Health Risk Assessment of POPs and Heavy Metals in Hong Kong Residents based on Their Concentrations in Selected Food Items and Different Human Tissues (Blood Plasma and Adipose Tissues)

QIN Yanyan

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Principal Supervisor: Prof. WONG Ming Hung

Hong Kong Baptist University

December 2010
ABSTRACT

This study aimed to investigate the levels of persistent organic pollutants (POPs) and heavy metals in different food items and human samples (blood and adipose tissues) collected from Hong Kong, and the co-exposure effects of POPs and heavy metals based on human cell-lines and mice models.

Food items were collected from Hong Kong markets and were classified into five food groups (meat, edible oil, nut, milk and wine). Naphthalene, pp-DDE, beta-, gamma-HCH, PBDE 47 and Pb were detected in most of the food items, with goose liver accumulating the highest levels of PAHs (47.3 ng/g wet wt), DDTs (25.1), HCHs (13.4), PCBs (4.20), PBDEs (479 pg/g wet wt) among all studied food items. Significant (p<0.01 or 0.05) correlations were found in the meat and nut groups between lipid contents and concentrations of PAHs (meat: r=0.878), HCHs (meat: r=0.753), DDTs (meat: r=0.937; nut: r=0.968) and PCBs (meat: r=0.832; nut: r=0.946). Concentrations of DDTs, HCHs and PCBs in vegetable oils were lower, but HCHs in fish oil were higher, when compared with other countries. The concentrations of PAHs, DDTs, PCBs, PBDEs and heavy metals were all below safety guidelines which indicated the selected food items were safe for human consumption.

Human blood and adipose tissues were collected to determine the body burdens of POPs and heavy metals in the Hong Kong population. Naphthalene, phenanthrene, pp-DDE, beta-, gamma-HCH, PCB-118 and PBDE-47 were detected in 100% of the human blood plasma and adipose tissues. Human blood was collected from 111 healthy residents by the Hong Kong Red Cross. Residents from a wide age
range (20-60) of both genders, were considered to fully reflect the average levels of POPs and heavy metals in the general population. Compared with other countries, the levels of DDTs and HCHs in human blood were comparatively higher. In addition, females accumulated significantly higher (p<0.01 or 0.05) concentrations of acenaphthylene (female: 78.0 ng/g lipid; male: 38.8), anthracene (10.8; 6.82), fluoranthene (106; 88.5), pp-DDE (942; 756), pp-DDT (71.7; 54.7), PCB-183 (2.35; 2.03), BDE-99 (0.09; 0.04) than males, while males accumulated significantly higher (p<0.01 or 0.05) pyrene (female: 184 ng/g lipid; male: 212), Fe (female: 0.92 mg/L; male: 1.28), Hg (1.01 μg/L; 1.73) and Pb (23.4; 31.6) than females.

Compared with healthy females, patients with uterine leiomyomas (UL) accumulated significantly higher (p<0.01 or 0.05) concentrations of DDTs (patient: 1968 ng/g lipid; healthy control: 1235), HCHs (286; 107), PCBs (191; 126), PAHs (1835; 876), PBDEs (13.1; 5.36), As (0.59 μg/kg lipid; 0.32), Cd (0.38; 0.27), Pb (5.24; 3.36) and Hg (9.12; 5.94) in adipose tissues, which indicated that these pollutants may link with UL disease. Smokers accumulated significantly higher (p<0.01 or 0.05) acenaphthene, benzo(a)pyrene, pp-DDE, pp-DDT, PCB-138, BDE-47, -99, Cd and Pb than non-smokers in human blood. Positive correlations (p<0.05) were found in human blood and adipose tissues between pollutants (DDTs, PCBs, PBDEs, As, Cd, Pb and Hg), with respect to seafood diet habit, BMI and age. It indicated these factors can influence the body loadings of these pollutants.

Synergistic or additive effects of BaP and metals (As, Cd, Hg and Pb) were observed in human cell-lines (HepG2 and KERTr cell lines) and mice models under
in vitro and in vivo conditions respectively. Based on the results of EROD (induction of CYP1A1) and IL-8 (induction of IL-8) assays in human HepG2 and KERTr cells, BaP+metals groups (CYP1A1: BaP+As: 6.44 pmol/min/mg protein, As: 0.29; BaP+Cd: 3.74, Cd: 0.30; BaP+Hg: 1.77, Hg: 0.29; BaP+Pb: 4.99, Pb: 0.28; IL-8: BaP+Hg: 145 pg/ml; Hg: 94) showed significantly higher (p<0.05) induction of CYP1A1 and IL-8 than single metal or BaP group (CYP1A1: 1.44 pmol/min/mg protein; IL-8: 97.3 pg/ml). As for mice models, the groups of BaP+As and BaP+Hg induced significantly higher (p<0.05) CYP1A1 mRNA and MT protein than BaP, As, Hg and control groups, in both female and male mice livers through dietary exposure to these pollutants. This indicated that BaP and metals (As, Pb, Cd and Hg) had synergistic or additive effects on human cells and mice models. Moreover, the damages of mice liver, kidney and the female reproductive system via examination of tissue slices indicated they may be the main target organs of POPs and heavy metals.

The present study revealed that food and human specimens collected from Hong Kong were more contaminated with DDTs and HCHs than other countries because of their high usage in the past and possibly illegal usage, leading to high background levels in the region. The high concentrations of DDTs, HCHs, Pb and Hg found in food and human blood, together with even higher levels of these pollutants in adipose tissues of patients with UL indicated exposure to POPs and heavy metals continuously via dietary may influence human health.
TABLE OF CONTENTS

DECLARATION ................................................................................................................. i

ABSTRACT ......................................................................................................................... ii

ACKNOWLEDGEMENTS ................................................................................................. v

TABLE OF CONTENTS ................................................................................................... vi

LIST OF TABLES ............................................................................................................... x

LIST OF FIGURES .......................................................................................................... xii

LIST OF ABBREVIATIONS ............................................................................................... xiv

CHAPTER 1 GENERAL INTRODUCTION ......................................................... 1
  1.1 Research Background ............................................................................................ 1
  1.1.1 Persistent Organic Pollutants ........................................................................... 1
  1.1.2 Heavy Metals .................................................................................................. 9
  1.1.3 Human Tissues as Indicators of Human Exposure to Environmental Chemicals ........................................................................................................... 12
  1.1.4 Toxicology Assessment of POPs and Heavy Metals Co-Exposure Effects on Human Cell-Lines ....................................................................................... 13
  1.1.5 Toxicology Assessment of POPs and Heavy Metals Co-Exposure Effects on Mice Models ................................................................................................. 15
  1.2 Research Objectives .............................................................................................. 16
  1.3 Framework ............................................................................................................. 17

CHAPTER 2 PERSISTENT ORGANIC POLLUTANTS AND HEAVY METALS IN FOOD ITEMS COLLECTED IN HONG KONG ......................... 20
  2.1 Introduction ............................................................................................................. 20
  2.2 Materials and Methods .......................................................................................... 22
  2.2.1 Sample Collection ............................................................................................ 22
  2.2.2 Chemicals and Apparatus ............................................................................... 24
  2.2.3 Chemical Analyses .......................................................................................... 24
  2.2.4 Statistical Analyses ......................................................................................... 28
  2.3 Results and Discussion ......................................................................................... 28
  2.3.1 PAHs in Food Items ......................................................................................... 29
2.3.2 OCPs in Food Items ................................................................. 30
2.3.3 PCBs in Food Items ............................................................... 34
2.3.4 PBDEs in Food Items ............................................................. 37
2.3.5 Correlations between Lipid Contents and POPs Levels .......... 40
2.3.6 Comparison of DDTs, HCHs, PCBs and PBDEs in Edible Oils in Different Countries ................................................. 40
2.3.7 Heavy Metals Concentrations in Food Items ......................... 45
2.4 Risk Assessment ..................................................................... 49
2.5 Conclusion ............................................................................ 51

CHAPTER 3 PERSISTENT ORGANIC POLLUTANTS AND
HEAVY METALS IN BLOOD PLASMA OF
HONG KONG RESIDENTS ................................. 53

3.1 Introduction ........................................................................ 53
3.2 Materials and Methods .......................................................... 55
  3.2.1 Sample Collection .............................................................. 55
  3.2.2 Chemicals and Apparatus .................................................. 56
  3.2.3 Analyses of POPs ............................................................... 56
  3.2.4 Analyses of Heavy Metals ................................................ 58
  3.2.5 Statistical Analyses ........................................................... 58
3.3 Results and Discussion ........................................................... 58
  3.3.1 Levels of POPs ................................................................. 58
  3.3.2 Comparison of POPs Levels in Different Countries .......... 61
  3.3.3 Levels of Heavy Metals .................................................... 69
  3.3.4 Factors that Influencing POPs and Heavy Metals Levels in Plasma .... 69
  3.3.5 Toxic and Health Effects of POPs and Heavy Metals in Human Plasma in Hong Kong .................................................. 80
3.4 Conclusion ............................................................................ 81

CHAPTER 4 PERSISTENT ORGANIC POLLUTANTS AND
HEAVY METALS IN ADIPOSE TISSUES OF
PATIENTS WITH UTERINE LEIOMYOMAS
COMPARED WITH HEALTHY FEMALES ..... 82

4.1 Introduction ........................................................................ 82
4.2 Materials and Methods ........................................................ 84
  4.2.1 Sample Collection ............................................................. 84
  4.2.2 Chemicals and Apparatus .................................................. 86
  4.2.3 Analyses of POPs .............................................................. 86
  4.2.4 Analyses of Heavy Metals ................................................ 87
CHAPTER 5 CO-EXPOSURE EFFECTS OF

BENZO(A)PYRENE AND HEAVY METALS

(CADMIUM, LEAD, ARSENIC AND MERCURY)

ON HUMAN HEPG2 AND KERATINOCYTE

CELL-LINES.................................................. 108

5.1 Introduction......................................................... 108
5.2 Materials and Methods............................................. 113
  5.2.1 Chemicals and Apparatus.................................... 113
  5.2.2 HepG2 and Keratinocyte Cells Cultures.................... 114
  5.2.3 Measurement of Cell Viability............................... 115
  5.2.4 EROD Assay.................................................. 116
  5.2.5 Metallothionein Bioassay................................... 117
  5.2.6 IL-8 ELISA Assay........................................... 118
  5.2.7 Real Time-PCR............................................... 118
  5.2.8 Statistical Analyses......................................... 119
5.3 Results and Discussion........................................... 119
  5.3.1 Effects of Metals and BaP on Human HepG2 and KERTr Cells Viabilities.............................................. 119
  5.3.2 HepG2 Cells.................................................. 120
  5.3.3 KERTr Cells.................................................. 121
5.4 Conclusion........................................................ 134

CHAPTER 6 CO-EXPOSURE EFFECTS OF

BENZO(A)PYRENE AND ARSENIC,

MERCURY ON MICE MODELS......................... 136
6.1 Introduction........................................................................................................... 136
6.2 Materials and Methods....................................................................................... 138
   6.2.1 Chemicals and Apparatus.............................................................................. 138
   6.2.2 Animals and Study Design.......................................................................... 138
   6.2.3 Real Time-PCR .......................................................................................... 139
   6.2.4 Metallothionein Bioassay............................................................................ 139
   6.2.5 Histological Analyses................................................................................ 141
   6.2.6 Statistical Analyses.................................................................................... 142
6.3 Results and Discussion...................................................................................... 142
   6.3.1 General Observations................................................................................ 142
   6.3.2 Weight Changes of Mice Organs (Liver, Kidney, Spleen, Ovary and Testis)......................................................................................................................... 143
   6.3.3 CYP1A1 mRNA Induction in Mice Livers through RT-PCR...................... 143
   6.3.4 MT Protein Induction in Mice Livers........................................................... 150
   6.3.5 Histology Changes in Mice Organs (Liver, Kidney, Spleen, Ovary and Testis)......................................................................................................................... 150
6.4 Conclusion ........................................................................................................ 162

CHAPTER 7 GENERAL DISCUSSION AND CONCLUSION

................................................................................................................................. 163
7.1 Introduction........................................................................................................... 163
7.2 Dietary Exposure to POPs and Heavy Metals in Hong Kong......................... 164
   7.2.1 Concentrations of POPs and Heavy Metals in Food Items Collected in
        Hong Kong....................................................................................................... 165
   7.2.2 Bioaccumulation........................................................................................ 166
7.3 Human Body Loadings of POPs and Heavy Metals with Their Implication to
    Human Health...................................................................................................... 167
7.4 Co-Exposure Effects of POPs and Heavy Metals on Human Cell Lines and
    Mice Models...................................................................................................... 176
7.5 Toxic Effects of POPs and Heavy Metals on Human Health from Dietary
    Exposure in Hong Kong...................................................................................... 178
7.6 Conclusion ......................................................................................................... 180
7.7 Limitations of the Research.............................................................................. 181
7.8 Future Work ..................................................................................................... 183

REFERENCES ......................................................................................................... 185

APPENDICES ........................................................................................................ 224

PUBLICATIONS .................................................................................................... 226

CURRICULUM VITAE ............................................................................................ 228