Persistent Organic Pollutants and Metabolism in Midlife Women

by

Amelia Grant-Alfieri

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Environmental Health Sciences)
in the University of Michigan
2023

Doctoral Committee:

Professor Stuart Batterman, Co-Chair
Associated Professor Sung Kyun Park, Co-Chair
Professor William Herman
Research Associate Professor Deborah Watkins

Amelia Grant-Alfieri

agrantal@umich.edu

ORCID iD: 0000-0002-5828-8751

DEDICATION

This work is dedicated to all defenders of the earth and human rights. Thank you to my parents, Ellen Grant and Tony Alfieri, who instilled in me a deep respect for the natural world. They also taught me the importance of hard work and of challenging the status quo for the greater good. I am grateful for the continued support from loved ones including my brother, Adrian Grant-Alfieri, and friends near and far. Finally, thank you to the current and former residents and parishioners of the West Grove in Miami, Florida.

ACKNOWLEDGEMENTS

I would like to thank Dr. Sung Kyun Park and Dr. Stuart Batterman who guided and supported me these past four years. Dr. Park's passion and patience showed me that scientists must lead with empathy. Dr. Batterman and Dr. Park modeled rigorous scientific practice and believed that I could meet their high standards. This work was also made possible by Dr. William Herman, Dr. Deborah Watkins, and Dr. Carrie Karvonen-Gutierrez, and Dr. Amila Devasurendra for their insights and support. Thank you to the Department of Environmental Health Sciences, the Environmental Toxicology and Epidemiology training grant community, the Center for Midlife Science, and the staff, faculty, and students across the School of Public Health.

Many funding sources supported my doctoral studies, including the National Institute of Environmental Health Sciences (NIEHS) Environmental Toxicology and Epidemiology training grant (T32 ES007062), the National Center for Research Resources and National Center for Advancing Translational Sciences UCSF-CTSI grant UL1 RR024131, NIEHS R01-ES026578, R01-ES026964 and P30-ES017885, and the Center for Disease Control and Prevention/National Institute for Occupational Safety and Health grant T42-OH008455. The Study of Women's Health Across the Nation (SWAN) has grant support from the National Institutes of Health (NIH), DHHS, through the National Institute on Aging, the National Institute of Nursing Research and NIH Office of Research on Women's Health (Grants U01NR004061; U01AG012505, U01AG012535, U01AG012531, U01AG012539, U01AG012546,

U01AG012553, U01AG012554, U01AG012495, and U19AG063720). This research was also supported by the SWAN Repository (U01AG017719).

Clinical Centers: *University of Michigan, Ann Arbor – Carrie Karvonen-Gutierrez, PI 2021 – present, Siobán Harlow, PI 2011 – 2021, MaryFran Sowers, PI 1994-2011; Massachusetts General Hospital, Boston, MA – Joel Finkelstein, PI 1999 – present; Robert Neer, PI 1994 – 1999; Rush University, Rush University Medical Center, Chicago, IL – Howard Kravitz, PI 2009 – present; Lynda Powell, PI 1994 – 2009; University of California, Davis/Kaiser – Ellen Gold, PI; University of California, Los Angeles – Gail Greendale, PI; Albert Einstein College of Medicine, Bronx, NY – Carol Derby, PI 2011 – present, Rachel Wildman, PI 2010 – 2011; Nanette Santoro, PI 2004 – 2010; University of Medicine and Dentistry – New Jersey Medical School, Newark – Gerson Weiss, PI 1994 – 2004; and the University of Pittsburgh, Pittsburgh, PA – Karen Matthews, PI.*

NIH Program Office: *National Institute on Aging, Bethesda, MD – Rosaly Correa-de-Araujo 2020 – present; Chhanda Dutta 2016- 2020; Winifred Rossi 2012–2016; Sherry Sherman 1994 – 2012; Marcia Ory 1994 – 2001; National Institute of Nursing Research, Bethesda, MD – Program Officers.*

Central Laboratory: *University of Michigan, Ann Arbor – Daniel McConnell (Central Ligand Assay Satellite Services).*

NIA Biorepository: *Rosaly Correa-de-Araujo 2019 – Present; SWAN Repository: University of Michigan, Ann Arbor – Siobán Harlow 2013 - 2018; Dan McConnell 2011 - 2013; MaryFran Sowers 2000 – 2011.*

Coordinating Center: *University of Pittsburgh, Pittsburgh, PA – Maria Mori Brooks, PI 2012 - present; Kim Sutton-Tyrrell, PI 2001 – 2012; New England Research Institutes, Watertown, MA - Sonja McKinlay, PI 1995 – 2001.*

Steering Committee: Susan Johnson, Current Chair

Chris Gallagher, Former Chair

We thank the study staff at each site and all the women who participated in SWAN.

TABLE OF CONTENTS

DEDICATION ii

ACKNOWLEDGEMENTS iii

LIST OF TABLES ix

LIST OF FIGURES xiv

ABSTRACT xvii

Chapter I. Persistent Organic Pollutants and Metabolism 1

Importance and Prevalence of Type 2 Diabetes 1

Adipose Tissue, Endocrine Factors, and Type 2 Diabetes 2

Endocrine Disrupting POPs: PCBs, PBDEs, OCPs 4

Polychlorinated biphenyls (PCBs) 5

Polybrominated diphenyl ethers (PBDEs) 6

Organochlorine pesticides (OCPs) 6

The Role of Adiposity in the Storage and Effects of POPs 7

Disparities in POPs Exposure and Risk of T2D 8

Effects of POPs on Adipocytes and Adipokines 9

Biological Mechanisms Linking POPs and T2D 11

Endocrine Disruption 11

Mitochondrial Dysfunction 13

Cross-sectional Studies 15

Case-control Studies 16

Prospective Studies 16

POP Mixtures in Prospective Studies 17

Summary of Epidemiological Findings 18

References 20

Chapter II. Changes in Adipose Tissue and Circulating Concentrations of Persistent Organic Pollutants in The Study of Women's Health Across the Nation 41

Abstract 41

Introduction 43

Methods 45

Study Population 45

POP Measurements 47
Covariates 50
Statistical Analysis 51
Results 52
Participant Characteristics 52
Longitudinal Trends of Serum POPs concentrations 1999-2011 53
Changes in Waist Circumference and Serum POPs 56
Discussion 59
Strengths and Limitations 62
References 64
Supplementary Materials 70
Chapter III. Associations of Organochlorine Pesticide, Polybrominated Diphenyl Ether, and Polychlorinated Biphenyl Mixtures with Incident Diabetes in The Study of Women's Health Across the Nation 77
Abstract 77
Introduction 79
Methods 81
Study Population 81
POPs Measurements 83
Diabetes Incidence 84
Covariates 84
Statistical Analysis 86
Results 88
Participant Characteristics 88
Exposure to POPs 90
POPs and Diabetes Incidence 90
Discussion 94
POPs and Diabetes Development 94
Strengths and Limitations 99
Conclusion 101
References 102
Supplementary Materials 110
Chapter IV. Adipokines and Mixtures of Organochlorine Pesticides, Polybrominated Diphenyl Ethers, and Polychlorinated Biphenyls in The Study of Women's Health Across the Nation 130
Abstract 130
Introduction 132
Methods 134
Study Population 134
POP Measurements 135
Adipokine Measurements 136
Covariates 136
Statistical Analysis 137

Results 139
Participant Characteristics 139
Individual POPs and Adipokines 142
POP Mixtures and Adipokines 144
DDT Metabolites and Adipokines 145
Discussion 149
POPs and Adipokines 149
Strengths and Limitations 154
Conclusion 155
References 156
Supplemental Materials 164
Chapter V. Conclusions 206
Overall Conclusions 206
Summary of Findings 207
Aim 1 207
Aim 2 208
Aim 3 209
Public Health Implications 210
The Health of Aging Women 210
Policy and Regulation 210
Climate Change-related Exposure Exacerbation 211
Mixtures 212
Future Directions 212
Expanding the Longitudinal SWAN MPS Substudy 212
Expanded Mixtures Analysis 213
Consideration of Additional Biomarkers 213
References 215

LIST OF TABLES

Table 2.1. Characteristics of participants in the SWAN MPS longitudinal substudy (n = 65 for PCBs; n = 59 for OCPs)……..……..54

Table 2.2. Average and range of waist circumference change (inches) between visits in the SWAN MPS longitudinal Substudy (n = 181 for PCBs; n = 151 for OCPs)…….……...55

Table 2.3. Median (IQR) Serum POP Concentrations (ng/g lipid) in the SWAN MPS longitudinal substudy (n = 181 for PCBs; n = 151 for OCPs)…….…….56

Table S2.1. Detection frequencies (DF) of PCBs, PBDEs, and OCPs in SWAN MPS longitudinal substudy.…….……..72

Table S2.2. Availability of POPs observations at two or more timepoints in the SWAN MPS longitudinal substudy…….……..…….……...74

Table S2.3. Percent change (95% CI) in the difference in covariate-adjusted lipid-standardized POP concentration associated with a one-inch (2.54 cm) increase in the difference in waist circumference in the SWAN MPS longitudinal Substudy.……...75

Table S2.4. Percent change (95% CI) in the difference in serum POP concentration associated with a one-inch increase in the difference in waist circumference in the SWAN MPS longitudinal substudy. Comparison of three methods to calculate serum POP concentrations. All models were adjusted for race/ethnicity, age, parity, study site, and the number of visits elapsed between observations.……..……..……..76

Table 3.1. Characteristics of SWAN MPS Participants Free of Diabetes at Baseline, 1999/2000……..……..……..…….……..89

Table 3.2. Serum POP Concentrations (ng/g lipid) among SWAN MPS Participants Free of Diabetes at Baseline, 1999/2000……..……..……..…..91

Table 3.3. Hazard Ratio (95% CI) of incident diabetes associated with serum POP concentrations, 1999-2016. Model adjusted for age, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, fish/ shellfish intake, lipids, and joint stabilized inverse probability weights. Second and third tertiles of serum POP concentrations were analyzed relative to the first tertile.…..……..…..93

Table 3.4. Hazard Ratio (95% CI) of incident diabetes associated with DDT metabolites, 1999-2016. Model adjusted for age, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, fish/shellfish intake, lipids, and joint stabilized inverse probability weights. Moderate and high quantiles of exposure were analyzed relative to non-detection (n = 1040)……..……..94

Table S3.1. Detection frequencies (DF) of PCBs, PBDEs, and OCPs in SWAN MPS at baseline (1999/2000)……..……..…….…….…….....113

Table S3.2. Hazard Ratio (95% CI) of incident diabetes associated with serum POP concentrations, 1999-2016. Model is unadjusted. Second and third tertiles of serum POP concentrations were analyzed relative to the first tertile……..……..…..117

Table S3.3. Hazard Ratio (95% CI) of incident diabetes associated with serum POP concentrations, 1999-2016. Model adjusted for age, race/ethnicity, site, education, financial strain, and joint stabilized inverse probability weights. Second and third tertiles of serum POP concentrations were analyzed relative to the first tertile……..……..…..118

Table S3.4. Hazard Ratio (95% CI) of incident diabetes associated with serum POP concentrations, 1999-2016. Model adjusted for age, race/ethnicity, site, education, financial strain, lipids, and joint stabilized inverse probability weights. Second and third tertiles of serum POP concentrations were analyzed relative to the first tertile…….….119

Table S3.5. Hazard Ratio (95% CI) of incident diabetes associated with serum POP concentrations, 1999-2016. Model adjusted for age, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, fish/ shellfish intake, lipids, waist circumference, and joint stabilized inverse probability weights. Second and third tertiles of serum POP concentrations were analyzed relative to the first tertile……..……..……..……..……...120

Table S3.6. Hazard Ratio (95% CI) of incident diabetes associated with DDT metabolites, 1999-2016. Model adjusted for age, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, fish/shellfish intake, lipids, waist circumference, and joint stabilized inverse probability weights. Moderate and high exposure quantiles were analyzed relative to non-detect (n = 1040)…….….121

Table S3.7. Hazard Ratio (95% CI) of incident diabetes associated with the detection of DDT metabolites, 1999-2016. Model adjusted for age, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, fish/shellfish intake, lipids, and joint stabilized inverse probability weights (n = 1040)…...122

Table S3.8. Hazard Ratio (95% CI) of incident diabetes associated with the detection of DDT metabolites, 1999-2016. Model adjusted for age, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, fish/ shellfish intake, lipids, waist circumference, and joint stabilized inverse probability weights (n = 1040) ……..……..……..…….....123

Table S3.9. Hazard Ratio (95% CI) of incident diabetes associated with serum POP mixture characterized using two and three K-means clusters, 1999-2016. Model adjusted for age, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, fish/shellfish intake, lipids, and joint stabilized inverse probability weights……..……..……..…….....126

Table S3.10. Hazard Ratio (95% CI) of incident diabetes associated with serum POP mixture characterized using two and three K-means clusters, 1999-2016. Model adjusted for age, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, fish/shellfish intake, lipids, waist circumference, and joint stabilized inverse probability weights……..……..……...…..…127

Table 4.1. Characteristics of SWAN MPS participants, 1999/2000 (n = 872) ……..141

Table S4.1. Detection frequencies (DF) of PCBs, PBDEs, and OCPs in SWAN MPS, 1999/2000……..……..……..……..……...165

Table S4.2. Serum concentrations of POPs (ng/g lipid) in SWAN MPS, 1999/2000 (n = 872) ……..……..……..……..…….169

Table S4.3. Median serum adipokine concentrations by waist circumference change (gain vs. stable) between 1999/2000 and 2002/03……..……..…..…..…170

Table S4.4. Serum concentrations of POPs (ng/g lipid) in SWAN MPS, 1999/2000….……171

Table S4.5. Serum concentrations of DDT metabolites (ng/g lipid) among SWAN MPS participants at baseline (n = 1,116) ……..……..……173

Table S4.6. Percent change (95% CI) in serum concentrations of leptin associated with an increase in serum POP concentrations in SWAN MPS. Models were adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and lipids……..……..……..…….……...174

Table S4.7. Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with an increase in serum POP concentrations in SWAN MPS. Models were adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and lipids……..……...…….175

Table S4.8. Percent change (95% CI) in serum concentrations of leptin associated with an increase in serum POP concentrations in SWAN MPS. Models were unadjusted……176

Table S4.9. Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with an increase in serum POP concentrations in SWAN MPS. Models were unadjusted.……..……..……..……..177

Table S4.10. Percent change (95% CI) in serum concentrations of leptin associated with an increase in serum POP concentrations in SWAN MPS. Models were adjusted for age, race/ethnicity, site, education, and financial strain……..…….……..178

Table S4.11. Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with an increase in serum POP concentrations in SWAN MPS. Models were adjusted for age, race/ethnicity, site, education, and financial strain.……179

Table S4.12. Percent change (95% CI) in serum concentrations of leptin associated with an increase in serum POP concentrations in SWAN MPS. Models were adjusted for age, race/ethnicity, site, education, financial strain, and lipids……..…….……180

Table S4.13. Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with an increase in serum POP concentrations in SWAN MPS. Models were adjusted for age, race/ethnicity, site, education, financial strain, and lipids.….181

Table S4.14. Percent change (95% CI) in serum concentrations of leptin associated with an increase in serum POP concentrations in SWAN MPS. Models were adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, lipids, and waist circumference……..……..……....182

Table S4.15. Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with an increase in serum POP concentrations in SWAN MPS. Models were adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, lipids, and waist circumference……..…….183

Table S4.16. Percent change (95% CI) in serum concentrations of leptin associated with an increase in serum POP concentrations by change in waist circumference in SWAN MPS. Models were adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and lipids……..…….……..184

Table S4.17. Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with an increase in serum POP concentrations by change in waist circumference in SWAN MPS. Models were adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and lipids……..186

Table S4.18. Percent change (95% CI) in serum concentrations of leptin associated with an increase in serum POP concentrations by change in waist circumference in SWAN MPS. Models were adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, lipids, and waist circumference…….……..188

Table S4.19. Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with an increase in serum POP concentrations by change in waist circumference in SWAN MPS. Models were adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, lipids, and waist circumference……..……..……..…….…...190

Table S4.20. Association between exposure to a two-cluster POP mixture and serum concentrations of leptin and high molecular weight adiponectin. Estimates of above-average exposure relative to below-average exposure. Model 3 was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and lipids. Model 4 was additionally adjusted for waist circumference at baseline…….196

Table S4.21. Association between exposure to a four-cluster POP mixture and serum concentrations of leptin and high molecular weight adiponectin. Estimates are relative to below-average exposure. Model 3 was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and lipids. Model 4 was additionally adjusted for waist circumference at baseline……..……198

Table S4.22. Association between exposure to a three-cluster POP mixture and serum concentrations of leptin and high molecular weight adiponectin. Estimates are relative to below-average exposure. Model 3 was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and lipids. Model 4 was additionally adjusted for waist circumference at baseline……..……199

Table S4.23. Association between exposure to a three-cluster POP mixture and serum concentrations of leptin and high molecular weight adiponectin in SWAN MPS. Estimates are relative to below-average exposure. Model 3 was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and lipids. Model 4 was additionally adjusted for waist circumference at baseline…….……204

Table S4.24. Association between exposure to a three-cluster POP mixture and serum concentrations of leptin and high molecular weight adiponectin in SWAN MPS. Estimates are relative to below-average exposure. Model 3 was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and lipids. Model 4 was additionally adjusted for waist circumference at baseline…….……205

LIST OF FIGURES

Figure 1.1. Structure of PCB 153……..……..…….…..5

Figure 1.2. Structure of PBDE 47……..……..…….…....6

Figure 1.3. Structure of DDT……..……..…….…..…….6

Figure 1.4. Sources and United States regulations of polychlorinated biphenyl ethers, organochlorine pesticides, and polybrominated diphenyl ethers……..…….…..7

Figure 1.5. Conceptual model of the relationship between lipophilic persistent organic pollutants in serum and adipose tissue following the loss or gain of adipose tissue……..…….8

Figure 1.6. Conceptual model of the mechanisms linking POPs exposure and T2D…..…..…..14

Figure 2.1. Percent change (95% CI) in the difference in covariate-adjusted lipid-standardized POP concentration associated with a one-inch (2.54 cm) increase in the difference in waist circumference in the SWAN MPS longitudinal substudy. (n = 181 for PCBs; n = 151 for OCPs). Models were adjusted for race/ethnicity, age, parity, study site, visit, and number of visits elapsed between observations……..…….……..….58

Figure S2.1. Study design of the Study of Women's Health Across the Nation Multi-Pollutant Study (SWAN MPS) ……..……..…….…….……...70

Figure S2.2. Study design of the SWAN MPS longitudinal substudy……..…….……71

Figure S2.3. Directed Acyclic Graph (DAG) of the association between adipose tissue and serum concentrations of POPs……..……..……...……...….73

Figure S3.1. Study design of the Study of Women's Health Across the Nation Multi-Pollutant Study (SWAN MPS) ……..…….…..…...112

Figure S3.2. Correlation heatmap of serum POP concentrations in SWAN MPS at baseline, 1999/2000 (n = 809) ……..……..……..……..…..…...114

Figure S3.3. Elbow method identification of k exposure clusters of the POP mixture in SWAN MPS……..……..……..……...……..……..115

Figure S3.4. Silhouette method identification of k exposure clusters of the POP mixture in SWAN MPS……..……..……..…….…..116

Figure S3.5. Distributions of serum POP concentrations in three K-means clusters in SWAN MPS (n = 809) ……..……..……..…….…..124

Figure S3.6. Distributions of serum POP concentrations in two K-means clusters in SWAN MPS (n=809) ……..……..……..…….125

Figure S3.7. Individual Contributions of POP Mixture Components to Overall Joint Effect on Diabetes Risk in SWAN MPS (n = 809). Model adjusted for age, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, fish/shellfish intake, lipids, and joint stabilized inverse probability weights…..128

Figure S3.8. Individual Contributions of POP Mixture Components to Overall Joint Effect on Diabetes Risk in SWAN MPS (n = 809). Model adjusted for age, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, fish/shellfish intake, lipids, waist circumference, and joint stabilized inverse probability weights……..……..……..…….129

Figure 4.1. Percent change (95% CI) in serum concentrations of leptin associated with serum concentrations of select POPs in SWAN MPS. Model was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and lipids……..……..…….……....143

Figure 4.2. Percent change (95% CI) in serum concentrations of leptin associated with a one interquartile range increase in serum concentrations of select POPs by waist circumference change from 1999/2000 to 2002/2003 in SWAN MPS. Model was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and lipids……..……..…….…….....146

Figure 4.3. Overall joint effect of exposure to the POP mixture on serum concentrations of leptin in SWAN MPS. Model was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and lipids…….……147

Figure 4.4. Overall joint effect of exposure to the POP mixture on serum concentrations of high molecular weight adiponectin in SWAN MPS. Model was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and lipids……..……..…….....148

Figure S4.1. Study design of the Study of Women's Health Across the Nation Multi-Pollutant Study (SWAN MPS) ……..……..…….……..164

Figure S4.2. Spearman correlation matrix of serum concentrations of POPs (ng/g lipid).…..166

Figure S4.3. Results of Elbow analysis of serum concentrations of POPs (ng/g lipid) in SWAN MPS (1999/2000) that informed selection of optimal number of K-means clusters…..…....167

Figure S4.4. Results of Silhouette analysis of serum concentrations of POPs (ng/g lipid) in SWAN MPS (1999/2000) that informed selection of optimal number of K-means clusters……..……..……..……..……..168

Figure S4.5. Directed acyclic graph (DAG) illustrating the role of adiposity change in the association between serum concentrations of persistent organic pollutants (POPs) and serum levels of adipokines……..……..……..……172

Figure S4.6. K-means Clusters (k = 3) of serum concentrations of POPs (ng/g lipid) in SWAN MPS. Concentrations were scaled, centered, and log-transformed……..192

Figure S4.7. Percent change (95% CI) in serum concentrations of leptin associated with exposure to a three-cluster POP mixture by waist circumference change (gain vs. stable) between 1999/2000 and 2002/03 in SWAN MPS……..……..193

Figure S4.8. Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with exposure to a three-cluster POP mixture by waist circumference change between 1999/2000 and 2002/03 in SWAN MPS……...194

Figure S4.9. K-means Clusters (k = 2) of serum concentrations of POPs (ng/g lipid) in SWAN MPS. Concentrations were scaled, centered, and log-transformed……..…195

Figure S4.10. K-means Clusters (k = 4) of Serum POP Concentrations in SWAN MPS……197

Figure S4.11. Association between serum concentrations of leptin and individual POP mixture components in SWAN MPS……..……..…….200

Figure S4.12. Association between serum concentrations of high molecular weight adiponectin and individual POP mixture components in SWAN MPS……...201

Figure S4.13. Percent change (95% CI) in serum concentrations of leptin associated with p,p'-DDE exposure by waist circumference change (gain vs. stable) between 1999/2000 and 2002/03 in SWAN MPS……..……..…….……..202

Figure S4.14. Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with p,p'-DDE exposure by waist circumference change (gain vs. stable) between 1999/2000 and 2002/03 in SWAN MPS……..……..….203

ABSTRACT

Many environmental chemicals are known or suspected risk factors for metabolic disease, including persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs). These chemicals persist in the environment following agricultural, industrial, and commercial use in electronics, plastics, pesticides and pesticide extenders, fabrics, and furniture. PCBs and many OCPs were banned by the U.S. and other high-income countries in the 1970s and 1980s. In 2013, the U.S. banned many classes of PBDEs. Despite these phase-outs, humans are still exposed to POPs primarily via diet and, in the case of PCBs and PBDEs, indoor air and dust. Due to their lipophilicity, POPs are sequestered in adipose tissue, where they may impair adipocyte functioning. Disruption of adipokine hormones has been linked to cardiometabolic diseases. Changes in adiposity may redistribute POPs between serum and adipose tissue. The menopausal transition entails rapidly changing adiposity.

This dissertation explored potential impacts of POPs on metabolism. The following aims were assessed in a longitudinal, multi-ethnic, multi-site cohort of U.S. midlife women, the Study of Women's Health Across the Nation. The three aims explored different facets of visceral adipose tissue, as it relates to internal exposure to POPs and metabolic health.

Aim 1 Changes in adiposity may affect the sequestration and circulation of POPs. Few studies have repeated measures of POPs. We evaluated the relationship between changes in central adiposity and changes in circulating concentrations of PCBs and OCPs in 75 women over a twelve-year period (1999-2011). After multivariable adjustment, a one-inch increase in the

difference in waist circumference between visits was associated with a 4.9% decrease in the difference in serum concentration of PCB 194 (95% CI: -8.0%, -1.6%). No associations were observed for other PCBs or OCPs. PCB 194, a highly lipophilic congener, may be sequestered in adipose tissue following adiposity gain. The study's main strength was measuring POPs at multiple timepoints. The study was limited by its small sample. Future research should investigate intra-person variation in serum POP concentrations and predictors of temporal trends.

Aim 2 Little is known about the joint effect of exposure to mixtures of POPs on diabetes risk. Few studies have evaluated diabetes risk associated with PBDEs. We explored the association between serum concentrations of POPs, individually and as mixtures, and diabetes development over 18 years (1999-2016) in 1,400 women. Using single pollutant models, most POPs were not identified as being significantly associated with diabetes risk. After multivariable adjustment, the hazard ratio (HR) and 95% CI for diabetes associated with tertiles of exposure (T2 or T3), relative to the first tertile, was 1.7 at T2 (1.0, 2.8) and 1.5 at T3 (0.84, 2.7) for hexachlorobenzene and 1.9 at T2 (1.1, 3.3) and 1.6 at T3 (0.88, 2.9) for PCB 123. We observed no association between diabetes risk and moderate and high levels of exposure to p,p'-DDE, p,p'-DDT, and p,p'-DDD, relative to non-detection. Using Quantile Based G-Computation to account for co-pollutant confounding between POP mixture components, we observed no significant overall joint effect of the mixture on incident diabetes (HR = 1.04 [0.53, 2.07]). This is the first prospective diabetes study to use a mixtures approach. Furthermore, this is one of few studies that analyzed PBDEs in addition to PCBs and OCPs. More research is needed on the diabetogenic effects of PBDEs, ideally in a more highly exposed population.

Aim 3 Little is known about the relationship between POPs, especially as mixtures, and adipokines. We investigated the relationship between serum concentrations of POPs measured in

1999/2000, individually and as mixtures, and serum levels of leptin and high molecular weight (HMW) adiponectin, a biologically active form of adiponectin, measured in 2002/2003 among 1,400 women. After multivariable adjustment, no significant associations were observed for the overall joint effect of the POP mixture on adipokines using Bayesian Kernel Machine Regression (BKMR) models. Based on single pollutant analyses, a one-interquartile range (IQR) higher PCB 194 concentration was associated with 9.0% lower (95% CI: -13.2%, -4.7%) leptin and 4.1% higher (95% CI: 0.35%, 7.9%) HMW adiponectin. A one-IQR higher PCB 180 concentration was associated with 5.2% lower (95% CI: -9.6%, -0.6%) leptin. This is the first study of POPs and adipokines in adults not restricted to patients with obesity. Another study strength was our attempt to improve the validity of exposure measurement between POP and adipokine collection timepoints; limiting our interpretation to women with stable adiposity, we controlled for a loss or gain of adipose tissue that could alter serum concentrations of POPs and adipokines. This study highlights the potential complexity of mechanisms underlying POPs and adipokines.

Overall, this dissertation does not provide consistent evidence of an association between POP mixtures and incident diabetes or adipokines in U.S. midlife women. Discordant results between single pollutant and mixtures models may have been due to co-pollutant confounding, individual POPs affecting risk in different directions, and/or compound-specific mechanistic activity. Aims 2 and 3 are notable for their analysis of mixtures of PCBs, OCPs, and PBDEs using numerous modeling approaches. In all aims, the role of serum lipids and visceral adiposity were carefully considered. Another strength was the representation of women from multiple urban areas across the U.S. and a focus on Chinese and Japanese women who have been historically underrepresented in U.S. studies. The dissertation leverages SWAN's original design to characterize changes throughout the menopausal transition including changes in

adiposity. Moving forward, research is needed on the effects of POPs, especially PBDEs, on the metabolic functioning of adipose tissue. Further research is also warranted on intra-person variation in serum POP concentrations and factors affecting trends in serum concentrations.

Chapter I. Persistent Organic Pollutants and Metabolism

Importance and Prevalence of Type 2 Diabetes

Diabetes is a common and growing cause of morbidity and mortality linked to lifestyle and environmental factors. In 2021, diabetes caused 6.7 million deaths worldwide.[1] The number of people with diabetes is predicted to increase 46% from 537 million cases (1 in 10 adults) in 2021 to 784 million cases (1 in 8 adults) in 2045, and the rise is most rapid in low- and middle-income countries.[1,2] Approximately 4 in 5 people with diabetes reside in low- and middle-income countries and many are undiagnosed.[1] Diabetes risk increases with age.[3] By 2030, it is approximated that 180 million adults ages 45-64 will be living with diabetes worldwide.[4] More than 95% of people with diabetes have type 2 diabetes.[2] Approximately 1 in 10 U.S. adults (over 35 million) has been diagnosed with Type 2 Diabetes (T2D), 1 in 3 are prediabetic, and 1 in 5 are undiagnosed.[5,6] If diabetes is undiagnosed, it goes untreated, which increases risk of complications including those related to SARS-CoV-2 infection and the risk of death.[7,8]

T2D is characterized by insulin resistance, the inability of glucose to be used by the body to produce energy, and the subsequent accumulation of glucose in the blood. It is diagnosed as having a fasting plasma glucose (FPG) level ≥ 126 mg/dL, a 2-hour blood glucose level ≥ 200 mg/dL, or a hemoglobin A1C level ≥ 6.5%.[9] Risk of T2D increases with obesity, lack of exercise, and family history, however genetics only explain 10% of cases.[10] Increasingly, environmental exposures are explored as additional risk factors. People with T2D are at higher risk of cardiovascular disease (CVD), stroke, kidney failure, certain types of cancer, and death.[11]

Treating diabetes and diabetes-related conditions incurs enormous medical costs that, on average, are 2.3 times greater than the costs of caring for people without diabetes.[11] Globally, diabetes was responsible for 966 billion USD in expenditures in 2021, an increase of 316% over 15 years.[1] In the U.S., the excess medical costs of diabetes totaled $9,601 per person in 2017.[5] Better characterization of environmental risk factors for Type 2 Diabetes and their etiologies can contribute to reducing medical costs and, more importantly, morbidity and mortality.

Adipose Tissue, Endocrine Factors, and Type 2 Diabetes

The relationship between adiposity, metabolic health, and T2D is complex. Obesity is a known risk factor for T2D as well as other comorbidities like CVD.[12] Rates of obesity have risen globally.[13] In 2018, among U.S. adults aged 20 and older, 73.6% were overweight or obese and 42.5% were obese.[14] The relationship between obesity and T2D varies by age. Patients diagnosed with T2D at an older age are less obese and have lower functioning beta cells.[15] In addition, there is a wide variation in T2D risk among people with similar degrees of obesity.[16] Among obese individuals, insulin resistance (a pre-diabetic condition) can vary 6-fold, and 75–80% may never develop T2D.[17,18] Chronic inflammation in adipose tissue may determine whether someone becomes either metabolically healthy and obese or metabolically unhealthy and lean.[19,20] In addition to inflammatory factors, endocrine factors released from adipocytes, such as sex hormones and adipokines, may play a role in T2D pathogenesis.

The endocrine disrupting mechanisms underlying metabolic disruption in adipocytes are sex-specific.[21,22,23,24] Peroxisome proliferator-activated receptor gamma (PPAR-γ) may regulate the expression of adipocyte-specific genes and affect insulin sensitivity via sex hormones.[21] In women, estradiol may reduce adiposity and increase insulin sensitivity; however, this relationship may differ by menopausal status. After menopause, adipocytes become the main site

of estradiol production, which leads to high correlation between estradiol and body fat.[21] Testosterone levels in women, particularly postmenopausal women, are associated with decreased insulin sensitivity, increased visceral adiposity, and higher risk of T2D.[21,25,26,27]

Adipokines

Leptin and adiponectin are peptide hormones released by adipocytes, also known as adipokines. Unlike other endocrine factors released by adipocytes, leptin and adiponectin are only expressed in adipose tissues and not in other organs.[28] They are primarily produced and released by white adipose tissue (WAT), which stores energy.[28] For this reason, adipokine secretion depends on adipocyte energy status.[28] Leptin and adiponectin work together to maintain glucose homeostasis and insulin sensitivity.[28] Soluble leptin receptors (sOB-R) are the primary binders and transporters of leptin that enable its bioavailability and activity.[29] High molecular weight (HMW) adiponectin is the most active form of adiponectin and, compared to total adiponectin, is more relevant to cardiometabolic disease.[30,31,32]

A poor adipokine profile -- the combination of higher leptin and lower adiponectin -- is associated with cardiometabolic disorders including T2D. The lack or under-expression of adiponectin is associated with reduced insulin production and secretion, greater insulin resistance, and cardiometabolic disease.[33,34] Balance is critical, as too little leptin is associated with thyroid and immune dysfunction, obesity, and insulin resistance.[35,36] This may be due to the fact that leptin can also help to reduce food consumption by increasing satiety, and in turn helping to manage adiposity and metabolism.[28,37] However, in individuals with obesity, leptin levels are higher than in individuals without obesity because their bodies are resistant to the beneficial metabolic effects of leptin.[28,37] A cycle commences; increased adiposity leads to leptin resistance, which leads to disrupted leptin signaling and a subsequent increase in food intake.[37]

Yet, obesity, or lack thereof, may not always be the cause of dysfunctional, or healthy, adipocytes. Interestingly, even in obese animals, over-expressed adiponectin was associated with healthy insulin sensitivity.[38] Adiponectin and leptin may be responsible for changes in metabolism regardless of obesity.[38,39]

The menopausal transition is characterized by accelerated gains in fat mass and losses of lean mass.[40] These changes in body composition have been associated with a poor adipokine profile: low adiponectin and high leptin levels.[41] However, little is known about adipokine changes following gains in lean mass among post-menopausal women.[41] The menopausal transition is also marked by changes in estradiol.[40] Changes in estradiol affect energy homeostasis pathways including food intake and energy expenditure, regulation of adipose tissue lipid storage and metabolism, and insulin sensitivity.[40] After menopause, adipose tissue becomes the main producer of estrogens by the enzyme aromatase. In women with obesity, aromatase activity is elevated, which leads to greater estrogen production and blood levels.[42] Inflammation in adipose tissue may induce the aromatase enzyme to produce more estrogen.[42] During the menopausal transition, dysfunctional adipose tissue has been linked to the deregulation of estrogen.[42] The majority of studies on adipokines and menopause address endometrial cancer or breast cancer given the central role of obesity-related endocrine disruption in these diseases.[42–44]

Endocrine Disrupting POPs: PCBs, PBDEs, OCPs

Persistent organic pollutants (POPs) are polyhalogenated organic compounds that confer effects as a mixture. Highly lipophilic, they persist in the environment and animals, more specifically in adipose tissue. Three main types of POPs include polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs) as illustrated in Figure 4. Research on environmental and biological fate, exposure, and

epidemiology has focused on PCBs and OCPs. Globally, production of OCPs and PCBs peaked in the 1960s and 1970s whereas production of PBDEs peaked in the 1990s and 2000s.[45] In Asia, OCP continued widely through the 2000s.[46] Most recently, China and other countries outside Europe and the U.S. have been responsible for the majority of environmental releases of decaBDEs and pentaBDEs.[45]

The POPs attributable health and economic burden is large. In the European Union in 2010, dichlorodiphenyldichloroethylene (DDE) alone may have been responsible for €834 million to 16.6 billion in annual healthcare costs related to adult diabetes.[47] Reducing PCB-153 and DDE exposure by 25% may lead to 8% lower diabetes prevalence among elderly Europeans, according to 2010 data.[48] The Stockholm Convention on Persistent Organic Pollutants (signed in 2001, implemented in 2004) was a global effort to reduce or eliminate POPs. Biomonitoring studies confirm that concentrations of POPs in humans are generally decreasing.[49,50]

Polychlorinated biphenyls (PCBs)

PCBs are characterized by two linked benzene rings with 1 to 10 hydrogen atoms substituted by chlorine atoms.[51] PCBs were used in industrial and commercial settings as coolant, dielectric, and heat-transfer fluids as well as paint and plastic additives. In the U.S., PCBs were produced from the 1930s until 1979, when they were banned for causing cancer. There are 209 PCB congeners, classified by the number of chlorines. PCBs were commonly sold as mixtures of congeners, such as Aroclor™ manufactured by Monsanto.[51] Half-lives range from months to over 30 years in humans, with greater persistence exhibited by higher-chlorinated congeners, especially PCB 138 and 153.[52] PCBs enter the environment during manufacturing, use, or improper storage. Once

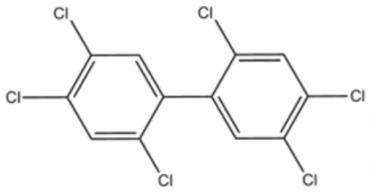


Figure 1.1. Structure of PCB 153.

released, they persist and can cycle between air, water, and soil.[51] Humans are primarily exposed through fatty animal-based foods.[53]

Polybrominated diphenyl ethers (PBDEs)

PBDEs are brominated hydrocarbons with 1 to 10 bromine atoms attached to a diphenyl ether molecule.[54] They are used as flame retardants applied to industrial and consumer products like textiles, furniture, and electronic devices. There are 209 PBDE congeners, determined by the number of bromines. PBDEs with more bromine atoms are less flammable and therefore more commonly used in commercial products, sold as congener mixtures. In the U.S., they have been used widely since the 1970s. They can be released into the environment during manufacturing or during disposal of products containing PBDEs.[55] Lower-brominated PBDEs may have the potential for long-range transport in air and are more likely to bioaccumulate than higher-brominated PBDEs.[56] The main human exposure pathways are diet and indoor dust inhalation and ingestion.[57] In humans, elimination time is not well characterized; studies suggest half-lives of days to three months, with lower-brominated congeners persisting longer than higher-brominated ones, sequestered in adipose tissue.[56]

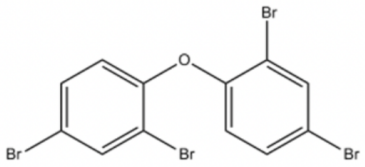


Figure 1.2. Structure of PBDE 47.

Organochlorine pesticides (OCPs)

OCPs are defined by chlorine substituted aliphatic or aromatic rings. Although most agricultural uses of OCPs were banned by many high-income countries (including the U.S. in the 1970s), their use has been rising in low- and middle-income countries.[58] They enter the environment after agricultural application, as leakage from

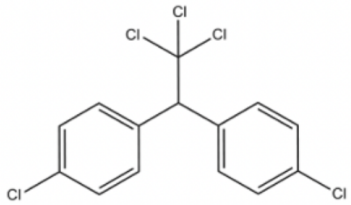


Figure 1.3. Structure of DDT.

landfills, or as industrial emissions and can be transported long distances in air and water.[59] Humans are most commonly exposed by eating animal-based fatty foods.[53] Common OCPs include dichlorodiphenyltrichloroethane (DDT), chlordane, heptachlor, endrin, aldrin, dieldrin, lindane, mirex, and hexachlorobenzene (HCB). Half-lives in humans range from days to decades in the case of endrin and DDT, respectively.[60,61]

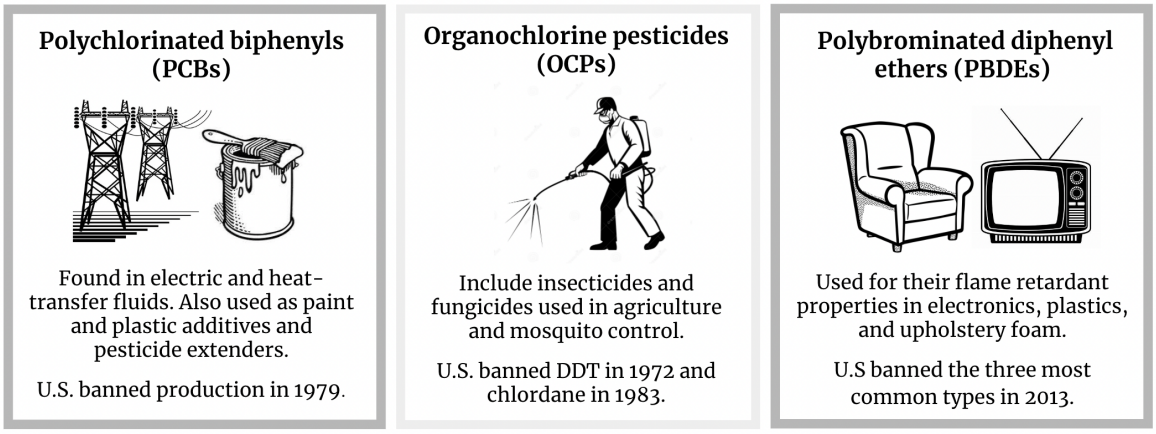


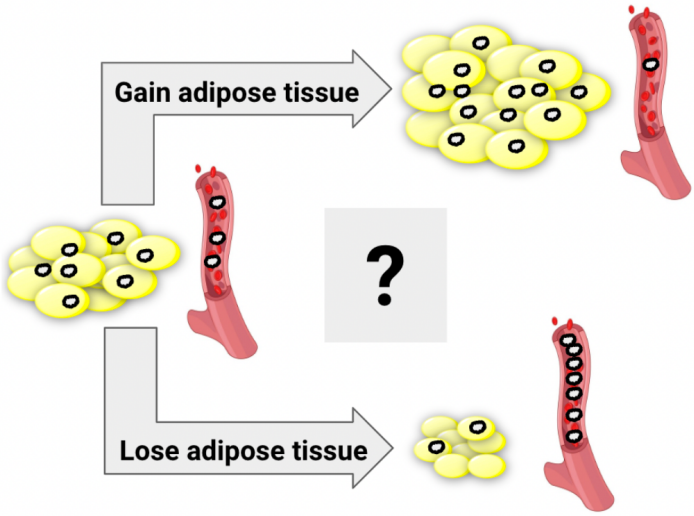
Figure 1.4. Sources and United States regulations of polychlorinated biphenyl ethers, organochlorine pesticides, and polybrominated diphenyl ethers.

The Role of Adiposity in the Storage and Effects of POPs

POPs are endocrine disrupting chemicals (EDCs), or more specifically metabolism disrupting chemicals, that accumulate in adipose tissue and circulate in blood bound to lipids.[62,63] Even after external exposure to POPs ceases, POPs stored in adipose tissue may continue to serve as an internal source of exposure. Obesity and weight change affect the sequestration of POPs and their circulation in blood.[20,64–72] Lipolysis of adipocytes and associated weight loss cause the release of POPs from adipose tissue and increased serum concentrations; in contrast, weight gain reduces serum concentrations and increases storage in adipose tissue (Figure 5).[20,59,63,73–75] POPs in adipose tissue may affect adipocytes in ways that increase the risk of

metabolic disease.[76,77] Adipose tissue may both protect from and exacerbate the deleterious health effects of POPs.[78]

Figure 1.5. Conceptual model of the relationship between lipophilic persistent organic pollutants in serum and adipose tissue following the loss or gain of adipose tissue.



Disparities in POPs Exposure and Risk of T2D

Racial and ethnic minority populations in the U.S. bear a greater burden of POPs exposure and a greater risk of T2D. [79] American Indian or Alaska Native adults have the highest rates of diagnosed diabetes (14.7%) among all U.S. racial/ethnic groups, followed by Hispanics (12.5%) and non-Hispanic Blacks (11.7%).[11] Furthermore, in non-White populations, associations between POPs and T2D are stronger than in White populations.[80] Some postulate that racial/ethnic and economic disparities are exacerbated by differential exposures to POPs.[21,81] The coalescence of these exposure and disease disparities is an issue of environmental justice. With this in mind, race/ethnicity may confound the relationship between POPs and T2D.

Women during the menopausal transition may be at greater risk of T2D and related complications relative to men and to women of different age groups. T2D increases the risk of coronary heart disease (CHD) more so in women (3-7 times) than men (2-3 times).[82] Although

the U.S. has seen a decline in CHD over the last few decades, women with T2D and mid-life women have not experienced these trends.[83,84] Menopause is a critical period of changes in hormones and adiposity that may increase T2D risk.[25,85,86] Sex differences in hormones may be responsible in part for a greater risk in women.[87] In addition, mid-life women, more specifically, uniquely experience rapid changes in hormone levels (declining estrogen and rising follicle stimulating hormone) during the two years leading up to and after the final menstrual period, after which levels plateau.[88] These hormone changes are less drastic in obese women.[88]

Furthermore, women may have higher serum concentrations of POPs. Serum concentrations tend to be higher in women than men despite clearance via childbirth and breastfeeding.[89,90] In addition, women may have a stronger relationship between POPs and obesity because their greater fat composition, on average, might extend the storage of POPs. POPs affect women during the menopausal transition and have been associated with early age at menopause, yet research is lacking.[91] As women lose or gain weight throughout the menopausal transition, POPs may be released or further sequestered in adipose tissue. Studies of the metabolic health effects of POPs in mid-life women must consider menopausal characteristics including underlying endocrine mechanisms unique to this life stage.

Effects of POPs on Adipocytes and Adipokines

Endocrine disrupting POPs can alter adipogenesis, adipose tissue growth, and the production and release of adipokines. They alter homeostatic metabolic setpoints, disrupt appetite controls, perturb lipid homeostasis to promote adipocyte hypertrophy, stimulate adipogenic pathways that enhance adipocyte hyperplasia, or alter adipocyte differentiation.[92,93] In vivo studies have observed that adult animals chronically exposed to human-relevant doses of POPs mixtures are more likely to experience low-grade inflammation,[94] increased visceral

fat,[95,94] and weight gain.[96,97] In humans, visceral fat was also positively associated with adult PCB exposure.[98] Post-menopausal, non-diabetic, obese women with higher plasma concentrations of POPs, compared to lower concentrations, may have lower insulin sensitivity and poorer cardiometabolic profiles.[99] The relationship between PCBs and adipose tissue may be stronger in women than men, yet few adult studies exist.[100] Of the few existing studies that assess mixtures of non-dioxin-like POPs and adiposity, most focus on the early life or prenatal exposure developmental window.[101,102,103,104] Moreover, the literature on POPs and adipokines is limited.

Epidemiological studies of POPs and adipokines have been limited and have had inconsistent findings. All prospective studies and most cross-sectional studies have been conducted in a patient population with obesity or in birth cohorts of women and children. Many studies indicate null or inverse associations between POPs and both adiponectin and leptin. Women and girls may more vulnerable to harmful changes in adipokines.[1,272,105–111] Exposure to summary measures of PCBs was inversely associated with leptin.[112] Studies of individual POPs found no associations between leptin and PCBs, HCB, or PBDEs.[108,111,113] For DDE specifically, evidence has suggested both inverse and null associations with leptin.[108,113,114] Adiponectin and PCBs have demonstrated inverse associations among women with obesity[106,107] as well as null associations in more general populations.[105,112,114] PBDEs had positive, marginally significant associations with adiponectin.[114] With the exception of one study in newborn girls, null associations were observed between both HCB and DDE and adiponectin. [105,110,114]

Compared to the epidemiological literature, in vivo and in vitro studies more consistently observed positive effects on leptin[115–123] yet similarly inconsistent effects on adiponectin. Mature adipocytes exposed to POPs (PCB-101, 126, 153, 180, or DDE) increased[118,121] or did not affect leptin levels.[122] Exposing preadipocytes to DDE increased leptin and adiponectin.[119,120]

Preadipocytes treated with PCB 153 demonstrated a reduction in leptin gene expression and no significant change in adiponectin expression.[123] Treatment of preadipocytes with a PBDE replacement increased fat tissue and expression of adiponectin.[124] Animal studies suggest that PCBs lead to hypothyroidism and increased leptin but differ in their findings on adiponectin.[115,116,125] To our knowledge, only one animal study has evaluated PBDEs and adiponectin, finding a negative effect among males and no effect among females.[117]

Biological Mechanisms Linking POPs and T2D

Endocrine Disruption

Endocrine disruption is the most likely biological mechanism to explain the relationship between POPs and T2D. POPs behave like hormones, which often display non-linear, non-monotonic dose-response relationships.[126] Hormones produce large biological effects at low doses. Hormones demonstrate linear effects within a low-dose range, up to a dose that occupies 10% of receptors, above which a higher dose does not translate to a greater effect.[126,127]

Experimental studies suggest that POPs alter adipose tissue and increase the risk of T2D in a non-monotonic, non-linear dose-response relationship. Low-dose POPs mixtures are obesogenic and impair insulin sensitivity and glucose homeostasis, with stronger effects seen following weight loss. [94,95,128,129,130] Adult animals that chronically ingest POPs (obtaining a body burden similar to those in humans approximately ages 40 to 50) develop insulin resistance and visceral obesity among other conditions.[95] Chronic consumption of POPs-contaminated fish may lead to increased visceral obesity and accelerated insulin resistance and glucose intolerance, yet the dose-response relationship is unclear, possibly due to the beneficial effects of fish or to the existence of a non-linear dose-response curve.[94,128] In vivo results are supported by evidence that

adipocytes treated with a mixture of OCPs and PCBs may develop reduced insulin action with a non-monotonic, non-linear dose-response relationship similar to that exhibited by hormones.[95]

POPs may be hormone antagonists or agonists, acting synergistically or additively as a mixture to amplify or nullify their effects.[131] The endocrine effects of PCBs and OCPs have been studied more than the effects of PBDEs. OCPs are well-known EDCs, conferring effects such as altered thyroid hormone levels, hormone-related cancers, and T2D. EDCs can act as agonists or antagonists of nuclear receptors, non-nuclear steroid membrane receptors, orphan receptors (besides AHR), and nonsteroid receptors and influence or be metabolized by endogenous hormone enzymatic pathways.[132] Activation or inhibition of hepatic or adipose nuclear receptors (PXR, CAR, PPARs, FXR) is associated with dysregulated and altered energy metabolism and obesity.[133] It is unclear which endocrine disrupting characteristics of POPs drive the development of T2D, although multiple hormone pathways may be involved. Estrogens, androgens, thyroid hormone, and glucocorticoid hormone are involved in glucose homeostasis and lipid metabolism.[134] Furthermore, sex steroids play vital roles in the pathogenesis of cardiometabolic disorders with sex-specific effects.[21]

In women, OCPs have both estrogenic and anti-estrogenic effects.[59]PCBs also have estrogenic and anti-estrogenic effects[59,51] as well as disruptions to thyroid hormone signaling.[135]

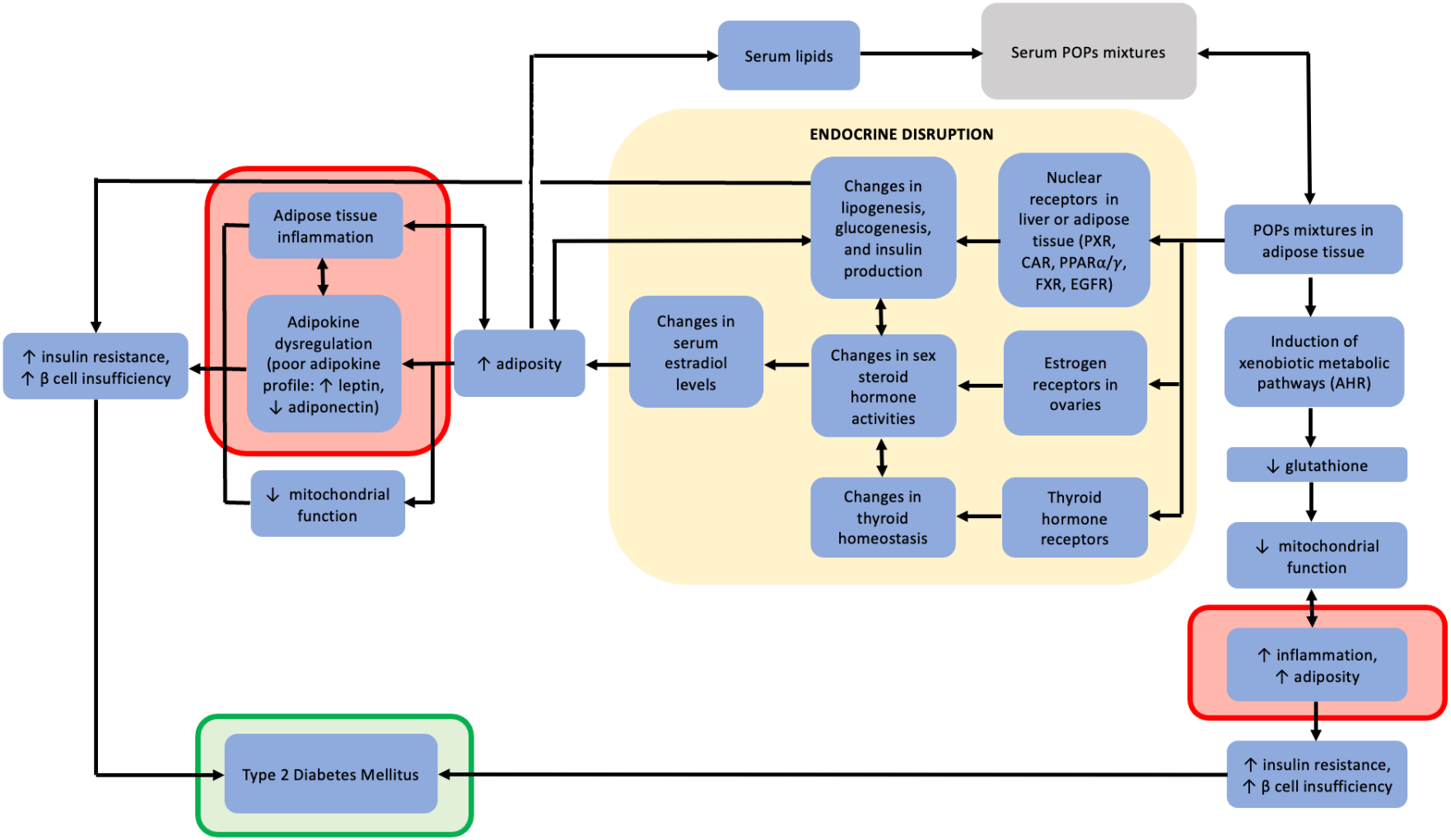
Through disrupted hormone axes, POPs may upset the healthy functioning of adipose tissue. Potential pathways are illustrated in Figure 6. POPs may inhibit adipose cell differentiation and instead encourage a combination of many small immature cells alongside large existing mature cells.[59,136,137] If adipose tissue in this state experiences excess energy intake, lipids rise in the blood and other organs, leading to increased ectopic fat (and possibly obesity), insulin resistance, and T2D.[59,136,137] As discussed in Section 4, growing adipose tissue

may sequester POPs, in turn reducing serum concentrations, while also releasing adipokines, which may play a role in the development of insulin resistance and T2D.

Mitochondrial Dysfunction

Mitochondrial dysfunction is another proposed mechanism linking POPs and T2D, as depicted in Figure 6. It is characterized by low levels of blood glutathione (GSH). Mitochondria are involved in the pathogenesis of insulin resistance and T2D.[138,139] High concentrations of DDT and PCBs may induce mitochondrial toxicity.[140,141,142] Yet, minimal evidence suggests that current background concentrations of POPs could affect mitochondria, possibly via chronic low-grade inflammation, even in non-obese people.[95,143] Moreover, there is insufficient evidence that mitochondrial dysfunction could explain the inverted u-shaped dose-response curve observed in numerous epidemiological studies. For this to be true, more studies would have to show that GSH is protective at high levels.[144] Considering the available scientific literature, endocrine disruption is a more plausible biological mechanism than mitochondrial dysfunction.

Figure 1.6. Conceptual model of the mechanisms linking POPs exposure and T2D, adapted from Wahlang et al. (2018) and Lee et al. (2014).



Human Studies of the Relationship between POPs and T2D

Cross-sectional Studies

Cross-sectional studies suggest that OCPs and PCBs are associated with increased T2D prevalence, although the strength and shape of such relationships may differ by compound, sex, and BMI.[76,145–152] Women may be at greater risk.[151,153,154,155] The largest studies have found strong linear relationships between T2D prevalence and serum concentrations of individual POPs (trans-chlordane, trans-nonachlor, oxychlordane, pp-DDE, PCB-126, PCB-153) with stronger effects observed when using a summary POPs measure and among obese or overweight individuals; pp-DDT may have a non-linear relationship with T2D.[76,146,147] Smaller cross-sectional studies provide less consistent results.[114,147,154–156]

Only three cross-sectional studies have focused on underrepresented populations.[157,158,159] Among Mexican Americans, pp-DDE exhibited a linear relationship with T2D whereas other OCPs did not exhibit dose-response relationships (trans-nonachlor, pp-DDT, beta-HCH, and oxychlordane) or had no effect (HCB and dieldrin).[157] Among Native Americans, high concentrations of individual POPs (pp-DDE, HCB, PCB-153, and PCB-74) in addition to a summary measure of 101 PCBs were positively associated with T2D.[158] Among First Nations men and women in Canada, increased exposure to a mixture of pp-DDE and 8 PCB congeners was associated with increased prevalence of T2D.[159] Racial and ethnic minorities are often not prioritized let alone included in the literature, but considering the disproportionate burden of POPs exposure and T2D experienced by these communities, it is vital to understand their risk.

Cross-sectional studies suggest that adiposity and POPs have a synergistic effect on T2D, although the studies are somewhat contradictory and fail to elucidate a dose-response curve. Overall, the design of cross-sectional studies could produce false associations between POPs and

obesity because increasing obesity over time could increase sequestration of POPs. In order to supplement data from a single blood draw, it may be possible to simulate accumulated life-time concentrations of PCBs at previous time points.[153] However, true prospective studies are required to elucidate any temporal relationship between POPs and T2D.

Case-control Studies

Few case-control studies have addressed POPs and T2D. Some have reported strong associations between T2D and OCPs and PCBs.[145,160] One major limitation is the measurement of serum POPs after T2D diagnosis because it prevents assessment of a temporal relationship even though the long half-lives of POPs may reflect decades-old exposure. Case-control studies are also limited by potential reverse causation, that T2D could affect levels of POPs in serum.

Prospective Studies

Prospective studies have reported mixed results yet suggest that mid-life women may be particularly susceptible. Among women during mid-life (mean age of 59 at baseline), T2D risk was strongly associated with higher serum concentrations of HCBs while concentrations of DDT, DDE, and PCBs (individual and summary measures) were positively but not significantly associated. Exposure to PCBs at high concentrations (much higher than U.S. background levels) was associated with increased T2D incidence, with women at significantly greater risk than men.[87,161,162,163] Among young adults ages 18 to 30, POPs exhibited non-linear relationships with risk of T2D with the strongest associations seen at low-doses of OCPs, highly chlorinated PCBs, and PBB-153; analysis using a summary measure of 31 POPs revealed a clearer inverse u-shaped dose-response curve.[164] However these findings could be spurious, possibly due to the small sample size or to the execution of numerous statistical tests.

Among mid-life and elderly individuals, exposure to OCPs, namely DDE and trans-nonachlor, were significant predictors of T2D while the effects of PCBs and PBDEs were inconsistent.[114,165–167] Charles et al. (2022) was notable for multiple serum measurements of POPs and a temporal analysis pre- and post-diabetes diagnosis; they identified positive associations between T2D and OCPs up to seven years before diagnosis with estimates strengthening when using serum collected at timepoints increasing closer to diagnosis.[166] Many prospective studies have been conducted in mid-life and older populations, yet only two examined mid-life women specifically; one skewed post-menopausal[161] and the other used dietary data to estimate exposure rather than serum samples.[168] No prospective study has investigated how mid-life changes in adipose tissue may act along the path from POPs exposure to T2D nor how the menopausal transition impacts women's risk.

Observational Studies of PBDEs

There is limited epidemiological literature on diabetes risk associated with PBDEs and other brominated flame retardants. Magliano et al. (2021) found no association between multiple PBDEs and diabetes.[169] Lee et al. (2010) found a positive association between wet weight concentrations of PBB 153, yet the association disappeared after concentrations were standardized for serum lipids.[164] Ongono et al. (2019) found positive associations between dietary estimates of exposure to multiple PBDEs and diabetes.[168] Zhang et al. (2016) observed a positive association between PBDEs and blood glucose but did not observe an association with insulin resistance.[170] Additional epidemiological research on PBDEs and diabetes is needed.

POP Mixtures in Prospective Studies

Only two diabetes studies have analyzed POP mixtures and none have incorporated PBDE exposure. The first study used Bayesian Kernel Machine Regression (BKMR) models to

show that exposure to a PCB mixture was associated with increased diabetes prevalence in a linear fashion.[171] Employing Weighted Quantile Sum (WQS) regression, the second study found that exposure to a mixture of PCBs and OCPs was associated with an increased prevalence of metabolic syndrome driven primarily by the effects OCPs.[172] More mixtures research is needed to more comprehensively understand the relationship between POPs and metabolism.

Although not a mixtures approach, summing exposure to individual PCBs has been common practice; however, it does not account for confounding among PCB congeners nor how PCBs may interact with OCPs and PBDEs through shared biological pathways. Summary scores for young adult exposure to 32 POPs (23 PCBs and 8 OCPs, excluding PBDEs due to low detection) were associated with greater disruption of glucose homeostasis after 18 years of follow up, with the strongest associations seen among individuals ages 48-55 after adjusting for BMI.[173] Using sum of rank measures of PCBs and OCPs, it was observed that increasing POPs within a lower dose range were associated with increased risk of T2D whereas increasing POPs within a higher dose range were associated with only a slight increase in T2D risk, suggesting non-linearity.[174]

Summary of Epidemiological Findings

Epidemiological studies point to an association between POPs and T2D and a synergistic relationship between POPs and obesity, although the evidence is inconsistent. Mid-life women may be at greater risk of T2D due to POPs exposure as well as changing adiposity. Across studies, the shapes of dose-response curves and the statistical significance for individual POPs vary. This does not negate the existence of an inverted U-shaped dose-response curve. Rather, these puzzling findings may be explained by different exposure distributions between study populations. If the concentration in the reference category is different across studies (and if there

is a wide range of concentrations in the reference category), then the risk estimates will be very different across studies. For example, if a population is exposed to a range of POPs concentrations, all of which are extremely high, as in some occupational settings, it would be unsurprising if POPs were found to have no significant association with T2D because even the referent "low" level category is considered "high" in non-occupational settings. An inverted U-shaped dose-response curve is biologically plausible, yet evidence in humans is lacking. Furthermore, only two studies have analyzed the effects of POP mixtures on T2D and no studies of adipokines have employed mixtures methods. Mixtures research is necessary to characterize complex exposures more accurately and assess their cumulative effects on metabolic processes.

Dissertation Overview

The objective of this dissertation was to evaluate visceral adipose tissue as a time-varying internal source of POPs as well as the potential metabolic impacts of POPs throughout the menopausal transition. It consists of the following three aims, all of which were conducted in the Study of Women's Health Across the Nation (SWAN) Multi-Pollutant Study (MPS). Aim 1 investigated the association between the difference in waist circumference and the difference in serum concentrations of OCPs and PCBs across four visits between 1999 and 2011 in 75 women. Aim 2 analyzed the association of exposure to an overall mixture of serum concentrations of OCPs, PCBs, and PBDEs measured in 1999/2000, as well as individual mixture components, and incident diabetes through 2016 in 1,400 women. Aim 3 assessed the relationship between serum concentrations of OCPs, PCBs, and PBDEs in 1999/2000, individually and as mixtures, and levels of leptin and HMW adiponectin in 2002/2003. This research has the potential to inform chemical policies and regulations worldwide, assessment of climate change-related health impacts, and clinical considerations related to metabolic risk factors in aging women.

References

1. International Diabetes Federation. *Diabetes Around the World 2021*. https://diabetesatlas.org/idfawp/resource-files/2021/11/IDFDA10-global-fact-sheet.pdf (2021).
2. World Health Organization. *Diabetes*. https://www.who.int/news-room/fact-sheets/detail/diabetes (2022).
3. Centers for Disease Control and Prevention. *Prevalence of Both Diagnosed and Undiagnosed Diabetes*. https://www.cdc.gov/diabetes/data/statistics-report/diagnosed-undiagnosed-diabetes.html (2022).
4. Wild, S., Roglic, G., Green, A., Sicree, R. & King, H. Global Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care* **27**, 1047–1053 (2004).
5. Centers for Disease Control and Prevention. *By the Numbers: Diabetes in America*. https://www.cdc.gov/diabetes/health-equity/diabetes-by-the-numbers.html (2022).
6. Centers for Disease Control and Prevention. *Diabetes Infographics*. https://www.cdc.gov/diabetes/library/socialmedia/infographics.html (2023).
7. Vargas-Vázquez, A. *et al.* Impact of undiagnosed type 2 diabetes and pre-diabetes on severity and mortality for SARS-CoV-2 infection. *BMJ Open Diabetes Res. Care* **9**, e002026 (2021).
8. Chatterjee, S., Khunti, K. & Davies, M. J. Type 2 diabetes. *The Lancet* **389**, 2239–2251 (2017).

9. Harvard Medical School. Type 2 Diabetes Mellitus. https://www.health.harvard.edu/a_to_z/type-2-diabetes-mellitus-a-to-z (2018).

10. Billings, L. K. & Florez, J. C. The genetics of type 2 diabetes: what have we learned from GWAS?: Genetics of type 2 diabetes. *Ann. N. Y. Acad. Sci.* **1212**, 59–77 (2010).

11. Centers for Disease Control and Prevention. *Diabetes Report Card 2019*. https://www.cdc.gov/diabetes/library/reports/reportcard.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fdiabetes%2Flibrary%2Freports%2Fcongress.html (2019).

12. Centers for Disease Control and Prevention. The Health Effects of Overweight and Obesity. https://www.cdc.gov/healthyweight/effects/index.html (2020).

13. World Health Organization. Obesity and Overweight. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (2020).

14. Center for Disease Control and Prevention: National Center for Health Statistics. Obesity and Overweight. https://www.cdc.gov/nchs/fastats/obesity-overweight.htm (2021).

15. Biggs, M. L. Association Between Adiposity in Midlife and Older Age and Risk of Diabetes in Older Adults. *JAMA* **303**, 2504 (2010).

16. Sims, E. A. Are there persons who are obese, but metabolically healthy? *Metabolism* **50**, 1499–1504 (2001).

17. McLaughlin, T., Abbasi, F., Lamendola, C. & Reaven, G. Heterogeneity in the prevalence of risk factors for cardiovascular disease and type 2 diabetes mellitus in obese individuals: effect of differences in insulin sensitivity. *Arch. Intern. Med.* **167**, 642–648 (2007).

18. Gregg, E. W., Cheng, Y. J., Narayan, K. M. V., Thompson, T. J. & Williamson, D. F. The relative contributions of different levels of overweight and obesity to the increased prevalence of diabetes in the United States: 1976–2004. *Prev. Med.* **45**, 348–352 (2007).

19. Blüher, M. The distinction of metabolically ‘healthy’ from ‘unhealthy’ obese individuals. *Curr. Opin. Lipidol.* **21**, 38–43 (2010).

20. Dirtu, A. C. *et al.* Dynamics of Organohalogenated Contaminants in Human Serum from Obese Individuals during One Year of Weight Loss Treatment. *Environ. Sci. Technol.* **47**, 12441–12449 (2013).

21. Liu, S. & Sun, Q. Sex differences, endogenous sex-hormone hormones, sex-hormone binding globulin, and exogenous disruptors in diabetes and related metabolic outcomes: 性别差异、内源性性激素、性激素结合球蛋白以及外源性干扰因子对糖尿病及其相关代谢结果的影响. *J. Diabetes* **10**, 428–441 (2018).

22. Ding, E. L. *et al.* Sex Hormone–Binding Globulin and Risk of Type 2 Diabetes in Women and Men. *N. Engl. J. Med.* **361**, 1152–1163 (2009).

23. Chen, B. H. *et al.* Sex Hormone–Binding Globulin and Risk of Clinical Diabetes in American Black, Hispanic, and Asian/Pacific Islander Postmenopausal Women. *Clin. Chem.* **58**, 1457–1466 (2012).

24. Perry, J. R. B. *et al.* Genetic evidence that raised sex hormone binding globulin (SHBG) levels reduce the risk of type 2 diabetes. *Hum. Mol. Genet.* **19**, 535–544 (2010).

25. Ding, E. L., Song, Y., Malik, V. S. & Liu, S. Sex Differences of Endogenous Sex Hormones and Risk of Type 2 Diabetes: A Systematic Review and Meta-analysis. *JAMA* **295**, 1288 (2006).

26. Andersson, B., Marin, P., Lissner, L., Vermeulen, A. & Bjorntorp, P. Testosterone Concentrations in Women and Men With NIDDM. *Diabetes Care* **17**, 405–411 (1994).

27. Phillips, G. B. *et al.* Association of hyperandrogenemia and hyperestrogenemia with type 2 diabetes in Hispanic postmenopausal women. *Diabetes Care* **23**, 74–79 (2000).

28. Scheja, L. & Heeren, J. The endocrine function of adipose tissues in health and cardiometabolic disease. *Nat. Rev. Endocrinol.* **15**, 507–524 (2019).

29. Schaab, M. & Kratzsch, J. The soluble leptin receptor. *Best Pract. Res. Clin. Endocrinol. Metab.* **29**, 661–670 (2015).

30. von Eynatten, M. *et al.* High-molecular weight adiponectin is independently associated with the extent of coronary artery disease in men. *Atherosclerosis* **199**, 123–128 (2008).

31. Nakashima, R. *et al.* Decreased Total and High Molecular Weight Adiponectin Are Independent Risk Factors for the Development of Type 2 Diabetes in Japanese-Americans. *J. Clin. Endocrinol. Metab.* **91**, 3873–3877 (2006).

32. Peake, P. W., Kriketos, A. D., Campbell, L. V., Shen, Y. & Charlesworth, J. A. The metabolism of isoforms of human adiponectin: studies in human subjects and in experimental animals. *Eur. J. Endocrinol.* **153**, 409–417 (2005).

33. Fang, H. & Judd, R. L. Adiponectin Regulation and Function. in *Comprehensive Physiology* (ed. Pollock, D. M.) 1031–1063 (John Wiley & Sons, Inc., 2018). doi:10.1002/cphy.c170046.

34. Dunmore, S. J. & Brown, J. E. P. The role of adipokines in β-cell failure of type 2 diabetes. *J. Endocrinol.* **216**, T37–T45 (2013).

35. Farooqi, I. S. & O'Rahilly, S. 20 YEARS OF LEPTIN: Human disorders of leptin action. *J. Endocrinol.* **223**, T63–T70 (2014).

36. Friedman, J. The long road to leptin. *J. Clin. Invest.* **126**, 4727–4734 (2016).

37. Izquierdo, A. G., Crujeiras, A. B., Casanueva, F. F. & Carreira, M. C. Leptin, Obesity, and Leptin Resistance: Where Are We 25 Years Later? *Nutrients* **11**, 2704 (2019).

38. Kim, J.-Y. *et al.* Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *J. Clin. Invest.* **117**, 2621–2637 (2007).

39. Ebenibo, S., Edeoga, C., Owei, I. & Dagogo-Jack, S. Basal and Dynamic Leptin Secretion: Association with Cardiometabolic Risk and Body Weight Trajectories in African-Americans and European-Americans. *Front. Endocrinol.* **9**, 12 (2018).

40. Greendale, G. A. *et al.* Changes in body composition and weight during the menopause transition. *JCI Insight* **4**, 124865 (2019).

41. Chapman-Lopez, T. *et al.* The influence of resistance training on adipokines in post-menopausal women: A brief review. *Sports Med. Health Sci.* **4**, 219–224 (2022).

42. Sánchez-Jiménez, F., Pérez-Pérez, A., De La Cruz-Merino, L. & Sánchez-Margalet, V. Obesity and Breast Cancer: Role of Leptin. *Front. Oncol.* **9**, 596 (2019).

43. Ding, S., Madu, C. O. & Lu, Y. The Impact of Hormonal Imbalances Associated with Obesity on the Incidence of Endometrial Cancer in Postmenopausal Women. *J. Cancer* **11**, 5456–5465 (2020).

44. Zimta *et al.* Molecular Links between Central Obesity and Breast Cancer. *Int. J. Mol. Sci.* **20**, 5364 (2019).

45. Li, L. *et al.* What do we know about the production and release of persistent organic pollutants in the global environment? *Environ. Sci. Adv.* **2**, 55–68 (2023).

46. FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS REGIONAL OFFICE FOR ASIA AND THE PACIFIC. *PROCEEDINGS: Asia Regional Workshop on the Implementation, Monitoring and Observance of the International Code of*

Conduct on the Distribution and Use of Pesticides: http://assets.apppc.fao.org.s3-eu-west-1.amazonaws.com/public/documents/20141212/1140573518738_Report_of_WSP_1_2014 11060356--1.67%20MB_201412120854--1.67%20MB.pdf (2005).

47. Legler, J. *et al.* Obesity, Diabetes, and Associated Costs of Exposure to Endocrine-Disrupting Chemicals in the European Union. *J. Clin. Endocrinol. Metab.* **100**, 1278–1288 (2015).
48. Trasande, L., Lampa, E., Lind, L. & Lind, P. M. Population attributable risks and costs of diabetogenic chemical exposures in the elderly. *J. Epidemiol. Community Health* **71**, 111–114 (2017).
49. Norén, K. & Meironyté, D. Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20–30 years. *Chemosphere* **40**, 1111–1123 (2000).
50. Porta, M. *et al.* Monitoring concentrations of persistent organic pollutants in the general population: The international experience. *Environ. Int.* **34**, 546–561 (2008).
51. Agency for Toxic Substances and Disease Registry. *Toxicological Profile for Polychlorinated Biphenyls (PCBs)*. https://www.atsdr.cdc.gov/toxprofiles/tp17.pdf.
52. Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Polychlorinated Biphenyls (PCBs)*. https://www.atsdr.cdc.gov/ToxProfiles/tp17.pdf (2000).
53. Fisher, B. E. Most unwanted. *Environ. Health Perspect.* **107**, (1999).
54. Environmental Protection Agency. *Technical Fact Sheet – Polybrominated Diphenyl Ethers (PBDEs)*. https://www.epa.gov/sites/production/files/2014-03/documents/ffrrofactsheet_contaminant_perchlorate_january2014_final_0.pdf (2017).

55. Darnerud, P. O., Eriksen, G. S., Jóhannesson, T., Larsen, P. B. & Viluksela, M. Polybrominated diphenyl ethers: occurrence, dietary exposure, and toxicology. *Environ. Health Perspect.* **109**, 49–68 (2001).

56. Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Polybrominated Diphenyl Ethers (PBDEs)*. https://www.atsdr.cdc.gov/toxprofiles/tp207-c6.pdf.

57. Agency for Toxic Substances and Disease Registry. *Toxicological Profile for Polybrominated Diphenyl Ethers (PBDEs)*. https://www.atsdr.cdc.gov/toxprofiles/tp207-c6.pdf.

58. Jayaraj, R., Megha, P. & Sreedev, P. Review Article. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. *Interdiscip. Toxicol.* **9**, 90–100 (2016).

59. Lee, D.-H., Porta, M., Jacobs, D. R. & Vandenberg, L. N. Chlorinated Persistent Organic Pollutants, Obesity, and Type 2 Diabetes. *Endocr. Rev.* **35**, 557–601 (2014).

60. Agency for Toxic Substances and Disease Registry. *Toxicological Profile for DDT, DDE, and DDD*. https://www.atsdr.cdc.gov/toxprofiles/tp35-c3.pdf.

61. Agency for Toxic Substances and Disease Registry. *Toxicological Profile for Endrin*. https://www.atsdr.cdc.gov/ToxProfiles/tp89-c3.pdf.

62. Heindel, J. J. *et al.* Metabolism disrupting chemicals and metabolic disorders. *Reprod. Toxicol.* **68**, 3–33 (2017).

63. Yu, G. W., Laseter, J. & Mylander, C. Persistent Organic Pollutants in Serum and Several Different Fat Compartments in Humans. *J. Environ. Public Health* **2011**, 1–8 (2011).

64. Sjödin, A. *et al.* Polybrominated Diphenyl Ethers, Polychlorinated Biphenyls, and 2,2-Bis(4-chlorophenyl)-1,1-dichloroethene in 7- and 9-Year-Old Children and Their Mothers in the Center for the Health Assessment of Mothers and Children of Salinas Cohort. *Environ. Sci. Technol.* **52**, 2287–2294 (2018).

65. Stubleski, J., Lind, L., Salihovic, S., Lind, P. M. & Kärrman, A. Longitudinal changes in persistent organic pollutants (POPs) from 2001 to 2009 in a sample of elderly Swedish men and women. *Environ. Res.* **165**, 193–200 (2018).

66. Fénichel, P. *et al.* Sustained bloodstream release of persistent organic pollutants induced by extensive weight loss after bariatric surgery: Implications for women of childbearing age. *Environ. Int.* **151**, 106400 (2021).

67. Dirinck, E. *et al.* Pivotal Role for the Visceral Fat Compartment in the Release of Persistent Organic Pollutants During Weight Loss. *J. Clin. Endocrinol. Metab.* **100**, 4463–4471 (2015).

68. Dirinck, E. L. *et al.* Endocrine-disrupting polychlorinated biphenyls in metabolically healthy and unhealthy obese subjects before and after weight loss: difference at the start but not at the finish. *Am. J. Clin. Nutr.* **103**, 989–998 (2016).

69. Chevrier, J. *et al.* Body weight loss increases plasma and adipose tissue concentrations of potentially toxic pollutants in obese individuals. *Int. J. Obes.* **24**, 1272–1278 (2000).

70. Imbeault, P., Tremblay, A., Simoneau, J.-A. & Joanisse, D. R. Weight loss-induced rise in plasma pollutant is associated with reduced skeletal muscle oxidative capacity. *Am. J. Physiol.-Endocrinol. Metab.* **282**, E574–E579 (2002).

71. He, F., Zuo, L., Ward, E. & Arciero, P. J. Serum Polychlorinated Biphenyls Increase and Oxidative Stress Decreases with a Protein-Pacing Caloric Restriction Diet in Obese Men and Women. *Int. J. Environ. Res. Public. Health* **14**, E59 (2017).

72. Jansen, A. *et al.* Increased levels of persistent organic pollutants in serum one year after a great weight loss in humans: Are the levels exceeding health based guideline values? *Sci. Total Environ.* **622–623**, 1317–1326 (2018).

73. Irigaray, P., Newby, J. A., Lacomme, S. & Belpomme, D. Overweight/obesity and cancer genesis: More than a biological link. *Biomed. Pharmacother.* **61**, 665–678 (2007).

74. Louis, C., Tinant, G., Mignolet, E., Thomé, J.-P. & Debier, C. PCB-153 Shows Different Dynamics of Mobilisation from Differentiated Rat Adipocytes during Lipolysis in Comparison with PCB-28 and PCB-118. *PLoS ONE* **9**, e106495 (2014).

75. Lim, J. S., Son, H.-K., Park, S.-K., Jacobs, D. R. & Lee, D.-H. Inverse associations between long-term weight change and serum concentrations of persistent organic pollutants. *Int. J. Obes.* **35**, 744–747 (2011).

76. Lee, D.-H., Jacobs, D. R. & Porta, M. Could low-level background exposure to persistent organic pollutants contribute to the social burden of type 2 diabetes? *J. Epidemiol. Community Health* **60**, 1006–1008 (2006).

77. Porta, M. Persistent organic pollutants and the burden of diabetes. *The Lancet* **368**, 558–559 (2006).

78. La Merrill, M. *et al.* Toxicological Function of Adipose Tissue: Focus on Persistent Organic Pollutants. *Environ. Health Perspect.* **121**, 162–169 (2013).

79. Ruiz, D., Becerra, M., Jagai, J. S., Ard, K. & Sargis, R. M. Disparities in Environmental Exposures to Endocrine-Disrupting Chemicals and Diabetes Risk in Vulnerable Populations. *Diabetes Care* **41**, 193–205 (2018).

80. Song, Y. *et al.* Endocrine-disrupting chemicals, risk of type 2 diabetes, and diabetes-related metabolic traits: A systematic review and meta-analysis: 内分泌干扰物、2型糖尿病危险因素以及糖尿病相关代谢特征:一项系统回顾与meta分析. *J. Diabetes* **8**, 516–532 (2016).

81. Sargis, R. M. & Simmons, R. A. Environmental neglect: endocrine disruptors as underappreciated but potentially modifiable diabetes risk factors. *Diabetologia* **62**, 1811–1822 (2019).

82. Mosca, L. *et al.* Cardiovascular Disease in Women: A Statement for Healthcare Professionals From the American Heart Association. *Circulation* **96**, 2468–2482 (1997).

83. Gu, K. Diabetes and Decline in Heart Disease Mortality in US Adults. *JAMA* **281**, 1291 (1999).

84. Towfighi, A. Sex-Specific Trends in Midlife Coronary Heart Disease Risk and Prevalence. *Arch. Intern. Med.* **169**, 1762 (2009).

85. Ding, E. L. *et al.* Plasma sex steroid hormones and risk of developing type 2 diabetes in women: a prospective study. *Diabetologia* **50**, 2076–2084 (2007).

86. Li, C., Ford, E. S., Li, B., Giles, W. H. & Liu, S. Association of Testosterone and Sex Hormone-Binding Globulin With Metabolic Syndrome and Insulin Resistance in Men. *Diabetes Care* **33**, 1618–1624 (2010).

87. Wang, S.-L., Tsai, P.-C., Yang, C.-Y. & Leon Guo, Y. Increased Risk of Diabetes and Polychlorinated Biphenyls and Dioxins: A 24-year follow-up study of the Yucheng cohort. *Diabetes Care* **31**, 1574–1579 (2008).

88. Randolph, J. F. *et al.* Change in Follicle-Stimulating Hormone and Estradiol Across the Menopausal Transition: Effect of Age at the Final Menstrual Period. *J. Clin. Endocrinol. Metab.* **96**, 746–754 (2011).

89. Vallack, H. W. *et al.* Controlling persistent organic pollutants–what next? *Environ. Toxicol. Pharmacol.* **6**, 143–175 (1998).

90. Fernández-Rodríguez, M. *et al.* Levels and predictors of persistent organic pollutants in an adult population from four Spanish regions. *Sci. Total Environ.* **538**, 152–161 (2015).

91. Grindler, N. M. *et al.* Persistent Organic Pollutants and Early Menopause in U.S. Women. *PLOS ONE* **10**, e0116057 (2015).

92. Grün, F. *et al.* Endocrine-Disrupting Organotin Compounds Are Potent Inducers of Adipogenesis in Vertebrates. *Mol. Endocrinol.* **20**, 2141–2155 (2006).

93. Richter, C. A. *et al.* In vivo effects of bisphenol A in laboratory rodent studies. *Reprod. Toxicol.* **24**, 199–224 (2007).

94. Ibrahim, M. M. *et al.* Chronic Consumption of Farmed Salmon Containing Persistent Organic Pollutants Causes Insulin Resistance and Obesity in Mice. *PLoS ONE* **6**, e25170 (2011).

95. Ruzzin, J. *et al.* Persistent Organic Pollutant Exposure Leads to Insulin Resistance Syndrome. *Environ. Health Perspect.* **118**, 465–471 (2010).

96. Lyche, J. L. *et al.* Natural Mixtures of Persistent Organic Pollutants (POP) Increase Weight Gain, Advance Puberty, and Induce Changes in Gene Expression Associated with Steroid

Hormones and Obesity in Female Zebrafish. *J. Toxicol. Environ. Health A* **73**, 1032–1057 (2010).

97. Lyche, J. L. *et al.* Natural mixtures of POPs affected body weight gain and induced transcription of genes involved in weight regulation and insulin signaling. *Aquat. Toxicol.* **102**, 197–204 (2011).

98. Dirinck, E. L. *et al.* Exposure to Persistent Organic Pollutants: Relationship With Abnormal Glucose Metabolism and Visceral Adiposity. *Diabetes Care* **37**, 1951–1958 (2014).

99. Gauthier, M.-S. *et al.* The Metabolically Healthy But Obese Phenotype Is Associated With Lower Plasma Levels of Persistent Organic Pollutants as Compared to the Metabolically Abnormal Obese Phenotype. *J. Clin. Endocrinol. Metab.* **99**, E1061–E1066 (2014).

100. Tang-Péronard, J. L. *et al.* Association between prenatal polychlorinated biphenyl exposure and obesity development at ages 5 and 7 y: a prospective cohort study of 656 children from the Faroe Islands. *Am. J. Clin. Nutr.* **99**, 5–13 (2014).

101. Braun, J. M. Early-life exposure to EDCs: role in childhood obesity and neurodevelopment. *Nat. Rev. Endocrinol.* **13**, 161–173 (2017).

102. Biemann, R., Fischer, B. & Navarrete Santos, A. Adipogenic Effects of a Combination of the Endocrine-Disrupting Compounds Bisphenol A, Diethylhexylphthalate, and Tributyltin. *Obes. Facts* **7**, 48–56 (2014).

103. Kassotis, C. D. *et al.* Endocrine-Disrupting Activity of Hydraulic Fracturing Chemicals and Adverse Health Outcomes After Prenatal Exposure in Male Mice. *Endocrinology* **156**, 4458–4473 (2015).

104. Carnevali, O. *et al.* Dietary administration of EDC mixtures: A focus on fish lipid metabolism. *Aquat. Toxicol.* **185**, 95–104 (2017).

105. Debost-Legrand, A. *et al.* Prenatal exposure to persistent organic pollutants and organophosphate pesticides, and markers of glucose metabolism at birth. *Environ. Res.* **146**, 207–217 (2016).

106. Mullerova, D. *et al.* Negative association between plasma levels of adiponectin and polychlorinated biphenyl 153 in obese women under non-energy-restrictive regime. *Int. J. Obes. 2005* **32**, 1875–1878 (2008).

107. Lim, J. & Jee, S. H. Association between serum levels of adiponectin and polychlorinated biphenyls in Korean men and women. *Endocrine* **48**, 211–217 (2015).

108. Tang-Péronard, J. L. *et al.* Prenatal exposure to persistent organochlorine pollutants is associated with high insulin levels in 5-year-old girls. *Environ. Res.* **142**, 407–413 (2015).

109. Pereira-Fernandes, A. *et al.* Expression of Obesity Markers and Persistent Organic Pollutants Levels in Adipose Tissue of Obese Patients: Reinforcing the Obesogen Hypothesis? *PLoS ONE* **9**, e84816 (2014).

110. Berghuis, S. A. *et al.* Prenatal Environmental Exposure to Persistent Organic Pollutants and Reproductive Hormone Profile and Pubertal Development in Dutch Adolescents. *Int. J. Environ. Res. Public. Health* **19**, 9423 (2022).

111. Leijs, M. M. *et al.* Alterations in the programming of energy metabolism in adolescents with background exposure to dioxins, dl-PCBs and PBDEs. *PLOS ONE* **12**, e0184006 (2017).

112. Clair, H. B. *et al.* Liver Disease in a Residential Cohort With Elevated Polychlorinated Biphenyl Exposures. *Toxicol. Sci.* **164**, 39–49 (2018).

113. Burns, J. S. *et al.* Association Between Chlorinated Pesticides in the Serum of Prepubertal Russian Boys and Longitudinal Biomarkers of Metabolic Function. *Am. J. Epidemiol.* **180**, 909–919 (2014).

114. Turyk, M. *et al.* Persistent Organic Pollutants and Biomarkers of Diabetes Risk in a Cohort of Great Lakes Sport Caught Fish Consumers. *Environ. Res.* **140**, 335–344 (2015).

115. Ahmed, R. G., El-Gareib, A. W. & Shaker, H. M. Gestational 3,3',4,4',5-pentachlorobiphenyl (PCB 126) exposure disrupts fetoplacental unit: Fetal thyroid-cytokines dysfunction. *Life Sci.* **192**, 213–220 (2018).

116. Ahmed, R. G. Early weaning PCB 95 exposure alters the neonatal endocrine system: thyroid adipokine dysfunction. *J. Endocrinol.* **219**, 205–215 (2013).

117. Kozlova, E. V. *et al.* Developmental exposure to indoor flame retardants and hypothalamic molecular signatures: Sex-dependent reprogramming of lipid homeostasis. *Front. Endocrinol.* **13**, 997304 (2022).

118. Ferrante, M. C. *et al.* Polychlorinated biphenyls (PCB 101, PCB 153 and PCB 180) alter leptin signaling and lipid metabolism in differentiated 3T3-L1 adipocytes. *Toxicol. Appl. Pharmacol.* **279**, 401–408 (2014).

119. Mangum, L. H., Howell, G. E. & Chambers, J. E. Exposure to p,p′-DDE enhances differentiation of 3T3-L1 preadipocytes in a model of sub-optimal differentiation. *Toxicol. Lett.* **238**, 65–71 (2015).

120. Howell, G. & Mangum, L. Exposure to bioaccumulative organochlorine compounds alters adipogenesis, fatty acid uptake, and adipokine production in NIH3T3-L1 cells. *Toxicol. In Vitro* **25**, 394–402 (2011).

121. Taxvig, C. *et al.* Differential effects of environmental chemicals and food contaminants on adipogenesis, biomarker release and PPARγ activation. *Mol. Cell. Endocrinol.* **361**, 106–115 (2012).

122. Caron, A. *et al.* Effects of PCB126 on Adipose-to-Muscle Communication in an *in Vitro* Model. *Environ. Health Perspect.* **128**, 107002 (2020).

123. May, P. *et al.* In Vitro Cocktail Effects of PCB-DL (PCB118) and Bulky PCB (PCB153) with BaP on Adipogenesis and on Expression of Genes Involved in the Establishment of a Pro-Inflammatory State. *Int. J. Mol. Sci.* **19**, (2018).

124. Peshdary, V., Calzadilla, G., Landry, A., Sorisky, A. & Atlas, E. Dechlorane Plus increases adipogenesis in 3T3-L1 and human primary preadipocytes independent of peroxisome proliferator-activated receptor γ transcriptional activity. *Int. J. Obes. 2005* **43**, 545–555 (2019).

125. Wahlang, B. *et al.* Polychlorinated biphenyl 153 is a diet-dependent obesogen that worsens nonalcoholic fatty liver disease in male C57BL6/J mice. *J. Nutr. Biochem.* **24**, 1587–1595 (2013).

126. Vandenberg, L. N. *et al.* Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr. Rev.* **33**, 378–455 (2012).

127. Welshons, W. V. *et al.* Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. *Environ. Health Perspect.* **111**, 994–1006 (2003).

128. Ibrahim, M. M. *et al.* Metabolic impacts of high dietary exposure to persistent organic pollutants in mice. *Toxicol. Lett.* **215**, 8–15 (2012).

129. Baker, N. A. *et al.* Coplanar Polychlorinated Biphenyls Impair Glucose Homeostasis in Lean C57BL/6 Mice and Mitigate Beneficial Effects of Weight Loss on Glucose Homeostasis in Obese Mice. *Environ. Health Perspect.* **121**, 105–110 (2013).

130. Gray, S. L., Shaw, A. C., Gagne, A. X. & Chan, H. M. Chronic exposure to PCBs (Aroclor 1254) exacerbates obesity-induced insulin resistance and hyperinsulinemia in mice. *J. Toxicol. Environ. Health A* **76**, 701–715 (2013).

131. Woodruff, T. J., Zota, A. R. & Schwartz, J. M. Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. *Environ. Health Perspect.* **119**, 878–885 (2011).

132. Diamanti-Kandarakis, E. *et al.* Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocr. Rev.* **30**, 293–342 (2009).

133. Wahlang, B. Exposure to persistent organic pollutants: impact on women's health. *Rev. Environ. Health* **33**, 331–348 (2018).

134. Neel, B. A. & Sargis, R. M. The paradox of progress: environmental disruption of metabolism and the diabetes epidemic. *Diabetes* **60**, 1838–1848 (2011).

135. Giera, S., Bansal, R., Ortiz-Toro, T. M., Taub, D. G. & Zoeller, R. T. Individual polychlorinated biphenyl (PCB) congeners produce tissue- and gene-specific effects on thyroid hormone signaling during development. *Endocrinology* **152**, 2909–2919 (2011).

136. Abate, N. Adipocyte Maturation Arrest: A Determinant of Systemic Insulin Resistance to Glucose Disposal. *J. Clin. Endocrinol. Metab.* **97**, 760–763 (2012).

137. Danforth, E. Failure of adipocyte differentiation causes type II diabetes mellitus? *Nat. Genet.* **26**, 13 (2000).

138. Patti, M.-E. & Corvera, S. The role of mitochondria in the pathogenesis of type 2 diabetes. *Endocr. Rev.* **31**, 364–395 (2010).

139. Szendroedi, J., Phielix, E. & Roden, M. The role of mitochondria in insulin resistance and type 2 diabetes mellitus. *Nat. Rev. Endocrinol.* **8**, 92–103 (2011).

140. Bagchi, M. & Stohs, S. J. In vitro induction of reactive oxygen species by 2,3,7,8-tetrachlorodibenzo-p-dioxin, endrin, and lindane in rat peritoneal macrophages, and hepatic mitochondria and microsomes. *Free Radic. Biol. Med.* **14**, 11–18 (1993).

141. Pardini, R. S. Polychlorinated biphenyls (PCB): effect on mitochondrial enzyme systems. *Bull. Environ. Contam. Toxicol.* **6**, 539–545 (1971).

142. Pardini, R. S., Heidker, J. C., Baker, T. A. & Payne, B. Toxicology of various pesticides and their decomposition products on mitochondrial electron transport. *Arch. Environ. Contam. Toxicol.* **9**, 87–97 (1980).

143. López-Armada, M. J., Riveiro-Naveira, R. R., Vaamonde-García, C. & Valcárcel-Ares, M. N. Mitochondrial dysfunction and the inflammatory response. *Mitochondrion* **13**, 106–118 (2013).

144. Slezak, B. P. *et al.* Oxidative stress in female B6C3F1 mice following acute and subchronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Toxicol. Sci. Off. J. Soc. Toxicol.* **54**, 390–398 (2000).

145. Taylor, K. W. *et al.* Evaluation of the association between persistent organic pollutants (POPs) and diabetes in epidemiological studies: a national toxicology program workshop review. *Environ. Health Perspect.* **121**, 774–783 (2013).

146. Everett, C. J. *et al.* Association of a polychlorinated dibenzo-p-dioxin, a polychlorinated biphenyl, and DDT with diabetes in the 1999-2002 National Health and Nutrition Examination Survey. *Environ. Res.* **103**, 413–418 (2007).

147. Airaksinen, R. *et al.* Association between type 2 diabetes and exposure to persistent organic pollutants. *Diabetes Care* **34**, 1972–1979 (2011).

148. Ukropec, J. *et al.* High prevalence of prediabetes and diabetes in a population exposed to high levels of an organochlorine cocktail. *Diabetologia* **53**, 899–906 (2010).

149. Tanaka, T. *et al.* Congener-specific polychlorinated biphenyls and the prevalence of diabetes in the Saku Control Obesity Program (SCOP). *Endocr. J.* **58**, 589–596 (2011).

150. Uemura, H. *et al.* Associations of environmental exposure to dioxins with prevalent diabetes among general inhabitants in Japan. *Environ. Res.* **108**, 63–68 (2008).

151. Silverstone, A. E. *et al.* Polychlorinated biphenyl (PCB) exposure and diabetes: results from the Anniston Community Health Survey. *Environ. Health Perspect.* **120**, 727–732 (2012).

152. Jørgensen, M. E., Borch-Johnsen, K. & Bjerregaard, P. A cross-sectional study of the association between persistent organic pollutants and glucose intolerance among Greenland Inuit. *Diabetologia* **51**, 1416–1422 (2008).

153. Rylander, C., Sandanger, T. M., Nøst, T. H., Breivik, K. & Lund, E. Combining plasma measurements and mechanistic modeling to explore the effect of POPs on type 2 diabetes mellitus in Norwegian women. *Environ. Res.* **142**, 365–373 (2015).

154. Rignell-Hydbom, A., Rylander, L. & Hagmar, L. Exposure to persistent organochlorine pollutants and type 2 diabetes mellitus. *Hum. Exp. Toxicol.* **26**, 447–452 (2007).

155. Arrebola, J. P. *et al.* Relationship between serum concentrations of persistent organic pollutants and markers of insulin resistance in a cohort of women with a history of gestational diabetes mellitus. *Environ. Res.* **136**, 435–440 (2015).

156. Rylander, L., Rignell-Hydbom, A. & Hagmar, L. A cross-sectional study of the association between persistent organochlorine pollutants and diabetes. *Environ. Health* **4**, 28 (2005).

157. Cox, S., Niskar, A. S., Narayan, K. M. V. & Marcus, M. Prevalence of Self-Reported Diabetes and Exposure to Organochlorine Pesticides among Mexican Americans: Hispanic Health and Nutrition Examination Survey, 1982–1984. *Environ. Health Perspect.* **115**, 1747–1752 (2007).

158. Codru, N., Schymura, M. J., Negoita, S., Rej, R. & Carpenter, D. O. Diabetes in Relation to Serum Levels of Polychlorinated Biphenyls and Chlorinated Pesticides in Adult Native Americans. *Environ. Health Perspect.* **115**, 1442–1447 (2007).

159. Philibert, A., Schwartz, H. & Mergler, D. An Exploratory Study of Diabetes in a First Nation Community with Respect to Serum Concentrations of p,p'-DDE and PCBs and Fish Consumption. *Int. J. Environ. Res. Public. Health* **6**, 3179–3189 (2009).

160. Son, H.-K. *et al.* Strong associations between low-dose organochlorine pesticides and type 2 diabetes in Korea. *Environ. Int.* **36**, 410–414 (2010).

161. Wu, H. *et al.* Persistent organic pollutants and type 2 diabetes: a prospective analysis in the nurses' health study and meta-analysis. *Environ. Health Perspect.* **121**, 153–161 (2013).

162. Vasiliu, O., Cameron, L., Gardiner, J., Deguire, P. & Karmaus, W. Polybrominated biphenyls, polychlorinated biphenyls, body weight, and incidence of adult-onset diabetes mellitus. *Epidemiol. Camb. Mass* **17**, 352–359 (2006).

163. Bertazzi, P. A. *et al.* Health effects of dioxin exposure: a 20-year mortality study. *Am. J. Epidemiol.* **153**, 1031–1044 (2001).

164. Lee, D.-H. *et al.* Low dose of some persistent organic pollutants predicts type 2 diabetes: a nested case-control study. *Environ. Health Perspect.* **118**, 1235–1242 (2010).

165. Rignell-Hydbom, A. *et al.* Exposure to p,p'-DDE: a risk factor for type 2 diabetes. *PloS One* **4**, e7503 (2009).

166. Charles, D. *et al.* Longitudinal changes in concentrations of persistent organic pollutants (1986–2016) and their associations with type 2 diabetes mellitus. *Environ. Res.* **204**, 112129 (2022).

167. Lee, D.-H. *et al.* Low Dose Organochlorine Pesticides and Polychlorinated Biphenyls Predict Obesity, Dyslipidemia, and Insulin Resistance among People Free of Diabetes. *PLoS ONE* **6**, e15977 (2011).

168. Ongono, J. S. *et al.* Dietary exposure to brominated flame retardants and risk of type 2 diabetes in the French E3N cohort. *Environ. Int.* **123**, 54–60 (2019).

169. Magliano, D. J. *et al.* Exposure to persistent organic pollutants and the risk of type 2 diabetes: a case-cohort study. *Diabetes Metab.* **47**, 101234 (2021).

170. Zhang, Z. *et al.* Environmental exposure to BDE47 is associated with increased diabetes prevalence: Evidence from community-based case-control studies and an animal experiment. *Sci. Rep.* **6**, 27854 (2016).

171. Gasull, M. *et al.* Blood Concentrations of Persistent Organic Pollutants and Prediabetes and Diabetes in the General Population of Catalonia. *Environ. Sci. Technol.* **46**, 7799–7810 (2012).

172. Reina-Pérez, I. *et al.* Cross-sectional associations of persistent organic pollutants measured in adipose tissue and metabolic syndrome in clinically diagnosed middle-aged adults. *Environ. Res.* 115350 (2023) doi:10.1016/j.envres.2023.115350.

173. Suarez-Lopez, J. R., Lee, D.-H., Porta, M., Steffes, M. W. & Jacobs, D. R. Persistent organic pollutants in young adults and changes in glucose related metabolism over a 23-year follow-up. *Environ. Res.* **137**, 485–494 (2015).

174. Lee, D.-H. *et al.* Associations of persistent organic pollutants with abdominal obesity in the elderly: The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. *Environ. Int.* **40**, 170–178 (2012).

Chapter II. Changes in Adipose Tissue and Circulating Concentrations of Persistent Organic Pollutants in The Study of Women's Health Across the Nation

Amelia Grant-Alfieri[a], Amila Devasurendra[a], Stuart Batterman[a], Carrie Karvonen-Gutierrez[b], Sung Kyun Park[a,b]

Abstract

Background: Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are persistent organic pollutants (POPs) that bioaccumulate in adipose tissue and can negatively impact health via endocrine system disruption. In the United States beginning in the 1970s, regulations on the industrial production or commercial use of POPs have reduced external exposure. Yet, POPs exposure can be persistent within an individual because they can be released from adipose tissue and thus constitute a continued internal source of exposure.

Objectives: We investigated the relationship between change in central adiposity and changes in circulating concentrations of POPs over a twelve-year period during the midlife.

Methods: We measured serum concentrations of 34 PCBs and 19 OCPs at four timepoints (1999/2000, 2002/03, 2005/06, 2009/11) in a longitudinal cohort of midlife women, the Study of Women's Health Across the Nation. Linear mixed models were used to test the association

[a] Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, Michigan, USA
[b] Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan, USA

between a change in waist circumference and change in serum POP concentrations. PCB data was available for 65 women for a total of 181 observations. OCP data was available for 59 women for a total of 151 observations.

Results: After adjustment for covariates (study site, race/ethnicity, age at baseline, parity), a one-inch (2.54 cm) increase in the change in waist circumference from one visit to the next was associated with a 4.9% decrease in the change in serum concentration of PCB 194 (95% CI: -8.0%, -1.6%). No associations were observed for other PCB congeners or OCPs. Of all the POPs analyzed, PCB 194 has the greatest lipophilicity.

Conclusions: An increase in the difference in waist circumference over time was not associated with a change in the difference in serum concentrations of PCBs and OCPs with the exception of PCB 194. PCB 194 may be sequestered in adipose tissue following adiposity gain during the midlife in women. Further research is warranted on intra-person variation in serum POPs concentrations as well as other factors that may affect trends in serum concentrations.

Introduction

Persistent organic pollutants (POPs) include polyhalogenated organic compounds that can confer harmful health effects including neurological, immunological, hepatic, reproductive, and developmental toxicity as well as carcinogenesis.[1,2] Two important classes of POPs are polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs). PCBs encompass 209 congeners with between 1 and 10 chlorine atoms on a biphenyl (two benzene ring) structure.[1] PCBs were heavily produced in the United States (U.S.) from the 1930s until 1979 and used as dielectric, coolant, and heat-transfer fluids as well as paint and plastic additives. Many OCPs are defined by chlorine-substituted aliphatic or aromatic rings and other structures. Most uses of OCPs were banned by many high-income countries, including the U.S., in the 1970s and 1980s due to harmful ecological and neurological effects and possible carcinogenic effects.[3] Other countries later adopted regulations on OCPs such as Mexico, which banned DDT in 2000.[4] Due to rising food demands and pesticide resistance, the use of pesticides including OCPs has risen in countries across Asia as well as in parts of Europe and Africa, although the contribution of OCPs relative to total pesticide burden is difficult to determine.[4]

POPs persist in the environment and animals, especially in adipose tissue, and act as endocrine disrupting chemicals. The main pathway of human exposure to POPs is diet, through consumption of animal-based fatty foods.[1] Indoor air is another important exposure source of PCBs, settling on surfaces and accumulating in household dust.[5] Due to their lipophilic nature and very low elimination rates, even after external exposure ceases, POPs stored in adipose tissue continue to serve as an internal source of exposure.[6] The capacity and duration of POP storage in adipose tissue may vary by tissue type and POP lipophilicity.[7] Highly-chlorinated PCBs are more

likely to bioaccumulate than less-chlorinated PCBs. Obesity and weight change affect the sequestration of POPs and their circulation in blood, and in turn, may affect health outcomes.[8,9]

There is limited evidence regarding temporal trends in POPs. In the U.S. there are two longitudinal studies of general background exposure to PCBs and/or OCPs measured in serum at multiple timepoints.[10,11] Results are inconsistent. Sjödin and colleagues measured p,p'-DDE and 6 PCBs annually from 2009 to 2011 and found a decreasing trend ($p < 0.05$) for PCB 99 in mothers and for PCB 99, 118, 138/158, 153, and 180 in children. Marek and colleagues measured 209 PCBs in 2008/09 and 2009/10 and found that the median concentration of ∑PCBs increased by 6 ng/g lipid (5^{th}-95^{th} percentile: −115 to 164). Given the persistence and slow elimination of many POPs, with half-lives ranging from days to 30 years or more in the case of DDT metabolites and some PCBs,[12] multiple measurements may be necessary to characterize within-person changes over time. Most cohort studies with repeat serum measurements have focused on historically highly exposed populations, e.g. anglers and residents near contaminated industrial sites, and thus do not reflect background exposure levels of the broader U.S. population.[13–17] Longitudinal studies of longer duration have been conducted elsewhere, all in northern Europe, and found decreasing serum concentrations of OCPs and PCBs since the late 1980s with some OCPs exhibiting more stable trends.[18–20] Cross-sectional studies corroborate these findings and suggest that exposure to OCPs and PCBs will continue to wane in large part thanks to global efforts to ban or reduce production of these chemicals; yet, among older, and thus more highly exposed, individuals, serum concentrations of some PCBs and OCPs have been shown to increase with age.[21] Overall, there is insufficient evidence to support conclusions about intra-individual trends in serum concentrations of POPs and how trends may be influenced by changes in adiposity.

Weight and fat loss are associated with increased serum concentrations of POPs.[11,22–30] However, most studies assessed adults with obesity before and after a weight loss program (e.g. dietary intervention), bariatric surgery, or gastroplasty.[23,26–30] Few studies went further to include control individuals who were not obese and did not undergo weight loss, which would allow for greater discrimination of effects due to weight loss.[24,25] To our knowledge, only two studies represent general, non-obese, non-patient populations; one was conducted among the elderly in Sweden and the other among children in the U.S.[11,22] Studies are limited by a maximum of two serum POP samples and a lack of representativeness of individuals without obesity.

To better understand the relationship between POPs and adiposity, we must evaluate long-term trends in exposure during a life stage characterized by changes in body composition. The menopausal transition is widely understood as a time of rapid increases in fat mass and redistribution of adipose tissue.[31] The Study of Women's Health Across the Nation (SWAN) is one of the most carefully phenotyped populations with respect to the timing of the menopausal transition and various heath markers. This study investigated whether changes in waist circumference (WC), a measure of central adiposity, were associated with changes in serum concentrations of POPs among U.S. midlife women in a longitudinal substudy of SWAN.

Methods

Study Population

Participants were from the Study of Women's Health Across the Nation (SWAN) Multi-Pollutant Study (MPS), which is ancillary to the larger SWAN. SWAN is fully detailed in a previous study.[32] SWAN is a multi-site, multi-ethnic cohort study launched in 1996 to follow 3,302 premenopausal women between the ages of 42 and 52 through the menopausal transition. By design, women were recruited from seven clinical sites (Oakland, CA; Los Angeles, CA; Chicago,

IL; southeast, MI; Pittsburgh, PA; Boston, MA; Newark, NJ). Each site recruited a White sample and a site-specific non-White sample (Black, Chinese, Japanese, or Hispanic). Additional eligibility criteria included having an intact uterus and ovary, reporting a menstrual period within the past three months, and not using hormone medications in the last three months. Across a total of 17 follow-up visits from 1996 to the present day, SWAN has collected data on metabolic and reproductive biomarkers and health outcomes, in addition to socio-demographic, lifestyle, and other risk factors. The institutional review board at each participating site approved the study protocol and all participants provided written, signed informed consent at each study visit.

The SWAN MPS characterizes longitudinal environmental exposures in a subset of SWAN women. The sample design of the SWAN MPS is detailed in Figure S2.1. Environmental exposure data was collected from biobanked specimens from 1999/2000 at SWAN Visit 3 (V03 or baseline). After further excluding participants with insufficient serum or urine samples, the final MPS study totaled 1,400 women at V03 (1999/2000). A subset of 75 women were analyzed for serum concentrations of POPs at three additional timepoints for a total of 300 samples across four visits: V03 (1999/2000), Visit 6 (V06, 2002/03), Visit 9 (V09, 2005/06), and Visit 12 (V12, 2009/11). The substudy sampling procedure is detailed in Figure S2.2. Due to limited resources, three study sites were selected in attempts to best capture racial/ethnic and geographic variations: Boston, MA, southeast MI, and Oakland, CA. To maximize representativeness, we conducted random sampling to attain an equal distribution of women with above-, below-, or at-average WC change (ΔWC) over the study period, after omitting those in the top and bottom 2% of ΔWC. We oversampled certain racial/ethnic groups to attain a composition of 50% White, 25% Black, and 25% Chinese. The substudy is a unique longitudinal cohort designed to assess environmental exposures over time in relation to changing WC, a proxy for central adiposity. We

analyzed POP observations for individuals with a minimum of two timepoints that met POP quality assurance criteria. Consequently, PCB data was available for 65 women for a total of 181 observations, and OCP data was available for 59 women for a total of 151 observations.

POP Measurements

Serum concentrations of PBDEs, PCBs, and OCPs were measured using fasting blood samples collected from the 75 women in the SWAN MPS substudy at V03 (1999/2000), V06 (2002/03), V09 (2005/06), and V12 (2009/11). Samples were processed by the Organic Chemistry Lab in the Department of Environmental Health Sciences at the University of Michigan, Ann Arbor, Michigan, USA for 34 PCBs, 14 PBDEs, and 19 OCPs including DDT metabolites, chlordanes, and hexachlorobenzene (HCB) (see Table S2.1 for the complete list). Typically, the more chlorinated PCB homologues are more persistent,[1] whereas PBDE congeners with higher numbers reflect lower bromination and lesser persistence.[33]

Target analyte concentrations (ng/g sample) were measured by gas chromatography-mass spectrometry (GC/MS) using the following procedures. Two vials of serum, nominally slightly over 0.5mL each, were withdrawn from the SWAN repository. The samples had been previously stored at -80°C for 8-20 years after sample collection. Given the stability of analytes at this temperature, loss of sample integrity was not expected to be an issue. The two samples were combined to obtain a larger volume desired for analysis, weighed, spiked with surrogate standards $^{13}C_{12}$-PCB-208 and $^{-13}C_{12}$-BDE-139 prepared at 10 ng/mL, and 1mL of 6M hydrochloric acid was added. Liquid-liquid extractions used sequential additions of 6 mL methanol-isopropanol (1:1), 6 mL of hexane/methyl t-butyl ether (1:1), and 3 mL of hexane/MTBE to the sample, followed by cleanup using a column packed with 0.1 g of silica (top layer) and 1 g of silica (lower layer). The column was eluted with hexane/DCM (8 mL, 1:1 v/v), and concentrated to 0.5 mL under nitrogen

flow, dissolved with 0.5 mL of n-nonane, evaporated to 0.25 mL, and transferred to a conical insert placed in a GC-vial. The cleaned extracts were spiked with 15 µL of an internal standard containing 11 OCPs, 10 BDEs, and 21 PCBs, all labeled compounds at a concentration of 7.5 ng/mL, then the vial sealed and placed into the autosampler for analysis. Surrogate and internal standards were from Cambridge Isotope Labs (Andover, MA, USA). Analytes were identified and quantified by GC/MS (5890/5973, Agilent Industries, Palo Alto, CA, USA) using 2 µL splitless injections, a capillary DB-5MS column (30 m length, 0.25 mm ID, 0.25 µm film thickness, J&W Scientific, Folsom, CA, USA), negative chemical ionization, the two most abundant ions, and separate runs for OCPs, BDEs, and PCBs with GC temperature programs optimized to separate compounds. Calibrations used authentic standards that spanned the expected concentration range of the target analytes. If individual peaks could not be identified (several PCBs fell into crowded windows), a mixture was reported and not used in the analysis.

Quality control/quality assurance measures performed in each analytical sequence included the use of ^{13}C labeled internal and surrogate standards, blank checks, drift checks, recovery checks, and performance checks using a standard reference material (SRM 1957 "Organic contaminants in non-fortified human serum," NIST, Gaithersburg, MD, USA). Acceptance criteria for drift checks was variation less than 10%, and $r^2 > 0.999$ for linearity. Spike recoveries ranged from 88−95%. PCB surrogate recoveries ranged from 76% to 103%. Method detection limits (MDLs), based on 7 replicate low concentration measurements, ranged from 0.0062-0.092, 0.0041-0.513, and 0.072-0.462 ng/mL for OCPs, PCBS, and PBDEs, respectively.

POPs congeners detected in at least 70% of samples at all four visits were included in analyses of individual congeners. Concentrations below the MDL were replaced with MDL/$\sqrt{2}$.

PBDEs were not included due to low detection frequencies. PBDEs with relatively high detection frequencies (20-50%) were PBDE 47, 153 and 154. Detection frequencies for OCPs, PCBs, and PBDEs are presented in Table S2.1.

Lipid-standardized POP concentrations are preferable to wet weight concentrations because POPs are lipophilic, circulate bound to serum lipids, and distribute in the body mainly according to a tissue's lipid content.[34,35] Serum lipids including total cholesterol and triglycerides for lipid standardization of POP concentrations were measured by enzymatic methods using Hitachi 747-200 analyzer (Boehringer Mannheim Diagnostics, Indianapolis IN). The total lipid concentration was imputed using linear regression to replace missing observations of total cholesterol (n = 1) and triglycerides (n = 10). Figure S2.3 illustrates the relationships between adipose tissue, serum lipids, and serum POPs.

Standardizing serum POP concentrations by serum lipid levels has been the most common method employed in studies of serum POPs. We compared two methods of lipid standardization: traditional standardization (Method 1) and covariate-adjusted standardization (Method 2).[36] Method 1 eliminates the influence of recent fat intake on serum lipids and facilitates the comparisons of exposure between individuals. O'Brien and colleagues recommended traditional standardization in addition to model adjustment for serum lipids when serum POPs are the exposure of interest. However, in our study, it is the outcome. Serum lipid levels are affected by the gain or loss of adipose tissue, which is our exposure of interest. Method 2 allows for between-person variation in adiposity-based serum lipids due to participant characteristics. Method 2 is preferred over Method 1 because standardizing serum POPs with serum lipids may lead to a spurious association between adipose tissue loss/gain and lipid-

standardized serum POP levels. We further compared Methods 1 and 2, shown below, to models of wet weight serum POPs (Method 3).

Method 1: $S = C / OTL \times 102.6$

$OTL = (2.27 * TC) + TG + 62.3$ [37]

Method 2: $AS = C / (OTL / FTL)$

where S is the lipid-standardized serum POP concentration (ng/g lipid); C is the wet weight serum POP concentration (ng/g); OTL is the observed total lipid concentration (mg/dl); TC is total cholesterol (mg/dl); TG is triglycerides (mg/dl); AS is the covariate-adjusted standardized serum POP concentration (ng/g); and FTL is the fitted concentration of total lipids (ng). A linear mixed model was used to calculate the natural-log-transformed FTL adjusting for WC, age, study site, and race/ethnicity with a random effect for each woman.

Our outcome of primary interest is the proportional change in POP concentration between visits (ΔPOP) using the natural log transformation to normalize the distributions. As shown below,

$\Delta lnPOP = ln(POP_{v+1} / POP_v)$,

where POP_v is the POP concentration (ng/g lipid) at visit v and POP_{v+1} is the POP concentration at the next available timepoint.

Waist Circumference (WC)

WC is a marker of central (visceral) adiposity assessed at each study visit by a trained technician. WC was measured to the nearest 0.1 cm at the narrowest part of the torso. We converted the units from centimeters to inches to aid interpretation. ΔWC between visits was calculated.

Covariates

Potential confounders of the relationship between ΔPOP and ΔWC were selected a priori and included age at baseline (1999/2000), study site, race/ethnicity, and parity. Age is important

given that bans and phase-outs of many POPs have increased the likelihood that older women have a greater body burden.[18,38] Study sites included Oakland, CA, Boston, MA, and southeast MI. Race/ethnicity included Chinese, White, and Black. Race is not used as a proxy for biological or genetic differences yet is included because it is implicit in systems, policies, and institutions that shape individuals' environments and experiences. Study site and race/ethnicity can influence lifestyle and access to resources (i.e. diet, built environment) that affect both POPs exposure and WC. Furthermore, historical contamination differs by site.[39] Differences in lifestyle, resources, and past contamination may result from structural racism (institutional or systemic racism), "a system in which public policies, institutional practices, cultural representations, and other norms work in various, often reinforcing ways to perpetuate racial group inequity." [40] Parity is also important to consider because breastfeeding is an elimination route of POPs,[41] and parity may be associated with adiposity changes later in life.[42,43] Parity was defined as nulliparous or parous based on live births and stillbirths. Uniform protocols were used to collect covariate data across study sites.

Statistical Analysis

The distributions of 34 PCB congeners and 19 OCPs were characterized using the median and interquartile range (IQR). We reported concentrations using traditional lipid-standardization to facilitate comparison with previous studies. An intraclass correlation coefficient (ICC) was calculated to assess how much of the temporal variability in serum POP measurements exists between subjects. A high ICC indicates that the inter-subject variability exceeds within-subject variability. We examined time-dependent and time-independent participant characteristics. T-tests and ANOVAs were performed to analyze WC and serum POP concentrations by participant characteristics.

Linear mixed models were constructed with random intercepts for each participant to examine the association between a change in WC and a change in serum POP concentrations. We fit a crude model of ΔPOP that included ΔWC and visit (V03 serving as the reference) to capture time trends and other visit effects. Serum concentrations of POPs were transformed using the natural log to approximate a normal distribution. In the final model, we adjusted for age at baseline, race/ethnicity, study site, parity, and the number of visits elapsed between observations.

We compared models using traditional POP lipid-standardization with those using wet weight concentrations and covariate-adjusted standardization. Ultimately, we used covariate-adjusted standardization in crude and adjusted models, as shown below,

$$\Delta ln(POP)_{ij} = \beta_0 + \beta_1 \Delta WC + \beta_2 V06_i + \beta_3 V09_i + \beta_4 V12_i + \beta_5 White_i + \beta_6 Chinese_i + \beta_7 Parous_i + \beta_8 Oakland_i + \beta_9 Michigan_i + \beta_{10} Age_i + \beta_{11} T_i + b_{0j} + \varepsilon_{ij},$$

where $\Delta ln(POP)_{ij}$ is the proportional change in POP concentration between visits (ng/g) for the ith subject with the jth observation; ΔWC is the change in WC (inches) between visits; V06 is 2002/03, V09 is 2005/06, and V12 is 2009/11; Age is age at baseline (years); and T is the number of visits elapsed between observations. Statistical analyses were conducted using Rstudio (2022.12.0.353), Integrated Development Environment for R, Posit Software, PBC, Boston, MA.

Results

Participant Characteristics

The characteristics of SWAN women at baseline (or first available timepoint, in the case of sociodemographic characteristics) are presented in Table 2.1. Characteristics vary slightly depending on the POP type (and therefore sample) for which data is available at two or more timepoints. The median age at baseline was 49 years (IQR: 47-50). Most women were from Oakland, CA followed by Boston, MA. In terms of race/ethnicity, approximately half were

White. White and Black women were recruited from Boston, MA and southeast MI whereas Chinese and White women were recruited from Oakland, CA. The median WC at baseline was approximately 32 inches (81.28 cm) (IQR: 30-37). Few women (17%) were nulliparous.

The ΔWC between visits is reported in Table 2.2. Among women with available PCB data, the average ΔWC was positive between timepoints up to Visit 9 and negative for intervals that included Visit 12. For example, the mean ΔWC was 0.52 inches (1.32 cm) (range: -2.48, 5.39) from Visit 3 to 6 and -0.77 inches (-1.96 cm) (range: -4.65, 3.35) from Visit 9 to 12. Among women with available OCP data, the average ΔWC showed similar trends. The sample size and composition of the OCP and PCB subgroups differed slightly.

Longitudinal Trends of Serum POPs concentrations 1999-2011

Serum concentrations of POPs across follow-up visits are displayed in Table 2.3 for compounds with detection frequencies >70%. PCBs were detected at the highest frequencies with many congeners detected in all samples across the four visits. For OCPs, only trans-chlordane, p,p'-DDE, and p,p'-DDD were detected at frequencies greater than 70% across all visits. Detection frequencies for all compounds are presented in Table S2.1.

Median concentrations of POPs were relatively stable over the four timepoints. Across most congeners, the median concentrations of OCPs and PCBs increased from visit 3 to 6, decreased from visit 6 to 9 and then increased slightly from visit 9 to 12. This trend must be interpreted carefully because many women do not have POP observations at all timepoints. Intraclass correlation coefficients were low (range: 0.01-0.32), indicating low similarity and highly variable concentrations for a woman over time.

Table 2.1. Characteristics of participants in the SWAN MPS longitudinal substudy (n = 65 for PCBs; n = 59 for OCPs).

	PCBs (n = 65)	**OCPs (n = 59)**
Study Site		
Boston, MA	19 (29%)	18 (31%)
Oakland, CA	31 (48%)	28 (47%)
SE Michigan	15 (23%)	13 (22%)
Race/Ethnicity		
Black	15 (23%)	13 (22%)
Chinese	17 (26%)	16 (27%)
White	33 (51%)	30 (51%)
Age at baseline (yr)	49 (47-50)	49 (47-50)
Waist Circumference at Baseline (in)	32.56 (30.31-37.40)	32.32 (30.24-37.42)
Parous (Y/N)	54 (83%)	49 (83%)

Characteristics at baseline or the first available visit for women with at least two observations of serum POPs. Median (IQR: 25^{th}-75^{th} percentile) or Frequency (%).

Table 2.2. Average and range of waist circumference change (inches) between visits in the SWAN MPS longitudinal Substudy (n = 181 for PCBs; n = 151 for OCPs).

	Visit 3 (1999/2000) – Visit 6 (2002/03)	**Visit 6 (2002/03) – Visit 9 (2005/06)**	**Visit 9 (2005/06) – Visit 12 (2009/11)**	**Visit 3 (1999/2000) – Visit 9 (2005/06)**	**Visit 3 (1999/2000) – Visit 12 (2009/11)**	**Visit 6 (2002/03) – Visit 12 (2009/11)**
PCB Group	**N = 26**	**N = 24**	**N = 35**	**N = 18**	**N = 4**	**N = 9**
	0.52 (-2.48, 5.39)	0.49 (-3.27, 4.72)	-0.77 (-4.65, 3.35)	1.38 (-4.92, 8.15)	-0.61 (-5.31, 4.09)	-2.12 (-7.83, 1.50)
OCP Group	**N = 17**	**N = 15**	**N = 25**	**N = 19**	**N = 4**	**N = 12**
	0.58 (-2.24, 5.39)	0.54 (-3.27, 4.72)	-0.49 (-4.65, 3.35)	1.80 (-4.92, 8.15)	-2.27 (-5.31, 3.50)	-1.98 (-7.83, 1.50)

Included women with at least two observations of serum POPs. Mean (Range). 1 in. = 2.54 cm.

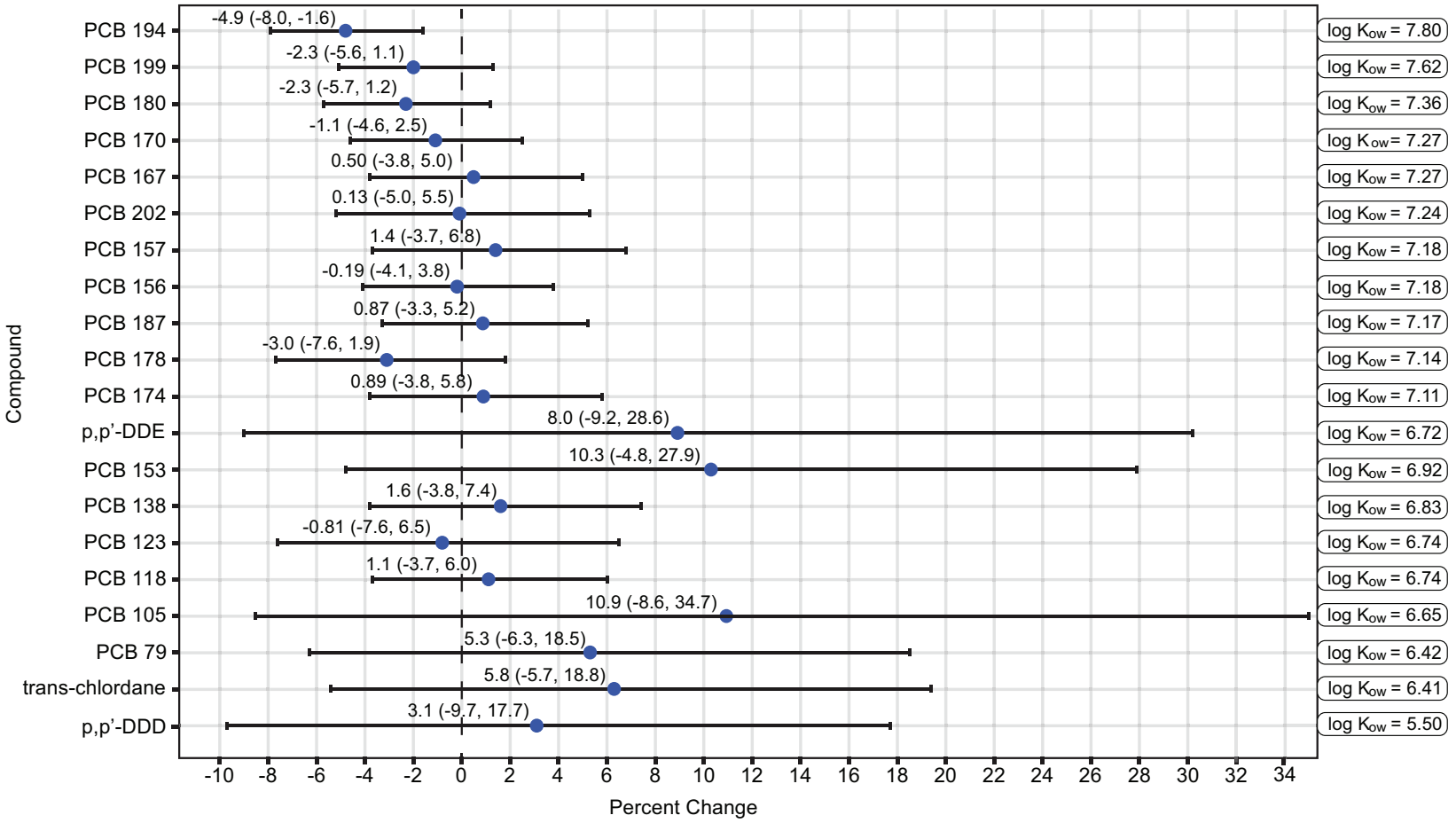
Changes in Waist Circumference and Serum POPs

As depicted in Figure 2.1, after adjusting for confounding factors, a one-inch (2.54 cm) increase in the difference in WC between visits was associated with a 4.9% decrease in the difference in a serum concentration of PCB 194 (95% CI: -8.0%, -1.6%) (Figure 2.1). No associations were observed for other PCB congeners, all of which have a lower degree of lipid solubility,[44–46] or for the OCPs (p,p'-DDE, p,p'-DDD, or trans-chlordane). Similar findings were observed in unadjusted models [Table S2.3]. We compared our results using the three approaches to calculating serum POPs concentrations: wet weight (ng/g serum), traditional lipid-standardization (ng/g lipid), and covariate-adjusted lipid-standardization (unitless) [Table S2.4]. The effect estimate for PCB 194 was smallest when using wet weight POP concentrations [-4.0% (95% CI: -7.2%, -0.58%)], whereas it was largest when using traditional lipid-standardization [-5.5% (95% CI: -8.6%, -2.3%)]. Regardless of the method, our conclusions for PCBs and OCPs remain unchanged.

Table 2.3. Median (IQR) Serum POP Concentrations (ng/g lipid) in the SWAN MPS longitudinal substudy (n = 181 for PCBs; n = 151 for OCPs).

	DF (%)	ng/g lipid	DF (%)	ng/g lipid	DF (%)	ng/g lipid	DF (%)	ng/g lipid
OCPs	**Visit 3 (1999/2000),** N = 40		**Visit 6 (2002/03)**, N = 31		**Visit 9 (2005/06)**, N = 39		**Visit 12 (2009/11)**, N = 41	
p,p'-DDD	92.9	44.40 (12.76, 116.33)	100	65.57 (20.46, 120.57)	90.7	58.83 (15.20, 140.49)	93.0	39.67 (13.75, 120.22)
p,p'-DDE	90.5	54.15 (23.37, 85.80)	91.4	50.72 (23.32, 133.25)	83.7	32.70 (13.38, 103.59)	88.4	62.73 (24.61, 114.78)
trans-chlordane	88.1	5.30 (1.71, 9.32)	94.3	4.78 (2.43, 11.07)	81.4	4.88 (1.46, 6.66)	86.1	5.15 (2.37, 10.94)
PCBs	**Visit 3 (1999/2000)**, N = 48		**Visit 6 (2002/03)**, N = 37		**Visit 9 (2005/06)**, N = 48		**Visit 12 (2009/11)**, N = 48	
PCB 79	100	460.10 (199.68, 1,215.12)	100	665.70 (173.61, 1,458.56)	100	289.48 (152.47, 896.86)	100	650.48 (188.44, 1,393.93)
PCB 105	88.0	43.53 (17.26, 88.61)	84.6	44.61 (15.91, 114.55)	74.0	26.70 (6.14, 67.30)	82.0	41.96 (11.19, 88.27)
PCB 118	100	424.14 (337.77, 544.01)	100	410.51 (298.22, 620.63)	100	346.64 (281.34, 485.88)	100	409.71 (265.68, 610.37)
PCB 123	100	59.66 (43.07, 83.89)	97.4	53.52 (39.94, 65.92)	96.0	47.43 (37.52, 74.40)	98.0	60.01 (41.16, 87.90)
PCB 138	100	533.52 (429.75, 706.37)	100	524.23 (403.09, 813.69)	100	435.41 (346.67, 562.35)	100	539.50 (339.58, 841.59)
PCB 153	100	160.58 (106.26, 232.52)	100	176.17 (103.59, 323.93)	94.0	122.87 (85.81, 217.67)	90.0	157.55 (83.29, 252.38)
PCB 156	100	19.42 (15.52, 24.35)	100	20.42 (15.02, 30.67)	100	15.71 (13.27, 20.86)	100	19.26 (13.22, 25.72)
PCB 157	96.0	4.15 (3.05, 5.52)	100	4.77 (3.12, 6.19)	94.0	3.64 (2.54, 5.08)	88.0	3.98 (2.72, 4.96)
PCB 167	100	9.30 (7.48, 11.74)	100	9.58 (7.42, 13.14)	100	7.52 (6.10, 10.97)	100	9.03 (6.38, 13.17)
PCB 170	100	26.61 (21.90, 35.43)	100	28.84 (24.02, 39.97)	100	23.03 (17.62, 29.55)	100	26.52 (18.56, 35.77)
PCB 174	100	31.06 (24.60, 42.31)	100	30.56 (23.30, 47.83)	100	25.14 (21.38, 33.52)	98.0	27.33 (20.09, 45.96)
PCB 178	100	9.49 (7.30, 12.24)	100	9.06 (7.42, 13.02)	98.0	7.36 (6.30, 11.08)	100	8.34 (6.48, 12.10)
PCB 180	100	54.14 (46.07, 68.60)	100	56.94 (44.87, 74.82)	100	46.61 (34.94, 63.54)	100	51.08 (37.67, 73.21)
PCB 187	100	48.21 (40.75, 63.50)	100	51.86 (38.36, 72.67)	100	40.18 (33.70, 57.89)	100	50.07 (34.99, 68.66)
PCB 194	98.0	6.36 (5.13, 9.00)	97.4	6.67 (5.10, 9.86)	92.0	5.80 (4.54, 7.69)	90.0	6.06 (4.68, 8.31)
PCB 199	90.0	10.64 (8.55, 13.29)	92.3	11.18 (8.61, 15.05)	82.0	8.81 (6.75, 11.55)	86.0	10.00 (7.49, 12.79)
PCB 202	100	10.16 (7.99, 12.16)	92.3	9.10 (7.21, 15.64)	92.0	8.14 (6.75, 11.60)	90.0	9.43 (5.99, 12.79)

Figure 2.1. Percent change (95% CI) in the difference in covariate-adjusted lipid-standardized POP concentration associated with a one-inch (2.54 cm) increase in the difference in waist circumference in the SWAN MPS longitudinal substudy. (n = 181 for PCBs; n = 151 for OCPs). Models were adjusted for race/ethnicity, age at baseline, parity, study site, visit, and number of visits elapsed between observations. K_{ow} is the octanol/water partition coefficient, which represents the degree of lipid solubility. All PCB log K_{ow} values are from IARC (2018). Log K_{ow} values for p,p'-DDE and p,p'-DDD are from Han et al. (2011). Log K_{ow} values for trans-chlordane are from Ellington and Stancil (1988).



Discussion

This study contributes valuable information about changes in circulating concentrations POPs in women as they undergo changes in adiposity throughout the midlife. Fluctuations in WC detected across the four study visits (1999 - 2011) reinforce our decision to leverage repeat measures available at all visits as opposed to an overall trend.

Changes in Waist Circumference and Serum POPs

Overall, an increase in ΔWC was associated with a diminished change in serum concentration of PCB 194. No statistically significant relationship was detected for other PCBs and OCPs. Previous epidemiological studies suggest that adipose tissue is a reservoir for POPs which when gained or lost may sequester or release POPs, respectively.[11,22–25,29]

Lipid solubility may explain why associations were seen for PCB 194 and not for other congeners. POPs with higher degrees of lipophilicity, or chlorination in the case of PCBs, have had stronger inverse associations with adipose tissue gain.[23,24,47] Of the POPs present in this analysis, PCB 194 has the highest lipid solubility ($\log K_{ow}$ of 7.80, Table 3).[44] POPs may be stored preferentially in visceral, compared to subcutaneous, adipose tissue which means that weight loss may prompt larger releases of POPs from visceral fat compartments and subsequent distribution patterns may depend on lipophilicity.[26,48] Animal studies highlight the importance of this research, demonstrating that fat loss leads to elevated POP concentrations in blood and lipid-rich tissue like the brain and liver, which can have toxic effects.[48,49]

Our analyses utilized several modeling strategies, including traditional lipid-standardized (Method 1), covariate-adjusted standardized (Method 2), and wet weight POPs (Method 3). When comparing results for these different approaches, we found that associations for some higher-chlorinated PCBs were strongest and closest to reaching statistical significance when

using Method 1 and weakest when using wet weight concentrations (Table S2.4). This may suggest that serum lipids suppress the true effect of change in WC and failure to account for predictors of serum lipids may overestimate the true effect. On the other hand, it is possible that using covariate-adjusted lipid standardized serum POP concentrations may have led to model over-adjustment. Methods 2 and 3 do not account for within-subject variation, however the use of fasting serum samples may sufficiently reduce measurement error due to recent dietary fat intake. Although none of these approaches fully address these issues, our findings were consistent regardless of modeling techniques.

Our findings also suggest that midlife women exhibited large within-person variability of serum POP concentrations between 1999 and 2011. This observation aligns in part with a previous finding of wide intra-person variation in serum PCBs and metabolites.[10] Other longitudinal studies did not report intra-person correlation.[11,22] Our findings suggest the importance of characterizing intra-person variation of serum POPs, particularly in light of changes in potential predictors of variability such as gain/loss of adipose tissue, which is rapid and common during certain life stages. Understanding intra-person variation is required to more accurately quantify POP exposure to evaluate the temporal association between POP exposure and onset of disease.

Two longitudinal studies have investigated the relationship between weight loss and serum POPs in general non-obese, non-patient populations such as ours.[11,22] Sjodin and colleagues found that among children ages 7 to 9, a one-kilogram increase in body weight was associated with a decrease in serum PCB concentrations ranging from -0.5% to -0.7%, depending on the congener, and a 2.4% decrease in serum p,p'-DDE concentrations. Stubleski and colleagues conducted a study similar to ours, assessing the relationship between the percent

change in weight and percent change in serum POP concentrations. Increases and decreases in weight change of 1% were associated with a smaller change in the serum concentration of 14 PCBs, HCB, and trans-nonachlor among Swedish men and women from age 70 to 75. The strongest association was between a 1% difference in weight change and PCB 194 (β = -4.9, SE = 2.0, p = 0.016), which supports our finding. The inverse relationship between weight change and change in serum POPs could be explained by adiposity loss increasing serum POPs, followed by metabolism and excretion of POPs, which decrease serum levels, somewhat offsetting the prior increase.[50] Due to these counteracting processes, people who lose adiposity may experience a smaller reduction in serum POP levels than people who gain or maintain adiposity.[22]

Losing weight or fat mass has been associated with increased serum concentrations of POPs in several studies before and after weight loss regimens or surgery.[23–25,27,29] One study found increased serum OCPs but not PCBs,[28] and two studies suggested that the magnitude of the increase may differ by sex.[23,28] Weight loss studies of women observed increased serum p,p′-DDE, HCB, and PCBs, with PCB 153 displaying the greatest increase.[26,30] Increases in serum PCBs may be more pronounced in women who lose more visceral than subcutaneous fat.[26] The present study specifically investigated visceral (central) adiposity for this reason.

Our results have some inconsistencies with the literature. PCB 138, PCB 153 and p,p'-DDE were relatively abundant in this and previous studies; yet, unlike prior studies, we did not find inverse associations between serum concentrations of these compounds and ΔWC. Our finding for PCB 194 is consistent with the literature. However, caution must be taken when comparing previous findings with ours. We analyzed differences in the between-visit changes of both outcome and predictor whereas most studies analyzed differences in a continuous outcome

before and after weight loss. Additionally, a one-inch (2.54 cm) increase in ΔWC is much smaller than the ΔWC following weight loss interventions in previous studies: 15 cm[27] and 32.4 cm[23] in women after 12 months. This distinction may have limited the impact of adiposity change on circulating POP concentrations. Our community-based population may also differ from the women (and men) with obesity who participate in weight loss programs or undergo surgery. Less evidence is available for non-obese, non-patient populations, although such studies drew a similar conclusion that natural weight gain is associated with decreased serum POP concentrations. However, in contrast to our study, studies of weight loss program participants and patients identified significant effects for more congeners.[11,22] Our finding of significance for a single congener could be due to random chance. Furthermore, the effects of adiposity loss on serum POP concentrations may differ from the effects of adiposity gain.

Strengths and Limitations

A study strength is the repeated measurement of serum POPs which allow us to investigate intra-individual changes in exposure over twelve years. In terms of assessing changing adiposity, WC was the most appropriate proxy for visceral fat mass, considering the invasiveness of collecting adipose tissue and the high degree of missingness for body composition scan data.[26] Our models were made more robust by our careful consideration of the role of serum lipids; we compared three treatments of serum POPs using wet weight concentrations, traditional lipid-standardization, and covariate-adjusted standardization.[36] Another strength is the representation of women from multiple urban areas across the U.S. and a focus on Chinese women who have been historically underrepresented in U.S. studies. The study leverages SWAN's original design to characterize changes throughout the menopausal transition which include changes in adiposity. As our data suggest, one measurement of serum POPs may

not be sufficient to characterize an individual's exposure, especially if adipose tissue serves as time-varying internal source of POPs exposure.

This study is limited by its small sample. A larger sample size would enable the power necessary to perform analyses stratified by key mechanistic factors, e.g. menopausal status, obesity status, or metabolic dysfunction. In addition, the study design decision to exclude women in the top and bottom 2% of WC change could have biased results toward the null. We did not adjust for diet due to the restriction of food frequency questionnaire data to limited timepoints. Furthermore, foods such as fish and dairy known to contain high levels of PCBs and OCPs are not necessarily associated with loss or gain in WC.[51] Our oversampling of Black and Chinese women expanded our understanding of these issues in a more diverse population. A larger sample size would allow investigations of interactions between site and race/ethnicity. Our results may not be generalizable to all SWAN women because the 75 substudy women resided in only three of seven study locations and were required to meet additional eligibility criteria. Finally, we did not assess the potential impact of unmeasured confounders.

In summary, among U.S. midlife women between 1999 and 2011, an increase in the ΔWC over time was not associated with a decrease in the change in serum concentrations of PCBs or OCPs with the exception of PCB 194. Future research should engage a larger study population with POPs measured at multiple timepoints to better understand trends within and between individuals. Last, studies should evaluate potential racial/ethnic and place-based disparities in the relationship between changes in adipose tissue and serum POPs.

References

1. Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Polychlorinated Biphenyls (PCBs)*. https://www.atsdr.cdc.gov/ToxProfiles/tp17.pdf (2000).
2. Jayaraj, R., Megha, P. & Sreedev, P. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. *Interdiscip. Toxicol.* **9**, 90–100 (2016).
3. *Chemical Factsheets*. https://www.cdc.gov/biomonitoring/chemical_factsheets.html.
4. Sharma, A. *et al.* Worldwide pesticide usage and its impacts on ecosystem. *SN Appl. Sci.* **1**, 1446 (2019).
5. Kraft, M. *et al.* Inhalation Exposure to PCB from Contaminated Indoor Air—How Much Is Absorbed into the Blood? *Pollutants* **1**, 181–193 (2021).
6. Lee, Y.-M., Kim, K.-S., Jacobs, D. R. & Lee, D.-H. Persistent organic pollutants in adipose tissue should be considered in obesity research. *Obes. Rev. Off. J. Int. Assoc. Study Obes.* **18**, 129–139 (2017).
7. Barrett, J. R. POPs vs. Fat: Persistent Organic Pollutant Toxicity Targets and Is Modulated by Adipose Tissue. *Environ. Health Perspect.* **121**, a61 (2013).
8. Hong, N.-S. *et al.* The association between obesity and mortality in the elderly differs by serum concentrations of persistent organic pollutants: a possible explanation for the obesity paradox. *Int. J. Obes.* **36**, 1170–1175 (2012).
9. La Merrill, M. *et al.* Toxicological Function of Adipose Tissue: Focus on Persistent Organic Pollutants. *Environ. Health Perspect.* **121**, 162–169 (2013).

10. Marek, R. F., Thorne, P. S., DeWall, J. & Hornbuckle, K. C. Variability in PCB and OH-PCB Serum Levels in Children and Their Mothers in Urban and Rural U.S. Communities. *Environ. Sci. Technol.* **48**, 13459–13467 (2014).
11. Sjödin, A. *et al.* Polybrominated Diphenyl Ethers, Polychlorinated Biphenyls, and 2,2-Bis(4-chlorophenyl)-1,1-dichloroethene in 7- and 9-Year-Old Children and Their Mothers in the Center for the Health Assessment of Mothers and Children of Salinas Cohort. *Environ. Sci. Technol.* **52**, 2287–2294 (2018).
12. Agency for Toxic Substances and Disease Registry. *Toxicological Profiles*. https://www.atsdr.cdc.gov/toxprofiledocs/index.html.
13. Humphrey, H. E. & Budd, M. L. Michigan's fisheater cohorts: a prospective history of exposure. *Toxicol. Ind. Health* **12**, 499–505 (1996).
14. Karmaus, W. & Zhu, X. Maternal concentration of polychlorinated biphenyls and dichlorodiphenyl dichlorethylene and birth weight in Michigan fish eaters: a cohort study. *Environ. Health* **3**, 1 (2004).
15. Tanner, E. M. *et al.* A Longitudinal Study of Polychlorinated biphenyls and Neuropsychological Function Among Older Adults from New York State. *Int. J. Hyg. Environ. Health* **223**, 1–9 (2020).
16. Birnbaum, L. S., Dutton, N. D., Cusack, C., Mennemeyer, S. T. & Pavuk, M. Anniston Community Health Survey: Follow-Up and Dioxin Analyses (ACHS-II) - Methods. *Environ. Sci. Pollut. Res. Int.* **23**, 2014–2021 (2016).
17. Chang, C.-J. *et al.* Serum concentrations of polybrominated biphenyls (PBBs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in the

Michigan PBB Registry 40 years after the PBB contamination incident. *Environ. Int.* **137**, 105526 (2020).

18. Nøst, T. H. *et al.* Persistent organic pollutants in Norwegian men from 1979 to 2007: intraindividual changes, age-period-cohort effects, and model predictions. *Environ. Health Perspect.* **121**, 1292–1298 (2013).
19. Charles, D. *et al.* Longitudinal changes in concentrations of persistent organic pollutants (1986–2016) and their associations with type 2 diabetes mellitus. *Environ. Res.* **204**, 112129 (2022).
20. Donat-Vargas, C. *et al.* Association between dietary intakes of PCBs and the risk of obesity: the SUN project. *J. Epidemiol. Community Health* **68**, 834–841 (2014).
21. Porta, M. *et al.* Monitoring concentrations of persistent organic pollutants in the general population: The international experience. *Environ. Int.* **34**, 546–561 (2008).
22. Stubleski, J., Lind, L., Salihovic, S., Lind, P. M. & Kärrman, A. Longitudinal changes in persistent organic pollutants (POPs) from 2001 to 2009 in a sample of elderly Swedish men and women. *Environ. Res.* **165**, 193–200 (2018).
23. Fénichel, P. *et al.* Sustained bloodstream release of persistent organic pollutants induced by extensive weight loss after bariatric surgery: Implications for women of childbearing age. *Environ. Int.* **151**, 106400 (2021).
24. Dirtu, A. C. *et al.* Dynamics of Organohalogenated Contaminants in Human Serum from Obese Individuals during One Year of Weight Loss Treatment. *Environ. Sci. Technol.* **47**, 12441–12449 (2013).
25. Chevrier, J. *et al.* Body weight loss increases plasma and adipose tissue concentrations of potentially toxic pollutants in obese individuals. *Int. J. Obes.* **24**, 1272–1278 (2000).

26. Dirinck, E. *et al.* Pivotal Role for the Visceral Fat Compartment in the Release of Persistent Organic Pollutants During Weight Loss. *J. Clin. Endocrinol. Metab.* **100**, 4463–4471 (2015).
27. Dirinck, E. L. *et al.* Endocrine-disrupting polychlorinated biphenyls in metabolically healthy and unhealthy obese subjects before and after weight loss: difference at the start but not at the finish. *Am. J. Clin. Nutr.* **103**, 989–998 (2016).
28. Imbeault, P., Tremblay, A., Simoneau, J.-A. & Joanisse, D. R. Weight loss-induced rise in plasma pollutant is associated with reduced skeletal muscle oxidative capacity. *Am. J. Physiol.-Endocrinol. Metab.* **282**, E574–E579 (2002).
29. He, F., Zuo, L., Ward, E. & Arciero, P. J. Serum Polychlorinated Biphenyls Increase and Oxidative Stress Decreases with a Protein-Pacing Caloric Restriction Diet in Obese Men and Women. *Int. J. Environ. Res. Public. Health* **14**, E59 (2017).
30. Jansen, A. *et al.* Increased levels of persistent organic pollutants in serum one year after a great weight loss in humans: Are the levels exceeding health based guideline values? *Sci. Total Environ.* **622–623**, 1317–1326 (2018).
31. Greendale, G. A. *et al.* Changes in body composition and weight during the menopause transition. *JCI Insight* **4**, 124865 (2019).
32. Sowers, M. *et al.* SWAN: A Multicenter, Multiethnic, Community-Based Cohort Study of Women and the Menopausal Transition. *Sybil Crawford* (2000).
33. Agency for Toxic Substances and Disease Registry. *Toxicological Profile for Polybrominated Diphenyl Ethers (PBDEs)*. https://www.atsdr.cdc.gov/toxprofiles/tp207.pdf (2017).
34. Heindel, J. J. *et al.* Metabolism disrupting chemicals and metabolic disorders. *Reprod. Toxicol.* **68**, 3–33 (2017).

35. Yu, G. W., Laseter, J. & Mylander, C. Persistent Organic Pollutants in Serum and Several Different Fat Compartments in Humans. *J. Environ. Public Health* **2011**, 1–8 (2011).

36. O'Brien, K. M., Upson, K., Cook, N. R. & Weinberg, C. R. Environmental Chemicals in Urine and Blood: Improving Methods for Creatinine and Lipid Adjustment. *Environ. Health Perspect.* **124**, 220–227 (2016).

37. Centers for Disease Control and Prevention (CDC). *Serum PCBs and Persistent Pesticides*. https://www.cdc.gov/nchs/data/nhanes/nhanes_01_02/l28poc_b_met_pcb_pesticides.pdf. (2001).

38. Laden, F. *et al.* Predictors of plasma concentrations of DDE and PCBs in a group of U.S. women. *Environ. Health Perspect.* **107**, 75–81 (1999).

39. Wattigney, W. A., Irvin-Barnwell, E., Pavuk, M. & Ragin-Wilson, A. Regional Variation in Human Exposure to Persistent Organic Pollutants in the United States, NHANES. *J. Environ. Public Health* **2015**, 1–12 (2015).

40. The Aspen Institute. *Glossary for Understanding the Dismantling Structural Racism/Promoting Racial Equity Analysis*. https://assets.aspeninstitute.org/content/uploads/files/content/docs/rcc/RCC-Structural-Racism-Glossary.pdf. (2017).

41. Bjermo, H. *et al.* Fish intake and breastfeeding time are associated with serum concentrations of organochlorines in a Swedish population. *Environ. Int.* **51**, 88–96 (2013).

42. Li, W. *et al.* Association between parity and obesity patterns in a middle-aged and older Chinese population: a cross-sectional analysis in the Tongji-Dongfeng cohort study. *Nutr. Metab.* **13**, 72 (2016).

43. Taghdir, M. *et al.* Association between parity and obesity: a cross sectional study on 6,447 Iranian females. *J. Prev. Med. Hyg.* **61**, E476–E481 (2020).

44. International Agency for Research on Cancer (IARC). *Polychlorinated biphenyls*. https://monographs.iarc.who.int/wp-content/uploads/2018/06/mono107-001.pdf (2018).

45. Han, S. *et al.* Determination of n-octanol/water partition coefficient for DDT-related compounds by RP-HPLC with a novel dual-point retention time correction. *Chemosphere* **83**, 131–136 (2011).

46. Ellington, J. J. & Stancil, F. E. *Octanol/Water Partition Coefficients for Evaluation of Hazardous Waste Land Disposal: Selected Chemicals*. https://nepis.epa.gov/Exe/ZyPDF.cgi/9100S8E6.PDF?Dockey=9100S8E6.PDF (1988).

47. De Roos, A. J., Ulrich, C. M., Sjodin, A. & McTiernan, A. Adiposity, body composition, and weight change in relation to organochlorine pollutant plasma concentrations. *J. Expo. Sci. Environ. Epidemiol.* **22**, 617–624 (2012).

48. Jandacek, R. J. *et al.* Effects of yo-yo diet, caloric restriction, and olestra on tissue distribution of hexachlorobenzene. *Am. J. Physiol.-Gastrointest. Liver Physiol.* **288**, G292–G299 (2005).

49. Ohmiya, Y. & Nakai, K. Effect of starvation on excretion, distribution and metabolism of DDT in mice. *Tohoku J. Exp. Med.* **122**, 143–153 (1977).

50. Li, L. & Wania, F. Mechanistic Pharmacokinetic Modeling of the Bioamplification of Persistent Lipophilic Organic Pollutants in Humans during Weight Loss. *Environ. Sci. Technol.* **51**, 5563–5571 (2017).

51. Guo, W. *et al.* Persistent Organic Pollutants in Food: Contamination Sources, Health Effects and Detection Methods. *Int. J. Environ. Res. Public. Health* **16**, 4361 (2019).

Supplementary Materials

Figure S2.1 Study design of the Study of Women's Health Across the Nation Multi-Pollutant Study (SWAN MPS).

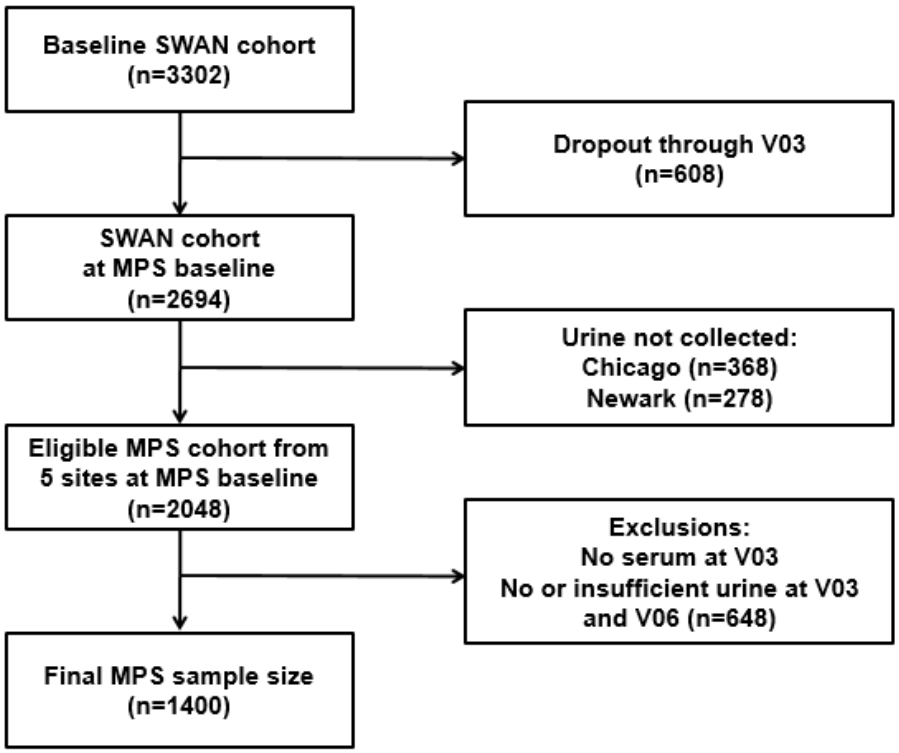


Figure S2.2 Study design of the SWAN MPS longitudinal substudy.

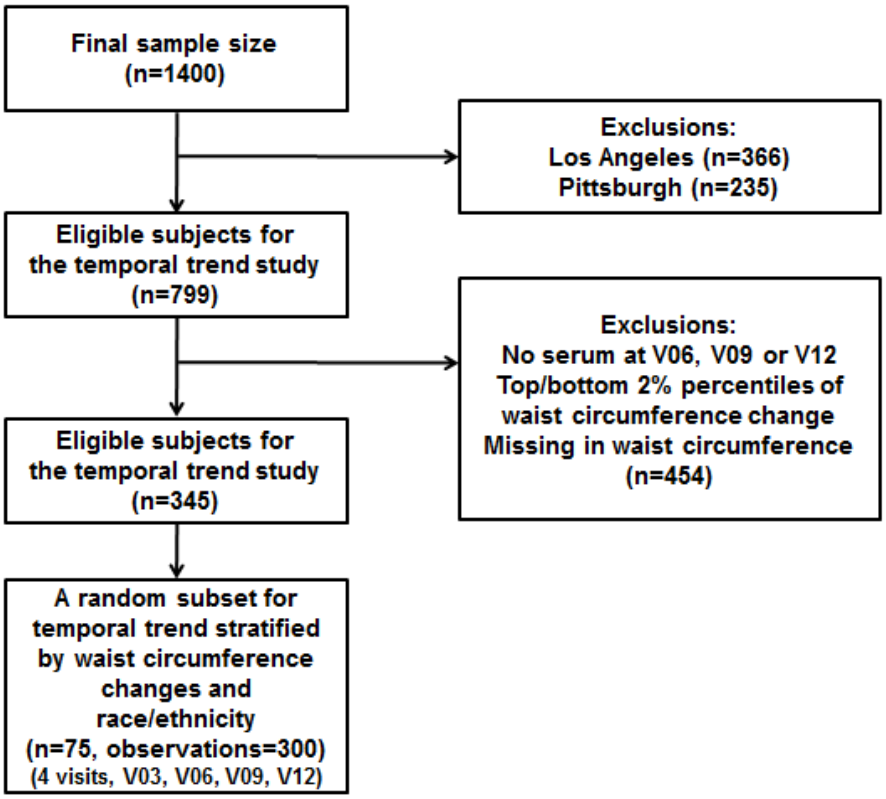


Table S2.1: Detection frequencies (DF) of PCBs, PBDEs, and OCPs in SWAN MPS longitudinal substudy.

		DF (%)			
PCB		**Visit 3 (1999/2000)**	**Visit 6 (2002/03)**	**Visit 9 (2005/06)**	**Visit 12 (2009/11)**
Tetra	77	10.0	10.3	4.0	8.0
	78	2.0	2.6	4.0	4.0
	79	100	100	100	100
	81	0.0	0.0	0.0	2.0
	114	30.0	33.3	22.0	22.0
Penta	105	88.0	84.6	74.0	82.0
	87/110/111	0.0	0.0	0.0	2.0
	118	100	100	100	100
	123	100	97.4	96.0	98.0
	126	34.0	38.5	34.0	24.0
Hexa	138	100	100	100	100
	149	38.0	41.0	36.0	36.0
	153	100	100	94.0	90.0
	156	100	100	100	100
	157	96.0	100	94.0	88.0
	162	2.0	0.0	2.0	6.0
	167	100	100	100	100
	169	0.0	0.0	0.0	0.0
Hepta	170	100	100	100	100
	174	100	100	100	98.0
	178	100	100	98.0	100
	180	100	100	100	100
	187	100	100	100	100
	188	0.0	0.0	0.0	0.0
	189	0.0	0.0	0.0	0.0
Octa	194	98.0	97.4	92.0	90.0
	195	10.0	10.3	6.0	4.0
	199	90.0	92.3	82.0	86.0
	202	100	92.3	92.0	90.0
	203	0.0	0.0	0.0	0.0
	205	0.0	0.0	0.0	0.0
Nona	206	16.0	12.8	16.0	6.0
	208	48.0	53.9	44.0	50.0
Deca	209	32.0	30.8	32.0	22.0

	DF (%)			
OCP	**Visit 3 (1999/2000)**	**Visit 6 (2002/03)**	**Visit 9 (2005/06)**	**Visit 12 (2009/11)**
p,p'-DDD	92.9	100	90.7	93.0
p,p'-DDT	26.2	37.1	32.6	30.2
o,p'-DDT	7.1	2.9	4.7	0.0
p,p'-DDE	90.5	91.4	83.7	88.4
aldrin	7.1	8.6	0.0	11.6
dieldrin	23.8	25.7	27.9	18.6
endrin	0.0	2.9	7.0	2.3
cis-chlordane	40.5	31.4	20.9	32.6
trans-chlordane	88.1	94.3	81.4	86.1
heptachlor	11.9	8.6	14.0	18.6
cis-nonachlor	2.4	0.0	2.3	2.3
trans-nonachlor	28.6	20.0	14.0	20.9
mirex	0.0	8.6	4.7	4.7
oxychlordane	21.4	34.3	18.6	25.6
c/t-heptachlorepoxide	21.4	22.9	11.6	16.3
α-HCH	35.7	20.0	20.9	27.9
β-HCH	19.1	22.9	20.9	23.3
γ-HCH	9.5	25.7	11.6	18.6
HCB	59.5	71.4	65.1	62.8
PBDE				
17	35.1	13.2	19.6	27.6
28	0.0	0.0	0.0	0.0
47	21.1	35.9	33.9	36.2
66	0.0	0.0	0.0	0.0
71	0.0	0.0	0.0	0.0
85	0.0	0.0	0.0	0.0
99	19.3	32.1	21.4	13.8
100	3.5	1.9	3.6	3.5
138	0.0	0.0	0.0	0.0
153	29.8	28.3	33.9	44.8
154	45.6	49.1	37.5	36.2
183	0.0	0.0	0.0	0.0
190	0.0	0.0	0.0	0.0
209	0.0	0.0	0.0	0.0

Figure S2.3 Directed Acyclic Graph (DAG) of the association between adipose tissue and serum concentrations of POPs adapted from O'Brien et al. (2016). Measured variables are outlined in solid lines and unmeasured variables in dashed lines.

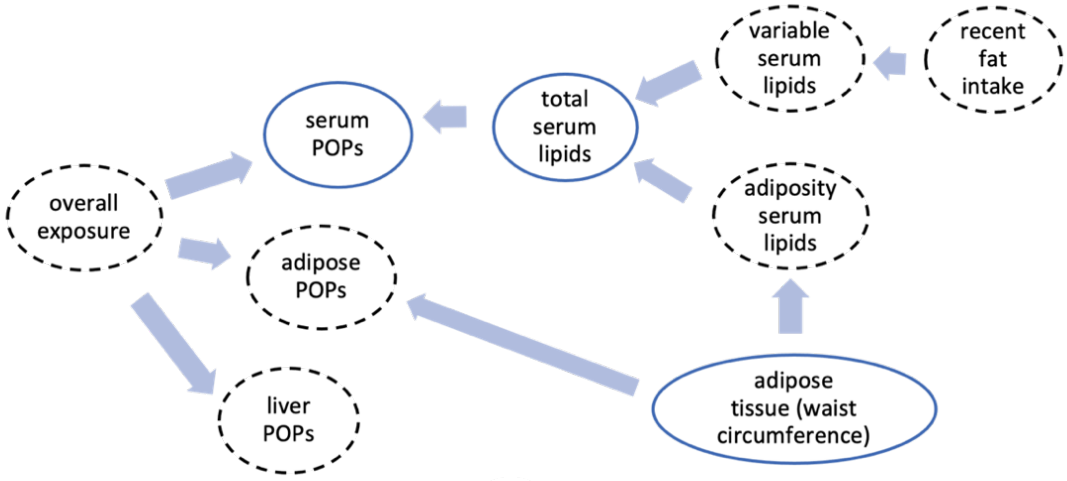


Table S2.2: Availability of POPs observations at two or more timepoints in the SWAN MPS longitudinal substudy**.**

No. of Visits	Visit 3 (1999/2000)	Visit 6 (2002/03)	Visit 9 (2005/06)	Visit 12 (2009/11)	PCBs (n)	OCPs (n)
Four	X	X	X	X	11	5
Three	X	X	X		5	2
		X	X	X	5	4
	X		X	X	13	11
	X	X		X	6	6
Two	X	X			4	4
	X		X		5	8
	X			X	4	4
		X	X		3	4
			X	X	6	5
		X		X	3	6
Total					65	59
PCB Total	48	37	48	48	181	
OCP Total	40	31	39	41		151

Table S2.3. Percent change (95% CI) in the difference in covariate-adjusted lipid-standardized POP concentration associated with a one-inch (2.54 cm) increase in the difference in waist circumference in the SWAN MPS longitudinal Substudy.

	Unadjusted Model [a]			Adjusted Model [b]			log K_{ow} [c]
Compound	**% Change**	**95% CI**	**p-value**	**% Change**	**95% CI**	**p-value**	
p,p'-DDE	7.5	-9.3, 27.4	0.40	8.0	-9.2, 28.6	0.35	6.72
p,p'-DDD	3.5	-9.1, 17.9	0.60	3.1	-9.7, 17.7	0.65	5.50
trans-chlordane	6.9	-4.5, 19.7	0.24	5.8	-5.7, 18.8	0.30	6.41
PCB 79	5.2	-6.2, 17.8	0.38	5.3	-6.3, 18.5	0.38	6.42
PCB 105	10.6	-8.3, 33.4	0.29	10.9	-8.6, 34.7	0.29	6.65
PCB 118	1.5	-3.1, 6.3	0.53	1.1	-3.7, 6.0	0.66	6.74
PCB 123	-0.51	-7.2, 6.7	0.88	-0.81	-7.6, 6.5	0.82	6.74
PCB 138	2.2	-3.1, 7.8	0.42	1.6	-3.8, 7.4	0.56	6.83
PCB 153	10.8	-3.9, 27.8	0.16	10.3	-4.8, 27.9	0.19	6.92
PCB 156	0.16	-3.6, 4.1	0.93	-0.19	-4.1, 3.8	0.92	7.18
PCB 157	2.0	-3.0, 7.2	0.44	1.4	-3.7, 6.8	0.59	7.18
PCB 167	0.93	-3.3, 5.3	0.66	0.50	-3.8, 5.0	0.82	7.27
PCB 170	-0.78	-4.3, 2.8	0.66	-1.1	-4.6, 2.5	0.54	7.27
PCB 174	1.3	-3.3, 6.1	0.59	0.89	-3.8, 5.8	0.71	7.11
PCB 178	-3.0	-7.6, 1.8	0.21	-3.0	-7.6, 1.9	0.21	7.14
PCB 180	-1.9	-5.2, 1.6	0.28	-2.3	-5.7, 1.2	0.20	7.36
PCB 187	1.2	-2.8, 5.5	0.55	0.87	-3.3, 5.2	0.69	7.17
PCB 194	-4.6	-7.8, -1.4	0.006	-4.9	-8.0, -1.6	0.004	7.80
PCB 199	-2.0	-5.2, 1.4	0.24	-2.3	-5.6, 1.1	0.23	7.62
PCB 202	0.27	-4.7, 5.5	0.91	0.13	-5.0, 5.5	0.97	7.24

[a] Includes follow-up visit; [b]Adjusted for race/ethnicity, age at baseline, parity, study site, and number of visits elapsed between observations, in addition to the follow-up visit. [c] log K_{ow} is the octanol/water partition coefficient, which represents the degree of lipid solubility. All PCB log K_{ow} values are from IARC (2018). Log K_{ow} values for p,p'-DDE and p,p'-DDD are from Han et al. (2011). The log K_{ow} value for trans-chlordane is from Ellington and Stancil (1988).

Table S2.4 Percent change (95% CI) in the difference in serum POP concentration associated with a one-inch increase in the difference in waist circumference in the SWAN MPS longitudinal substudy. Comparison of three methods to calculate serum POP concentrations. All models were adjusted for race/ethnicity, age at baseline, parity, study site, and the number of visits elapsed between observations.

	Adjusted Models								
	Covariate-adjusted standardized POPs			Traditional lipid-standardized POPs			Wet weight POPs		
Compound	**% Change**	**95% CI**	**p-value**	**% Change**	**95% CI**	**p-value**	**% Change**	**95% CI**	**p-value**
p,p'-DDE	8.0	-9.2, 28.6	0.35	7.4	-9.8, 27.8	0.36	3.3	-14.1, 24.2	0.73
p,p'-DDD	3.1	-9.7, 17.7	0.65	2.5	-10.2, 17.0	0.74	-1.2	-14.3, 13.8	0.86
trans-chlordane	5.8	-5.7, 18.8	0.30	5.2	-6.2, 18.0	0.34	6.2	-6.0, 19.9	0.33
PCB 79	5.3	-6.3, 18.5	0.38	4.6	-7.0, 17.7	0.45	6.3	-5.4, 19.6	0.30
PCB 105	10.9	-8.6, 34.7	0.29	10.2	-9.2, 33.8	0.32	12.0	-7.7, 35.9	0.25
PCB 118	1.1	-3.7, 6.0	0.66	0.38	-4.3, 5.3	0.88	2.0	-2.7, 7.0	0.40
PCB 123	-0.81	-7.6, 6.5	0.82	-1.5	-8.3, 5.8	0.66	0.14	-6.8, 7.6	0.97
PCB 138	1.6	-3.8, 7.4	0.56	0.94	-4.5, 6.6	0.74	2.6	-2.9, 8.4	0.35
PCB 153	10.3	-4.8, 27.9	0.19	9.6	-5.4, 27.0	0.20	11.4	-3.9, 29.1	0.15
PCB 156	-0.19	-4.1, 3.8	0.92	-0.87	-4.7, 3.1	0.66	0.77	-3.1, 4.8	0.70
PCB 157	1.4	-3.7, 6.8	0.59	0.72	-4.3, 6.0	0.70	2.4	-2.7, 7.8	0.36
PCB 167	0.50	-3.8, 5.0	0.82	-0.18	-4.4, 4.3	0.93	1.5	-2.8, 5.9	0.50
PCB 170	-1.1	-4.6, 2.5	0.54	-1.8	-5.3, 1.8	0.32	-0.17	-3.7, 3.5	0.93
PCB 174	0.89	-3.8, 5.8	0.71	0.21	-4.5, 5.1	0.92	1.9	-2.8, 6.7	0.44
PCB 178	-3.0	-7.6, 1.9	0.21	-3.6	-8.2, 1.2	0.08	-2.1	-6.7, 2.8	0.39
PCB 180	-2.3	-5.7, 1.2	0.20	-2.9	-6.3, 0.56	0.10	-1.3	-4.8, 2.2	0.45
PCB 187	0.87	-3.3, 5.2	0.69	0.19	-3.9, 4.5	0.93	1.8	-2.3, 6.1	0.38
PCB 194	-4.9	-8.0, -1.6	0.004	-5.5	-8.6, -2.3	0.003	-4.0	-7.2, -0.58	0.02
PCB 199	-2.3	-5.6, 1.1	0.23	-2.9	-6.2, 0.38	0.14	-1.4	-4.7, 2.1	0.42
PCB 202	0.13	-5.0, 5.5	0.97	-0.55	-5.6, 4.8	0.77	1.1	-4.0, 6.4	0.68

Chapter III. Associations of Organochlorine Pesticide, Polybrominated Diphenyl Ether, and Polychlorinated Biphenyl Mixtures with Incident Diabetes in The Study of Women's Health Across the Nation

Amelia Grant-Alfieri[a], William H Herman[b,c], Deborah J. Watkins[a], Stuart Batterman[a], Carrie Karvonen-Gutierrez[b], Sung Kyun Park[a,b]

Abstract

Background: Organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), and polychlorinated biphenyls (PCBs) are persistent organic pollutants (POPs) that can negatively impact metabolic health through pathways including endocrine disruption. Few studies have evaluated diabetes risk associated with PBDEs. Little is known about the joint effect of exposure to mixtures of POPs on diabetes risk.

Objectives: We investigated the relationship between POPs, individually and as mixtures, and diabetes development over an 18-year period (1999-2016) in midlife women.

Methods: We measured lipid-standardized serum concentrations of 14 PCBs, 4 OCPs, and 1

[a] Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, Michigan, USA
[b] Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan, USA
[c] Department of Internal Medicine, Division of Metabolism, Endocrinology, and Diabetes, University of Michigan Medical School, Ann Arbor, Michigan, USA

PBDE in 1,040 midlife women from the Study of Women's Health Across the Nation. Cox proportional hazards models were used to test the association between serum concentrations of POPs in 1999/2000 and incident diabetes assessed at twelve timepoints. Diabetes was defined by the presence of one or more of the following: 1) use of an anti-diabetic medication at any visit; 2) fasting glucose ≥7 mmol/l at two consecutive visits while not taking corticosteroids including glucocorticoids; and 3) any two visits with self-reported diabetes and at least one visit with fasting glucose ≥7 mmol/l. K-means Clustering and Quantile-Based G-Computation (QBGC) were used to evaluate associations with the POP mixture.

Results: For most mixture components, both single pollutant and mixtures analyses indicated null associations with diabetes risk, however results did not align for all POPs. After multivariable adjustment, the HR (95% CI) for diabetes associated with tertiles of exposure (T2/T3) relative to the first tertile (T1), was 1.7 at T2 (1.0, 2.8) and 1.5 at T3 (0.84, 2.7) for HCB and 1.9 at T2 (1.1, 3.3) and 1.6 at T3 (0.88, 2.9) for PCB 123. After additional adjustment for waist circumference, PCB 138 was inversely associated with diabetes (T2 HR = 0.77 [0.45, 1.33]; T3 HR = 0.50 [0.26, 0.97]; p for trend = 0.037). No significant association was found for PBDE 47 and diabetes. QBGC identified no significant association for the overall joint effect of the POP mixture on incident diabetes (HR = 1.04 [0.53, 2.07]).

Conclusion: Exposure to a mixture of PCBs, OCPs, and PBDEs was not associated with incident diabetes in midlife U.S. women, although some individual POPs demonstrated significant yet inconsistent associations with diabetes risk. While we did not find consistent relationships between incident diabetes and serum POP concentrations, we recommend exploring non-linear and non-monotonic dose-response dynamics. More research is needed on the diabetogenic effects of PBDEs.

Introduction

Type 2 Diabetes (T2D) is common, its prevalence is growing, and it is a leading cause of morbidity and mortality. It has been linked to both lifestyle and environmental factors. Diabetes was responsible for 6.7 million deaths worldwide in 2021.[1] Global diabetes prevalence is projected to rise 46% from 1 in 10 adults in 2021 to 1 in 8 adults in 2045, with growth most rapid in low- and middle-income countries.[1,2] T2D comprises over 95% of diabetes cases.[2] In the United States (U.S.), approximately 1 in 10 adults have been diagnosed with T2D, 1 in 3 have prediabetes, and 1 in 5 are undiagnosed.[3,4] People with T2D are at higher risk of cardiovascular disease, stroke, kidney failure, cancer, complications from SARS-CoV-2 infection, and death.[5–7] T2D is characterized by insulin resistance, the inability of glucose to be used by the body to produce energy, and the subsequent accumulation of glucose in the blood. Risk of T2D increases with obesity, lack of exercise, and family history, however genetics only explain 10% of cases.[8] Increasingly, environmental chemical exposures are being explored as risk factors for T2D.

Persistent organic pollutants (POPs) are a broad class of chemicals that include, among others, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs). PCBs have been used in dielectric and coolant fluids, paint, plastics, and pesticides. PCBs and OCPs were banned by many high-income countries, including the U.S., in the 1970s and 1980s due to health risks at high doses.[9] Additional countries have since banned OCPs, although other countries continue to utilize them.[10] PBDEs have been used as flame retardants that have been manufactured in several commercial mixtures including decaBDE used in electronics, octaBDEs in plastics, and pentaBDEs in upholstery foam.[11] By 2013, the U.S. prohibited the manufacture and import of all three types.[11]

POPs can be highly persistent in the human body and can act as endocrine disruptors.[12–14] The main exposure pathway is diet, primarily animal-based fatty foods.[15] Indoor air is another source of PCBs and PBDEs, which accumulate in dust and then may be ingested.[11,16] High lipophilicity allows POPs to accumulate in adipose tissue, where they can induce adipocyte dysfunction regardless of obesity.[17] Hormonal receptor-mediated mechanisms including low-grade inflammation in adipose tissue have been linked to insulin resistance and T2D.[18,19]

The relationship between diabetes and PCBs and OCPs is a popular topic of research, however, the findings are contradictory, often differing by compound and lipid-standardization of serum POP concentrations. In the case of PCBs, prospective study results have conflicted, many finding PCBs to increase diabetes risk [20–23] and others finding no link [24–28]. Prospective studies of OCPs suggest compound-specific positive associations with diabetes risk[24,26–29] or no associations.[23,25] HCB and p,p'-DDE were repeatedly found to be associated with diabetes risk, however, the direction of effect and shape of dose-response curves were contradictory.[12,15,17,18] One limitation challenging the comparison of some prospective studies is the failure to standardize serum POP concentrations by serum lipids.[21,22,29,30]

Few studies have investigated the association between PBDEs and diabetes, and of those that do, most focus on gestational diabetes. Studies relevant to non-gestational diabetes are contradictory with inconsistent dose-response curves. The most robust prospective studies found no associations after serum lipid adjustment.[25,27] In a highly exposed cohort, another flame retardant, polybrominated biphenyl (PBB) 153, was found to have no association with diabetes.[22] PBDE exposure calculated using dietary intake and food contamination data, rather than serum concentrations, was found to have a positive, non-linear association with diabetes risk.[31]

Given the conflicting literature and the reality of simultaneous and correlated exposures, it is critical to evaluate the overall effect of mixtures of PCBs, OCPs, and PBDEs. To our knowledge, only two studies, both cross-sectional, have employed mixtures methods.[32,33] PCBs and OCPs mixtures in adipose tissue were linked to higher prevalence of metabolic syndrome.[32] Serum mixtures of PCBs were associated with greater prevalence of diabetes.[33]

Women may be at greater risk of diabetes due to POPs exposure.[21,22] Midlife and older women may be at a particularly increased risk of disrupted glucose homeostasis associated with chronic exposure to POPs, independent of body mass index (BMI), due to compounded effects of multi-decade POPs exposure and possible interactions of POPs with aging-related physiological processes.[34] The Study of Women's Health Across the Nation (SWAN) is one of the most carefully phenotyped population samples with respect to the menopausal transition and numerous heath markers. This study investigated whether serum concentrations of PCBs, PBDEs, and OCPs, individually and as mixtures, were associated with incident diabetes among U.S. midlife women in SWAN.

Methods

Study Population

The SWAN Multi-Pollutant Study (MPS) is part of the larger, ongoing SWAN, which has been detailed previously.[35] In short, SWAN is a multi-site, multi-ethnic cohort study launched in 1996 to follow 3,302 premenopausal women between the ages of 42 and 52 through the menopausal transition. Across 15 follow-up visits from 1996/97 to 2015/16, SWAN collected data on metabolic and reproductive biomarkers and health outcomes, in addition to socio-demographic, lifestyle, and other risk factors. SWAN retained approximately 75% of living participants over the

study period.[36] The institutional review board at each site approved the study protocol and all participants provided written, signed informed consent at each study visit.

The MPS characterizes environmental exposures in a subset of SWAN. The design of SWAN MPS is illustrated in Supplemental Figure S3.1. Environmental exposure data were collected from biobanked specimens at the MPS baseline visit (1999/2000). After further excluding participants with insufficient serum or urine samples, the final MPS totaled 1,400 women from Boston, MA, Los Angeles, CA, Oakland, CA, Pittsburgh, PA, and southeast MI, which limited the sample to White, Black, Japanese, and Chinese women. Only women with POPs data meeting surrogate recovery and other quality assurance criteria were included. Of the 1,400 participants, this requirement excluded 166 women with OCP data, 405 with PCB data, and 281 with PBDE data, in turn creating three subsamples by POP type. We excluded women for insufficient serum lipids (cholesterol) information: two from the PCB subgroup, two from the PBDE subgroup, and one from the OCP subgroup. We excluded women who had diabetes at or before 1999/2000: 67 from the OCP subgroup, 52 from the PCB group, and 60 from the PBDE group. Additionally, we omitted participants lacking key covariate information, leaving 1,071 women in the OCP subgroup, 866 women in the PCB subgroup, and 972 women in the PBDE subgroup. To compute inverse probability weights (IPW) to account for potential selection bias, we omitted participants missing IPW predictors. Details about IPW are described in the Supplemental Methods. After restricting to observations with IPW, the final OCP subsample was reduced to 1,040 (102 cases of incident diabetes), the PCB subsample to 838 (85 cases), and the PBDE subsample to 943 (92 cases). Mixtures analyses were conducted in a slightly reduced population of 809 women who had complete observations for PCBs, OCPs, *and* PBDEs. We conducted all analyses in populations with and without IPW.

POPs Measurements

Serum concentrations of PBDEs, PCBs, and OCPs were measured using blood samples collected from the 1,400 women in the SWAN MPS at baseline (1999/2000). We analyzed serum samples for 34 PCB congeners, 14 PBDEs, and 19 OCPs including DDT metabolites, chlordanes, and hexachlorobenzene (HCB) among others (see Supplemental Table S3.1 for the complete list). Laboratory procedures have been detailed previously.[37] Concentrations below the method detection limit (MDL) were replaced with MDL/$\sqrt{2}$. We retained compounds detected in at least 70% of samples except for p,p'-DDT, p,p'-DDE, and p,p'-DDD. Notwithstanding the low detection frequencies of DDT metabolites, approximately 40%, their high lipophilicity and persistence, endocrine disruption and carcinogenicity, and continued use in vector control worldwide warranted special consideration.[38]

Lipid-standardized POP concentrations are preferable to wet weight concentrations because POPs are lipophilic, circulate bound to lipids, and distribute in the body according to a tissue's lipid content.[39,40] Standardizing serum POP concentrations by serum lipids has been the most common method employed in studies of serum POPs because it eliminates the influence of recent fat intake on serum lipids and enables the comparison of exposure across individuals.[41] Total cholesterol and triglycerides were measured by enzymatic methods Hitachi 747-200 analyzer, Boehringer Mannheim Diagnostics, Indianapolis IN). The total lipid concentration was imputed using linear regression to address missing observations of total cholesterol and triglycerides. O'Brien and colleagues (2016) recommended traditional standardization in addition to model adjustment for serum lipids when serum POPs are the exposure of interest.[41]

$$S = C / OTL \times 102.6$$

$$OTL = (2.27 * TC) + TG + 62.3$$

where S is lipid-standardized serum POP concentration (ng/g lipid); C is wet weight serum POP concentration (ng/g); OTL is observed total lipid concentration (mg/dl);[42] TC is total cholesterol concentration (mg/dl); and TG is triglycerides concentration(mg/dl).

Diabetes Incidence

From 1999/2000 (baseline) through 2015/16, participants were defined as having incident diabetes if they met one or more of the following criteria: (1) use of an anti-diabetic medication at any visit; (2) fasting glucose ≥7 mmol/l at two consecutive visits while not taking corticosteroids including glucocorticoids; and (3) any two visits with self-reported diabetes and at least one visit with fasting glucose ≥7 mmol/l. Most if not all the diabetes cases in this population of midlife women are likely T2D. See Supplemental Methods for additional information on incident diabetes ascertainment.

Covariates

Potential confounders of the relationship between serum POP concentrations and diabetes incidence were selected a priori. Age can influence exposure especially for historical contaminants such as PCBs, OCPs, and PBDEs. In addition, T2D risk increases with age, notably after 45 years of age.[43] Study sites included Los Angeles, CA, Oakland, CA, Pittsburgh, PA, Boston, MA, and Southeast MI. Self-reported race/ethnicity, financial hardship, and education were assessed through an initial self-administered questionnaire in 1996/97. Race/ethnicity included Chinese, Japanese, white, and Black. White and Black women were recruited from Boston, Southeast MI, and Pittsburgh; Chinese and White women from Oakland; Japanese and White women from Los Angeles. Race was not used as a proxy for biological or genetic differences yet was included because it is implicit in systems, policies, and institutions that shape individuals' environments and experiences.[44] Race/ethnicity, study site, education, and financial strain can influence lifestyle and

access to resources. Differences in lifestyle, resources, and historical contamination may result from structural (institutional or systemic) racism, "a system in which public policies, institutional practices, cultural representations, and other norms work in various, often reinforcing ways to perpetuate racial group inequity".[44] Dietary intake of meat, high fat dairy, fish and shellfish were collected in 1996/97 using a semiquantitative adaptation of the Block Food Frequency Questionnaire. Weekly dietary intake frequency was calculated by summing food group items. Fish/shellfish included shellfish and fish. High fat dairy intake included whole/chocolate milk, cottage cheese, regular cheese/cheese spread, yogurt/frozen yogurt, butter, margarine, ice cream, cream/half-and-half, and cheese dishes. Meat intake included breakfast sausage/bacon, ground beef/hamburger, steak/roast beef, liver, pork chops, hot dogs, and lunch meats.

At baseline (1999/2000), age, smoking status, alcohol consumption, and menopausal status were self-reported. Smoking status was defined as never, current, or past. Alcohol consumption was categorized as 0, <1, 1-7, or >7 drinks per week. Menopausal status was categorized as i) pre-menopause or early peri-menopause, ii) late peri-menopause or post-menopause due to surgical or natural processes, or iii) unknown due to hormone therapy. Baseline waist circumference (WC) was measured to the nearest 0.1 cm around the narrowest part of the torso. WC was the most appropriate proxy for visceral fat mass, considering the invasiveness of collecting adipose tissue and the high degree of missingness for body composition scan data,[45] and may better predict diabetes incidence.[46] Physical activity during the prior 12 months was scored on a scale from 3 to 15 with 15 indicating the highest level of activity, as previously detailed.[36] If women were missing physical activity observations from baseline (1999/2000), we used observations from 1997/99. Parity was defined as parous or nulliparous based on live and stillbirths. Uniform protocols were used to collect covariate data across study sites.

Statistical Analysis

The distributions of 34 PCB congeners, 19 OCPs, and 14 PBDEs were characterized using the median and various quantiles. We reported the 25th and 75th percentiles because we analyze the effects of serum POPs per interquartile range (IQR), and the 33rd and 66th percentiles because they serve as cutoffs in defining tertiles (T1, T2, T3). We reported concentrations using traditional lipid-standardization to facilitate comparison with previous studies. We visualized Spearman correlations between serum POP concentrations using a heatmap.

Cox proportional hazard models were constructed to evaluate the association between serum POP concentrations and diabetes incidence over an 18-year follow-up period. Serum POP concentrations, standardized using traditional lipid-standardization, were the independent variables.[41] We treated serum POP concentrations as continuous, interpreting hazard ratios (HRs) and 95% confidence intervals (CIs) per IQR increase. POPs were not log-transformed because there was no clear evidence of log-linear dose-response relationships. To explore non-linearity and non-monotonicity, we assessed POP tertiles. We also assessed DDT metabolites, which have been widely studied, especially p,p'-DDE. However, a detection frequency (DF) <50% dictated categorical variables. Binary exposure was defined as detect/non-detect. A 3-level exposure variable treated non-detection as the reference group and divided detectable concentrations at the median to create moderate and high groups.

In single pollutant and mixture analyses, final models were adjusted for age, study site, race/ethnicity, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, fish/shellfish intake, and serum lipids (Model 3). We first investigated crude models (Model 0) followed by models adjusted for inverse probability weights as well as demographic and socioeconomic covariates (Model 1). Model 2 further adjusted for serum lipids

as recommended when the exposures of interest are serum concentrations of lipid-soluble chemicals.[41] Considering the role of WC, we additionally adjusted for baseline WC in Model 4. However, this may have led to over-adjustment, therefore Model 3 was chosen as our main model. Menopausal status in 1999/2000 and change in WC between 1997/99 and 1999/2000 were explored as potential confounders and ultimately not included (data not shown). All models were adjusted for joint stabilized inverse probability weights (IPWs). IPW lessens the impact of selection bias on diabetes risk estimates that may arise from selective participation into SWAN and into MPS (Supplemental Methods).

Model 0	=	*Unadjusted*
Model 1	=	*Joint stabilized inverse probability weights + demographic and socioeconomic variables: age, race/ethnicity, study site, education, and financial strain*
Model 2	=	*Model 1 + serum lipids*
Model 3 (final)	=	*Model 2 + behavioral and reproductive variables: alcohol consumption, smoking status, parity, physical activity, high fat dairy intake, meat intake, and fish/shellfish intake*
Model 4	=	*Model 3 + waist circumference*

We analyzed the effect of POP mixtures using K-means Clustering and Quantile-Based G-Computation (QBGC). The strong positive correlations between POP concentrations informed our decision to use a mixtures approach (Figure S3.2). K-means Clustering categorizes overall POP exposure into distinct homogenous subgroups, or k clusters, and we used cluster membership as the exposure in Cox models.[47] We used silhouette and elbow methods to select the number of clusters (Figures S3.3 and S3.4). We selected three clusters (low, medium, and high) for our primary analysis to allow for better comparison with tertile analyses. Two-cluster sensitivity analysis was performed. We used the 'kmeans' function from the R 'stats' package.[48]

Finally, we used QBGC to quantify the overall mixture effect. QBGC combines the strengths of the Weighted Quantile Sums regression (WQS) and the causal effect estimation method, G-Computation.[49] Compared to WQS, QBGC does not assume directional homogeneity, linearity, or additivity of mixture components. Thus, using QBGC, we computed a weighted index of POPs based on tertiles of exposure. The scaling allowed for comparability across POP mixture components. We then built Cox proportional hazards models using a single weighted index exposure term. The overall mixture effect was computed as the sum of estimated regression coefficients of all POP variables and can be interpreted as the HR of incident diabetes associated with a one-tertile increase in the serum concentrations of all POPs after controlling for covariates. The R package 'qgcomp' was used in this analysis.[50]

Results

Participant Characteristics

The characteristics of women at SWAN MPS baseline (or first available timepoint, in the case of sociodemographic characteristics) are presented in Table 3.1. The median age at baseline was 49 years (IQR: 47-51). Approximately one quarter of women were from Los Angeles, another quarter from Oakland, and those remaining were relatively evenly distributed among Pittsburgh, Boston, and southeast MI. In terms of race/ethnicity, approximately half were White. Half of the participants attained a college education at minimum. Most women reported little to no alcohol use and no history of smoking. Approximately 30% of women experienced moderate or high financial strain. The median WC at baseline was 81 cm (IQR: 73-93). Approximately 80% of women were parous. Between 1999 and 2016, 10% of women developed diabetes with 85 cases in the PCB subsample, 92 in the PBDE subsample, and 102 in the OCP subsample.

Table 3.1. Characteristics of SWAN MPS Participants Free of Diabetes at Baseline, 1999/2000.

	PCB Subsample	PBDE Subsample	OCP Subsample
Characteristic	**N = 838**[1]	**N = 943**[1]	**N = 1,040**[1]
Developed Diabetes	85 (10%)	92 (9.8%)	102 (9.8%)
Age at Baseline (yr)	49.0 (47.0, 51.0)	49.0 (47.0, 51.0)	49.0 (47.0, 51.0)
Race/Ethnicity			
Black	152 (18%)	169 (18%)	191 (18%)
White	441 (53%)	495 (52%)	548 (53%)
Chinese	116 (14%)	135 (14%)	141 (14%)
Japanese	129 (15%)	144 (15%)	160 (15%)
Study Site			
Boston	126 (15%)	144 (15%)	157 (15%)
Los Angeles	224 (27%)	251 (27%)	290 (28%)
Oakland	208 (25%)	236 (25%)	246 (24%)
Pittsburgh	145 (17%)	159 (17%)	180 (17%)
SE Michigan	135 (16%)	153 (16%)	167 (16%)
Educational Attainment			
High School or Less	137 (16%)	160 (17%)	179 (17%)
Beyond High School	261 (31%)	285 (30%)	316 (30%)
College	199 (24%)	231 (24%)	253 (24%)
Beyond College	241 (29%)	267 (28%)	292 (28%)
Menopause Status			
Pre-menopause or early peri-menopause	524 (63%)	590 (63%)	649 (62%)
Late peri-menopause or surgical/natural post-menopause	182 (22%)	202 (21%)	218 (21%)
Unknown	132 (16%)	151 (16%)	173 (17%)
Alcohol Consumption at Baseline			
None	425 (51%)	483 (51%)	529 (51%)
<1 drink per week	210 (25%)	227 (24%)	257 (25%)
1-7 drinks per week	145 (17%)	163 (17%)	179 (17%)
>7 drinks per week	58 (6.9%)	70 (7.4%)	75 (7.2%)
Smoking Status at Baseline			
Never	539 (64%)	604 (64%)	667 (64%)
Current	80 (9.5%)	89 (9.4%)	98 (9.4%)
Past Only	219 (26%)	250 (27%)	275 (26%)
Financial Strain, Somewhat or Very Hard	240 (29%)	272 (29%)	303 (29%)
Physical Activity Score (3 to 15), not including work	7.8 (6.7, 8.9)	7.9 (6.7, 9.0)	7.9 (6.7, 9.0)
Parous (live or still birth)	662 (79%)	751 (80%)	826 (79%)
Total Serum Lipid Concentration (mg/dl) at Baseline	613.2 (544.9, 699.4)	610.5 (545.0, 695.9)	611.8 (545.8, 698.6)
Waist Circumference (cm)	81.3 (73.3, 93.2)	81.1 (73.0, 92.8)	81.1 (73.1, 93.2)
High Fat Dairy Consumption (Frequency per Week)	10.6 (5.7, 19.0)	10.8 (5.8, 19.1)	10.8 (5.8, 19.3)
Meat Consumption (Frequency per Week)	3.5 (2.0, 5.6)	3.5 (1.8, 5.6)	3.5 (2.0, 5.5)
Fish and Shellfish Consumption (Frequency per Week)	1.3 (0.79, 2.5)	1.5 (0.79, 2.5)	1.5 (0.79, 2.5)
[1] Median (IQR) or Frequency (%)			

Exposure to POPs

Serum concentrations of POPs at baseline (1999/2000) are displayed in Table 3.2. PCBs were detected at the highest frequencies. 14 PCBs, HCB, cis-chlordane, trans-chlordane, trans-nonachlor, and PBDE 47 met DF criteria (Table A.1). PCB 118, 138, 153, and 180 had the highest median (IQR) concentrations of 263.4 ng/g lipid (138.4, 402.8), 366.0 (176.0, 643.4), and 215.6 (120.8, 353.4), respectively. PCB 105, PCB 180, and PBDE 47 also had relatively elevated median (IQR) concentrations of 73.8 (38.3, 122.4), 39.1 (24.6, 60.1), and 38.5 (22.8, 80.7), respectively.

POPs and Diabetes Incidence

Single pollutant models identified few statistically significant results and included both positive and negative associations. In models fully adjusted for IPW and confounders including serum lipids (Model 3), a one-IQR increase in serum concentrations of HCB and PCB 123, 157, 167, 180, 187, and 194 was associated with increased diabetes risk with HRs (95% CI) of 1.04 (0.99, 1.09), 1.10 (1.00, 1.20), 1.06 (1.00, 1.12), 1.07 (1.01, 1.14), 1.07 (1.01, 1.14), 1.08 (1.00, 1.16), and 1.09 (1.00, 1.18), respectively (Table 3.3). Analysis using tertiles of serum POP concentrations suggest positive and negative associations with diabetes risk as well as non-linearity (Table 3.3). After multivariable adjustment, (Model 3), HCB and PCB 123 were associated with increased diabetes risk with HRs (95% CI) of 1.67 (1.00, 2.79) and 1.87 (1.06, 3.29), respectively, when comparing T2 to T1. No significant effects were observed when comparing T3 to T1 of HCB and PCB 123, although HRs were above 1.0, suggesting a positive, non-linear relationship. Neither HCB nor PCB 123 had a significant trend across tertiles.

Table 3.2. Serum POP Concentrations (ng/g lipid) among SWAN MPS Participants Free of Diabetes at Baseline, 1999/2000

	DF (%)	P25	P33	P50	P66	P75
PCBs (n = 838)						
PCB 105	99.3	38.3	47.6	73.8	98.1	122.4
PCB 118	95.3	138.4	178.7	263.4	348.7	402.8
PCB 123	91.7	13.1	16.7	30.7	46.1	60.7
PCB 138	100.0	176.0	247.8	366.0	534.0	643.4
PCB 153	100.0	120.8	151.9	215.6	288.7	353.4
PCB 156	97.2	3.9	4.6	6.3	9.4	11.9
PCB 157	85.7	1.4	1.8	2.6	3.6	4.4
PCB 167	98.8	3.6	4.4	6.2	8.2	9.6
PCB 170	99.7	12.2	14.1	19.0	25.2	28.6
PCB 174	98.5	11.3	14.3	21.1	27.6	33.1
PCB 178	82.1	3.8	4.7	6.6	8.8	10.2
PCB 180	94.9	24.6	29.1	39.1	52.7	60.1
PCB 187	99.8	19.7	24.5	34.4	45.6	53.7
PCB 194	71.8	<LOD	3.5	4.8	6.3	7.3
PBDEs (n = 943)						
PBDE 47	72.8	<LOD	24.6	38.5	57.1	80.7
OCPs (n = 1,040)						
cis-chlordane	78.4	6.4	8.0	10.8	14.2	16.5
HCB	97.1	9.3	10.3	12.9	16.2	18.3
trans-chlordane	96.1	7.0	8.9	12.7	17.0	20.6
trans-nonachlor	72.0	<LOD	6.7	10.0	13.6	16.5
p,p'-DDT	39.6	<LOD	<LOD	<LOD	5.2	9.3
p,p'-DDD	38.9	<LOD	<LOD	<LOD	11.3	25.7
p,p'-DDE	38.8	<LOD	<LOD	<LOD	11.7	23.1

Compared to unadjusted results (Table S3.2), controlling for demographic and socioeconomic factors and IPW shifted most estimates away from the null, in a negative direction, whereas the positive association for PCB 123 remained similar and the positive association for HCB became stronger (Table S3.3). Additional adjustment for serum lipids (Model 2) shifted estimates slightly in a positive direction (Table S3.4). Further adjustment for lifestyle factors and parity (Model 3) shifted estimates in a positive direction and made many continuous POP estimates newly significant (Table 3.3). Additional adjustment for WC (Model

4) resulted in similar positive associations, although PCB and PBDE 47 estimates were slightly attenuated while PCB 138 and OCP estimates shifted away from the null (Table S3.5). T3 of PCB 138 was associated with lower diabetes risk (HR = 0.50, 95% CI: 0.26, 0.97), compared to T1 (p for trend = 0.037). Effect estimates for other compounds did not reach significance regardless of adjustment for WC. Potential overadjustment in Model 3 would not explain the null associations between individual POPs and diabetes risk because controlling for lipids, lifestyle factors, and parity shifted estimates in a positive direction (Tables S3.3, S3.4, and S3.5).

Moderate and high exposures to p,p'-DDE, p,p'-DDT, and p,p'-DDD were not significantly associated with diabetes risk, relative to non-detection (Table 3.4). Further adjustment for WC did not impact estimates (Table S3.6). Analyses of binary exposure to DDT metabolites (detection vs. non-detection) produced similar null results (Tables S3.7 and S3.8).

The distributions of serum POP concentrations in three K-means clusters are illustrated in Figure S3.5. Three-cluster analysis showed that relative to below-average exposure, average and above-average exposure were not associated with diabetes risk with HRs (95% CI) of 0.75 (0.42, 1.31) and 0.71 (0.38, 1.31), respectively (Table S3.9). We also depicted the distributions of serum POPs in two clusters (Figure S3.6). Additional adjustment for WC strengthened the protective effects of above-average PCB exposure and below-average OCP exposure (Table S3.10).

QBGC survival analysis suggested a null overall joint effect of the POP mixture. After multivariable adjustment, a one-tertile increase in the serum concentrations of all POPs was not associated with diabetes risk (HR = 1.04 [95% CI: 0.53, 2.07]). Further adjustment for baseline WC did not significantly impact the estimate (HR = 1.15 [95% CI: 0.57, 2.29]). Examining the contributions (weights) of individual compounds on the overall joint effect of the POP mixture, it appeared that the direction of effect was compound dependent (Figures S3.7 and S3.8).

Table 3.3. **Hazard Ratio (95% CI) of incident diabetes associated with serum POP concentrations, 1999-2016**. Model adjusted for age at baseline, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, and fish/shellfish intake, serum lipids at baseline, and joint stabilized inverse probability weights. Second and third tertiles of serum POP concentrations were analyzed relative to the first tertile.

Compound	Continuous (per IQR)	p	Tertile 2	Tertile 3	p for trend
OCPs (n = 1040)					
cis-chlordane	1.01 (0.99, 1.02)	0.4963	0.88 (0.52, 1.46)	0.96 (0.58, 1.59)	0.8713
HCB	1.04 (0.99, 1.09)	0.0894	1.67 (1.00, 2.79)	1.49 (0.84, 2.66)	0.1584
trans-chlordane	0.99 (0.97, 1.01)	0.1819	1.41 (0.84, 2.38)	1.30 (0.75, 2.24)	0.3399
trans-nonachlor	0.98 (0.94, 1.03)	0.4753	0.92 (0.55, 1.54)	1.13 (0.67, 1.89)	0.6481
PBDEs (n = 943)					
PBDE 47	1.01 (1.00, 1.02)	0.2446	0.98 (0.56, 1.72)	1.37 (0.78, 2.43)	0.2772
PCBs (n = 838)					
PCB 105	1.06 (0.93, 1.20)	0.3667	1.03 (0.59, 1.80)	0.81 (0.44, 1.48)	0.5031
PCB 118	1.06 (0.93, 1.20)	0.3904	0.91 (0.52, 1.59)	0.85 (0.47, 1.53)	0.5819
PCB 123	1.10 (1.00, 1.20)*	0.0472	1.87 (1.06, 3.29)*	1.60 (0.88, 2.92)	0.1180
PCB 138	1.06 (0.97, 1.17)	0.1936	0.83 (0.48, 1.43)	0.72 (0.40, 1.28)	0.2607
PCB 153	1.06 (0.97, 1.16)	0.2194	1.05 (0.61, 1.82)	0.72 (0.39, 1.33)	0.3068
PCB 156	0.92 (0.69, 1.23)	0.5791	0.91 (0.53, 1.57)	0.68 (0.38, 1.21)	0.1881
PCB 157	1.06 (1.00, 1.12)*	0.0400	0.71 (0.40, 1.23)	0.72 (0.39, 1.33)	0.2744
PCB 167	1.07 (1.01, 1.14)*	0.0268	0.84 (0.50, 1.42)	0.88 (0.48, 1.62)	0.6585
PCB 170	1.00 (0.98, 1.01)	0.5618	0.95 (0.55, 1.64)	0.77 (0.43, 1.35)	0.3636
PCB 174	1.05 (0.95, 1.18)	0.3388	0.98 (0.57, 1.68)	0.77 (0.42, 1.43)	0.4111
PCB 178	1.05 (0.93, 1.19)	0.4242	0.93 (0.53, 1.61)	0.77 (0.43, 1.37)	0.3742
PCB 180	1.07 (1.01, 1.14)*	0.0276	0.67 (0.38, 1.19)	0.80 (0.47, 1.38)	0.4101
PCB 187	1.08 (1.00, 1.16)*	0.0382	0.98 (0.57, 1.71)	0.84 (0.47, 1.50)	0.5598
PCB 194	1.09 (1.00, 1.18)	0.0514	0.81 (0.46, 1.45)	0.96 (0.56, 1.65)	0.8687

* *indicates a p-value < 0.05*

Table 3.4. **Hazard Ratio (95% CI) of incident diabetes associated with DDT metabolites, 1999-2016**. Model adjusted for age at baseline, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, and fish/shellfish intake, serum lipids at baseline, and joint stabilized inverse probability weights. Moderate and high quantiles of exposure were analyzed relative to non-detection. (n = 1040)

Metabolite	Moderate Exposure	High Exposure	p for trend
p,p'-DDD	0.93 (0.53, 1.64)	0.96 (0.56, 1.65)	0.836
p,p'-DDE	0.80 (0.44, 1.45)	1.35 (0.84, 2.17)	0.381
p,p'-DDT	1.16 (0.70, 1.92)	1.11 (0.63, 1.97)	0.614

Discussion

POPs and Diabetes Development

This study attempted to elucidate the potentially complex dose-response dynamics between POPs and diabetes by investigating the effects of individual pollutants and the overall mixture, carefully considering the role of serum lipids and WC, and conducting both continuous and tertile exposure analyses in attempts to elucidate dose-response dynamics. We did not find a consistent relationship, although some single pollutant models showed positive and negative relationships, as might be expected from multiple comparisons. The failure to reach significance at one or both tertiles impaired our ability to draw conclusions about dose-response shape. K-means Clustering results align with those from single pollutant tertile models of PCB exposure, although statistical significance is lacking. There is precedence for dose-response curves that plateau or resemble an inverted U.[20,27,33] ΣPCBs and trans-nonachlor have been associated with a non-linear increase in diabetes risk.[20,27] HCB was associated with a non-linear decrease in risk.[27] Tan et al. (2022) identified a positive, non-linear association for PCB 118 yet an overall positive linear association for ΣPCBs. The mechanism underlying a non-linear association is unclear,

although endocrine disruption is one proposed explanation. At low doses, endocrine disruptors can exert effects not seen at higher doses, thus displaying non-monotonic relationships.[19]

The mechanisms linking POPs, insulin resistance, and T2D are complex in part because they involve adipose tissue, a major storage site for POPs. People with similar adiposity can have wide variation in insulin resistance and T2D risk.[51–53] The dysfunction of visceral adipose tissue has been implicated in the development of metabolic disease including T2D.[54–56] Evidence in vivo and in vitro (in adipocytes) indicates that adipose tissue is an insulin-sensitive tissue.[57,58] It is critical to look beyond the adipose tissue mass to consider POPs stored inside the tissue.[19,55,56]

Lipophilicity allows many POPs to accumulate in adipose tissue where they may exert endocrine disrupting and/or inflammatory effects.[19] Inflammation in adipose tissue rather than tissue mass itself may better predict metabolic health.[59,60] Insulin resistance and T2D have been characterized by chronic low-grade inflammation in adipose tissue.[19] Low-dose POPs exposure has pro-inflammatory effects in adipose tissue.[17] Endocrine mechanisms linking POPs and T2D include the disruption of estrogen, androgen, thyroid hormone, and glucocorticoid homeostasis.[18,26] Additional mechanisms may include oxidative stress and mitochondrial dysfunction.[18,19] In mitochondrial dysfunction, diacylglycerol and fatty acid metabolites accumulate and suppress insulin signaling, leading to insulin resistance.[26] In vivo studies of OCPs and PCBs support a link between exposure and insulin resistance.[57,58,61,62]

In our study, although we sampled POPs in serum rather than adipose tissue, we hypothesized that insulin resistance was likely to occur via mechanisms in adipose tissue. The association between serum POPs and incident diabetes is influenced by the facts that i) POPs are more likely to accumulate in visceral adipose tissue and ii) visceral fat is more metabolically active compared to subcutaneous fat.[63] For these reasons, we conducted a sensitivity analysis for

additional WC adjustment (Model 4). In single pollutant analyses, adjustment for WC may have attenuated PCB and PBDE estimates because they are more lipid soluble compared to OCPs. It is important to note that individual POPs differ in characteristics beyond lipophilicity, such as toxicity and half-life. The is important to note that the POPs we examined differ in lipophilicity, half-lives, and toxicity. In addition to insulin resistance, pancreatic beta-cell dysfunction has been proposed as an explanation for the increasing risk of diabetes. Both observational and in vivo studies have linked low-dose POPs exposure to T2D via beta cell dysfunction.[64,65]

The literature on POPs and T2D -- including prospective, cross-sectional, in vivo, and in vitro studies – demonstrates strong evidence for a relationship between low-dose exposure to POPs and T2D, according to one critical review,[17] and moderate evidence for a relationship between exposure to *p*,*p'*-DDE and T2D risk, according to another systematic review.[18] A meta-analysis of chlordane-specific research concluded that exposure to trans-chlordane among other compounds increased risk of T2D.[66] According to the Endocrine Society's Scientific Statement on EDCs, there is moderate to strong evidence that EDCs including POPs have diabetogenic effects, which manifest in a non-monotonic manner.[13] Overall, cross-sectional more consistently found positive associations whereas prospective studies were less consistent in the shape and strength of dose-response relationships. Given that the overwhelming evidence remains cross-sectional in design, causality cannot be inferred.[13]

Observational studies of PCBs and OCPs and diabetes risk have been contradictory. In the case of PCBs, results from prospective studies have conflicted, many finding PCBs to increase diabetes risk[20–22] and others finding no relationship.[24–27,30] Two such PCB studies found significant positive relationships occurring only in women, whose ages ranged from 20 to >60 years.[21,22] A study of insulin sensitivity found that among people who never developed diabetes,

lipid-adjusted ΣPCBs but not OCPs were associated with reduced insulin sensitivity, most prominently among the 48-55 age group; no effect was seen among participants who developed diabetes for any age group.[34] Prospective studies of OCPs suggest positive associations that vary in strength and significance by compound[24,26,27,29,30] while another found no association.[25] HCB and p,p'-DDE were repeatedly associated with diabetes incidence, however, the direction of effect and shape of dose-response curves have been contradictory.[24,27,29,30] A noteworthy prospective study found no significant associations between PCBs or OCPs in adipose tissue and 16-year T2D incidence although the results suggested a harmful effect.[67] Most cross-sectional studies suggest positive relationships with linear or non-linear dose-response relationships depending upon the compound. One notable cross-sectional study assessed serum PCBs and OCPs at multiple timepoints and found increased risk of T2D up to 7 years before diagnosis with the effect strength increasing as the time between serum collection and diagnosis decreased.[26]

In our study, mixtures approaches were useful for comparison purposes but did not contribute significantly to our conclusions beyond what was indicated by single pollutant analyses. It is difficult to contextualize our findings in the literature as only two such studies have been conducted. Gasull (2012) found a positive, linear association between a PCB mixture and diabetes prevalence using Bayesian Kernel Machine Regression (BKMR) modeling.[68] Perez (2023) expanded mixture analysis to include PCBs and OCPs; using Weighted Quantile Sum (WQS) regression models, they found a positive association between the POP mixture and prevalence of metabolic syndrome driven by OCPs.[32] Neither BKMR nor WQS can be used for time-to-event analysis. Single pollutant analysis also revealed that HCB and γ-HCH were associated with increased fasting blood glucose. Neither of the existing mixtures studies

evaluated PBDEs.[32,68] Notwithstanding, it remains difficult to explain our null findings relative to positive associations in mixtures and single pollutant studies.

The epidemiological literature on T2D is less developed with respect to PBDEs. The two most robust prospective studies found no associations after serum lipids adjustment.[25,27] Exposure to ΣPBDEs, based on dietary estimates rather than serum concentrations, had a positive, non-linear association with diabetes risk.[31] Animal studies suggest that PBDEs and other brominated flame retardants may increase diabetes risk, possibly involving the PPARγ receptor.[69] In vivo, PBDE 47 [70] and PBDE 209 [69] increased fasting glucose, with PBDE 209 demonstrating an inverse U-shaped dose-response with HOMA-IR. In vitro, PBDE-47 and 85 increased glucose stimulated insulin secretion.[71]

The long follow-up period after POP exposure measurement is another factor that may obscure the relationship between POPs and diabetes risk. Research shows that although a longer follow-up time has numerous advantages, the value of baseline serum concentrations may decrease with an increasing follow-up duration, thus reducing our chances of uncovering a relationship between serum POPs and diabetes.[17] One prospective study of midlife participants ages 50-59 with exposure measured in 1995-2000 found no association between the risk of diabetes and PCB 153 yet found a positive association for p,p'-DDE.[28] Although this study had a similar timing of exposure, a shorter follow-up period of 11 years may have benefitted in elucidating an association especially for pp-DDE, which would have likely declined in serum over a longer follow-up period.[28] Effect size may decrease as the time between serum collection and diagnosis increases.[26] The timing of exposure matters because exposure measurement error may increase over time as menopausal changes in adiposity affect serum concentrations of lipophilic POPs. This may render insufficient a single serum POP measurement. Our ability to

evaluate potential differences in effect size by follow-up time was impaired by low case counts. Other studies with long follow-up periods comparable to our study assessed participants highly exposed to contaminated food sources between 1976 and 1983, meaning that the findings were less representativeness of risk in the general population (Vasiliu 2006, Wang 2008).

The level of POP exposure among SWAN women may contribute to the discrepancy between our findings and the literature. Participants in SWAN MPS and Lee et al. (2010) had similar serum POP concentrations; in both studies, concentrations were higher than in NHANES 1999-2004.[72] Compared to participants in Wu et al. (2013), SWAN reported lower levels of PCB 180 and HCB yet higher levels of PCB 118, 138, and 153. A prospective study of menopausal and post-menopausal women measured POPs in 1989-1990 and concentrations far exceeded those measured a decade later in SWAN MPS women; after 19 years of follow-up, they found null associations for PCBs and DDT metabolites yet found a positive linear association for HCB (Wu 2013). Investigating POPs in the low-dose range, such as PCB 194, we may find that disease risk increases more linearly compared to POPs in the high-dose range that may demonstrate a more non-linear risk curve.[17]Differences in exposure distributions across studies may not fully account for incongruent results, nevertheless they limit our ability to draw rigorous conclusions regarding dose-response dynamics.

Strengths and Limitations

This is the first prospective study of diabetes to employ a mixtures approach. Our study is one of few to analyze PBDEs in addition to PCBs and OCPs. We employ QBGC, a rigorous, outcome-based mixtures method that can account for co-pollutant confounding and interactions in a highly correlated mixture. Compared to similar approaches, QBGC does not assume directional homogeneity, linearity, or additivity of mixture components. The study leveraged

SWAN's original design to understand metabolic changes throughout the menopausal transition. The prospective design enabled the investigation of diabetes risk over 18 years and allowed us to account for selection bias at multiple timepoints using IPW. Our approach was strengthened by careful consideration of potential biological mechanisms involving serum lipids and adipose tissue. We calculated serum POP concentrations using traditional lipid-standardization to account for recent fat intake and adjusted models for serum lipids to reduce bias.[41] We examined the role of WC in sensitivity analyses. Furthermore, our study represents women from multiple urban areas with a focus on Chinese and Japanese women who have been historically underrepresented in U.S. studies.

This study is primarily limited by the small number of diabetes cases and the single measure of serum POPs. A larger sample size would have equipped us with the power necessary to stratify by potential mechanistic characteristics such as menopausal status or obesity status. Our oversampling of Black, Japanese, and Chinese women expanded our understanding of POPs exposure and diabetes risk in a more diverse population. Notwithstanding, a larger sample would have allowed us to investigate the interaction of race/ethnicity with serum POPs and study site. Differences in the timing of serum collection with respect to diabetes diagnosis could have affected risk estimates. Robust analysis of time-varying hazard ratios was prohibited by small case numbers at early follow-up visits. These findings may only be generalizable to populations similar to U.S. midlife women residing in greater metropolitan areas. K-means Clustering is limited by uncertainty in cluster membership. K-means Clustering and QBGC do not produce results that are perfectly comparable. Finally, in the single pollutant analyses, we did not address multiple comparisons.

Conclusion

This study examined incident diabetes and exposure to POPs individually and as a mixture. Overall, we conclude that exposure to a mixture of serum POPs was not associated with incident diabetes in midlife U.S. women, although some individual POPs demonstrated inconsistent yet significant associations with diabetes risk. In this study, mixtures analyses did not contribute substantial information beyond the single pollutant approach. Nevertheless, a mixtures approach is valuable to validate and contextualize single pollutant findings and is necessary to evaluate the overall effect of a mixture. Additional studies are needed on the effects of PBDEs on insulin resistance, perhaps in a younger population. In addition, we recommend that repeated POP measures and a larger study sample are warranted to elucidate this association and provide insight into mechanisms of action.

References

1. International Diabetes Federation. *Diabetes Around the World 2021*. https://diabetesatlas.org/idfawp/resource-files/2021/11/IDFDA10-global-fact-sheet.pdf (2021).
2. World Health Organization. *Diabetes*. https://www.who.int/news-room/fact-sheets/detail/diabetes (2022).
3. Centers for Disease Control and Prevention. *By the Numbers: Diabetes in America*. https://www.cdc.gov/diabetes/health-equity/diabetes-by-the-numbers.html (2022).
4. Centers for Disease Control and Prevention. *Diabetes Infographics*. https://www.cdc.gov/diabetes/library/socialmedia/infographics.html (2023).
5. Chatterjee, S., Khunti, K. & Davies, M. J. Type 2 diabetes. *The Lancet* **389**, 2239–2251 (2017).
6. Vargas-Vázquez, A. *et al.* Impact of undiagnosed type 2 diabetes and pre-diabetes on severity and mortality for SARS-CoV-2 infection. *BMJ Open Diabetes Res. Care* **9**, e002026 (2021).
7. Centers for Disease Control and Prevention. *Diabetes Report Card 2021*. https://www.cdc.gov/diabetes/library/reports/reportcard.html (2021).
8. Billings, L. K. & Florez, J. C. The genetics of type 2 diabetes: what have we learned from GWAS?: Genetics of type 2 diabetes. *Ann. N. Y. Acad. Sci.* **1212**, 59–77 (2010).
9. *Chemical Factsheets*. https://www.cdc.gov/biomonitoring/chemical_factsheets.html.

10. Sharma, A. *et al.* Worldwide pesticide usage and its impacts on ecosystem. *SN Appl. Sci.* **1**, 1446 (2019).

11. Agency for Toxic Substances and Disease Registry. *Toxicological Profile for Polybrominated Diphenyl Ethers (PBDEs)*. https://www.atsdr.cdc.gov/toxprofiles/tp207.pdf (2017).

12. Wahlang, B. Exposure to persistent organic pollutants: impact on women's health. *Rev. Environ. Health* **33**, 331–348 (2018).

13. Gore, A. C. *et al.* EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. *Endocr. Rev.* **36**, E1–E150 (2015).

14. Bonefeld-Jørgensen, E. C. *et al.* Biomonitoring and Hormone-Disrupting Effect Biomarkers of Persistent Organic Pollutants *In Vitro* and *Ex Vivo*. *Basic Clin. Pharmacol. Toxicol.* **115**, 118–128 (2014).

15. Agency for Toxic Substances and Disease Registry. *Toxicological Profile for Polychlorinated Biphenyls (PCBs)*. https://www.atsdr.cdc.gov/toxprofiles/tp17.pdf (2000).

16. Kraft, M. *et al.* Inhalation Exposure to PCB from Contaminated Indoor Air—How Much Is Absorbed into the Blood? *Pollutants* **1**, 181–193 (2021).

17. Lee, Y.-M., Jacobs Jr., D. R. & Lee, D.-H. Persistent Organic Pollutants and Type 2 Diabetes: A Critical Review of Review Articles. *Front. Endocrinol.* **9**, 712 (2018).

18. Lind, P. M. & Lind, L. Endocrine-disrupting chemicals and risk of diabetes: an evidence-based review. *Diabetologia* **61**, 1495–1502 (2018).

19. Lee, D.-H., Porta, M., Jacobs, D. R. & Vandenberg, L. N. Chlorinated Persistent Organic Pollutants, Obesity, and Type 2 Diabetes. *Endocr. Rev.* **35**, 557–601 (2014).

20. Lee, D.-H. *et al.* Low Dose Organochlorine Pesticides and Polychlorinated Biphenyls Predict Obesity, Dyslipidemia, and Insulin Resistance among People Free of Diabetes. *PLoS ONE* **6**, e15977 (2011).

21. Wang, S.-L., Tsai, P.-C., Yang, C.-Y. & Leon Guo, Y. Increased Risk of Diabetes and Polychlorinated Biphenyls and Dioxins: A 24-year follow-up study of the Yucheng cohort. *Diabetes Care* **31**, 1574–1579 (2008).

22. Vasiliu, O., Cameron, L., Gardiner, J., DeGuire, P. & Karmaus, W. Polybrominated Biphenyls, Polychlorinated Biphenyls, Body Weight, and Incidence of Adult-Onset Diabetes Mellitus. *Epidemiology* **17**, 352–359 (2006).

23. Wolf, K. *et al.* Persistent organic pollutants and the incidence of type 2 diabetes in the CARLA and KORA cohort studies. *Environ. Int.* **129**, 221–228 (2019).

24. Wu, H. *et al.* Persistent organic pollutants and type 2 diabetes: a prospective analysis in the nurses' health study and meta-analysis. *Environ. Health Perspect.* **121**, 153–161 (2013).

25. Magliano, D. J. *et al.* Exposure to persistent organic pollutants and the risk of type 2 diabetes: a case-cohort study. *Diabetes Metab.* **47**, 101234 (2021).

26. Charles, D. *et al.* Longitudinal changes in concentrations of persistent organic pollutants (1986–2016) and their associations with type 2 diabetes mellitus. *Environ. Res.* **204**, 112129 (2022).

27. Lee, D.-H. *et al.* Low dose of some persistent organic pollutants predicts type 2 diabetes: a nested case-control study. *Environ. Health Perspect.* **118**, 1235–1242 (2010).

28. Rignell-Hydbom, A., Rylander, L. & Hagmar, L. Exposure to persistent organochlorine pollutants and type 2 diabetes mellitus. *Hum. Exp. Toxicol.* **26**, 447–452 (2007).

29. Turyk, M., Anderson, H. A., Knobeloch, L., Imm, P. & Persky, V. W. Prevalence of diabetes and body burdens of polychlorinated biphenyls, polybrominated diphenyl ethers, and p,p′-diphenyldichloroethene in Great Lakes sport fish consumers. *Chemosphere* **75**, 674–679 (2009).

30. Rignell-Hydbom, A. *et al.* Exposure to p,p'-DDE: a risk factor for type 2 diabetes. *PloS One* **4**, e7503 (2009).

31. Ongono, J. S. *et al.* Dietary exposure to brominated flame retardants and risk of type 2 diabetes in the French E3N cohort. *Environ. Int.* **123**, 54–60 (2019).

32. Reina-Pérez, I. *et al.* Cross-sectional associations of persistent organic pollutants measured in adipose tissue and metabolic syndrome in clinically diagnosed middle-aged adults. *Environ. Res.* 115350 (2023) doi:10.1016/j.envres.2023.115350.

33. Tan, Q. *et al.* Associations of polychlorinated biphenyls exposure with plasma glucose and diabetes in general Chinese population: The mediating effect of lipid peroxidation. *Environ. Pollut.* **308**, 119660 (2022).

34. Suarez-Lopez, J. R., Lee, D.-H., Porta, M., Steffes, M. W. & Jacobs, D. R. Persistent organic pollutants in young adults and changes in glucose related metabolism over a 23-year follow-up. *Environ. Res.* **137**, 485–494 (2015).

35. Sowers, M. *et al.* SWAN: A Multicenter, Multiethnic, Community-Based Cohort Study of Women and the Menopausal Transition. *Menopause: Biology and Pathobiology* (2000).

36. Wang, X., Karvonen-Gutierrez, C. A., Mukherjee, B., Herman, W. H. & Park, S. K. Urinary metals and adipokines in midlife women: The Study of Women's Health Across the nation (SWAN). *Environ. Res.* 110426 (2020) doi:10.1016/j.envres.2020.110426.

37. Grant-Alfieri, A., Devasurendra, A., Batterman, S., Karvonen-Gutierrez, C. & Park, S. K. Changes in adipose tissue and circulating concentrations of persistent organic pollutants in midlife women. *Under Review* (2023).

38. U.S. Environmental Protection Agency. *DDT: A Brief History and Status*. https://www.epa.gov/ingredients-used-pesticide-products/ddt-brief-history-and-status (2023).

39. Heindel, J. J. *et al.* Metabolism disrupting chemicals and metabolic disorders. *Reprod. Toxicol.* **68**, 3–33 (2017).

40. Yu, G. W., Laseter, J. & Mylander, C. Persistent Organic Pollutants in Serum and Several Different Fat Compartments in Humans. *J. Environ. Public Health* **2011**, 1–8 (2011).

41. O'Brien, K. M., Upson, K., Cook, N. R. & Weinberg, C. R. Environmental Chemicals in Urine and Blood: Improving Methods for Creatinine and Lipid Adjustment. *Environ. Health Perspect.* **124**, 220–227 (2016).

42. Centers for Disease Control and Prevention. *Serum PCBs and Persistent Pesticides*. https://www.cdc.gov/nchs/data/nhanes/nhanes_01_02/l28poc_b_met_pcb_pesticides.pdf.

43. Centers for Disease Control and Prevention. *Diabetes Risk Factors*. https://www.cdc.gov/diabetes/basics/risk-factors.html.

44. The Aspen Institute. *Glossary for Understanding the Dismantling Structural Racism/Promoting Racial Equity Analysis*. https://assets.aspeninstitute.org/content/uploads/files/content/docs/rcc/RCC-Structural-Racism-Glossary.pdf. (2017).

45. Dirinck, E. *et al.* Pivotal Role for the Visceral Fat Compartment in the Release of Persistent Organic Pollutants During Weight Loss. *J. Clin. Endocrinol. Metab.* **100**, 4463–4471 (2015).

46. Fan, Y. *et al.* Waist Circumference and its Changes Are More Strongly Associated with the Risk of Type 2 Diabetes than Body Mass Index and Changes in Body Weight in Chinese Adults. *J. Nutr.* **150**, 1259–1265 (2020).

47. Hartigan, J. A. & Wong, M. A. Algorithm AS 136: A K-Means Clustering Algorithm. *Appl. Stat.* **28**, 100 (1979).

48. R Core Team. R: A language and environment for statistical computing. (2022).

49. Keil Alexander P. *et al.* A Quantile-Based g-Computation Approach to Addressing the Effects of Exposure Mixtures. *Environ. Health Perspect.* **128**, 047004.

50. Keil, A. qgcomp: Quantile G-Computation. (2022).

51. Sims, E. A. Are there persons who are obese, but metabolically healthy? *Metabolism* **50**, 1499–1504 (2001).

52. McLaughlin, T. Heterogeneity in the Prevalence of Risk Factors for Cardiovascular Disease and Type 2 Diabetes Mellitus in Obese Individuals: Effect of Differences in Insulin Sensitivity. *Arch. Intern. Med.* **167**, 642 (2007).

53. Gregg, E. W., Cheng, Y. J., Narayan, K. M. V., Thompson, T. J. & Williamson, D. F. The relative contributions of different levels of overweight and obesity to the increased prevalence of diabetes in the United States: 1976–2004. *Prev. Med.* **45**, 348–352 (2007).

54. Lamat, H. *et al.* Metabolic syndrome and pesticides: A systematic review and meta-analysis. *Environ. Pollut.* **305**, 119288 (2022).

55. Barrett, J. R. POPs vs. Fat: Persistent Organic Pollutant Toxicity Targets and Is Modulated by Adipose Tissue. *Environ. Health Perspect.* **121**, a61 (2013).

56. La Merrill, M. *et al.* Toxicological Function of Adipose Tissue: Focus on Persistent Organic Pollutants. *Environ. Health Perspect.* **121**, 162–169 (2013).

57. Enan, E., Liu, P. & Matsumura, F. TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) causes reduction in glucose uptake through glucose transporters on the plasma membrane of the guinea pig adipocyte. *J. Environ. Sci. Health Part B* **27**, 495–510 (1992).

58. Ruzzin, J. *et al.* Persistent Organic Pollutant Exposure Leads to Insulin Resistance Syndrome. *Environ. Health Perspect.* **118**, 465–471 (2010).

59. Dirtu, A. C. *et al.* Dynamics of Organohalogenated Contaminants in Human Serum from Obese Individuals during One Year of Weight Loss Treatment. *Environ. Sci. Technol.* **47**, 12441–12449 (2013).

60. Blüher, M. The distinction of metabolically 'healthy' from 'unhealthy' obese individuals. *Curr. Opin. Lipidol.* **21**, 38–43 (2010).

61. Gray, S. L., Shaw, A. C., Gagne, A. X. & Chan, H. M. Chronic exposure to PCBs (Aroclor 1254) exacerbates obesity-induced insulin resistance and hyperinsulinemia in mice. *J. Toxicol. Environ. Health A* **76**, 701–715 (2013).

62. Yau, E. T. & Mennear, J. H. The inhibitoty effect of DDT on insulin secretion in mice. *Toxicol. Appl. Pharmacol.* **39**, 81–88 (1977).

63. Dirinck, E. L. *et al.* Exposure to Persistent Organic Pollutants: Relationship With Abnormal Glucose Metabolism and Visceral Adiposity. *Diabetes Care* **37**, 1951–1958 (2014).

64. Lee, Y.-M. *et al.* Low-Dose Persistent Organic Pollutants Impair Insulin Secretory Function of Pancreatic β-Cells: Human and In Vitro Evidence. *Diabetes* **66**, 2669–2680 (2017).

65. Hectors, T. L. M. *et al.* Environmental pollutants and type 2 diabetes: a review of mechanisms that can disrupt beta cell function. *Diabetologia* **54**, 1273–1290 (2011).

66. Mendes, V. *et al.* The association between environmental exposures to chlordanes, adiposity and diabetes-related features: a systematic review and meta-analysis. *Sci. Rep.* **11**, 14546 (2021).

67. Barrios-Rodríguez, R. *et al.* Associations of accumulated selected persistent organic pollutants in adipose tissue with insulin sensitivity and risk of incident type-2 diabetes. *Environ. Int.* **155**, 106607 (2021).

68. Gasull, M. *et al.* Blood Concentrations of Persistent Organic Pollutants and Prediabetes and Diabetes in the General Population of Catalonia. *Environ. Sci. Technol.* **46**, 7799–7810 (2012).

69. Alimu, A. *et al.* Decabromodiphenyl ether causes insulin resistance and glucose and lipid metabolism disorders in mice. *World J. Diabetes* **12**, 1267–1281 (2021).

70. Zhang, Z. *et al.* Environmental exposure to BDE47 is associated with increased diabetes prevalence: Evidence from community-based case-control studies and an animal experiment. *Sci. Rep.* **6**, 27854 (2016).

71. Karandrea, S., Yin, H., Liang, X. & Heart, E. A. BDE-47 and BDE-85 stimulate insulin secretion in INS-1 832/13 pancreatic β-cells through the thyroid receptor and Akt. *Environ. Toxicol. Pharmacol.* **56**, 29–34 (2017).

72. Centers for Disease Control and Prevention. *National Report on Human Exposure to Environmental Chemicals: Biomonitoring Data Tables for Environmental Chemicals*. https://www.cdc.gov/exposurereport/data_tables.html.

Supplementary Materials

Methods

Incident diabetes ascertainment

At each SWAN visit, incident diabetes was defined by the participant meeting one or more of the following criteria: (1) use of an anti-diabetic medication at any visit; (2) fasting glucose ≥7 mmol/l at two consecutive visits while not on corticosteroids including glucocorticoids; and (3) any two visits with self-reported diabetes and at least one visit with fasting glucose ≥7 mmol/l. Among women who used anti-diabetic medication, the visit of diabetes incidence was defined as (i) the first visit at which medication use was reported or (ii) the first visit prior to medication commencement at which serum glucose was ≥7 mmol/L or diabetes diagnosis was self-reported. Among women who did not use anti-diabetic medication, the visit of diabetes incidence was defined as the first visit at which serum glucose was ≥7 mmol/L while not on corticosteroids.

Inverse probability weighting

Bias may have occurred if exposure to POPs, diabetes risk factors, or potential confounders influenced the selection of participants into SWAN MPS either through (i) loss to follow up from the initial SWAN visit (1996/97) to the third SWAN visit (1999/2000) or (ii) selection into the MPS from the larger SWAN study population. POPs were measured in serum samples collected in 1999/2000 from a subsample of 1,400 SWAN participants designated the MPS. Therefore, we were unable to determine whether women who were censored between

1996/7 and 1999/2000 or who did not participate in MPS were more highly exposed to POPs or at greater risk of developing diabetes later. Participants in SWAN MPS may have been different from the source population. To reduce these two types of bias, we used inverse probability weighting (IPW).

IPW created a pseudo population representing participants at risk of diabetes at the time of POP measurement (1999/2000) in the original SWAN population; data from original SWAN participants was used to weight observations from MPS participants. The probability of continuation in SWAN from 1996/97 to 1999/2000 (P_1) was modeled separately from the probability of selection into MPS (P_2). P_1 was estimated using a logistic regression model adjusted for age, study site, race/ethnicity, education, smoking status, body mass index, waist circumference, diabetes medication use, overall health, and stroke. The reciprocal of this cumulative probability (W_1) was the weight of remaining a participant free of diabetes in 1999/2000. W_1 was then stabilized to minimize the variance, producing SW_1. P_2 was estimated using a logistic regression model adjusted for age, study site, race/ethnicity, education, smoking status, metabolic syndrome, and diastolic blood pressure. The reciprocal of this probability (W_2) was the weight of being selected into MPS in 1999/2000. W_2 was then stabilized to minimize the variance, producing SW_2. Finally, we calculated the joint IPW, $SW_{MPS} = SW_1 * SW_2$, as the inverse of the probability of participation in the SWAN MPS.

Figure S3.1 Study design of the Study of Women's Health Across the Nation Multi-Pollutant Study (SWAN MPS).

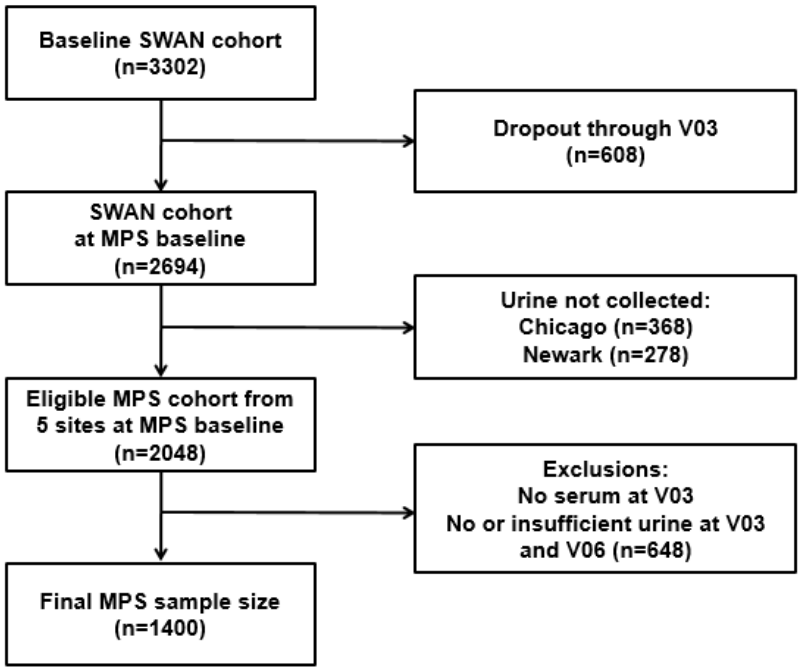


Table S3.1: Detection frequencies (DF) of PCBs, PBDEs, and OCPs in SWAN MPS at baseline (1999/2000).

PCB		DF (%)
Tetra	77	5.28
	78	0.00
	79	2.01
	81	0.00
	114	9.61
Penta	105	99.26
	87/110/111	0.00
	118	95.35
	123	91.66
	126	12.57
Hexa	138	100.00
	149	59.66
	153	100.00
	156	97.25
	157	85.74
	162	0.53
	167	98.84
	169	0.42
Hepta	170	99.68
	174	98.52
	178	82.15
	180	94.93
	187	99.79
	188	0.00
	189	0.11
Octa	194	71.81
	195	6.55
	199	61.25
	202	64.84
	203	0.00
	205	0.00
Nona	206	11.93
	208	32.00
Deca	209	20.59

OCP	DF (%)
p,p'-DDD	38.94
p,p'-DDT	39.61
o,p'-DDT	16.50
p,p'-DDE	38.78
aldrin	0.25
dieldrin	2.09
endrin	2.60
cis-chlordane	78.39
trans-chlordane	96.06
heptachlor	6.37
cis-nonachlor	3.27
trans-nonachlor	72.03
mirex	16.83
oxychlordane	9.72
c/t-heptachlorepoxide	13.32
α-HCH	5.53
β-HCH	22.36
γ-HCH	9.55
HCB	97.15

PBDE	DF (%)
17	5.73
28	4.23
47	72.84
66	0.28
71	0.00
85	1.60
99	20.39
100	12.69
138	0.85
153	15.13
154	21.15
183	1.13
190	2.35
209	0.94

Figure S3.2. Correlation heatmap of serum POP concentrations in SWAN MPS at baseline, 1999/2000 (n = 809).

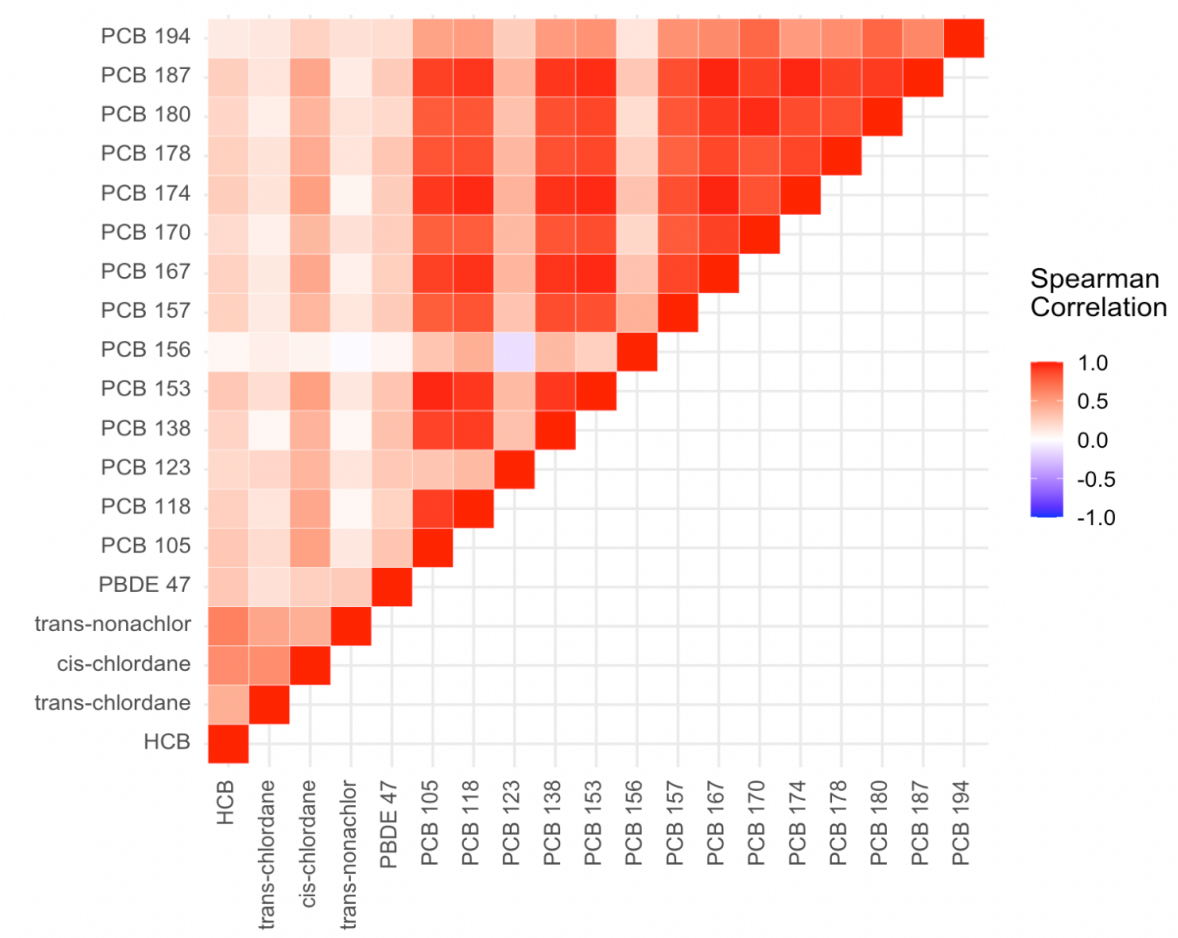


Figure S3.3. Elbow method identification of k exposure clusters of the POP mixture in SWAN MPS.

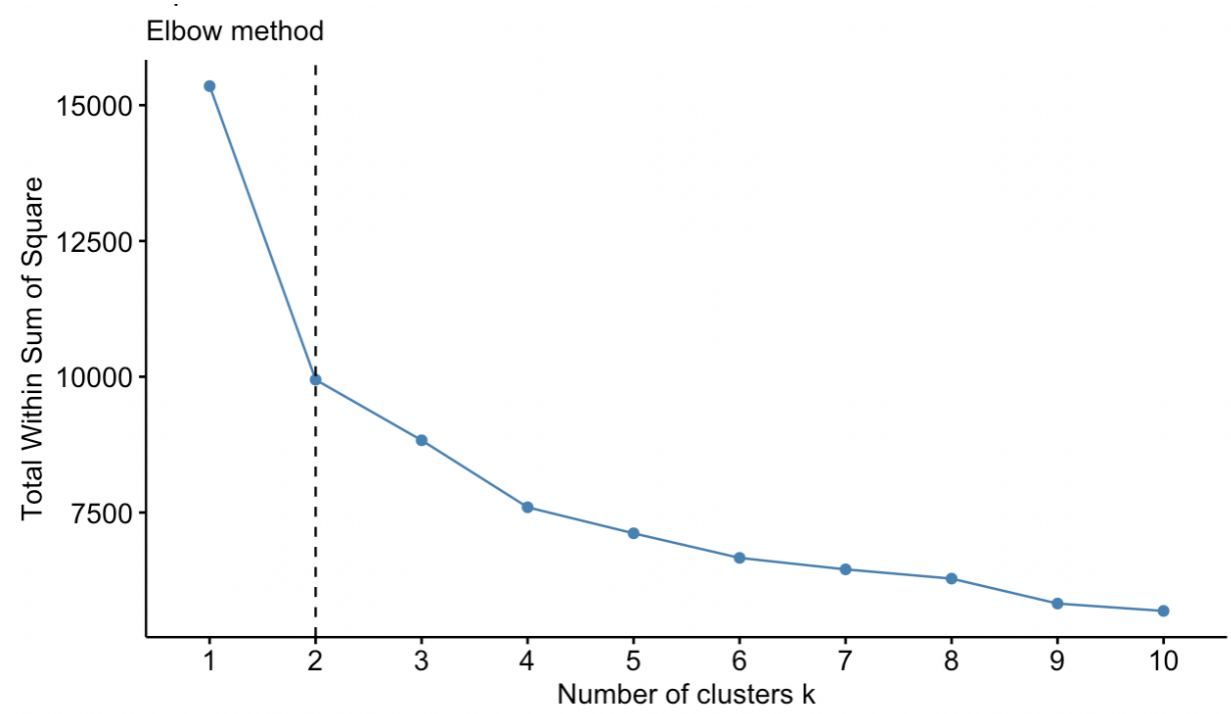


Figure S3.4. Silhouette method identification of k exposure clusters of the POP mixture in SWAN MPS.

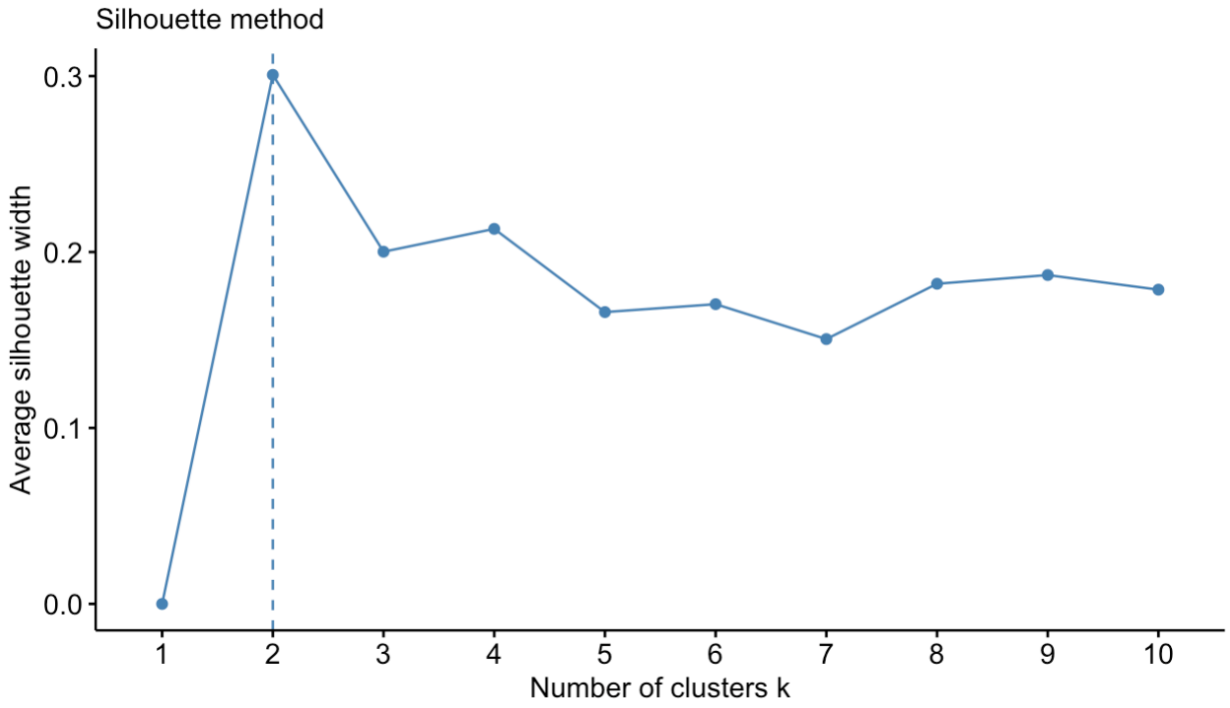


Table S3.2 Hazard Ratio (95% CI) of incident diabetes associated with serum POP concentrations, 1999-2016. Model is unadjusted. Second and third tertiles of serum POP concentrations were analyzed relative to the first tertile.

Compound	Continuous (per IQR)	p	Tertile 2	Tertile 3	p for trend
OCPs (n = 1040)					
cis-chlordane	1.01 (0.99, 1.03)	0.5385	0.81 (0.5, 1.29)	0.82 (0.51, 1.31)	0.3964
HCB	1.05 (0.97, 1.14)	0.2227	1.44 (0.89, 2.32)	1.13 (0.69, 1.87)	0.6405
trans-chlordane	0.98 (0.88, 1.1)	0.7474	1.29 (0.8, 2.07)	1.13 (0.69, 1.84)	0.6372
trans-nonachlor	1 (0.92, 1.07)	0.9145	1.04 (0.64, 1.7)	1.2 (0.75, 1.93)	0.4470
PBDEs (n = 943)					
PBDE 47	1.01 (1, 1.02)	0.1930	1 (0.6, 1.68)	1.24 (0.76, 2.04)	0.3816
PCBs (n = 838)					
PCB 105	1.05 (0.89, 1.25)	0.5276	0.96 (0.58, 1.59)	0.75 (0.44, 1.28)	0.2959
PCB 118	1.05 (0.89, 1.23)	0.5780	0.89 (0.53, 1.47)	0.78 (0.46, 1.32)	0.3549
PCB 123	1.09 (0.96, 1.24)	0.2005	1.87 (1.09, 3.19)	1.36 (0.77, 2.41)	0.3120
PCB 138	1.06 (0.92, 1.22)	0.4198	0.82 (0.5, 1.36)	0.68 (0.4, 1.15)	0.1495
PCB 153	1.06 (0.92, 1.22)	0.4320	0.99 (0.6, 1.63)	0.71 (0.41, 1.21)	0.2172
PCB 156	0.92 (0.74, 1.14)	0.4474	0.71 (0.43, 1.18)	0.65 (0.39, 1.09)	0.0961
PCB 157	1.05 (0.95, 1.16)	0.3030	0.75 (0.45, 1.24)	0.69 (0.41, 1.15)	0.1464
PCB 167	1.06 (0.95, 1.19)	0.2811	0.85 (0.51, 1.42)	0.76 (0.45, 1.28)	0.3007
PCB 170	1 (0.96, 1.03)	0.8532	0.98 (0.59, 1.61)	0.72 (0.42, 1.24)	0.2476
PCB 174	1.05 (0.9, 1.22)	0.5513	1.02 (0.62, 1.67)	0.72 (0.42, 1.24)	0.2500
PCB 178	1.05 (0.89, 1.22)	0.5777	1.09 (0.66, 1.81)	0.79 (0.46, 1.35)	0.3974
PCB 180	1.06 (0.96, 1.17)	0.2328	0.73 (0.44, 1.23)	0.72 (0.43, 1.2)	0.1947
PCB 187	1.07 (0.96, 1.2)	0.2269	1 (0.6, 1.67)	0.81 (0.48, 1.37)	0.4365
PCB 194	1.07 (0.96, 1.2)	0.2239	0.73 (0.43, 1.22)	0.82 (0.49, 1.35)	0.4074

Table S3.3 Hazard Ratio (95% CI) of incident diabetes associated with serum POP concentrations, 1999-2016. Model adjusted for age at baseline, race/ethnicity, site, education, financial strain, and joint stabilized inverse probability weights. Second and third tertiles of serum POP concentrations were analyzed relative to the first tertile.

Compound	Continuous (per IQR)	p	Tertile 2	Tertile 3	p for trend
OCPs (n = 1040)					
cis-chlordane	1.01 (0.99, 1.02)	0.5077	0.84 (0.51, 1.38)	0.84 (0.52, 1.36)	0.4834
HCB	1.02 (0.98, 1.05)	0.3040	1.55 (0.95, 2.52)	1.28 (0.76, 2.14)	0.0810
trans-chlordane	0.99 (0.96, 1.01)	0.2815	1.3 (0.79, 2.14)	1.2 (0.71, 2.01)	0.3001
trans-nonachlor	0.98 (0.93, 1.03)	0.4852	0.99 (0.6, 1.64)	1.05 (0.64, 1.72)	0.9659
PBDEs (n = 943)					
PBDE 47	1.01 (1, 1.02)	0.0928	0.89 (0.52, 1.53)	1.2 (0.71, 2.04)	0.6739
PCBs (n = 838)					
PCB 105	0.99 (0.88, 1.11)	0.8594	1.02 (0.6, 1.75)	0.74 (0.43, 1.29)	0.9340
PCB 118	1 (0.9, 1.1)	0.9263	0.95 (0.56, 1.62)	0.79 (0.46, 1.36)	0.8521
PCB 123	1.03 (0.96, 1.1)	0.3950	1.8 (1.04, 3.11)	1.36 (0.77, 2.42)	0.0362
PCB 138	1 (0.93, 1.08)	0.9636	0.84 (0.49, 1.44)	0.67 (0.39, 1.15)	0.5177
PCB 153	0.99 (0.9, 1.09)	0.8977	1.02 (0.6, 1.74)	0.68 (0.39, 1.18)	0.9308
PCB 156	0.91 (0.72, 1.14)	0.4056	0.83 (0.48, 1.43)	0.65 (0.38, 1.12)	0.4959
PCB 157	1.01 (0.97, 1.05)	0.7058	0.72 (0.42, 1.24)	0.72 (0.41, 1.24)	0.2409
PCB 167	1.01 (0.96, 1.06)	0.6443	0.8 (0.47, 1.35)	0.79 (0.46, 1.35)	0.3962
PCB 170	0.99 (0.96, 1.02)	0.5857	0.84 (0.49, 1.44)	0.69 (0.41, 1.19)	0.5300
PCB 174	0.99 (0.89, 1.1)	0.8525	0.99 (0.59, 1.67)	0.72 (0.41, 1.26)	0.9653
PCB 178	0.98 (0.86, 1.12)	0.7667	0.95 (0.55, 1.64)	0.73 (0.42, 1.26)	0.8612
PCB 180	1.01 (0.96, 1.06)	0.6048	0.63 (0.36, 1.1)	0.71 (0.42, 1.18)	0.1011
PCB 187	1.01 (0.96, 1.07)	0.6637	0.96 (0.56, 1.66)	0.77 (0.45, 1.32)	0.8937
PCB 194	1.02 (0.95, 1.09)	0.6426	0.69 (0.39, 1.21)	0.81 (0.49, 1.34)	0.1961

Table S3.4 Hazard Ratio (95% CI) of incident diabetes associated with serum POP concentrations, 1999-2016. Model adjusted for age at baseline, race/ethnicity, site, education, financial strain, serum lipids, and joint stabilized inverse probability weights. Second and third tertiles of serum POP concentrations were analyzed relative to the first tertile.

Compound	Continuous (per IQR)	p	Tertile 2	Tertile 3	p for trend
OCPs (n = 1040)					
cis-chlordane	1.01 (0.99, 1.02)	0.4846	0.87 (0.53, 1.45)	0.9 (0.55, 1.48)	0.6053
HCB	1.02 (0.99, 1.05)	0.1370	1.66 (1, 2.75)	1.4 (0.82, 2.4)	0.0480
trans-chlordane	0.99 (0.97, 1.01)	0.1779	1.37 (0.82, 2.31)	1.28 (0.75, 2.2)	0.2286
trans-nonachlor	0.98 (0.94, 1.03)	0.4883	0.95 (0.58, 1.58)	1.04 (0.63, 1.72)	0.8541
PBDEs (n = 943)					
PBDE 47	1.01 (1, 1.02)	0.1265	0.93 (0.53, 1.63)	1.28 (0.74, 2.23)	0.8053
PCBs (n = 838)					
PCB 105	1 (0.91, 1.1)	0.9700	1.05 (0.61, 1.8)	0.79 (0.45, 1.38)	0.8619
PCB 118	1.01 (0.93, 1.09)	0.8337	0.98 (0.57, 1.67)	0.86 (0.49, 1.49)	0.9328
PCB 123	1.04 (0.97, 1.11)	0.2612	1.76 (1.01, 3.06)	1.4 (0.79, 2.49)	0.0451
PCB 138	1.01 (0.95, 1.08)	0.7584	0.86 (0.5, 1.47)	0.71 (0.41, 1.23)	0.5781
PCB 153	1 (0.93, 1.08)	0.9048	1.04 (0.61, 1.77)	0.72 (0.41, 1.27)	0.8924
PCB 156	0.92 (0.74, 1.15)	0.4867	0.86 (0.5, 1.49)	0.68 (0.39, 1.19)	0.5939
PCB 157	1.01 (0.98, 1.05)	0.4926	0.74 (0.43, 1.27)	0.76 (0.43, 1.33)	0.2714
PCB 167	1.02 (0.98, 1.06)	0.3926	0.81 (0.48, 1.37)	0.85 (0.49, 1.47)	0.4265
PCB 170	0.99 (0.97, 1.02)	0.5391	0.86 (0.5, 1.47)	0.73 (0.42, 1.26)	0.5843
PCB 174	1 (0.92, 1.09)	0.9640	1.01 (0.6, 1.72)	0.77 (0.43, 1.37)	0.9653
PCB 178	0.99 (0.89, 1.1)	0.8803	0.98 (0.57, 1.69)	0.78 (0.44, 1.36)	0.9351
PCB 180	1.02 (0.97, 1.06)	0.4343	0.64 (0.37, 1.13)	0.75 (0.45, 1.26)	0.1256
PCB 187	1.02 (0.97, 1.07)	0.4080	0.98 (0.57, 1.69)	0.83 (0.48, 1.44)	0.9402
PCB 194	1.02 (0.96, 1.09)	0.5104	0.71 (0.4, 1.25)	0.84 (0.5, 1.4)	0.2369

Table S3.5 Hazard Ratio (95% CI) of incident diabetes associated with serum POP concentrations, 1999-2016. Model adjusted for age at baseline, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, and fish/shellfish intake, waist circumference at baseline, serum lipids at baseline, and joint stabilized inverse probability weights. Second and third tertiles of serum POP concentrations were analyzed relative to the first tertile.

Compound	Continuous (per IQR)	p	Tertile 2	Tertile 3	p for trend
OCPs (n = 1040)					
cis-chlordane	1.01 (0.99, 1.02)	0.4506	0.84 (0.5, 1.43)	1 (0.62, 1.63)	0.9933
HCB	1.05 (1.01, 1.10)	0.0288	1.75 (1.02, 2.99)	1.6 (0.88, 2.92)	0.1132
trans-chlordane	0.98 (0.96, 1.01)	0.2335	1.52 (0.88, 2.6)	1.38 (0.8, 2.4)	0.2411
trans-nonachlor	0.99 (0.96, 1.03)	0.6860	0.98 (0.58, 1.67)	1.2 (0.69, 2.07)	0.5295
PBDEs (n = 943)					
PBDE 47	1.00 (0.98, 1.01)	0.4651	0.9 (0.51, 1.61)	1.13 (0.63, 2.03)	0.6709
PCBs (n = 838)					
PCB 105	1.03 (0.83, 1.27)	0.7794	0.86 (0.47, 1.55)	0.67 (0.36, 1.27)	0.2199
PCB 118	1.03 (0.84, 1.26)	0.7779	0.78 (0.44, 1.38)	0.73 (0.39, 1.37)	0.3207
PCB 123	1.11 (1.01, 1.22)	0.0327	1.92 (1.07, 3.44)	1.41 (0.75, 2.66)	0.2838
PCB 138	1.03 (0.87, 1.23)	0.7032	0.77 (0.45, 1.33)	0.50 (0.26, 0.97)	0.0372
PCB 153	1.05 (0.93, 1.19)	0.3991	0.85 (0.49, 1.49)	0.62 (0.32, 1.18)	0.1417
PCB 156	0.86 (0.61, 1.22)	0.4021	0.97 (0.57, 1.64)	0.56 (0.30, 1.07)	0.0736
PCB 157	1.06 (1.00, 1.12)	0.0398	0.60 (0.34, 1.07)	0.66 (0.34, 1.26)	0.1833
PCB 167	1.06 (0.99, 1.15)	0.0955	0.67 (0.38, 1.16)	0.75 (0.40, 1.40)	0.3313
PCB 170	1.00 (0.99, 1.01)	0.8557	0.86 (0.49, 1.51)	0.84 (0.47, 1.50)	0.5419
PCB 174	1.04 (0.87, 1.23)	0.6981	0.86 (0.50, 1.50)	0.65 (0.34, 1.25)	0.1948
PCB 178	1.06 (0.94, 1.19)	0.3460	0.90 (0.50, 1.59)	0.76 (0.42, 1.38)	0.3742
PCB 180	1.08 (1.02, 1.15)	0.0063	0.64 (0.36, 1.16)	0.89 (0.51, 1.56)	0.6281
PCB 187	1.07 (1.00, 1.16)	0.0653	0.80 (0.45, 1.42)	0.75 (0.41, 1.36)	0.3390
PCB 194	1.13 (1.04, 1.22)	0.0028	0.99 (0.54, 1.81)	1.45 (0.78, 2.68)	0.2757

Table S3.6. **Hazard Ratio (95% CI) of incident diabetes associated with DDT metabolites, 1999-2016**. Model adjusted for age at baseline, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, and fish/shellfish intake, serum lipids and waist circumference at baseline, and joint stabilized inverse probability weights. Moderate and high exposure quantiles were analyzed relative to non-detect. (n = 1040)

Metabolite	Moderate Exposure	High Exposure	p for trend
p,p'-DDD	0.88 (0.49, 1.58)	0.90 (0.52, 1.59)	0.661
p,p'-DDE	0.63 (0.33, 1.19)	1.33 (0.83, 2.12)	0.554
p,p'-DDT	1.14 (0.68, 1.92)	1.12 (0.67, 1.89)	0.592

Table S3.7. Hazard Ratio (95% CI) of incident diabetes associated with the detection of DDT metabolites, 1999-2016. Model adjusted for age at baseline, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, and fish/shellfish intake, serum lipids at baseline, and joint stabilized inverse probability weights. (n = 1040)

Metabolite	HR (95% CI)	p
p,p'-DDD	0.95 (0.62, 1.44)	0.7964
p,p'-DDE	1.05 (0.69, 1.60)	0.8146
p,p'-DDT	1.14 (0.75, 1.73)	0.5403

Table S3.8. **Hazard Ratio (95% CI) of incident diabetes associated with the detection of DDT metabolites, 1999-2016**. Model adjusted for age at baseline, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, and fish/shellfish intake, serum lipids and waist circumference at baseline, and joint stabilized inverse probability weights. (n = 1040)

Metabolite	HR (95% CI)	p
p,p'-DDD	0.89 (0.57, 1.39)	0.6137
p,p'-DDE	0.93 (0.60, 1.44)	0.7360
p,p'-DDT	1.13 (0.75, 1.72)	0.5531

Figure S3.5. Distributions of serum POP concentrations in three K-means clusters in SWAN MPS (n = 809)

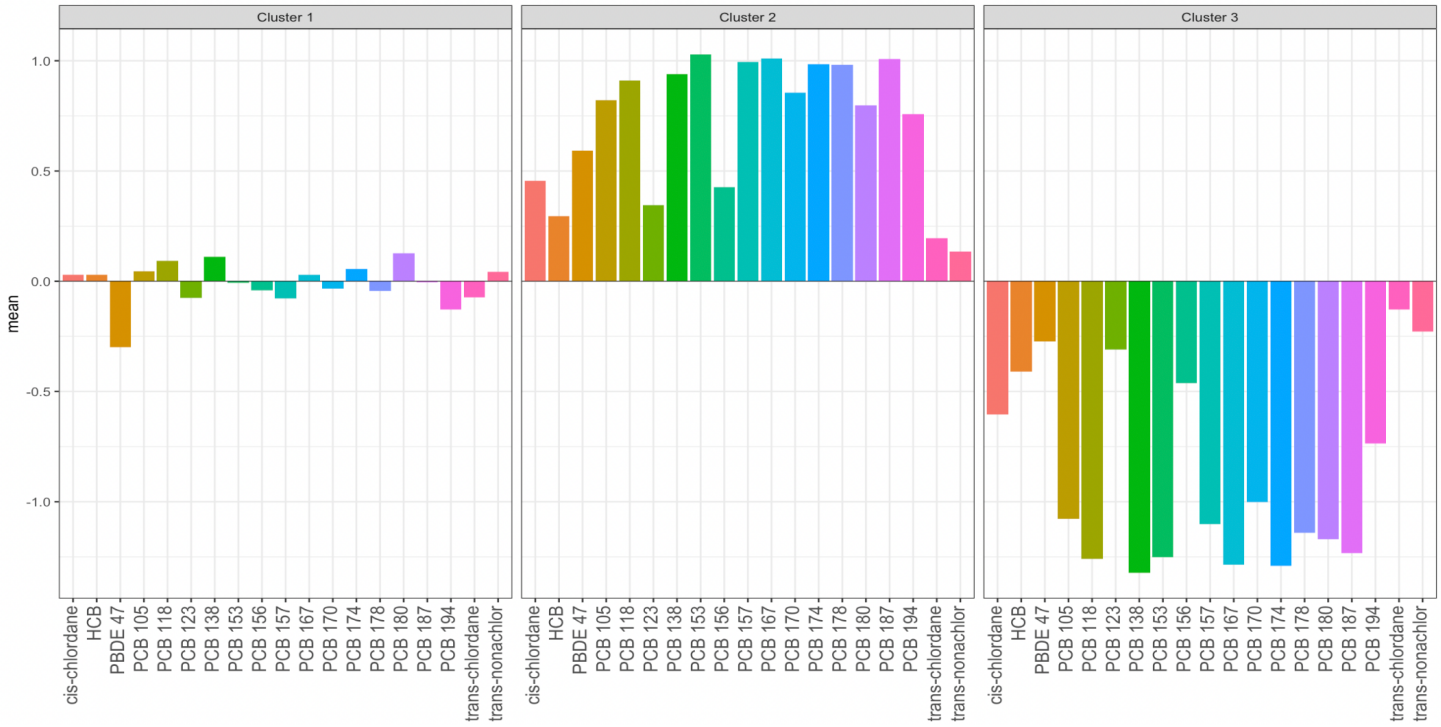


Figure S3.6. Distributions of serum POP concentrations in two K-means clusters in SWAN MPS (n=809)

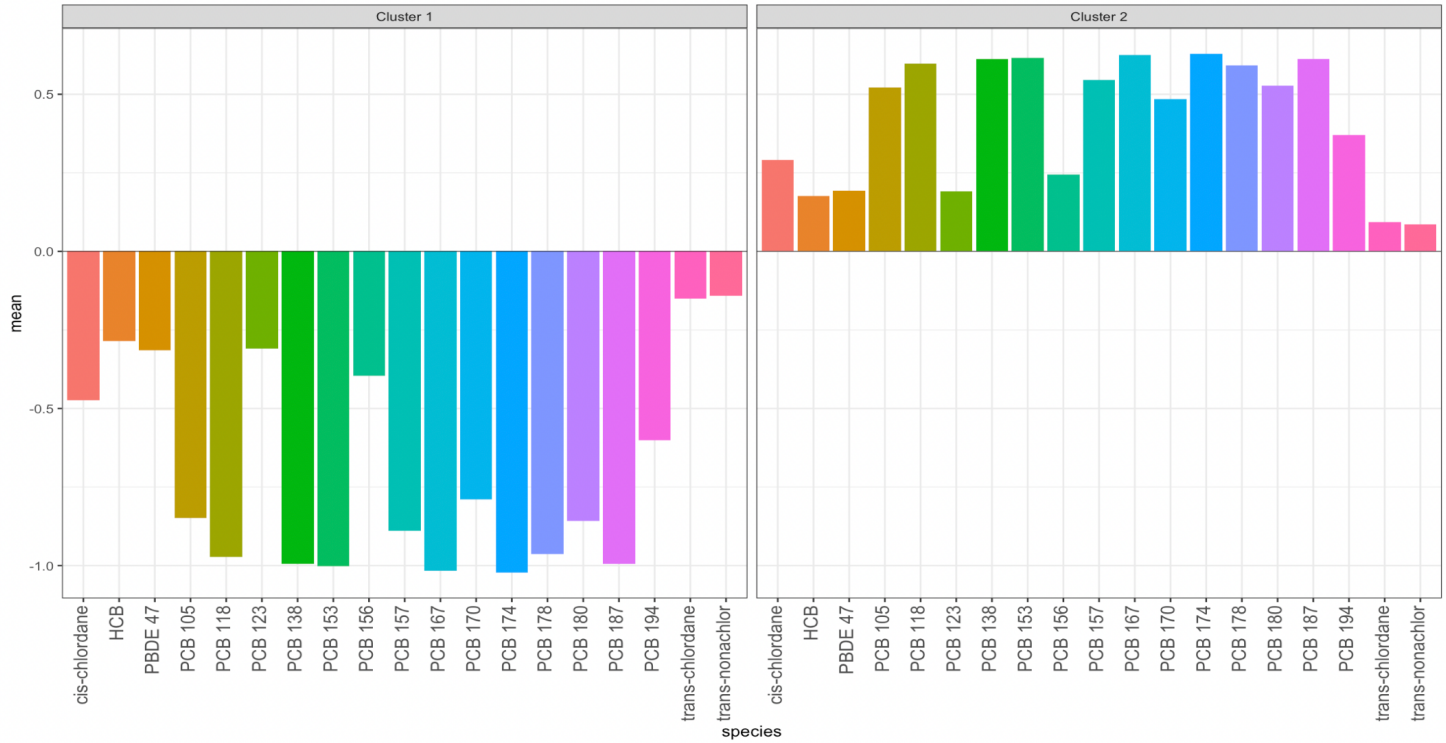


Table S3.9. Hazard Ratio (95% CI) of incident diabetes associated with serum POP mixture characterized using two and three K-means clusters, 1999-2016. Model adjusted for age at baseline, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, and fish/shellfish intake, serum lipids at baseline, and joint stabilized inverse probability weights.

No. of clusters	Exposure Cluster (reference = below average)	HR (95% CI)	p
2	All Above Average	0.75 (0.47, 1.21)	0.2416
3	All Average except PBDE 47 below average	0.75 (0.42, 1.31)	0.3054
	All Above Average	0.71 (0.38, 1.31)	0.2768

Table S3.10. Hazard Ratio (95% CI) of incident diabetes associated with serum POP mixture characterized using two and three K-means clusters, 1999-2016. Model adjusted for age at baseline, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, and fish/shellfish intake, serum lipids and waist circumference at baseline, and joint stabilized inverse probability weights.

No. of clusters	Exposure Cluster (reference = below average)	HR (95% CI)	p
2	All Above Average	0.62 (0.37, 1.02)	0.0600
3	All Average except PBDE 47 below average	0.64 (0.36, 1.13)	0.1223
	All Above Average	0.61 (0.32, 1.16)	0.1320

Figure S3.7. Individual Contributions of POP Mixture Components to Overall Joint Effect on Diabetes Risk in SWAN MPS (n = 809). Model adjusted for age at baseline, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, and fish/shellfish intake, serum lipids at baseline, and joint stabilized inverse probability weights.

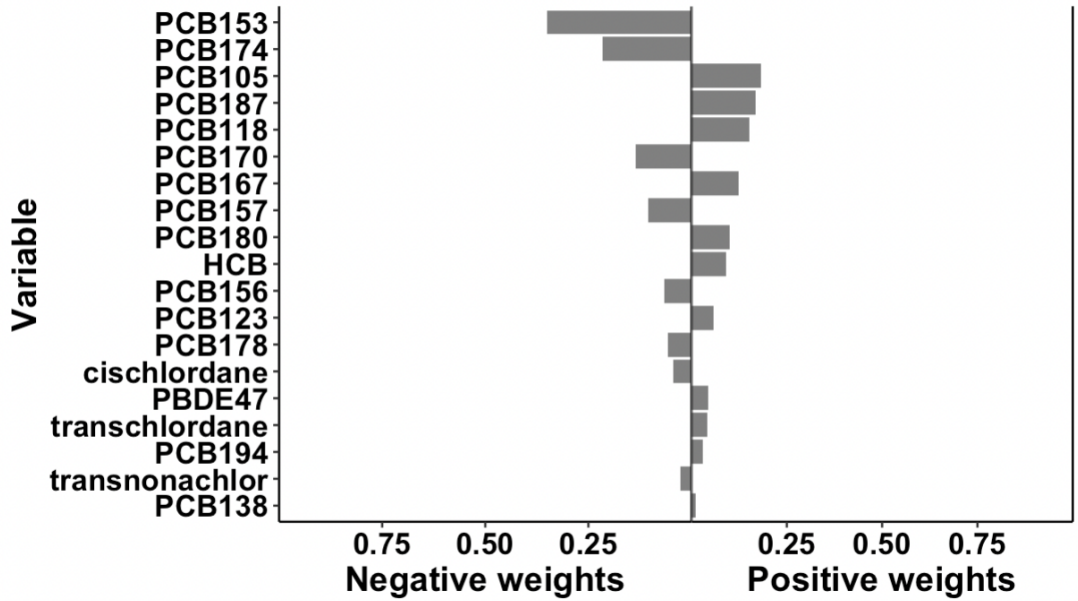
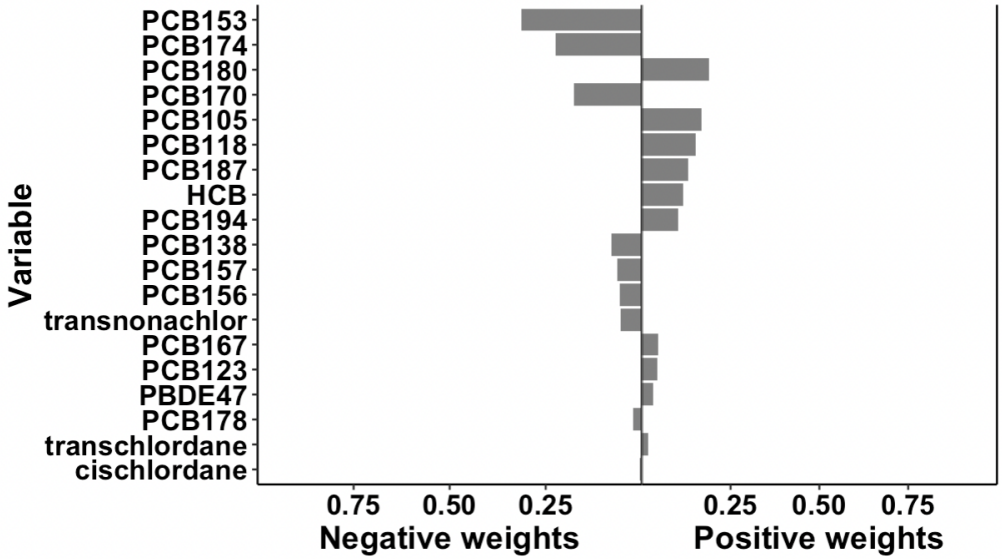


Figure S3.8. Individual Contributions of POP Mixture Components to Overall Joint Effect on Diabetes Risk in SWAN MPS (n = 809). Model adjusted for age at baseline, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, and fish/shellfish intake, serum lipids and waist circumference at baseline, and joint stabilized inverse probability weights.



Chapter IV. Adipokines and Mixtures of Organochlorine Pesticides, Polybrominated Diphenyl Ethers, and Polychlorinated Biphenyls in The Study of Women's Health Across the Nation

Amelia Grant-Alfieri[a], Deborah J. Watkins[a], William H. Herman[b,c], Stuart Batterman[a], Carrie Karvonen-Gutierrez[b], Sung Kyun Park[a,b]

Abstract

Background: Organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), and polychlorinated biphenyls (PCBs) are persistent organic pollutants (POPs) that are sequestered in adipose tissue. Adipose dysfunction, more specifically the disruption of hormones called adipokines, can lead to cardiometabolic diseases. Little is known about the relationship between POPs, especially as mixtures, and adipokines.

Objectives: We investigated the relationship between POPs and adipokines in midlife women.

Methods: We measured lipid-standardized serum concentrations of 14 PCBs, 4 OCPs, and 1 PBDE in 1,400 midlife women aged 45-56 years from the Study of Women's Health Across the

[a] Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, Michigan, USA
[b] Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan, USA
[c] Department of Internal Medicine, Division of Metabolism, Endocrinology, and Diabetes, University of Michigan Medical School, Ann Arbor, Michigan, USA

Nation in 1999/2000. Multivariate linear regression models tested the association between serum concentrations of individual POPs and serum levels of leptin and high molecular weight (HMW) adiponectin in 2002/2003. Mixtures methods – K-means Clustering and Bayesian Kernel Machine Regression (BKMR) – also were employed.

Results: After adjustment for age, site, race/ethnicity, education, financial strain, alcohol consumption, smoking, parity, exercise, menopausal status, and serum lipids, no significant associations were observed for the overall joint effect of the POP mixture on leptin and HMW adiponectin using BKMR. Single pollutant analyses indicated significant associations for some PCBs. A one-interquartile range (IQR) higher PCB 194 concentration was associated with 9.0% lower (95% CI: -13.2%, -4.7%) leptin and 4.1% higher (0.35%, 7.9%) HMW adiponectin. A one-IQR higher PCB 180 concentration was associated with 5.2% lower (-9.6%, -0.6%) leptin. K-means Cluster analysis did not identify an association between POPs and HMW adiponectin yet found a positive, non-linear association with leptin.

Conclusion: Exposure to the overall POP mixture was not associated with leptin or HMW adiponectin. Null associations determined using BKMR may be explained by individual POPs exerting effects in different directions. Mixture analysis is valuable to contextualize single pollutant findings. More research is needed on the effects of POPs on inflammatory, endocrine, and metabolic functioning of adipose tissue.

Introduction

Adipose tissue is an endocrine organ vital to regulate energy expenditure, appetite, glucose homeostasis, insulin sensitivity, and inflammation.[1] Adipocytes release peptide hormones known as adipokines.[1] Two main adipokines, leptin and adiponectin, work together to maintain metabolic homeostasis.[1] Unlike other endocrine factors released by adipocytes, the secretion of leptin and adiponectin is specific to adipose tissues although their effects can be exerted on multiple organs.[1] They are primarily produced and released by white adipose tissue, the main site of energy storage in the body.[1] High molecular weight (HMW) adiponectin is the most biologically active form of adiponectin and, compared to total adiponectin, is a more useful marker in predicting cardiometabolic disease.[2–4] Leptin is pro-inflammatory whereas adiponectin is anti-inflammatory.[5] Elevated leptin and lower adiponectin have been associated with cardiometabolic disorders including T2D.[6–11] As adipose tissue increases, leptin is upregulated and adiponectin is downregulated. However, obesity does not always underly the harmful metabolic effects of adipokines.[12,13] Maintaining a balance of adipokines is key; the absence or very low levels of leptin also is associated with thyroid and immune dysfunction, obesity, and insulin resistance.[14,15]

Increasingly, environmental chemicals have been investigated as risk factors of cardiometabolic disease. Persistent organic pollutants (POPs) are examples of harmful environmental chemicals and include polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs). PCBs are industrial chemicals banned, along with many OCPs, by many high-income countries, including the U.S., in the 1970s and 1980s due to health risks at high doses.[16] PBDEs function as flame retardants that have been used in electronics, plastics, fabrics, and upholstery foam.[17] The U.S. banned the most

common classes of PBDEs in 2013. In some countries, some POPs, notably PBDEs, continued to be produced and released at least through the 2010s.[18] POPs are lipophilic and, thus, highly persistent. Humans are primarily exposed via diet, primarily fatty animal-based foods.[19] Indoor air and dust are additional sources of PCBs and PBDEs.[20,21] Due to their lipophilicity, POPs are heavily sequestered in adipose tissue,[22,23] where they may have the potential to impair adipocyte functioning. Although external exposure to PCBs, OCPs, and PBDEs has greatly diminished over the past few decades, aging and the menopausal transition can prompt the loss or gain of adipose tissue that may lead to the release POPs from adipose tissue into serum or the sequestration of POPs from serum into adipose tissue, respectively.[24–27]

Epidemiological evidence on the association between POPs and adipokines is limited. All prospective studies and most cross-sectional studies reflect patients with obesity or pregnant women and children in birth cohorts.[28–34] Overall, the epidemiological evidence is inconsistent with many studies suggesting null or inverse associations between POPs and both adiponectin and leptin. Research focused on women is needed considering that leptin levels are higher in women than in men, independent of adiposity.[35] In addition, women and girls may be more susceptible to unfavorable changes in adiponectin.[30,32,36] Sex-specific effects may be due to the androgen antagonistic behavior of non-dioxin-like endocrine disrupting chemicals.[36] To our knowledge, no study of adipokines has explored the effect of POP mixtures. We examined the associations of POPs – as individual pollutants, exposure clusters, and the overall mixture – with levels of HMW adiponectin and leptin among U.S. midlife women in the Study of Women's Health Across the Nation (SWAN). SWAN is one of the most carefully phenotyped prospective studies of U.S. women with respect to the menopausal transition and numerous heath markers.

Methods

Study Population

The SWAN Multi-Pollutant Study (MPS) is part of the larger multi-site, multi-ethnic SWAN cohort detailed previously.[37] Beginning in 1996/1997, the study followed 3,302 premenopausal women between the ages of 42 and 52 through the menopausal transition.[37] Women were recruited from seven sites across the U.S.: Boston, Massachusetts (MA), Los Angeles, California (CA), Oakland, CA, Pittsburgh, Pennsylvania (PA), Southeast Michigan (MI), Newark, New Jersey, and Chicago, Illinois. SWAN continues to collect data on metabolic and reproductive biomarkers and health outcomes, in addition to socio-demographic, lifestyle, and other risk factors. The institutional review board at each site approved the study protocol, and all participants provided written, signed, informed consent at each visit.

The MPS investigated environmental exposures in a subset of SWAN (see Figure S4.1). Environmental exposure data was collected from biobanked specimens at the MPS baseline visit (1999/2000). Only participants with serum and urine samples were included for further analysis. The final MPS included 1,400 women from five of the seven study sites. Black and white participants were recruited from Southeast MI, Boston, and Pittsburgh; Japanese and white participants from Los Angeles; Chinese and white participants from Oakland. Only participants with POPs data attaining surrogate recovery and other quality assurance criteria were included, which excluded 166, 405, and 281 participants with OCP, PCB, and PBDE measurements, respectively. We excluded participants for insufficient serum lipids (cholesterol) information: two from the PCB subgroup, two from the PBDE subgroup, and one from the OCP subgroup. Lastly, participants lacking covariate information were excluded. The final included 1,116 women in the OCP subgroup, 900 women in the PCB subgroup, and 1,011 women in the PBDE

subgroup. Mixtures analyses were conducted in a slightly reduced sample of 872 women who had complete observations for PCBs, OCPs, *and* PBDEs.

POP Measurements

Concentrations of PBDEs, PCBs, and OCPs were measured using serum collected from 1,400 women in the SWAN MPS in 1999/2000. Serum was analyzed for 34 PCBs, 14 PBDEs, and 19 OCPs including DDT metabolites, chlordanes, and hexachlorobenzene (HCB) among others (Table S4.1). Laboratory procedures were detailed previously.[38] With the exceptions of p,p'-DDE, p,p'-DDT, and p,p'-DDD, compounds detected in at least 70% of samples were retained, and those below detection were replaced with MDL/$\sqrt{2}$. Although DDT metabolites p,p'-DDE, p,p'-DDT, and p,p'-DDD had detection frequencies of approximately 40%, their high persistence, demonstrated endocrine disruption and carcinogenicity, and continued global use in vector control warranted exclusive investigation.[39]

Lipid-standardized POP concentrations are preferred over wet weight concentrations because POPs are lipophilic, circulate bound to lipids, and distribute in organs according to tissue lipid content.[22,23] Standardizing serum POP concentrations by serum lipids is the most common method to handle serum POPs because it reduces measurement error and allows for the comparison of exposure across individuals. We measured serum lipids including total cholesterol and triglycerides using enzymatic methods with the Hitachi 747-200 analyzer (Boehringer Mannheim Diagnostics, Indianapolis IN). Missing observations of total cholesterol and triglycerides were handled by imputation of the total lipid concentration using linear regression.

$$S = C / OTL \times 102.6$$

$$OTL = (2.27 * TC) + TG + 62.3$$

where S is lipid-standardized serum POP concentration (ng/g lipid); C is wet weight serum POP concentration (ng/g); OTL is observed total lipid concentration (mg/dl);[40] TC is total cholesterol concentration (mg/dl); and TG is triglycerides concentration (mg/dl).

Adipokine Measurements

HMW adiponectin and leptin were assayed in duplicate at the University of Michigan using 12-hour fasted serum samples collected in 2002/03. The assays used commercially available colorimetric enzyme immunoassay kits (Millipore, St. Charles, Missouri). The mean coefficient of variation for duplicate samples was 8.1% for HMW adiponectin and 4.0% for leptin. The lower limit of detection was 0.5 ng/ml for both HMW adiponectin and leptin.

Covariates

Potential confounders of the relationship between serum POP concentrations and adipokines were selected a priori. Age can influence metabolism as well as exposure, especially historical exposure to now-regulated POPs. Race/ethnicity included Chinese, Japanese, Black, and white. Race was not a proxy for biological or genetic differences, rather it was included because it is ingrained in systems that shape people's environments and experiences. Differences in lifestyle, resources, and historical contamination may result from structural racism (institutional or systemic racism), "a system in which public policies, institutional practices, cultural representations, and other norms work in various, often reinforcing ways to perpetuate racial group inequity".[41] Information on study site, education, financial hardship, parity, smoking status, alcohol consumption, physical activity, menopausal status, and waist circumference have been described previously.[42] Change in WC (ΔWC) between 1999/2000 and 2002/03 was calculated and categorized as gain ($\Delta WC \geq 2$ cm), stable ($-2 \text{ cm} < \Delta WC < 2$ cm) or loss ($\Delta WC \leq -2$ cm).[43–45] Uniform protocols were used to collect covariate data across sites and visits.

Statistical Analysis

The distributions of 34 PCB congeners, 19 OCPs, and 14 PBDEs were characterized using the median and various quantiles. We reported the 25th and 75th percentiles because we analyzed the effects of POPs per interquartile range (IQR) increase. We reported the 33rd and 66th percentiles because they were cutoffs in defining tertiles of serum POPs. We reported concentrations using traditional lipid-standardization to facilitate comparison with previous studies. We performed T-tests and ANOVAs to analyze serum POP and adipokine concentrations by participant characteristics. We visualized Spearman correlations between serum concentrations of POPs.

Multivariable linear regression models were constructed to evaluate the association between lipid-standardized serum concentrations of POPs and serum concentrations of leptin and HMW adiponectin. First, we analyzed single pollutants. We treated POP concentrations as continuous, interpreting the change in adipokines per IQR increase in POPs. To explore non-linearity and non-monotonicity, we analyzed tertiles of POP exposure. For DDT metabolites, which had detection frequencies of approximately 40%, exposure was categorized as binary (detect vs. non-detect). A three-level exposure variable was created by dividing detectable concentrations at the median to create moderate and high groups relative to non-detection.

Next, we analyzed the effect of POP mixtures using K-means Clustering and Bayesian Kernel Machine Regression (BKMR). Strong positive correlations between POP concentrations dictated a mixtures approach (Figure S4.2). We used K-means Clustering to categorize overall POP exposure into k clusters, treating cluster membership as our exposure variable. POP concentrations were log-transformed prior to clustering. Elbow and silhouette methods were used to select the number of clusters (Figures S4.3 and S4.4). We chose three clusters for our primary analysis to allow for better comparison with single pollutant tertile analyses. We also performed

sensitivity analyses using two and four clusters. K-means can identify distinct homogenous subgroups and enables the classification of low, medium, and high exposure. Given our interest in the dose-response relationship between POPs and adipokines, K-means was better suited to our research question. We used the 'kmeans' function from the R 'stats' package.[46]

Finally, we used BKMR to quantify the overall mixture effect while accounting for co-pollutant confounding and interactions. Considering that our primary research question is the overall mixture effect, BKMR was the most appropriate method. BKMR can estimate overall effect, independent effect, two-way interaction between chemicals, and visualize dose-response relationships.[47] POP concentrations and continuous covariates were standardized by subtracting the mean and by dividing by the standard deviation. We report the overall effect as the change in the mean concentrations of HMW adiponectin and leptin when all POP mixture components are fixed at specific decile relative to when all components are fixed at their medians and when all covariates are held constant.[47] We report independent effects as the changes in the mean concentrations of HMW adiponectin and leptin when a single POP mixture component is fixed at its 90th percentile compared to its 10th percentile, while the remaining mixture components are fixed at the median and all covariates are held constant.[47] Finally, we report two-way interactive effects as the differences in adipokines associated with a single POP mixture component when other components are fixed at the 90th percentiles, compared to when all components are fixed at the 10th percentiles.[47] The R package 'bkmr' was used in this analysis.[48]

In single pollutant and mixture analyses, final models were adjusted for age, study site, race/ethnicity, education, financial strain, alcohol consumption, smoking status, parity, physical activity, menopausal status, and serum lipids, all of which were selected *a priori* (Model 3). First, we constructed crude models (Model 0). Next, in Model 1, we only controlled for

demographic and socioeconomic factors. In Model 2, we adjusted for baseline serum lipids in addition to calculating lipid-standardized serum POP concentrations to account for measurement error and to ensure that confounding is controlled and that indirect (backdoor) pathways connecting POPs and adipokines are blocked. This dual approach was recommended over traditional standardization alone.[49] We also performed sensitivity analyses for serum lipids and waist circumference in Models 2 and 4, respectively. The inclusion of waist circumference may have led to over-adjustment, therefore Model 3 was chosen as our main model. Change in WC between 1999/2000 and 2002/03 was explored as a potential effect modifier (Figure S4.5). Serum concentrations of POPs may be affected by changes in visceral adiposity.[24–27] Changes in Women with stable WC between 1999/2000 and 2002/03 may have greater biomarker validity of serum POP concentrations in relation to an outcome measured in 2002/03.

Model 0	=	*Unadjusted*
Model 1	=	*Demographic and socioeconomic variables: age, race/ethnicity, study site, education, and financial strain*
Model 2	=	*Model 1 + serum lipids*
Model 3 (final)	=	*Model 2 + behavioral and reproductive variables: alcohol consumption, smoking status, physical activity, parity, and menopausal status*
Model 4	=	*Model 3 + waist circumference*

Results

Participant Characteristics

Table 4.1 presents the characteristics of women at SWAN MPS baseline (or first available timepoint, in the case of sociodemographic characteristics) with complete observations of PCBs, PBDEs, and OCPs. The median age in 1999/2000 was 49 years (IQR: 47-51). Half of the participants were white. Most participants consumed little to no alcohol and never smoked. The

sample was well educated and of higher socioeconomic status, with half of participants attaining a college education at minimum and one-third experiencing moderate or high financial strain. At baseline, 63% of participants were pre-menopausal or in the early phase of peri-menopause. The median WC in 1999/2000 was 81 cm (IQR: 73-93). Between 1999/2000 and 2002/03, most participants experienced stable or increased WC.

Concentrations of serum POPs and adipokines are reported for the 872 women in the mixture subsample with complete observations of PCBs, PBDEs, and OCPs detected at a minimum frequency of 70% (Table S4.2). Median concentrations of HMW adiponectin and leptin were 5.58 ug/ml (IQR: 3.14, 9.59) and 19.30 ng/ml (IQR: 10.32, 33.50), respectively. As expected, median concentrations of leptin were higher among women who gained WC between 1999/2000 and 2002/03 compared to those with stable WC (Table S4.3). Concentrations of POPs with DFs ≥70% are reported in Table A.4. Overall, 14 PCBs, PBDE 47, HCB, cis-chlordane, trans-chlordane, and trans-nonachlor met DF criteria. Table S4.1 presents the DFs for all 67 POPs measured. PCB 118, 138, and 153 had the highest median concentrations of 255.4 ng/g lipid (IQR: 133.0, 398.5), 355.5 (IQR: 169.3, 616.2), and 212.0 (IQR: 118.9, 336.0), respectively. Concentrations were similar between the mixture and POP-specific subsamples (Tables S4.2 and S4.4). DDT metabolites are presented separately in Table S4.6 because they had a DF < 70%. Of the 19 POPs analyzed, 18 were positively correlated (Figure S4.2).

Table 4.1. Characteristics of SWAN MPS participants, 1999/2000 (n = 872).

Characteristic	Median (IQR) or Frequency (%)
Age (years)	49 (47, 51)
Study Site	
Boston	127 (15%)
Los Angeles	240 (28%)
Oakland	207 (24%)
Pittsburgh	148 (17%)
SE Michigan	150 (17%)
Race/Ethnicity	
Black	168 (19%)
White	448 (51%)
Chinese	116 (13%)
Japanese	140 (16%)
Educational Attainment	
high school or less	156 (18%)
beyond high school	274 (31%)
college	205 (24%)
beyond college	237 (27%)
Parous	696 (80%)
Physical Activity Score (3 to 15), not including occupation	7.85 (6.70, 8.90)
Menopause Status	
late peri-menopause or surgical or natural post-menopause	182 (21%)
pre-menopause or early peri-menopause	549 (63%)
unknown	141 (16%)
Total Serum Lipids (mg/dl)	614.46 (544.81, 709.04)
Waist Circumference (cm)	81.30 (73.30, 93.32)
Change in Waist Circumference 1999/2000 - 2002/03 [a]	
gain	383 (44%)
stable	314 (36%)
loss	175 (20%)
Alcohol Consumption	
>7 Drinks per Week	55 (6.3%)
1-7 Drinks per Week	144 (17%)
<1 Drink per Week	211 (24%)
None	462 (53%)
Smoking Status	
Current	91 (10%)
Never	547 (63%)
Past Only	234 (27%)
Financial Strain	
Not Hard	608 (70%)
Somewhat or Very Hard	264 (30%)

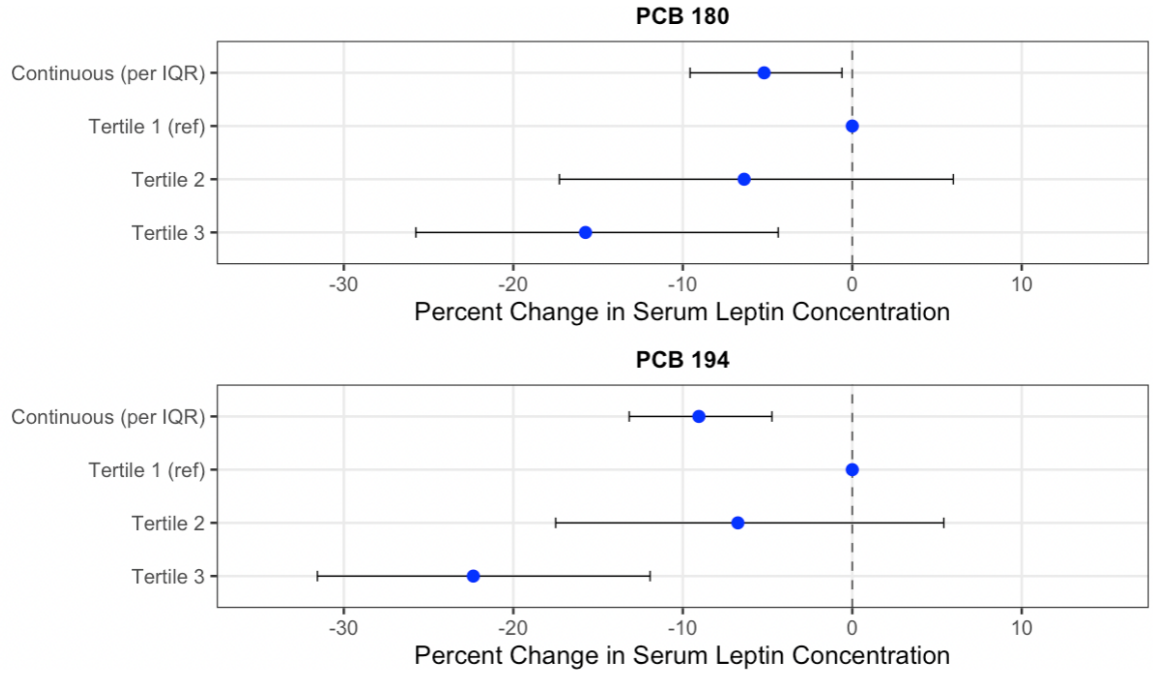
IQR = interquartile range. [a] Waist Circumference (WC) Gain = $\Delta WC \geq 2$ cm; Stable = $-2 \text{ cm} < \Delta WC < 2\text{cm}$; Loss = $\Delta WC \leq -2$cm. Menopausal status unknown due to hormone therapy or hysterectomy.

Individual POPs and Adipokines

After controlling for covariates including serum lipids (Model 3), for most individual POPs, associations with leptin and HMW adiponectin failed to reach statistical significance (Tables S4.6 and S4.7). Exceptions included inverse linear associations between PCB 170, 180, and 194 and leptin and a linear positive association between PCB 194 and HMW adiponectin. Increasing PCB 194 from the 25th to 75th percentile was associated with a 4.1% increase (95% CI: 0.35%, 7.9%) in HMW adiponectin. Tertile analysis indicated a similar linear association (2nd: 5.3% [-4.4%, 15.9%]; 3rd: 10.0% [-0.34%, 21.5%]; p for trend = 0.059). At the highest exposure tertile relative to the lowest, PCB 170 was associated with a 13.0% decrease in leptin (95% CI: -23.3%, -1.3%; p for trend = 0.032), PCB 180 with a 15.7% decrease in leptin (95% CI: -25.7%, -4.4%; p for trend = 0.008), and PCB 194 with a 22.4% decrease in leptin (95% CI: -31.6%, -11.9%; p for trend < 0.001). Continuous and tertile analysis of POPs and leptin using Model 3 produced similar conclusions (Figure 4.1).

The results for crude models are presented in Tables S4.8 and S4.9 for leptin and HMW adiponectin, respectively. Adjustment for demographic and socioeconomic covariates (Model 1) strengthened positive associations between leptin and OCPs and inverse associations with PBDE and PCBs (Table S4.10); in contrast, for HMW adiponectin, OCP and PBDE estimates remained similar while positive estimates for PCB were strengthened (Table S4.11). Adjustment for baseline lipids (Model 2) attenuated estimates of both leptin and HMW adiponectin (Tables S4.12 and S4.13). Adjustment for lifestyle and reproductive factors (Model 3) did not alter most associations between POPs and both leptin and HMW (Tables S4.6 and S4.7). Adjustment for baseline WC (Model 4) attenuated the association between POPs and leptin (Table S4.14) whereas, the effect of adjustment on HMW adiponectin was inconsistent (Table S4.15).

Figure 4.1. Percent change (95% CI) in serum concentrations of leptin associated with serum concentrations of select POPs in SWAN MPS. Model was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and serum lipids.



By stratifying the analysis by gain or maintenance of WC between 1999/2000 and 2002/03, we observed that high exposure to POPs was associated with lower leptin among women with stable WC with two exceptions. PCB 194 was associated with lower leptin regardless of WC change. Findings for PCB 105 suggested an association with higher leptin among women who gained WC (2nd: 20.8% [95% CI: 1.3%, 44.1%]; 3rd: 18.3% [-0.9%, 41.4%]; p for trend = 0.063) and lower leptin among women with stable WC (2nd: -4.8% [95% CI: -22.9%, 17.4%]; 3rd: -13.1% [-30.4%, 8.5%]; p for trend = 0.21) (Table S4.16). Figure 4.2 illustrates stronger inverse associations for HCB and some highly-chlorinated PCBs in the stable WC group relative to WC gain group. Among women with stable WC, high exposure to PBDE 47, trans-nonachlor, and trans-chlordane were associated with 18.6% (95% CI: -30.5%, -4.8%; p for trend = 0.01), 16.5% (-28.1%, -3.1%; p for trend = 0.015), and 14.0% (-26.1%, 0.11%; p for

trend = 0.053) reductions in HMW adiponectin (Table S4.17) Further adjustment for WC at baseline attenuated PCB estimates for both leptin and HMW adiponectin yet did not alter notably the estimates for OCPs or PBDE 47 (Tables S4.18 and S4.19).

POP Mixtures and Adipokines

Above-average, average, and below-average exposure clusters were created using K-means Clustering (Figure S4.6). Clusters reflect POP correlations; PCBs had strong positive correlations with each other, with the exceptions of PCB 123 and 156, whereas OCPs and PBDE 47 had weakly or moderately positive correlations with other POPs (Figure S4.2). After adjustment for confounders, average exposure to the POP mixture was positively associated with leptin, compared to below-average exposure, suggesting non-linearity. This overall positive association appeared to be driven by women who gained WC (Figure S4.7). Exposure to K-means clusters of POPs was not associated with HMW Adiponectin (Figure S4.8).

Limiting our interpretation to women to those with stable WC, we found no association between POPs and leptin. Two-cluster sensitivity analyses found that above-average exposure to the POP mixture, relative to below-average exposure, was not associated with adipokines (Figure S4.9; Table S4.20). Sensitivity analyses using four clusters indicated a positive association between leptin and average exposure, compared to below-average exposure (Figure S4.10; Table S4.21). All associations using two-, three-, and four-clusters were attenuated and no longer significant after further adjustment for WC at baseline (Tables S4.20, S4.21, S4.22). In addition, regardless of adjustment for WC, HMW adiponectin was not associated with POP exposure characterized using two-, three-, or four-clusters (Tables S4.20, S4.21, S4.22).

Using BKMR, we observed that cumulative exposure to the POP mixture was not associated with leptin or HMW adiponectin, after adjustment for confounders. The mean

concentrations of HMW adiponectin and leptin did not change when POP mixture components were fixed at various deciles and compared to when mixture components were fixed at their medians, holding covariates constant (Figures 4.3 and 4.4). Upon examining the contributions of each mixture component, it appeared that higher concentrations of PCB 194 was marginally associated with lower log-leptin and higher PCB 118 significantly associated with lower log-HMW adiponectin when other POPs were fixed to their medians and covariates were held constant (Figures S4.11 and S4.12).

DDT Metabolites and Adipokines

Exposure to p,p'-DDE, p,p'-DDD, and p,p'-DDT were not associated with leptin or HMW adiponectin. However, findings suggest positive effects; the detection of p,p-DDE was associated with a 7.3% (95% CI: -2.2%, 17.7%) higher in leptin and a 5.1% (-2.2%, 13.0%) higher in HMW adiponectin (Figures S4.13 and S4.14). Positive trends remained following further adjustment for baseline WC. Table S4.23 presents findings from binary analysis. Similarly, high and moderate exposure to p,p'-DDE, relative to non-detection, reinforced a potential positive association with leptin and HMW adiponectin, although suggest a non-linear dose-response relationship (Table S4.24). For p,p'-DDE, among women with stable WC between 1999/2000 and 2002/03, the association with leptin was weaker and association with HMW adiponectin was stronger compared to women who gained WC, although these differences were not statistically significant (Figures S4.13 and S4.14).

Figure 4.2. Percent change (95% CI) in serum concentrations of leptin associated with a one interquartile range increase in serum concentrations of select POPs by waist circumference change from 1999/2000 to 2002/2003 in SWAN MPS. Model was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and serum lipids.

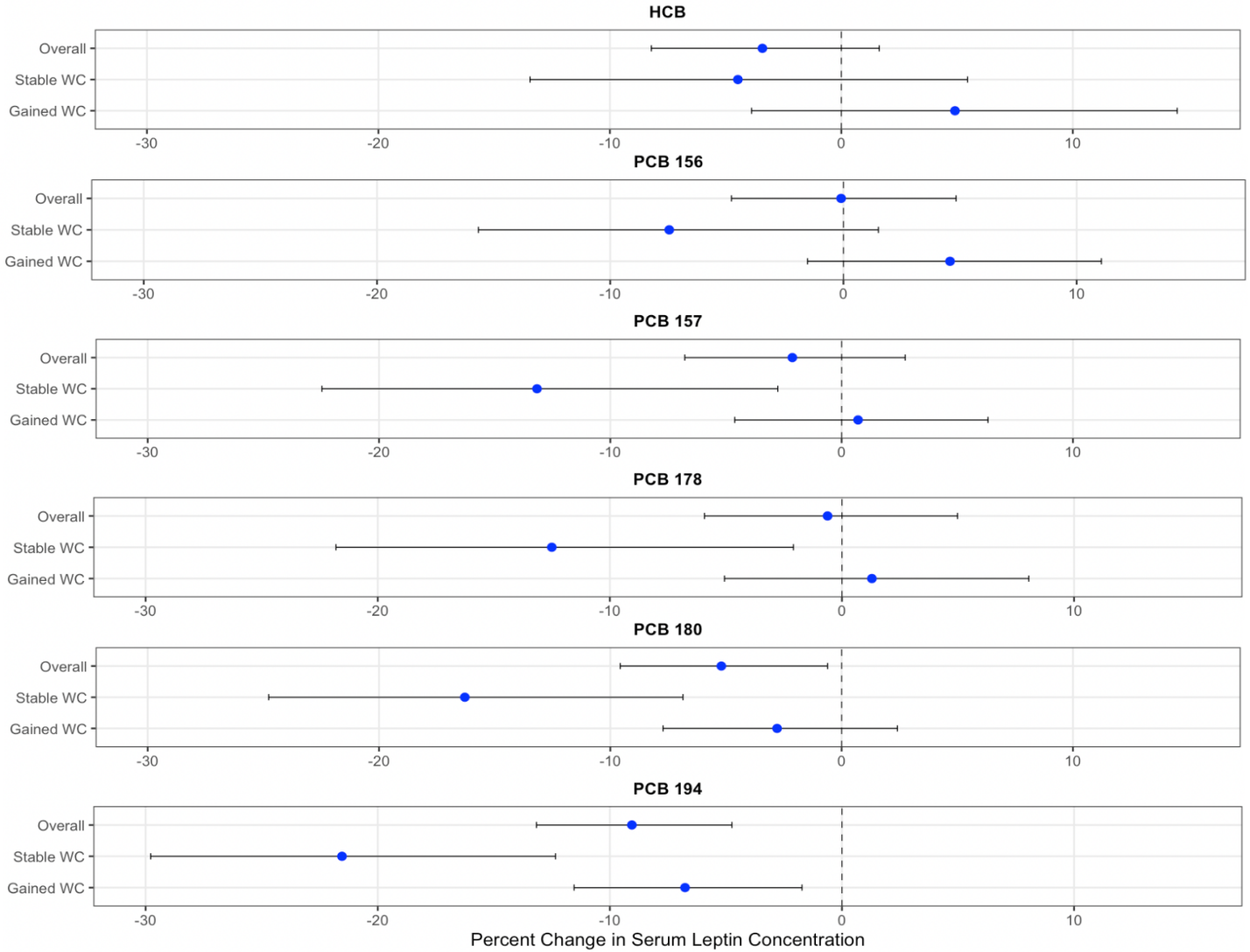


Figure 4.3. Overall joint effect of exposure to the POP mixture on serum concentrations of leptin in SWAN MPS. Model was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and serum lipids.

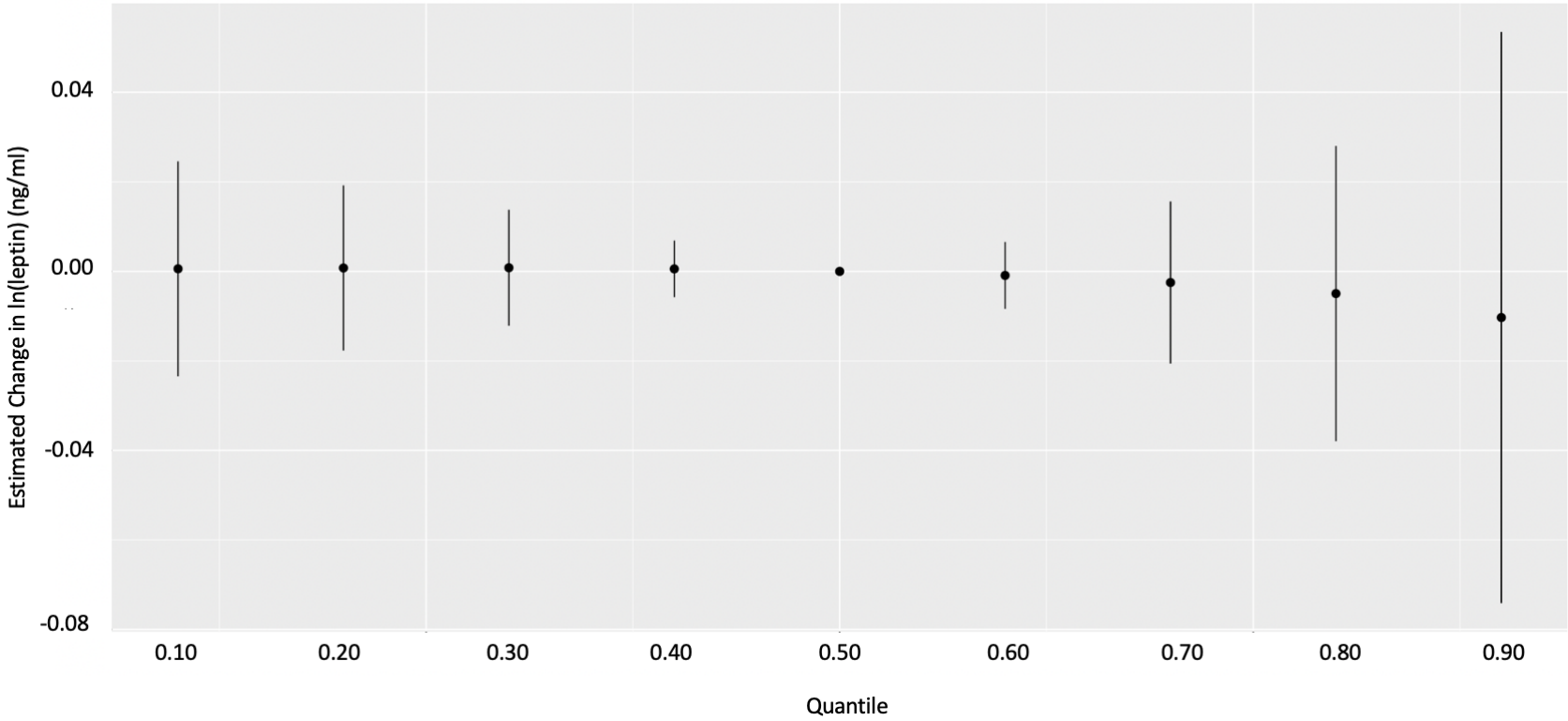
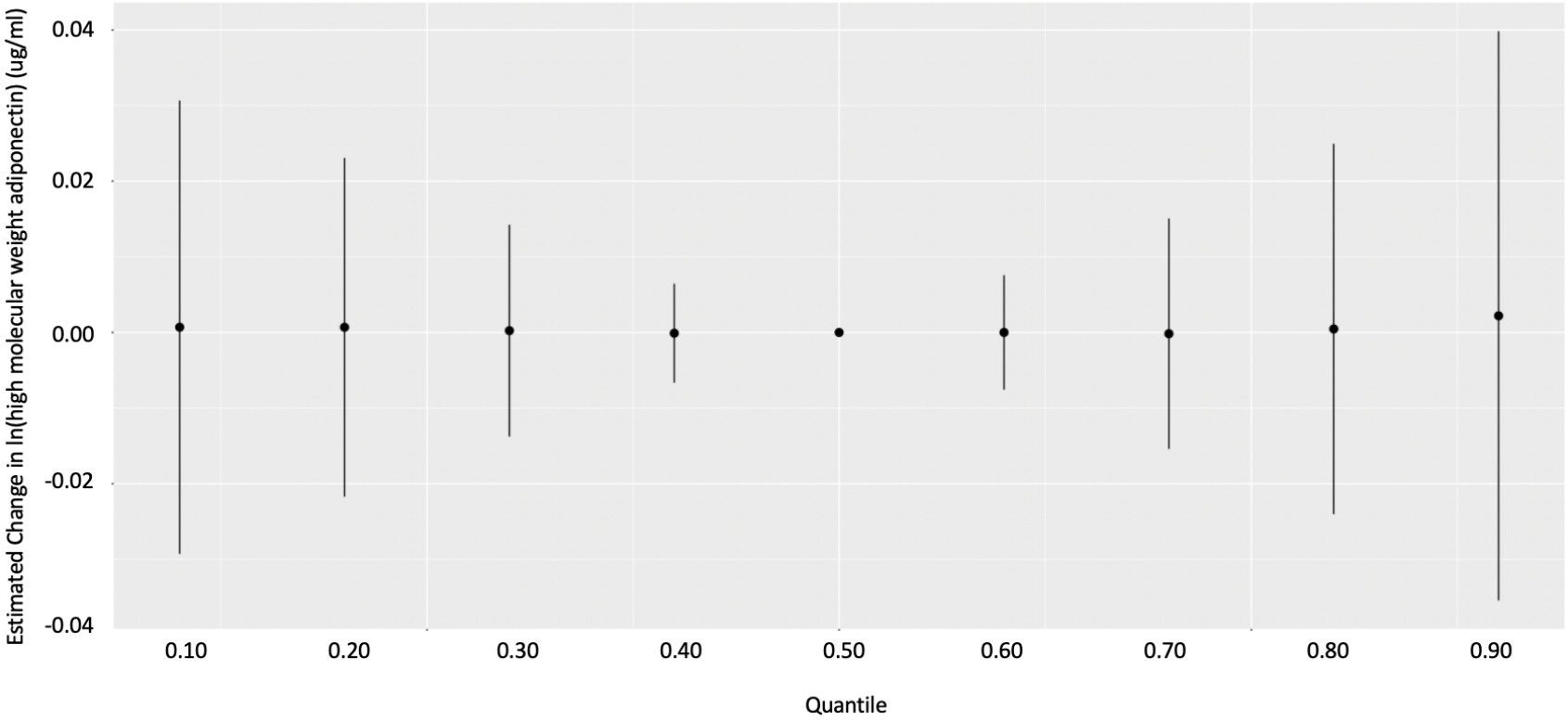


Figure 4.4. Overall joint effect of exposure to the POP mixture on serum concentrations of high molecular weight adiponectin in SWAN MPS. Model was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and serum lipids.



Discussion

POPs and Adipokines

To our knowledge, this study is the first to investigate the overall effect of POP mixtures on adipokines. Our evidence suggests that leptin and HMW adiponectin are not associated with exposure to the overall POP mixture. When analyzed individually, most POPs were not associated with adipokines. Exceptions include inverse relationships between leptin and highly-chlorinated PCB 170, 180, and 194. PCB 194 was also positively associated with HMW adiponectin. When considered jointly as a mixture, individual POPs exerting effects in different directions may account for the overall null effect we detected.

Leptin was not associated with most PCBs, PBDE 47, and OCPs, including DDT metabolites. Serum concentrations in the highest tertile of PCB 170, 180, and 194 were associated with a 13.0%, 15.7%, and 22.4% lower levels of leptin, relative to the lowest tertile. These findings are somewhat consistent with the literature. Summary exposure to PCBs including PCB 156, 180, and 194 has been inversely associated with leptin.[50] Yet, other studies identified no associations between leptin and PCBs,[29,31] HCB,[31,51] or PBDEs.[29] For DDE, the literature for leptin suggests null[31,52] or inverse[51] associations. To our knowledge, observational studies do not support a positive association between PCBs and leptin. While the epidemiological evidence is inconsistent, in vivo and in vitro studies have demonstrated a positive and consistent association between POPs and leptin.[53–61] For this reason, as well as evidence for the positive association between POPs and adiposity, our single pollutant findings were somewhat unexpected. Our findings, however, must be interpreted with caution given that we did not account for multiple comparisons.

HMW adiponectin was not associated with PBDE 47, OCPs, including DDT metabolites, and all but one PCB congener. A one-IQR increase in PCB 194 was associated with a 4% increase in HMW Adiponectin. Except for one study in newborn girls, null associations were observed between DDE and adiponectin.[28,30,52] PCB 138 and 153 were associated with lower adiponectin among women and people with obesity.[32,36] PBDEs including PBDE 47 were marginally associated with higher adiponectin.[52] Several studies have reported null associations between adiponectin and PCBs[30,50,52] or HCB.[30] Overall, the literature on HMW adiponectin is inconsistent. Furthermore, adiponectin may have less robust results, compared to leptin, because visceral adiposity may be more relevant to circulating leptin levels while subcutaneous adiposity may be especially relevant for serum adiponectin.[1] This study focused on visceral adiposity because of the preferential accumulation of POPs in visceral adipose tissue.[25] Furthermore, access to measures of subcutaneous adiposity was limited.

Although the evidence on adipokines and POPs is inconsistent, the mechanisms linking POPs and leptin may differ from those related to HMW adiponectin or that mechanisms are more compound-specific. From Model 2, we see that demographic and socioeconomic factors do not explain the positive associations between leptin and OCPs, the inverse associations between leptin and PCBs and PBDE-47, nor the positive association between HMW adiponectin and PCBs. We observed that serum lipids explained in part the inverse association between POPs and leptin as well as the positive association between POPs and HMW. In other words, serum lipids may be involved in one or more biological pathways that lower leptin and raise adiponectin. Furthermore, we found that the negative association between POPs and leptin was partly attributed to WC, yet the role of WC in pathways related to HMW is less consistent and differs by compound. This suggests that visceral adiposity may be involved in a mechanism that

lowers leptin and may be less or differently involved in mechanisms related to adiponectin. This may be possible, considering evidence that gene expression of leptin and adiponectin were higher in subcutaneous adipose tissue (SAT) compared to visceral adipose tissue (VAT) and that the expression of Peroxisome proliferator-activated receptor gamma (PPAR-γ) was higher in SAT than VAT.[34] PPAR-γ is a nuclear receptor involved in metabolic homeostasis, and PPAR-γ antagonists have been associated with metabolic disease.[62] Endocrine disrupting chemicals can bind to nuclear receptors and alter adipocyte function.[63] The differential expression of PPAR-γ, leptin, and adiponectin in different adipose tissue depots and our sole consideration of visceral adiposity may have contributed to our somewhat unexpected results. Moreover, differential affinities of POP compounds for aryl hydrocarbon receptor (AHR) may have contributed to differences in effects on adipose tissue function and adipokine expression by POP type or congener.[64]

The role of adiposity and adiposity change in the relationship between POPs and adipokines are crucial to consider.[32,36] As previously described, we found that adjustment for baseline WC attenuated negative associations for leptin. This may have occurred because POPs are stored in adipose tissue and visceral adiposity closely correlates with leptin. Limiting our analysis to women with stable WC -- to improve the temporal validity of exposure biomarkers -- reinforced the inverse association between highly-chlorinated PCBs and leptin. In contrast, K-means clustering modeling showed that the positive association between leptin and above-average exposure to POPs was more robust among women who gained WC. This could be explained by an increase in the sequestration of POPs in adipocytes, which may more directly impact adipokine levels and which was not reflected by serum POPs measurements (Figure S4.5). Prior studies of POPs and adipokines did not investigate adiposity trajectories.[28–33,36,50–

[52,65] In one study, the loss of adipose tissue post-bariatric surgery was associated with decreased serum levels of leptin in women and increased serum levels of adiponectin in men and women.[33] Sex-specific effects may be due to endocrine disruption. Furthermore, if the reverse associations hold true, it would be expected that as adipose tissue increases in women, serum levels of POPs and adiponectin decrease while serum levels of leptin increase.

Attempts to elucidate the mechanisms linking adiposity, lipophilic POPs, and endocrine disruption are further complicated by the menopausal transition. The menopausal transition is characterized by accelerated gains in fat mass and losses of lean mass as well as changes in the female sex hormone estradiol.[66] Changes in estradiol affect energy homeostasis pathways including food intake and energy expenditure, regulation of adipose tissue lipid storage and metabolism, and insulin sensitivity.[66] In addition to its pro-inflammatory functions, leptin regulates satiety by suppressing appetite.[1] With this in mind, the inverse association we observed between leptin and select POPs may be explained by unknown, potentially bidirectional, relationships involving hormonal and other metabolic changes occurring in midlife women. At the minimum, future research should consider changing WC in longitudinal studies with a single POPs measurement.

K-means Clustering produced an inverted U-shaped dose-response curve for leptin. Exposure to average levels of the POP mixture, compared to below average exposure, was associated with a 16% increase in leptin. Non-linearity aligns with some PCB estimates from single pollutant models, however none achieved significance. Leptin estimates from K-means Clustering and BKMR differed in shape and direction. Yet, results from K-means Clustering and BKMR should be compared with caution. K-means Clustering is an unsupervised approach that creates exposure groupings based on the homogeneity of mixture components; by doing so,

misclassification can lead to certain POPs dominating a cluster. On the other hand, BKMR is a supervised method that analyzes the effects of increasing the concentration of each mixture component by one decile.

Comparing the results from BKMR analysis of individual mixture components and initial single pollutant analyses, both suggested that leptin was inversely associated with HCB, PCB 180, and PCB 194. Associations between leptin and other compounds including PCB 118, 157 and PCB 174 did not align across BKMR and single pollutant analyses. For HMW adiponectin, both BKMR and single pollutant analysis suggested a negative relationship with PCB 118 and a positive relationship with PCB 123. Associations between HMW adiponectin and other compounds including PCB 180, 187 and PCB 194 did not align across BKMR and single pollutant analyses. Misalignment may be due to collinearity and co-pollutant confounding that was not considered when analyzing POPs individually. Although, for example, we might expect that results for PCBs with stronger positive correlations with other PCBs (i.e. PCB 118, 157, 174, and 180) would exhibit less alignment between single pollutant and mixture methods (Figure S4.2). However, this may be an over-simplification. It is possible that effects vary because POPs are unevenly distributed within and among adipocytes, which may not be dictated by waist circumference and which cannot be captured by serum concentrations alone.[56]

The measurement of POPs in serum rather than the dose administered to adipocytes may also explain in part the misalignment between epidemiological studies and in vitro studies. Inharmonious results between epidemiological studies and in vivo studies are slightly more challenging to explain. However, few in vivo studies have been conducted and all examined fetal adipokine levels.[53–55] The metabolism of a woman during the menopausal transition may not be comparable to those of a fetal rodent.

Strengths and Limitations

Our study was limited by single measurements of POPs and adipokines in serum. Future studies would benefit from repeated measures of both POPs and adipokines to characterize temporal trends and explore the relevance of timing in exposure-response. We carefully considered changing visceral adiposity as a potential predictor of serum POP concentrations at subsequent unmeasured visits. We stratified analyses based on whether WC increased or remained stable between the time of POP measurement and adipokine measurement. Women with stable WC may have improved validity of serum POP biomarkers relative to those who gained WC. In addition, K-means Clustering is limited by uncertainty in cluster membership. For this reason, among others, we employed BKMR, a more robust, outcome-based, mixture method that accounts for co-pollutant confounding and interactions in the highly correlated POP mixture. BKMR allowed us to analyze effects associated with a simultaneous change in all components of the POP mixture. Finally, the possibility remains that unmeasured confounders could have impacted our results. We controlled for carefully selected confounders yet it is important to acknowledge that exposure to environmental mixtures is not limited to POPs. Co-occurring pollutants or other environmental factors may interact to impact adipokines.

This study is notable for its analysis of mixtures of PCBs, OCPs, and PBDEs. To our knowledge, it is the first study of adults not limited to patients with obesity. The rich data collected from the Study of Women's Health Across the Nation enabled the exploration of adipokines in women, and, more specifically, women during the menopausal transition, a period of rapidly changing adiposity.

Conclusion

In conclusion, joint cumulative exposure to POP mixtures was not associated with leptin or HMW adiponectin among U.S. midlife women. Null associations identified by BKMR may be explained by the absence of a mechanism operating at the concentrations observed in this study or because individual POPs exert effects in different directions. Mixture analysis is valuable to contextualize and validate single pollutant findings. It is also necessary to evaluate the overall effect of a mixture, which is the more relevant exposure of interest given humans' constant exposure to multitudes of environmental contaminants. Future studies should measure POPs and adipokines at multiple timepoints. To better understand potential mechanisms occurring in adipose tissue, future researchers should expand exposure biomarkers to include POP concentrations in adipose tissue. They should carefully consider the influence of changing adiposity over time. The findings contribute to the growing body of evidence on the role of POPs in adipose and metabolic dysfunction in women.

References

1. Scheja, L. & Heeren, J. The endocrine function of adipose tissues in health and cardiometabolic disease. *Nat. Rev. Endocrinol.* **15**, 507–524 (2019).
2. von Eynatten, M. *et al.* High-molecular weight adiponectin is independently associated with the extent of coronary artery disease in men. *Atherosclerosis* **199**, 123–128 (2008).
3. Nakashima, R. *et al.* Decreased Total and High Molecular Weight Adiponectin Are Independent Risk Factors for the Development of Type 2 Diabetes in Japanese-Americans. *J. Clin. Endocrinol. Metab.* **91**, 3873–3877 (2006).
4. Peake, P. W., Kriketos, A. D., Campbell, L. V., Shen, Y. & Charlesworth, J. A. The metabolism of isoforms of human adiponectin: studies in human subjects and in experimental animals. *Eur. J. Endocrinol.* **153**, 409–417 (2005).
5. Ouchi, N., Parker, J. L., Lugus, J. J. & Walsh, K. Adipokines in inflammation and metabolic disease. *Nat. Rev. Immunol.* **11**, 85–97 (2011).
6. Hou, N. & Luo, J.-D. Leptin and cardiovascular diseases: Leptin and cardiovascular diseases. *Clin. Exp. Pharmacol. Physiol.* **38**, 905–913 (2011).
7. Liu, J. *et al.* Leptinemia and its association with stroke and coronary heart disease in the Jackson Heart Study: Leptinemia, stroke and coronary heart disease in Jackson Heart Study cohort. *Clin. Endocrinol. (Oxf.)* **72**, 32–37 (2010).
8. Sierra-Johnson, J. *et al.* Relation of Increased Leptin Concentrations to History of Myocardial Infarction and Stroke in the United States Population††All the analysis, interpretation,

and/or conclusion reached in this study are the work of the authors and not of the National Center for Health Statistics, Hyattsville, Maryland. *Am. J. Cardiol.* **100**, 234–239 (2007).

9. Wannamethee, S. G. *et al.* Plasma leptin: Associations with metabolic, inflammatory and haemostatic risk factors for cardiovascular disease. *Atherosclerosis* **191**, 418–426 (2007).
10. Fang, H. & Judd, R. L. Adiponectin Regulation and Function. in *Comprehensive Physiology* (ed. Pollock, D. M.) 1031–1063 (John Wiley & Sons, Inc., 2018). doi:10.1002/cphy.c170046.
11. Dunmore, S. J. & Brown, J. E. P. The role of adipokines in β-cell failure of type 2 diabetes. *J. Endocrinol.* **216**, T37–T45 (2013).
12. Ebenibo, S., Edeoga, C., Owei, I. & Dagogo-Jack, S. Basal and Dynamic Leptin Secretion: Association with Cardiometabolic Risk and Body Weight Trajectories in African-Americans and European-Americans. *Front. Endocrinol.* **9**, 12 (2018).
13. Kim, J.-Y. *et al.* Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *J. Clin. Invest.* **117**, 2621–2637 (2007).
14. Farooqi, I. S. & O'Rahilly, S. 20 YEARS OF LEPTIN: Human disorders of leptin action. *J. Endocrinol.* **223**, T63–T70 (2014).
15. Friedman, J. The long road to leptin. *J. Clin. Invest.* **126**, 4727–4734 (2016).
16. *Chemical Factsheets*. https://www.cdc.gov/biomonitoring/chemical_factsheets.html.
17. Agency for Toxic Substances and Disease Registry. *Toxicological Profile for Polybrominated Diphenyl Ethers (PBDEs)*. https://www.atsdr.cdc.gov/toxprofiles/tp207.pdf (2017).
18. Li, L. *et al.* What do we know about the production and release of persistent organic pollutants in the global environment? *Environ. Sci. Adv.* **2**, 55–68 (2023).

19. Fisher, B. E. Most unwanted. *Environ. Health Perspect.* **107**, (1999).

20. Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Polychlorinated Biphenyls (PCBs)*. https://www.atsdr.cdc.gov/ToxProfiles/tp17.pdf (2000).

21. Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Polybrominated Diphenyl Ethers (PBDEs)*. https://www.atsdr.cdc.gov/toxprofiles/tp207-c6.pdf.

22. Yu, G. W., Laseter, J. & Mylander, C. Persistent Organic Pollutants in Serum and Several Different Fat Compartments in Humans. *J. Environ. Public Health* **2011**, 1–8 (2011).

23. Heindel, J. J. *et al.* Metabolism disrupting chemicals and metabolic disorders. *Reprod. Toxicol.* **68**, 3–33 (2017).

24. Jansen, A. *et al.* Increased levels of persistent organic pollutants in serum one year after a great weight loss in humans: Are the levels exceeding health based guideline values? *Sci. Total Environ.* **622–623**, 1317–1326 (2018).

25. Dirinck, E. *et al.* Pivotal Role for the Visceral Fat Compartment in the Release of Persistent Organic Pollutants During Weight Loss. *J. Clin. Endocrinol. Metab.* **100**, 4463–4471 (2015).

26. Stubleski, J., Lind, L., Salihovic, S., Lind, P. M. & Kärrman, A. Longitudinal changes in persistent organic pollutants (POPs) from 2001 to 2009 in a sample of elderly Swedish men and women. *Environ. Res.* **165**, 193–200 (2018).

27. Sjödin, A. *et al.* Polybrominated Diphenyl Ethers, Polychlorinated Biphenyls, and 2,2-Bis(4-chlorophenyl)-1,1-dichloroethene in 7- and 9-Year-Old Children and Their Mothers in the Center for the Health Assessment of Mothers and Children of Salinas Cohort. *Environ. Sci. Technol.* **52**, 2287–2294 (2018).

28. Berghuis, S. A. *et al.* Prenatal Environmental Exposure to Persistent Organic Pollutants and Reproductive Hormone Profile and Pubertal Development in Dutch Adolescents. *Int. J. Environ. Res. Public. Health* **19**, 9423 (2022).

29. Leijs, M. M. *et al.* Alterations in the programming of energy metabolism in adolescents with background exposure to dioxins, dl-PCBs and PBDEs. *PLOS ONE* **12**, e0184006 (2017).

30. Debost-Legrand, A. *et al.* Prenatal exposure to persistent organic pollutants and organophosphate pesticides, and markers of glucose metabolism at birth. *Environ. Res.* **146**, 207–217 (2016).

31. Tang-Péronard, J. L. *et al.* Prenatal exposure to persistent organochlorine pollutants is associated with high insulin levels in 5-year-old girls. *Environ. Res.* **142**, 407–413 (2015).

32. Mullerova, D. *et al.* Negative association between plasma levels of adiponectin and polychlorinated biphenyl 153 in obese women under non-energy-restrictive regime. *Int. J. Obes. 2005* **32**, 1875–1878 (2008).

33. Jansen, A. *et al.* Do changes in persistent organic pollutants after bariatric surgery cause endocrine disruption? *Chemosphere* **313**, 137461 (2023).

34. Pereira-Fernandes, A. *et al.* Expression of Obesity Markers and Persistent Organic Pollutants Levels in Adipose Tissue of Obese Patients: Reinforcing the Obesogen Hypothesis? *PLoS ONE* **9**, e84816 (2014).

35. Sinha, M. K. & Caro, J. F. Clinical Aspects of Leptin. in *Vitamins & Hormones* vol. 54 1–30 (Elsevier, 1998).

36. Lim, J. & Jee, S. H. Association between serum levels of adiponectin and polychlorinated biphenyls in Korean men and women. *Endocrine* **48**, 211–217 (2015).

37. Sowers, M. *et al.* SWAN: A Multicenter, Multiethnic, Community-Based Cohort Study of Women and the Menopausal Transition. *Menopause: Biology and Pathobiology* (2000).

38. Grant-Alfieri, A., Devasurendra, A., Batterman, S., Karvonen-Gutierrez, C. & Park, S. K. Changes in adipose tissue and circulating concentrations of persistent organic pollutants in midlife women. *Under Review* (2023).

39. U.S. Environmental Protection Agency. *DDT: A Brief History and Status*. https://www.epa.gov/ingredients-used-pesticide-products/ddt-brief-history-and-status (2023).

40. Centers for Disease Control and Prevention. *Serum PCBs and Persistent Pesticides*. https://www.cdc.gov/nchs/data/nhanes/nhanes_01_02/l28poc_b_met_pcb_pesticides.pdf.

41. The Aspen Institute. *Glossary for Understanding the Dismantling Structural Racism/Promoting Racial Equity Analysis*. https://www.aspeninstitute.org/wp-content/uploads/files/content/docs/rcc/RCC-Structural-Racism-Glossary.pdf (2017).

42. Grant-Alfieri, A. *et al.* Associations of organochlorine pesticide, polybrominated diphenyl ether, and polychlorinated biphenyl mixtures with incident diabetes in midlife women. (2023).

43. Yuan, Y. *et al.* Analysis of Changes in Weight, Waist Circumference, or Both, and All-Cause Mortality in Chinese Adults. *JAMA Netw. Open* **5**, e2225876 (2022).

44. Hosseinpanah, F., Barzin, M., Mirmiran, P. & Azizi, F. Effect of changes in waist circumference on metabolic syndrome over a 6.6-year follow-up in Tehran. *Eur. J. Clin. Nutr.* **64**, 879–886 (2010).

45. Wang, L. *et al.* A prospective study of waist circumference trajectories and incident cardiovascular disease in China: the Kailuan Cohort Study. *Am. J. Clin. Nutr.* **113**, 338–347 (2021).

46. R Core Team. R: A language and environment for statistical computing. (2022).

47. Bobb, J. F., Claus Henn, B., Valeri, L. & Coull, B. A. Statistical software for analyzing the health effects of multiple concurrent exposures via Bayesian kernel machine regression. *Environ. Health* **17**, 67 (2018).

48. Bobb, J. F. bkmr: Bayesian Kernel Machine Regression. (2022).

49. O'Brien, K. M., Upson, K., Cook, N. R. & Weinberg, C. R. Environmental Chemicals in Urine and Blood: Improving Methods for Creatinine and Lipid Adjustment. *Environ. Health Perspect.* **124**, 220–227 (2016).

50. Clair, H. B. *et al.* Liver Disease in a Residential Cohort With Elevated Polychlorinated Biphenyl Exposures. *Toxicol. Sci.* **164**, 39–49 (2018).

51. Burns, J. S. *et al.* Association Between Chlorinated Pesticides in the Serum of Prepubertal Russian Boys and Longitudinal Biomarkers of Metabolic Function. *Am. J. Epidemiol.* **180**, 909–919 (2014).

52. Turyk, M. *et al.* Persistent Organic Pollutants and Biomarkers of Diabetes Risk in a Cohort of Great Lakes Sport Caught Fish Consumers. *Environ. Res.* **140**, 335–344 (2015).

53. Ahmed, R. G. Early weaning PCB 95 exposure alters the neonatal endocrine system: thyroid adipokine dysfunction. *J. Endocrinol.* **219**, 205–215 (2013).

54. Ahmed, R. G., El-Gareib, A. W. & Shaker, H. M. Gestational 3,3',4,4',5-pentachlorobiphenyl (PCB 126) exposure disrupts fetoplacental unit: Fetal thyroid-cytokines dysfunction. *Life Sci.* **192**, 213–220 (2018).

55. Kozlova, E. V. *et al.* Developmental exposure to indoor flame retardants and hypothalamic molecular signatures: Sex-dependent reprogramming of lipid homeostasis. *Front. Endocrinol.* **13**, 997304 (2022).

56. Ferrante, M. C. *et al.* Polychlorinated biphenyls (PCB 101, PCB 153 and PCB 180) alter leptin signaling and lipid metabolism in differentiated 3T3-L1 adipocytes. *Toxicol. Appl. Pharmacol.* **279**, 401–408 (2014).

57. Mangum, L. H., Howell, G. E. & Chambers, J. E. Exposure to p,p′-DDE enhances differentiation of 3T3-L1 preadipocytes in a model of sub-optimal differentiation. *Toxicol. Lett.* **238**, 65–71 (2015).

58. Caron, A. *et al.* Effects of PCB126 on Adipose-to-Muscle Communication in an *in Vitro* Model. *Environ. Health Perspect.* **128**, 107002 (2020).

59. May, P. *et al.* In Vitro Cocktail Effects of PCB-DL (PCB118) and Bulky PCB (PCB153) with BaP on Adipogenesis and on Expression of Genes Involved in the Establishment of a Pro-Inflammatory State. *Int. J. Mol. Sci.* **19**, (2018).

60. Taxvig, C. *et al.* Differential effects of environmental chemicals and food contaminants on adipogenesis, biomarker release and PPARγ activation. *Mol. Cell. Endocrinol.* **361**, 106–115 (2012).

61. Howell, G. & Mangum, L. Exposure to bioaccumulative organochlorine compounds alters adipogenesis, fatty acid uptake, and adipokine production in NIH3T3-L1 cells. *Toxicol. In Vitro* **25**, 394–402 (2011).

62. Hernandez-Quiles, M., Broekema, M. F. & Kalkhoven, E. PPARgamma in Metabolism, Immunity, and Cancer: Unified and Diverse Mechanisms of Action. *Front. Endocrinol.* **12**, 624112 (2021).

63. Darbre, P. D. Endocrine Disruptors and Obesity. *Curr. Obes. Rep.* **6**, 18–27 (2017).

64. Arsenescu, V., Arsenescu, R. I., King, V., Swanson, H. & Cassis, L. A. Polychlorinated Biphenyl-77 Induces Adipocyte Differentiation and Proinflammatory Adipokines and Promotes Obesity and Atherosclerosis. *Environ. Health Perspect.* **116**, 761–768 (2008).

65. Li, W. *et al.* Association between parity and obesity patterns in a middle-aged and older Chinese population: a cross-sectional analysis in the Tongji-Dongfeng cohort study. *Nutr. Metab.* **13**, 72 (2016).

66. Greendale, G. A. *et al.* Changes in body composition and weight during the menopause transition. *JCI Insight* **4**, 124865 (2019).

Supplemental Materials

Figure S4.1 Study design of the Study of Women's Health Across the Nation Multi-Pollutant Study (SWAN MPS).

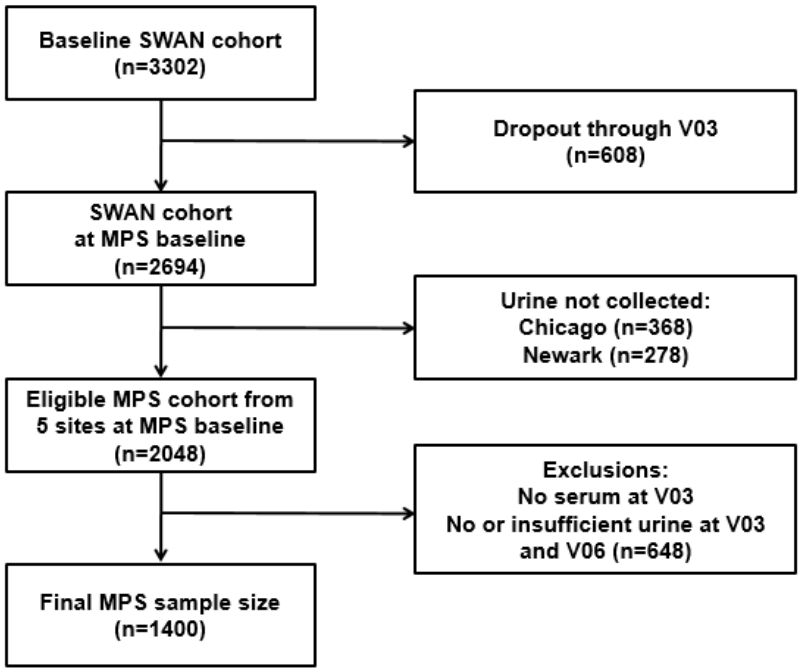


Table S4.1 Detection frequencies (DF) of PCBs, PBDEs, and OCPs in SWAN MPS, 1999/2000.

PCB		**DF (%)**
Tetra	77	5.28
	78	0.00
	79	2.01
	81	0.00
	114	9.61
Penta	105	99.26
	87/110/111	0.00
	118	95.35
	123	91.66
	126	12.57
Hexa	138	100.00
	149	59.66
	153	100.00
	156	97.25
	157	85.74
	162	0.53
	167	98.84
	169	0.42
Hepta	170	99.68
	174	98.52
	178	82.15
	180	94.93
	187	99.79
	188	0.00
	189	0.11
Octa	194	71.81
	195	6.55
	199	61.25
	202	64.84
	203	0.00
	205	0.00
Nona	206	11.93
	208	32.00
Deca	209	20.59

OCP	**DF (%)**
p,p'-DDD	38.94
p,p'-DDT	39.61
o,p'-DDT	16.50
p,p'-DDE	38.78
aldrin	0.25
dieldrin	2.09
endrin	2.60
cis-chlordane	78.39
trans-chlordane	96.06
heptachlor	6.37
cis-nonachlor	3.27
trans-nonachlor	72.03
mirex	16.83
oxychlordane	9.72
c/t-heptachlorepoxide	13.32
α-HCH	5.53
β-HCH	22.36
γ-HCH	9.55
HCB	97.15

PBDE	**DF (%)**
17	5.73
28	4.23
47	72.84
66	0.28
71	0.00
85	1.60
99	20.39
100	12.69
138	0.85
153	15.13
154	21.15
183	1.13
190	2.35
209	0.94

Figure S4.2 Spearman correlation matrix of serum concentrations of POPs (ng/g lipid).

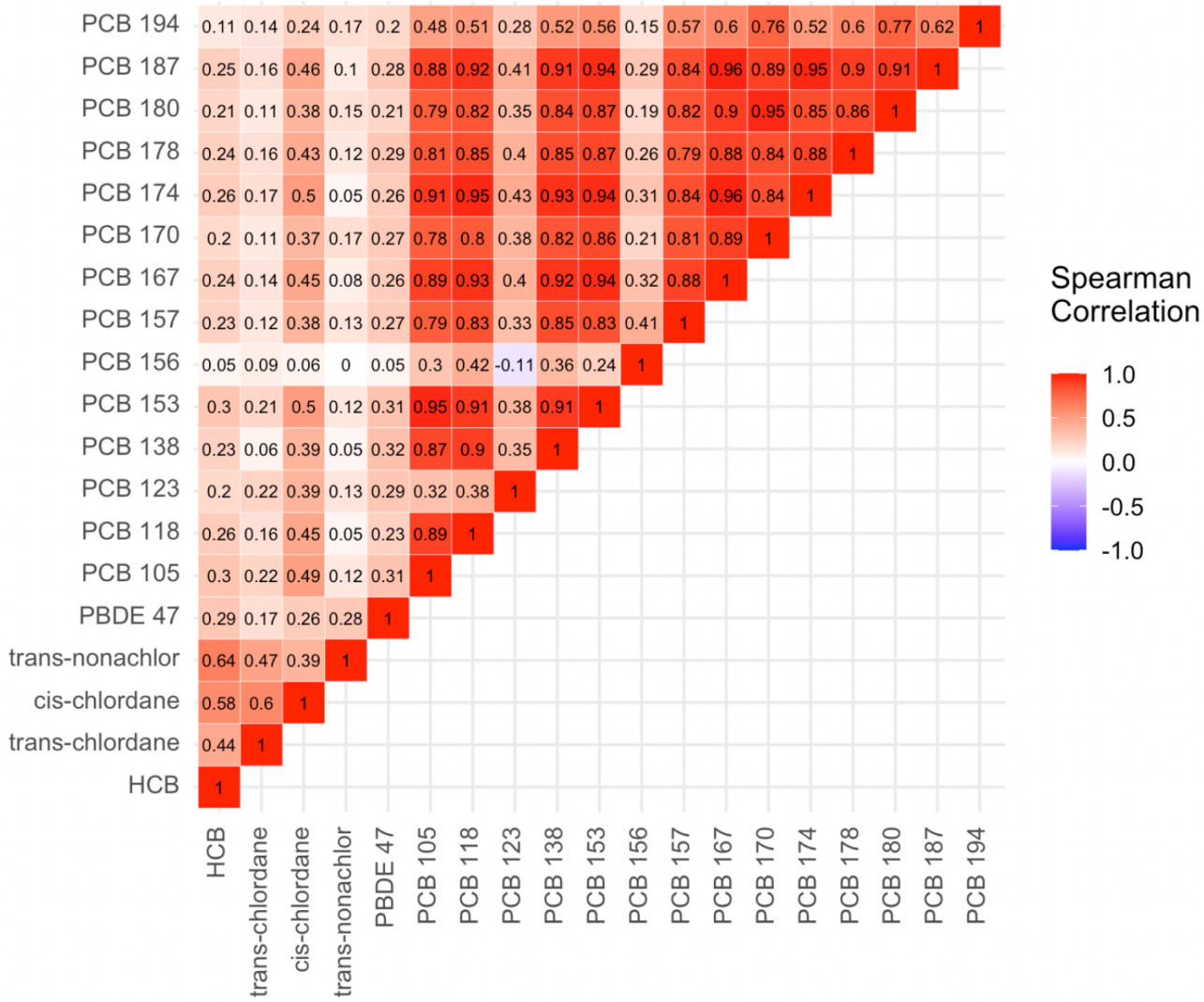


Figure S4.3 Results of Elbow analysis of serum concentrations of POPs (ng/g lipid) in SWAN MPS (1999/2000) that informed selection of optimal number of K-means clusters.

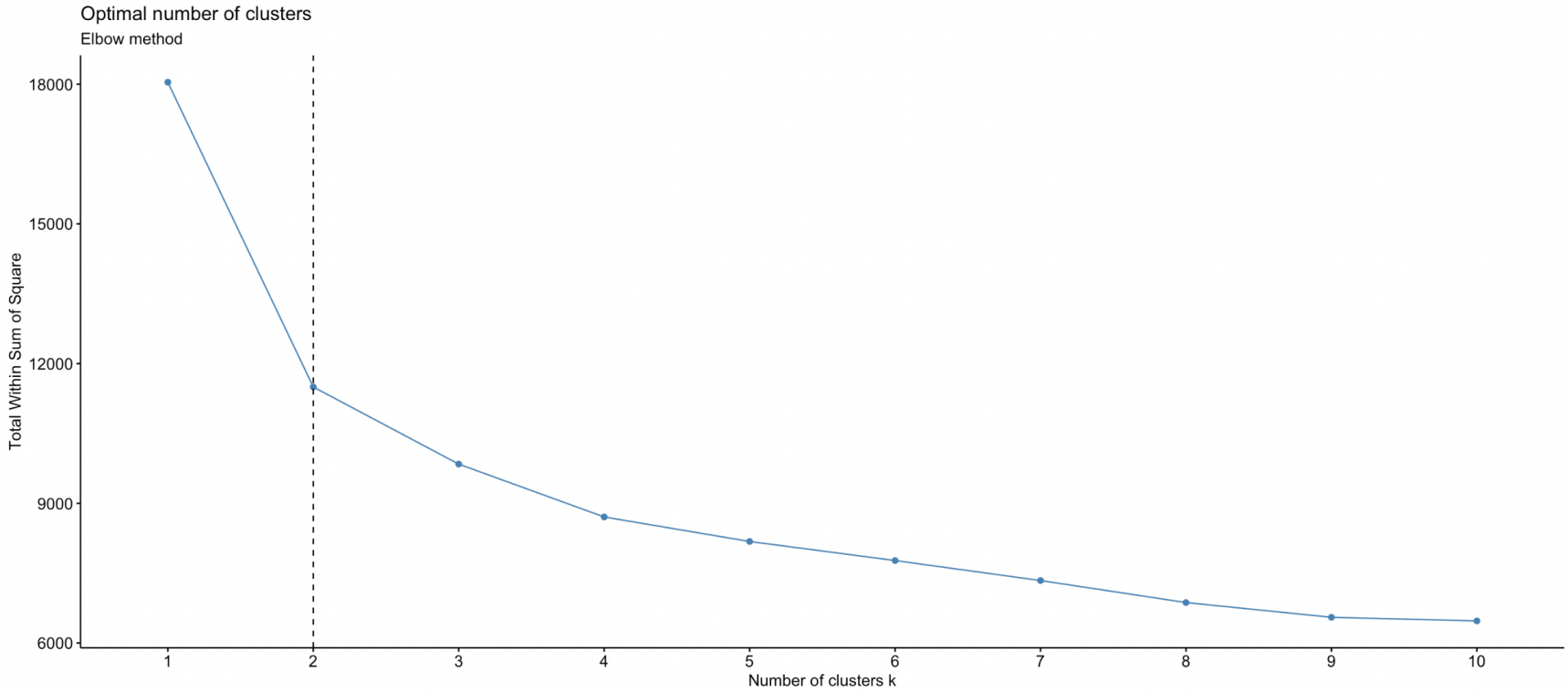


Figure S4.4 Results of Silhouette analysis of serum concentrations of POPs (ng/g lipid) in SWAN MPS (1999/2000) that informed selection of optimal number of K-means clusters.

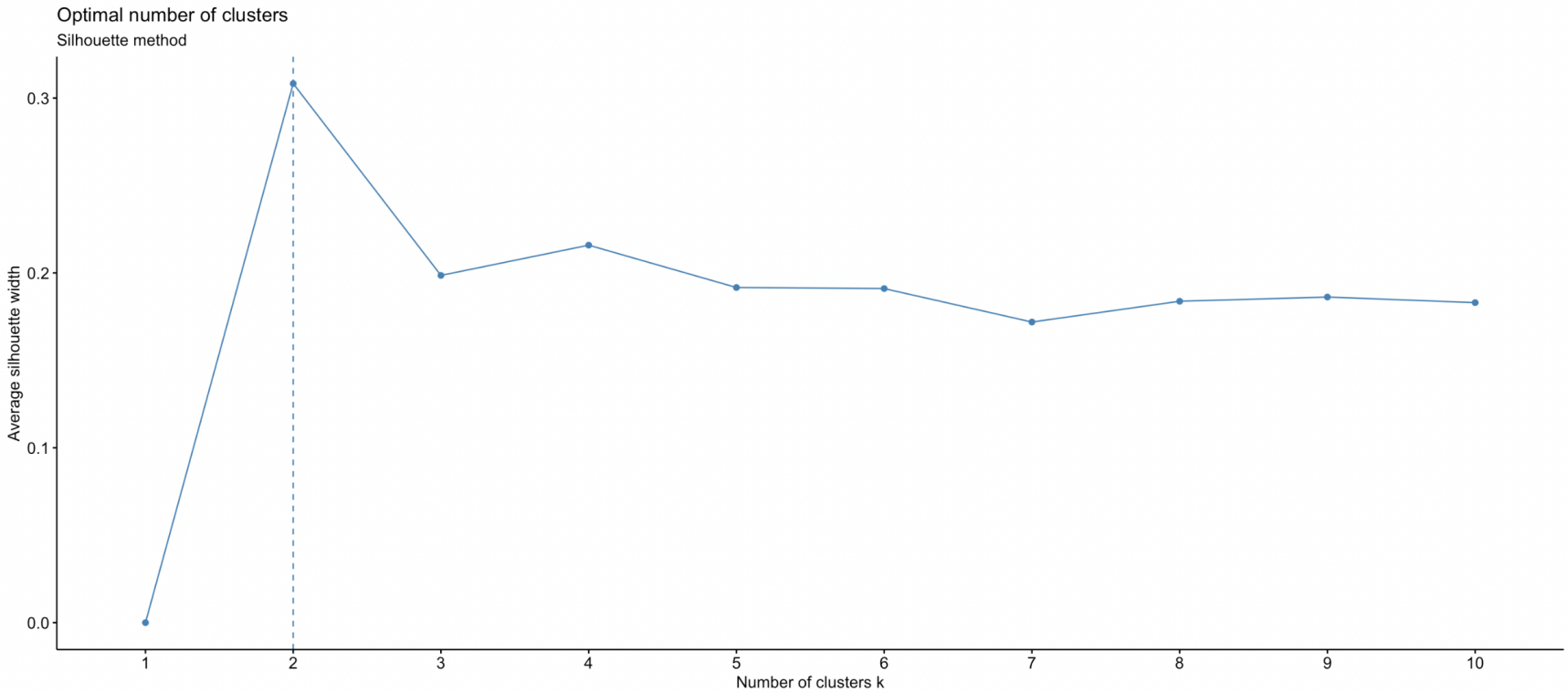


Table S4.2 Serum concentrations of POPs (ng/g lipid) in SWAN MPS, 1999/2000 (n = 872).

	DF (%)	P25	P33	P50	P66	P75
PCBs						
PCB 105	99.3	37.6	46.7	70.1	96.2	118.8
PCB 118	95.3	133.0	173.0	255.4	341.1	398.5
PCB 123	91.7	13.6	17.4	31.0	47.6	61.0
PCB 138	100.0	169.3	237.7	355.5	515.2	616.2
PCB 153	100.0	118.9	146.4	212.0	281.1	335.9
PCB 156	97.2	3.9	4.6	6.0	8.7	11.0
PCB 157	85.7	1.4	1.8	2.6	3.6	4.3
PCB 167	98.8	3.4	4.3	6.0	8.2	9.4
PCB 170	99.7	11.8	13.7	18.6	24.9	27.9
PCB 174	98.5	11.2	13.8	20.3	26.9	32.0
PCB 178	82.1	3.7	4.5	6.5	8.6	10.0
PCB 180	94.9	23.9	28.5	38.5	51.9	59.4
PCB 187	99.8	19.3	23.7	33.9	45.3	52.4
PCB 194	71.8	<MDL	3.5	4.7	6.1	7.2
PBDEs						
PBDE 47	72.8	<MDL	22.7	34.9	52.0	67.9
OCPs						
cis-chlordane	78.4	5.7	7.1	10.0	13.2	15.6
HCB	97.1	9.2	10.3	13.6	17.3	19.4
trans-chlordane	96.1	5.8	7.6	11.8	15.8	19.8
trans-nonachlor	72.0	<MDL	8.5	12.2	15.7	18.6

Table S4.3 Median serum adipokine concentrations by waist circumference change (gain vs. stable) between 1999/2000 and 2002/03.

	OCP Subsample		**PCB Subsample**		**PBDE Subsample**	
	Stable [a] (n = 389)	Gain [b] (n = 499)	Stable (n = 322)	Gain (n = 396)	Stable (n = 356)	Gain (n = 453)
Leptin (ng/ml)	14.43	24.25	14.76	24.13	14.40	24.11
HMW Adiponectin (ug/ml)	6.42	5.21	5.91	4.99	6.43	5.19

WC = waist circumference. [a] Stable = -2 cm < ΔWC < 2 cm; [b] Gain = ΔWC ≥ 2 cm

Table S4.4 Serum concentrations of POPs (ng/g lipid) in SWAN MPS, 1999/2000.

	DF (%)	P25	P33	P50	P66	P75
PCBs (n = 900)						
PCB 105	99.26	38.29	47.55	71.99	98.12	122.75
PCB 118	95.35	136.4	177.9	261.86	352.88	409.11
PCB 123	91.66	13.17	16.9	30.74	47.13	60.77
PCB 138	100.00	176.38	241.44	363.11	530.66	638.39
PCB 153	100.00	120.81	150.15	215.01	288.09	349.19
PCB 156	97.25	3.96	4.68	6.31	9.31	11.78
PCB 157	85.74	1.44	1.83	2.66	3.68	4.44
PCB 167	98.84	3.53	4.36	6.18	8.31	9.72
PCB 170	99.68	11.97	14.03	19	25.19	28.36
PCB 174	98.52	11.35	14.19	21.03	27.58	32.98
PCB 178	82.15	3.8	4.67	6.58	8.82	10.18
PCB 180	94.93	24.31	29.01	39.93	52.72	60.09
PCB 187	99.79	19.84	24.52	34.43	45.94	53.68
PCB 194	71.81	<MDL	3.58	4.8	6.22	7.26
PBDEs (n = 1,011)						
PBDE 47	72.84	<MDL	24.36	37.99	57.34	77.92
OCPs (n = 1,116)						
cis-chlordane	78.39	6.43	8.03	10.84	14.16	16.46
HCB	97.15	9.25	10.26	12.88	16.29	18.41
trans-chlordane	96.06	7.07	9.08	12.62	17.02	20.62
trans-nonachlor	72.03	<MDL	6.63	9.92	13.6	16.68
p,p'-DDT	38.78	<MDL	<MDL	<MDL	5	8.8
p,p'-DDD	39.61	<MDL	<MDL	<MDL	12.41	26.23
p,p'-DDE	38.94	<MDL	<MDL	<MDL	11.54	22.36

Figure S4.5 Directed acyclic graph (DAG) illustrating the role of adiposity change in the association between serum concentrations of persistent organic pollutants (POPs) and serum levels of adipokines. Measured variables are outlined in solid lines and unmeasured variables in dashed lines.

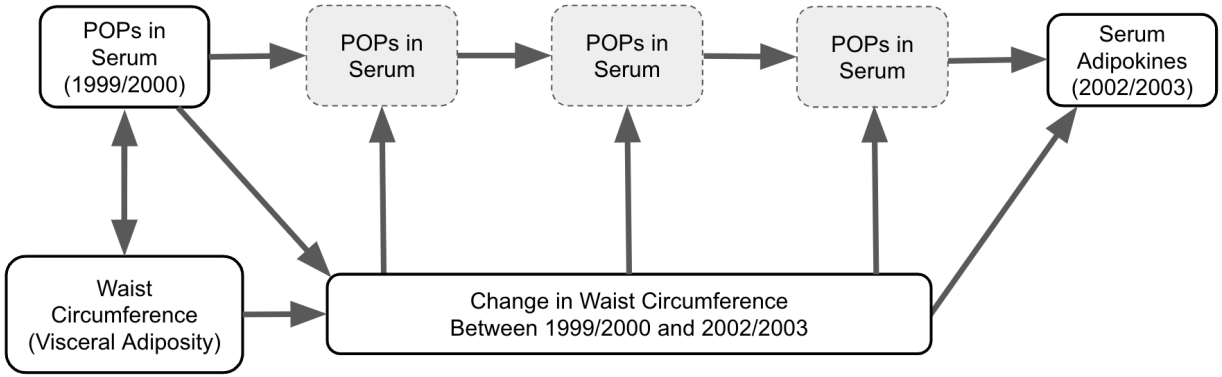


Table S4.5 Serum Concentrations of DDT Metabolites (ng/g lipid) among SWAN MPS Participants at Baseline (n = 1,116)

DDT Metabolites	DF (%)	P25	P33	P50	P66	P75
p,p'-DDT	38.78	<MDL	<MDL	<MDL	5	8.8
p,p'-DDD	39.61	<MDL	<MDL	<MDL	12.41	26.23
p,p'-DDE	38.94	<MDL	<MDL	<MDL	11.54	22.36

Table S4.6 Percent change (95% CI) in serum concentrations of leptin associated with an increase in serum POP concentrations in SWAN MPS. Models were adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and serum lipids.

Compound	Continuous (per IQR)	p	Tertile 2	Tertile 3	p for trend
OCPs (n = 1,116)					
trans-nonachlor	-0.3 (-1.87, 1.29)	0.7069	-5.01 (-15.06, 6.21)	-4.11 (-14.39, 7.41)	0.4644
Cis-chlordane	0.02 (-0.67, 0.71)	0.9627	-1.93 (-12.42, 9.81)	-7.41 (-17.54, 3.96)	0.1916
trans-chlordane	0.01 (-0.33, 0.36)	0.9389	-5.83 (-15.81, 5.34)	3.57 (-7.55, 16.02)	0.5550
HCB	-3.41 (-8.21, 1.64)	0.1825	-4.78 (-14.87, 6.5)	-4.61 (-14.82, 6.81)	0.4120
PBDEs (n = 1,011)					
PBDE 47	-0.34 (-0.88, 0.19)	0.2113	7.43 (-4.54, 20.9)	-1 (-12.05, 11.43)	0.8703
PCBs (n = 900)					
PCB 105	1 (-4.72, 7.06)	0.7380	7.65 (-4.92, 21.87)	3.85 (-8.44, 17.79)	0.5409
PCB 118	2.52 (-3.24, 8.62)	0.3992	1.36 (-10.47, 14.75)	1.15 (-10.97, 14.92)	0.8567
PCB 123	0.22 (-4.71, 5.41)	0.9308	6.56 (-5.86, 20.62)	-0.71 (-12.44, 12.58)	0.9201
PCB 138	0.61 (-4.83, 6.37)	0.8291	7.8 (-4.74, 21.99)	0.48 (-11.45, 14)	0.9116
PCB 153	0.5 (-4.97, 6.29)	0.8613	5.56 (-6.77, 19.53)	1.19 (-10.85, 14.87)	0.8324
PCB 156	-0.1 (-4.8, 4.83)	0.9677	-14.3 (-24.25, -3.04)	-3.45 (-14.82, 9.44)	0.5928
PCB 157	-2.13 (-6.78, 2.75)	0.3855	11.81 (-1.2, 26.54)	-9.1 (-19.83, 3.06)	0.1564
PCB 167	0.8 (-4.33, 6.19)	0.7660	5.5 (-6.86, 19.49)	-5.29 (-16.59, 7.54)	0.4271
PCB 170	-0.34 (-0.86, 0.17)	0.1939	0.28 (-11.4, 13.49)	-13.03 (-23.34, -1.34)	0.0322
PCB 174	1.29 (-4.27, 7.18)	0.6557	3.9 (-8.18, 17.57)	3.51 (-8.94, 17.67)	0.5875
PCB 178	-0.62 (-5.92, 4.98)	0.8248	0.81 (-10.98, 14.17)	-4.32 (-15.72, 8.63)	0.5042
PCB 180	-5.2 (-9.57, -0.61)	0.0270	-6.38 (-17.28, 5.96)	-15.74 (-25.75, -4.37)	0.0083
PCB 187	-0.4 (-5.65, 5.15)	0.8858	8.74 (-3.94, 23.1)	-4.7 (-15.99, 8.1)	0.4933
PCB 194	-9.05 (-13.16, -4.74)	0.0001	-6.75 (-17.5, 5.4)	-22.36 (-31.56, -11.93)	0.0001

Table S4.7 Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with an increase in serum POP concentrations in SWAN MPS. Models were adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and serum lipids.

Compound	Continuous (per IQR)	p	Tertile 2	Tertile 3	p for trend
OCPs (n = 1,116)					
trans-nonachlor	-0.6 (-1.81, 0.63)	0.3370	2.9 (-5.64, 12.21)	-7.35 (-15.15, 1.17)	0.0935
Cis-chlordane	0.06 (-0.48, 0.59)	0.8366	6.18 (-2.75, 15.93)	5.41 (-3.66, 15.34)	0.2536
trans-chlordane	-0.1 (-0.37, 0.17)	0.4509	-0.96 (-9.22, 8.06)	-4.71 (-12.76, 4.09)	0.2859
HCB	-0.9 (-4.74, 3.11)	0.6560	6.65 (-2.22, 16.31)	-5.3 (-13.24, 3.38)	0.2285
PBDEs (n = 1,011)					
PBDE 47	0.28 (-0.14, 0.7)	0.1885	-8.28 (-16.26, 0.47)	-6.47 (-14.62, 2.47)	0.1506
PCBs (n = 900)					
PCB 105	-0.35 (-4.78, 4.28)	0.8782	-2.52 (-11.51, 7.38)	-1.11 (-10.36, 9.09)	0.8149
PCB 118	-2.45 (-6.74, 2.05)	0.2809	-2.3 (-11.29, 7.61)	-7.99 (-16.69, 1.61)	0.1033
PCB 123	1.64 (-2.28, 5.71)	0.4188	-1.28 (-10.37, 8.73)	3.08 (-6.54, 13.69)	0.5477
PCB 138	-0.93 (-5.13, 3.46)	0.6727	1.04 (-8.25, 11.27)	0.11 (-9.28, 10.47)	0.9773
PCB 153	-0.17 (-4.43, 4.28)	0.9385	0.47 (-8.8, 10.69)	0.55 (-8.91, 10.99)	0.9121
PCB 156	-1.04 (-4.69, 2.74)	0.5844	1.66 (-7.7, 11.96)	-1.29 (-10.5, 8.88)	0.7933
PCB 157	0.98 (-2.78, 4.89)	0.6143	-0.4 (-9.6, 9.74)	5 (-4.84, 15.85)	0.3384
PCB 167	-0.08 (-4.06, 4.06)	0.9679	1.16 (-8.21, 11.49)	-0.72 (-10.08, 9.63)	0.8953
PCB 170	0.36 (-0.05, 0.76)	0.0841	2.56 (-6.89, 12.97)	7.83 (-2.29, 18.99)	0.1353
PCB 174	-1.42 (-5.66, 3.02)	0.5255	0.34 (-8.87, 10.48)	-4.93 (-13.96, 5.05)	0.3328
PCB 178	0.29 (-3.9, 4.67)	0.8930	2.73 (-6.76, 13.2)	1.12 (-8.4, 11.63)	0.8154
PCB 180	2.29 (-1.41, 6.14)	0.2283	-0.5 (-9.66, 9.6)	8.06 (-2.11, 19.28)	0.1297
PCB 187	0.27 (-3.87, 4.6)	0.8990	-0.03 (-9.26, 10.13)	2.08 (-7.49, 12.65)	0.6871
PCB 194	4.06 (0.35, 7.9)	0.0320	5.3 (-4.35, 15.92)	10.03 (-0.34, 21.47)	0.0587

Table S4.8 Percent change (95% CI) in serum concentrations of leptin associated with an increase in serum POP concentrations in SWAN MPS. Models were unadjusted.

Compound	Continuous (per IQR)	p	Tertile 2	Tertile 3	p for trend
OCPs (n = 1,116)					
trans-nonachlor	-0.5 (-2.29, 1.34)	0.5934	-8.18 (-19.2, 4.35)	-6.71 (-17.97, 6.11)	0.2877
Cis-chlordane	-0.19 (-0.97, 0.61)	0.6475	-5.82 (-17.14, 7.05)	-12.47 (-23.04, -0.45)	0.0427
trans-chlordane	-0.02 (-0.42, 0.39)	0.9326	-1.97 (-13.78, 11.47)	4.35 (-8.28, 18.72)	0.5161
HCB	-6.66 (-11.88, -1.13)	0.0191	-8.9 (-19.87, 3.58)	-15.07 (-25.25, -3.51)	0.0122
PBDEs (n = 1,011)					
PBDE 47	-0.3 (-0.91, 0.31)	0.3381	7.96 (-5.61, 23.48)	-5.63 (-17.5, 7.95)	0.3983
PCBs (n = 900)					
PCB 105	0.05 (-6.23, 6.76)	0.9873	10.13 (-4.46, 26.95)	2.56 (-10.96, 18.12)	0.7227
PCB 118	1.46 (-4.82, 8.16)	0.6568	3.56 (-10.2, 19.42)	-0.43 (-13.54, 14.67)	0.9532
PCB 123	-1.23 (-6.69, 4.55)	0.6700	3.73 (-9.99, 19.55)	-8.42 (-20.5, 5.51)	0.2253
PCB 138	-0.53 (-6.52, 5.84)	0.8659	8.06 (-6.24, 24.55)	-1.9 (-14.84, 13.01)	0.7949
PCB 153	-0.95 (-6.93, 5.41)	0.7627	7.92 (-6.39, 24.42)	-1.06 (-14.08, 13.93)	0.8864
PCB 156	2.02 (-3.36, 7.7)	0.4691	-16.69 (-27.66, -4.06)	-0.5 (-13.73, 14.76)	0.9545
PCB 157	-0.43 (-5.7, 5.13)	0.8759	19.26 (3.54, 37.36)	-6.29 (-18.59, 7.87)	0.3721
PCB 167	0.53 (-5.13, 6.52)	0.8591	8.37 (-6.03, 24.98)	-4.84 (-17.3, 9.5)	0.4920
PCB 170	-0.41 (-1, 0.18)	0.1737	-2.02 (-14.95, 12.87)	-15.62 (-26.71, -2.85)	0.0188
PCB 174	-0.27 (-6.32, 6.17)	0.9318	4.53 (-9.32, 20.49)	1.12 (-12.29, 16.57)	0.8788
PCB 178	-2.06 (-7.9, 4.14)	0.5063	0.56 (-12.77, 15.93)	-8.09 (-20.22, 5.88)	0.2426
PCB 180	-5.7 (-10.55, -0.59)	0.0297	-6.41 (-18.74, 7.8)	-19.23 (-29.83, -7.03)	0.0030
PCB 187	-2.12 (-7.84, 3.95)	0.4847	9.87 (-4.68, 26.63)	-8.95 (-20.89, 4.79)	0.1946
PCB 194	-10.07 (-14.63, -5.27)	0.0001	-5.16 (-17.59, 9.14)	-25.47 (-35.3, -14.14)	0.0001

Table S4.9 Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with an increase in serum POP concentrations in SWAN MPS. Models were unadjusted.

Compound	Continuous (per IQR)	p	Tertile 2	Tertile 3	p for trend
OCPs (n = 1,116)					
trans-nonachlor	-0.92 (-2.25, 0.43)	0.1815	5.25 (-4.24, 15.68)	-10.30 (-18.44, -1.36)	0.0267
Cis-chlordane	0.13 (-0.45, 0.73)	0.6552	11.16 (1.10, 22.22)	12.19 (1.99, 23.42)	0.0182
trans-chlordane	-0.11 (-0.41, 0.19)	0.4594	2.23 (-7.06, 12.44)	-2.84 (-11.71, 6.92)	0.5533
HCB	1.65 (-2.61, 6.09)	0.4549	11.24 (1.13, 22.35)	2.55 (-6.71, 12.75)	0.6001
PBDEs (n = 1,011)					
PBDE 47	0.34 (-0.12, 0.79)	0.1487	-9.09 (-17.71, 0.43)	-3.20 (-12.38, 6.95)	0.5246
PCBs (n = 900)					
PCB 105	1.02 (-3.76, 6.05)	0.6812	-3.43 (-13.18, 7.41)	2.13 (-8.12, 13.53)	0.6985
PCB 118	-0.60 (-5.24, 4.27)	0.8052	-0.83 (-10.85, 10.33)	-3.56 (-13.22, 7.19)	0.5016
PCB 123	1.99 (-2.25, 6.42)	0.3633	-1.60 (-11.52, 9.42)	6.05 (-4.61, 17.91)	0.2784
PCB 138	0.45 (-4.11, 5.22)	0.8512	0.94 (-9.24, 12.26)	4.54 (-5.97, 16.22)	0.4120
PCB 153	1.26 (-3.35, 6.09)	0.5982	2.17 (-8.15, 13.65)	5.06 (-5.47, 16.76)	0.3595
PCB 156	0.42 (-3.57, 4.58)	0.8392	6.21 (-4.48, 18.09)	1.67 (-8.66, 13.17)	0.7652
PCB 157	1.65 (-2.40, 5.87)	0.4308	-2.91 (-12.69, 7.97)	7.82 (-3.00, 19.85)	0.1641
PCB 167	0.97 (-3.31, 5.44)	0.6626	-0.43 (-10.52, 10.80)	3.03 (-7.25, 14.46)	0.5784
PCB 170	0.27 (-0.18, 0.72)	0.2389	0.51 (-9.61, 11.75)	9.48 (-1.49, 21.68)	0.0944
PCB 174	0.53 (-4.07, 5.35)	0.8239	-0.24 (-10.31, 10.95)	0.39 (-9.75, 11.66)	0.9434
PCB 178	1.12 (-3.42, 5.87)	0.6357	0.94 (-9.26, 12.28)	4.54 (-5.97, 16.22)	0.4115
PCB 180	2.49 (-1.48, 6.63)	0.2228	-0.76 (-10.74, 10.33)	11.53 (0.36, 23.94)	0.0440
PCB 187	0.66 (-3.78, 5.29)	0.7759	-1.70 (-11.64, 9.35)	4.87 (-5.62, 16.54)	0.3780
PCB 194	4.14 (0.13, 8.30)	0.0429	4.43 (-6.07, 16.10)	11.69 (0.39, 24.27)	0.0424

Table S4.10 Percent change (95% CI) in serum concentrations of leptin associated with an increase in serum POP concentrations in SWAN MPS. Models were adjusted for age, race/ethnicity, site, education, and financial strain.

Compound	Continuous (per IQR)	p	Tertile 2	Tertile 3	p for trend
OCPs (n = 1116)					
cis-chlordane	-0.02 (-0.74, 0.7)	0.9559	-8.75 (-18.82, 2.58)	-17.01 (-26.21, -6.66)	0.0019
HCB	-5.99 (-10.8, -0.93)	0.0211	-9.59 (-19.56, 1.61)	-12.03 (-21.72, -1.15)	0.0312
trans-chlordane	0.06 (-0.3, 0.43)	0.7313	-8.63 (-18.75, 2.74)	-2.76 (-13.55, 9.37)	0.6370
trans-nonachlor	0 (-1.65, 1.68)	0.9989	-5.73 (-16.1, 5.92)	-3.48 (-14.25, 8.64)	0.5499
PBDEs (n = 1,011)					
PBDE 47	-0.38 (-0.94, 0.18)	0.1864	3.01 (-8.98, 16.59)	-6.56 (-17.43, 5.74)	0.2807
PCBs (n = 900)					
PCB 105	-2.87 (-8.49, 3.1)	0.3395	8.4 (-4.92, 23.6)	-2.19 (-14.11, 11.39)	0.7387
PCB 118	-1.28 (-6.93, 4.71)	0.6683	3.27 (-9.4, 17.72)	-4.62 (-16.25, 8.61)	0.4779
PCB 123	-2.72 (-7.7, 2.52)	0.3026	4.31 (-8.43, 18.83)	-9.28 (-20.38, 3.38)	0.1450
PCB 138	-2.75 (-8.14, 2.97)	0.3391	8.09 (-5.14, 23.17)	-6.16 (-17.62, 6.88)	0.3433
PCB 153	-3.57 (-8.93, 2.1)	0.2130	6.37 (-6.71, 21.27)	-6.36 (-17.75, 6.6)	0.3231
PCB 156	-1.03 (-5.86, 4.05)	0.6863	-17.65 (-27.67, -6.24)	-4.5 (-16.24, 8.87)	0.5033
PCB 157	-4.16 (-8.85, 0.78)	0.0977	16.11 (2.01, 32.17)	-12.81 (-23.42, -0.73)	0.0422
PCB 167	-2.28 (-7.36, 3.08)	0.3968	7.2 (-5.96, 22.22)	-10.74 (-21.57, 1.59)	0.0871
PCB 170	-0.28 (-0.82, 0.27)	0.3231	-1.06 (-13.12, 12.68)	-18.76 (-28.65, -7.5)	0.0019
PCB 174	-2.89 (-8.32, 2.87)	0.3190	3.93 (-8.8, 18.43)	-4.58 (-16.28, 8.76)	0.4821
PCB 178	-3.89 (-9.17, 1.69)	0.1686	2.25 (-10.29, 16.56)	-9.97 (-20.95, 2.55)	0.1147
PCB 180	-7.49 (-11.88, -2.88)	0.0018	-7.89 (-19.1, 4.87)	-22.07 (-31.54, -11.29)	0.0002
PCB 187	-4.14 (-9.31, 1.33)	0.1359	10.48 (-3.03, 25.87)	-11.81 (-22.49, 0.34)	0.0583
PCB 194	-11 (-15.17, -6.62)	0.0000	-8.78 (-19.79, 3.74)	-27.19 (-36.15, -16.96)	0.0000

Table S4.11 Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with an increase in serum POP concentrations in SWAN MPS. Models were adjusted for age, race/ethnicity, site, education, and financial strain.

Compound	Continuous (per IQR)	p	Tertile 2	Tertile 3	p for trend
OCPs (n = 1116)					
cis-chlordane	0.11 (-0.45, 0.66)	0.7088	11.15 (1.6, 21.59)	15.59 (5.62, 26.5)	0.0017
HCB	1.92 (-2.11, 6.11)	0.3565	11.04 (1.51, 21.45)	1.77 (-6.94, 11.31)	0.6941
trans-chlordane	-0.13 (-0.41, 0.15)	0.3627	1.28 (-7.46, 10.84)	0.48 (-8.21, 9.98)	0.9173
trans-nonachlor	-0.77 (-2.03, 0.5)	0.2323	3.04 (-5.76, 12.67)	-7.59 (-15.59, 1.18)	0.0931
PBDEs (n = 1,011)					
PBDE 47	0.31 (-0.12, 0.75)	0.1533	-7.12 (-15.47, 2.05)	-2.05 (-10.85, 7.61)	0.6699
PCBs (n = 900)					
PCB 105	3.24 (-1.36, 8.05)	0.1707	-2.44 (-11.74, 7.85)	5.63 (-4.35, 16.66)	0.2797
PCB 118	1.24 (-3.21, 5.9)	0.5912	-2.18 (-11.5, 8.12)	-0.02 (-9.48, 10.42)	0.9949
PCB 123	3.97 (-0.11, 8.22)	0.0570	0.07 (-9.41, 10.55)	10.02 (-0.42, 21.56)	0.0610
PCB 138	2.13 (-2.23, 6.68)	0.3447	1.25 (-8.37, 11.88)	7.39 (-2.79, 18.63)	0.1615
PCB 153	3.39 (-1.03, 8.01)	0.1347	0.88 (-8.74, 11.51)	9.2 (-1.1, 20.57)	0.0822
PCB 156	0.42 (-3.35, 4.33)	0.8318	5.41 (-4.58, 16.45)	1.41 (-8.3, 12.14)	0.7901
PCB 157	2.95 (-0.93, 6.97)	0.1383	-1.87 (-11.17, 8.41)	10.71 (0.2, 22.33)	0.0470
PCB 167	2.93 (-1.18, 7.21)	0.1659	1.04 (-8.61, 11.72)	7.54 (-2.61, 18.75)	0.1514
PCB 170	0.34 (-0.08, 0.76)	0.1107	4.32 (-5.56, 15.24)	15.63 (4.69, 27.71)	0.0043
PCB 174	2.35 (-2.05, 6.96)	0.3002	1.06 (-8.54, 11.67)	3.93 (-5.96, 14.86)	0.4501
PCB 178	3.31 (-1.05, 7.86)	0.1390	3.05 (-6.76, 13.9)	9.18 (-1.16, 20.59)	0.0840
PCB 180	4.54 (0.72, 8.5)	0.0196	1.66 (-7.96, 12.27)	16.74 (5.72, 28.91)	0.0024
PCB 187	3.56 (-0.74, 8.04)	0.1060	-0.11 (-9.61, 10.39)	10.64 (0.21, 22.14)	0.0458
PCB 194	5.8 (1.96, 9.78)	0.0029	6.63 (-3.43, 17.74)	15.67 (4.53, 28)	0.0049

Table S4.12 Percent change (95% CI) in serum concentrations of leptin associated with an increase in serum POP concentrations in SWAN MPS. Models were adjusted for age, race/ethnicity, site, education, financial strain, and serum lipids.

Compound	Continuous (per IQR)	p	Tertile 2	Tertile 3	p for trend
OCPs (n = 1116)					
cis-chlordane	0.06 (-0.64, 0.78)	0.8594	-4.36 (-14.84, 7.42)	-9.33 (-19.51, 2.14)	0.1072
HCB	-3.12 (-8.06, 2.09)	0.2361	-6.35 (-16.55, 5.1)	-5.93 (-16.27, 5.68)	0.3024
trans-chlordane	0.07 (-0.29, 0.43)	0.6965	-6.86 (-16.99, 4.52)	3.56 (-7.85, 16.38)	0.5684
trans-nonachlor	-0.11 (-1.72, 1.53)	0.8974	-7.69 (-17.67, 3.5)	-5.66 (-16.01, 5.96)	0.3199
PBDEs (n = 1,011)					
PBDE 47	-0.36 (-0.91, 0.19)	0.2001	3.74 (-8.1, 17.1)	-3.13 (-14.22, 9.39)	0.6077
PCBs (n = 900)					
PCB 105	0.87 (-4.94, 7.03)	0.7760	8.52 (-4.53, 23.36)	4.71 (-7.93, 19.07)	0.4748
PCB 118	3.04 (-2.84, 9.29)	0.3181	3.6 (-8.84, 17.73)	4.67 (-8.08, 19.19)	0.4869
PCB 123	0.11 (-4.96, 5.45)	0.9659	5.61 (-7.03, 19.97)	-2.64 (-14.47, 10.81)	0.6952
PCB 138	0.43 (-5.09, 6.28)	0.8811	7.9 (-5.03, 22.59)	0.45 (-11.7, 14.27)	0.9250
PCB 153	-0.02 (-5.56, 5.83)	0.9935	6.41 (-6.4, 20.97)	1.41 (-10.87, 15.38)	0.8153
PCB 156	0.85 (-3.99, 5.94)	0.7356	-14.85 (-25.01, -3.3)	-0.67 (-12.66, 12.96)	0.9381
PCB 157	-2.17 (-6.89, 2.79)	0.3845	13.82 (0.23, 29.25)	-8.17 (-19.23, 4.4)	0.2204
PCB 167	0.91 (-4.3, 6.4)	0.7379	6.45 (-6.37, 21.03)	-3.56 (-15.23, 9.72)	0.6074
PCB 170	-0.28 (-0.81, 0.26)	0.3089	-0.36 (-12.28, 13.18)	-13.8 (-24.19, -1.98)	0.0252
PCB 174	1.26 (-4.4, 7.27)	0.6695	3.59 (-8.82, 17.68)	5.34 (-7.61, 20.1)	0.4341
PCB 178	-0.81 (-6.21, 4.9)	0.7754	3.43 (-9.01, 17.57)	-2.44 (-14.3, 11.06)	0.7183
PCB 180	-5.79 (-10.19, -1.16)	0.0149	-6.61 (-17.77, 6.07)	-16.82 (-26.86, -5.4)	0.0052
PCB 187	-0.97 (-6.28, 4.64)	0.7293	9.8 (-3.38, 24.77)	-4.64 (-16.17, 8.49)	0.5060
PCB 194	-10 (-14.14, -5.66)	0.0000	-8.82 (-19.6, 3.4)	-25.3 (-34.32, -15.04)	0.0000

Table S4.13 Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with an increase in serum POP concentrations in SWAN MPS. Models were adjusted for age, race/ethnicity, site, education, financial strain, and serum lipids.

Compound	Continuous (per IQR)	p	Tertile 2	Tertile 3	p for trend
OCPs (n = 1116)					
cis-chlordane	0.03 (-0.51, 0.58)	0.9050	6.68 (-2.37, 16.58)	6.99 (-2.31, 17.18)	0.1470
HCB	-0.81 (-4.7, 3.24)	0.6910	7.55 (-1.5, 17.42)	-4.23 (-12.36, 4.66)	0.3452
trans-chlordane	-0.14 (-0.41, 0.14)	0.3281	-0.4 (-8.8, 8.77)	-4.85 (-12.97, 4.03)	0.2772
trans-nonachlor	-0.68 (-1.91, 0.56)	0.2814	4.89 (-3.87, 14.46)	-5.78 (-13.76, 2.95)	0.1971
PBDEs (n = 1,011)					
PBDE 47	0.3 (-0.12, 0.72)	0.1645	-7.68 (-15.77, 1.19)	-5.02 (-13.37, 4.13)	0.2733
PCBs (n = 900)					
PCB 105	0.2 (-4.23, 4.83)	0.9307	-2.52 (-11.6, 7.48)	0.09 (-9.26, 10.4)	0.9922
PCB 118	-2.16 (-6.45, 2.32)	0.3392	-2.43 (-11.48, 7.55)	-7.42 (-16.13, 2.2)	0.1288
PCB 123	1.65 (-2.29, 5.76)	0.4176	-0.91 (-10.09, 9.2)	4 (-5.77, 14.79)	0.4403
PCB 138	-0.47 (-4.67, 3.91)	0.8291	1.4 (-8.01, 11.76)	1.63 (-7.89, 12.13)	0.7456
PCB 153	0.46 (-3.8, 4.92)	0.8350	0.85 (-8.55, 11.2)	2.49 (-7.11, 13.09)	0.6255
PCB 156	-1.09 (-4.73, 2.69)	0.5670	2.58 (-6.93, 13.06)	-1.79 (-11, 8.37)	0.7137
PCB 157	1.26 (-2.48, 5.15)	0.5142	-0.2 (-9.46, 10.01)	5.97 (-3.95, 16.91)	0.2545
PCB 167	0.35 (-3.63, 4.48)	0.8669	1.64 (-7.84, 12.1)	0.8 (-8.65, 11.23)	0.8682
PCB 170	0.34 (-0.07, 0.75)	0.1003	3.73 (-5.89, 14.32)	10.17 (-0.12, 21.52)	0.0537
PCB 174	-1.02 (-5.27, 3.42)	0.6479	1.33 (-8.05, 11.68)	-4.12 (-13.23, 5.95)	0.4227
PCB 178	0.74 (-3.47, 5.13)	0.7350	2.1 (-7.4, 12.58)	2.32 (-7.31, 12.94)	0.6471
PCB 180	3 (-0.7, 6.83)	0.1135	0.52 (-8.79, 10.77)	10.7 (0.35, 22.12)	0.0447
PCB 187	0.9 (-3.25, 5.23)	0.6744	0.4 (-8.93, 10.7)	3.69 (-6.04, 14.42)	0.4760
PCB 194	4.83 (1.11, 8.69)	0.0107	6.67 (-3.16, 17.49)	13.27 (2.61, 25.05)	0.0137

Table S4.14 Percent change (95% CI) in serum concentrations of leptin associated with an increase in serum POP concentrations in SWAN MPS. Models were adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, serum lipids, and waist circumference at baseline.

Compound	Continuous (per IQR)	p	Tertile 2	Tertile 3	p for trend
OCPs (n = 1,116)					
trans-nonachlor	-0.2 (-1.5, 1.12)	0.7649	-5.7 (-14.05, 3.46)	-2.56 (-11.32, 7.07)	0.5810
Cis-chlordane	0.03 (-0.54, 0.6)	0.9296	-4.53 (-13.09, 4.88)	-5.51 (-14.17, 4.04)	0.2498
trans-chlordane	0 (-0.29, 0.29)	0.9856	-7.27 (-15.52, 1.77)	-1.96 (-10.79, 7.75)	0.6701
HCB	-3.61 (-7.6, 0.55)	0.0884	-10.9 (-18.81, -2.21)	-7.08 (-15.39, 2.06)	0.1244
PBDEs (n = 1,011)					
PBDE 47	-0.23 (-0.67, 0.22)	0.3211	-0.7 (-10, 9.56)	-7.03 (-15.74, 2.58)	0.1463
PCBs (n = 900)					
PCB 105	0.92 (-3.84, 5.91)	0.7113	1.42 (-8.51, 12.42)	0.53 (-9.44, 11.6)	0.9160
PCB 118	1.85 (-2.91, 6.85)	0.4526	-1.43 (-11.06, 9.25)	-0.47 (-10.46, 10.63)	0.9223
PCB 123	1.19 (-2.95, 5.51)	0.5787	3.6 (-6.52, 14.81)	-0.7 (-10.51, 10.2)	0.9018
PCB 138	0.54 (-3.99, 5.28)	0.8192	1.84 (-8.09, 12.85)	-2.19 (-11.91, 8.61)	0.6915
PCB 153	1.05 (-3.53, 5.85)	0.6583	-1.15 (-10.83, 9.6)	0.16 (-9.83, 11.25)	0.9835
PCB 156	0.03 (-3.88, 4.11)	0.9873	-6.03 (-15.22, 4.16)	-0.76 (-10.57, 10.13)	0.8934
PCB 157	-0.02 (-3.98, 4.1)	0.9910	7.83 (-2.71, 19.52)	-4.51 (-13.98, 6)	0.4181
PCB 167	1.11 (-3.17, 5.57)	0.6180	0.29 (-9.56, 11.22)	-4.93 (-14.43, 5.62)	0.3558
PCB 170	-0.16 (-0.59, 0.27)	0.4559	0.94 (-8.92, 11.87)	-5.65 (-15.06, 4.8)	0.2858
PCB 174	1.37 (-3.26, 6.23)	0.5685	2.43 (-7.54, 13.48)	1.24 (-8.97, 12.58)	0.8095
PCB 178	1.31 (-3.19, 6.03)	0.5740	1.22 (-8.7, 12.21)	-2.67 (-12.38, 8.12)	0.6240
PCB 180	-1.6 (-5.4, 2.36)	0.4237	-3.75 (-13.16, 6.67)	-7.91 (-17.13, 2.33)	0.1260
PCB 187	0.6 (-3.81, 5.22)	0.7937	4.14 (-6.05, 15.43)	-4.4 (-13.89, 6.14)	0.4245
PCB 194	-3.12 (-6.84, 0.75)	0.1127	-1.07 (-10.71, 9.6)	-6.51 (-16, 4.04)	0.2191

Table S4.15 Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with an increase in serum POP concentrations in SWAN MPS. Models were adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, serum lipids, and waist circumference at baseline.

Compound	Continuous (per IQR)	p	Tertile 2	Tertile 3	p for trend
OCPs (n = 1,116)					
trans-nonachlor	-0.64 (-1.81, 0.56)	0.2944	3.16 (-5.15, 12.19)	-7.86 (-15.39, 0.34)	0.0636
Cis-chlordane	0.05 (-0.46, 0.57)	0.8410	7.17 (-1.58, 16.7)	4.67 (-4.07, 14.22)	0.3087
trans-chlordane	-0.1 (-0.36, 0.16)	0.4536	-0.43 (-8.5, 8.35)	-2.91 (-10.88, 5.79)	0.5019
HCB	-0.82 (-4.56, 3.06)	0.6728	9.13 (0.32, 18.71)	-4.44 (-12.21, 4.03)	0.3006
PBDEs (n = 1,011)					
PBDE 47	0.24 (-0.16, 0.64)	0.2437	-5.9 (-13.9, 2.84)	-4.54 (-12.65, 4.34)	0.3067
PCBs (n = 900)					
PCB 105	-0.33 (-4.62, 4.17)	0.8843	-0.53 (-9.46, 9.28)	-0.02 (-9.11, 9.99)	0.9953
PCB 118	-2.23 (-6.41, 2.13)	0.3114	-1.37 (-10.19, 8.32)	-7.49 (-15.99, 1.87)	0.1184
PCB 123	1.3 (-2.49, 5.24)	0.5055	-0.33 (-9.26, 9.46)	3.08 (-6.27, 13.35)	0.5349
PCB 138	-0.91 (-4.99, 3.35)	0.6719	3.02 (-6.19, 13.14)	1.03 (-8.18, 11.17)	0.8205
PCB 153	-0.36 (-4.49, 3.95)	0.8685	2.75 (-6.48, 12.89)	0.9 (-8.32, 11.05)	0.8406
PCB 156	-1.09 (-4.63, 2.58)	0.5567	-1.5 (-10.34, 8.22)	-2.21 (-11.09, 7.55)	0.6451
PCB 157	0.25 (-3.38, 4.02)	0.8933	0.84 (-8.22, 10.8)	3.25 (-6.16, 13.61)	0.5145
PCB 167	-0.19 (-4.05, 3.83)	0.9256	2.92 (-6.35, 13.12)	-0.84 (-9.93, 9.16)	0.8817
PCB 170	0.3 (-0.1, 0.69)	0.1401	2.33 (-6.84, 12.4)	4.9 (-4.7, 15.46)	0.3288
PCB 174	-1.44 (-5.56, 2.86)	0.5052	0.82 (-8.17, 10.69)	-4.21 (-13.05, 5.54)	0.3985
PCB 178	-0.36 (-4.41, 3.86)	0.8651	2.59 (-6.62, 12.72)	0.54 (-8.66, 10.66)	0.9021
PCB 180	1.01 (-2.56, 4.71)	0.5836	-1.42 (-10.26, 8.28)	4.87 (-4.76, 15.47)	0.3434
PCB 187	-0.06 (-4.07, 4.11)	0.9753	1.45 (-7.66, 11.46)	1.97 (-7.32, 12.2)	0.6858
PCB 194	1.87 (-1.71, 5.58)	0.3100	3.21 (-6.01, 13.33)	3.31 (-6.3, 13.92)	0.5111

Table S4.16 Percent change (95% CI) in serum concentrations of leptin associated with an increase in serum POP concentrations by change in waist circumference in SWAN MPS. Models were adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and serum lipids at baseline.

Leptin % Change (95% CI)						
Compound	**ΔWC V3-V6**	**Continuous (per IQR)**	**p**	**Tertile 2**	**Tertile 3**	**p for trend**
trans-nonachlor	Stable	-1.31 (-4.04, 1.5)	0.3577	-13.1 (-28.4, 5.47)	-4.02 (-20.48, 15.86)	0.7093
	Gain	-2.22 (-9.09, 5.15)	0.5448	0.66 (-13.79, 17.53)	-0.49 (-14.95, 16.43)	0.9567
Cis-chlordane	Stable	-0.57 (-1.76, 0.63)	0.3483	-6.7 (-23.33, 13.54)	-19.22 (-33.31, -2.15)	0.0280
	Gain	1.2 (-1.91, 4.41)	0.4546	-1.99 (-16.08, 14.46)	4.08 (-11.44, 22.34)	0.6286
trans-chlordane	Stable	-0.23 (-0.8, 0.35)	0.4329	5.48 (-13.2, 28.18)	3.4 (-14.51, 25.06)	0.7361
	Gain	5.53 (-2.5, 14.23)	0.1829	-3.12 (-17.31, 13.51)	6.9 (-8.57, 24.99)	0.3888
HCB	Stable	-4.47 (-13.45, 5.45)	0.3651	-0.17 (-18, 21.55)	-2.85 (-19.84, 17.74)	0.7593
	Gain	4.91 (-3.88, 14.51)	0.2835	-6.94 (-20.35, 8.72)	-2.89 (-16.89, 13.46)	0.6908
PBDE 47	Stable	0.07 (-0.66, 0.8)	0.8564	8.76 (-10.84, 32.66)	9.77 (-9.91, 33.74)	0.3488
	Gain	-0.41 (-1.3, 0.48)	0.3646	5.82 (-10.06, 24.5)	-7.5 (-21.96, 9.64)	0.3836
PCB 105	Stable	-7.4 (-17.4, 3.82)	0.1889	-4.84 (-22.88, 17.43)	-13.14 (-30.44, 8.46)	0.2148
	Gain	3.6 (-3.37, 11.08)	0.3201	20.82 (1.32, 44.06)	18.35 (-0.92, 41.37)	0.0625
PCB 118	Stable	-5.34 (-15.27, 5.76)	0.3329	-8.51 (-25.7, 12.65)	-13.17 (-31.05, 9.34)	0.2261
	Gain	4.64 (-2.52, 12.32)	0.2104	11.85 (-6.44, 33.73)	10.09 (-7.71, 31.31)	0.2816
PCB 123	Stable	3.82 (-6.29, 15.03)	0.4736	-0.88 (-19.89, 22.65)	2.82 (-16.46, 26.55)	0.7898
	Gain	-1.97 (-7.69, 4.11)	0.5177	9.89 (-7.75, 30.9)	-3.8 (-19.53, 15)	0.6777
PCB 138	Stable	-7.75 (-18.61, 4.55)	0.2076	0.2 (-18.41, 23.06)	-9.18 (-27.01, 13.01)	0.3944
	Gain	2.04 (-4.38, 8.89)	0.5431	13.18 (-5.26, 35.22)	7.87 (-9.55, 28.64)	0.3955
PCB 153	Stable	-7.7 (-18, 3.9)	0.1855	1 (-18.13, 24.58)	-9.15 (-27.12, 13.25)	0.3960
	Gain	1.86 (-4.38, 8.51)	0.5683	9.85 (-7.99, 31.15)	5.24 (-12.01, 25.87)	0.5693
PCB 156	Stable	-7.47 (-15.65, 1.5)	0.1009	-16.88 (-32.87, 2.91)	-12.46 (-29.05, 8.02)	0.2249
	Gain	4.57 (-1.54, 11.06)	0.1465	-2.15 (-17.87, 16.57)	12.1 (-6.58, 34.51)	0.2110
PCB 157	Stable	-13.17 (-22.47, -2.76)	0.0151	8.53 (-11.62, 33.27)	-18.79 (-34.42, 0.55)	0.0610
	Gain	0.71 (-4.62, 6.33)	0.7996	10.68 (-7.54, 32.48)	-3.97 (-19.75, 14.9)	0.6555
PCB 167	Stable	-5.49 (-15.77, 6.05)	0.3377	3.52 (-16.05, 27.66)	-10.19 (-27.98, 12)	0.3465
	Gain	1.92 (-3.93, 8.12)	0.5291	7.59 (-9.95, 28.55)	2.09 (-14.48, 21.87)	0.7995
PCB 170	Stable	-0.23 (-0.73, 0.28)	0.3746	-6.62 (-24.18, 15.02)	-21.09 (-36.6, -1.77)	0.0343
	Gain	-2.14 (-6.84, 2.8)	0.3899	-4.12 (-19.83, 14.65)	-7.66 (-22.75, 10.37)	0.3807
PCB 174	Stable	-7.31 (-18.15, 4.97)	0.2325	-3.86 (-22.05, 18.57)	-11.63 (-29.64, 10.98)	0.2901
	Gain	2.46 (-3.83, 9.16)	0.4531	12.99 (-5.31, 34.83)	12.26 (-6.2, 34.36)	0.2058
PCB 178	Stable	-12.5 (-21.8, -2.09)	0.0206	4.24 (-15.13, 28.05)	-16.77 (-33.46, 4.11)	0.1200
	Gain	1.29 (-5.06, 8.05)	0.6987	-4.93 (-20.52, 13.74)	0.2 (-15.92, 19.42)	0.9935

PCB 180	Stable	-16.29 (-24.77, -6.86)	0.0012	-6.57 (-24.11, 15.03)	-25.73 (-40.27, -7.65)	0.0080
	Gain	-2.79 (-7.72, 2.41)	0.2879	-11.15 (-25.63, 6.15)	-8.01 (-22.84, 9.66)	0.3432
PCB 187	Stable	-9.64 (-19.5, 1.43)	0.0867	12.49 (-8.39, 38.12)	-16.33 (-32.54, 3.78)	0.1159
	Gain	1.74 (-4.28, 8.14)	0.5797	4.84 (-12.09, 25.04)	5.33 (-11.78, 25.77)	0.5616
PCB 194	Stable	-21.54 (-29.78, -12.34)	0.0000	-4.66 (-22.53, 17.34)	-27.04 (-41.06, -9.7)	0.0038
	Gain	-6.76 (-11.54, -1.72)	0.0095	-13.16 (-26.87, 3.12)	-22.36 (-35.11, -7.11)	0.0059

Table S4.17 Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with an increase in serum POP concentrations by change in waist circumference in SWAN MPS. Models were adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and serum lipids at baseline.

HMW Adiponectin % Change (95% CI)						
Compound	**ΔWC V3-V6**	**Continuous (per IQR)**	**p**	**Tertile 2**	**Tertile 3**	**p for trend**
trans-nonachlor	Stable	-0.94 (-3.15, 1.32)	0.4135	7.89 (-7.45, 25.78)	-16.53 (-28.09, -3.11)	0.0146
	Gain	1.71 (-4.24, 8.04)	0.5812	-3.12 (-14.79, 10.16)	0.08 (-12.14, 14)	0.9848
Cis-chlordane	Stable	0.49 (-0.47, 1.47)	0.3169	-4.17 (-18.22, 12.29)	3.16 (-11.63, 20.43)	0.6664
	Gain	2.73 (0.11, 5.42)	0.0412	10.93 (-2.43, 26.13)	8.22 (-5.32, 23.7)	0.2459
trans-chlordane	Stable	-0.04 (-0.5, 0.43)	0.8812	-10.27 (-23.21, 4.86)	-14.01 (-26.13, 0.11)	0.0532
	Gain	2.77 (-3.76, 9.76)	0.4148	7 (-6.17, 22.03)	6.07 (-6.83, 20.76)	0.3828
HCB	Stable	-3.4 (-10.77, 4.58)	0.3934	0.74 (-13.94, 17.93)	-9.5 (-22.41, 5.55)	0.1823
	Gain	-0.8 (-7.75, 6.68)	0.8289	4.98 (-7.72, 19.44)	-1.32 (-13.27, 12.28)	0.8654
PBDE 47	Stable	0.03 (-0.55, 0.62)	0.9085	-17.5 (-29.59, -3.34)	-18.64 (-30.51, -4.76)	0.0097
	Gain	0.25 (-0.48, 0.99)	0.5085	-2.8 (-14.97, 11.1)	2.05 (-11.26, 17.36)	0.7852
PCB 105	Stable	-0.34 (-9.13, 9.31)	0.9428	-3.83 (-18.84, 13.95)	-4.55 (-20.22, 14.18)	0.6087
	Gain	-1.53 (-7, 4.26)	0.5964	-2.46 (-15.64, 12.77)	-2.77 (-16.03, 12.58)	0.7055
PCB 118	Stable	-1.25 (-9.7, 7.98)	0.7823	-2.7 (-17.68, 15.01)	-13.74 (-28.33, 3.82)	0.1252
	Gain	-2.73 (-8.22, 3.08)	0.3499	-2.03 (-15.38, 13.44)	-5.45 (-18.19, 9.26)	0.4481
PCB 123	Stable	0.95 (-7.06, 9.64)	0.8234	5.75 (-10.91, 25.53)	2.14 (-13.59, 20.73)	0.8117
	Gain	1.74 (-3.15, 6.87)	0.4931	0.39 (-13.05, 15.91)	1 (-12.78, 16.96)	0.8943
PCB 138	Stable	0.39 (-9.26, 11.08)	0.9391	2.63 (-13.03, 21.12)	-4.26 (-19.73, 14.18)	0.6377
	Gain	-1.27 (-6.39, 4.13)	0.6377	4.7 (-9.52, 21.16)	2.38 (-11.4, 18.3)	0.7466
PCB 153	Stable	0.39 (-8.76, 10.46)	0.9363	-2.58 (-17.76, 15.39)	-4.13 (-19.74, 14.53)	0.6415
	Gain	-0.95 (-5.96, 4.32)	0.7171	0.68 (-12.94, 16.44)	0.3 (-13.4, 16.16)	0.9677
PCB 156	Stable	0.99 (-6.29, 8.84)	0.7965	-11.81 (-25.77, 4.77)	-4.64 (-19.52, 12.98)	0.6004
	Gain	-1.65 (-6.39, 3.33)	0.5103	5.9 (-8.29, 22.27)	0.72 (-13.28, 16.99)	0.9416
PCB 157	Stable	4.61 (-4.58, 14.69)	0.3375	1.31 (-14.31, 19.78)	4.23 (-12.44, 24.08)	0.6418
	Gain	-0.08 (-4.43, 4.47)	0.9722	1.42 (-12.51, 17.58)	2.9 (-11.21, 19.25)	0.7043
PCB 167	Stable	1.91 (-7.13, 11.83)	0.6901	4.27 (-11.96, 23.5)	-0.06 (-16.38, 19.45)	0.9996
	Gain	-1.07 (-5.74, 3.84)	0.6633	-0.36 (-13.88, 15.28)	-5 (-17.83, 9.83)	0.4945
PCB 170	Stable	0.37 (-0.04, 0.78)	0.0772	14.17 (-3.53, 35.13)	10.36 (-7.54, 31.74)	0.2797
	Gain	0.22 (-3.75, 4.35)	0.9157	-0.41 (-13.99, 15.32)	0.56 (-13.12, 16.4)	0.9409
PCB 174	Stable	0.7 (-8.93, 11.33)	0.8925	-2.41 (-17.58, 15.57)	-7.11 (-22.7, 11.62)	0.4334
	Gain	-2.13 (-7.08, 3.08)	0.4162	0.65 (-12.93, 16.36)	-6.27 (-19.12, 8.62)	0.3917
PCB 178	Stable	-1.69 (-10.27, 7.72)	0.7153	6.32 (-9.99, 25.59)	-3.85 (-19.79, 15.26)	0.6964
	Gain	-0.57 (-5.7, 4.84)	0.8340	-1.04 (-14.55, 14.62)	-1.24 (-14.47, 14.03)	0.8632

PCB 180	Stable	4.7 (-4.06, 14.27)	0.3034	3.46 (-12.68, 22.58)	6.31 (-11, 26.98)	0.4994
	Gain	0.55 (-3.65, 4.94)	0.8004	1.37 (-12.4, 17.3)	3.96 (-10.01, 20.09)	0.5986
PCB 187	Stable	1.58 (-7.49, 11.54)	0.7424	-3.9 (-18.7, 13.6)	3.84 (-12.87, 23.77)	0.6825
	Gain	-0.67 (-5.51, 4.42)	0.7914	3.82 (-10.13, 19.94)	-1.25 (-14.6, 14.18)	0.8764
PCB 194	Stable	3.98 (-5.15, 13.99)	0.4060	9.22 (-7.83, 29.43)	3.36 (-13.17, 23.05)	0.7229
	Gain	1.79 (-2.54, 6.31)	0.4238	13.63 (-1.36, 30.9)	13.78 (-1.83, 31.88)	0.0859

Table S4.18 Percent change (95% CI) in serum concentrations of leptin associated with an increase in serum POP concentrations by change in waist circumference in SWAN MPS. Models were adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, serum lipids, and waist circumference at baseline.

Leptin % Change (95% CI)						
Compound	**ΔWC V3-V6**	**Continuous (per IQR)**	**p**	**Tertile 2**	**Tertile 3**	**p for trend**
trans-nonachlor	Stable	-1.39 (-3.61, 0.88)	0.2286	-17.99 (-29.88, -4.08)	-10.81 (-23.42, 3.87)	0.1668
	Gain	-1.35 (-6.78, 4.41)	0.6393	-2.71 (-13.77, 9.77)	-1.54 (-12.87, 11.27)	0.7876
Cis-chlordane	Stable	-0.45 (-1.42, 0.53)	0.3667	-9.32 (-22.67, 6.33)	-18.09 (-29.88, -4.31)	0.0121
	Gain	0.38 (-2.04, 2.85)	0.7632	-5.28 (-16.05, 6.87)	3.13 (-9.05, 16.93)	0.6341
trans-chlordane	Stable	-0.19 (-0.65, 0.28)	0.4291	5.28 (-10.09, 23.27)	-8.04 (-21.23, 7.36)	0.2837
	Gain	1.91 (-4.2, 8.42)	0.5482	-4.02 (-15.16, 8.58)	1.53 (-10.13, 14.69)	0.7898
HCB	Stable	-4.8 (-12.14, 3.14)	0.2295	-11.33 (-24.46, 4.08)	-9.57 (-22.63, 5.69)	0.2349
	Gain	-0.49 (-7.08, 6.56)	0.8877	-9.67 (-19.95, 1.94)	-8.93 (-19.32, 2.79)	0.1219
PBDE 47	Stable	0.16 (-0.43, 0.75)	0.5945	-4.4 (-18.71, 12.44)	-0.77 (-15.52, 16.56)	0.9157
	Gain	-0.09 (-0.78, 0.61)	0.8095	2.09 (-10.09, 15.9)	-4.31 (-16.21, 9.27)	0.5254
PCB 105	Stable	-5.77 (-14.15, 3.42)	0.2113	-6.67 (-21.32, 10.69)	-13.83 (-28.05, 3.19)	0.1061
	Gain	2.1 (-3.34, 7.84)	0.4573	6.42 (-7.45, 22.37)	7.71 (-6.42, 23.97)	0.3006
PCB 118	Stable	-3.52 (-11.85, 5.59)	0.4365	-5.71 (-20.39, 11.68)	-11.34 (-26.5, 6.94)	0.2082
	Gain	2.21 (-3.32, 8.06)	0.4408	-0.56 (-13.65, 14.51)	2.57 (-10.72, 17.84)	0.7215
PCB 123	Stable	-2.59 (-10.43, 5.95)	0.5414	-5.63 (-20.63, 12.2)	-7.77 (-22.15, 9.27)	0.3515
	Gain	0.67 (-3.98, 5.54)	0.7817	10.75 (-3.43, 27.02)	1.34 (-11.91, 16.57)	0.8443
PCB 138	Stable	-5.43 (-14.59, 4.72)	0.2843	-6.33 (-20.78, 10.74)	-8.09 (-23.06, 9.79)	0.3484
	Gain	1.81 (-3.25, 7.12)	0.4909	9.19 (-5.03, 25.55)	3.01 (-10.29, 18.27)	0.6691
PCB 153	Stable	-5.79 (-14.44, 3.74)	0.2262	-5.33 (-20.21, 12.33)	-8.87 (-23.82, 9.02)	0.3094
	Gain	1.64 (-3.28, 6.81)	0.5205	-1.67 (-14.5, 13.08)	2.26 (-11.14, 17.69)	0.7582
PCB 156	Stable	-3.19 (-10.26, 4.44)	0.4027	-8.83 (-23.46, 8.59)	-2.92 (-18.28, 15.34)	0.7537
	Gain	1.9 (-2.82, 6.84)	0.4368	1.21 (-11.81, 16.15)	5.98 (-8.18, 22.32)	0.4241
PCB 157	Stable	-8.85 (-16.92, 0.01)	0.0511	7.17 (-9.38, 26.74)	-11.76 (-25.93, 5.13)	0.1715
	Gain	1.63 (-2.61, 6.05)	0.4581	6.94 (-7.14, 23.16)	-1.42 (-14.37, 13.5)	0.8410
PCB 167	Stable	-4.85 (-13.35, 4.49)	0.2986	0.66 (-15.14, 19.38)	-8.03 (-23.16, 10.08)	0.3660
	Gain	1.53 (-3.06, 6.34)	0.5204	-0.6 (-13.59, 14.34)	-0.99 (-13.83, 13.77)	0.8884
PCB 170	Stable	-0.12 (-0.53, 0.29)	0.5745	-9.34 (-23.51, 7.45)	-15.6 (-29.42, 0.92)	0.0635
	Gain	0.26 (-3.55, 4.23)	0.8940	-2.07 (-14.89, 12.68)	-2.4 (-15.16, 12.27)	0.7326
PCB 174	Stable	-5.58 (-14.67, 4.48)	0.2675	-6.86 (-21.46, 10.44)	-11.47 (-26.43, 6.54)	0.1963
	Gain	1.85 (-3.08, 7.04)	0.4689	8.56 (-5.51, 24.72)	4.92 (-8.9, 20.84)	0.5019
PCB 178	Stable	-10.63 (-18.44, -2.07)	0.0166	0.85 (-14.67, 19.19)	-16.41 (-30.3, 0.26)	0.0598
	Gain	3.06 (-2.02, 8.42)	0.2434	-1.52 (-14.43, 13.34)	3.13 (-10.12, 18.34)	0.6681

PCB 180	Stable	-11.03 (-18.52, -2.85)	0.0096	-9.25 (-23.45, 7.58)	-17.47 (-31, -1.29)	0.0360
	Gain	-0.03 (-4.05, 4.16)	0.9879	-6.23 (-18.46, 7.84)	-2.49 (-15.07, 11.95)	0.7096
PCB 187	Stable	-7.26 (-15.6, 1.89)	0.1176	9.18 (-7.66, 29.08)	-11.9 (-26.1, 5.03)	0.1722
	Gain	1.8 (-2.95, 6.78)	0.4642	-0.59 (-13.43, 14.16)	1.27 (-11.89, 16.38)	0.8624
PCB 194	Stable	-13.49 (-21.17, -5.07)	0.0024	2.96 (-13.19, 22.12)	-14.57 (-28.4, 1.94)	0.0779
	Gain	-1.06 (-5.16, 3.21)	0.6210	-8.24 (-19.91, 5.12)	-2.4 (-15.55, 12.81)	0.7235

Table S4.19 Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with an increase in serum POP concentrations by change in waist circumference in SWAN MPS. Models were adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, serum lipids, and waist circumference at baseline.

HMW Adiponectin % Change (95% CI)						
Compound	**ΔWC V3-V6**	**Continuous (per IQR)**	**p**	**Tertile 2**	**Tertile 3**	**p for trend**
trans-nonachlor	Stable	-0.9 (-3.01, 1.25)	0.4085	10.52 (-4.52, 27.93)	-13.95 (-25.36, -0.78)	0.0316
	Gain	1.43 (-4.35, 7.57)	0.6349	-2.1 (-13.61, 10.95)	0.41 (-11.55, 13.97)	0.9683
Cis-chlordane	Stable	0.44 (-0.48, 1.37)	0.3486	-3.01 (-16.61, 12.81)	2.55 (-11.51, 18.85)	0.7160
	Gain	2.99 (0.44, 5.61)	0.0217	12.12 (-1.05, 27.04)	8.53 (-4.7, 23.61)	0.2167
trans-chlordane	Stable	-0.05 (-0.49, 0.39)	0.8136	-10.2 (-22.6, 4.2)	-9.75 (-22, 4.42)	0.1715
	Gain	3.9 (-2.54, 10.78)	0.2417	7.32 (-5.56, 21.95)	7.79 (-5, 22.3)	0.2509
HCB	Stable	-3.25 (-10.3, 4.34)	0.3913	5.94 (-8.88, 23.18)	-6.71 (-19.44, 8.03)	0.2998
	Gain	0.84 (-6.08, 8.26)	0.8181	5.95 (-6.56, 20.13)	0.64 (-11.26, 14.14)	0.8943
PBDE 47	Stable	0 (-0.56, 0.56)	0.9875	-13.17 (-25.47, 1.18)	-15.32 (-27.23, -1.45)	0.0308
	Gain	0.15 (-0.57, 0.87)	0.6829	-1.75 (-13.76, 11.93)	1.02 (-11.85, 15.77)	0.8903
PCB 105	Stable	-1.1 (-9.41, 7.96)	0.8047	-3 (-17.43, 13.95)	-4.22 (-19.2, 13.55)	0.6188
	Gain	-1.07 (-6.42, 4.58)	0.7041	1.64 (-11.82, 17.14)	0.24 (-13.11, 15.65)	0.9730
PCB 118	Stable	-2.08 (-10.05, 6.59)	0.6276	-3.99 (-18.08, 12.52)	-14.53 (-28.31, 1.89)	0.0846
	Gain	-2.01 (-7.39, 3.69)	0.4825	1.74 (-11.83, 17.41)	-3.28 (-15.99, 11.35)	0.6447
PCB 123	Stable	3.86 (-4.01, 12.39)	0.3471	8.08 (-8.15, 27.18)	7.19 (-8.6, 25.7)	0.3986
	Gain	0.88 (-3.84, 5.84)	0.7194	0.14 (-12.91, 15.14)	-0.67 (-13.88, 14.56)	0.9264
PCB 138	Stable	-0.71 (-9.8, 9.3)	0.8853	5.78 (-9.61, 23.8)	-4.77 (-19.41, 12.53)	0.5839
	Gain	-1.2 (-6.18, 4.04)	0.6475	5.92 (-8.09, 22.06)	3.92 (-9.7, 19.59)	0.5890
PCB 153	Stable	-0.52 (-9.15, 8.94)	0.9110	0.24 (-14.66, 17.75)	-4.26 (-19.12, 13.33)	0.6147
	Gain	-0.89 (-5.75, 4.23)	0.7293	4.36 (-9.44, 20.27)	1.23 (-12.22, 16.75)	0.8621
PCB 156	Stable	-1.03 (-7.84, 6.29)	0.7770	-15.46 (-28.22, -0.44)	-9.05 (-22.59, 6.86)	0.2667
	Gain	-0.83 (-5.49, 4.06)	0.7342	4.76 (-8.91, 20.48)	2.55 (-11.35, 18.62)	0.7437
PCB 157	Stable	2.38 (-6.22, 11.76)	0.5995	1.89 (-13.09, 19.45)	0.41 (-14.95, 18.53)	0.9590
	Gain	-0.37 (-4.59, 4.03)	0.8669	2.55 (-11.17, 18.39)	2.03 (-11.59, 17.75)	0.7836
PCB 167	Stable	1.61 (-6.96, 10.97)	0.7229	5.59 (-10.08, 23.98)	-1.11 (-16.5, 17.11)	0.9057
	Gain	-0.95 (-5.5, 3.82)	0.6909	2.21 (-11.32, 17.81)	-4.06 (-16.67, 10.46)	0.5774
PCB 170	Stable	0.32 (-0.07, 0.71)	0.1067	15.7 (-1.38, 35.75)	7.08 (-9.49, 26.69)	0.4299
	Gain	-0.56 (-4.4, 3.43)	0.7799	-1.08 (-14.22, 14.06)	-1.21 (-14.31, 13.89)	0.8660
PCB 174	Stable	-0.13 (-9.21, 9.86)	0.9791	-1.03 (-15.7, 16.2)	-7.19 (-22.03, 10.49)	0.4067
	Gain	-1.95 (-6.77, 3.12)	0.4451	1.94 (-11.46, 17.38)	-4.22 (-17.03, 10.56)	0.5582
PCB 178	Stable	-2.61 (-10.7, 6.22)	0.5507	7.91 (-7.86, 26.37)	-4.04 (-19.19, 13.96)	0.6702
	Gain	-1.12 (-6.08, 4.11)	0.6690	-2.15 (-15.16, 12.86)	-2.15 (-14.91, 12.52)	0.7571

PCB 180	Stable	1.89 (-6.28, 10.77)	0.6615	4.82 (-10.77, 23.14)	1.37 (-14.43, 20.09)	0.8676
	Gain	-0.35 (-4.41, 3.89)	0.8707	-0.37 (-13.56, 14.84)	2.04 (-11.32, 17.42)	0.7807
PCB 187	Stable	0.42 (-8.12, 9.75)	0.9264	-2.61 (-16.93, 14.17)	1.49 (-14.11, 19.92)	0.8691
	Gain	-0.69 (-5.4, 4.25)	0.7793	5.62 (-8.21, 21.52)	0.01 (-13.16, 15.16)	0.9850
PCB 194	Stable	-0.61 (-9.05, 8.61)	0.8922	5.46 (-10.26, 23.94)	-3.82 (-18.62, 13.68)	0.6359
	Gain	-0.14 (-4.34, 4.24)	0.9493	11.67 (-2.71, 28.18)	5.87 (-8.59, 22.62)	0.4306

Figure S4.6 K-means Clusters (k = 3) of serum concentrations of POPs (ng/g lipid) in SWAN MPS. Concentrations were scaled, centered, and log-transformed.

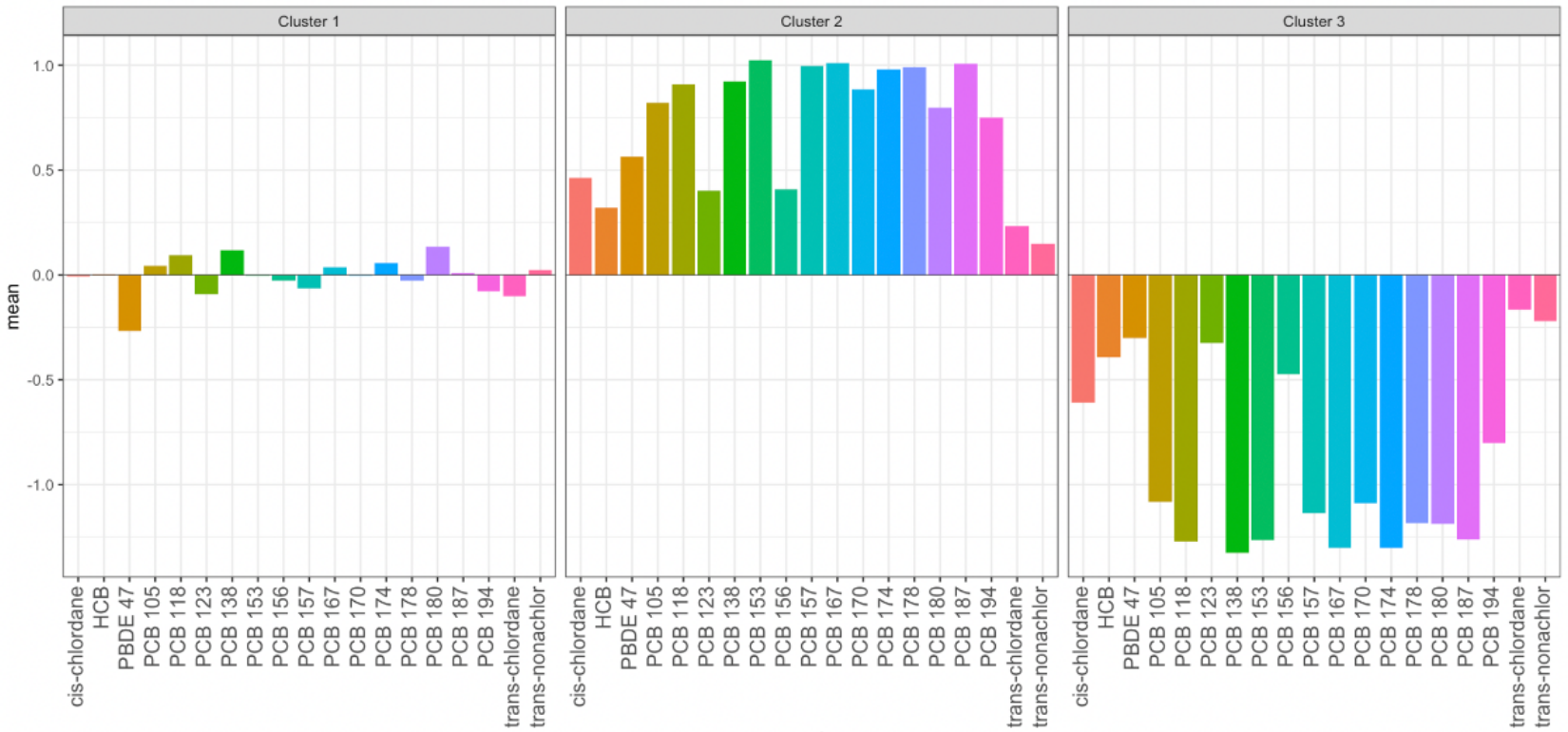
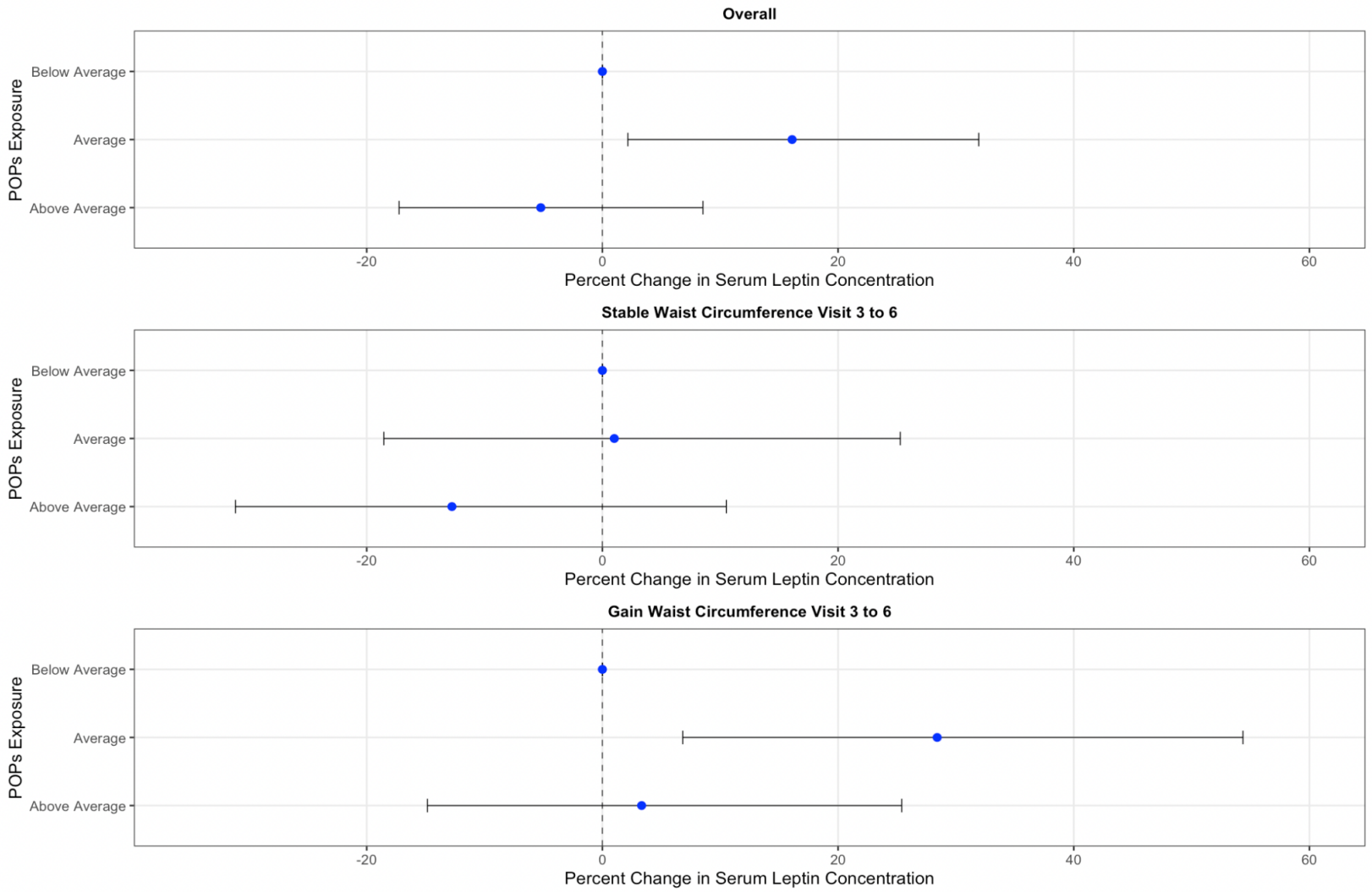
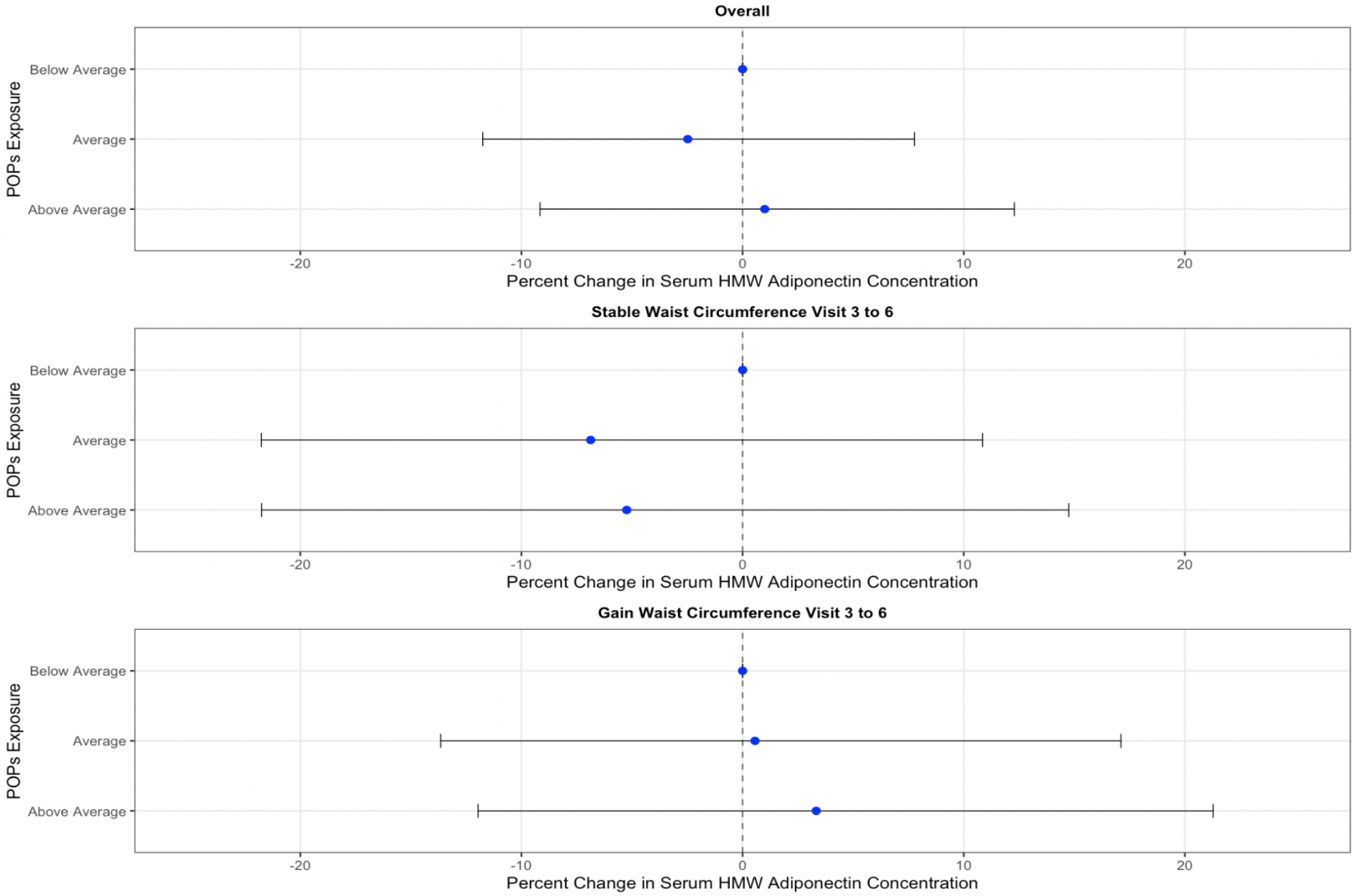


Figure S4.7 Percent change (95% CI) in serum concentrations of leptin associated with exposure to a three-cluster POP mixture by waist circumference change (gain vs. stable) between 1999/2000 and 2002/03 in SWAN MPS.



WC = waist circumference. Stable = -2 cm < ΔWC < 2 cm; Gain = ΔWC ≥ 2 cm

Figure S4.8 Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with exposure to a three-cluster POP mixture by waist circumference change between 1999/2000 and 2002/03 in SWAN MPS.



WC = waist circumference. Stable = -2 cm < ΔWC < 2 cm; Gain = ΔWC ≥ 2 cm

Figure S4.9 K-means Clusters (k = 2) of serum concentrations of POPs (ng/g lipid) in SWAN MPS. Concentrations were scaled, centered, and log-transformed.

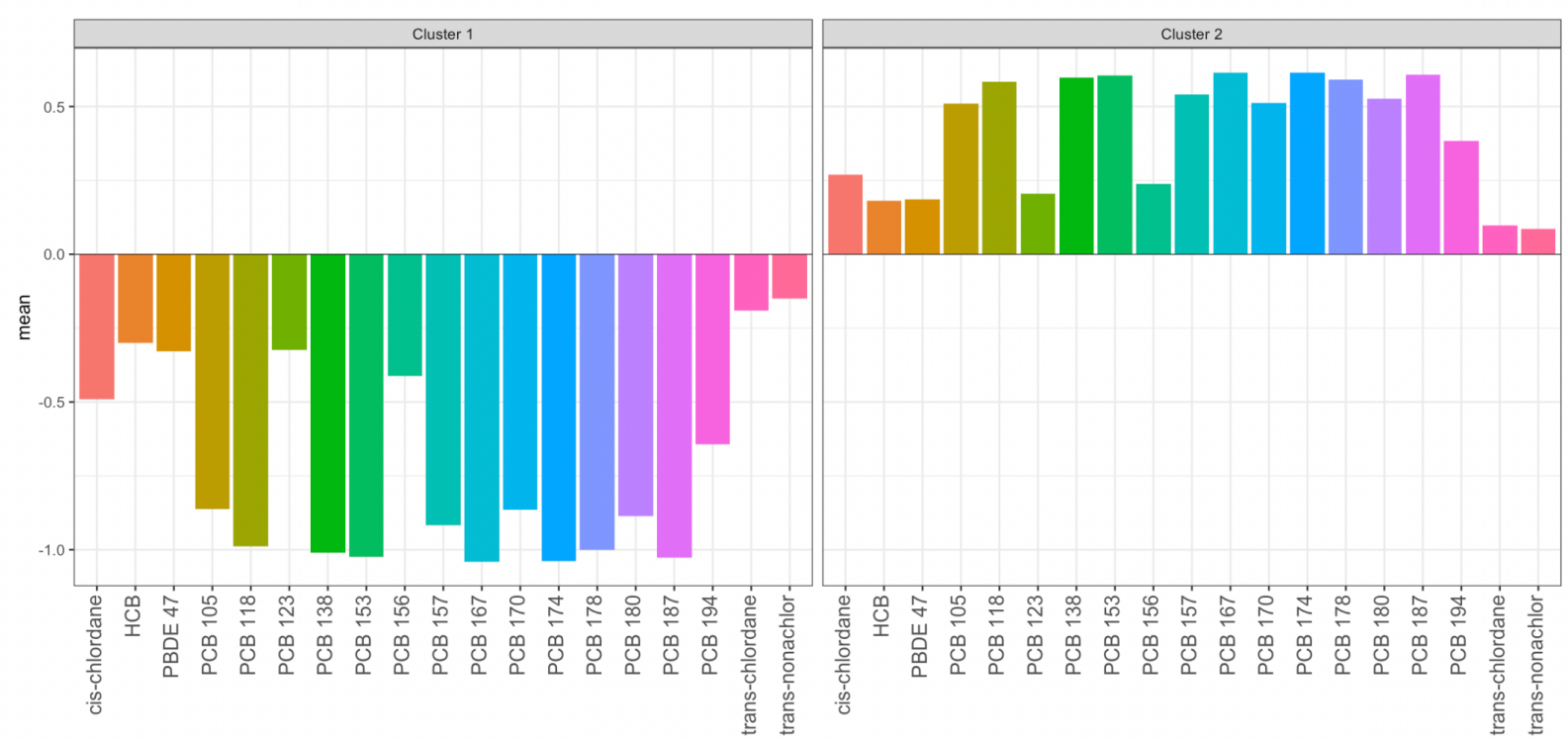


Table S4.20 Association between exposure to a two-cluster POP mixture and serum concentrations of leptin and high molecular weight adiponectin. Estimates of above-average exposure relative to below-average exposure. Model 3 was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and serum lipids. Model 4 was additionally adjusted for waist circumference at baseline.

	Leptin		**HMW Adiponectin**	
Model	**% Change (95% CI)**	**p**	**% Change (95% CI)**	**p**
3	-1.99 (-12, 9.16)	0.7149	2.85 (-5.4, 11.82)	0.5106
4	-1.74 (-10.15, 7.45)	0.6999	2.76 (-5.25, 11.45)	0.5109

Figure S4.10 K-means Clusters (k = 4) of Serum POP Concentrations in SWAN MPS

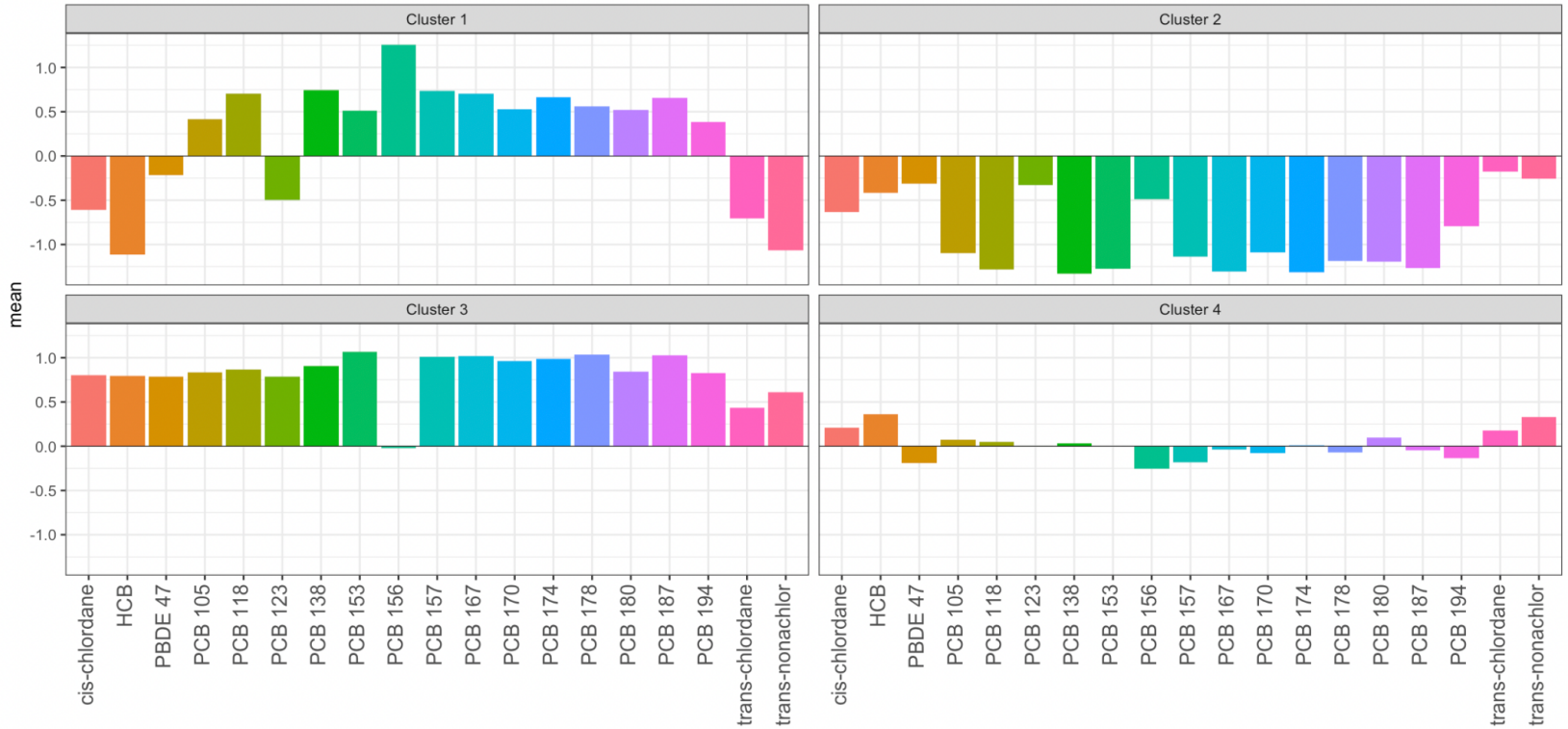


Table S4.21 Association between exposure to a four-cluster POP mixture and serum concentrations of leptin and high molecular weight adiponectin. Estimates are relative to below-average exposure. Model 3 was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and serum lipids. Model 4 was additionally adjusted for waist circumference at baseline.

		Leptin		**HMW Adiponectin**	
Model	**Cluster (2nd is ref)**	**% Change (95% CI)**	**p**	**% Change (95% CI)**	**p**
3	Average	15.6 (1.09, 32.2)	0.0345	-4.04 (-13.58, 6.55)	0.4403
	Above Average	-7.1 (-19.97, 7.84)	0.3332	0.62 (-10.44, 13.04)	0.9175
	Other	6.01 (-9.58, 24.29)	0.4724	-0.94 (-12.51, 12.15)	0.8811
4	Average	8.48 (-3, 21.33)	0.1542	-1.95 (-11.45, 8.56)	0.7046
	Above Average	-2.52 (-13.92, 10.38)	0.6870	-1.01 (-11.6, 10.85)	0.8605
	Other	6.97 (-6.3, 22.13)	0.3191	-1.25 (-12.47, 11.41)	0.8386

Table S4.22 Association between exposure to a three-cluster POP mixture and serum concentrations of leptin and high molecular weight adiponectin. Estimates are relative to below-average exposure. Model 3 was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and serum lipids. Model 4 was additionally adjusted for waist circumference at baseline.

		Leptin		**HMW Adiponectin**	
Model	**Cluster (2nd is ref)**	**% Change (95% CI)**	**p**	**% Change (95% CI)**	**p**
3	Average	16.1 (2.16, 31.94)	0.0224	-2.48 (-11.75, 7.77)	0.6223
	Above Average	-5.23 (-17.25, 8.53)	0.4376	1 (-9.16, 12.29)	0.8540
4	Average	10.36 (-0.79, 22.77)	0.0702	-0.79 (-9.97, 9.33)	0.8732
	Above Average	-2.92 (-13.28, 8.69)	0.6074	0.18 (-9.62, 11.04)	0.9733

Figure S4.11 Association between serum concentrations of leptin and individual POP mixture components in SWAN MPS.

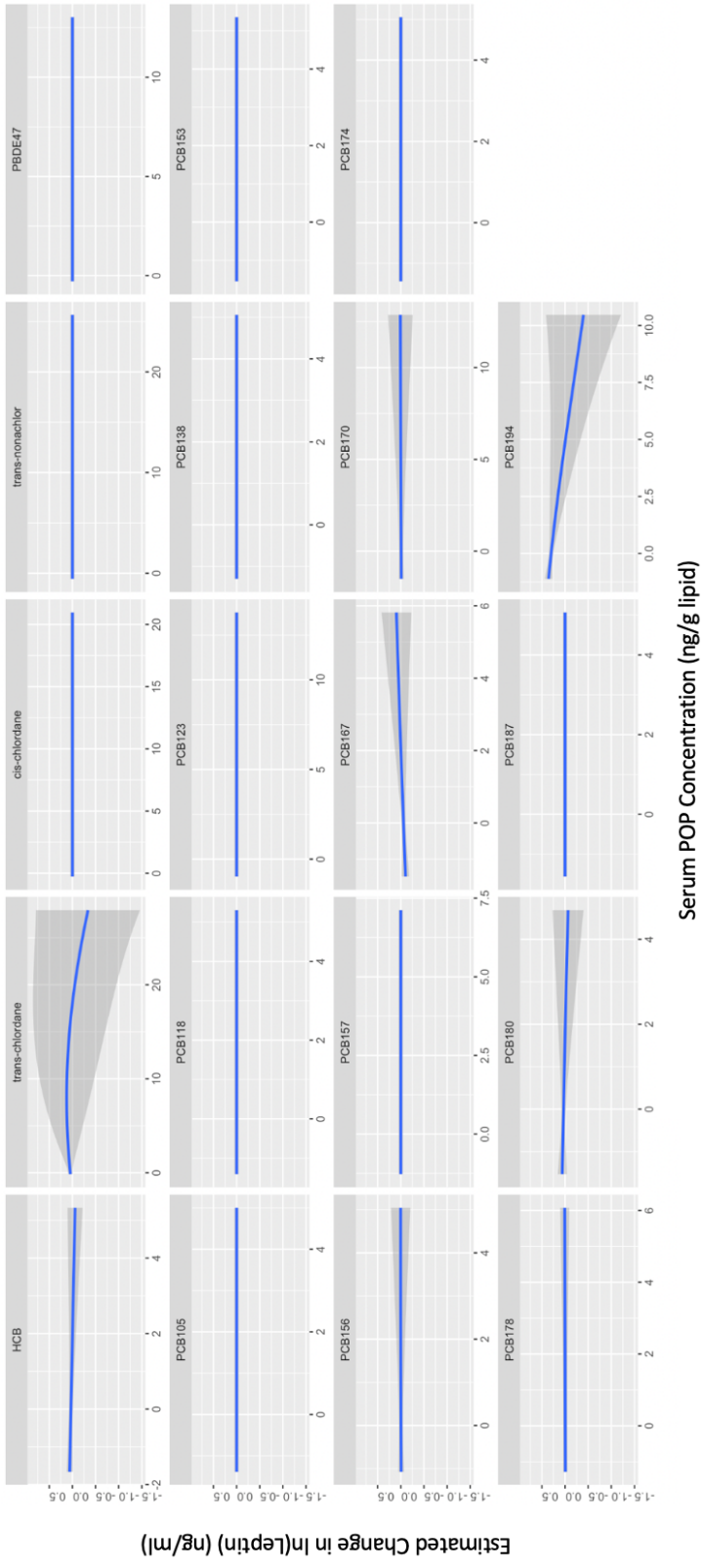


Figure S4.12 Association between serum concentrations of high molecular weight adiponectin and individual POP mixture components in SWAN MPS.

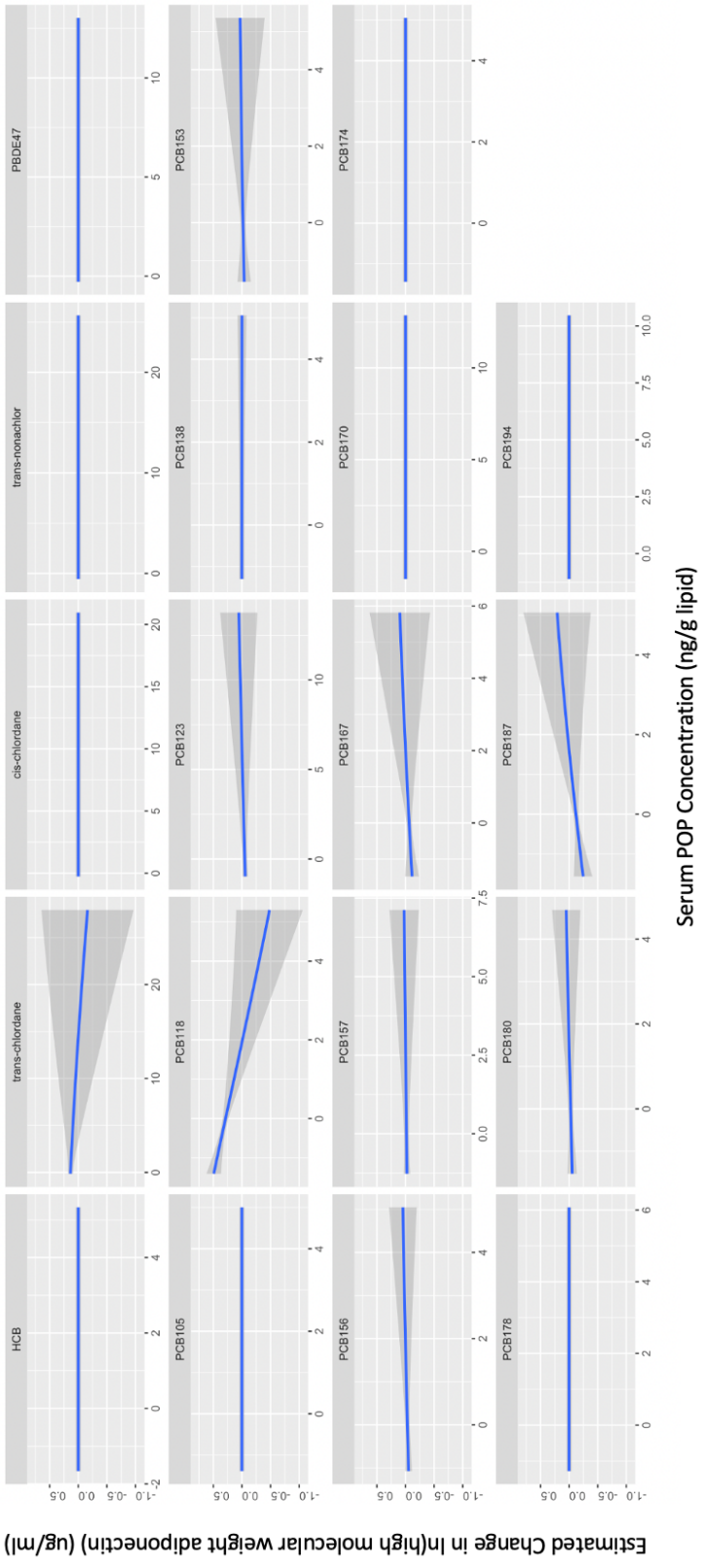
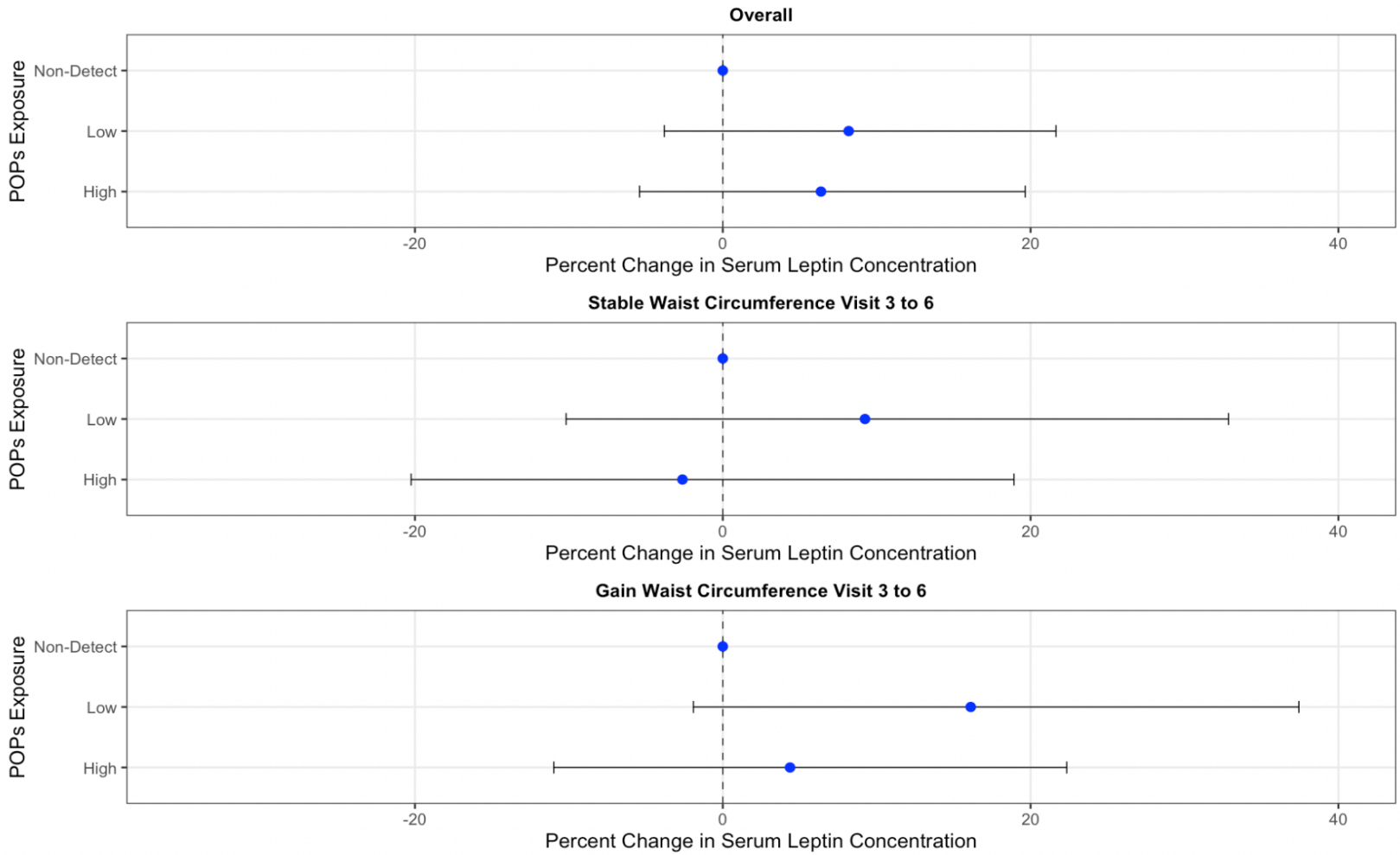
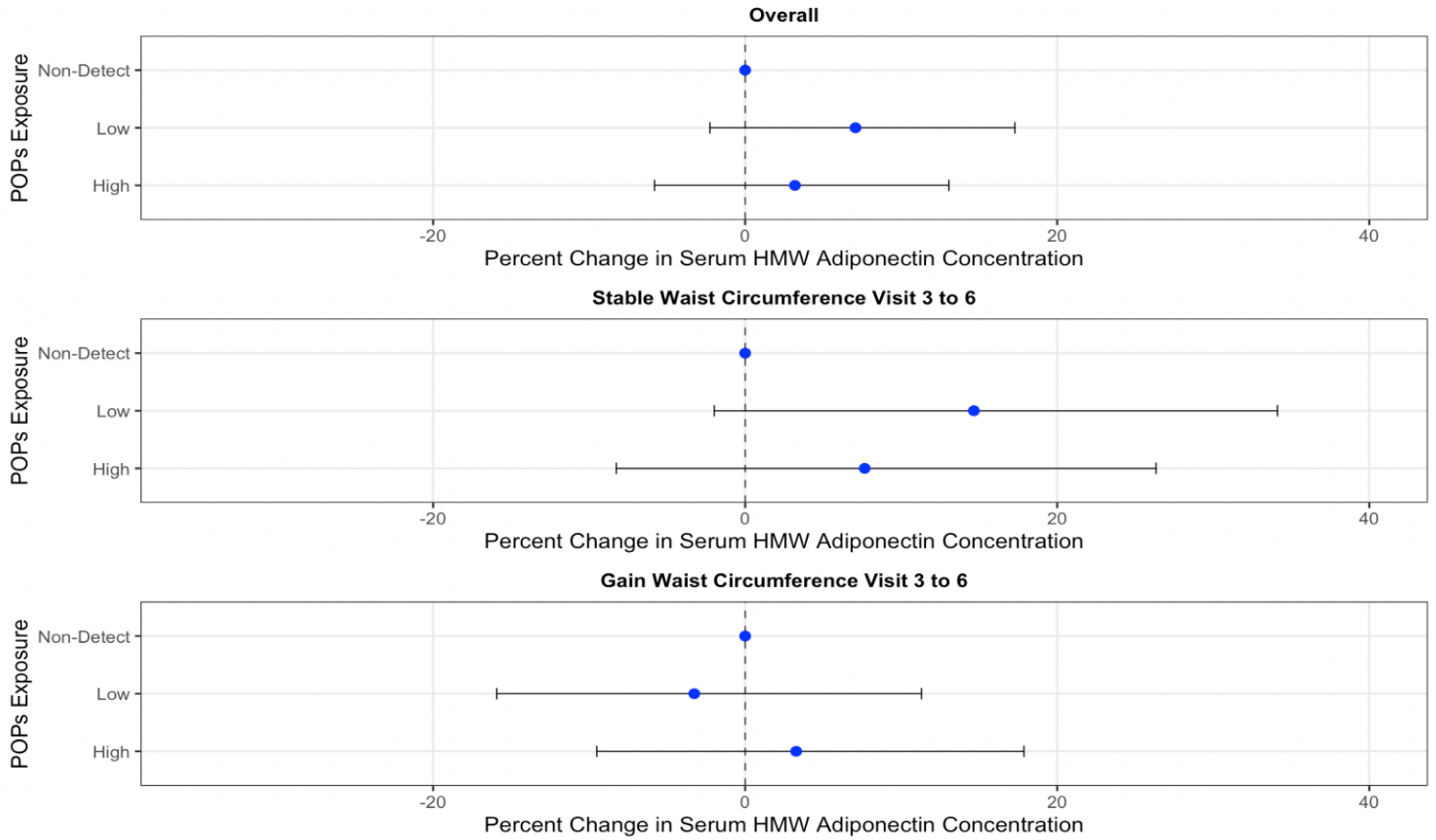


Figure S4.13 Percent change (95% CI) in serum concentrations of leptin associated with p,p'-DDE exposure by waist circumference change (gain vs. stable) between 1999/2000 and 2002/03 in SWAN MPS.



WC = waist circumference. Stable = -2 cm < ΔWC < 2 cm; Gain = ΔWC ≥ 2 cm

Figure S4.14 Percent change (95% CI) in serum concentrations of high molecular weight adiponectin associated with p,p'-DDE exposure by waist circumference change (gain vs. stable) between 1999/2000 and 2002/03 in SWAN MPS.



WC = waist circumference. Stable = -2 cm < ΔWC < 2 cm; Gain = ΔWC ≥ 2 cm

Table S4.23 Association between exposure to a three-cluster POP mixture and serum concentrations of leptin and high molecular weight adiponectin in SWAN MPS. Estimates are relative to below-average exposure. Model 3 was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and serum lipids. Model 4 was additionally adjusted for waist circumference at baseline.

	Model	**Leptin**		**HMW Adiponectin**	
POP		**% Change (95% CI)**	**p**	**% Change (95% CI)**	**p**
p,p'-DDT	3	2.95 (-6.32, 13.14)	0.5457	3.45 (-3.86, 11.32)	0.3647
	4	-0.12 (-7.65, 8.03)	0.9765	4.53 (-2.64, 12.24)	0.2217
p,p'-DDE	3	7.28 (-2.23, 17.71)	0.1382	5.12 (-2.19, 12.98)	0.1751
	4	3.57 (-4.13, 11.88)	0.3736	6.41 (-0.78, 14.12)	0.0820
p,p'-DDD	3	3.98 (-5.22, 14.07)	0.4089	-3.68 (-10.37, 3.51)	0.3073
	4	3.35 (-4.3, 11.62)	0.4006	-3.48 (-9.99, 3.49)	0.3200

Table S4.24 Association between exposure to a three-cluster POP mixture and serum concentrations of leptin and high molecular weight adiponectin in SWAN MPS. Estimates are relative to below-average exposure. Model 3 was adjusted for age, race/ethnicity, site, smoking, alcohol, parity, education, financial strain, exercise, menopausal status, and serum lipids. Model 4 was additionally adjusted for waist circumference at baseline.

			Leptin		**HMW Adiponectin**	
POP	**Model**	**"Tertile" (ref: <MDL)**	**% Change (95% CI)**	**p**	**% Change (95% CI)**	**p**
p,p'-DDT	3	2nd	2.13 (-9.25, 14.94)	0.7264	0.16 (-8.62, 9.78)	0.9725
		3rd	3.84 (-8.02, 17.22)	0.5428	7.09 (-2.53, 17.66)	0.1542
	4	2nd	-1.03 (-10.28, 9.18)	0.8364	1.25 (-7.37, 10.67)	0.7839
		3rd	0.87 (-8.8, 11.56)	0.8666	8.17 (-1.27, 18.5)	0.0922
p,p'-DDE	3	2nd	8.18 (-3.8, 21.65)	0.1894	7.07 (-2.26, 17.29)	0.1420
		3rd	6.38 (-5.41, 19.65)	0.3022	3.19 (-5.81, 13.06)	0.4997
	4	2nd	0.77 (-8.61, 11.11)	0.8776	9.75 (0.46, 19.91)	0.0396
		3rd	6.44 (-3.46, 17.35)	0.2106	3.18 (-5.55, 12.71)	0.4883
p,p'-DDD	3	2nd	6.64 (-5.14, 19.89)	0.2820	-1.47 (-10.03, 7.92)	0.7503
		3rd	1.36 (-9.89, 14.01)	0.8222	-5.87 (-14.09, 3.14)	0.1948
	4	2nd	6.09 (-3.74, 16.92)	0.2339	-1.29 (-9.62, 7.81)	0.7730
		3rd	0.66 (-8.7, 10.99)	0.8945	-5.65 (-13.65, 3.09)	0.1988

Chapter V. Conclusions

Overall Conclusions

This dissertation thoroughly analyzed PCB, OCP, and PBDE mixtures and several outcomes related to metabolism in U.S. midlife women from the Study of Women's Health Across the Nation (SWAN). This body of work represents women from five U.S metropolitan areas with a focus on Chinese and Japanese women who have been historically underrepresented in U.S. studies. All three aims leveraged the original design of SWAN to understand metabolic changes throughout the menopausal transition. Using multiple mixtures approaches, no significant associations were identified between overall exposure to POP mixtures and risk of Type 2 Diabetes nor levels of high molecular weight (HMW) adiponectin or leptin. Results from single pollutant analyses were not all consistent with results from mixtures analyses. Inconsistent effects observed between mixture components and statistical approaches may have been due to i) compound-specific mechanisms causing effects in different directions, ii) uneven distribution of POPs within and among adipocytes, and/or iii) differential involvement of adipokines or endocrine disruption in visceral adipose tissue compared to other adipose tissue. Mechanisms regulating the healthy functioning of adipocytes and metabolism are complex. No significant relationship was observed between changes in visceral adiposity and changes in individual serum concentrations of POPs. Nevertheless, the high degree of intra-person temporal variability in serum POP concentrations emphasizes a need for repeated exposure measurements in epidemiological studies of POPs more broadly. Although external exposure to POPs has greatly diminished over the past four decades, the drastic physiological changes occurring during the

menopausal transition further compel the evaluation of exposure beyond a single timepoint. Limited by a single POP measurement, Aim 3 attempted to improve exposure measurement validity by restricting interpretation to women with stable waist circumference. Aim 2 also may have been impacted by diminished exposure measurement validity over a long follow-up period, however, missingness of repeated waist circumference measurements prevented such analysis. All aims carefully considered the role of serum lipids and visceral adiposity in the relationship between POPs and metabolism. This dissertation elevates the metabolic health of midlife women of diverse racial and ethnic backgrounds and the value of mixtures approaches to reflect real-world exposure to environmental contaminants.

Summary of Findings

Aim 1

Aim 1 explored the difference in serum concentrations of PCBs and OCPs associated with a change in visceral adiposity between 1999 and 2011 in 75 midlife women. Changes in visceral adiposity, such as those experienced during the menopausal transition, may release POPs stored in adipose tissue into the bloodstream or further sequester them. Unlike previous studies, this study included repeated measurement of serum POPs which allowed us to investigate intra-individual changes in exposure over time. We also carefully considered the role of serum lipids, comparing three approaches to calculate serum concentrations of POPs: i) wet weight, ii) traditional lipid-standardization, and iii) covariate-adjusted standardization. Aim 1 is primarily limited by its small sample size.

An increase in the difference in waist circumference over time was not associated with a change in the difference in serum concentrations of PCBs and OCPs apart from PCB 194. After adjustment for study site, race/ethnicity, age, and parity, a one-inch (2.54 cm) greater difference

in waist circumference between visits was associated with a 4.9% smaller difference in serum concentration of PCB 194 (95% CI: -8.0%, -1.6%). Of the POPs analyzed, PCB 194 has the greatest lipophilicity. PCB 194 may be sequestered in adipose tissue following adiposity gain during the midlife in women.

Aim 2

Aim 2 assessed diabetes risk associated with exposure to mixtures of POPs among 1,400 midlife women between 1999 and 2016. This study has novelty and strengths. It is the first prospective study to use a mixtures approach to analyze the effect of PBDEs in addition to PCBs and OCPs on diabetes incidence. We employed QBGC, a rigorous, outcome-based mixtures method that can account for co-pollutant confounding and interactions in a highly correlated mixture while not assuming directional homogeneity, linearity, or additivity of mixture components. Furthermore, this is one of few studies that analyzed PBDEs in addition to PCBs and OCPs. In Aim 2, the low case count prevented further investigation of interactions with or effect modification by race/ethnicity or geographic location.

Mixtures analysis using Quantile Based G-Computation (QBGC) identified no significant association for the overall joint effect of the POP mixture on incident diabetes (HR = 1.04 [0.53, 2.07]). K-means three-cluster analysis demonstrated that average and above-average exposure, relative to below-average exposure, were not associated with diabetes risk with HRs of 0.75 (95% CI: 0.42, 1.31) and 0.71 (95% CI: 0.38, 1.31), respectively. Using single pollutant models, most POPs were not significantly associated with diabetes risk. After adjustment for age, race/ethnicity, site, education, financial strain, alcohol, smoking, parity, physical activity, high fat dairy intake, meat intake, fish/shellfish intake, and serum lipids, the HR (95% CI) for diabetes associated with tertiles of exposure (T2 or T3), relative to the first tertile, was 1.7 at T2 (1.0, 2.8)

and 1.5 at T3 (0.84, 2.7) for hexachlorobenzene and 1.9 at T2 (1.1, 3.3) and 1.6 at T3 (0.88, 2.9) for PCB 123. After additional adjustment for waist circumference, PCB 138 was inversely associated with diabetes (HR at T2 = 0.77 [0.45, 1.33]; HR at T3 = 0.50 [0.26, 0.97]; p for trend = 0.037). We observed no association between diabetes risk exposure to PBDE 47, p,p'-DDE, p,p'-DDT, and p,p'-DDD.

Aim 3

Aim 3 evaluated the relationship between mixtures of POPs and adipokines, specifically leptin and HMW adiponectin in 1,400 midlife women. The menopausal transition is a life stage characterized by changes in adipose tissue. Unlike previous studies, of which there are few, this study employed mixtures methods to analyze the joint effects of exposure to PCBs, OCPs, and PBDEs. We employed Bayesian Kernel Machine Regression (BKMR) to quantify the overall mixture effect while accounting for co-pollutant confounding. This is also the first study of POPs and adipokines in an adult population not limited to patients with obesity. We explored how to improve the validity of exposure measurement between POP and adipokine collection timepoints by limiting our interpretation to women with stable adiposity; the loss or gain of adipose tissue, a storage site for POPs, may alter serum concentrations of POPs.

No significant associations were observed for the overall joint effect of the POP mixture on leptin or high molecular weight (HMW) adiponectin using BKMR, after adjusting for age, site, race/ethnicity, education, financial strain, alcohol consumption, smoking, parity, exercise, menopausal status, and serum lipids. Single pollutant analyses indicated significant associations for few highly-chlorinated PCBs. K-means cluster analysis did not identify an association between POPs and HMW adiponectin yet found a positive, non-linear association with leptin. Stratifying models by gain or maintenance of waist circumference (WC) between 1999/2000 and

2002/2003, we found no association between POPs and leptin among women with stable WC. Single pollutant models demonstrated that a one-interquartile range (IQR) higher PCB 194 concentration was associated with 9.0% lower (95% CI: -13.2%, -4.7%) leptin and 4.1% higher (0.35%, 7.9%) HMW adiponectin. A one-IQR higher PCB 180 concentration was associated with 5.2% lower (-9.6%, -0.6%) leptin. Limiting single pollutant modeling to women with stable WC, and therefore greater exposure measurement validity, high levels of most POPs were associated with lower leptin and HMW adiponectin. Exposure to p,p'-DDE, p,p'-DDD, and p,p'-DDT were not significantly associated with leptin or HMW adiponectin although findings suggested positive effects. Aim 3 was limited by the measurement of adipokines at a single timepoint.

Public Health Implications

The Health of Aging Women

Metabolic research in midlife and aging women is important given the increasing rates of diabetes, the physiological changes unique to the menopausal transition, and the reality that this population has been historically understudied. In 2021, one in seven U.S. adults had diabetes.[1] Sex-specific diabetes rates are relatively equivalent, however, women with diabetes are more likely to have pre-existing depression and are at higher risk of cardiovascular complications and blindness.[2] Throughout the menopausal transition, women experience adverse changes in body fat distribution, lipids, metabolic function, and cardiovascular health.[3] It is essential to understand how environmental factors, including but not limited to POPs, may impact endocrine, inflammatory, and metabolic processes in aging women. In addition, this dissertation emphasizes the importance of research that represents women from multiple racial/ethnic groups.

Policy and Regulation

Research on the relationship between POPs and metabolism is important to inform policies and regulations addressing chemical production and use. The U.S. and many high-income countries have implemented POP regulations. Yet, even in the U.S., regulations are not all encompassing, commonly regulating a single chemical at a time. Furthermore, as compounds become regulated, chemical manufacturers introduce new chemicals onto the market. Novel brominated flame retardants (NBFRs) are an emerging group of chemicals that replaced penta-, octa-, and deca-brominated BDEs after their production and use were banned by the EPA in 2012.[4,5] In one study, at least 19 of the 52 NBRFs studied exhibited persistence and long-range transport potential similar to that of POPs or PBDEs.[6] Worldwide, NBFRs have been found extensively in indoor dust, air, consumer goods, and food with indoor occurrence being the least understood.[7] The questions explored in this dissertation are important to ask about similar emerging chemicals including NBRFs to inform regulation.

Climate Change-related Exposure Exacerbation

Climate change has the potential to exacerbate POPs exposure worldwide as more intense and frequent floods remobilize and redistribute historical toxicants.[8,9] The wide array of potential health consequences are yet to be fully understood.[8,9] Health risks are especially dire for communities near operational or historical chemical facilities, from which chemical releases can be triggered by extreme weather effects.[10] The inundation or erosion of historic coastal landfills also may release chemical contaminants.[11] The extent of exposure exacerbation is unclear. Nonetheless, it is also important to recognize that climate change impacts will be most severe in low- and middle-income countries that have contributed relatively little emissions yet will bear the brunt of associated health effects. Furthermore, chronic health effects are likely to be

underreported and poorly monitored in the aftermath of a natural disaster with disproportionate harm experienced by lower-resourced communities.[9]

Mixtures

Mixtures research is vital given that human exposures to environmental chemicals typically occur as a mixture. This dissertation illustrates that components of a mixture can have similar or different modes of action and health effects. It is important to leverage statistical methods that enable the analysis of cumulative effects while accounting for co-pollutant confounding and without assuming directional homogeneity. As this dissertation models, mixtures approaches must be selected carefully to align with the research question. In addition, as discussed previously in relation to climate change, mixtures of chemicals, metals, bio-toxins, and sewage in floodwater will differ depending on whether the affected area is densely urban, industrial, and/or rural.[9] It will become increasingly necessary to expand our conception of mixtures beyond chemicals to include pathogens, climate factors, and built environment characteristics. Finally, the mixtures analyses conducted in this dissertation are necessary to shift the regulatory paradigm away from single chemicals toward classes of chemicals and, hopefully in the future, to set standards that account for the effects of a compound as part of a more realistic environmental mixture.

Future Directions

Expanding the Longitudinal SWAN MPS Substudy

Longitudinal exposure studies are important to shed light on how the body burden of POPs may change over time and may vary person to person. Although Aim 1 was limited in size, it suggests variability in serum concentrations over time, possibly in part due to changes in waist circumference. Although Aim 1 could not address effect modification or interactions involving

race/ethnicity, site, or other predictors due to its size, it nevertheless contributes to the limited body of literature with repeated POP measurements. Future research should examine intra-person variation in serum POPs concentrations and predictors of temporal trends in serum concentrations.

Expanded Mixtures Analysis

With additional exposure biomarker measurements, this research could be expanded beyond mixtures of PCBs, PBDEs, and OCPs to include NBFRs, per- and poly-fluoroalkyl substances, phthalates, parabens, and phenols. Analyzing a larger mixture of endocrine disrupting chemicals could provide a more realistic picture of potential metabolic effects.

Consideration of Additional Biomarkers

Moving forward, more research is needed on the effects of POPs on inflammatory, endocrine, and metabolic functioning of adipose tissue. Future studies could leverage additional biomarkers that have already been collected by SWAN MPS. For example, Aim 3 could be expanded to model the effects of POPs on MCP-1, IL-6, CRP, fibrinogen, tissue polypeptide antigen, and plasminogen activator inhibitor-1, which are inflammatory biomarkers involved in adipose tissue functioning and/or cardiometabolic disease. Additionally interested in endocrine disrupting mechanisms of environmental chemicals, future SWAN research could explore sex hormones as outcomes. Sex steroid hormones have been associated with adipose tissue and metabolism via sex-specific mechansims.[12–15] In women, estradiol was linked to reduced adiposity and improved insulin sensitivity.[12] The production and metabolic effects of hormones may differ by menopausal status. After menopause, adipocytes become the main producers of estradiol.[12] In addition, testosterone was associated with insulin sensitivity, increased visceral adiposity, and higher risk of T2D with stronger effects in postmenopausal women.[12,16–18]

In addition, SWAN MPS could be expanded to analyze additional biomarkers of exposure and inflammation. In terms of exposure, NBFRs could be analyzed in biobanked serum samples. In addition, SWAN MPS could measure the following inflammatory biomarkers related to adipose tissue: IL-1, IL-10, TNF-alpha, resistin, angiotensinogen, visfatin, retinol-binding protein-4, and serum amyloid protein. In conceptualizing future studies, it is important to remember that metabolic changes have multifactorial causes. Environmental chemical exposures, although complex, comprise few of numerous risk factors. Similarly, inflammation and insulin resistance involving adipose tissue are only some of many potential biological pathways linking exposure to environmental chemicals and metabolic health.

References

1. International Diabetes Federation. *Diabetes Around the World 2021*. https://diabetesatlas.org/idfawp/resource-files/2021/11/IDFDA10-global-fact-sheet.pdf (2021).
2. U.S. Department of Health and Human Services Office on Womens Health. *Diabetes*. https://www.womenshealth.gov/a-z-topics/diabetes#:~:text=About%2015%20million%20women%20in%20the%20United%20States,about%201%20in%20every%209%20adult%20women.%201.
3. El Khoudary, S. R. *et al.* The menopause transition and women's health at midlife: a progress report from the Study of Women's Health Across the Nation (SWAN). *Menopause* **Publish Ahead of Print**, (2019).
4. Ali, N., Harrad, S., Muenhor, D., Neels, H. & Covaci, A. Analytical characteristics and determination of major novel brominated flame retardants (NBFRs) in indoor dust. *Analytical and Bioanalytical Chemistry* (2011) doi:10.1007/s00216-011-4966-7.
5. Environmental Protection Agency. *Significant New Use Rules on Certain Chemical Substances*. https://www.federalregister.gov/documents/2012/10/05/2012-23993/significant-new-use-rules-on-certain-chemical-substances (2012).
6. Kuramochi, H., Takigami, H., Scheringer, M. & Sakai, S. Estimation of physicochemical properties of 52 non-PBDE brominated flame retardants and evaluation of their overall persistence and long-range transport potential. *Sci. Total Environ.* **491–492**, 108–117 (2014).

7. Zuiderveen, E. A. R., Slootweg, J. C. & de Boer, J. Novel brominated flame retardants - A review of their occurrence in indoor air, dust, consumer goods and food. *Chemosphere* **255**, 126816 (2020).

8. Crawford, S. E. *et al.* Remobilization of pollutants during extreme flood events poses severe risks to human and environmental health. *J. Hazard. Mater.* **421**, 126691 (2022).

9. Erickson, T. B., Brooks, J., Nilles, E. J., Pham, P. N. & Vinck, P. Environmental health effects attributed to toxic and infectious agents following hurricanes, cyclones, flash floods and major hydrometeorological events. *J. Toxicol. Environ. Health Part B* **22**, 157–171 (2019).

10. Anenberg, S. C. & Kalman, C. Extreme Weather, Chemical Facilities, and Vulnerable Communities in the U.S. Gulf Coast: A Disastrous Combination. *GeoHealth* **3**, 122–126 (2019).

11. Brand, J. H. & Spencer, K. L. Will flooding or erosion of historic landfills result in a significant release of soluble contaminants to the coastal zone? *Sci. Total Environ.* **724**, 138150 (2020).

12. Liu, S. & Sun, Q. Sex differences, endogenous sex-hormone hormones, sex-hormone binding globulin, and exogenous disruptors in diabetes and related metabolic outcomes: 性别差异、内源性性激素、性激素结合球蛋白以及外源性干扰因子对糖尿病及其相关代谢结果的影响. *J. Diabetes* **10**, 428–441 (2018).

13. Ding, E. L. *et al.* Sex Hormone–Binding Globulin and Risk of Type 2 Diabetes in Women and Men. *N. Engl. J. Med.* **361**, 1152–1163 (2009).

14. Chen, B. H. *et al.* Sex Hormone–Binding Globulin and Risk of Clinical Diabetes in American Black, Hispanic, and Asian/Pacific Islander Postmenopausal Women. *Clin. Chem.* **58**, 1457–1466 (2012).
15. Perry, J. R. B. *et al.* Genetic evidence that raised sex hormone binding globulin (SHBG) levels reduce the risk of type 2 diabetes. *Hum. Mol. Genet.* **19**, 535–544 (2010).
16. Ding, E. L., Song, Y., Malik, V. S. & Liu, S. Sex Differences of Endogenous Sex Hormones and Risk of Type 2 Diabetes: A Systematic Review and Meta-analysis. *JAMA* **295**, 1288 (2006).
17. Andersson, B., Marin, P., Lissner, L., Vermeulen, A. & Bjorntorp, P. Testosterone Concentrations in Women and Men With NIDDM. *Diabetes Care* **17**, 405–411 (1994).
18. Phillips, G. B. *et al.* Association of hyperandrogenemia and hyperestrogenemia with type 2 diabetes in Hispanic postmenopausal women. *Diabetes Care* **23**, 74–79 (2000).