxy-2-methyl

benzothiazole, is similar to 2-hydroxy-benzothiazole that was recently found in indoor dust samples and associated with rubber tires.¹⁰³ It is not known whether the substituted benzothiazole found in toenails is used in the rubber pads or soles in tennis shoes. Another particular compound, octocrylene, is a common UV filter used in various sunscreen lotions that has become a suspect endocrine disrupting compound.^{99, 104} As with the musks, these compounds may be incorporated, in part, from topical application.

Table 34. Tentatively-Identified Unsuspected Compounds by Full-scan Mass Spectra in Toenail Samples from Set 3

Tentatively-Identified Compound or before Methylation	GC Time	Extract or Eluate Fraction	Possible Origin - Commercial Uses	CAS Number	Possible Effects
Methyl-2-amino-benzoate likely as: Methyl-2-amino-benzoic acid	8.56	pH<3, methylated, DCM	In essential (Neroli) oil derived from citrus (Methyl Anthranilate)	134-20-3	Estrogenic
4-Methoxy-benzoate likely as: 4-Methoxy-benzoic acid or 4-Hydroxy-benzoic acid	9.37	pH<3, methylated, DCM	Food additive or flavoring agent Occurs in anise (flavoring agent) Natural/synthetic anti-oxidant	121-98-2 100-09-4 99-96-7	
Dimethyl quinazoline	9.75	DCM, methylated	Possible drug intermediate	703-63-9	
Methyl 2-(dimethylamino)-benzoate likely as: 2-dimethylamino-benzoic acid	9.95	pH<3, methylated, DCM	Widely sold-related to essential (Neroli) oil	10072-05-6 610-16-2	
Methyl 2-(methylamino)-benzoate likely as: 2-methylamino-benzoic acid	10.19	pH<3, methylated, DCM	Food flavor or fragrance Food flavor or fragrance	85-91-6 119-68-6	
6-Methoxy-2-Methyl-Benzothiazole or as: (6-Hydroxy-2-Methyl-Benzothiazole)	15.25	DCM, methylated	Widely sold	2941-72-2 19989-66-3	
2-(acetyl amino)-benzoate likely as: 2-(acetyl amino)-benzoic acid	16.45	pH<3, Early GPC, DCM, methylated	Food flavor or fragrance Drug intermediate or fragrance	2719-08-6 89-52-1	
Benzophenone	17.44	DCM, methylated	UV filter, derivatives are in sunscreen	119-61-9	
Anisalacetone	18.52	DCM, methylated	A flavoring agent	943-88-4	
2-Anilino-4-methyl-quinoline	31.95	DCM, methylated	Produced, uses unknown	10562-04-6	
Octocrylene	46.87	DCM, methylated	UV Filter, in sun-blocking lotions	6197-30-4	

House cats as possible “sentinels” of home-related pollutants for humans?

During the past decade, researchers were still studying why the flame retardant PBDE levels in biomonitors had increased exponentially since the 1980s, doubling every

five years, and why PBDE concentrations in people from the US were about 20 times higher than those in people from Europe.⁵¹ PBDEs were not only in adults but in new born children and often even higher in toddlers and young children. Because PBDEs have been used in percent levels in home furnishings of mattresses, couches, and electronics, Stapleton et al. (2004)¹⁰⁵ analyzed house dust and dryer lint samples from 17 homes and found PBDEs in every sample, the total ranging from 0.8 to 30 µg/g. Jones-Otazo et al. (2005)⁵⁰ concluded, “Inadvertent ingestion of house dust is the largest contributor to exposure of toddlers through to adults.” Meanwhile, a dramatic increase in feline hyperthyroidism for indoor cats, possibly affected by PBDEs, prompted Dye et al. (2007)¹⁰⁶ to measure PBDEs in house cat serum as a possible “sentinel” for human exposure. Although they did not also measure PBDEs in humans living with the cats, they found total PBDEs in their 23 cat samples ranged 20- to 100-fold greater than median PBDE levels in US adults reported by others.¹⁰⁶ Lacking a way to measure lipid in the small cat serum samples without sacrificing part of their sample, they reported total PBDEs averaged 4.3 ng/mL-blood for young cats, 10.5 ng/mL for older cats without hyperthyroidism, and 12.7 ng/mL for cats with hyperthyroidism.¹⁰⁶ Assuming lipid in cat serum is < 1%, total PBDEs in lipid-adjusted concentrations likely approached or exceeded 1000 ng/g-lipid. However, there was too much variability in their small number of samples to directly associate total PBDEs with hyperthyroidism.

Within the past year, researchers of cats in California,¹⁰⁷ Pakistan,¹⁰⁸ and Sweden¹⁰⁹ have reported PBDEs in cat serum. Their lipid-based PBDE concentrations are compared with one cat toenail sample in this study (Table 35). Although we

Table 35. Lipid-Based Concentrations (ng/g-lipid) of PBDEs in Cat Toenails and Extant Cat Serum

	Toenails	SERUM from CA House Cats			SERUM - Pakistan House Cats			SERUM
Date Sampled:	19T-4	n = 26			n = 20			Pool of 16
Sample Submitter	Apr 2010	2008-2010			2012			SWEDEN
Mass (g):	M-cat	Guo et al. (2012)			Ali et al. (2013)			Norrgran et al.
Lipid (%):	0.120							Cats w/Hyper-
	1.12	Mean	Lowest	Highest	Mean	Lowest	Highest	thyroidism
ANALYTE NAME	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
Tri-BDE 17	---	0.9	---	4.6				
Tri-BDE 28	17	4.5	0.4	47.5				
Tetra-BDE 49	---							
Tetra-BDE 47	---	830	49.3	4,941	28	< 0.5	525	250
Tetra-BDE 66	---	2.2	---	12.9				
Penta-BDE 100	---	93	7.8	826				Est. 25
Penta-BDE 99	1,910	1,884	176	13,554	36	< 0.4	665	450
Penta-BDE 85	71	21	0.7	126				
Hexa-BDE 154	174	155	13.1	1,046	1.8	< 0.2	16	Est. 40
Hexa-BDE 153	311	293	21.2	1,695	3.9	0.35	45	Est. 80
Hexa-BDE 139	41							
Hexa-BDE 138	40							
Hepta-BDE 183	17	20	3	74.4				Est. 30
Octa-BDE 197	4	29	4.5	153				
Nona-BDE 207	---	122	13.2	1,391				
Nona-BDE 206	---	20	---	318				
Deca-BDE 209	283	961	23.2	14,276				500
Bis-TriBr-PE	---				1.6	< 1	9	

Est. Estimated from Figure 1 in Norrgran et al. (2012).

--- Not Detected at Specified Detection Limit or < [Blank Avg + 3SD]

BLANK SPACE Denotes Not Targeted for Analysis

analyzed only one sample, cat toenail PBDE concentrations detected above the blanks compare closely to the mean concentrations found in the serum of cats from California.¹⁰⁷

Penta-BDE 99 was highest in the cat toenail sample, consistent with that reported in all PBDE-cat-related studies, and sufficiently high to exceed the increased blank level for PBDE 99 (but not for PBDE 47) in Set 4 toenail samples. In humans and

many other animals, PBDE 99 is typically lower, more transient and more readily metabolized. PBDE 153 (311 ng/g-lipid) in the cat toenail sample compared closely to the California cat serum mean (293 ng/g-lipid).

In Table 36, extant lipid-adjusted concentrations of several POPs pesticides from the California and Pakistan cat studies were compared with the single cat toenail sample from this work. Oxychlordane and p,p'-DDE were again both relatively low in the cat toenail compared with that in serum from California cats, perhaps in a similar way as previously discussed for human toenails. In the context of a "sentinel," the higher

Table 36. Lipid-Based Concentrations (ng/g lipid) of Pesticides in Cat Toenails and Extant Cat Serum

	TOENAILS	SERUM from CA House Cats			SERUM - Pakistan House Cats		
	19T-4	n = 26			n = 20		
Date Sampled:	Apr 2010	2008-2010			2012		
Sample Submitter	M-cat	Guo et al. (2012)			Ali et al. (2013)		
Mass (g):	0.120						
Lipid (%):	1.12	Mean	Lowest	Highest	Mean	Lowest	Highest
ANALYTE NAME	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
Hexachlorobenzene	---	7.0	4.0	14.1	3.7	< 2	6.9
Pentachloroanisole	85						
Methyl (ether) Triclosan	---						
gamma-HCH (Lindane)	13						
beta-HCH	---	0.3	---	2.6			
Dieldrin	---						
Chlordanes:							
Oxychlordane	23	111	---	2,108			
Heptachlor Epoxide	28						
trans-Chlordane	1,210						
cis-Chlordane	734						
trans-Nonachlor	846	318	---	6,315			
cis-Nonachlor	71						
DDE and DDTs:							
o,p'-DDE	---						
p,p'-DDE	60	530	83	1,746	282	< 1	2,150
o,p'-DDT	---	0.03	---	0.5			
p,p'-DDT	---	58	---	365			

--- Not Detected or not (detected) above method blank

BLANK SPACE Denotes Not Targeted for Analysis

chlordanes concentrations in the cat toenail indicate exposure in the home to chlordanes. Because extant lipid-adjusted concentrations of PCB congeners were available for comparison from the California cats study, comparison was made with concentrations of the majority of PCB congeners found in the one cat toenail (Table 33). Concentrations of the most persistent and higher-chlorinated PCB congeners in the cat toenail are within the range of that found in California cat serum samples. However, the more transient PCB congeners in mammals were detected and noticeably higher in the one cat toenail than in the CA cat serum samples. In this work, based on the example with chlordanes and PBDEs, if PCBs had been used in the home construction, it is likely that a cat would be a possible “sentinel” to indicate PCB exposure in its toenails.

Summary

Our hypothesis that the toenail offers a non-invasive biomonitoring sampling matrix for measuring chronic environmental organic pollutant exposure has been shown to be valid and even advantageous for compounds that are chronically exposed but transient and less likely to be effectively measured in traditional serum samples. Toenail samples are easy to collect, can be stored for years at room temperature, and are safe to ship or process without worry of biohazards like viruses. A nail’s strong keratin structure helps shield it from most external contamination and its slow growth integrates chronic internal exposure from the blood stream. Alternating hydrophilic/lipophilic layers within the nail plate enables incorporation of both lipophilic and hydrophilic organic compounds.

Table 37. Lipid-Based Concentrations (ng/g-lipid) of PCBs in Cat Toenails and Extant Cat Serum

	TOENAILS	SERUM from CA House Cats			SERUM - Pakistan House Cats		
Date Sampled:	19T-4 Apr 2010	n = 26 2008-2010 Guo et al. (2012)			n = 20 2012 Ali et al. (2013)		
Sample Submitter	M-cat						
Mass (g):	0.120						
Lipid (%):	1.12	Mean	Lowest	Highest	Mean	Lowest	Highest
ANALYTE NAME	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
< Tri-PCB 18 >	94						
< Tri-PCB 31 > -28 *	78						
< Tetra-PCB 52 * >	66						
< Tetra-PCB 44 >	42						
Tetra-PCB 74	38	4.1	---	11.5			
< Tetra-PCB 70 * >	122						
Tetra-PCB 66	56	3.8	---	14.9			
< << Penta-PCB 95 * >> >	186						
<< Penta-PCB 84 >>	42						
< Penta-PCB 101 * >	256	17	---	41.2	6.9	1.5	25
[Penta-PCB 99]	84	17	---	43.7			
< Penta-PCB 97 >	87						
< Penta-PCB 87 >	146						
<< Penta-PCB 110 >>	187						
[Penta-PCB 118 *]	208	18	---	42.9	7.0	1.7	26
[Penta-PCB 105 *]	188	3.0	---	11.3			
<< Hexa-PCB 135 >>	34						
[Hexa-PCB 153 */168]	83	49	---	158	7.4	< 5	27
[Hexa-PCB 138 */158]	88	26	---	91.3	10.4	3.5	33
[Hexa-PCB 156 *]	17	2.1	---	6.8			
[Hepta-PCB 187]	26	16	---	49.6			
[Hepta-PCB 183]	13	9.2	---	65.4			
[Hepta-PCB 180 */193]	9	24	---	78.4			
[Hepta-PCB 170 *]	14	4.9	---	19.8			
[Octa-PCB 194]	---	5.0	---	16.0			
SUM: 52 PCB congeners	2,502	--- Not Detected or not (detected) above method blank					
		BLANK SPACE Denotes Not Targeted for Analysis					
	KEY:						
< >	Less Persistent (2,5-ring Cl substituents)						
<< >>	Less Persistent (2,3,6-ring Cl substituents)						
[]	More Persistent (no vicinal H-substituents)						

Metabolites formed within the human body, e.g. oxychlordane, were remarkably low in toenail samples and less appropriate for toenail biomonitoring. Toenail concentrations of POPs pesticides and persistent PCB congeners that are likely in steady state in the body, including hexachlorobenzene, beta-hexachlorocyclohexane, heptanona-PCBs, and chlordanes for those living in newer homes matched geometric means of CDC's latest national serum survey, but with sample mass 10-20 times less than serum, toenail biomonitoring analysis could be more difficult to achieve detection limits near < 0.1 ng/g wet weight or < 10 ng/g-lipid.

Following the Needham speciation approach, compounds favored by toenail biomonitoring include those chronically or episodically exposed, including chlordanes associated with pre-1989 homes treated for termites, more transient pesticides (DDT), elusive but potentially neurotoxic tri-hexa-PCBs with chlorines 2,5- and/or 2,3,6-substituted, PBDEs including penta-BDE 99, and, if not topically applied, triclosan. With further steps to reduce method background and/or improve method recovery, it may be possible to monitor bisphenol A and tetrabromobisphenol A. For biomonitoring of infants or young children, pooling several toenail samples from the individual might provide sufficient mass for optimum analysis. Although the small mass of toenail clippings requires an optimized method of sample preparation with minimal lab contamination, no sample carryover, and sensitive analytical instrument(s), its use appears to be justified especially for measuring chronic exposure to elusive but potentially toxic compounds. A faster and more productive analytical method should be possible using a mill with small, individualized stainless steel vessels to pulverize and

reduce pre-rinsed toenail samples to extremely small pieces with high surface area to enable rapid but mild digestion for subsequent extraction and cleanup. Great care would be necessary to avoid any lab dust getting into the vessels or any nail powder or aerosol contaminating the lab or any other vessel. Once extracted, trace-level analytical technique with few steps, minimal size, quality, and volume of solvents and reagents, high performance chromatographic columns, and instruments (GC/HRMS and LC/MS/MS) would suffice.

CHAPTER 5. LIMITATIONS AND FUTURE RESEARCH

Improve Toenail Digestion and Extraction

Toenails were not fully digested or dissolved by the various mild chemical and biochemical reagents used and resulting concentrations of partially digested extracts were probably lower than if the toenail matrix was completely digested and dissolved for solvent extraction. One way to improve digesting the nail is to first pulverize it into smaller pieces first, ideally using a commercial miniature mill grinder, such as the one made by Fritsch in Germany and recently used for nail and hair samples.³⁰ For chemical digestion, a mild basic buffer (at or below pH 11) may help and may not break down too many target analytes. Some phenolics will begin as anions, so a later pH 5 or pH 3 extraction may be appropriate to extract any polar phenolic compounds. Once digested, there is a need to avoid or minimize a resulting emulsion after digestion with solvent extraction as it drastically slows down the initial extraction process before preparing for flash GPC. An additional centrifugation step should be tried to assess effectiveness. For any future PBDE analyses with toenail samples, the results from Set 4 using Proteinase K enzyme show the need to either find a different source of that enzyme that has very low background of PBDEs or avoid the use of Proteinase K entirely. Concerning the redox reagent DTT for use in nail digestion, it should be tested with all target analytes, especially those with poor method recoveries, ie. phthalate metabolites.

Target Compounds with Shorter-Half Lives whose Exposures Might be Integrated in Nails

Bisphenol A is a very important compound to justify targeting and to achieve a consistently low lab background. In principle, an ideal method for BPA would use a miniaturized method with low volumes of reagents and solvents, relatively few procedures exposed to lab aerosol and dust, and ideally, a choice of highly sensitive instruments, a GC/HRMS that will presume a methylation step and an HPLC/MS/MS tandem system that can measure both free BPA and the BPA-glucuronide and sulfate conjugated forms for a complete sum of BPA in the nail matrix. In that same group, include tetrabromobisphenol A to evaluate and optimize better method recoveries. Chlorinated and brominated BPA compounds could also be included because the chlorinated BPAs and BPA were reported in adipose tissue of women from Spain.¹¹⁰ Brominated BPAs were identified in trace ($\mu\text{g/L}$) levels in local Culligan commercial bottled water.¹¹¹ If lab background of BPA for toenail samples can only be reduced partially, that may necessitate pooling two or three dates of toenail clippings from the same person to double or triple the sample mass (to 0.5-1 g) in order to overcome the lab background limitations.

If targeted, phthalate metabolites should be analyzed with a relatively modern HPLC/MS/MS. Any future method for phthalate metabolites in nail samples will also have to optimize the purity of the deconjugating enzyme to avoid possible lab background of phthalates being then incidentally and unintentionally converted to the phthalate metabolite and causing false positives.

With the use of an HPLC/MS/MS operated in negative ion electrospray mode but with different ions and somewhat different chromatography, several new analytes could be targeted with a modified or separate method for certain perfluoroalkyl organic compounds. For example, two of the most common compounds would complement very recent data reported by Chinese researchers in small biomonitoring studies using nail and other matrices.^{29, 30} The obvious two most common perfluoro-alkyl organics would be perfluorooctane sulfonate or PFOS and perfluorooctanoic acid or PFOA. One of the challenges again with these perfluorinated compounds would be to avoid lab background from Teflon or related perfluorinated plastic tubing, Teflon septa, and from other unforeseen seals or lubricating items.

Continue to Search for and Identify Unsuspected Compounds

Unsuspected analytes should be considered for human biomonitoring research by certain broad-based GC or HPLC/full scan MS acquisition data. Instead of GC/MS with EI mass spectra that match a mass spectral data base, identifying HPLC/MS data typically requires another MS stage after a collision cell to generate diagnostic ion fragments and ion losses. Unknowns often remain unknown until accurate mass measurements (from a specialized HPLC/MS/MS) provide diagnostic elemental formulas for further identification. With the integration of various possible exposures during two to three months of nail growth, it is quite possible that even polar and transient compounds and/or conjugated metabolites could be detected and identified by a thorough, broad-based type of GC/MS and/or HPLC/MS analysis.

Future Toenail Biomonitoring Research – Validate with a Diverse Population, Target Compounds with Known or Suspected Exposures, and Other Matrices

Concerning any and all further toenail biomonitoring research and evaluating whether a toenail sample is a suitable noninvasive matrix, it is hoped that researchers continue in the spirit of the late Larry Needham, who effectively advocated a “speciation” approach to human biomonitoring for the ultimate good of society.⁶ Needham’s approach advocated to find the best or suitable matrices for a specific chemical or, if necessary, for its metabolite. This preliminary toenail study evaluated whether a variety of organic compounds of varying persistency could be measured in toenails at or often below 1 ng/g-nail. This work also demonstrated that, on a lipid-adjusted basis, some compounds either matched or exceeded concentrations measured in other matrices used for biomonitoring indicating some potential for integrating exposures.

Future research is necessary to validate toenails as a biomonitor by testing samples from a diverse population of age, gender, race, body mass index, occupations, diets, geographic location, and other details. Validation might also include a study with known or relatively controlled exposures and exposure pathways based on work, home, or life-style. Timing or estimating exposures with toenails will not be very predictable compared with that using hair linear growth rate, but the generally lower exogenous contamination in toenails compared with hair should definitely be a positive factor for its use in biomonitoring. Ideally, at least some compounds will be present at sufficiently

high concentrations for replicate analysis on the same individual's sample, perhaps on each foot separately.

BIBLIOGRAPHY

1. Needham, L. L.; Calafat, A. M.; Barr, D. B., Uses and issues of biomonitoring. *International Journal of Hygiene and Environmental Health* **2007**, *210*, (3–4), 229-238.
2. CDC, Fourth Report on Human Exposure to Environmental Chemicals. In U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. <http://www.cdc.gov/exposurereport/>; Atlanta, GA, 2009.
3. Carson, R., *Silent Spring (Drawings by Lois and Louis Darling.)*. Houghton Mifflin: Boston, 1962; p 368.
4. Angerer, J.; Ewers, U.; Wilhelm, M., Human biomonitoring: State of the art. *International Journal of Hygiene and Environmental Health* **2007**, *210*, (3–4), 201-228.
5. Erickson, M. D., Introduction: PCB properties, uses, occurrence, and regulatory history. In *PCBs - Recent Advances in Environmental Toxicology and Health Effects*, Robertson, L. W.; Hansen, L. G., Eds. University Press of Kentucky: Lexington, KY, 2001; pp xi-xxx.
6. Needham, L.; Patterson, D., Jr.; Barr, D.; Grainger, J.; Calafat, A., Uses of speciation techniques in biomonitoring for assessing human exposure to organic environmental chemicals. *Anal Bioanal Chem* **2005**, *381*, (2), 397-404.
7. Kutz, F. W.; Wood, P. H.; Bottimore, D. P., Organochlorine Pesticides and Polychlorinated Biphenyls in Human Adipose Tissue. In *Reviews of Environmental Contamination and Toxicology*, Ware, G. W., Ed. Springer-Verlag: New York, 1991; Vol. 120, pp 1-82.
8. Esteban, M.; Castaño, A., Non-invasive matrices in human biomonitoring: A review. *Environment International* **2009**, *35*, (2), 438-449.
9. Stellman, S. D.; Djordjevic, M. V.; Muscat, J. E.; Gong, L.; Bernstein, D.; Citron, M. L.; White, A.; Kemeny, M.; Busch, E.; Nafziger, A. N., Relative abundance of organochlorine pesticides and polychlorinated biphenyls in adipose tissue and serum of women in Long Island, New York. *Cancer Epidemiology Biomarkers & Prevention* **1998**, *7*, (6), 489-496.
10. Norén, K.; Weistrand, C.; Karpe, F., Distribution of PCB Congeners, DDE, Hexachlorobenzene, and Methylsulfonyl Metabolites of PCB and DDE Among Various Fractions of Human Blood Plasma. *Arch. Environ. Contam. Toxicol.* **1999**, *37*, (3), 408-414.
11. Yu, G. W.; Laseter, J.; Mylander, C., Persistent Organic Pollutants in Serum and Several Different Fat Compartments in Humans. *Journal of Environmental and Public Health* **2011**, 2011.

12. Rönn, M.; Lind, L.; Bavel, B. v.; Salihovic, S.; Michaëlsson, K.; Lind, P. M., Circulating levels of persistent organic pollutants associate in divergent ways to fat mass measured by DXA in humans. *Chemosphere* **2011**, *85*, (3), 335-343.
13. Lakind, J. S.; Wilkins, A. A.; Bates, M. N., Human breast biomonitoring and environmental chemicals: use of breast tissues and fluids in breast cancer etiologic research. *J Expos Sci Environ Epidemiol* **2007**, *17*, (6), 525-540.
14. Fukata H, O. M., Osada H, Todaka E, Mori C., Necessity to measure PCBs and organochlorine pesticide concentrations in human umbilical cords for fetal exposure assessment. *Environmental Health Perspectives* **2005**, *113*, (3), 297-303.
15. Duty, S. M.; Ackerman, R. M.; Calafat, A. M.; Hauser, R., Personal Care Product Use Predicts Urinary Concentrations of Some Phthalate Monoesters. *Environmental Health Perspectives* **2005**, *113*, (11), 1530-1535.
16. Sukumar, A., Human Nails as a Biomarker of Element Exposure. *Rev Environ Contam Toxicol* **2006**, *185*, 141-177.
17. Nichols, T. A.; Morris, J. S.; Mason, M. M.; Spate, V. L.; Baskett, C. K.; Cheng, T. P.; Tharp, C. J.; Scott, J. A.; Horsman, T. L.; Colbert, J. W.; Rawson, A. E.; Karagas, M. R.; Stannard, V., The study of human nails as an intake monitor for arsenic using neutron activation analysis. *Journal of Radioanalytical and Nuclear Chemistry* **1998**, *236*, (1-2), 51-57.
18. Morris, J. S.; Spate, V. L.; Ngwenyama, R. A., Determinants of selenium in the toenail biomonitor. *Journal of Radioanalytical and Nuclear Chemistry* **2006**, *269*, (2), 283-290.
19. Mandal, B. K.; Ogra, Y.; Suzuki, K. T., Speciation of arsenic in human nail and hair from arsenic-affected area by HPLC-inductively coupled argon plasma mass spectrometry. *Toxicology and Applied Pharmacology* **2003**, *189*, (2), 73-83.
20. Slotnick, M. J., Toenails for Biomonitoring of Environmental Exposures. In *Encyclopedia of Environmental Health*, Editor-in-Chief: Jerome, O. N., Ed. Elsevier: Burlington, 2011; pp 360-366.
21. Brockman, J.; Raymond, L.; Ralston, C.; Robertson, J. D.; Bodkin, N.; Sharp, N.; Ralston, N. C., The Nail as a Noninvasive Indicator of Methylmercury Exposures and Mercury/Selenium Molar Ratios in Brain, Kidney, and Livers of Long-Evans Rats. *Biol Trace Elem Res* **2011**, *144*, (1-3), 812-820.
22. Karagas, M. R.; Morris, J. S.; Weiss, J. E.; Spate, V.; Baskett, C.; Greenberg, E. R., Toenail samples as an indicator of drinking water arsenic exposure. *Cancer Epidemiology Biomarkers & Prevention* **1996**, *5*, (10), 849-852.
23. Hussein Were, F.; Njue, W.; Murungi, J.; Wanjau, R., Use of human nails as bio-indicators of heavy metals environmental exposure among school age children in Kenya. *Science of The Total Environment* **2008**, *393*, (2-3), 376-384.
24. Kitahara, T. O., H., The extraction and characterization of human nail keratin. *J. Dermatol. Sci.* **1991**, *2*, 402-406.
25. Palmeri, A.; Pichini, S.; Pacifici, R.; Zuccaro, P.; Lopez, A., Drugs in Nails: Physiology, Pharmacokinetics and Forensic Toxicology. *Clinical Pharmacokinetics* **2000**, *38*, (2), 95-110.

26. Kobayashi, Y.; Miyamoto, M.; Sugibayashi, K.; Morimoto, Y., Drug Permeation through the Three Layers of the Human Nail Plate. *Journal of Pharmacy and Pharmacology* **1999**, *51*, (3), 271-278.
27. Kobayashi, Y.; Komatsu, T.; Sumi, M.; Numajiri, S.; Miyamoto, M.; Kobayashi, D.; Sugibayashi, K.; Morimoto, Y., In vitro permeation of several drugs through the human nail plate: relationship between physicochemical properties and nail permeability of drugs. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences* **2004**, *21*, (4), 471-477.
28. Debruyne, D.; Coquerel, A., Pharmacokinetics of antifungal agents in onychomycoses. *Clinical Pharmacokinetics* **2001**, *40*, (6), 441-472.
29. Liu, W.; Xu, L.; Li, X.; Jin, Y. H.; Sasaki, K.; Saito, N.; Sato, I.; Tsuda, S., Human Nails Analysis as Biomarker of Exposure to Perfluoroalkyl Compounds. *Environmental Science & Technology* **2011**, *45*, (19), 8144-8150.
30. Li, J.; Guo, F.; Wang, Y.; Zhang, J.; Zhong, Y.; Zhao, Y.; Wu, Y., Can nail, hair and urine be used for biomonitoring of human exposure to perfluorooctane sulfonate and perfluorooctanoic acid? *Environment International* **2013**, *53*, (0), 47-52.
31. Srogi, K., Testing for Drugs in Hair – a Review of Chromatographic Procedures. *Microchimica Acta* **2006**, *154*, (3), 191-212.
32. Covaci, A.; Schepens, P., Chromatographic aspects of the analysis of selected persistent organochlorine pollutants in human hair. *Chromatographia* **2001**, *53*, (1), S366-S371.
33. Covaci, A.; Tutudaki, M.; Tsatsakis, A. M.; Schepens, P., Hair analysis: another approach for the assessment of human exposure to selected persistent organochlorine pollutants. *Chemosphere* **2002**, *46*, (3), 413-418.
34. Altshul, L.; Covaci, A.; Hauser, R., The Relationship between Levels of PCBs and Pesticides in Human Hair and Blood: Preliminary Results. *Environmental Health Perspectives* **2004**, *112*, (11), 1193-1199.
35. Turusov, V.; Rakitsky, V.; Tomatis, L., Dichlorodiphenyltrichloroethane (DDT): ubiquity, persistence, and risks. *Environmental Health Perspectives* **2002**, *110*, (2), 125-128.
36. López-Carrillo, L.; Torres-Arreola, L.; Torres-Sánchez, L.; Espinosa-Torres, F.; Jiménez, C.; Cebrián, M.; Waliszewski, S.; Saldate, O., Is DDT use a public health problem in Mexico? *Environmental Health Perspectives* **1996**, *104*, (6), 584-588.
37. Bitman, J.; Cecil, H. C.; Harris, S. J.; Feil, V. J., Estrogenic activity of o,p'-DDT metabolites and related compounds. *Journal of Agricultural and Food Chemistry* **1978**, *26*, (1), 149-151.
38. Danzo, B. J., Environmental Xenobiotics May Disrupt Normal Endocrine Function by Interfering with the Binding of Physiological Ligands to Steroid Receptors and Binding Proteins. *Environmental Health Perspectives* **1997**, *105*, (3), 294-301.
39. Colborn, T.; Saal, F. S. v.; Soto, A. M., Developmental Effects of Endocrine-Disrupting Chemicals in Wildlife and Humans. *Environmental Health Perspectives* **1993**, *101*, (5), 378-384.

40. Kelce, W. R.; Wilson, E. M., Environmental antiandrogens: developmental effects, molecular mechanisms, and clinical implications. *J Mol Med* **1997**, *75*, (3), 198-207.
41. Colborn, T. E.; Dumanoski, D.; Myers, J. P., *Our Stolen Future: Are We Threatening our Fertility, Intelligence, and Survival? — A Scientific Detective Story*. Penguin Books: New York, 1997.
42. Schettler, T.; Solomon, G.; Valenti, M.; Huddle, A., *Generations at Risk – Reproductive Health and the Environment*. MIT Press: Cambridge, MA, 1999.
43. Barr, J. R.; Maggio, V. L.; Barr, D. B.; Turner, W. E.; Sjödin, A.; Sandau, C. D.; Pirkle, J. L.; Needham, L. L.; Patterson Jr, D. G., New high-resolution mass spectrometric approach for the measurement of polychlorinated biphenyls and organochlorine pesticides in human serum. *Journal of Chromatography B* **2003**, *794*, (1), 137-148.
44. Meerts, I. A. T. M.; Letcher, R. J.; Hoving, S.; Marsh, G.; Bergman, Å.; Lemmen, J. G.; Burg, B. v. d.; Brouwer, A., In Vitro Estrogenicity of Polybrominated Diphenyl Ethers, Hydroxylated PBDEs, and Polybrominated Bisphenol A Compounds. *Environmental Health Perspectives* **2001**, *109*, (4), 399-407.
45. Meerts, I. A. T. M.; van Zanden, J. J.; Luijckx, E. A. C.; van Leeuwen-Bol, I.; Marsh, G.; Jakobsson, E.; Bergman, Å.; Brouwer, A., Potent Competitive Interactions of Some Brominated Flame Retardants and Related Compounds with Human Transthyretin in Vitro. *Toxicological Sciences* **2000**, *56*, (1), 95-104.
46. Hale, R. C.; La Guardia, M. J.; Harvey, E.; Mainor, T. M., Potential role of fire retardant-treated polyurethane foam as a source of brominated diphenyl ethers to the US environment. *Chemosphere* **2002**, *46*, (5), 729-735.
47. Boxall, B., Manufacturers to phase out toxic flame retardant; In a deal with federal regulators, use of deca will end by 2013. *Los Angeles Times* 2009, p A.57.
48. Gandhi, N.; Bhavsar, S. P.; Gewurtz, S. B.; Tomy, G. T., Can biotransformation of BDE-209 in lake trout cause bioaccumulation of more toxic, lower-brominated PBDEs (BDE-47, -99) over the long term? *Environment International* **2011**, *37*, (1), 170-177.
49. de Wit, C. A., An overview of brominated flame retardants in the environment. *Chemosphere* **2002**, *46*, (5), 583-624.
50. Jones-Otazo, H. A.; Clarke, J. P.; Diamond, M. L.; Archbold, J. A.; Ferguson, G.; Harner, T.; Richardson, G. M.; Ryan, J. J.; Wilford, B., Is House Dust the Missing Exposure Pathway for PBDEs? An Analysis of the Urban Fate and Human Exposure to PBDEs. *Environmental Science & Technology* **2005**, *39*, (14), 5121-5130.
51. Hites, R. A., Polybrominated Diphenyl Ethers in the Environment and in People: A Meta-Analysis of Concentrations. *Environmental Science & Technology* **2004**, *38*, (4), 945-956.
52. Petreas, M.; She, J.; Brown, F. R.; Winkler, J.; Windham, G.; Rogers, E.; Zhao, G.; Bhatia, R.; Charles, M. J., High Body Burdens of 2,2',4,4'-Tetrabromodiphenyl Ether (BDE-47) in California Women. *Environmental Health Perspectives* **2003**, *111*, (9), 1175-1179.
53. D'Havé, H.; Covaci, A.; Scheirs, J.; Schepens, P.; Verhagen, R.; De Coen, W., Hair as an indicator of endogenous tissue levels of brominated flame retardants in mammals. *Environmental Science & Technology* **2005**, *39*, (16), 6016-6020.

54. Hauser, R.; Meeker, J. D.; Singh, N. P.; Silva, M. J.; Ryan, L.; Duty, S.; Calafat, A. M., DNA damage in human sperm is related to urinary levels of phthalate monoester and oxidative metabolites. *Human Reproduction* **2007**, *22*, (3), 688-695.
55. Anderson, W. A. C.; Castle, L.; Scotter, M. J.; Massey, R. C.; Springall, C., A biomarker approach to measuring human dietary exposure to certain phthalate diesters. *Food Additives & Contaminants* **2001**, *18*, (12), 1068-1074.
56. Silva, M.; Barr, D.; Reidy, J.; Kato, K.; Malek, N.; Hodge, C.; Hurtz, D., III; Calafat, A.; Needham, L.; Brock, J., Glucuronidation patterns of common urinary and serum monoester phthalate metabolites. *Arch Toxicol* **2003**, *77*, (10), 561-567.
57. Blount, B. C.; Silva, M. J.; Caudill, S. P.; Needham, L. L.; Pirkle, J. L.; Sampson, E. J.; Lucier, G. W.; Jackson, R. J.; Brock, J. W., Levels of Seven Urinary Phthalate Metabolites in a Human Reference Population. *Environmental Health Perspectives* **2000**, *108*, (10), 979-982.
58. Högberg, J.; Hanberg, A.; Berglund, M.; Skerfving, S.; Remberger, M.; Calafat, A. M.; Filipsson, A. F.; Jansson, B.; Johansson, N.; Appelgren, M.; Håkansson, H., Phthalate Diesters and Their Metabolites in Human Breast Milk, Blood or Serum, and Urine as Biomarkers of Exposure in Vulnerable Populations. *Environmental Health Perspectives* **2008**, *116*, (3), 334-339.
59. Peterman, P. H.; Delfino, J. J.; Dube, D. J.; Gibson, T. A.; Priznar, F. J., Chloro-organic compounds in the Lower Fox River, Wisconsin. In *Hydrocarbons and Halogenated Hydrocarbons in the Aquatic Environment*, Afghan, B. K.; Mackay, D., Eds. Plenum Press: New York, 1980; pp 145-160.
60. Krishnan, A. V.; Stathis, P.; Permuth, S. F.; Tokes, L.; Feldman, D., Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology* **1993**, *132*, (6), 2279-86.
61. vom Saal, F. S.; Nagel, S. C.; Timms, B. G.; Welshons, W. V., Implications for human health of the extensive bisphenol A literature showing adverse effects at low doses: A response to attempts to mislead the public. *Toxicology* **2005**, *212*, (2-3), 244-252.
62. Rubin, B. S.; Lenkowski, J. R.; Schaeberle, C. M.; Vandenberg, L. N.; Ronsheim, P. M.; Soto, A. M., Evidence of Altered Brain Sexual Differentiation in Mice Exposed Perinatally to Low, Environmentally Relevant Levels of Bisphenol A. *Endocrinology* **2006**, *147*, (8), 3681-3691.
63. vom Saal, F. S., Bisphenol A Eliminates Brain and Behavior Sex Dimorphisms in Mice: How Low Can You Go? *Endocrinology* **2006**, *147*, (8), 3679-3680.
64. Calafat, A. M.; Kuklenyik, Z.; Reidy, J. A.; Caudill, S. P.; Ekong, J.; Needham, L. L., Urinary Concentrations of Bisphenol A and 4-Nonylphenol in a Human Reference Population. *Environmental Health Perspectives* **2005**, *113*, (4), 391-395.
65. Engelhart, D. A.; Lavins, E. S.; Sutheimer, C. A., Detection of Drugs of Abuse in Nails. *Journal of Analytical Toxicology* **1998**, *22*, (4), 314-318.
66. Schramm, K. W., Hair-biomonitoring of organic pollutants. *Chemosphere* **2008**, *72*, (8), 1103-1111.

67. Rüegg, U. T.; Rudinger, J., [10] Reductive cleavage of cystine disulfides with tributylphosphine. In *Methods in Enzymology*, Hirs, C. H. W.; Serge, N. T., Eds. Academic Press: 1977; Vol. Volume 47, pp 111-116.
68. Stalling, D. L.; Tindle, R. C.; Johnson, J. L., Cleanup of pesticide and polychlorinated biphenyl residues in fish extracts by gel permeation chromatography. **1972**.
69. Peterman, P.; Feltz, K.; Orazio, C.; Echols, K., Basic Alumina Flash Chromatographic Separation of Bulk *Ortho*-PCBs from Non-*Ortho*-PCBs, PBDEs, PCDFs, PCDDs, PCDTs, OCPs, and PCTs. *Organohalogen Compounds* **2006**, 68, 2458-2461.
70. Lampe, M. A.; Burlingame, A.; Whitney, J.; Williams, M. L.; Brown, B. E.; Roitman, E.; Elias, P. M., Human stratum corneum lipids: characterization and regional variations. *Journal of lipid research* **1983**, 24, (2), 120-130.
71. Yadav, G. D.; Krishnan, M. S., An Ecofriendly Catalytic Route for the Preparation of Perfumery Grade Methyl Anthranilate from Anthranilic Acid and Methanol. *Organic Process Research & Development* **1998**, 2, (2), 86-95.
72. Ye, X.; Zhou, X.; Hennings, R.; Kramer, J.; Calafat, A. M., Potential External Contamination with Bisphenol A and Other Ubiquitous Organic Environmental Chemicals during Biomonitoring Analysis: An Elusive Laboratory Challenge. *Environmental Health Perspectives* **2013**.
73. Czuczwa, J. M.; Alford-Stevens, A., Optimized gel permeation chromatographic cleanup for soil, sediment, wastes, and oily waste extracts for determination of semivolatile organic pollutants and PCBs. *Journal - Association of Official Analytical Chemists* **1989**, 72, (5), 752-9.
74. Juhl, U.; Witte, I.; Butte, W., Metabolism of pentachlorophenol to tetrachlorohydroquinone by human liver homogenate. *Bull Environ Contam Toxicol* **1985**, 35, (5), 596-601.
75. Aronson, K. J.; Miller, A. B.; Woolcott, C. G.; Sterns, E. E.; McCready, D. R.; Lickley, L. A.; Fish, E. B.; Hiraki, G. Y.; Holloway, C.; Ross, T.; Hanna, W. M.; SenGupta, S. K.; Weber, J.-P., Breast Adipose Tissue Concentrations of Polychlorinated Biphenyls and Other Organochlorines and Breast Cancer Risk. *Cancer Epidemiology Biomarkers & Prevention* **2000**, 9, (1), 55-63.
76. Brown, J. F., Jr.; Lawton, R. W., Factors controlling the distribution and levels of PCBs after occupational exposure. In *PCBs - Recent Advances in Environmental Toxicology and Health Effects*, Robertson, L. W.; Hansen, L. G., Eds. University Press of Kentucky: Lexington, KY, 2001; pp 103-109.
77. Hansen, L. G., Identification of steady state and episodic PCB congeners from multiple pathway exposures. In *PCBs - Recent Advances in Environmental Toxicology and Health Effects*, Robertson, L. W.; Hansen, L. G., Eds. University Press of Kentucky: Lexington, KY, 2001; pp 47-56.
78. Hansen, L. G., Stepping backward to improve assessment of PCB congener toxicities. *Environmental Health Perspectives* **1998**, 106, (Suppl 1), 171.
79. Schechter, A.; Stanley, J.; Boggess, K.; Masuda, Y.; Mes, J.; Wolff, M.; Fürst, P.; Fürst, C.; Wilson-Yang, K.; Chisholm, B., Polychlorinated biphenyl levels in the tissues of

- exposed and nonexposed humans. *Environmental health perspectives* **1994**, *102*, (Suppl 1), 149.
80. Darras, V. M., Endocrine disrupting polyhalogenated organic pollutants interfere with thyroid hormone signalling in the developing brain. *Cerebellum* **2008**, *7*, (1), 26-37.
 81. Fonnum, F.; Mariussen, E., Mechanisms involved in the neurotoxic effects of environmental toxicants such as polychlorinated biphenyls and brominated flame retardants. *Journal of Neurochemistry* **2009**, *111*, (6), 1327-1347.
 82. Mariussen, E.; Fonnum, F., Neurochemical Targets and Behavioral Effects of Organohalogen Compounds: An Update. *Critical Reviews in Toxicology* **2006**, *36*, (3), 253-89.
 83. Langeveld, W. T.; Meijer, M.; Westerink, R. H. S., Differential Effects of 20 Non-Dioxin-Like PCBs on Basal and Depolarization-Evoked Intracellular Calcium Levels in PC12 Cells. *Toxicological Sciences* **2012**, *126*, (2), 487-496.
 84. Lehmler, H.-J.; Robertson, L. W.; Garrison, A. W.; Kodavanti, P. R. S., Effects of PCB 84 enantiomers on [3H]-phorbol ester binding in rat cerebellar granule cells and $^{45}\text{Ca}^{2+}$ -uptake in rat cerebellum. *Toxicology Letters* **2005**, *156*, (3), 391-400.
 85. Mitchell, M. M.; Woods, R.; Chi, L.-H.; Schmidt, R. J.; Pessah, I. N.; Kostyniak, P. J.; LaSalle, J. M., Levels of select PCB and PBDE congeners in human postmortem brain reveal possible environmental involvement in 15q11-q13 duplication autism spectrum disorder. *Environmental and Molecular Mutagenesis* **2012**, *53*, (8), 589-598.
 86. Simon, T.; Britt, J. K.; James, R. C., Development of a neurotoxic equivalence scheme of relative potency for assessing the risk of PCB mixtures. *Regulatory Toxicology and Pharmacology* **2007**, *48*, (2), 148-170.
 87. Wolff, M. S.; Camann, D.; Gammon, M.; Stellman, S. D., Proposed PCB Congener Groupings for Epidemiological Studies. *Environmental Health Perspectives* **1997**, *105*, (1), 13-14.
 88. Wong, P. W.; Garcia, E. F.; Pessah, I. N., ortho-Substituted PCB95 alters intracellular calcium signaling and causes cellular acidification in PC12 cells by an immunophilin-dependent mechanism. *Journal of Neurochemistry* **2001**, *76*, (2), 450-463.
 89. Pessah, I. N.; Hansen, L. G.; Albertson, T. E.; Garner, C. E.; Ta, T. A.; Do, Z.; Kim, K. H.; Wong, P. W., Structure-activity relationship for noncoplanar polychlorinated biphenyl congeners toward the ryanodine receptor- Ca^{2+} channel complex type 1 (RyR1). *Chemical research in toxicology* **2006**, *19*, (1), 92-101.
 90. Johnson-Restrepo, B.; Kannan, K.; Rapaport, D. P.; Rodan, B. D., Polybrominated diphenyl ethers and polychlorinated biphenyls in human adipose tissue from New York. *Environmental science & technology* **2005**, *39*, (14), 5177-5182.
 91. Petreas, M.; Nelson, D.; Brown, F. R.; Goldberg, D.; Hurley, S.; Reynolds, P., High concentrations of polybrominated diphenylethers (PBDEs) in breast adipose tissue of California women. *Environment International* **2011**, *37*, (1), 190-197.
 92. Daniels, J. L.; Pan, I. J.; Jones, R.; Anderson, S.; Patterson, D. G., Jr.; Needham, L. L.; Sjodin, A., Individual characteristics associated with PBDE levels in U.S. human milk samples. *Environ Health Perspect* **2010**, *118*, (1), 155-60.
 93. Malarvannan, G.; Isobe, T.; Covaci, A.; Prudente, M.; Tanabe, S., Accumulation of brominated flame retardants and polychlorinated biphenyls in human breast milk and

scalp hair from the Philippines: Levels, distribution and profiles. *Science of The Total Environment* **2013**, 442, (0), 366-379.

94. Eriksson, P.; Fischer, C.; Fredriksson, A., Polybrominated Diphenyl Ethers, A Group of Brominated Flame Retardants, Can Interact with Polychlorinated Biphenyls in Enhancing Developmental Neurobehavioral Defects. *Toxicological Sciences* **2006**, 94, (2), 302-309.
95. Kim, K. H.; Bose, D. D.; Ghogha, A.; Riehl, J.; Zhang, R.; Barnhart, C. D.; Lein, P. J.; Pessah, I. N., Para-and ortho-substitutions are key determinants of polybrominated diphenyl ether activity toward ryanodine receptors and neurotoxicity. *Environmental health perspectives* **2011**, 119, (4), 519.
96. Hoh, E.; Zhu, L.; Hites, R. A., Novel flame retardants, 1,2-bis(2,4,6-tribromophenoxy)ethane and 2,3,4,5,6-pentabromoethylbenzene, in United States' environmental samples. *Environ Sci Technol* **2005**, 39, (8), 2472-7.
97. Kannan, K.; Reiner, J. L.; Yun, S. H.; Perrotta, E. E.; Tao, L.; Johnson-Restrepo, B.; Rodan, B. D., Polycyclic musk compounds in higher trophic level aquatic organisms and humans from the United States. *Chemosphere* **2005**, 61, (5), 693-700.
98. Reiner, J. L.; Wong, C. M.; Arcaro, K. F.; Kannan, K., Synthetic Musk Fragrances in Human Milk from the United States. *Environmental Science & Technology* **2007**, 41, (11), 3815-3820.
99. Schlumpf, M.; Kypke, K.; Wittassek, M.; Angerer, J.; Mascher, H.; Mascher, D.; Vökt, C.; Birchler, M.; Lichtensteiger, W., Exposure patterns of UV filters, fragrances, parabens, phthalates, organochlor pesticides, PBDEs, and PCBs in human milk: Correlation of UV filters with use of cosmetics. *Chemosphere* **2010**, 81, (10), 1171-1183.
100. Wang, H.; Zhang, J.; Gao, F.; Yang, Y.; Duan, H.; Wu, Y.; Berset, J.-D.; Shao, B., Simultaneous analysis of synthetic musks and triclosan in human breast milk by gas chromatography tandem mass spectrometry. *Journal of Chromatography B* **2011**, 879, (21), 1861-1869.
101. Calafat, A. M.; Ye, X.; Wong, L. Y.; Reidy, J. A.; Needham, L. L., Urinary concentrations of triclosan in the U.S. population: 2003-2004. *Environ Health Perspect* **2008**, 116, (3), 303-7.
102. Geens, T.; Neels, H.; Covaci, A., Distribution of bisphenol-A, triclosan and n-nonylphenol in human adipose tissue, liver and brain. *Chemosphere* **2012**, 87, (7), 796.
103. Wang, L.; Asimakopoulos, A. G.; Moon, H.-B.; Nakata, H.; Kannan, K., Benzotriazole, Benzothiazole, and Benzophenone Compounds in Indoor Dust from the United States and East Asian Countries. *Environmental Science & Technology* **2013**.
104. Matsumoto, H.; Adachi, S.; Suzuki, Y., Estrogenic Activity of Ultraviolet Absorbers and the Related Compounds. *YAKUGAKU ZASSHI* **2005**, 125, (8), 643-652.
105. Stapleton, H. M.; Dodder, N. G.; Offenberg, J. H.; Schantz, M. M.; Wise, S. A., Polybrominated Diphenyl Ethers in House Dust and Clothes Dryer Lint. *Environmental Science & Technology* **2004**, 39, (4), 925-931.
106. Dye, J. A.; Venier, M.; Zhu, L.; Ward, C. R.; Hites, R. A.; Birnbaum, L. S., Elevated PBDE Levels in Pet Cats: Sentinels for Humans? *Environmental Science & Technology* **2007**, 41, (18), 6350-6356.

107. Guo, W.; Park, J.-S.; Wang, Y.; Gardner, S.; Baek, C.; Petreas, M.; Hooper, K., High polybrominated diphenyl ether levels in California house cats: House dust a primary source? *Environmental Toxicology and Chemistry* **2012**, *31*, (2), 301-306.
108. Ali, N.; Malik, R. N.; Mehdi, T.; Eqani, S. A. M. A. S.; Javeed, A.; Neels, H.; Covaci, A., Organohalogenated contaminants (OHCs) in the serum and hair of pet cats and dogs: Biosentinels of indoor pollution. *Science of The Total Environment* **2013**, *449*, (0), 29-36.
109. Norrgran, J.; Jones, B.; Lindquist, N.-G.; Bergman, Å., Decabromobiphenyl, Polybrominated Diphenyl Ethers, and Brominated Phenolic Compounds in Serum of Cats Diagnosed With the Endocrine Disease Feline Hyperthyroidism. *Arch. Environ. Contam. Toxicol.* **2012**, *63*, (1), 161-168.
110. Fernandez, M. F.; Arrebola, J. P.; Taoufik, J.; Navalón, A.; Ballesteros, O.; Pulgar, R.; Vilchez, J. L.; Olea, N., Bisphenol-A and chlorinated derivatives in adipose tissue of women. *Reproductive Toxicology* **2007**, *24*, (2), 259-264.
111. Peterman, P. H.; Orazio, C. E.; Gale, R. W. In *Detection of Tetrabromobisphenol A and Formation of Brominated 13C-bisphenol A's in Commercial Drinking Water Stored in Reusable Polycarbonate Containers*, Spring 2000 ACS Meeting, San Francisco, CA, 2000; American Chemical Society, Environmental Division: San Francisco, CA, 2000.

VITA

Paul Herbert Peterman was born on April 21, 1948 and grew up in the Northwoods of Wisconsin in Rhinelander. Encouraging and enthusiastic high school teachers especially a chemistry teacher contributed to achieving Salutatorian and later double-majoring in chemistry and history at Augsburg College, Minneapolis following Bethany Jr. College in Mankato, MN. A summer job as a lab tech at the local paper mill gave Paul insight to environmental pollution. With a low draft number during the Vietnam War, Paul was drafted and served stateside for two years in the US Army. The War piqued his interest for an M.A. degree or more in non-Western history at the University of Wisconsin-Madison in 1975, but a professor denouncing his term papers as lab reports and a summer job in Madison at an environmental testing lab analyzing animal samples from Michigan for a flame retardant (hexabromobiphenyl) wrongly fed to dairy cattle and learning mass spectrometry all contributed to a new career. While using GC/MS to investigate for trace-level pollutants in Fox River samples downstream from paper mills, Paul identified bisphenol A, chlorinated bisphenol A derivatives, and initially unknown compounds, later identified as PCB replacements used in carbonless copy paper, that became the focus of his graduate research for an M.S. (1983) in the Water Chemistry Program of the University while still working full time at the WI State Lab of Hygiene on campus. Dr. Joseph Delfino, with half-time appointments to both agencies, was the major reason for Paul's M.S. degree. Delfino's leaving and an environmental research job opportunity using GC/MS and later with high resolution MS led Paul to Columbia,

MO at the Dept. of Interior's "Fish Pesticide" Lab now a part of the US Geological Survey. In a similar way to several planets aligning together or God's help, a combination of things allowed and encouraged Paul on the Ph. D path at Mizzou. First, the University of Missouri Chemistry Department was willing to enroll non-traditional graduate students, Dr. Robertson with eclectic research interests accepted Paul in his research group and suggested toenail for biomonitoring, a US Geological Survey more family-friendly policy of flex-time began, an immediate supervisor (Dr. Carl Orazio), who had already graduated after going as a non-traditional student at Mizzou, took over and encouraged Paul including participating in an EU workshop on PBDEs in Brussels in 2003 and meeting Dr. Adrian Covaci, a small side research collaboration with Dr. Fred vom Saal and his student, now Dr. Kembra Howdeshell, about bisphenol A leaching from plastic animal cages wetted research interest, and after a divorce in 1994, marrying Tina Lunsford Peterman (Ph.D in 2010 from Mizzou with three daughters), whose genuine love of learning since a freshman in 1998 has inspired and encouraged Paul through these graduate school years. Now, all three daughters are or have been in college and have also added encouragement.