Graduate School in Biomedical, Clinical and Experimental Sciences

UNIVERSITÀ DEGLI STUDI DI MILANO
FACOLTÀ DI MEDICINA E CHIRUGIA

Exposure Assessment of Persistent Organic Pollutants and Associated Health Risks among Italian Population

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2011/2012

Exposure Assessment of Persistent Organic Pollutants and Associated Health Risks among Italian Population

by

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A thesis submitted in fulfillment of the requirements for the degree of

Doctor of Philosophy (PhD)

in
Occupational Medicine and Industrial Hygiene

XXV Cycle

Graduate School in Biomedical, Clinical and Experimental Sciences,
Department of Health Sciences

Università degli Studi di Milano,

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Milan
December, 2012

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Università degli Studi di Milano a thesis entitled: ***Exposure Assessment of Persistent Organic Pollutants and Associated Health Risks among Italian Population,*** in fulfillment of the requirements for the degree of Doctor of Philosophy (Occupational Medicine and Industrial Hygiene) of the Università degli Studi di Milano.

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Date: 6-II-2013

DECLARATION

AND

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I, **Ezra Jonathan Mrema**, declare that this thesis is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

Signature .....

ACKNOWLEDGEMENTS

Prays, glory and honour is to Almighty God for his mercy, grace and blessings to me throughout the whole period of my study. I have been encouraged and motivated so much with these verses: “I can do ALL things through him who gives me strength (Phillipians 4:13)” and “Rise up this matter is in your hands. We will support you, so take courage and do it (Ezra 10:4).” These motivated me to undertake this big commitment which I have finally managed to accomplish.

A number of individuals have contributed a lot towards my postgraduate training and make this work a great success and thus they deserve to be acknowledged. First, I feel greatly indebted to all members of my family including my parents, my father the late Jonathan Nakisa Mrema and my mother Salome Ndesanjo Macha for the foundation of education laid on me despite of their poor economic situation; my wife Candida Flavian Materu, my sons Evan and Joel who accepted and afforded to have me away, even during the times when they needed me the most; my brother and sisters (Frank, Ainess, Happiness, Luza and Violet); grandfathers (the late Nakisa Nguruko Mrema & Ndesanjo Mtuche Macha) and grandmothers (the late Magdalena Mrema and Awishana Ndelaiyona Maliha), uncles and aunts whose encouragement and moral supports are greatly honoured. *Asanteni sana!!*

I acknowledge the mentorship of Prof. Claudio Colosio the Director of International Centre for Rural Health (ICRH), a WHO Collaborating Centre, for his significant contributions in developing my professional and academic career. He provided a considerable support at each and every stage of my work since conceiving to finalization of my PhD project. The conducive and amicable learning environment he created is a secret of all this success. He was not tired in providing support whenever needed. He assisted in translation of application package which made my life easier during the application process. Even where I was about to stuck in the embassy in processing my application his endless efforts were made to push and ensure my application went through. *Grazie mille sei molto gentile!!* Once he had this offer for non–EU fellow he did not hesitate to share it with his colleagues. Prof. Leslie London of University of Capetown, South Africa is acknowledged for availing this information to Dr. Aiwerasia Vera Ngowi the Head of Environmental and Occupational Health (DEOH) of Muhimbili University of Health and Allied Sciences (MUHAS), Tanzania

who in turn encouraged me to apply for the post. It is through this networking which enabled me to get this opportunity. Thank you very much madam!!

Prof. Antonio Colombi the then Coordinator of the PhD and the Director of Department of Occupational and Environmental Health of Università degli Studi di Milano (UNIMI) for providing me with the invitation to participate in the proficiency test for admission to PhD programme in Occupational Health. I also extend my thanks to other member of academic staff including Prof. Pier Alberto Bertazzi, Prof. Giovanni Costa, Prof. Gabri Brambila, Prof. Angelo Moretto and Dr. Dario Consonni for providing academic support during the period of my training.

Administration of Muhimbili University of Health and Allied Sciences (MUHAS) is acknowledged for giving me the 3 years study leave which allowed me to take part in this study. Thanks to other members of DEOH for their moral support and encouragement during the whole period of study. To mention them these are Dr. Larama Rongo, Dr. Simon Mamuya, Mr. Yahya Kishashu, Ms. Jane Mlimbila, Ms Gloria Sakwari, Mr. Mohamed Hussein, Mr. Hurbert Kaijage and Mr. Denis Ryemamu. Ms. Else an Italian lady working in Tanzania is acknowledged for her wonderful day and night work of translating my academic certificates and other documents required for registration to the course. *Grazie mille signorina!!*

Dr. Federico Maria Rubino was quiet instrumental and his time to time willingness to provide valuable technical and professional support to my work is highly appreciated and honoured. I thank Dr. Luca Neri for his initial assistance in various aspects of statistical analysis of my data. Dr. Raffaella Zanzottera a librarian at Clinica del Lavoro Luigi Devoto is acknowledged for her wonderful and tireless assistance in searching and availing references not available online whenever she was requested. I also extend my thanks to my fellow postgraduate students: Ramin Tabibi, Dr. Stefan Mandic-Rajcevic and Lorenzo Fugnoli who willingly contributed their experiences during the entirely period of my study. Thanks are also due to those whom we worked together especially at International Centre for Rural Health (ICRH) including Dr. Maryam Sokooti, Giulia Rabozzi, Maria Grazia Troja Martinazzoli, Lucia Zanini, Gaia Varischi, Giorgio Vienello and Marco Pitton. All have played a crucial role in one way or another towards the success of my study.

Other ICHR team members especially Signora Giovanna Radaelli, Chiara Somaruga, Fracesca Velere and Illaria Bollina are highly appreciated for their tireless in providing commucations and organization of my traveling and stay at Milan (communication, hospitality, information on how to live in Region of Lombardy, flight and accommodation arrangements).

Thanks are also extended to Prof. Claudio Minoia and Dr. Roberta Turci of the Laboratory for Environmental and Toxicological Testing, Salvatore Maugeri Foundation, Pavia Italy, for providing analytical facilities which enabled all analyses of investigated persistent organic pollutants reported in this Thesis.

Many sincere thanks are also due to all study subjects who willingly consented to take part to the study. Definitely without them this work would not be possible. I also recognize and extend thanks to some International Scientists for accepting the invitations of my supervisor to revise our project anticipated to be run in Tanzania as a continuation of this work. Among them include Melissa Perry, The Professor of Epidemiology and Biostatistics based at The George Washington University, United States of America and Luigi Cocco, The Professor of Occupational Medicine and Epidemiology, University of Cagliari, Italy. Professor Mary Gulumian from School of Pathology, University of the Witwatersrand, South Africa is highly acknowledged for her generous invitation to Risk Assessment course which enriched my skills and knowledge in this area.

I feel particularly indebted to The Ministry of Health of Italy for providing me with financial support for the first two years of my study through the then Department of Occupational and Environmental Health of Università degli Studi di Milano within REALEXPO project. Professor Paolo Carrer is highly thanked for soliciting funds which enable me to finalize my final year of my training.

Last but not least, I acknowledge all administrative and technical staff of the Università degli Studi di Milano for their cooperation during the course of my studies. Specifically Ms. Raffaella Saurgnani, Samantha Astro, Rosemary Bentoglio, Teresa Costantiello and Antonella Astori are acknowledged for providing all administrative arrangements required prior and during the whole period of the study.

DEDICATION

To my family especially my mother Salome Ndesanjo Macha, my wife Candida Flavian Materu and our children Evan and Joel

ABSTRACT

Persistent Organic Pollutants is a group of structurally diverse man-made chemicals characterized by a high bio-persistence and the ability to travel a long distance on the planetary scale. This class of chemicals includes polychlorinated biphenyls, organochlorinated pesticides such as DDT and its metabolites and other chemicals such as polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzo furans. These chemicals are widely distributed in the environment as some of them have long been employed in agriculture and in public health as pesticides, others as industrial chemicals and others are unavoidable by-products of industrial processes. These chemicals can be a source of health risk to human.

Despite extensive use of OCPs such DDT in Italy in the 1940s to 1970s especially for public health control of malaria mosquitoes and PCBs in industry, in energy production and in industrial commodities, information on their exposure levels among the general population is limited. This study aims at determining the levels of OCPs and of PCB congeners among the residents from Italians living in three different places: Novafeltria (a village in Central Italy), Pavia (a mid-sized town) and Milan (the second-largest city in Italy) and at exploring the relationship with places of residence, gender, age and individual body size of their accumulation in the population.

To measure PCBs concentrations, blood samples were collected from 372 consented subjects whereas to measure OCPs blood samples were collected from 137 subjects. Thirty six PCB congeners and eight OCPs were measured in blood serum by gas-chromatography-mass spectrometry. To normalize PCBs and OCPs concentrations to total blood lipid concentrations (a commonly employed technique to take into account the lipophilic character of the compounds), serum concentrations of triglycerides and total cholesterol were measured by standard clinical chemistry techniques. Personal information such as gender, age, height and weight, dietary habits, education, residence and occupation information were collected through a questionnaire.

Statistical analyses were employed to highlight variation in analytes levels with respects to residence, age, gender and body mass index. The analytes concentrations were summarized for the overall population of three sites and separately for the individual sites, for gender, for age groups and for BMI categories.

The PCB congeners 138, 153 and 180 were the most frequently detected in overall population sample and were the major contributors of total PCB burden. PCB 153 had the highest median level in each site. Total PCB level differed significantly between the study sites ($p < 0.0001$) with median concentrations of 837, 1355 and 2062 pmol/g lipid, respectively in Novafeltria, Pavia and Milan. No evidence was found for the difference in distribution of total PCB levels by genders. Total DL–PCB differed significantly ($p < 0.0001$) between the sites (median 110, 51 and 167 pmol/g lipid, respectively) and genders of Novafeltria and Pavia ($p = 0.011$ and 0.009, respectively). PCB 138, 153, 170 and 180 differed significantly between the sites ($p < 0.0001$) with higher values in Milan population. In overall population total PCB, PCB 138, 153, 156, 170 and 180 correlated positively with age (r for linear correlations range between 0.320–0.569, $p < 0.0001$). In Novafeltria the r correlations range between 0.545–0.670 and 0.516–0.666 in Pavia. In Milan, r correlations with age range between 0.327–0.417 for total PCB, congener 138, 153 and 180. With exception of PCB 170 there was no evidence of significant difference in distribution of most abundant PCB congeners and total PCB across the BMI categories.

Among the OCPs, *p,p'*-DDE and HCB were the most abundant and major contributors of total OCP concentration. Their levels differed significantly between the three towns with a trend Milan > Novafeltria > Pavia ($p < 0.0001$). Females had significantly higher concentrations of HCB and *p,p'*-DDE than males in overall population sample. HCB concentrations were significantly higher in females than in males of Milan ($p = 0.029$). We observed positive correlations of *p,p'*-DDE and HCB with age in Novafeltria subjects ($r = 0.468$, $p = 0.004$). Total OCP concentrations differed significantly across BMI categories ($p = 0.018$) in overall population.

We have demonstrated a pattern of distribution of the levels of the main PCB congeners and OCPs in a fairly large population in Italy. Generally our study provides information on PCBs and OCPs exposure among the Italian general population and provides indications for further investigations.

Keywords: *Persistent organic pollutants; human exposure; serum; DDT; DDE; DDD; HCB; HCH; PCBs; DL–PCBs; total PCBs.*

RIASSUNTO

I contaminanti organici persistenti costituiscono un gruppo di sostanze chimiche artificiali strutturalmente tra loro diverse e caratterizzate, quale elemento comune, da un'elevata bio-persistenza e dalla capacità di essere trasportate per lunghe distanze su scala planetaria, lontano dal luogo originario di emissione nell'ambiente. A questa classe di sostanze chimiche appartengono, tra gli altri, i bifenili poli-clorurati, i pesticidi organo-clorurati, tra cui il DDT e i suoi metaboliti, e altri prodotti chimici come le dibenzo-*p*-diossine e i dibenzo-furani policlorurati. Queste sostanze chimiche sono ormai ampiamente distribuite nell'ambiente, in quanto alcuni di loro sono stati a lungo impiegati in agricoltura e nella sanità pubblica come pesticidi (il DDT e gli altri insetticidi clorurati), altri come prodotti chimici industriali (i bifenili poli–clorurati) e altri sono inevitabili sottoprodotti di processi industriali (le dibenzo-*p*-diossine e i dibenzo–furani policlorurati). Per le loro caratteristiche tossicologiche, queste sostanze chimiche possono essere fonte di rischio per la salute umana.

In Italia si è fatto largo uso di insetticidi della famiglia organo clorurata (DDT e prodotti simili) dagli anni '40 agli anni '70 del 1900, soprattutto per fini di salute pubblica, per l'eradicazione di vettori (zanzare) della malaria e dei PCB, prodotti in Italia per il settore manifatturiero (produzione di dispositivi elettrici e termici), nella produzione di energia (uso di trasformatori e condensatori) e come materiali industriali in diversi comparti. Ciò nonostante, le informazioni sui livelli di esposizione della popolazione generale rimangono limitate. Questo studio si è proposto di misurare i livelli di alcuni insetticidi organo-clorurati e dei PCB nella popolazione generale italiana in tre luoghi diversi: Novafeltria (un paese del Centro Italia), Pavia (una città di medie dimensioni) e Milano (la seconda città più grande in Italia) e ad esplorare la relazione del loro accumulo nella popolazione con i luoghi di residenza, il sesso, l'età e la costituzione individuale.

Per misurare le concentrazioni di PCB, i campioni di sangue sono stati raccolti campioni di sangue da 372 soggetti che hanno fornito il consenso informato e per misurare gli insetticidi organo-clorurati sono stati raccolti i campioni da 137 soggetti. Nel siero sono stati misurati 36 congeneri dei PCB e 8 insetticidi organo-clorurati mediante gas-cromatografia-spettrometria di massa. Per normalizzare le concentrazioni dei PCB e degli insetticidi organo-clorurati alle concentrazioni totali di lipidi nel sangue

(una tecnica comunemente impiegata per tener conto del carattere lipofilico dei composti), sono stati misurate le concentrazioni sieriche di trigliceridi e colesterolo totale mediante tecniche standard di chimica clinica. Le informazioni personali quali il sesso, l'età, l'altezza e il peso, le abitudini alimentari, l'istruzione, la residenza e l'occupazione le informazioni sono state raccolte attraverso un questionario.

Sono state impiegate analisi statistiche per evidenziare eventuali relazioni dei livelli degli analiti con caratteristiche dei soggetti indagati quali la residenza, l'età, il sesso e l'indice di massa corporea. Le concentrazioni degli analiti sono state indagate in termini sintetici per la popolazione complessiva dei 3 siti, e separatamente per i singoli siti, per sesso, per età e per le categorie di BMI.

I PCB congeneri 138, 153 e 180 sono stati quelli più frequentemente rilevati nei campioni e sono risultati quelli maggioritari nel concorrere alla dose totale di PCB. Il PCB 153 ha il più alto livello medio in ciascun sito. Il livello totale di PCB differisce in modo significativo tra questi siti ($p < 0,0001$) con concentrazioni mediane di 837, 1355 e 2062 pmol/g di lipidi, rispettivamente a Novafeltria, a Pavia e in Milano. Non sono state dimostrate differenze nella distribuzione dei livelli di PCB totali tra i due sessi. La somma dei PCB diossino-simili differiva in modo significativo ($p < 0,0001$) tra i siti (media 111 e 167 pmol/g di lipidi, rispettivamente) e tra i due sessi a Novafeltria e a Pavia ($p = 0,011$ e 0,009, rispettivamente). I PCB 138, 153, 170 e 180 differivano in modo significativo tra i luoghi di residenza ($p < 0,0001$), con valori più elevati nella popolazione di Milano. In generale, nella popolazione totale esaminata i livelli dei PCB totali e dei congeneri 138, 153, 156, 170 e 180 correlano positivamente con l'età (coefficienti r di correlazione compresi tra 0,320 e 0,569, $p < 0,0001$). A Novafeltria i coefficienti r risultano compresi tra 0,545 e 0,670 e tra 0,516 e 0,666 a Pavia. A Milano, i coefficienti r per le correlazioni con età sono risultati compresi tra 0,327 e 0,417 per i PCB totali e per i congeneri 138, 153 e 180. Ad eccezione del PCB 170 non è stata osservata alcuna differenza significativa nella distribuzione dei più abbondanti congeneri di PCB e PCB totali tra le diverse categorie di BMI (individui 'più o meno grassi').

Tra gli insetticidi organo-clorurati, il *p,p'*-DDE e l'HCB contribuiscono in misura magiore al carico corporeo totale per la classe di composti. I loro livelli differiscono in modo significativo tra le tre città, con un trend di Milano > Novafeltria > Pavia ($p <$

0,0001). Considerando l’intero campione, le femmine hanno concentrazioni significativamente più alte di HCB e *p,p'*-DDE rispetto ai maschi. Le concentrazioni di HCB sono significativamente più alte nelle femmine rispetto ai maschi di Milano (p = 0,029). Abbiamo osservato correlazioni positive tra la concentrazione di *p,p'*-DDE e HCB e l’età solo nei soggetti di Novafeltria (r = 0,468, p = 0,004). Considerando l’intera popolazione studiata, le concentrazioni dell’insetticida organo clorurati totali differiscono in modo significativo tra le categorie di BMI (p = 0,018).

Abbiamo misurato il *pattern* dei principali congeneri dei PCB e degli insetticidi organo-clorurati in una campione abbastanza esteso della popolazione italiana. In generale il nostro studio fornisce informazioni sui livelli di esposizione a PCB e ad insetticidi organo-clorurati e fornisce indicazioni per ulteriori indagini.

Parole chiave: *Contaminanti organici persistenti; esposizione; siero; DDT; DDE; DDD; HCB; HCH; PCB; PCB diossino–simili; PCB totali.*

List of Articles

The research activities carried out during this project have brought to the publication of the scientific articles listed below:

1. **Mrema EJ**, Rubino FM, Brambilla G, Moretto A, Tsatsakis AM, Colosio C. Persistent organochlorinated pesticides and mechanisms of their toxicity. Toxicology 2012. doi:pii: S0300-483X (12) 00412-X.
2. **EJ Mrema**, FM Rubino, S Mandic-Rajcevic, E Sturchio, R Turci, A Osculati, G Brambilla, C Minoia and C Colosio. Exposure to priority organo-chlorine contaminants in the Italian general population. Part 1. Eight priority organochlorinated pesticides (OCPs) in serum (2012, Accepted for Publication to Human and Experimental Toxicology, in press).
3. **EJ Mrema**, FM Rubino, S Mandic-Rajcevic, E Sturchio, R Turci, A Osculati, G Brambilla, C Minoia and C Colosio. Exposure to priority organo-chlorine contaminants in the Italian general population. Part 2. Fifteen priority polychlorinated biphenyl congeners (PCBs) in blood serum (2012, Accepted for Publication to Human and Experimental Toxicology, in press).
4. **Mrema, E.J**., R. Turci, F. Rubino, L. Fugnoli, M. Pitton, S. Mandic-Rajcevic, C. Colosio and C. Minoia. Serum Levels of Polychlorinated biphenyls (PCBs) and Organochorinated Pesticides (OCPs) among individuals of general population in three Italian Geographic Regions. (Poster presentation at The 47th Congress of the European Societies of Toxicology (EUROTOX 2011) held in Paris, France, 28–31 August 2011. Published in Toxicology Letters 205S (2011) S60–S179. doi:10.1016/j.toxlet.2011.05.472.
5. **Ezra J. Mrema**, Federico Rubino and Claudio Colosio. Chapter 238. Pesticide Residue – Organochlorine (2012, Accepted for Publication by Elsevier in Encyclopedia of Food Safety).
6. **Mrema EJ**, Rubino FM and Colosio C. Obsolete Pesticides – A Threat to Environment, Biodiversity and Human Health (2012, In press, Proceedings of the NATO ASI School on Obsolete Pesticides – *L. Simeonov, F. Macaev and B. Simeonova (Eds.)*, Environmental Security Assessment and Management of Obsolete Pesticides in South East Europe, © *Springer Science+Business Media B.V. 2012*. Science for Peace and Security Programme).

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LIST OF ABBREVIATIONS

ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism and Excretion
AEE	Aerial exposure to endosulfan
AhR	Aromatic hydrocarbon receptor
AP-1	Activating protein-1
AR	Androgen Receptors
ARE	Antioxidant response element
ARNT	Aryl hydrocarbon receptor nuclear translocator
ATSDR	Agency for Toxic Substances and Disease Registry
BCA	Breast cancer
BMI	Body Mass Index
Bad	Bcl-2 associated death promoter
Bax	Bcl-2 associated X protein
Bcl-2	B-cell lymphoma 2 protein
b.wt	Body weight
CAR	Constitutive androstane receptor
CGC	Cerebellar granule cells
CN	Cortical neurons
CYP1A1	Cytochrome P450, family 1, subfamily A, polypeptide 1
CYP1A2	Cytochrome P450, family 1, subfamily A, polypeptide 2
CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1
CYP450	Cytochrome P450
DDA	bis(p-chlorophenyl)acetic acid
DDT	Dichlorodiphenyltrichloroethane
DEOH	Department of Environmental and Occupational Health
DES	Diethylstilbestrol
DNA	Deoxyribonucleic Acid
DL–PCBs	Dioxin-like polychlorinated biphenyls
EC50	Concentration which produced half of the maximum effect
ER	Estrogen receptor
EU	European Union
ERK1/2	Extracellular signal regulated kinases 1 and 2
FSH	Follicle Stimulating Hormone
GABAA	Gamma butyric acid type A
GC–MS	Gas Chromatograph–Mass Spectrometry
GJI	Gap junction inhibition
GR	Glucocorticoid receptor
GSH	Glutathione
HCB	Hexachlorobenzene
HER1	Human epidermal growth factor receptor
$HO^{\bullet}$	Hydroxyl radical
Hpr	Human progesterone
IARC	International Agency for Research on Cancer
IC_{50}	Concentration at which 50% of cell are not viable
ICRH	International Centre for Rural Health
IFCS	Intergovernmental Forum on Chemical Safety
IGF-IR	Insulin-like growth factor-insulin receptor
IGFs	Insulin-like growth factors
IPCS	International Programme on Chemical Safety
IUPAC	International Union of Pure and Applied Chemistry
$^{\bullet}NO$	Nitrogen oxide radical
16α–OHE	16α-hydroxyestrone
2–OHE	2-hydroxyestrone
6CpG	Cytosine Guanine base pair
JNK	c-Jun N-terminal kinases
KW	Kruskal Wallis
LDH	Lactate dehydrogenase
LH	Luteinizing Hormone

LRTAP	Long range transboundary air pollution
LoD	Limit of detection
MAPKs	Mitogen-activated protein kinases
MCF–7	Michigan Cancer Foundation–7
mRNA	Messenger ribonucleic acid
MUHAS	Muhimbili University of Health and Allied Sciences
Mwt	Molecular weight
NAC	N-acetylcysteine
NDL–PCBs	Non dioxin like polychlorinated byphenyls
NF-$_K$B	Nuclear factor kappa B
NRs	Nuclear Receptors
o,p'-DDD	1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p* chlorophenyl)ethane
o,p'-DDE	1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethylene
o,p'-DDT	1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p* chlorophenyl)-ethane
$O_2^{\bullet-}$	Superoxide radical
OCPs	Organochlorinated pesticides
OCs	Organochlorine compounds
OR	Odds Ratio
p,p'-DDD	1,1-dichloro-2, 2-bis (4-chlorophenyl)ethane
p,p'-DDE	1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene
p,p'-DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane
p53	Tumour protein 53
PAH	Poly Aromatic Hydrocarbon
PCBs	Polychlorinated biphenyls
PBDE	Polybrominated diphenyl ether
PCDDs	Polychlorinated dibenzo-*p*-dioxins
PCDFs	Polychlorinated dibenzofurans
PeCB	Pentachlorobenzene
PI3K	Phosphatidylinositol 3-kinase
POPs	Persistent Organic Pollutants
PR	Progesterone Receptor
PTDI	Provisional Tolerable Daily Intake
PXR	Rodent pregnane X receptor
R^2	Coefficient of determination
ROS	Reactive oxygen species
RR	Relative Risk
SAPKs	Stress-activated protein kinases
SIM	Selected ion monitoring
SMR	Sexual Maturity Rating
SOD	Superoxide dismutase
SPSS	Statistical Package for Social Sciences
STAT	Signal transducers and activators of transcription
TC	Total Cholesterol
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TEF	Toxic Equivalent Factor
TEQ	Toxic Equivalent
TG	Triglycerides
TL	Total Lipid
TRs	Thyroid hormone receptors
UN	United Nations
UNEP	United Nations Environmental Programme
UNIMI	Università degli Studi di Milano
USA	United States of America
WHO	World Health Organization
XRE	Xenobiotic response element
β-HCH	Beta-hexachlorocyclohexane

LIST OF UNITS

˚C	Degree celsius
eV	Electron volts
g/L	Grams per litre
kg/m^2	Kilograms per square metre
mg/mL	Milligrams per millilitre
mL/min	Millilitres per minute
ng/g lipid	Nanograms per gram serum lipid
pg/g lipid	Picograms per gram serum lipid
pmol/g lipid	Picomoles per gram of serum lipid
ppm	Parts per million
µg/kg lipid	Micrograms per kilogram serum lipid
µg/ml	Micrograms per milliliter
µL	Microlitre
ng TEQ kg^{-1}	Nanograms toxic equivalent per kilogram
TEQ pg/g fat	Toxic equivalent picograms per gram of fat

CHAPTER ONE

INTRODUCTION

1.1 General Introduction

1.1.1 Characteristics of Persistent Organic Pollutants

Persistent Organic Pollutants (POPs) represent a group of structurally diverse man-made and naturally chemicals characterized by environmental persistence and ability to accumulate in the food chain. According to UNEP POPs are defined as "*chemical substances that persist in the environment, bio-accumulate through the food web, and pose a risk of causing adverse effects to human health and the environment* (UNEP 1995). Once released in the environment, they break down very slowly in air, water, soil and in living organisms. These chemicals are thus difficult to be degraded in the environment by chemical, biological and photolysis processes.

POPs are subject to long range trans-boundary air pollution (LRTAP) transport where they are carried over long distances via the atmospheric transport. They migrate from warmer regions to the colder polar ones where they condense, precipitate and deposited again on earth surface as dry or wet deposit. POPs can also be transported for long distance by river, ocean currents and as contaminants in wildlife. DDT for example has been found to have this ability to cross international border and travel long distances in air and water. As a result DDT and its metabolite are found in Arctic air, sediment and snow with substantial accumulations in food chain, mammals and human in these regions. Even the body burden of the flocks of migratory birds is a sizeable contribution to LRTAP (WHO, 2003). Hexachlorobenzene is widely distributed in the environment due to long range transport via atmospheric or oceanic system. It has been detected in air, water, sediment, soil and biota from around the world. Its bioaccumulation and biomagnification through food chain has been reported. Polychlorinated biphenyls (PCBs) are semi volatile and resistance to degradation. These characteristics predispose them to long range transport. They are found in many parts of the world including those with no PCB production or use have even been reported, such as in Arctic regions. Analyses of food and breast milk show that PCBs are still present in those regions.

Many POPs have been employed in agriculture and public health as pesticides (DDT, lindane, hexachlorobenzene), others were used in industry as raw materials (polychlorinated biphenyls) or as by-products of production processes (dioxins and

furans). In particular, the introduction of synthetic organic chemicals in the second half of the 20th century boosted the capability to counter crop- and food-spoiling organisms and eradicate or control parasite-borne diseases such as malaria, both improving the quality of life of large populations in temperate and semi-tropical areas and allowing better utilization of agricultural areas. Because of their persistence they are widely distributed in the environment and human exposure to them is unavoidable. This is evidenced as they are detected in human tissues such as such as blood (whole blood, cord blood, serum and plasma), adipose tissues (from autopsies and biopsies), breast milk, muscles and hair (Appenzeller and Tsatsakis, 2012; Covaci *et al.*, 2002; Dewailly *et al.*, 1999; Jakszyn *et al.*, 2009; Mrema *et al.*, 2011; Rappolt and Hale, 1968; Tsang *et al.*, 2011; Tsatsakis and Tutudaki, 2004; Tsatsakis *et al.*, 1998, 2008; Tutudaki *et al.*, 2003).

Human exposure begins during early prenatal and continues during the breast-feeding neonatal periods, which are critical stages for the development and differentiation of sensitive body organs and systems. In fact, these chemicals cross the placenta to the foetus and are secreted into breast milk (Perera *et al*., 2005). In adults the dietary exposure route accounts for more than 90% of total organochlorine compounds (OCs) burden for the general population, whilst workers are exposed mainly through inhalation and skin contact.

Many POPs are highly toxic and can turn up in the food chain where bioaccumulation occurs in fatty tissues of animals and human as they are soluble in these tissues. Low environmental levels of POPs can lead to high levels in the body tissues as their concentration can become magnified up to 70,000 times higher than the background levels. Exposure to these persistent chemicals has been associated with health effects including cancer (Aronson *et al*., 2000; Cohn *et al.*, 2007; Mathur *et al.*, 2002; Recio-Vega *et al.*, 2011), reproductive defects (Nicolopoulou and Stamanti, 2001) and behavioral changes (Zala and Penn, 2004). These effects are believed to be related to POPs' ability to disrupt the functions of certain hormones, enzymes, growth factors, neurotransmitters and to interfere with the expression of some key genes involved in metabolism of steroids and xenobiotics (Gourounti *et al.*, 2008). Early ecological and human health concern led to intensive campaign against their use, leading to a gradual ban or restriction of their production and use in most countries starting in the early

1970s and finally ending with the Stockholm Protocol in 2001 (UNEP, 2001; http://www.pops.int).

1.1.2 Classes of Persistent Organic Pollutants

It was since the publication of Rachel Carson's book '*Silent Spring*' (Carson, 1962) when ecological and health concerns of uncontrolled application of pesticides quickly rose especially when highly populated regions of developed countries started to suffer the effects of widespread contamination as a threat to their own food sources. Industrialized countries started to limit application of pesticides, to ban their production and to enforce limits to their presence as ubiquitous contaminants of water and food.

In May 1995, United Nations Environmental Programme (UNEP) Governing Council initiated a global action to reduce and eliminate the release of POPs. Following this, the Intergovernmental Forum on Chemical Safety (IFCS) and the International Programme on Chemical Safety (IPCS) produced the initial list of 12 POPs for assessment based on their properties and the urgency for action. These compounds were named as "*the dirty dozen*" or "*poisons without passports*" and were listed in Annex A, B and C (**Table 1.1**). Of the more ecological and human health concerns compounds are OCPs such as DDT (IPCS, 1990) and lindane (IPCS, 1991). Others are industrial chemicals such as polychlorinated biphenyls (IPCS, 2003) and hexachlorobenzene (IPCS, 1997). Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzo furans are also of more concern (IPCS, 1989) with no known uses but unintentionally produced via several technological processes (Ritter *et al.*, 1995) such as photochemical and thermal processes or as byproducts in municipal waste incineration (Abad *et al.*, 2000), pesticide manufacturing (Masunaga *et al.*, 2001) and in chlorine bleaching of pulp and paper (Swanson *et al.*, 1988).

After a long elaboration and negotiations, an international environmental treaty, the Stockholm Convention, was signed in 23 May 2001 to eliminate or restrict the production and use of some priority persistent organic pollutants (POPs). It entered into force on 17 May 2004 with ratification by initial 128 parties and 151 signatories. As of April, 2011, there are 173 parties to the Convention and by October 2011 three more parties were added (http://chm.pops.int/). Parties to the Convention agreed to periodically reviewing the list to add more compounds or classes of compounds, when they meet criteria for persistence and trans-boundary threat. As a result a second set of nine new POPs was added to the list during its 4th meeting held in Geneva on 8 May 2009. Of these, four are pesticides, three isomers of hexachlorocyclohexane (HCH) and chlordecone (also known as kepone). Others are brominated flame retardants and

perfluorinated organic acids. In May 2011 technical endosulfan and its related isomers were added to the list (UNEP, 2011).

Due to the efficacy of some organochlorinated pesticides, such as DDT and lindane, in fighting parasite-borne diseases such as malaria and scabies, respectively, exemptions allowing to continue production from registered parties and use for specific purposes are embedded in Annex B, the enforcement of which is however a task of the individual countries.

Table 1.1. An extended list of POPs banned or restricted by the Stockholm Convention.

Chemical	Pesticides	Industrial Chemicals	Byproduct	Annex
Aldrin[a]	+			A
Chlordane[a]	+			A
Chlordecone[b]	+			A
DDT[a]	+			B
Dieldrin[a]	+			A
Endrin[a]	+			A
Heptachlor[a]	+			A
Lindane[b]	+			A
Mirex[a]	+			A
Technical endosulfan and its related isomers[c]	+			A
Toxaphene[a]	+			A
Hexabromobiphenyl[b]		+		A
Hexabromodiphenyl ether and heptabromodiphenyl ether (commercial octabromodiphenyl ether)[b]		+		A
Perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride[b]		+		B
Tetrabromodiphenyl ether and pentabromodiphenyl ether (commercial pentabromodiphenyl ether)[b]		+		A
Chlorinated dioxins[a]			+	A
Chlorinated furans[a]			+	A
Alpha hexachlorocyclohexane[b]	+	+		A
Beta hexachlorocyclohexane[b]	+	+		A
Pentachlorobenzene[b]	+	+		A & C
Polychlorinated biphenyls[a]		+	+	A
Hexachlorobenzene[a]	+	+	+	A

[a] Present in the initial "Dirty Dozen" list produced in 2001; [b]Added by the Fourth Conference of Parties, May 2009; [c]Added by Fifth Conference of Parties, May 2011; ***Annex A*** *– Intentionally produced chemicals that need to be eliminated****; Annex B*** *– Intentionally produced chemicals with restrictions****, Annex C*** *– Unintentionally produced chemicals.*

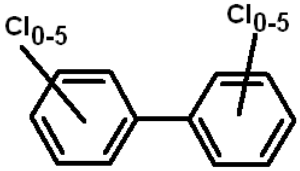
Some of the categories listed in **Table 1.1** above overlap. For example mirex which was produced primarily for use as a pesticide has also been used as a fire retardant; PCBs which were produced as industrial chemicals are also generated as unwanted by-products whereas hexachlorobenzene fits into all three categories i.e. as pesticide, industrial chemical and by-product.

Among the several existing POPs, in this study only polychlorinated biphenyls (PCBs) and organochlorinated pesticides (OCPs) have been addressed.

1.1.3 Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) constitute a group of 209 chemically related compounds which were used extensively in industrial applications and in commercial products. Their chemical formula is $C_{12}H_{10-x}Cl_x$ with 1 to 10 chlorine atoms substituting hydrogen atoms in the nucleus of biphenyl ring (**Table 1.2**). In terms of structure-toxicity relationship, PCB congeners are categorized as coplanar and non-coplanar congeners. The former contain one or no chlorine attached to *ortho* position while the latter has one or more than two chlorine atoms attached to *ortho* position of biphenyl ring as illustrated in **Figure 1.1**. The coplanar groups have a fairly rigid structure with the two phenyl rings in the same plane. This gives the molecule a similar structure to polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and allows it to interact in the same way with biological targets at the molecular level. For this reason coplanar congeners are referred to as dioxin–like (DL) PCBs while the non-coplanar are non–dioxin like (NDL) PCBs.

Table 1.2. The general schematic structure of 209 polychlorinated biphenyl (PCB) congeners

Polychlorobiphenyls	PCB Homologues	Formula	Mwt	N° of Congeners
	biphenyl	$C_{12}H_{10}Cl_0$	154.21	1
	monochlorobiphenyl	$C_{12}H_9Cl_1$	188.66	3
	dichlorobiphenyl	$C_{12}H_8Cl_2$	223.10	12
	trichlorobiphenyl	$C_{12}H_7Cl_3$	257.55	24
	tetrachlorobiphenyl	$C_{12}H_6Cl_4$	291.99	42
	pentachlorobiphenyl	$C_{12}H_5Cl_5$	326.44	46
	hexachlorobiphenyl	$C_{12}H_4Cl_6$	360.88	42
	heptachlorobiphenyl	$C_{12}H_3Cl_7$	395,33	24
	octachlorobiphenyl	$C_{12}H_2Cl_8$	429.77	12
	nonachlorobiphenyl	$C_{12}H_1Cl_9$	464.22	3
	pentachlorobiphenyl	$C_{12}H_0Cl_{10}$	498.66	1

Note: Biphenyl is not PCB congener due to its lack of chlorine atom but it is still included in the list.

The DL–PCBs act similarly like the most toxic dioxin 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (2,3,7,8-TCDD), since their planar configuration allows binding to the recognition site of the aromatic hydrocarbon receptor (AhR), thus causing biological effects similar to those of dioxins (Safe, 1993). The bound AhR dissociates from the inactive dimeric form to the 'activated' monomeric agonist-bound form which translocates from the citosol to the cell nucleus. The monomeric form binds specific DNA-binding proteins causing them to dissociate from the gene sequence and causing transcription or inhibition of the transcription of downstream gene elements, among which are catalytic or signaling proteins with a biological role in challenging such biological phenomena as exposure to xenobiotics (by enhancing the transcription of phase I and II metabolizing enzymes) or endogenous oxidative stress (by enhancing transcription of antioxidant enzymes).

As a consequence, substances such as PCBs with limited if any potential to be biotransformed into reactive intermediates can enhance biotransformation of other xenobiotics, such as PAH, into reactive potentially genotoxic and carcinogenic metabolites. Moreover, the action of some PCBs as structural mimics of natural estrogen receptor-binding agonists (endocrine disruption) can enhance cell proliferation and thus increase risk of estrogen-dependant cancer.

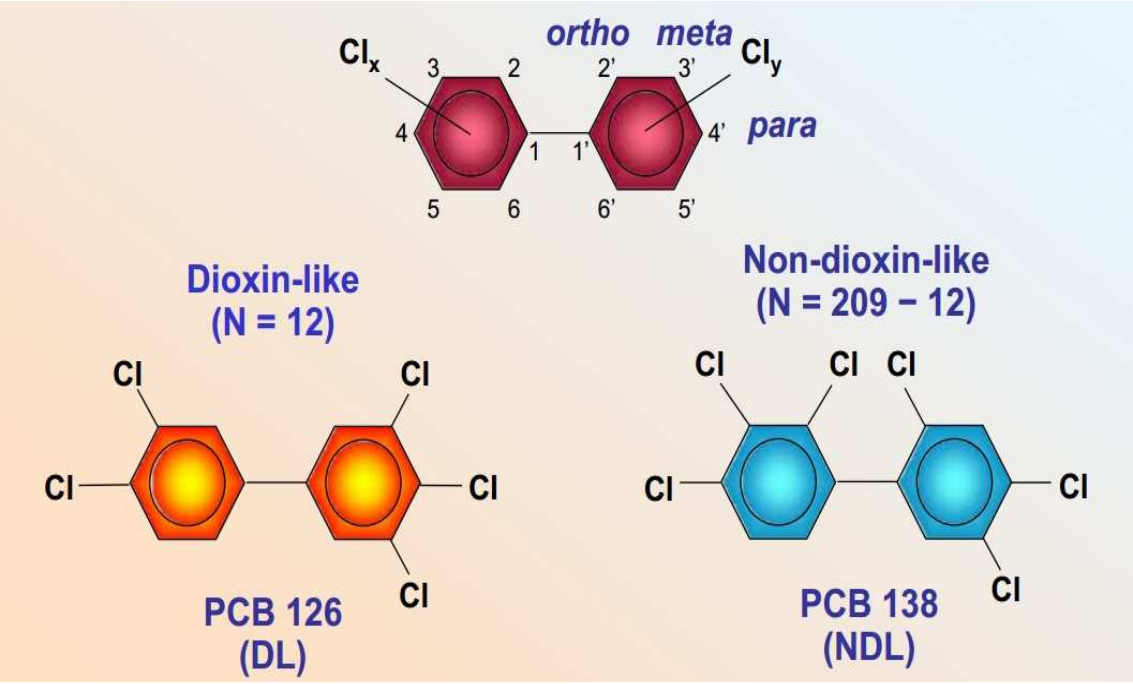


Figure 1.1. Illustration of the generalized structure of the two classes of PCB congeners.

Complex mixtures of PCBs (branded as Aroclor in the USA and as Apirolio or Fenclor in Italy) found large industrial applications mainly as dielectric fluids in high voltage appliances such as capacitors and transformers and as non flammable heat-transport fluids in heat exchangers. Globally total PCBs production was ~1.3 million tonnes between 1930 and 1993. In Italy it is estimated at around 150,000 metric tonnes from 1938 to 1984, with production in the 1970s between 2,500 and 5,000 metric tonnes per year from Caffaro the main producing plant in Northern Italy (Ruzzenenti, 2001). Another estimate gives figures of 31,092 metric tonnes being produced between 1958 and 1983 (Breivik *et al.*, 2002). In 1977 production and use of PCBs was banned by USA and in 2001 by the Stockholm Convention on Persistent Organic Pollutants (UNEP, 1999).

Since environmental exposure to PCBs is always due to complex and variable mixtures, toxic equivalent (TEQ) concept was developed to assist health risk assessment due to PCBs exposure by using toxic equivalent factors (TEFs). TEF indicates the degree of toxicity compared to 2,3,7,8–TCDD, the most toxic POP which is given a reference value of 1 (Ahlborg *et al.*, 1994; Safe, 1990). Other dioxin–like compounds such as PCDDs, PCDFs and some PCB congeners have TEFs which span from 1 (*i.e.*, a compound as toxic as 2,3,7,8–TCDD) to 5 x 10^{-6} (*i.e.*, a compound which is *approx*. a million times less toxic than 2,3,7,8–TCDD).

It has to be noted that not all PCB congeners have TEFs. Some criteria have been set to qualify a compound to the TEF concept (Van den Berg *et al.*, 1998, 2006) as outlined below:

a. It must have a structural relationship with PCDDs and PCDFs
b. It must bind to aromatic hydrocarbon receptor (AhR)
c. It has to elicit AhR-mediated biochemical and toxic responses
d. It has to persist and accumulate in the food chain.

Thus DL–PCBs fulfil these criteria and have been assigned TEFs. These include four non-*ortho* PCBs (nPCB 77, 81, 126, 169) and eight mono-*ortho* PCBs (mPCB 101, 114, 118, 123, 156, 157, 167 and 187). In 1994 Ahlborg *et al*. assigned TEFs also to two di-*ortho* PCBs (PCB 170 and 180) where in the revisions by Van den Berg *et al.* (1998, 2006) they were not considered.

A TEF weighted sum of lipid adjusted concentrations is calculated to obtain TEQ as shown in the equation 1 below:

$$TEQ_{DL\text{-}PCB} = \sum(nPCB_i \times TEF_i) + \sum(mPCB_i \times TEF_i) \quad (1)$$

The toxicity of specific PCB congeners depends on its planarity, the number of chlorine atoms attached to biphenyl rings and biotransformation rate. Majority of their adverse effects are thought to be mediated through aryl hydrocarbon receptor (AhR) activation. For instance the coplanar non-*ortho*-substituted PCBs activate AhR and AhR dependent signal transduction pathways (van den Berg *et al.*, 1998) and elicit dioxin-like effects such as anti estrogenic activity (Buchanan *et al.*, 2000, 2002; Oenga *et al.*, 2004; Safe and Wormke, 2003). The modes of these anti estrogenic activities may include repression of 17β-estradiol (E2)-dependent gene expression by interactions of activated AhR with DNA regions of E2 responsive gene promoters (Oenga *et al.*, 2004; Safe and Wormke, 2003), inhibition of E2-induced cell cycle proteins and uterine epithelial mitogenesis (Buchanan *et al.*, 2002; Wang *et al.*, 1998) or effects of PCBs on E2 metabolism (Pang *et al*., 1999; van Duursen *et al*., 2003). Non coplanar *ortho*-substituted PCBs have been shown to elicit AhR-independent effects such as neurotoxicity, (anti) estrogenicity and tumour promotion in MCF–7 cells (Brouwer *et al.*, 1999; Hansen 1998; Machala *et al.*, 2003; Robertson and Hansen, 2001; Safe, 1992; Wolff and Toniolo, 1995). However their modes of action are not clear.

Low molecular-weight PCBs have been found to elicit estrogenic activity both *in vitro* and *in vivo* as observed in several studies (Arcaro *et al.*, 1999; Nesaretnam and Darbre, 1997; Rogers and Denison, 2000; Rose *et al*., 2002) but with exception of DL-PCB 77, which elicited anti estrogenicity *in vivo* and also in some *in vitro* models (Ramamoorthy *et al*., 1999). On the other hand, some highly substituted PCBs such as the most prevalent di-*ortho* substituted PCBs (PCB 138, 153 and 180) have been reported to be antiestrogenic (Bonenfeld-Jorgensen *et al*., 2001; Plísková *et al.*, 2005).

Like other organochlorines, PCBs are strong inducers of key genes involved in metabolism of steroids and xenobiotics. PCB congeners that are sterically similar to dioxin induce *CYP1A1* and *CYP1A2* activities by binding with the aryl hydrocarbon receptor while congeners with at least two *para* and two *ortho* substitutions exhibit phenobarbital-like effects that induce *CYP2B1* and *CYP2B2* activities. Due to this

complex array of potential PCB effects, congener specific analysis is recommended to capture all potential effects which could be missed by evaluating only total PCB.

The most health-threatening PCBs congeners include those which are coded as 77, 126, 169, 105, 118, 128, 138,156, 170, 87, 99, 101, 153,180, 183, 194, 18, 44, 49, 52, 70, 74, 151, 177, 187, 201, 37, 81, 114, 119, 123, 157, 158, 167, 168 and 189 classified based on their environmental occurrence, abundance and potential toxicity (McFarland and Clarke, 1989). International Agency for Research on Cancer (IARC) classified PCBs as probably carcinogenic to human under group 2A. Agency for Toxic Substances and Disease Registry (ATSDR) concluded that PCBs which contain 60% Cl by weight are clearly carcinogenic. A non-*ortho* PCB 126 which has a high contribution to PCB TEQ (TEF = 0.1) has recently been established as a carcinogenic to humans (Consonni *et al.*, 2012).

1.1.4 Organochlorinated Pesticides

Organo-chlorinated pesticides (OCPs) are a structurally heterogeneous class of chlorinated organic compounds (OCs) which contain several chlorine atoms per molecule. Among OCPs of prominent concern are dichlorodiphenyltrichloroethane (DDT) and its metabolites, chlordane, dieldrin, aldrin, endosulfan, hexachlorobenzene (HCB) and hexachlorocyclohexane (HCH) isomers such as lindane (**Figure 1.2**). These chemicals are widely distributed in the environment. They were used since the mid-1940s as insecticides in agriculture and in public health for the control of parasite-borne diseases. Some compounds such as DDT is still used in tropical countries, to control malaria vectors through indoor residual spraying (Bouwman *et al.*, 2011) and lindane for treatment of lice and scabies (Engeler, 2009).

Their high content of chlorine in a mostly rigid carbon atom scaffold makes the molecules highly soluble in the lipid compartment of living organisms and poorly degradable in the environment by physical, chemical and biological processes (Mrema *et al.*, 2012a). The consequence of both properties, together with widespread and high-intensity use as pesticides for at least 30 years led to exposure to these chemicals of virtually every person and living organism on earth at different magnitude (Carson, 1962; Mrema *et al.*, 2012a). Due to their long environmental and biological half-lives, most banned OCPs are still present at trace levels in several foods, and thus represent a source of exposure for the general population in all the corners of the world, with

significant geographically based differences (Darnerud *et al.*, 2006; Wang *et al*., 2007; UNEP, 2003; WHO, 2003). Main sources of exposure are some sea foods, milk and dairy products, meat and poultry, fats and oils.

The insecticide DDT is produced as a technical mixture of 3 isomeric forms, the most prevalent form being the *para,para* (*p,p'*)-isomer; *ortho,ortho* (*o,o'*)- and *ortho,para* (*o,p'*)- isomers being present in minor and variable amounts. Its main by- and bio-transformation products are dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD). DDT was initially used in public health control of lice and malaria mosquitoes during the Second World War and afterwards it was used as pesticide in agriculture. Globally DDT production was ~1.8 million tonnes since 1940s (ATSDR, 2002) and more than 40,000 tonnes were used in agriculture annually (Geisz *et al.*, 2008). It is estimated that 600,000 tonnes of DDT were used domestically in USA 30 years prior to its ban. Over 80% of the quantity of the pesticide used in 1970–72 was applied to cotton crops and the remainder was used on peanut and soybean crops. The global production was reported to drop to 3,314 tonnes in 2009; the production is meant for use in control of malaria and leishmaniasis (UNEP, 2010a).

Complex and variable mixtures of hexachlorocyclohexane (HCH) products are produced by photochemical chlorination of benzene. HCH exists in 9 stereoisomeric forms, which include (+)- and (–)-alpha- (α), beta- (β), gamma- (γ), delta- (δ), epsilon- (ε), zeta-(ζ), eta- (η) and theta- (θ) isomers. Technical-grade HCH is comprised of 60-70% α-HCH, 5–12% β-HCH, 10–15% γ-HCH, 6–10% δ-HCH, and 3–4% ε-HCH. Purified HCH isomers, as well as technical-grade HCH are used either as fungicides or in the synthesis of other chemicals.

Lindane (γ-isomer) was used as seed treatment for barley, corn, oats, rye, sorghum and wheat to protect seeds for sowing from moulds and ants during storage before sowing and in the ground before sprouting. The estimation of global production between 1950 and 2000 was 600,000 tonnes and the majority was used in agriculture. In the past, γ-HCH was also used in veterinary products to control mites, lice and other pests, but recent data suggest that no products are currently registered, at least in the United States for this use. It was also used as an insecticide to treat fruit, vegetables, forest crops, animals and animal premises. It is also currently used in treatment of scabies and lice in humans in form of lotion, cream or shampoo, especially in tropical countries. β-HCH was widely used on cotton plants during 1960s and 1970s.

Hexachlorobenzene, a fully chlorinated aromatic hydrocarbon, was widely used as fungicide. Some amounts of HCB are formed as a by-product during the manufacture of solvents and other pesticides. The chemical was also used to make fireworks, ammunition and synthetic rubber. Small amounts can be produced by burning of city wastes. Its environmental half-life in soil and in air is of *approx.* 3–6 years and 0.63–6.28 years, respectively (ATSDR, 2002). In human the half-life of HCB is thought to be extremely long in the range of 6 years (To-Figueras *et al.*, 2000).

Endosulfan is synthesized via Diels-Alder addition of hexachloro-cyclopentadiene and cis-butene-1,4-diol in xylene. The annual production is estimated to range between 18,000 and 20,000 tonnes worldwide (UNEP, 2010b). In USA, 400 tonnes are estimated to be used annually for domestic purpose. The main uses include control of pests in vegetables, fruits, cereal grains and cotton, ornamental shrubs, trees, vines and ornamental plants. It was also used to control ectoparasite on beef and lactating cattle. In Africa it is commonly used in cotton production at a dose rate between 1,000 to 2,000 g/ha while in India it is used to control pests on cashew plantations at the dose rate of 1,200 g/ha. The estimated annual production in India is 9,500 tonnes, of which 4,500 to 5,000 tonnes are consumed domestically.

These OCPs can constitute a source of health risk (ATSDR, 2005; Mrema *et al.*, 2012b; Porta *et al.*, 2008; UNEP, 2003; WHO, 2003). In experimental models, DDT isomers are capable of inducing alterations on reproduction and development due to their hormone-altering actions. *o,p'*-DDT has the strongest estrogen-like properties, *p,p'*-DDE has anti-androgenic properties. The hormone disrupting properties of *o,p'*-DDD (mitotane) lead to be used as anticancer drug for adrenocortical carcinoma. All HCH isomers can produce liver and kidney effects (ATSDR, 2005). In humans the most sensitive target organs of HCB are the liver, the ovary and the central nervous system (ATSDR, 2002).

The International Agency for Research on Cancer (IARC) has classified DDT, all HCH isomers and HCB as possibly carcinogenic to humans under group 2B (IARC, 1987, 1991). Apart from cancer risk, or possibly as one causal mechanism of OCP-triggered cancer, the endocrine disruption activity of these substances deserves attention. In particular, DDT isomers are capable of inducing alterations on human reproduction and development due to their hormone-altering actions. For instance, *o,p'*-DDT isomer has the strongest estrogen-like properties (ATSDR, 2002) whereas *p,p'*-DDE has anti-

androgenic properties which has been shown to alter the development of reproductive organs when administered perinatally to rats (Gray *et al.*, 2001).

Ubiquitous presence of DDT in human tissues has been demonstrated in hundreds of studies, most of which are collected and reviewed in monographs by United Nations (UN) agencies (IARC, 1991; ATSDR, 2002). The human specimens most commonly used for OCPs measurement are blood (serum), but also visceral and female breast adipose tissue from autopsies and from living subjects.

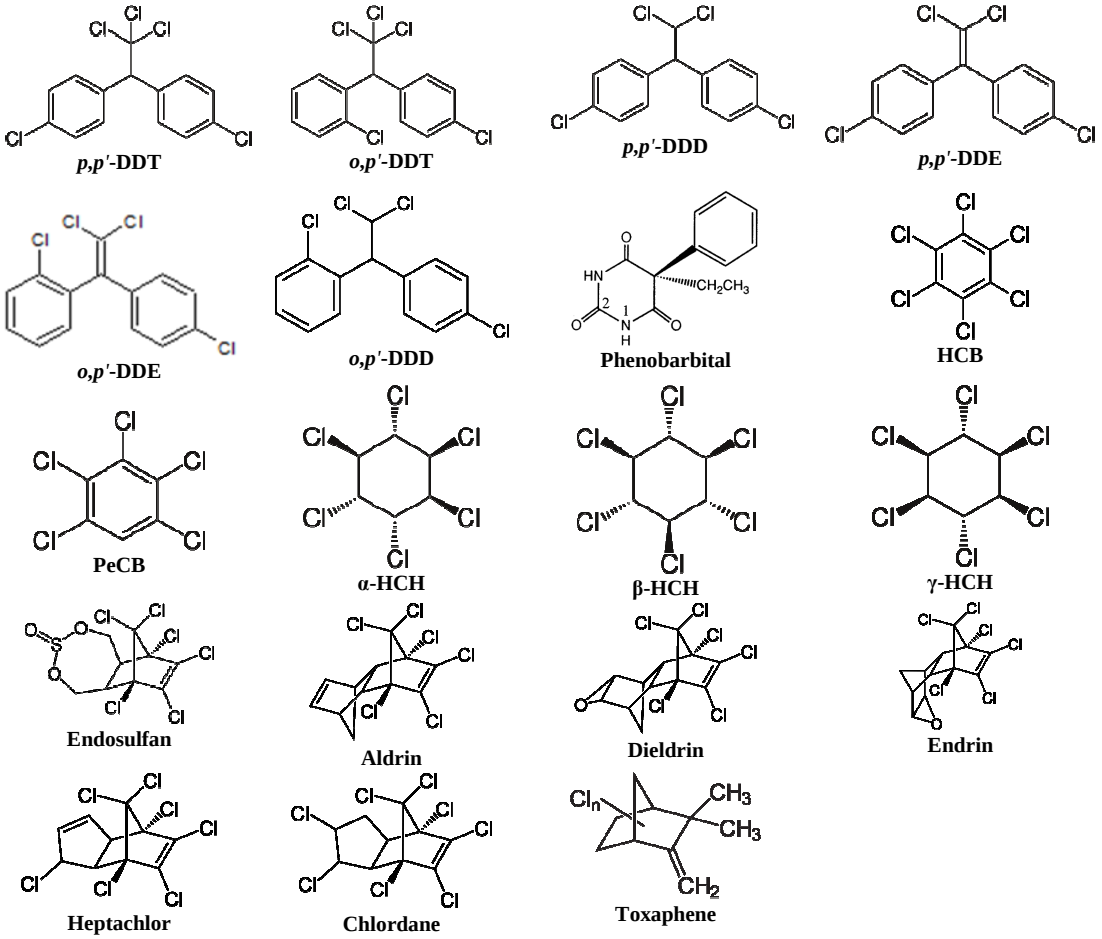


Figure 1.2. Molecular structure of phenobarbital (a prototypic CYP2B inducer) and the OCPs listed in the annexes of the Stockholm Convention.

1.2 Literature Review

1.2.1 Food as a Main Source of Exposure to POPs

People are exposed to POPs in several ways including inhalation, ingestion and skin contact. Inhalation and skin contact are the main routes of exposure in occupation settings. Other exposures occur through accidents like the explosion which occurred at a pesticide manufacturing plant in Seveso, Northern Italy in 1976 which exposed people to a large amount of dioxin (Mori and Todaka, 2011). Accidental exposure to PCBs and PCDFs was reported in Japan in 1968 and Taiwan in 1979 due to consumption of rice bran oil contaminated with PCBs that was used as a heat transfer medium in the manufacture of the oil. This led to the disease conditions known as Yusho, in Japan, and Yu-Cheng, in Taiwan (Aoki, 2001; Kuratsune *et al.*, 1987; Rogan *et al.*, 1988).

In people of general population, diet accounts for at least 90% of the total exposure to OCs (Duarte-Davidson and Jones, 1994; De Felip and Ingelido, 2008; Bilau *et al.*, 2008; Turci *et al.*, 2010b). As a consequence of widespread environmental contamination and biomagnification along the food chain, OCs are present in foods at levels which show significant variability among world countries and among different areas within individual countries, in particular in areas previously or currently used for intense agricultural activities and high levels of pesticide application. The dietary intake thus varies between countries and between populations groups within countries, as exemplified by the – data reported in **Tables 1.3** and **1.4**.

Table 1.3. Estimated dietary intake in nanograms per day (ng/day) of some POPs through seafood consumption by the general population of different countries (data adopted from Moon *et al.* (2009) and references therein).

Country	PCBs	DDTs	CHLs	HCHs	HCB	Authors
Indonesia	810	1100	10.0	18.0	10.0	Sudaryanto *et al.* (2007)
China	110	882	14.4	28.2	11.4	Yang *et al.* (2006)
Sweden	349	256	87.0	35.0	36.0	Darnerud *et al.* (2006)
Italy	–	197	–	13.0	4.6	Stefanelli *et al.* (2004)
Spain	–	–	–	–	12.4	Falcó *et al.* (2008)
South Korea	–	270	13.0	2.1	11.0	Moon *et al.* (2009)

Keys: Total PCBs, ∑PCB 8, 18, 28, 29, 44, 52, 87, 101, 105, 110, 118, 128, 138, 153, 170, 180,187, 194, 195, 200, 205, 206; Total DDTs, ∑*p,p′*-DDE, *p,p′*-DDD, *p,p′*-DDT, *o,p′*-DDD; CHLs, ∑oxy-chlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor; Total HCHs, ∑α-, β-, γ-HCH.

WHO reported the highest dietary intake of DDT in developing countries whereas Codex Alimentarius reported the highest estimation of lindane intake via food in Europe with a theoretical maximum daily intake of 0.742 mg, or 12 times the ADI. It has been

noted that due to the globalization of food trade almost all countries in the world may be subject to the impacts of POPs (Kaloyanova-Simeonova).

Table 1.4. Total DDT intake compared with the PTDI (Source: WHO, 2003).

Country	Year	Daily intake (ng/kg b.wt)	% of PTDI
Australia	1980	0.390	3.9
	1987	0.026	0.26
Egypt	1988	13.700	137.0
Finland	1984	0.041	0.42
	1986	0.026	0.26
Guatemala	1982	0.260	2.6
	1984	0.200	2.0
	1985	0.065	0.66
	1988	0.031	0.32
India	1981	3.900	39.0
	1983	3.600	36.0
Japan	1980	0.056	0.56
	1982	0.070	0.74
	1984	0.030	0.3
	1986	0.020	0.2
	1988	0.020	0.2
Netherlands	1984	0.004	
	1985	0.004	0.04
New Zealand	1982	0.003	0.03
Switzerland	1983	0.03	0.3
Thailand	1980	1.600	16.0
	1987	0.0008	0.008
United Kingdom	1980	0.050	0.5
	1981	0.035	0.36
	1985	0.05	0.5
United States	1980	0.360	3.6
	1982	0.033	0.34
	1985	0.036	0.36
	1986	0.019	0.2

Abbreviations: ADI, acceptable daily intake (0–0.02 mg/kg b.wt) (FAO/WHO 1984); PTDI, provisional tolerable daily intake (0–0.01 mg/kg b.wt) (FAO/WHO 2000).

Most food contamination occurs in meat and meat products, fish, milk and dairy products (Zuccato *et al.*, 1999). Deposition of airborne OCs on pasture is considered the primary pathway from which these pollutants enter the food chain, although in regions with highly contaminated soil, green vegetables may significantly contribute to human exposure. Food-producing animals accumulate OCs into the adipose tissue through ingestion of contaminated pasture or the feed derived from contaminated fatty materials.

OCs are thus transferred to animal milk and enter into human diet through milk and dairy products.

In the past phenoxyacetates, widely used herbicides were found to be contaminated by dioxin. In particular the formulation containing 2,4,5–trichlorophenoxy acid (2,4,5–T) was found to be contaminated by 2,3,7,8–tetrachlorodibenzo-*p*-dioxin (2,3,7,8–TCDD). This caused episodes of significant environmental contamination where the highest levels were observed in 1970s, when phenoxy herbicides found high levels of use in rice crops. Deliberate dispersion of unwillingly contaminated phenoxy herbicides had also occurred *e.g.* Agent Orange in South–Eastern Asia which was used by the U.S. military as part of its chemical warfare program.

Although fish and sea food is a small fraction of human diet, it is a major route of exposure for arctic populations who live on marine mammals, which are at the top of the marine trophic network (Bonde *et al.*, 2008). Low level of PCB intake from nine fish meals per month has been shown to exceed the tolerable monthly intake and thus can pose risk for human health (Carubelli *et al.*, 2007). Fish and other seafood have shown to represent principal means of contamination (Binelli and Provini, 2003). A significant association between levels of PCBs, DDT and DDE in human plasma and fish consumption was demonstrated and thus it was suggested that fish were the major source of exposure to these compounds among Swedish population (Asplund *et al.*, 1994; Svensson *et al.*, 1991). The study conducted by Fitzgerald *et al.* (2006) showed that a mono-*ortho* congener PCB 74 was related to rates of fish consumption among Native American population. In Sweden consumption of fatty fish from Baltic Sea represent a major source of PCBs, DDT and its metabolite *p,p'*-DDE. PCDDs/Fs and PCBs have been detected in home-produced eggs in Begium where they presented at levels of health concern for the consumers. Soil was the source of contamination by these chemicals (Van Overmeire *et al.*, 2009).

Intensive harvesting of Manila Clam and fishing in lagoon of Venice, Italy, was shown to present a risk to human health which was evidenced by presence of POPs in mother milk and blood serum of Venetians with different work exposure and food habits. This lagoon was reported to receive discharges of POPs such as polychlorinated biphenyls (Raccanelli *et al*., 2009). In 2008 Fattore *et al.* estimated the dietary intake of NDL–PCBs among Italian general population to be 24.6, 16.1 and 10.9 ng/kg-b.wt/day for

toddlers, children and adults, respectively. Fish and fishery products, milk and milk products were the major contributors.

Unlike other studies, Abballe *et al.* (2008) failed to establish correlation between NDL–PCB in human milk with fish-rich diet. The author thought that the inconsistency could be due to inverse correlation between consumption of fish and dairy products. However, the study showed the increase in PBDE 153 concentration with increase in fish consumption.

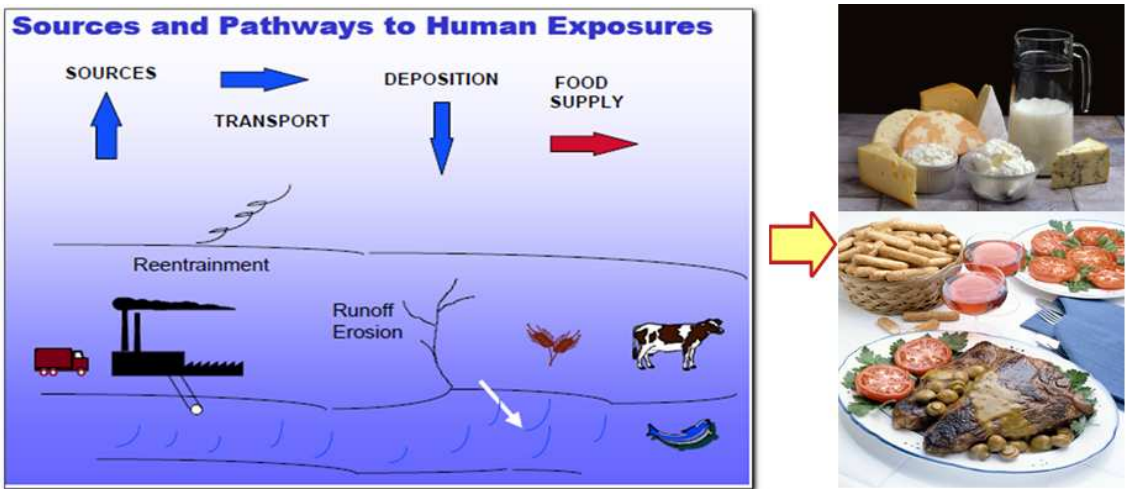


Figure 1.3. Sources and pathways of persistent organic pollutants to human exposures.

Human exposure of POPs can be well reflected in their presence in biological compartments such as human breast milk, hair, adipose tissue, breast fatty tissue and blood. Hence measuring the levels of POPs in these compartments provides a direct measure of the internal dose of these chemicals, to establish the relationship between these exposures and their health effects. Human breast milk analysis is limited only to lactating women. Measurement is usually performed in blood serum or in body fat, which are the transport and storage compartments of highly lipophilic compounds, respectively.

Blood is the least invasive and is the most easily accessible human specimen for such measurements. Adipose tissue specimens can only be obtained at autopsy (where detailed information can seldom be obtained) and from major surgery, for which ethical issues are highly prioritized. Stellman *et al.* (1998) showed that analysis of PCBs in either serum or adipose tissue provides comparable information on body burden. In their study they observed significant positive serum to adipose correlation for most analyzed

PCB congeners. Human hair is currently evaluated as an attractive matrix for measurement of inorganic and organic persistent pollutants since it is not invasive, easily available and inexpensive (Schramm, 2008). However, there are still limitations which prevent to use it as a valid monitoring tool for risk assessment. For these reasons in our study we measured the level of these pollutants in blood serum.

1.2.2 Relevant ADME Characteristics

Absorption. When organochlorine-contaminated food is consumed, OCs contaminants are rapidly absorbed since most of the OC pool is contained in the fatty fraction of food. When fat tissue is disrupted during cooking and by the digestion process of food, the OCs are readily absorbed from the small intestine and distributed by the blood circulation throughout the body. In vegetable crops, most of the very little content of OCs is embedded in the non-soluble fibre and lignin compartment and is only made bio-available if such component of vegetable food is disrupted by probiotic gut microbioma. Since even highly degraded vegetable fibre still retains a high absorptive activity for small lipophilic organics, it is generally considered that OCs embedded in the ligneous edible fraction of crop vegetables is eliminated with the stools.

Distribution. Since OCs are strongly soluble in body lipids, they accumulate in body tissues with high lipid contents such as the adipose tissue, the brain, the liver, the kidney and the myocardium. Although they are excreted to some extent from the liver into the bile, most are reabsorbed in the entero-hepatic recirculation. Thus most POPs have long elimination half-lives and body burden can be detected in fat years after the last exposure. Thereafter a continuous exchange between blood and tissues takes place, especially when strong remodeling of fatty tissue takes place as, for instance, in fast leaning and starving conditions, in severe disease states and in breastfeeding. In pregnant women these chemicals cross the placenta to the foetus. In lactating women OCs are secreted into breast milk thus transferring the mother’s burden to the young through breastfeeding (Perera *et al.*, 2005).

Metabolism or bio-transformation. In humans and other ‘upper’ organisms, most of the minimal bio-transformation of POPs and elimination from the body takes place slowly through the liver-kidney axis and, although they are excreted to some extent in the bile and most are reabsorbed in the entero-hepatic recirculation, especially PCBs can undergo anaerobic biotransformation by the gut microbioma.

DDT has a half-life of approximately 3.4 years in adipose tissue (Maroni *et al.*, 2000; Tordoir and van Sittert, 1994). DDE, a principal DDT metabolite, is more stable than DDT and is rarely excreted from the body but both are excreted in breast milk. Further hydroxylation of the chloro-phenyl ring system occurs in the positions *ortho*- to the chlorine atom (possibly by HO^+ electrophilic substitution) and *meta*- to the chlorine atom (possibly by $HO^{\cdot}$ radical mechanisms). Elaboration of the *gem*- dichloride ($=CCl_2$) function of DDE leads to stepwise *ipso*-substitution of the chlorine atoms and finally to the dichlorophenyl-acetic metabolites (**Figure 1.4**).

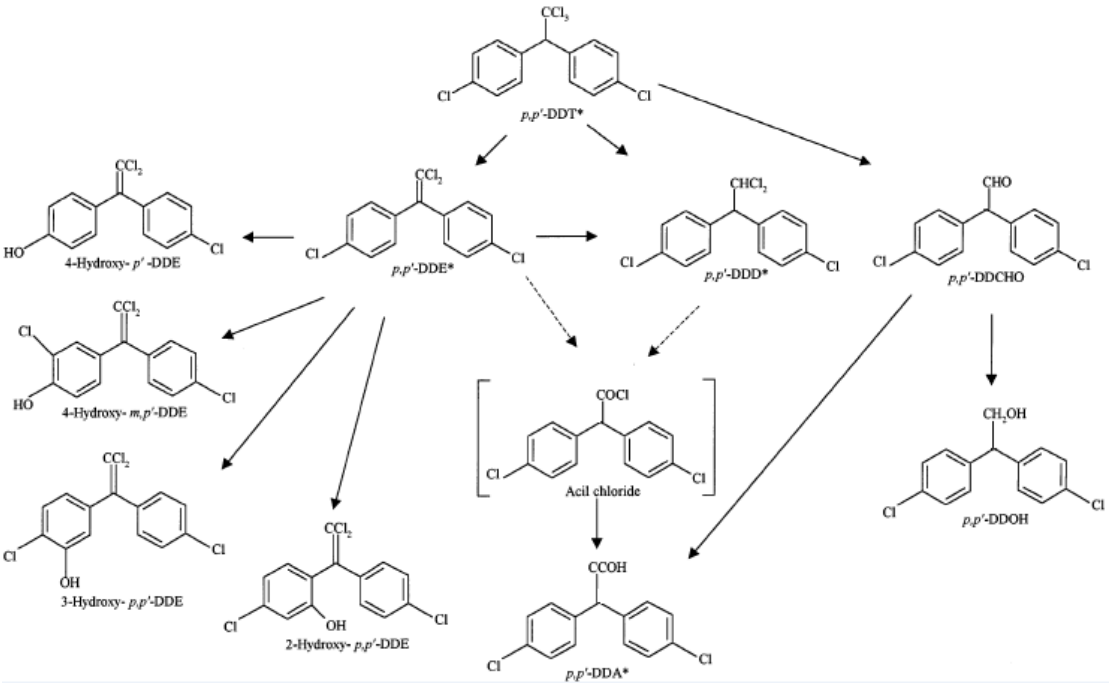


Figure 1.4. Proposed metabolic scheme of DDT in mammals (Source: Maroni *et al.*, 2000).

Dieldrin has a half-life of about 267 days in blood which is suspected to be shorter than those of heptachlor epoxide and α-, γ- and β- isomers of HCH (Maroni *et al.*, 2000; Tordoir and van Sittert, 1994). Comparing these three HCH isomers, β-HCH is eliminated very slowly from the body and thus has a significant contribution to the total HCH body burden. Its half-life in human blood is estimated to be about 7.2 years (Jung *et al.*, 1997). Half-life of HCB is thought to be extremely long in the range of 6 years (To-Figueras *et al.*, 2000).

PCB congeners have a half-life estimated between 4.6 to 41 years (Seegal *et al.*, 2011). In aerobic organisms, such as humans, PCBs are metabolized by mono-oxygenation through the arene-oxide pathway catalyzed by P450 isoenzymes (**Figure 1.5**). Highly chlorinated PCB congeners are relatively poorly metabolized and thus can remain in the

body for long periods of time. Less chlorinated PCBs are more easily metabolized and excreted faster from the body (ATSDR, 2000). Some hydroxylated metabolites of PCBs were found to be 10 times as estrogenic as its parent PCB, and equal to natural estrogen in potency, as observed by Arcaro *et al.* (1999).

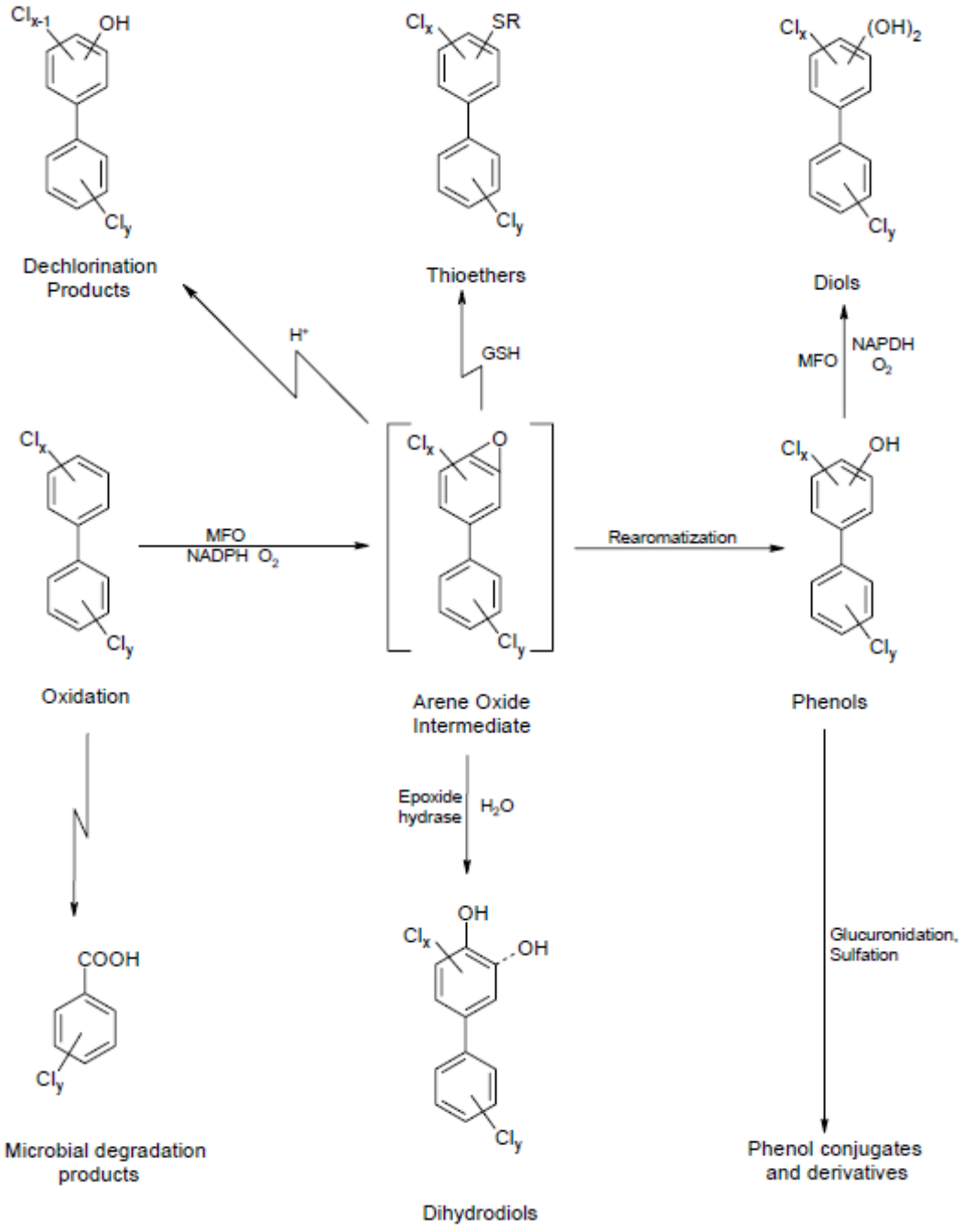


Figure 1.5. Proposed metabolic scheme of PCBs (Source: Safe, 1993 with some modifications).

Prolonged half-lives in the body have some exceptions: for example, estimated half-life of endrin is 24 hours while that of lindane and chlordane is between 10–20 days. Aldrin is readily metabolized to dieldrin so that residues are rarely found in food and animals

(Maroni *et al.*, 2000; Tordoir and van Sittert, 1994). Thus accumulation degree depends on metabolic rate where slowly metabolized OCs exhibits the highest accumulation rate and persists in the tissues for long periods of time.

Elimination. The major route of excretion of unmodified PCBs is through faeces, while the minor fraction of hydroxylated and conjugated metabolites is eliminated with the urine (ATSDR, 2000). Excretion of PCBs via the milk in humans is generally minor in comparison to faecal and urinary excretion (Shibamoto and Bjeldanes, 2009).

1.2.3 POPs concentrations in various body compartments

Organochlorine compounds are routinely detected as they are widely distributed in the environment and they accumulate in food chain. As stated in **Section 1.1** some organochlorine compounds such as DDT are still being used for agriculture and public health programs especially in developing countries. As a result people in these regions are still heavily exposed to these chemicals. Due to Long-Range Transboundary Air Pollution (LRTAP) effects and globalization of food trade, people in regions where these chemicals are not used are also exposed. The relative high levels have been found in human tissues and diet has been considered to be a major route of exposure as pointed out in **Sub-section 1.2.1**. In the subsequent sub-sections brief review of some studies is provided on the levels of POPs as measured in different body matrices and results are summarized in **Table 1.5–1.7**.

Levels of POPs in Breast Milk

Table 1.5 summarizes results of some studies which report the levels of OCs as detected in human breast milk in various countries. As pointed out earlier POPs are stored mainly in the adipose tissue; as such they can be easily excreted from mother to her new born via maternal milk. Zhao *et al.* (2007) measured levels of PCBs and OCPs in human milk from primiparous women residing in two regions with different exposure. In the exposed region the maternal milk were highly contaminated with the measured PCB as compared to unexposed region. OCPs levels were comparable for both milk and food samples in the two regions suggesting different sources of these pollutants. The PCBs level in foods collected from these regions could therefore reflect the mothers' exposure to these contaminants. Beta HCH and DDE are highly persistent thus account for their highest concentration observed in the milk.

The levels of PCBs detected among Iranian women were very high as compared to those from China. HCH was the major contributor to the total OCs burden in the human milk. There was a significant difference in DDT level between mothers who consumed one fish meal per week and those who consume more than one (Behrooz *et al.*, 2009). Among Polish women DDT and DDE concentrations were slightly higher than in other European countries. Çok *et al.* (2009) showed lower level of PCB among Turkish women as compared to Eastern Europe and German women while Bake *et al.* (2007) found similar levels of PCBs and OCPs as observed in other European countries like Finland, Germany, Sweden, Denmark and Norway.

Level of POPs in Adipose Tissue

Adipose tissue levels of POPs are considered to be the best indicator for life time environmental exposure in human due to lipophilic characteristic of POPs. Adipose tissue levels of POPs are expected to be much higher than in other matrix such as serum. The accumulation in human adipose tissue and excretion via lactation emphasizes the presence of these chemicals in the breast tissue**.** Tan *et al*. (2008) reported a level which showed that food consumption has the most significant role to account for level of POPs in adipose tissue. Fish and poultry were the routes of PCBs in mothers in Singapore while beta HCH came mainly from vegetables. An age dependent accumulation of POPs was found for beta HCH and PCB congeners. Lactation and gestation function as decontamination process for PCBs in the adipose tissue.

Shen *et al.* (2008) detected all twelve DL–PCBs and six PCBs indicators with total mean concentration of 32.2 and 154 ng/g lipids, respectively. PCB 105, 118 and 156 were dominant among DL–PCBs whereas PCB 153 concentration was the highest of all indicator PCBs. No correlation was observation between age and the levels of contaminants.

La Rocca *et al*. (2008) conducted a study to measure PCDDs/Fs and DL–PCBs among Italian obese subjects who underwent bariatric surgery to achieve weight loss. Abdominal adipose tissues were collected for analysis of these pollutants. The obese body burden was found to vary from 6 to 11 ng $TEQkg^{-1}$ b.wt exceeding the estimated steady state body burden 5 ng $TEQkg^{-1}$. The mean total DL–PCB was found to be 32161 pg/g lipid. Linear regression revealed a positive correlation between total TEQ level and age (r^2 = 0.83). In addition to OCPs and PCBs, Schiavone *et al.* (2010)

determined the levels of polybrominated diphenyl ethers (PBDEs), a new class of emerging POPs, which were found to range between 3.2 and 35.6 ng/g lipids in Italian adipose tissue.

Levels of POPs in Blood Serum/Plasma

Turci *et al.* (2006) determined the reference value of coplanar and non coplanar PCBs in blood serum collected from residents of two Italian cities; Novafeltria and Pavia. The mean values obtained for total PCB from these two cities were 2.48 and 3.93 μg/L respectively. Eight DL–PCBs were detected and the most abundant congeners were PCB 153, 138, 170 and 180. Age was shown to be a significant determinant of PCB concentration. Apostoli *et al.* (2005) assessed the reference values for PCBs level in serum of general population living in non-polluted areas in Brescia and found the mean total PCB of 5.15 ng/mL (~897 ng/g serum lipid). De Felip *et al.* (2008) observed an increased serum NDL–PCB body burden (430–470 ng/g lipid) among the two 55+ year age groups living in the vicinity of two incineration plants in Italy. These results are in agreement with the expected age-dependent increase.

Fourteen PCB congeners and 13 OCPs were analyzed from the blood samples of mothers from eight circumpolar countries. Significant differences among the groups were observed which can be accounted by dietary preferences and different contaminants deposition pattern across Arctic (Van Oostdam *et al.*, 2004).

Jakszyn *et al*. (2009) determined serum levels of *p,p′*-DDT, *p,p′*-DDE, β-HCH and HCB among health adults in Spain. The concentrations of all OCPs were positively associated with age and body mass index. No association was found between OCPs levels and dietary factors. The concentrations of *p,p′*-DDE and β-HCH were higher in southern regions while HCB levels were highs in the northern regions. These higher levels in the two regions were thought to be due to intensive past use of pesticides related to agricultural practices or industrial use, respectively.

Table 1.5. The mean concentration (ng/g fat) breast milk organochlorine compounds (OCs) in various countries.

Country	Region	Year of Sampling	PCBs	DDT	HCHs	HCB	Reference
Italy	Venice	1998–2001	6.87[d,n]	304[n,e]	–	38[n]	Abballe *et al*. (2008)
	Rome	1998–2001	6.81[d,n]	484[n,e]	–	51[n]	Abballe *et al*. (2008)
Latvia	Olaine	2004	141.85[a]	194[h]	53.5[k]	25.5	Bake *et al*. (2007)
Poland	Wielkopolska	2003	114.8[c]	1195[f]	20.0[i]	22.5	Szyrwińska and Lulek (2007)
China	Pingqiao	2003–2005	207.62[l]	1087.59[h]	256.93[j]	33.5	Zhao *et al*. (2007)
	Luqiao	2003–2005	377.8[l]	1448.43[h]	267.59[j]	46.53	Zhao *et al*. (2007)
Turkey	Seoul and Ichon	1997	6.70[l,m]	–	–	–	Yang *et al*. (2002)
	Instanbul	2007	19462[b,n]	–	–	–	Çok *et al*. (2009)
	Afyon	2007	12947[b,n]	–	–	–	Çok *et al*. (2009)
	K.Maraş	2007	10698[b,n]	–	–	–	Çok *et al*. (2009)
	Antalya	2007	25038[b,n]	–	–	–	Çok *et al*. (2009)
	Ankara	2007	18659[b,n]	–	–	–	Çok *et al*. (2009)
Iran	Nour	2006	1818[b]	2680[g]	3005[i]	629	Behrooz *et al.* (2009)
	Nour (countryside)	2006	992[b]	1571[g]	2588[i]	690	Behrooz *et al.* (2009)
	Noushahr	2006	1306[b]	3563[g]	5742[i]	1078	Behrooz *et al*. (2009)
	Noushahr (countryside)	2006	1888[b]	2322[g]	4548[i]	1493	Behrooz *et al.* (2009)
Czech Republic	Prague	1999–2001	848[c]	792[e]	35.7[k]	256	Černá *et al*. (2010)
	Kolín	1999–2001	877[c]	636[e]	25.4[k]	263	Černá *et al.* (2010)
	Kladno	1999–2001	432[c]	386[e]	–	119	Černá *et al*. (2010)

Keys: a, ∑PCB 28, 101, 138, 153,180; b, ∑PCBs 28, 52, 101, 138, 153, 180; c, ∑PCB 28, 52, 101, 118, 138, 153, 180; d, ∑PCB 105 ,114 ,118 ,123 ,156 ,157 ,167,189, 194; e, ∑*p,p'*-DDT, *p,p'*-DDE; f, ∑*p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD; g, ∑*p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDE; h, *p,p'*-DDE; i, ∑α-HCH, β-HCH, γ-HCH; ; j, ∑α-HCH, β-HCH, γ-HCH, δ-HCH; k, β-HCH; l, primiparae; m, TEQ pg/g fat; n, pg/g.

Table 1.6. The mean concentration of adipose tissue OCs in various countries.

Country	Region	Year of Sampling	PCBs	DDT	HCHs	HCB	Reference
Italy	Northern Italy, Brescia	2006	374.4[z,o]	202.0[z,x]	–	26.0[z]	Bergonzi *et al.* (2009)
	Central Italy, Siena	2005–2006	1550[z], 617[z,r]	2040[z,t]	73.9[z,x]	152[z]	Schiavone *et al.* (2010)
	Italian obese population	–	32161[b,p]	–	–	–	La Rocca *et al.* (2008)
Singapore		2004–2006	67.8[z,q]	641.19[z,u]	188.19[z,x]	19.6[z]	Tan *et al.* (2008)
Japan	Akita and Okinawa	1986–1988	–	2.4[w,y]	0.84[a,y]	–	Sasaki *et al.* (1991)
USA	Calfonia	–	–	20.9[z,v]	–		Rappolt and Hale (1968)
China	Zhejiang Province	2006	154[z,s], 32.8[z,q]	–	–		Shen *et al.* (2008)

Keys: o, ∑PCB PCB 28, 31, 52, 74, 99, 101, 105, 114, 118, 123, 128, 138, 146, 153, 156, 157, 167, 170, 172, 177, 180, 183, 187, 189, 194, 196, 201, 203, 206, 209; p, ∑DL-PCB 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189; q, ∑PCB 28, 31, 74, 99, 118, 132, 138, 153, 158, 180, 183, 187; r, ∑PCB 28, 52, 101, 118, 138, 153, 180; s, ∑PCB 28, 52, 101, 138, 153, 180; t, ∑*p,p′*-DDE, *o,p′*-DDE, *o,p′*-DDT, *p,p′*-DDT *o,p′*-DDD, *p,p′*-DDD; u, ∑*p,p′*-DDT, *p,p′*-DDD, *p,p′*-DDE; v, ∑*p,p′*-DDE, DDT; w, *p,p′*-DDE; x, ∑α-HCH, β-HCH, γ-HCH, δ-HCH; y, β-HCH; z, ng/g fat; a, ppm; b, pg/g.

Table 1.7. The mean concentration of serum OCs in various countries.

Country	Region	Year of Sampling	PCBs	DDT	HCHs	HCB	Reference
Italy	Northern Italy, Brescia	–	897[j,c]	–	–	–	Apostoli *et al.* (2005)
	Tuscany, Follonica	2005–2006	240[j,e]	–	–	–	De Felip *et al.* (2008)
	Novafeltria	–	2.48[k]				Turci *et al.* (2006)
	Pavia	–	3.93[k]	–	–	–	Turci *et al.* (2006)
Spain	North and South	1992–1996	–	934[j,g]	167.4[h,j]	379[j]	Jakszyn *et al.* (2009)
Canada	Nunavik	1994–1997	262[d]	262[f,i]	5.1[h,i]	36[j]	Van Oostdam *et al* (2004)
	Kitikmeot	1994–1997	181[d]	145[f,i]	9.6[h,i]	57[j]	Van Oostdam *et al.* (2004)
Finland	Lapland	1994–1997	152[d]	63[f,i]	7.2[h,i]	20[j]	Van Oostdam *et al.* (2004)
Greenland	Disco Bay	1994–1997	472[d]	436[f,i]	18[h,i]	80[j]	Van Oostdam *et al.* (2004)
Iceland	Reykjavik	1994–1997	233[d]	118[f,i]	32[h,i]	41[j]	Van Oostdam *et al* (2004)
Norway	Hummerfest, Kirkenes	1994–1997	175[d]	83[f,i]	8.1[h,i]	23[j]	Van Oostdam *et al.* (2004)
Russia	Nikel, Kola Penninsulla	1994–1997	235[d]	464[f,i]	223[h,i]	63[j]	Van Oostdam *et al* (2004)
Sweden	Kiruna Region, Lapland	1994–1997	225[d]	87[f,i]	9.2[h,i]	16[j]	Van Oostdam *et al.* (2004)
USA Alaska	Barrow/North Slope	1994–1997	66[d]	122[f,i]	7.9[h,i]	28[j]	Van Oostdam *et al.* (2004)

Keys: c, ∑PCB 28, 31, 52, 77, 81, 101, 105, 114, 118, 123, 126, 128, 167, 138, 153, 156, 157, 169, 170, 180, 189, 194, 206, 209; d, ∑PCB 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, 187; e, ∑PCB 28, 52, 101, 138, 153, 180; f, ∑*p,p'*-DDE, DDT; g, *p,p'*-DDE; h, β-HCH; i, µg/kg lipid; j, ng/g fat; k, ug/L.

1.2.4 Public Health Concerns regarding Persistent Organic Pollutants

Due to their environmental persistence, the ability to travel long distances in the atmosphere and the tendency to accumulate in the food chain, especially in the fat component of foods of animal origin, POPs can constitute a source of health risk. Because most of them bioaccumulate and remain preferentially in fat, their long-term effects are still a matter of public health concern. They are condemned for adverse health effects as stated in **Subsection 1.1.1**. These effects can be elicited via a number of mechanisms among others include disruption of endocrine system, oxidation stress and epigenetic. However most of the mechanisms are not clear thus a number of studies are ongoing trying to elucidate them.

1.2.5 Persistent organochlorinated pesticides and mechanisms of their toxicity

In this section we report an overview on available information of possible mechanisms of toxicity of some selected OCPs, which is taken from our recently published review article (*Toxicology* 2012. doi:pii: S0300-483X(12)00412 X.m10.1016/j.tox.2012.11.015).

1.0 Introduction

As pointed out earlier in **Section 1.1** above, human exposure to persistent organic pollutants has been associated with various health effects. These effects can be elicited via a number of mechanisms among others include disruption of endocrine system, oxidation stress and epigenetic. The mechanism of action involves toxicokinetic and toxicodynamic processes, at several levels between chemical exposure and the endpoints at individual level. Variation within humans can affect the endpoint at any toxicodynamic and/ or toxicokinetic steps (Mortensen and Euling, in press). Direct, non/genomic mechanisms of toxicity involve perturbation of the homeostatic redox level(s) of biological compartments (oxidative stress) and consequent cellular death through apoptosis. Indirect, genomic mechanisms of toxicity involve permanent modification of the transcription of gene elements by interference at the epigenetic level of DNA functioning.

Since little is known on the relationship among events that result in OCPs' toxicity in humans (Mortensen and Euling, in press), a number of studies are currently ongoing to investigate mechanisms responsible for OCPs' toxicity. A better knowledge on how

these compounds influence the development and progression of diseases is a crucial step towards addressing the emerging diseases and hence improving public health. In this review, we provide an overview on available information of possible mechanisms of toxicity of some selected OCPs and where possible a linkage is made to epidemiological data. We summarize findings of epidemiological studies and both *in vitro* and *in vivo* experimental studies (**Tables 1.1R**–**1.3R**).

The main OCPs for which we review available information are dichlorodiphenyl-trichloroethane (DDT) and its metabolites, hexachlorobenzene (HCB), beta-hexachlorohexacyclohexane (β–HCH) and endosulfan. These chemicals have been employed in agriculture and in public health as insecticides and biocides for several decades as detailed previously.

Table 1.1R. Summary for selected epidemiological studies showing the relationship between DDT/DDE exposure and related health effects.

Author	Effects assessed	OCP measured	OCP level (μg/L serum)	OCP level (μg/g lipid serum)	Results	P_{trend}
Cohn *et al.* (2007)	Breast cancer	DDT	Tertiles	Tertiles	OR, 95% CL	
			<8.09	<1.1	1.00	0.01
			8.09–13.9	1.1–1.8	2.8 (1.1–6.8)	
			>13.9	>1.8	5.4 (1.7–17.1)	
McGlynn *et al.* (2006)	Liver cancer	DDT	–	Quintiles	OR, 95% CL	
				<0.265	1.00	0.049
				0.265–0.382	1.3 (0.7–25)	
				0.383–0.521	1.4 (0.7–2.6)	
				0.522–0.787	1.4 (0.7–2.7)	
				>0.787	2.0 (1.1–3.9)	
McGlynn *et al.* (2006)	Liver cancer	DDT adjusted for DDE level	–	Quintiles	OR, 95% CL	
				<0.265	1.00	0.0024
				0.265–0.382	1.5 (0.8–2.7)	
				0.383–0.521	1.7 (0.9–3.3)	
				0.522–0.787	1.4 (1.0–4.3)	
				>0.787	3.8 (1.7–8.6)	
McGlynn *et al.* (2008)	Testicular germ tumours	DDE	–	Quartiles	RR, 95% CI	
				≤0.157	1.00	0.0002
				0.158–0250	1.01 (0.75–1.36)	
				0.251–0390	1.00 (0.73–1.38)	
				>0.390	1.71 (1.23–2.38)	

2.0 Mechanisms of OCPs toxicity

2.1 Endocrine activity

The endocrine system controls, balances and produces hormones and modulates their actions in the body through a network of activation and repression pathways at multiple levels of synthesis, secretion, transport, binding, action or elimination of natural hormones. Steroid hormones are involved in cell development, metabolism, protein synthesis, behaviour, reproduction and development. These responses are elicited through binding of hormones to their specific receptors such as nuclear receptors (NRs). Among the members of NRs are estrogen receptors (ERs), androgen receptor (AR), progesterone receptor (PR), glucocorticoid receptor (GR), constitutive androstane receptor (CAR), rodent pregname X receptor (PXR), mineralocorticoid receptor and thyroid hormone receptors (TRs). Aromatic hydrocarbon receptor (AhR) is a key regulator of the cellular response to xenobiotic exposure (Swedenborg *et al.*, 2009). NRs and AhR are involved in the induction of xenobiotic metabolizing enzymes.

In the absence of ligands NRs and AhR stay in the cytoplasm or in the cell nucleus in a complex form (Picard, 2006). In the presence of ligands, receptor-ligand binding occurs, followed by translocation of NRs/AhR to the nucleus. In the nucleus, a complex of aryl receptor nuclear translocator (ARNT) protein and co-activators is formed. The complex binds to the specific AhR-DNA recognition site and finally induces transcription of target genes. The ligand binding triggers conformational changes that lead to dissociation of the repressive complex, the recruitment of transcriptional co-activators and receptor dimerization (Hankinson, 2005). NRs homodimerize or heterodimerize with retinoid X receptor and AhR dimerizes with ARNT leading to gene transcription of NR or AhR target genes (Claessens and Gewirth, 2004; Swanson, 2002). NRs control transcription through binding to promoter regions of DNA.

Several chlorine atoms in the molecular structures of OCPs impart their highly lipophilic character and fairly rigid conformation. The chlorine atoms are poorly reactive towards nucleophilic displacement and elimination reactions and thus their biotransformation and biodegradation reactions are limited and are mostly confined, albeit to poor yield, only to anaerobic environment of sludges, but seldom in mammalian organisms. As a consequence, their interaction with biological systems is mostly limited to agonistic or antagonistic binding to the intracellular receptors for which natural hydrophobic substances, such as steroid derivatives, are the endogenous

ligands. Agonistic binding leads to recruitment of coactivators and thus increases transcriptional activity while antagonistic binding prevents coactivator recruitment and/or attracts corepressors, leading to decreased transcriptional activity of the receptors.

Many POPs including OCPs are known or suspected to be endocrine active. When present in the body they may interfere at several control points in the hormone signaling pathways. As a result, the response cascade of natural hormones can either be inhibited or excessively enhanced, at the wrong time, in the wrong tissue (Swedenborg *et al.*, 2009). Endocrine activity of OCPs can be due to direct binding with hormone receptors due to their conformational similarity with the receptor-binding portions of natural hormones, mainly of the steroid and diphenylether (thyroxine) structural groups. This is the case of aromatic polychlorinated substances such as DDT and its structural cognates, endosulfan and lindane. Other compounds indirectly alter hormonal pathway by directly inhibiting enzyme activities responsible for biosynthesis of the precursors of steroid hormones. In particular, the DDT analogue, mitotane, a pharmaceutical drug which is used to suppress cortisol production also causes a sharp raise in plasma cholesterol levels.

2.1.1 Interference of OCPs in estrogen hormone metabolism

17β-Estradiol is the main estrogen hormone, responsible for development of female sex characteristics such as growth of the breasts and suppression of body hair growth. Biotransformation of 17β–estradiol proceeds via two common metabolic pathways yielding catechol estrogen 2–hydroxyestrone (2–OHE), a weak estrogen; and 16α-hydroxyestrone (16α–OHE), a fully potent estrogen. Enzymes CYP1A1, CYP1B1, CYP1A2 and catechol-O-methyltransferase (COMT) are responsible for this metabolism. 16α–OHE, but not 2–OHE increases unscheduled DNA synthesis, uncontrolled cell division and increased anchorage-independent growth. Interrupting these pathways might lead to formation of more potent products as exemplified by some xenoestrogens. Exposure of cell cultures of MCF-7 to concentrations as low as 10^{-5}M of various OCPs such as DDT, lindane, chlordecone and endosulfan blocked the 2–OHE pathway and increase levels of 16α–OHE in estrogen positive (ER+) cells (Bradlow *et al.*, 1995). This interference in estrogen biotransformation led to increasing 16α–OHE/2–OHE ratio relative to the ratio in the untreated control cell cultures. This increasing ratio has been associated with breast and other cancers of animals (Telang *et*

al., 1992) (**Figure 1.1R**); possibly because of 16α-OHE has a strong estrogenic activity (Bradlow *et al.*, 1995). Levels of 16α-hydroxylation have been found increased by about 50% in human breast cancer as compared to control cases (Schneider *et al.*, 1982).

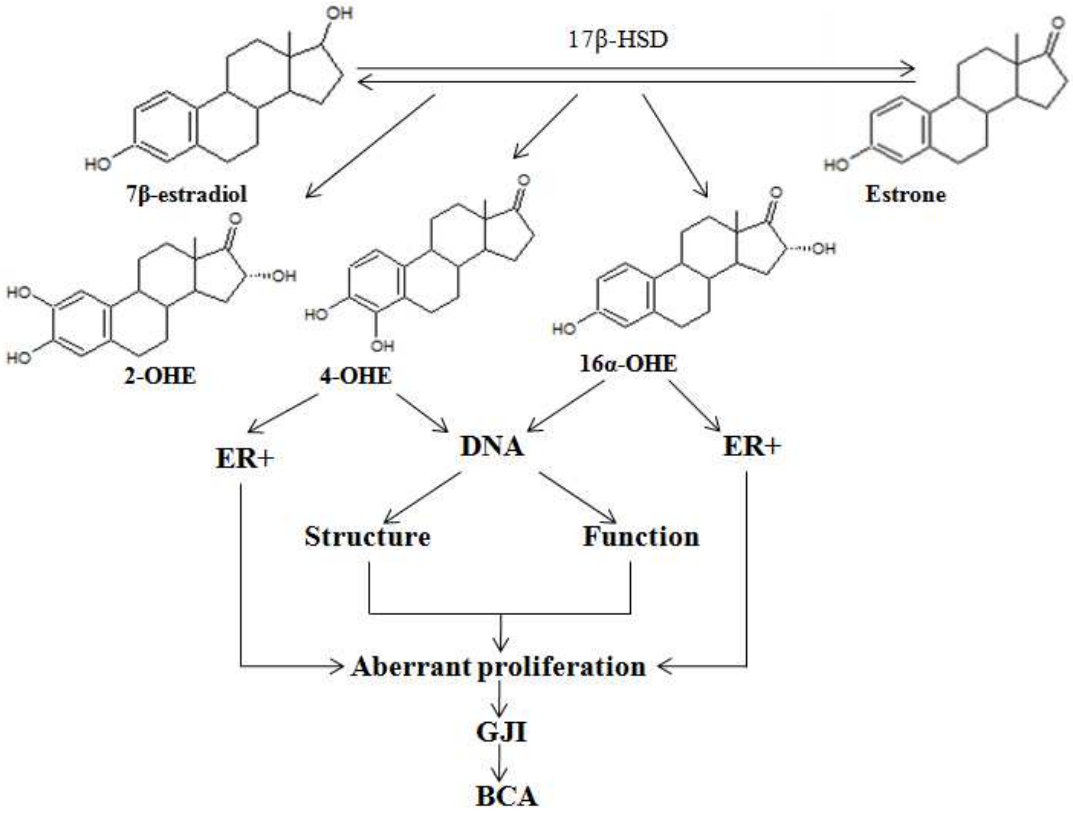


Figure 1.1R. Bifunctional pathways to breast cancer (BCA). *Abbreviations*: OHE, hydroxyestrone; ER, estrogen receptor; GJI, gap junction inhibition; 17β–HSD, 17 beta-hydroxysteroid dehydrogenase. 17β–estradiol metabolites affect cell proliferation and BCA development either directly via receptor-independent mechanisms involving structural/functional alterations in DNA, or indirectly via receptor-dependent mechanisms involving phenotypic growth regulation. These mechanisms upregulate aberrant proliferation and development of BCA. *Source*: Adapted from Davis *et al.* (1998) with some modifications.

CYP1A1 metabolizes estradiol and environmental contaminants into the corresponding hydroxylated derivatives through the highly reactive intermediates of arene-oxide connectivity (Hayes *et al.*, 1996). The expression of CYP1A1 is regulated by the aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor that activates the transcription of target genes, such as CYP1A1, CYP1A2, CYP1B1 and oncogenes (Androutsopoulos *et al.*, 2009b). DDT and its metabolites are capable of up regulating CYP1A activity (Van Tonder, 2011). According to Navas *et al.* (2004) CYP1A inducers have defined structures which make them capable of binding AhR. DDT has two aromatic rings linked by sp3 carbon and thus each of the rings can rotate along its single bond with the C2 carbon. This allows some degree of conformational mobility and in

principle the possibility to bind AhR but the rings cannot lie in the same plane, as in the estrogen molecules (**Figure 1.2R**). Lack of this molecular recognition motif greatly limits the affinity of AhR for DDT. In fact, the concentration of DDT employed in the Bradlow experiments is 10^{-5} M (10 μM), while the physiological concentration of steroid hormones such as estradiol is of at least four to six orders of magnitude lower (low-picomolar). On the other hand DDT and its metabolites as well as many other OCPs are known phenobarbital-type cytochrome P450 inducers as they have been shown to induce cytochrome P450 2B (CYP 2B) in rat liver or cultured rat hepatocytes (Campbell *et al.*, 1983; Li *et al.*, 1995; Lubet *et al.*, 1990, 1992; Yoshioka *et al.*, 1984). The enzyme activation upon exposure to these pesticides can thus lead to alterations in the endogenous levels of hormones and consequently compromise hormone signaling. In addition to xenobiotics metabolism, CYP1A1 has been shown to participate in the activation of dietary polyphenols (Androutsopoulos *et al.*, 2008, 2009a) and it is inhibited by various polyphenolics (Androutsopoulos *et al.*, 2010, 2011).

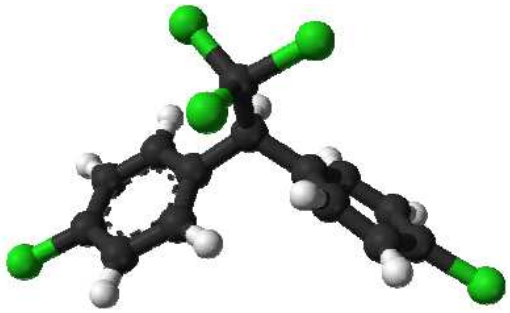


Figure 1.2R. The 3D molecular structure of DDT showing diphenyl rings which are not within the same plane.

2.1.2 DDT and its metabolites

DDT and its metabolite DDE exhibit hormonal activity in various tissues with mechanisms involving the steroidogenic pathway, receptor mediated changes in protein synthesis or antiandrogenic and estrogenic actions. Most of their endocrine effects result from the ability to mimic 17β–estradiol. *o,p′*-DDT is a well characterized DDT isomer; its intracellular mechanism of action is mediated through the ER pathway (Steinmetz *et al.*, 1996) described in **Sub-section 2.1** of this review. It has been shown to bind and activate the ER, to promote the expression of estrogen-dependent genes and to induce proliferation of ER-competent cells such as breast cancer cells (MCF-7) *in vitro* (Soto *et al.*, 1995). At 10 μM concentration, *o,p′*-DDT agonized both ERα– and ERβ–mediated

transcription a potency that is approximately 0.1% that of estradiol (Lemaire *et al*., 2006). A significant dose-dependent increase in cell number in MCF-7 cell culture was observed starting at 10^{-8} M and maximum response achieved at 10^{-6} M (Steinmetz *et al*., 1996). Enantiomer-specific estrogenic activity has been suggested (Hoekstra *et al*., 2001; McBlain, 1987; Wang *et al.*, 2009). At 5.26×10^{-5} M (S)-(–)-*o,p′*-DDT produced half of the maximum effect (EC_{50}) whereas (R)-(+)-*o,p′*-DDT had negligible estrogen activity (Hoekstra *et al*., 2001). The (+)-enantiomer exhibited estrogen-like response only at an environmentally unrealistic concentration of 10^{-3} M, and thus estrogenic activity of *o,p′*-DDT is due to the (–)-enantiomer.

Transient cotransfection assay of monkey kidney CV1 cells with human AR expression vector and a mouse mammary tumour virus promoter with a luciferase reporter vector revealed that androgen-induced AR transcriptional activity was inhibited by *p,p′*-DDE or by the potent antiandrogen, hydroxyflutamide at 0.2 µM (63.6 ppb) concentration. This level of *p,p′*-DDE is lower than 140 ppb found among residents living in DDT-treated homes (Bouwman *et al.*, 1991). *In utero* exposure to DDE has been shown to decrease anogenital distance (a morphologic quantitative indicator of feminization of male offspring) in male rat offspring, to increase retention of male nipple and to cause an alteration in expression of androgen receptor (Kelce *et al.*, 1995) suggesting that these abnormalities might be mediated at the level of the androgen receptor. In addition to anti-androgen activity, *p,p′*-DDE can activate the ER and induce cell proliferation (Kelce *et al.*, 1995). 17β-estradiol (1×10^{-8} M), *o,p′*-DDT (1×10^{-5} M) and β-HCH (1×10^{-5} M) similar showed gene expression profile of estrogen-responsive genes (i.e. *TFF1, PR, ER, BRCA1* and *CCND1*) in MCF-7 cell line, while *p,p′*-DDE (1×10^{-5} M) was a weak transcriptional inducer (Silva *et al.*, 2010).

In adult mice *p,p′*-DDT induced a dose-dependent effect on estrogen receptor activity in liver, brain, thymus and prostate of a transgenic mouse strain (ERE-tkLUC). The same effect was caused by *o,p′*-DDT, except in the liver. This study showed that DDT isomers modulate ER activity also in nonreproductive organs (Di Lorenzo *et al.*, 2002). At a dose known to interfere with animal fertility (50 µg/kg), *o,p′*-DDT significantly affected ER response. Treatment of rats with 50 or 100 mg/kg body weight *p,p′*-DDT (repeated doses for ten successive days) induced decrease in the number and motility of epididymal spermatozoa in a dose dependent manner and DDT induction of testosterone metabolism causing reduced testosterone levels (Ben Rhouma *et al.*, 2001). The same

treatment led to increased thyroid hormones and to a decrease in serum T4 levels and hypothyroidism (Tebourbi *et al.*, 2010). Given the very high doses used in the experiments, the results of this study are hardly comparable with effects forecasted at human environmental exposure levels. In fact, a cross-sectional study conducted among 781 young men did not find association between DDE levels (median: 2.7 µg/g serum lipid; range 0.1–56.1 µg/g serum lipid) and anogenital distance (Longnecker *et al.*, 2007). Also a nested case-control study by Torres-Sánchez *et al*. (2008) among 37 young men showed that higher DDE levels (10^{th}–90^{th} percentile: 0.21–5.89 µg/g serum lipid) significantly reduced the anogenital distance. A study by Bornman *et al.* (2010) on urogenital malformations in male newborns suggests increased rates of malformations among those whose mothers lived in DDT-treated homes. Although these studies do not provide sufficient information for determination of a quantitative risk to humans, biological plausibility is strengthened by animal findings (WHO, 2011).

Cohn *et al*. (2007) conducted an epidemiological study and showed an association, with a dose response relationship, between DDT exposure and development of breast cancer when exposure is prepubertal. This was the case for the younger women (<14 years of age) in second and third tertile of exposure who had DDT exposure in their childhood [2^{nd} tertile: 8.09–13.90 µg/l (~1.1–1.8 µg/g lipid), OR = 2.8, 95% CI, 1.1–6.8; 3^{rd} tertile: >13.90 µg/l, OR = 5.4, 95% CI, 1.7–17.1]. McGlynn *et al.* (2006) found significant increasing risk of liver cancer among individuals with values in the highest versus the lowest quintile of serum DDT concentration (geometric mean = 0.49 µg/g lipids; OR = 3.8; 95% CI, 1.7–8.6; p for trend = 0.0024) when analysis was adjusted for age, sex, hepatitis B surface antigen status, places of residence and DDE level. The lowest and the highest quintile of serum DDT concentrations were <0.265 and >0.787 µg/g lipids, respectively. However there was no evidence of significant association between liver cancer and serum DDE concentration (p = 0.51). McGlynn *et al.* (2008) showed an association between testicular germ cell carcinomas and DDE exposure (upper quartile: > 0.39 µg/g lipid, RR = 1.71; 95% CI, 1.23–2 38; **Table 1.1R**).

Several epidemiological data showed an association between DDT exposure and miscarriage or preterm birth (Dewailly *et al.*, 1993; Gladen *et al.*, 2003; Karmaus and Zhu, 2004). To understand the involvement of the AhR/CYP1A1 pathway in the mechanism of action of DDT and DDE, Wójtowicz *et al.* (2011) used human placenta explants cultures originated from normal pregnancy and cotreated them with 3.2 ng/mL

TCDD and 1, 10 or 100 ng/mL (*approx.* 0.18, 1.81, 18.10 µg/g lipid) of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE or *o,p'*-DDE for 24, 48 or 72 hours (these concentrations are known to occur in serum of pregnant women). Immunoblot analyses showed that the isomers of DDT and DDE inhibited the expression of CYP1A1 most effectively at 48 and/or 72 hours after treatment. In a separate experiment to study the expression of AhR, the placenta explants were cultured in presence of these isomers at 100 ng/mL for 1, 3, 6, 14, 24, 48 and 72 hours. The level of AhR protein decreased gradually with time starting after 3h of treatment in all treatment experiments, with exception of that with *o,p'*-DDE, where the decrease was delayed at 24 h after the start of the experiment. The author argued that as CYP1A1 is involved in metabolism and detoxification in the human placenta, any malfunction of this enzyme will disrupt the placental detoxification machinery. This may lead to an increased susceptibility of the foetus to environmental toxins and may be a risk factor for recurrent pregnancy loss. A cross sectional study conducted by Tsatsakis *et al.* (2009) among a Greek rural population, revealed a significant association between CYP1A1*2A polymorphism and miscarriages ($p = 0.012$). The investigated population had the median concentrations of 6.3, 2.9, 81.5, 3.1, 7.1, 5.3, 3.2 and 19.7 pg/mg for *α*-HCH, HCB, lindane, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD + *o,p'*-DDT and *p,p'*-DDT, respectively. The significant associations were also evident for other ailments such as chronic obstructive pneumonopathy ($p = 0.045$), peripheral circulatory problems (trend $p = 0.042$), arteritis ($p = 0.022$), allergies (trend $p = 0.046$), hemorrhoids (trend $p = 0.026$) and allergic dermatitis ($p = 0.0016$). Thus it was suggested that CYP1A1 may play a significant role in the incidence of several diseases among the population with long exposure to organochlorinated pesticides.

2.1.3 Endosulfan

Most studies investigating endosulfan estrogenicity *in vitro* show that its potency is 10^5 to 10^6 times lower than that of estradiol (**Table 1.2R**; Arcaro *et al*., 1998; Soto *et al*., 1994, 1995; Wade *et al.*, 1997). Briz *et al.* (2011) showed that endosulfan directly interacts with ERs in primary cultures of cortical neurons (CN) and cerebellar granule cells (CGC). It inhibited the binding of [^{3}H]–estradiol to the ER in both CN and CGC with a fairly high IC_{50} of 21.7 ± 4.2 and 35.0 ± 8.3 µM, respectively. These IC_{50} values are very close to that of α-endosulfan with recombinant human steroid receptors particularly hPR (20 µM) (Scippo *et al.*, 2004). Exposure of endosulfan at 10 µM for 5 h

increased Akt phosphorylation and activated ERK1/2 through a mechanism involving GABAA and glutamate receptors in CN. The observed alterations on ER-mediated signaling and ER levels in neurons were suggested to contribute to the neurotoxicity of endosulfan.

In vitro assay showed that endosulfan, dieldrin and lindane act as antagonists of androgen receptors (Andersen *et al.*, 2002; Li *et al.*, 2008; Nativelle-Serpentini et al., 2003). Endosulfan and dieldrin were shown to inhibit the aromatase enzyme (CYP19); the rate-limiting enzyme of estrogen synthesis from the non-aromatic precursor androstenedione. Like other organochlorines, endosulfan and dieldrin alter the estradiol metabolism by inducing CYP1 enzymes (Badawi *et al.*, 2000; Bradlow *et al.*, 1995). Endosulfan and dieldrin were shown to inhibit androgen response at 20 µM and thus acted as very weak antiandrogens. At 50 µM endosulfan reduced the aromatase activity in human placenta microsomes to 87% of the control level (i.e.1 µM of 4-Androsten-4-ol-3, 17-dione) and hence exhibited weak aromatase-inhibiting effect (Andersen *et al.*, 2002).

In vitro assay shows that dieldrin (5 µM) and endosulfan (1 µM) significantly increase cell proliferation and ER transactivation gene response in MCF–7 cells (Andersen *et al*., 2002). The maximum response occurred at 25 µM for both dieldrin and endosulfan. However, only endosulfan was shown to potentiate the 17β–estradiol induced proliferation when tested together with a concentration of 17β–estradiol causing a sub maximum response. Other authors have also shown the ability of endosulfan to promote MCF–7 cell proliferation *in vitro* (Grunfeld and Bonefeld-Jorgensen, 2004; Ibarluzea *et al.*, 2004; Soto *et al.*, 1994). However, there are no epidemiological studies linking exposure to endosulfan and increase of cancer risk in humans. *In vitro* studies examining the effects of endosulfan on androgen-responsive systems showed that endosulfan exhibited cytotoxic effects on testicular cells at a concentration of 20 µM or greater (Sinha *et al.*, 1999, 2001). These doses are environmentally irrelevant. *In vivo* assays have not demonstrated estrogenic effects (Gellert, 1978; Raizada *et al.*, 1991; Endosulfan caused alterations in testicular functions at very high doses. At doses of 2.5, 5 and 10 mg/kg administered orally to adult Druckrey rats for 5 days per week for 70 days, endosulfan was shown to increase testicular enzyme activities at all tested doses. Sperm count decreased in a dose-dependent manner (Sinha *et al.*, 1995).

Table 1.2R. Summary of selected *in vitro*, *in vivo* and epidemiology studies on the endocrine effects of endosulfan.

Author	Study type	Study sample/materials	Effects investigated	Observed effects
Soto *et al.* (1994)	*In vitro*	MCF–7 cell culture	Cell proliferation potential of pesticides	Endosulfan was 10^6 times less potent than 17β-estradiol. Effects observed at doses of 10 μM endosulfan or greater.
Soto *et al.* (1995)	*In vitro*	MCF–7 cell culture	Relative binding affinity of pesticides	Endosulfan hPR receptor binding affinity was 10^5 less than 17β-estradiol
Wade *et al.* (1997)	*In vitro*	MCF–7 cell culture	Cell proliferation potential of pesticides	Endosulfan was 10^5 less potent as compared to 17β-estradiol. Effect observed at the highest soluble dose.
Sinha *et al.* (1999)	*In vitro*	Rat testicular cells in culture	Cell viability and lactate dehydrogenase leakage	Endosulfan produced cytotoxic change. Effects observed at 20 μM of endosulfan at or above.
Sinha *et al.* (2001)	*In vitro*	Rat testicular cells in culture	Activity of polyol pathway enzymes	Increased aldose reductase activity and decreased sorbitol dehydrogenase activity at or >20 μM of endosulfan.
Grunfeld and Bonefeld-Jorgensen (2004)	*In vitro*	MCF–7 BUS cells	Human ERα and ERβ mRNA expression	Endosulfan decreased human ERα mRNA levels weakly with no effect on human EBβ expression.
Li *et al.* (2006)	*In vitro*	MCF–7 cell culture	Activation of MAPK, PI3-K, PKC, PKA and CaMKIV	At 20–75 μM endosulfan activated P13-K and MAPK. The effect observed at 10 nM of 17β-estradiol and DES.
Sinha *et al.* (1995)	*In vivo*	Adult Druckrey rats administered with repeat dose of endosulfan (0, 2.5, 5 or 10 mg/kg, 5 days per week for 70 days)	Testicular enzyme activities, sperm count, sperm morphology, and intra-testicular spermatid count	Increased testicular enzyme activities at all doses tested; sperm count decreased in a dose-dependent manner; decreased spermatid counts and reduced daily sperm production at the two highest test doses was reported.
Sinha *et al.* (1997)	*In vivo*	Weanling Druckrey rats administered with repeat dose of endosulfan (0, 2.5, 5 or 10 mg/kg, 5 days per week for 90 days)	Testicular enzyme activity, testes weights, sperm count, sperm abnormality, spermatid count and daily sperm production	No observed effects on testes weights; increased levels of testicular enzyme activity; sperm count decreased in a dose-dependent manner; decreased spermatid count and sperm production rate.
Raizada *et al.* (1991)	*In vivo*	Ovariectomized Wistar rats administered with repeat dose of endosulfan (1.5 mg/kg daily for 30 days and injected daily with estradiol 1 μg/rat i.p.)	Organs weights, glycogen content of organs, and histopathological changes in organs	No effects on weights of uterus, cervix, vagina and pituitary; no effects on glycogen content of the organs examined; no histopathological changes reported.
Shelby *et al.* (1996)	*In vivo*	Mice administered with repeat dose of endosulfan on postnatal days 17–19 at a dose of 10 mg/kg	Competitive binding of endosulfan to mouse estrogen receptor and uterine growth	No competitive inhibition of estrogen receptor observed; no increase in uterine wet mass; DES and estradiol significantly affected uterine growth.
Wade *et al.* (1997)	*In vivo*	18 days rats injected with 3 mg/kg/day endosulfan daily for 3 days	Uterine estrogen receptor binding	Estradiol binding inhibited at high doses (10^5 M endosulfan); no change in uterine weights, uterine peroxidase activity or numbers of uterine estrogen and progesterone receptors.
Saiyed *et al.* (2003)	Cohort	Male children lived in the village sprayed with endosulfan and children who lived in 20 km away from village	SMR assessment; serum testosterone, LH, and FSH; and endosulfan residues in blood	Sexual maturity delayed in the exposed population; controlled for age, only serum LH levels differed between the populations.
Ibarluzea *et al.* (2004)	Case–control	Breast cancer women (cases) and non-cancer women (controls)	Relationship between cancer diagnosis and the adipose pesticides.	Endosulfan residues were not associated with increased risk of breast cancer

Abbreviations: PI3-K, phosphatidylinositol-3-kinase; PKC, protein kinase C; PKA, protein kinase A; CaMKIV, calcium calmodulin-dependent kinase IV; AI, apoptotic index; MCF–7, Michigan Cancer Foundation-7; SMR, sexual maturity rating; LH, luteinizing hormone; FSH, follicle stimulating hormone; i.p, intraperitoneal injection; ER, estrogen receptor; hPR, human progesterone; DES, diethylbestrol.

Similar study conducted for 90 days using weaning Druckrey rats yielded similar results where spermatid count and sperm production rate decreased (Sinha *et al.*, 1997). Repeated dose of 3 mg/kg/day of endosulfan administered to immature rats for 3 days caused no change in uterine weights, uterine peroxidase activity, or numbers of uterine estrogen and progesterone receptors. Only at a high dose (10 μM) endosulfan inhibited estradiol binding (Wade *et al.*, 1997) as was in the case for *in vitro* studies discussed above.

Exposure of adult rats to 2.5, 5 or 10 mg/kg endosulfan for 10 weeks (5 day per week) or 3, 6 or 1000 mg/kg b.wt/day endosulfan have shown to cause reduction in intratesticular spermatid counts, sperm abnormalities, and changes in the marker enzymes of testicular activities, such as lactate dehydrogenase, sorbitol dehydrogenase, γ-glutamyl transpeptidase, and glucose-6-phosphate dehydrogenase (Khan and Sinha, 1996; Sinha *et al.*, 1995). Moreover exposure of pregnant rats to endosulfan at 1 mg/kg/ day from day 12 through parturition has shown to decrease spermatogenesis in offspring (Sinha *et al.*, 2001). It should be noted that these doses are orders of magnitude higher than those to which humans are usually exposed in the living environment and at the workplace.

Limited human data exist on the effect of endosulfan. A cohort study conducted by Saiyed *et al.* (2003) among male school children (10–19 years old) showed that endosulfan exposure was associated with delayed male sexual maturity and interfered with the male sex-hormone synthesis. Sexual maturity rating (SMR) scoring for development of pubic hair, testes, penis and serum testosterone level was positively related to age and negatively related to aerial exposure to endosulfan (AEE; $p < 0.01$). Serum LH levels were significantly positively related to AEE after controlling for age ($p < 0.01$). Serum endosulfan levels were significantly higher ($p < 0.001$) in the exposed population (mean 7.47 ± 1.19 ppb) than controls (1.37± 0.40 ppb). Moreover, a big proportion of exposed group (78%) had detectable serum endosulfan as compared to 29% of the controls. Although these findings match those expected on the basis of experimental evidences, nevertheless, it was not clear if endosulfan was the only chemical that could be linked to the observed associations.

Several cellular and molecular mechanisms of endosulfan toxicity have been proposed. These include mitochondrial dysfunction, induction of oxidative stress, modulation of activities of stress-responsive signal transduction pathways, activating protein-1(AP-1)

and antioxidant response element (ARE)-mediated transcription. Recently a model has been proposed in which endosulfan is shown to increase extracellular signal regulated kinases (ERK) 1/2 and p38 activities and, in turn, these proteins activate mitogen-activated protein kinases (MAPKs) and increase c-Jun phosphorylation. Phosphorylated c-Jun, in turn, increases AP-1 activity, which results in activation of ARE-mediated transcription (Song *et al.*, 2012). However, at the moment no firm conclusion can be drawn on these proposed mechanisms.

2.1.4 Hexachlorobenzene

Hexachlorobenzene is well known to induce porphyria through a free-radical generation mechanism (Mazzetti *et al*., 2004), due to its rather unique chemical characteristics of lipophilicity and possibly to conversion into the redox-active tetrachloro-1,4-benzoquinone. HCB is a weak agonist of the AhR protein (Hahn *et al.*, 1989). The most sensitive target organs are the liver, the ovary and the central nervous system (ATSDR, 2002). Unlike estrogen, HCB does not have a significant affinity for the ER thus suggesting other intracellular pathways than the one taken by endogenous estrogen in mediating these actions.

HCB can elicit tumorigenic activity through AhR-dependent and independent pathways. *In vivo* administration of HCB has shown to induce alterations in insulin-like growth factors (IGFs) signaling pathway in mammary gland and mammary tumors in rat only when co-administered with *N*-nitroso-*N*-methylurea (Randi *et al.*, 2006). Since the latter compound is a strong mutagen, HCB plays the role of a tumor cocarcinogen in rat mammary gland, as an inducer of cell proliferation and of c-Src kinase activity in MCF-7 breast cancer cells.

Garcia *et al.* (2010) used ERα (+) MCF-7 and ERα (-) MDA-MB-231 cell lines to investigate ability of HCB to promote cell proliferation and alter Insulin/IGF-I signaling pathway. At 0.005 and 0.05 µM, HCB significantly increased the proliferation of only MCF-7 cell lines, with no effect at 0.5 and 5 µM. In estrogen-depleted medium, HCB stimulated proliferation of MCF-7 cells only at the lower concentrations showing that the effect of HCB on cell proliferation was not dose responsive. Thus HCB stimulates proliferation in estrogen-sensitive cells in an ERα-dependent manner. At 0.005 and 0.05 µM, HCB increased the level of IGF-IR and IR in ERα (+) MCF–7 cell line. However at 0.5 and 5 µM, HCB did not alter the level of these receptors but increased CYP1A1 gene expression and induced apoptosis in MCF-7 cell lines, suggesting HCB effect on

apoptosis is AhR-dependent. At 0.005 and 0.05 µM of HCB did not induce the expression of CYP1A1 mRNA. On the other hand 0.005 µM of HCB led to c-Src activation signifying this effect is not AhR-dependent. At 0.005, 0.05, 0.5 and 5 µM, HCB increased IRS-1 phosphorylation with no effect in IRS-1 protein levels. IGF-I receptor is known to regulate proliferation, survival and differentiation in mammary gland. Insulin-like growth factor-insulin receptor (IGF-IR) pathway is involved in development of breast cancer and IR content is known to increase in human breast cancer whereas IRS-1 is the main intracellular substrate activated by IGF-I and insulin in human breast cancer cells. c-Src can promote growth of tumor cells, participating in or augmenting mitogenic signaling pathways that are initiated by extracellular growth factors or intracellular oncogenes (Biscardi *et al.,* 2000). As per authors' point of view, these results give a clue to the molecular mechanism of HCB in breast cancer development.

To investigate the effects of HCB, Pontillo *et al.* (2011) showed that at 0.05 µM HCB produced an early increase of c-Src, human epidermal growth factor receptor (HER1) activation, signal transducers and activators of transcription (STAT) 5b, ERK1/2, but not Akt, in ERα (-) MDA-MB-231 cell lines in a dose-dependent manner (0.005, 0.05, 0.5, and 5 µM).

Young rats (65 days of age) were administered 100 mg/kg b.wt of HCB three times a week (total dose of 1800 mg/kg b.wt over 45 day period) (Peña *et al.*, 2012). When the test animals reached oestrous phase they were sacrificed. In mammary glands, HCB increased c-Src and HER1 activation, c-Src/HER1 association; and STAT5b and ERK1/2 phosphorylation. Moreover HCB enhanced ERα phosphorylation and ERα/c-Src physical interaction. In tumours, HCB induced c-Src and HER1 activation, c-Src/HER1 association, as well as Akt and STAT5b phosphorylation. Additionally, HCB increased ERα protein content and decreased p-ERα levels and ERα/c-Src association. HCB increased serum 17-β estradiol and prolactin contents and decreased progesterone, FSH and LH levels in rats without tumours. This might be a consequence of a negative feedback on the pituitary hormones secretion, by E2. The opposite effect was observed in rats with tumors. These results indicate that HCB induces an estrogenic effect in mammary gland, increasing c-Src/HER1 and ERα signaling pathways. HCB stimulates c-Src/HER1 pathway, but decreases ERα activity in tumors, appearing to shift them towards a higher malignancy phenotype. The enhanced c-Src and HER1 activation

could be associated to the increase in the hyperplasia found in the mammary gland and the higher malignancy observed in tumors (Peña *et al.*, 2012). The synergism between c-Src and HER1 serves to upregulate the mitogenic activity of HER1 downstream effectors involved in tumorigenesis (Biscardi *et al.*, 2000). However, the very high doses tested significantly affect the relevance of this information for human environmental and occupational risk assessment.

2.1.5 β-Hexachlorocyclohexane

This isomer of HCH has all chlorine atoms in an equatorial position, and thus (***a***) while it lacks aromatic character, its molecular shape is more similar to that of strictly planar hexachlorobenzene than those of all other isomers and (***b***) it lacks any axial chlorine atom(s) which can be the site for 1,2-elimination by chemical mechanisms (**Figure 1.3R**). This is one reason why this isomer shows a higher bioaccumulation (half-life in human body of about 7.2 years) and a higher bioaccumulation factor of 5274 as stated earlier. Since this isomer is eliminated very slowly from human body, it makes a significant contribution to the total HCH body burden.

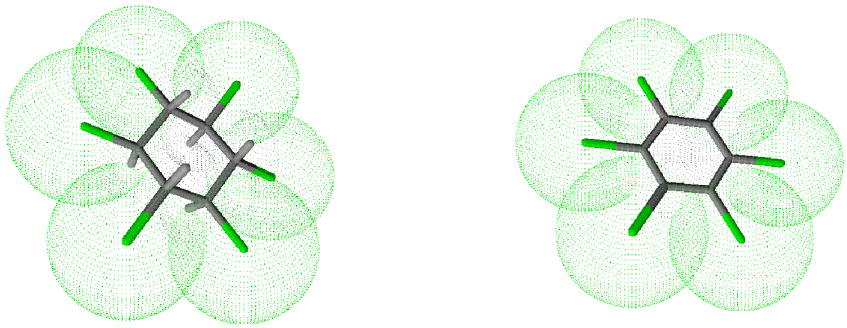


Figure 1.3R. 3D molecular shapes of: (**A**) beta-hexachlorocyclohexane (β-HCH or 1,2,3,4,5,6-HCH [1α,2β,3α,4β,5α,6β]); (**B**) hexachlorobenzene (HCB) (Plots by ACDLabs/3D, Advance Chemistry Development, 2007).

β-HCH (1µM) has mitogenic activity in MCF–7 cell lines *in vitro* (Coosen and van Velsen, 1989) but not in ERα-MDA-MB231 cell lines which express a non-functional ER (Steinmetz *et al.*, 1996). This result indicates that a functional ER is necessary to elicit the mitogenic activity.

In vivo assay with mouse xenograft showed that 10^{-8} M β-HCH significantly increased MCF-7 cell number and at 10^{-5} M maximal responses were achieved (Steinmetz *et al.*, 1996). Moreover β-HCH increased *pS2 gene* mRNA level. Like HCB, β-HCH does not compete with estradiol for binding to the ER (Coosen and van Velsen, 1989; Hatakeyama *et al.*, 2002; Steinmetz *et al.*, 1996). Its chemical structure characteristics

may not allow binding the classical ligand binding domain and thus fail to activate ER in the nucleus, but it shows affinity to the alternative pocket and consequently triggers the Src/Ras/ERK pathway (Silva *et al.*, 2010). Although unable to bind the ER, β-HCH is capable of activating ER target genes and the proliferation of estrogen-responsive cell lines (Silva *et al.*, 2007; Steinmetz *et al*., 1996) by other chemical and biochemical mechanisms.

Treatment of MCF-7 cells with 1×10^{-5} M β-HCH resulted in strong activation of the Src kinase, ERK1 and ERK2. Thus β-HCH has been shown to induce rapid and sustained Src/ERK signalling in MCF-7 cell lines lasting for at least 30 min, while this pathway was not significantly affected by *p,p′*-DDE (Silva *et al.*, 2010). Hatakeyama *et al.* (2002) observed similar activation of ERK1/ERK2 by β-HCH.

In epidemiological study Mussalo-Rauhamaa *et al.* (1990) found that β-HCH frequently occurs in breast fat of breast cancer patients with mean concentration of 0.13 ± 0.06 mg/kg fat. Controlling for age and parity, β-HCH remained a significant risk factor (OR, 10.51; 95% CI, 2.00–55.26). The cutoff point for the residue level in breast adipose tissue was > 0.1 mg/kg fat.

2.2 Oxidative stress, apoptosis and OCPs' toxicity

Apoptosis is the mechanism whereby damaged or unnecessary cells are naturally eliminated without causing damage to the surrounding tissue (Ameisen, 1996). This effect is accomplished by a partial, yet exhaustive digestion of cell structures (DNA, structural proteins, enzymes and lipids) prior to rupture of the cell membrane and release of products in the extracellular medium for clearance. When apoptosis is excessive or inappropriate, incomplete or insufficient, defections trigger pathological-related condition such as immunodeficiency, autoimmunity diseases, cancer (Alison and Sarraf, 1995; Gougeon *et al.*, 1996) and reproductive anomalies, such as those due to abnormal spermatogenesis (Allan *et al.*, 1992).

Oxidative stress, *i.e.*, a shift of the cell redox balance towards a more oxidative (more negative) value of the electrochemical potential with respect to that of the relevant cell phase, is one main trigger in the induction of apoptosis (Kannan *et al.*, 2000; Pérez-Maldonado *et al.*, 2005). Many pesticides share as a possible mechanism of toxicity the ability to trigger apoptosis through alterations in redox homeostasis generated by a decrease of antioxidant defenses and by accumulation of reactive oxygen species

(ROS). An over-production of ROS leads to processes such as oxidative modifications of redox signaling protein, oxidative DNA damage, endoplasmic reticulum stress and alterations in mitochondrial function which in turn trigger the activation of specific signaling cascades. Activation of stress-activated protein kinases (SAPKs) such as c-Jun N-terminal kinases (JNK) and of transcription-dependent p53 signaling cascades act as important sensors of electrophilic (and of oxidized) substances among which xenobiotic metabolites and induce apoptotic cell death. Pesticides also induce the activation of survival responses such as DNA repair mechanisms, mutagens-activated protein kinase/phosphatidylinositol 3-kinase (MAPK/PI3K) signaling cascades and up-regulation of antioxidant defenses in an attempt to cope with and counteract the deleterious effects of higher levels of intracellular reactive species which in turn trigger cell death pathways. In most cases apoptotic and survival signaling cascades are activated simultaneously in response to exposure to pesticides. **Table 1.3R** summarizes some studies on apoptotic effects of some OCPs.

DDT derivatives have been shown to induce neural cell death by apoptosis through the activation of mitogen-activated protein kinases (MAPKs) which play significant roles in controlling cell survival, proliferation, differentiation and cellular responses to various harmful signals (Shinomiya, N. and Shinomiya, M., 2003). β–HCH, *p,p′*-DDE and other OCPs can induce elevation of ROS such as $O_2^{\bullet-}$, $HO^{\bullet}$ and $^{\bullet}NO$ (Samanta and Chainy, 1997; Song *et al.*, 2008; Sujatha *et al.*, 2001).

Shi *et al.* (2009) hypothesized the signaling pathways involved in *p,p′*-DDE-induced apoptosis: in their model ROS generation plays critical role in the initiation of Sertoli cells apoptosis through two mechanisms. The first one is mitochondria-mediated pathway involving elevation of ROS, decrease in mitochondrial transmembrane potential along with the cytochrome c release from mitochondria into the cytosol and activation of the caspase-9 and -3. Other mechanism involves elevation of ROS, which resulted in activation of NF-*κ*B, expression of FasL and triggered FasL-dependent pathway (i.e. FasL/caspase-8/-3 signaling module). In their investigation, Song *et al.* (2008) found at levels above 30 µM, *p,p′*-DDE induced apoptotic cell death of cultured rat Sertoli cells in a pro-oxidant and mitochondria dependent manner by activating the intrinsic programmed cell death pathway. Moreover *p,p′*-DDE elevated the apoptotic rate of Sertoli cells *in vitro* (at >30 µM) and germinal cells *in vivo* (at >20 mg/kg b.wt) by mechanism suspected to involve FasL-dependent pathway (Shi *et al.*, 2009, 2010).

Table 1.3R. Summary of recent studies on the apoptotic effects of OCPs (DDT, DDE, methoxychlor, β-HCH and γ-HCH).

Author	Study type	Study sample/material	Effect investigated	Observed effects
Shi *et al.* (2009)	*In vitro*	Rat Sertoli cells from testes of 18 to 20 days old male Sprague-Dawley rats. The cells were incubated with 10, 30, 50 or 70 µM *p,p′*-DDE for 24 hours	Effects of *p,p′*-DDE on apoptosis, FasL, caspase-3, caspase-8 mRNA, procaspase-3, procaspase-8 and NF-$_K$B activation	Apoptotic cell death observed at >30 µM; FasL mRNA levels higher in a 50µM group; caspase-3 mRNA levels higher in 30 and 50 µM group; caspase-8 mRNA increased in different doses; FasL mRNA induced in 50 µM group, NAC attenuated this effect. Procaspase-3 and -8 significantly reduced over 30 and 10 µM, respectively; NF-ĸB activation enhanced with increase of dosage.
Shi *et al.* (2010)	*In vivo*	20-Day old male rats Prepubertal rats administered with 0, 20, 60, 100 mg/kg b. wt of *p,p′*-DDE for 10 days of treatment	Effects of *p,p′*-DDE on body weights, organs weights, apoptosis, GSH-Px activity, SOD activity, MDA level, Fas, FasL; caspase-3 and -8; procaspase-8, NF-$_K$B p65 proteins, caspase-3 and -8 activities in rat testis	No change in body weights and testis weights; selective degeneration of germ cells at the seminiferous tubules observed at >20 mg/kg b.wt; all tested doses induced MDA increase and decrease SOD and GSH-Px activity; mRNA level of Fas, FasL, caspase-3 and -8 elevated in 100mg/kg b.wt group; *p,p′*-DDE induced increase in FasL and reduction of procaspase-8; NF-ĸB p65 activated in 60 and 100mg/kg b.wt groups; caspase-3 and 8 activities increased in 100 mg/kg b.wt group.
Shi *et al.* (2011)	*In vitro*	Rat Sertoli cells from testes of 18–20 days old male rats treated with 10, 30, 50, 70 µM β-BHC to test the viability of cells. 50 µM was found to be the maximum tolerant dose. Thus 10, 30 and 50µM β-BHC (β-HCH) were used in subsequent experiments	Effect of β-BHC on apoptosis, ROS production, LDH leakage rate, SOD activity, MDA level, JNK/p38 MAPK protein levels, FasL, procaspase-3 and -8 protein levels, NF-$_K$B activation, NF-$_K$B p65 protein levels	Apoptotic rate increased in 30 and 50 µM β-BHC group and attenuated by NAC; ROS production increased in 30 and 50 µM group and neutralized by NAC; LDH leakage rate enhanced in 30 or 50 µM group, SOD activity lower in all groups, higher MDA level in all group; dose dependent phosphorylation of JNK1 and JNK2 observed, p38 MAPK phosphorylation not high in all groups; FasL increased in 30 and 50 µM group and attenuated by NAC; procaspase-3 and -8 reduced significantly at >30-µM; NF-$_K$B activation enhanced with dosage and neutralized with NAC; NF-$_K$B p65 increased in the 30, 50 µM groups and attenuated by NAC.
Song *et al.* (2008)	*In vitro*	Rat Sertoli cells from testes of 18 days old male Sprague-Dawley rats. The cells were treated with 10,30, 50 or 70 µM *p,p′*-DDE	Effects of *p,p′*-DDE on LDH leakage, ROS production, SOD activity and MDA level, $\Delta\Psi_m$, Bax family, cytochrome *c* translocation, procaspase-9 and -3 cleavage; effect of NAC on *p,p′*-DDE-induced apoptosis.	Apoptotic cell death induced at >30 µM, LDH leakage enhanced in 30 or 50 µM groups; apoptotic characters exhibited in 30 or 50 µM treated cells and blocked with NAC; ROS production increased in 50µM group; lower SOD activity in all groups; higher MDA level in 30 or 50 µM groups; loss of $\Delta\Psi_m$ induced with 10, 30 or 50; Bax and Bak significantly increased in cells treated with 30 or 50 µM. NAC had little effect on these changes; Bcl-w protein level declined significantly in 50µM group and neutralized by NAC; 30 or 50 µM induced cytochrome *c* translocation to cytosol, NAC rescued this translocation effectively. Procaspase-9 and -3 significantly decreased at over 30 µM.

Table 1.3R. Summary of recent studies on the apoptotic effects of OCPs (DDT, DDE, methoxychlor, β-HCH and γ-HCH).

Author	Study type	Study sample/material	Effect investigated	Observed effects
Pérez-Maldonado *et al.* (2004)	*In vitro* and *in vivo*	Human PBMC from volunteers (without DDT exposure) for *in vitro* study. DDT exposed and unexposed children for *in vivo*	Effects of DDT or its metabolites on induction of apoptosis in humans both *in vitro* and *in vivo.*	*o,p'*-DDT, *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD induced apoptosis of human PBMC *in vitro* at 20 µg/mL; blood level of DDT, DDE and DDD blood and the % apoptosis were higher in the exposed children than in the unexposed children.
Song *et al.* (2011)	*In vitro*	Rat Sertoli cells from testes of 18 days old male Sprague-Dawley rats treated with various 10, 30, 50 or 70 µM *p,p'*-DDE	Effect of NAC on *p,p'*-DDE-induced cellular viability reduction, ROS generation, MAPKs phosphorylation; effect of *p,p'*-DDE on mRNA levels of cytochrome c, bax, bak and bcl-w; effect of NAC, antinomycin D, SB202190 or SP600125 on *p,p'*-DDE-induced apoptosis.	Viability of cells reduced with 30, 50 or 70 µM treatments, NAC attenuated the cellular viability reduction induced by 50 µM; ROS production significantly increased by 50 µM treatment and neutralized with NAC; p38 and JNKs phosphorylation elevated after10, 30 or 50 µM treatments and inhibited by NAC; cytochrome c, bax and bak levels increased in 30 or 50 µM group and inhibited by antinomycin D; no significant changes of bcl-w observed; apoptotic cell death induced by 50 µM and attenuated with NAC or SB202190 or antinomycin D; SP600125 did not have any effect.
Saradha *et al.* (2009)	*In vivo*	Adult male Wistar rats (80–90 days old) administered with a single dose of lindane (5 mg/kg b.wt)	The effects of lindane: on the levels of cytochrome *c*, caspase-3 and-9, Fas and FasL; on localization of caspase-3 and Fas; on immunolocalization of FasL; on levels of NF-$_{\kappa}$B p65 in the cytoplasmic and nuclear extracts of the testis, localization of NF-$_{\kappa}$B p65, germ cell in the testis of rats by TUNEL assay.	Cytosolic cytochrome *c* and procapase-9 elevated as early as 6 h. A time-dependent elevation in procaspase-3, Fas, FasL levels observed. A time-dependent increase observed in colocalized expression of caspase-3 and Fas. This was the case for immunoreactivity of FasL. NF-$_{\kappa}$B p65 level in cytoplasmic extract and in nuclear extracts decreased and increased, respectively, in a time-dependent manner. The TUNEL-positive cells found in the peripheral region near the basement membrane of the seminiferous tubules. The number of apoptotic cells increased in a time-dependent manner following exposure to a single dose of lindane.
Vaithinathan *et al.* (2010)	*In vivo*	Adult male Wistar rats (80–90 days old) administered with a single dose of methoxychlor (50 mg/kg b.wt)	The effects of methoxychlor: on the levels of cytochrome *c*, caspase-3 and-9 (in adult rat testis), Fas, FasL, NF-$_{\kappa}$B (in testis cytoplasmic extract); on localization of caspase-3 and Fas; on immunolocalization of FasL and NF-$_{\kappa}$B; on localization of NF-$_{\kappa}$B p65 in the rats testis; on germ cell in the testis of rats by TUNEL assay.	Cytosolic cytochrome c and procaspase 9 elevated as early as 6 h. A time-dependent elevation in procaspase-3, Fas, FasL observed. NF-$_{\kappa}$B decreased in a time-dependent manner. NF-$_{\kappa}$B p65 changed from cytoplasm to nucleus of germ cells. A time-dependent increase in co-localization of Fas and Caspase 3 and in immunoreactivity of FasL observed. TUNEL-labeled germ cells increased in the peripheral regions in time-dependent manner after a single dose of methoxychlor. The number of TUNEL labeled germ cells increased progressively.

Abbreviations: Caspases, cysteinyl aspartate-specific proteinases; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde; NF-$_{\kappa}$B, nuclear factor kappa B; LDH, lactate dehydrogenase; $\Delta\Psi_{m,}$ mitochondrial membrane potential; MAPKs (s), mitogen-activated protein kinases; NAC, N-acetyl-l-cysteine; JNKs, c-Jun NH2-terminal kinase; PBMC, peripheral blood mononuclear; SB202190, p38 inhibitor; SP600125, JNK inhibitor; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; ROS, reactive oxygen species; b.wt, body weight.

Exposure to *p,p'*-DDE can enhance ROS production and oxidative stress, and then induce activation of NF-κB and expression of Fas-FasL. As a result, an intrinsic program of apoptotic death is stimulated in a target cell resulting to the activation of caspase 8. Ultimately, apoptosis of Sertoli cells and germinal cells is mediated by caspase 3, thereby disturbing the spermatogenesis (Shi *et al.*, 2010). Song *et al.* (2011) reported the induction of Sertoli cell apoptosis by *p,p'*-DDE at above 30 μM through oxidative stress-mediated p38 MAPK and mitochondria-related pathway. Methoxychlor, which was intended to replace DDT, has been shown to induce testicular apoptosis in rats following oral exposure to single doses of 50 mg/kg b.wt where apoptosis was induced through involvement of Fas-FasL and mitochondria-dependent pathways (Vaithinathan *et al.*, 2010). It is necessary to remark that the doses tested are unrealistically high if compared to those typical of occupational and environmental exposures.

Pérez-Maldonado *et al.* (2004) showed that *o,p'*-DDT, *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD can induce apoptosis of human peripheral blood mononuclear cells *in vitro* at 20 μg/mL. This level is 400-fold higher than the mean blood total DDT level (0.05 μg/mL) observed in children in their pilot study (Pérez-Maldonado *et al.*, 2004). The significant level of ROS, as detected by flow cytometry, was induced at 60 and 80 μg/mL (Pérez-Maldonado *et al.*, 2005). A marginal influence of DDT and DDE on percentage of apoptosis was observed ($p = 0.06$) confirmed by a weak positive association between apoptosis and DDT exposure. In the follow-up study Pérez-Maldonado *et al.* (2006) found significant association between the percentage of apoptotic cells and the blood levels of only DDE of exposed children recruited in 2003 ($p = 0.010$) and those recruited in 2004 ($p = 0.040$). The association between DDT or DDE exposure and DNA damage was significant ($p = 0.004$ and $p = 0.005$ respectively), whereas the association between DDT or DDE and oxidative DNA damage and that of oxidative damage and apoptosis were not significant.

An isomer of DDD, *o,p'*-DDD, marketed under the name Mitotane is used as an antineoplastic medication in the treatment of adrenocortical carcinoma. Mitotane alters steroid peripheral metabolism, directly suppresses the adrenal cortex through the controlled destruction of adrenal tissue, which leads to a decrease in cortisol production (Maher *et al.*, 1992; Wu *et al.*, 2006).

Exposure of Wistar rats to a single dose of lindane (γ-HCH; 5 mg/kg b.wt) induced testicular apoptosis through the involvement of Fas-FasL and mitochondria-dependent pathways (Saradha *et al.*, 2009). Shi *et al.* (2011) showed that concentrations >30 μM of β-HCH induced apoptotic cell death in rat Sertoli cells associated with increased expression of FasL levels which could lead to the Fas activation. These two genes are known to induce apoptosis. Moreover β-HCH treatment induced an increase of nuclear factor kappa B (NF-$_{\kappa}$B) p65. The latter can directly stimulate the expression of these genes. Oxidative stress has been reported to enhance NF-κB activation (Nakamura and Omaye, 2008). β-HCH has been shown to induce activation of caspase-8, that plays the role in transduction of death signal (Said *et al.*, 2004) and caspase-3, that initiates cell apoptosis (Khan *et al.*, 2000).

Moreover, Shi *et al.* (2011) showed that β-HCH induces increase in apoptotic rate of Sertoli cells by possible mechanisms of ROS/JNK/ FasL pathway. *In vitro* exposure to β-HCH in rat Sertoli cells can enhance ROS and oxidative stress, and then induce activation of JNKs and NF-κB and expression of FasL. Significant increase of FasL protein expression was observed with 30–50 μM β-HCH treatment. This increase could lead to activate the Fas system. Upon engagement of FasL to Fas, an intrinsic program of apoptotic death is stimulated in a target cell leading to the activation of caspase-8. Finally, apoptosis of Sertoli cells is mediated by caspase-3, thereby disturbing the spermatogenic process (Shi *et al.*, 2011; **Figure 1.4R**). These results led the authors hypothesize that ROS generation may play a critical role in the initiation of β-HCH-induced apoptosis by activation of the JNKs, translocation of NF-$_{\kappa}$B, expression of FasL and further activation of caspase cascade.

Yu *et al.* (2008) studied the individual effect of *p,p'*-DDE and β-HCH as well as the effect of their mixture on JNK and MAPK pathway in rat Sertoli cells. The latter were exposed to these OCPs at the final concentration of 10, 30 and 50 μM/L for 24 hours in each case. In both treatments, the expression of JNK and c-jun were elevated in a dose dependent manner after 24 hours of exposure. Liang *et al.* (2008) showed that at these levels *p,p'*-DDE, β-HCH and their mixture could induce apoptosis in rat Sertoli cells which was associated with activation of caspase-3 mediated by cleavage of caspase-8 and caspase-9. As stated for the case of *p,p'*-DDE above, these levels are also unrealistically high if compared even with the highest occupational exposures.

As for specific mechanisms, OCPs, which are themselves fairly un-reactive from the chemical point of view, are able to cause or trigger oxidative stress. However, little is known or hypothesized at the molecular level. Only the porphyrinogenic pesticide hexachlorobenzene is known for its ability to directly generate oxidative stress due to its unique property to act as an electron sink either as such or, more likely, through biotransformation products such as tetrachloro-1,4-benzoquinone (van Ommen and van Bladeren, 1989). That HCB is able to trigger apoptosis through the mitochondrial pathway has been demonstrated in the liver (Giribaldi *et al.*, 2011) and in the thyroid (Chiappini *et al.*, 2009).

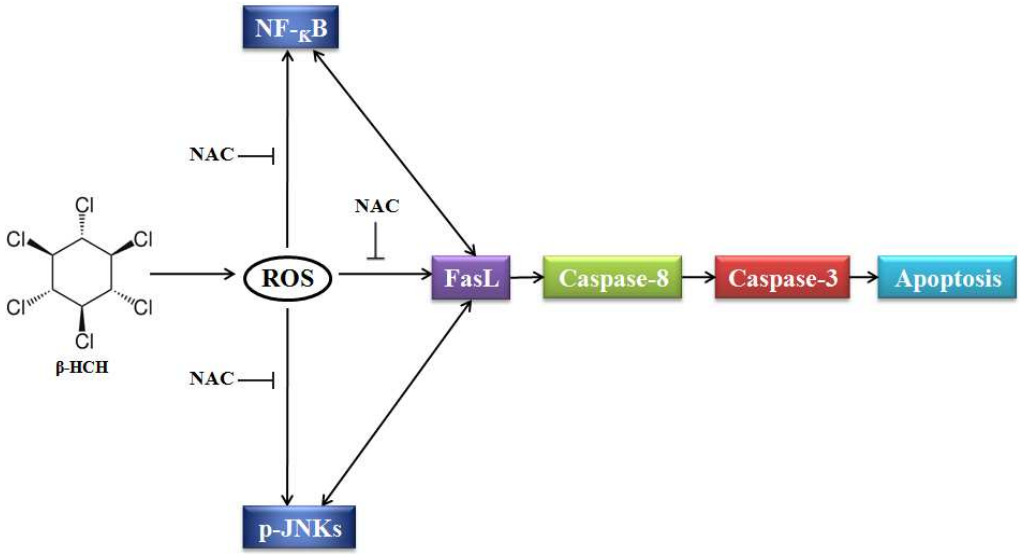


Figure 1.4R. β-HCH–induced signaling pathways leading to apoptosis. In this proposed model, ROS generation might be responsible for initiating the β-HCH–induced apoptosis of Sertoli cells. The increase of ROS could result in activation of JNKs and NF-κB, and expression of FasL, then triggered FasL-dependent pathway (FasL/caspase-8/caspase-3 signaling module). *N*-Acetyl-cystein (NAC) can inhibit triggers by protecting the sensor thiol residue of the signalling proteins. *Source*: Adapted from Shi *et al.* (2011) with some modifications.

2.3 Epigenetics and OCPs' toxicity

Epigenetics is an emerging field of study of heritable changes that influence chromosomal stability and gene expression while not directly affecting changes in DNA sequence, such as by point mutations or chromosome disruption and re-assembly (Rodenhiser and Mann, 2006). One main mechanism of epigenetic modulation of genetic expression is methylation at 5-position of cytosines in repeated CpG sequences, a modification which suppresses expression of downstream DNA sequences. Thus suppression of specific genes or re-activation of silenced genes determines the destiny of individual cells towards quiescence or proliferation, thus opening the way to

uncontrolled cell proliferation and cancer. Moreover, when epigenetic changes occur during certain stages of development, they become permanent and can be inherited by offsprings and disturbance of epigenetic modulation is thus thought to be an important mechanism in many diseases (Ozanne and Constancia, 2007).

Epigenetic effects have been hypothesized as a possible mechanism of POPs toxicity (Porta, 2006). For example, pregnant rats were treated with methoxychlor at the fairly high dose of 50–150 mg/kg during the sex-determining time window of pregnancy (days E7–E10). Gonads were collected from embryos in gestating mothers at E16 and from postnatal males showed a reduced germ cell to testis area at developmental age and an almost doubled fraction of apoptotic germ cells, although males were fertile and produced normal offspring (Cupp *et al.*, 2003). In a further experiment (Anway *et al*., 2005) exposition of pregnant rats to 100 mg/kg produced male offsprings with reduced sperm capacity and fertility and the compromised fertility trait was passed through the adult male germ line for four generations. Altered patterns of DNA methylation was demonstrated to occur in the germ cells of generations two and three.

In vitro and animal studies have suggested that exposure to endocrine active compounds, such as POPs, may adversely affect DNA methylation patterns (Anway and Skinner, 2006; Anway *et al.*, 2006). It has been proved that chemicals can alter the epigenetic marks and that epigenetic marks can be found in patients with the disease of concern or in diseased tissue (Baccarelli and Bollati, 2009).

DDT has been shown to affect DNA methylation in experimental animals as shown by Shutoh *et al.* (2009). DDT was found to alter the methylation pattern in the hypothalamus of young rats (3 weeks of age) exposed at a dosage of 0.06 mg/kg/day. The 6 CpG islands were considerably hypomethylated as compared with controls. Thus the authors speculated that under low level of oxidation stress, the DNA methylation machinery malfunctions and this leads to incomplete methylation of specific gene regions. Following pyrosequencing methylation analysis of the rat livers from the rats exposed to high dose of mixture of twelve OCPs (1.9 mg/kg/day) showed no decrease in the methylation of CpG sites which is contrary to other tested chemical mixtures (Desaulniers *et al.*, 2009).

To our knowledge only two epidemiological studies have been conducted to investigate exposure to POPs and DNA methylation levels in a human population. The first study was carried on Greenlandic Inuit and suggested a significant inverse linear relationship

between DNA methylation and plasma concentrations of DDT, DDE, β-HCH, oxychlordane, α-chlordane and mirex (Rusiecki *et al.*, 2008). This relationship was replicated by Kim *et al.* (2010) when they investigated the relationship between POPs exposure and DNA hypomethylation among healthy Koreans. These two studies demonstrated epigenetic changes related to environmental exposure although the two populations differ in magnitude of exposure, Greenland Inuit people being more exposed than Korean population.

3.0 Conclusions

The general population is exposed to OCPs through several ways, food being the major route of exposure. A few groups of the human population may show much higher levels of exposure than others due to the fact that they live in areas with a historical uncontrolled use of these pesticides in agriculture (typical examples being some areas of Central Asia) or that their diet mainly consists of bio-accumulating organisms, such as large marine mammals (the typical example is that of Arctic Inuits). In both cases the hazard is transmitted trans-generationally starting with breastfeeding of newborns and consequently the impairment of fertility can determine a demographic decline of some populations.

A number of adverse health effects such as endometriosis, infertility, immunotoxicity, neurotoxicity and spontaneous abortions, breast cancer, prostate cancer and neurodegenerative disorders have been suggested as a result of this exposure. OCPs may exert these effects through various mechanisms. Animal and *in vitro* studies using cell cultures are most widely used to evaluate toxicity of these chemicals. OCPs act as agonists on ERα and/or antagonists on ERβ and also as probably antagonists on androgenic receptors. These effects may contribute to the tumor promoting effects of these pesticides, which are observed in animals treated with high doses, which are not representative even of worst-case human exposure levels. Thus, *p,p'*-DDE and HCH have been shown to exhibit antiandrogenic effects by binding to ARs and competing with natural androgens, a characteristic which supports their estrogenic effect. Although HCB and β-HCH elicit estrogen-like responses, they do not compete with estradiol for binding to the ER like estrogen does. This suggest other intracellular pathways than the one used by endogenous estrogen in mediating these actions. They can elicit responses through AhR-dependent and independent pathways.

Many OCPs modulate or trigger apoptosis by redox signaling which involves alterations in antioxidant defenses and accumulation of ROS leading to oxidative stress. When not regulated, apoptosis can contribute to several diseases as immunodeficiency, autoimmunity and cancer. However, limited information exists on full mechanistic events involved in the induction of cell death or survival by these pesticides.

Although not extensively explored, epigenetics has been considered to be a potential mechanism of POPs toxicity. Few studies have addressed OCPs and epigenetic modification as evident in human studies where only two studies have been conducted to elucidate the involvement of OCPs in health effects at epigenetic level. However some of these studies have shown OCPs may operate at epigenetic level. Thus more studies (both animals and human) are needed to document evidence of involvement of pesticides at epigenetics level in eliciting adverse health effects to human.

It is also important to remark that a very common characteristics of laboratory studies is that they have been carried out testing unrealistically high doses, and this makes the extrapolation to environmental and occupational human exposures very critical. However they produce hypotheses that deserve an investigation through specifically addressed epidemiological and experimental studies. This is particularly important because most of the epidemiological studies we have evaluated are not conclusive, and often results of different epidemiological studies are conflicting.

However, despite the not fully conclusive picture, it is evident that, having in mind the toxic potential, the bioaccumulation and biomagnification properties, there is a need to perform specific interventions addressed at reducing exposure, especially of vulnerable population subgroups. Thus stringent control in food safety system should be put in place to prevent the occurrence of OCPs in food and feedstuffs.

The restriction of the use and the ban of most of OCPs in most developed countries limits the possible health threats they might pose. Cessation of direct application on animals or presence of OCPs in their feed, housing or pasture will help to control them. Removal of the contaminated items from the human food chain can be opted when acceptable limits have been exceeded. Withdrawal times can be appropriate means for growing (meat-type) animals, which do not deliver a product on a daily basis. Finally, continuing research is needed on these contaminants, their body burden, potential health effects and ways to reduce their bioavailability in food. Moreover, effective policies are needed to control their manufacture and release into the environment.

1.3 Rationale of the Study

The interest to measure PCBs contamination in the environment, food and human population of Italy started in the early 1970s, a few years after the discovery of PCBs as a new class of ubiquitous contaminants of the Baltic Sea (Jensen, 1966; Jensen *et al.*, 1969; Ahling and Jensen, 1970). Between 1971 and 1989 a group at the Istituto Superiore di Sanità, Rome expanded the interest into the measurement of chlorinated pesticides (Leoni, 1971; Leoni and Biocca, 1978; Leoni *et al.*, 1986), followed by other groups within the National agencies for health protection and in the academy which published their reports in Italian scientific journals and mainly starting from the early 1980s in the international literature (Galassi *et al.*, 1981; Martelli *et al.*, 1981). One main health concern was reproductive toxicity (Bercovici *et al.*, 1983; Rogan *et al.*, 1985; Leoni *et al.*, 1989) since some PCBs were reprotoxic in experimental animals.
In the 1980s several studies started establishing reference values of serum PCBs in the general population in countries with past or current use of PCBs. The aims were to confirm or refute the contribution of exposure to observed or suspected clusters of cancer incidence in towns where production plants were located or close to places where release into the environment had occurred.

A meta-analysis of 37 studies conducted between 1990 and 2003 established a mean reference value (0.9–56 μg/L) for total PCB in blood in the general population (Mangili *et al.*, 2004). Such levels are within the range reported in the toxicological assessment of PCBs performed in USA (4–19 ng/mL; ATSDR, 1997). In Brescia where the main PCB production plant was located, a mean value of 5.15 ng/mL for the total serum PCB level in the general population was measured in 2001–2003 (Apostoli *et al.*, 2005). Based on this finding, further investigations were proposed aimed at determining geographic distribution of PCB levels in the Italian population with larger study cohorts.

Among European countries, Italy used DDT extensively since 1945 up to the 1970s for controlling flies and mosquitoes (particularly malaria vectors). This led to improvement of health conditions especially in the Po river delta, in central–southern coastal and in insular Italy. The usage was estimated at 2,178 and 1,569.50 metric tonnes in 1970 and 1975, respectively. The usage declined in 1980s and 1990s where the estimates were 196.48, 103.20 and 54.72 metric tonnes in 1980, 1985 and 1990, respectively (WHO, 2003). Since human exposure to OCPs can bring about significant health effects

exposure measurement aimed at health risk assessment of these pollutants in the general population is still a matter of public health concern. Despite long-time use, measurements are mainly targeted at environmental contamination and only limited information is available on actual human burden of these pollutants in the Country. Between 1972 and 2011 about sixteen international published articles reported OCPs concentration in the Italian population as exemplified in **Table 4.1.** Such lack of detailed exposure data hinders adequate human risk assessment (De Felip and Ingelido, 2004).

This project aimed at improving existing knowledge on the levels of exposure of the Italian general population to concerning polychlorinated biphenyls and organochlorinated pesticides. In this Thesis results of serum PCB congeners and OCPs obtained within REALEXPO project among the residents from three Italian population subgroups from general population are reported. The relationship of these POPs with residence, gender, age and body mass index (BMI) was explored. In future the study will further investigate the relationship between the levels of POPs and the development of breast cancer among Italian female population.

1.4 Objectives of the Study

1.4.2 Broad Objective

To assess the human exposure to persistent organic pollutants and the associated health risks among Italian population.

1.4.3 Specific Objectives

1. To determine the concentrations of polychlorinated biphenyls and organochlorinated pesticides in human blood serum collected from residents from three Italian towns.
2. To assess the distribution of lipid-adjusted serum concentrations of polychlorinated biphenyls and organochlorinated pesticides among study subjects with respect to their places of residence.
3. To assess the gender distribution of lipid-adjusted serum concentrations of polychlorinated biphenyls and organochlorinated pesticides.
4. To compare the distribution of lipid-adjusted serum concentrations of polychlorinated biphenyls and organochlorinated pesticides among different age groups in the study subjects.
5. To evaluate the effect of body mass index in the distribution of lipid-adjusted serum levels of polychlorinated biphenyls and organochlorinated pesticides among the study subjects.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Study site and selection of study population

The project was run through three years from 2010 to 2012 as indicated in the work plan (**Annex I**). Our research group collected, in the frame of routine health checks of the general population, serum samples of subjects recruited from three Italian towns of Novafeltria (in Emilia Romagna region), Pavia and Milan in the region of Lombardy. Novafeltria is assumed to be a rural area (a non-industrialized area in Central Italy), Pavia a semi-urban town while Milan is clearly an urban area with past manufacturing activities. We thus expected to see variation of POPs pattern between these regions.

2.2 Selection criteria

The study subjects were recruited based on established inclusion criteria. These are absence of significant body weight loss (10% or more) a year before the study commenced, absence of dyslipidemia and other chronic metabolic diseases. The recruited subjects were also supposed to have neither history of occupational exposure nor accidental exposure to POPs from identified sources.

2.3 Ethical consideration

Informed consent was properly obtained from all study participants and the study was approved by the Institutional Ethical Committee of Ospedale San Paolo, Milano, Italy. Information about the nature of the study as well as possible risks and benefits was given to the study subjects. There were no monetary benefits. Confidentiality and privacy was assured throughout the study.

After securing informed consent recruited subjects filled in data collection forms regarding personal information like demographic information (age, gender, occupational and residential histories), anthropometric measurements (height and weight), lifestyle (usual diet, cigarette smoking, alcohol consumption, physical exercises) and use of medications. This information was collected at interview by using designed data collection forms (**Annex II**). After filling the forms the subjects were asked to donate a 10 mL of blood samples.

2.4 Blood sample collection

A 10 ml venous blood sample was obtained from each subject from the antecubital vein and collected in silicone-coated Vacutainer® (Becton & Dickinson, USA) without anticoagulants. Serum was separated within 24 hours after collection, kept at 4˚C until shipment and frozen at -20˚C upon receipt at the laboratory until analysis.

For the PCB study, selected information and serum samples were collected from a total number of 372 subjects where 163 from Novafeltria (a 7,000-inhabitant small town in Central Italy), 167 from Pavia (a 70,000-inhabitant small old city in Northern Italy) and 42 (the 1,300,000-inhabitant largest city in Northern Italy, the second-largest in Italy) from Milan (**Figure 2.1**). For the OCPs study, selected data were obtained from 137 subjects among them, 36, 59 and 42 subjects live in the respective towns.

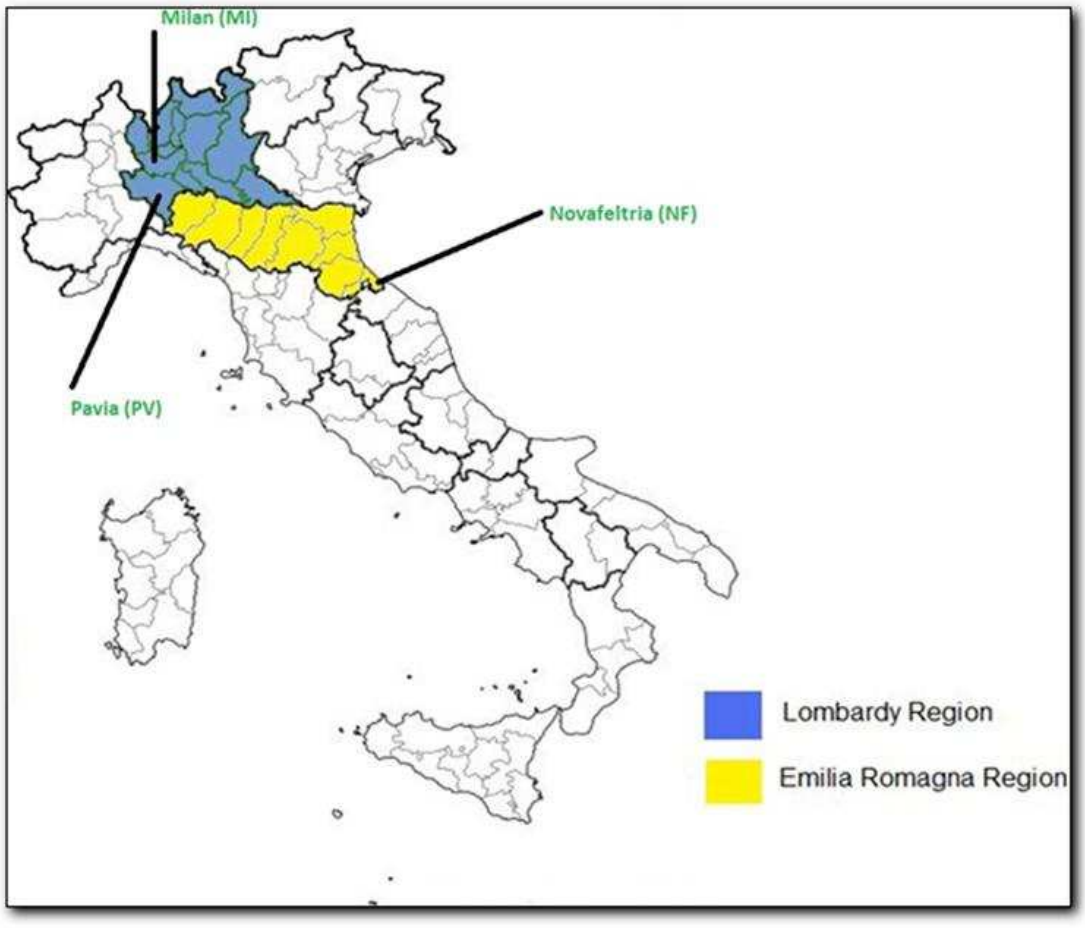


Figure 2.1. Map of Italy showing the study locations.

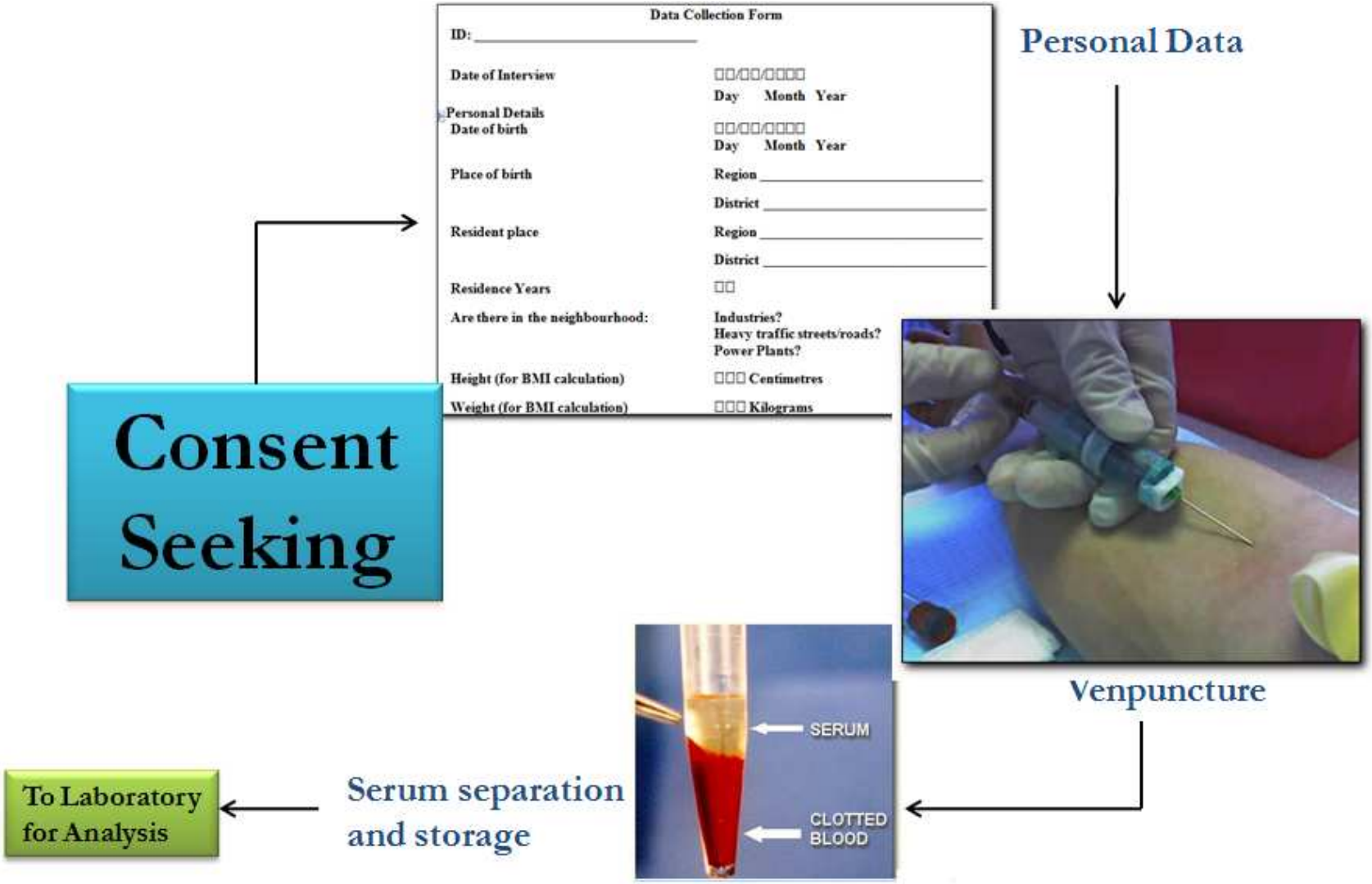


Figure 2.2. Schematic illustration of recruitment procedure of study subjects.

2.5 Laboratory analyses

All laboratory analyses were conducted at the Laboratory for Environmental and Toxicological Testing at Salvatore Maugeri Foundation in Pavia, Italy.

2.5.1 Determination of serum concentrations of triglycerides and total cholesterol

Serum concentrations of triglycerides and total cholesterol were measured in the serum sample by standard clinical chemistry techniques to define total lipid concentrations which were derived using the equation 2 reported in Philip *et al.* (1989).

Total Lipid (TL^a) = 2.27 x Total Cholesterol (TC^b) + Triglycerides (TG^c) + 0.623 (2)

[a] in g/L; [b] in g/L (normal range: 120–220 mg/100 mL); [c] in g/L (normal range: 40–170 mg/100 mL).

The individual POP concentrations were adjusted for total lipid to give better estimate of the body burden (Koppen *et al.*, 2002). Adjustment was computed by dividing the crude serum POP concentration (ng/mL) by total lipid concentration in serum (g/L) to yield OCP concentration in nanograms per gram of serum lipids which was finally converted to picomoles per gram serum lipid (pmol/g serum lipid). This allows comparison between congeners since there are substantial differences in molecular weight between the compounds (Fängström *et al*., 2005). The same conversion was applied to results published in the scientific literature which were used as reference in the interpretation of our own measurements. When results in the literature were expressed without lipid adjustment and no blood lipids levels were reported, an adjustment was nevertheless performed by taking the lower and upper levels of normal concentrations of cholesterol and triglycerides into equation 2 above.

2.5.2 Extraction and purification of PCBs and OCPs

Prior to extraction the blood serum was thawed at room temperature. Thereafter 2 mL of methanol was added to 2 mL of serum and solution was vortexed for 30 seconds to precipitate proteins. Lipophilic organic substances were extracted by adding to the sample 5 mL of a 1:1 (v/v) mixture of ethyl ether and hexane. This procedure was repeated twice. The combined organic layer was evaporated under a steam of nitrogen down to about 500 µL. This residue was purified on a Bond Elut® PCB (Supelco, Milano, Italy) solid-phase extraction cartridge. Activation of the cartridge was obtained by percolating 1 mL of hexane. The fraction extracted from serum was loaded and

eluted first with 3 mL hexane and then with 3 mL ethyl ether/hexane (1:1, v/v). The eluites were collected and dried in a stream of nitrogen. The dry residue was re-dissolved in 100 µL of hexane just prior to analysis. After extraction and clean up of extracts, the analytes were measured by gas chromatography–mass spectrometry (GC–MS).

2.5.3 PCBs and OCPs analyzed

We analyzed thirty six PCB congeners and eight OCPs in the overall study population which were selected based on their abundance, availability in the environment and potential toxicity.

Specifically 36, 34 and 15 PCB congeners were analyzed in serum samples of Novafeltria, Pavia and Milan, respectively. Due to differences in the number of measured congeners in the sites, 15 congeners commonly investigated in all sites were selected to facilitate comparison of total PCB levels among the sites (**Figure 2.4** and **Table 2.1**). For practical and economic reasons, it was decided to reduce the number of measured PCBs congeners to 15 in the Milan cohort, which was the last to be analyzed. The experience from previous studies conducted elsewhere and from the two cohorts from Pavia and Novafeltria which were analyzed earlier, showed that most of the fifteen congeners (such as PCBs 28, 52, 101, 118, 138, 153 and 180) are stable in the environment and may be good markers for human PCB exposure without the need to perform a much longer analysis.

The eight targeted OCPs were beta-hexacyclohexane (β-HCH); hexachlorobenzene (HCB), 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethylene (*o,p'*-DDE), 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene (*p,p'*-DDE), 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p* chlorophenyl)ethane (*o,p'*-DDD), 1,1-dichloro-2, 2-bis (4-chlorophenyl)ethane (*p,p'*-DDD), 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p* chlorophenyl)-ethane (*o,p'*-DDT) and 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (*p,p'*-DDT).

2.5.4 GC–MS Measurement of PCBs and OCPs

The analytical technique for determination of these compounds, limits of detection and accuracy of the method are described elsewhere (Turci *et al*., 2004, 2006, 2010a).

In brief, a Shimadzu (Shimadzu Deutschland GmbH, Duisburg, Germany) gas chromatograph mass spectrometer GCMS-QP5050A (**Figure 2.3**) equipped with an auto injector/auto-sampler AOC-20 was used to analyze the selected POPs. A 50m×0.2mm×0.5µm, J&W 100% dimethylpolysiloxane PONA capillary column was used with helium as carrier gas at a constant flow of 0.8 mL/min. Two microliters was injected in splitless mode with the split outlet opened after 0.7 min. Injector and detector temperatures was set at 270 and 280˚C, respectively. The oven temperatures program was: 80˚C for 1 min, 3˚C/min to 200˚C, then 5˚C /minutes to 300˚C and 6 minutes holds at 300˚C. The mass spectrometer operated in electron ionization mode (EI) at 70 eV. A SIM program was constructed for GC–MS acquisition and quantification. Two ions (M^+ and $[M+2]^+$ ions) were monitored for each level of chlorination. Retention time, masses and the abundance ratio of the chromatographic peaks of each analyte recorded by the two ions ($[M+2]^+$ confirmation ion to $M^{+\cdot}$ target ion) was used as identification and purity check criteria. Peak areas were measured by the GCMS Postrun Analysis program of the Shimadzu Workstation GCMS solution software for GCMS-QP5000 Series, which also calculated slopes, intercepts and the coefficients of correlation. Peak area ratios (analyte response/internal standard response) were plotted against amount ratios (analyte concentration/internal standard concentration) and standard calibration curves was obtained from linear regression analysis of the data. Minimum detectable amounts of POPs, expressed as Limit of Detections (LoDs) were in the range of 0.05–0.50 nanograms per millilitre (ng/mL).

Figure 2.3. GS–MS for laboratory analysis of selected persistent organic pollutants.

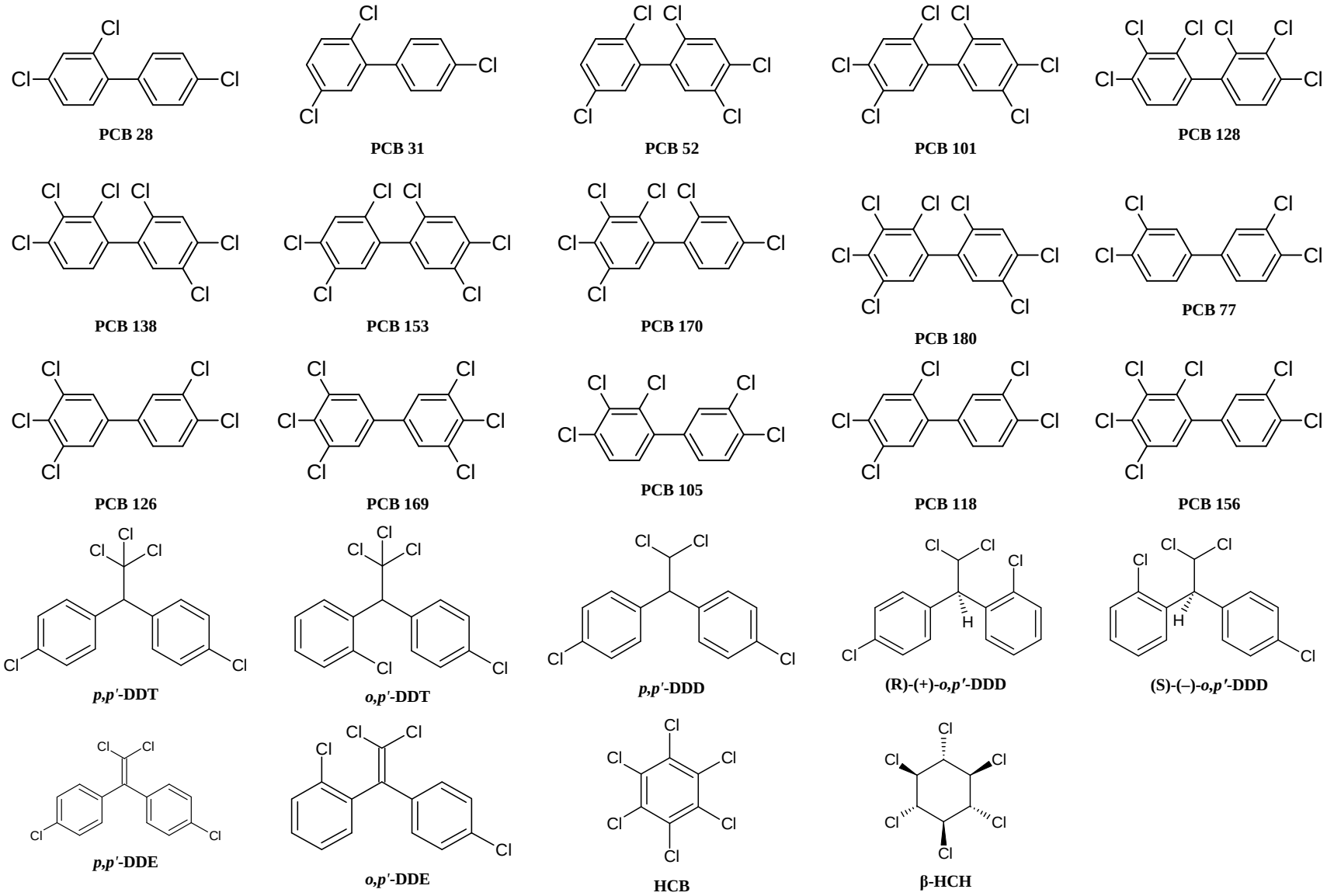
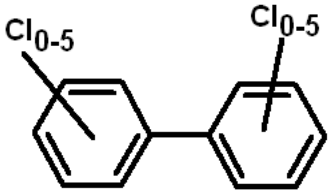


Figure 2.4. Molecular structures of the priority fifteen PCB congeners and eight OCPs commonly investigated in the three sites.

Table 2.1. The IUPAC numbers of thirty six investigated PCB congeners in the overall population.

General Structure	Classification	Formula	Mwt	IUPAC Nos. of PCB congeners
	trichlorobiphenyl	$C_{12}H_7Cl_3$	257.55	28, 31
	tetrachlorobiphenyl	$C_{12}H_6Cl_4$	291.99	44, 47, 49, 52, 61, 66, 70, 74, 77, 81
	pentachlorobiphenyl	$C_{12}H_5Cl_5$	326.44	99, 101, 105, 118, 126
	hexachlorobiphenyl	$C_{12}H_4Cl_6$	360.88	128, 138, 146, 149, 153, 156, 157, 167, 169
	heptachlorobiphenyl	$C_{12}H_3Cl_7$	395.33	170, 171, 172, 177, 180, 183, 187, 189, 190, 193

2.6 Statistical analyses

The data were analyzed by using the Statistical Package for Social Sciences (SPSS) software version 17.0 (SPSS Inc, Chicago, IL, USA) and custom Microsoft Excel©2003 spreadsheets. A bar chart was used to present prevalence of each OCP in the study population subgroups (**Figure 3.1**). All statistical analyses were performed on serum lipid adjusted concentrations expressed in pmol/g lipid.

Subjects with undetectable values were assigned half the detection limits (LoDs) before adjustment for lipids and calculation of the medians included these values. After lipid adjustment half LoDs differed for every sample due to differences in total lipid. The median concentrations were computed and reported for those analytes detectable in more than 50% of the population sample or 50% of the groups classified by sex, age, place of residence and body mass index.

Statistical analyses were conducted to assess inter-individual variability in analytes levels and to highlight differences with respects to sex, age, body mass index and residence. The dependent variable in this study is serum lipid-adjusted concentrations of analytes under investigation. The analytes concentrations were summarised for the whole sample and for the individual sites and separately for males and females using medians, minimum and maximum values.

Shapiro Wilks' test for normality was used to check the distribution of PCB congeners and OCPs. Since distribution of PCB congeners and OCPs levels among the subjects were non-symmetrical, all statistical tests were performed by non-parametric tests and medians were used as a measure of central tendency. Thus Kruskal Wallis (KW) Test was used to test the difference in concentration of individual organochlorine pesticides and PCB congeners between place of residence, age groups and BMI categories. The Mann–Whitney U test assessed the difference in analytes concentrations between the gender groups. The correlation analyses were performed by Spearman correlation test. Multivariate model were used to check the relationship between POPs and predictor variables under investigation. The variance explained by the model was estimated by using the determination coefficient, R^2. In most cases statistical tests were considered significant at the 0.05 level and in some cases at the 0.01 level.

As for OCPs statistical analyses were restricted to β-HCH, HCB, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDD and *o,p'*-DDT which were detected in more than 50% of the population sample.

CHAPTER THREE

RESULTS

According to the PhD work plan, all the results achieved have been discussed in conferences and congresses and have been submitted to Scientific Journals. Therefore, proceedings and presentations are published or have been accepted for publication (see the list of articles in page xii).

The two study populations are mostly composed of different subjects, although they were recruited in the same places. Only 122 subjects were common to both studies: 21 in Novafeltria, 59 in Pavia and 42 in Milan. All main characteristics of the individual subjects are collected in **Annex IV–VI**.

3.1 Organochlorinated pesticides study

3.1.1 Study population

The main characteristics of the study subjects are indicated in **Table 3.1**. Our study population was composed of 65 (47%) females and 71 (52%) males. The distribution of gender in Pavia and Milan was skewed with female and male subjects under represented, respectively. The mean body weight of the study subjects was 69.85 kg and mean BMI of 24.01 kg/m^2 which are consistent with the reported mean weight and mean BMI of a representative general Italian adult population of 18 years old or above (69.4 kg and 24.5 kg/m^2, respectively) (Gallus *et al.*, 2006). On average our study population had individuals who were in the normal or healthy range in terms of BMI classification.

Table 3.1. Main characteristics of study population – OCPs study.

Characteristic	All Sample n = 137	Novafeltria n = 36	Pavia n = 59	Milan n = 42
Female, n (%)	65 (47)	17 (47)	17 (29)	31 (74)
Male, n (%)	71 (52)	19 (53)	42 (71)	10 (24)
Age (years)	39.0 (19–70)	44.5 (22–59)	40.0 (20–70)	33.0 (19–69)
Body weight (kg)	68.0 (45–131)	70.0 (45–100)	72.5 (45–131)	60.0 (45–90)
BMI (kg/m^2)	23.52 (17.58–38.5)	23.31 (17.58–32.3)	24.56 (18.29–38.5)	21.70 (18.70–31.96)

Note: Genders are expressed as valid percentages (%) and absolute number (n); age, body weight and BMI are expressed as medians (minima and maxima).

Note: Twenty one subjects from Novafeltria were among those who were recruited for PCB study and 15 were newly recruited subjects. All 59 subjects from Pavia and 42 from Milan participated in the PCBs study.

3.1.2 Serum concentrations of OCPs and of their metabolites

A summary of the levels of individual OCPs in the subjects classified by residence and gender is reported in **Table 3.2**. To understand the complex pattern of OCPs, the targeted analytes were shrunk to chemically and metabolically analogous groups, by summing together the DDT-like compounds (DDT, DDE and DDD) with *p,p'*- and *o,p'*-chlorine substitution (*p,p'*-DDX and *o,p'*-DDX, respectively). Total OCP was computed as the sum of levels of all OCPs analyzed from an individual subject whereas total DDT included the summation of DDT related compounds (all isomers of DDT+DDE+DDD). This data reduction approach leads to six OCP groups (**Table 3.3**).

The most persistent isomer of HCH (β-HCH) was detected in 74 samples (54%) with median concentration of 123 pmol/g lipid (28–1023 pmol/g lipid). HCB and *p,p'*-DDE were the most frequently detected OCPs in the overall study population (99% and 97%, respectively) with median concentrations of 154 and 395 pmol/g lipid, respectively. *p,p'*-DDT and *o,p'*-DDE were the least detected and thus they were excluded from the analysis. The median levels of total OCP, total DDT, total *p,p'*-DDX and *o,p'*-DDX were 1756, 1229, 514 and 173 pmol/g lipid, respectively in the overall sample population (**Table 3.4**).

Table 3.2. Median, minimum, maximum and significance values of OCPs (in pmol/g serum lipid) between females and males subjects from 3 Italian population subgroups.

Analyte	Novafeltria						p value	Pavia						p value	Milan						p value
	Females (n=17)			Males (n=19)				Females (n=17)			Males (n=42)				Females (n=31)			Males (n=10)			
	Min.	**Med.**	Max.	Min.	**Med.**	Max.		Min.	**Med.**	Max.	Min.	**Med.**	Max.		Min.	**Med.**	Max.	Min.	**Med.**	Max.	
β-HCH	47	**65**	815	28	**59**	756	0.173	118	**327**	1023	109	**303**	860	0.987	39	**52**	84	40	**50**	75	0.484
HCB	7	**220**	2014	12	**189**	970	0.428	64	**86**	182	62	**100**	406	0.569	17	**627**	1215	323	**472**	834	0.029
p,p'-DDE	20	**538**	1348	33	**339**	1556	0.987	16	**143**	789	71	**173**	1854	0.770	15	**5363**	17531	1233	**3398**	8339	0.101
p,p'-DDD	11	**49**	1231	7	**31**	920	0.124	26	**116**	401	12	**86**	564	0.379	12	**16**	732	12	**16**	977	0.963
o,p'-DDT	16	**78**	1826	6	**118**	3378	0.899	12	**330**	1854	9	**336**	1343	0.913	11	**15**	1747	11	**14**	2495	0.939
o,p'-DDD	12	**26**	382	5	**21**	348	0.366	16	**81**	155	12	**71**	349	0.503	12	**16**	25	12	**15**	23	0.509
∑OCPs	216	**1371**	4534	525	**1378**	4865	0.862	689	**1233**	3050	502	**1352**	4726	0.907	126	**6155**	19363	1734	**4604**	9314	0.182
∑DDT	76	**876**	2834	331	**1144**	3956	0.887	161	**693**	2505	149	**769**	3530	0.960	59	**5495**	18103	1277	**4076**	8405	0.236

Note: Significant difference in distribution of HCB was observed between males and females in only Milan (p = 0.029, Females > Males). In Novafeltria and Pavia there was no evidence of significant difference in distribution of all analyzed OCPs between males and females.

Table 3.3. Main Results of the measurement of OCPs in the examined subjects (in pmol/g serum lipids).

Analyte	All Sample (n = 137)			Novafeltria (n = 36)			Pavia (n = 59)			Milan (n = 42)		
	Min	**Med**	Max.	Min.	**Med**	Max	Min	**Med**	Max.	Min.	**Med**	Max.
∑OCP	126	**1756**	19363	216	**1375**	4865	502	**1285**	4726	126	**5286**	19363
HCB	7	**154**	2014	7	**209**	2014	62	**100**	406	17	**571**	1215
β-HCH	28	**122**	1023	28	**65**	815	109	**323**	1023	39	**51**	84
∑DDT	59	**1229**	18103	76	**1055**	3956	149	**767**	3530	59	**4651**	18103
∑*p,p'*-DDX	30	**514**	18077	39	**576**	2248	86	**312**	2418	30	**4396**	18077
∑*o,p'*-DDX	23	**173**	3561	25	**157**	3561	28	**407**	2003	23	**30**	2512

Note: One subject from Milan was excluded from the analysis due to lack of most data. *Keys*: *p,p'*-DDX , ∑*p,p'*-DDE, *p,p'*-DDD; *o,p'*-DDX, ∑*o,p'*-DDD, *o,p'*-DDT; ∑DDT, (*p,p'*-DDX + *o,p'*-DDX); ∑OCP, (β-HCH + HCB + ∑DDT).

Table 3.4. Median, minimum and maximum concentrations (in pmol/g serum lipid) of OCPs/metabolites in selected 3 Italian population subgroups.

Analyte	All Subjects (n = 137)			Novafeltria (n = 36)			Pavia (n = 59)			Milan (n = 41)			p-Value
	Min.	**Med.**	Max.	Min.	**Med.**	Max.	Min.	Med.	Max.	Min.	Med.	Max.	
β-HCH	28	**123**	1023	28	**65**	815	109	323	1023	39	**51**	84	0.000
HCB	7	**154**	2014	7	**209**	2014	62	100	406	17	**571**	1215	0.000
p,p'-DDE	15	**395**	17531	20	**428**	1556	16	163	1854	15	**4380**	17531	0.000
o,p'-DDD	5	**22**	382	5	**22**	382	12	79	349	12	**16**	25	0.000
p,p'-DDD	7	**45**	1231	7	**35**	1231	12	110	564	12	**16**	977	0.001
o,p'-DDT	6	**109**	3378	6	**100**	3378	9	336	1854	11	**14**	2495	0.000
∑*p,p'*-DDX	30	**515**	18077	39	**576**	2248	86	312	2418	30	**4396**	18077	0.000
∑*o,p'*-DDX	23	**173**	3561	25	**157**	3561	28	407	2003	23	**30**	2512	0.000
∑DDT	59	**12305**	18103	76	**1055**	3956	149	767	3530	59	**4651**	18103	0.000
∑OCP	126	**1756**	19363	216	**1375**	4865	502	1285	4726	126	**5286**	19363	0.000

Note: One subject from Milan was excluded from this analysis due to lack of some important information.

3.1.3 Distribution of OCPs by place of residence

Novafeltria

The median total OCP and total DDT in Novafeltria (36 subjects) were 1375 and 1055 pmol/g lipid, respectively. HCB with a median concentration of 209 pmol/g lipid was found in all analyzed samples while β-HCH was detected in 11% of the samples. The main metabolite of DDT (*p,p'*-DDE) was the most abundant OCP in Novafeltria detected in 35 samples (median: 428 pmol/g lipid). *o,p'*-DDT and *o,p'*-DDD were each detected in 28 samples equivalent to 34% of 82 analyzed samples and 37% of 75 analyzed samples, respectively. *p,p'*-DDD was detected in 29 samples accounting for 29% of the analyzed samples (n = 84) in the overall sample population.

Pavia

The median total OCP and total DDT in Pavia (59 subjects) was 1285 and 767 pmol/g lipid, respectively. HCB and β-HCH were detected in all 59 samples with median concentration of 100 and 323 pmol/g lipid, respectively. This account for 44% (n = 135) and 80% (n = 74) of the overall samples with detectable levels of HCB and β-HCH, respectively. *p,p'*-DDE was detected in 58 accounting for 44% of subjects with detectable levels in the overall population (n = 133, median: 163 pmol/g lipid). *o,p'*-DDT, *o,p'*-DDD, *p,p'*-DDD and *o,p'*-DDE were detected in 62% (51/82), 63% (47/75), 55% (46/84) and 27% (3/11) of the samples with detectable levels of these OCPs, respectively.

Milan

The median total OCP and total DDT of 41 subjects (98%) in Milan was 5286 and 4651 pmol/g lipid, respectively. β-HCH, *o,p'*-DDE and *o,p'*-DDD were under detection limit in all samples whereas *p,p'*-DDT was detected in only one sample with a concentration of 395 pmol/g lipid. As in other sites, HCB and *p,p'*-DDE were the most frequent detected in 40 samples accounting for 30% of the samples analyzed in the overall population sample (n = 135, n = 133). *o,p'*-DDT, *p,p'*-DDD and *p,p'*-DDT were the least prevalent in this population subgroup.

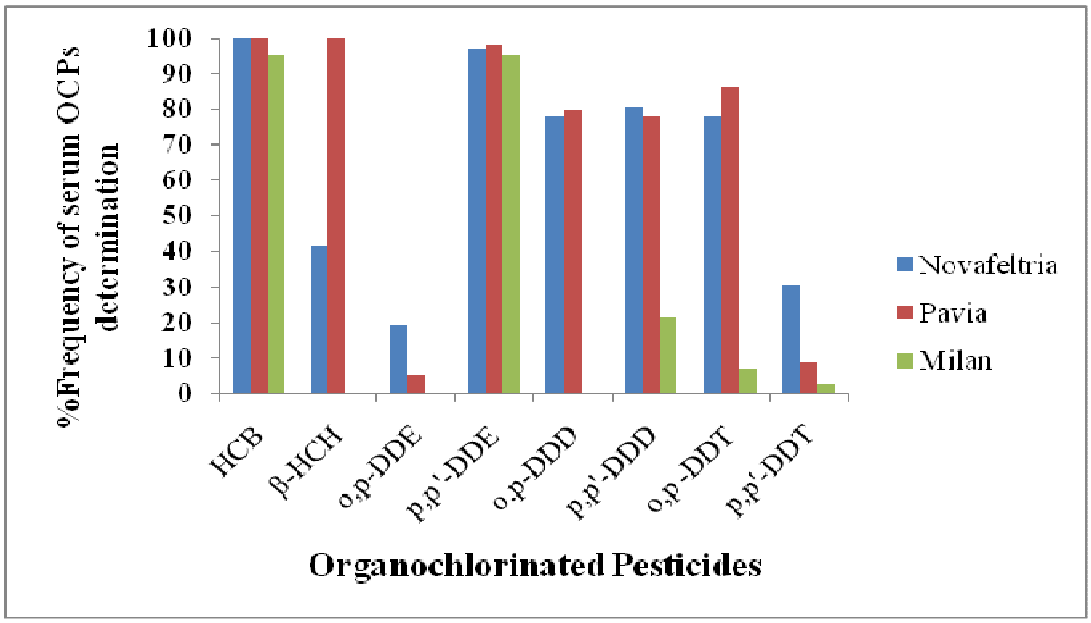


Figure 3.1. Percentage frequency of detection of individual OCP/metabolite in the three Italian population subgroups.

Comparison among places of residence

A trend of the median level of total OCP, total DDT, *p,p'*-DDE and HCB was observed among the residents in the investigated sites **(Figure 3.2**). Pavia showed the lowest median level for these OCPs while Milan the highest level. These levels were significant different across these sites (Milan > Novafeltria > Pavia, $p < 0.0001$). The highest median levels of total *o,p'*-DDX, β-HCH, *o,p'*-DDD, *p,p'*-DDD and *o,p'*-DDT were observed in Pavia and the lowest in Milan. The distribution of these OCPs differed significantly across the sites (Pavia > Novafeltria > Milan, $p < 0.001$**)**. Whereas β-HCH was the most frequently detected in Pavia and Novafeltria group, it was under detection limit in all Milan samples.

3.1.4 Distribution of OCPs by gender

In overall sample, females had significantly higher median total OCP than males (3050 and 1489 pmol/g lipids, respectively; $p < 0.0001$). Similar observation was seen for total DDT (medians: 2417 and 995 pmol/g lipid for females and males, respectively; $p = 0.001$).

The fungicide HCB was detected in 64 female samples (99%) while *p,p'*-DDE in 62 samples (95%) with median levels of 482 and 789 pmol/g lipid, respectively. These OCPs were detected in all 71 male subjects with median levels of 107 and 268 pmol/g lipids, respectively. The level of HCB and *p,p'*-DDE differed significantly between

females and males ($p < 0.0001$). Out of 65 female samples analyzed, *p,p′*-DDD was detected in 40 samples (62%), *o,p′*-DDD and *o,p′*-DDT were each detected in 31 samples (48%). *o,p′*-DDE and *p,p′*-DDT were the least abundant among female subjects. Gender distributions of total OCP, total DDT, *p,p′*-DDE, *p,p′*-DDD, HCB and β-HCH are illustrated in **Figure 3.3**.

Based on individual study site, β-HCH was detected in 15 subjects of Novafeltria 8 of them were females (53%) with median concentration of 65 pmol/g lipid. HCB was detected in all 17 women (median: 220 pmol/g lipid) whereas *p,p′*-DDE, *p,p′*-DDD and *o,p′*-DDT were found in 16, 14 and 13 subjects respectively, with median concentrations of 538, 49 and 78 pmol/g lipid, respectively.

Among male subjects of Novafetria 7 subjects (47%) had detectable concentrations of serum β–HCH, with a median of 59 pmol/g lipid. HCB and *p,p′*-DDE found in all 19 subjects with a median concentration of 189 and 339 pmol/g lipid, respectively. *p,p′*-DDD, *o,p′*-DDT and *o,p′*-DDD were detected in 15 subjects with median concentration of 31, 118 and pmol/g lipid, respectively. There was no evidence of significant difference in levels of all analyzed OCPs between males and females (**Table 3.2**).

In Pavia, β-HCH and HCB were detected in all 17 females (29%) with median concentrations of 327 and 86 pmol/g lipid, respectively. *p,p′*-DDE and *p,p′*-DDD were each detected in 16 subjects with median concentrations of 143 and 116 pmol/g lipid, respectively. *o,p′*-DDD and *o,p′*-DDT were detected in 14 and 15 subjects, respectively with a median concentration of 81 and 330 pmol/g lipid. In males group, β-HCH, HCB and *p,p′*-DDE were detected in all 42 subjects. The median concentrations of these OCPs were 303, 100 and 173 pmol/g lipid, respectively. *p,p′*-DDD, *o,p′*-DDT and *o,p′*-DDD detected in 30 (66%), 37 (73%) and 32 (68%) subjects, respectively with median concentrations of 86, 337 and 72 pmol/g lipid. As for Novafeltria, no evidence to account for the significant difference between males and females for all analyzed OCPs (**Table 3.2**).

In Milan HCB and *p,p′*-DDE were detected in 40 subjects of these 10 were male subjects (25%). Their median concentrations were 472 and 3397 pmol/g lipid, respectively. *o,p′*-DDT (median 14 pmol/g lipid) and *p,p′*-DDD (median 16 pmol/g lipid) were each detected in only two samples. Among female subjects of Milan, HCB and *p,p′*-DDE had median concentrations of 627 and 5363 pmol/g lipid, respectively. Contrary to Novafeltria and Pavia, significant difference in the distribution of HCB was

observed between males and females from Milan (p = 0.029), females having higher median level than males (**Table 3.2)**.

3.1.5 Distribution of OCPs by Subjects' Age

To evaluate the effect of age, subjects' ages were categorized into four 15-year groups (10–25, 26–40, 41–55 and 56–70). The analyzed OCPs were compared for the total content and the distribution across the age groups for the overall population and for the individual sites. No subject was younger than 19 years, while the majority of subjects aged between 26–40 and 41–55 (*approx.* 41 and 34%, respectively) and 14 were in the 56–70 years age. Compared to other sites Milan had younger subjects (**Table 3.5**).

The median concentration of total OCPs in the overall population sample were found to be 3060, 1678, 1536 and 1636 pmol/g lipid across these age groups, respectively. In Novafeltria the distribution of total OCPs (p = 0.015) and total DDT (p = 0.039) differed significantly across these age groups. However the significance value for total *p,p'*–DDX was very close but within the level of statistical significance (p = 0.051). The median concentrations along the respective age groups were 539, 1112, 1618 and 1782 pmol/g lipid (for total OCPs); 251, 375, 706 and 1091 pmol/g (for total *p,p'*-DDX) and 331, 781, 1458 and 1444 pmol/g lipid (for total DDT). The distribution of these OCP groups monotonically increased with subject age (r^2 = 0.181, p = 0.01; r^2 = 0.138, p = 0.026 and r^2 = 0.156, p = 0.017; for total OCP, total *p,p'*-DDX and total DDT, respectively). In Milan the distribution of these OCP groups were not statistically significant different across the age groups although their median levels increase consistently across age (5115, 5337, 5494 and 19363 pmol/g lipid for total OCP; 4428, 4693, 4999 and 18103 pmol/g lipid for total DDT; 4297, 4512, 4973 and 18077 pmol/g lipid for total *p,p'*-DDX).

As for individual analytes, the concentration of only β-HCH presented a statistically significant increasing trend across these age groups for only Pavia population subgroup (p = 0.027). The median concentrations of this compound for the four age groups 10–25, 26–40, 41–55 and 56–70 were 305, 401, 249 and 467 pmol/g lipid respectively. However correlation analysis did not reveal any significant correlation with age (r = 0.021, p = 0.874). No evidence for significant difference in distribution for other detected OCPs across these age groups as illustrated in **Figure 3.4**.

Table 3.5. Absolute frequency of study subjects in each age category for all subjects and for population of Novafeltria (NF), Pavia (PV) and Milan (MI).

Age Class	Description	All	NF	PV	MI
10–25	Born after 1995, never experienced direct exposure to DDT	20	3	5	12
26–40	Born 1970–1985; DDT ban in 1978; direct exposure to DDT unlikely	56	10	26	20
41–55	Born 1955–1960; may have experienced DDT use while in the child and youth age	46	21	17	8
56–70	Born 1940–1954; may have experienced DDT use while in the child and youth age	14	2	11	1
Total		136	36	59	41

3.1.6 Distribution of OCPs by BMI

The minimum and maximum BMI in our study population were 17.58 and 38.53 kg/m^2, respectively. The four main BMI categories and a total of 11 sub-classes as per international classification (http://www.indexmundi.com/italy/sex_ratio.html) reflect the status of the body fat in population studies, ranging from those underweight (BMI < 18.50 kg/m^2) to those severely obese (BMI ≥ 40 kg/m^2). Based on this classification our study group had 4 (3%) underweight individuals, 89 (65%) normal and 43 (32%) overweight (**Table 3.6**). None of the subjects fell into the severely underweight or severely obese.

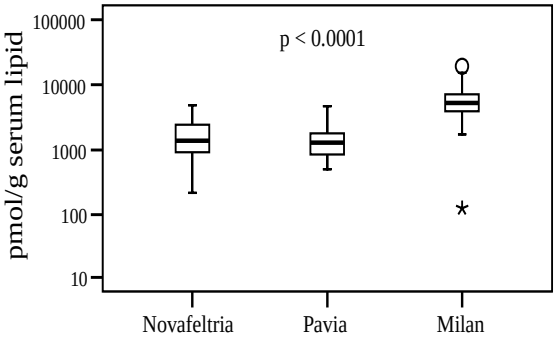
Table 3.6. Distribution of the examined subjects according to the International Classification of adult underweight, overweight and obesity measured by BMI.

Classification	**BMI (kg/m^2)**		**All Subjects**	**Novafeltria Subjects**	**Pavia Subjects**	**Milan Subjects**
	Principal cut-off points	**Additional cut-off points**	23.52 (17.58–38.5)	23.31 (17.58–32.3)	24.56 (18.29–38.5)	21.70 (18.70–31.96)
Underweight	<18.50	<18.50	4	3	1	–
Normal range	18.50–24.99	18.50–22.99	55	14	15	26
		23.00–24.99	34	8	20	6
Overweight	≥25.00	≥25.00	43	11	23	9
Total			136	36	59	41

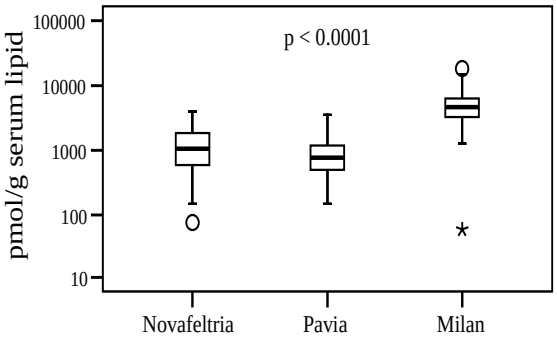
Figure 3.5 illustrates the distribution of these OCPs across the main BMI categories. Statistical test was performed to assess the influence of BMI in individual OCP level and total OCPs levels in both entire data set and individual population subgroups.

Considering the entire data set, significant difference was observed in distribution of total OCP and total DDT across the 4 categories namely <18.50, 18.50–22.99, 23.00–24.99 and ≥25.00 kg/m^2 (p = 0.018 and 0.023, respectively). The median levels of total OCP in these categories were 1097, 3050, 1359, 1855 pmol/g lipid (for total OCP) and 696, 2497, 963 and 1307 pmol/g lipid (for total DDT). However when analyses were restricted to individual sites, the significance value of *p,p'*-DDE fell exactly on the limit of significance level (p = 0.050) for Pavia group. Otherwise there was no evidence of significant difference in distribution of all other individual OCPs and total OCP across the BMI categories in all investigated population subgroups.

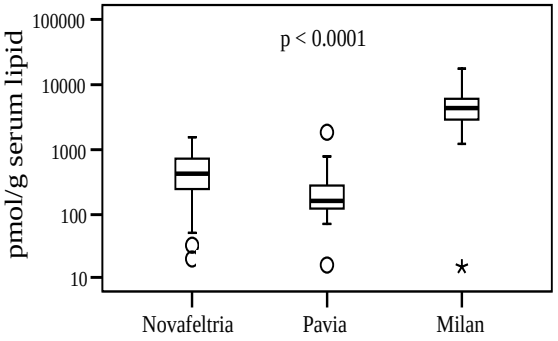
Total OCP



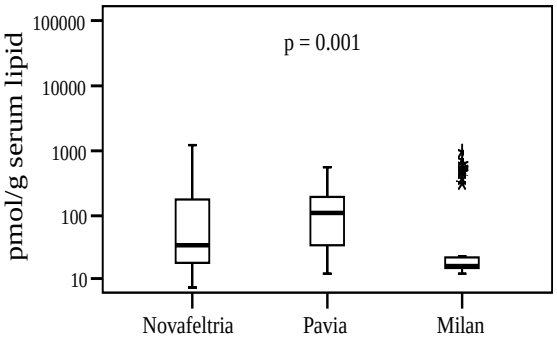
Total DDT



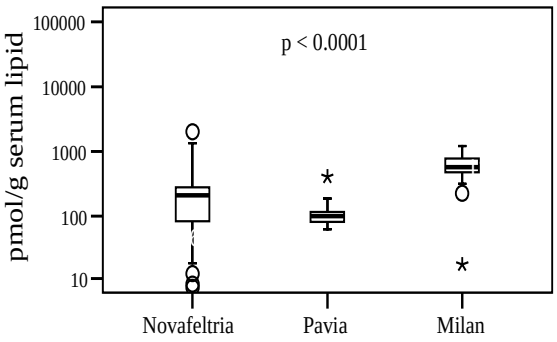
p,p'- DDE



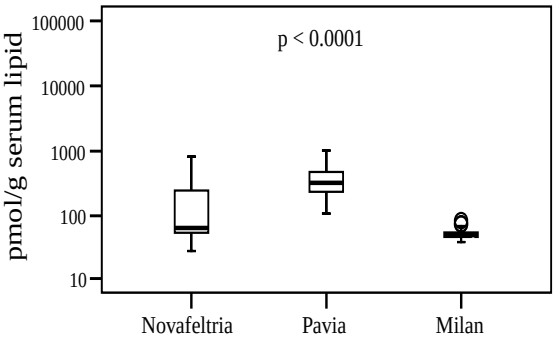
p,p'- DDD



HCB



β - HCH



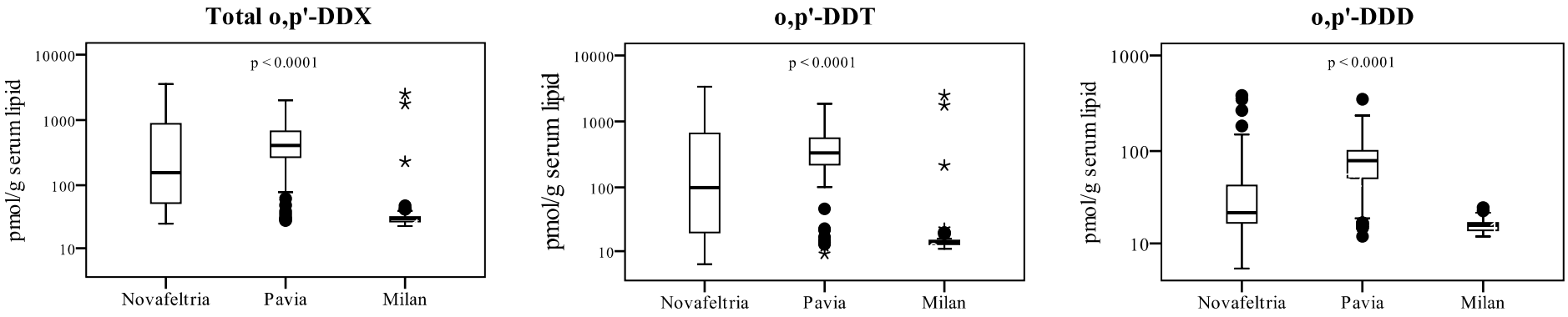


Figure 3.2. Distribution of OCPs concentration expressed in pmol/g serum lipid (logarithmic scale) among the study population stratified by geographical locations. Displayed results are for total OCP, total DDT and some individual OCPs with significantly different distribution across the investigated study sites. *Keys*: O, mild outliers; *, extreme outliers.

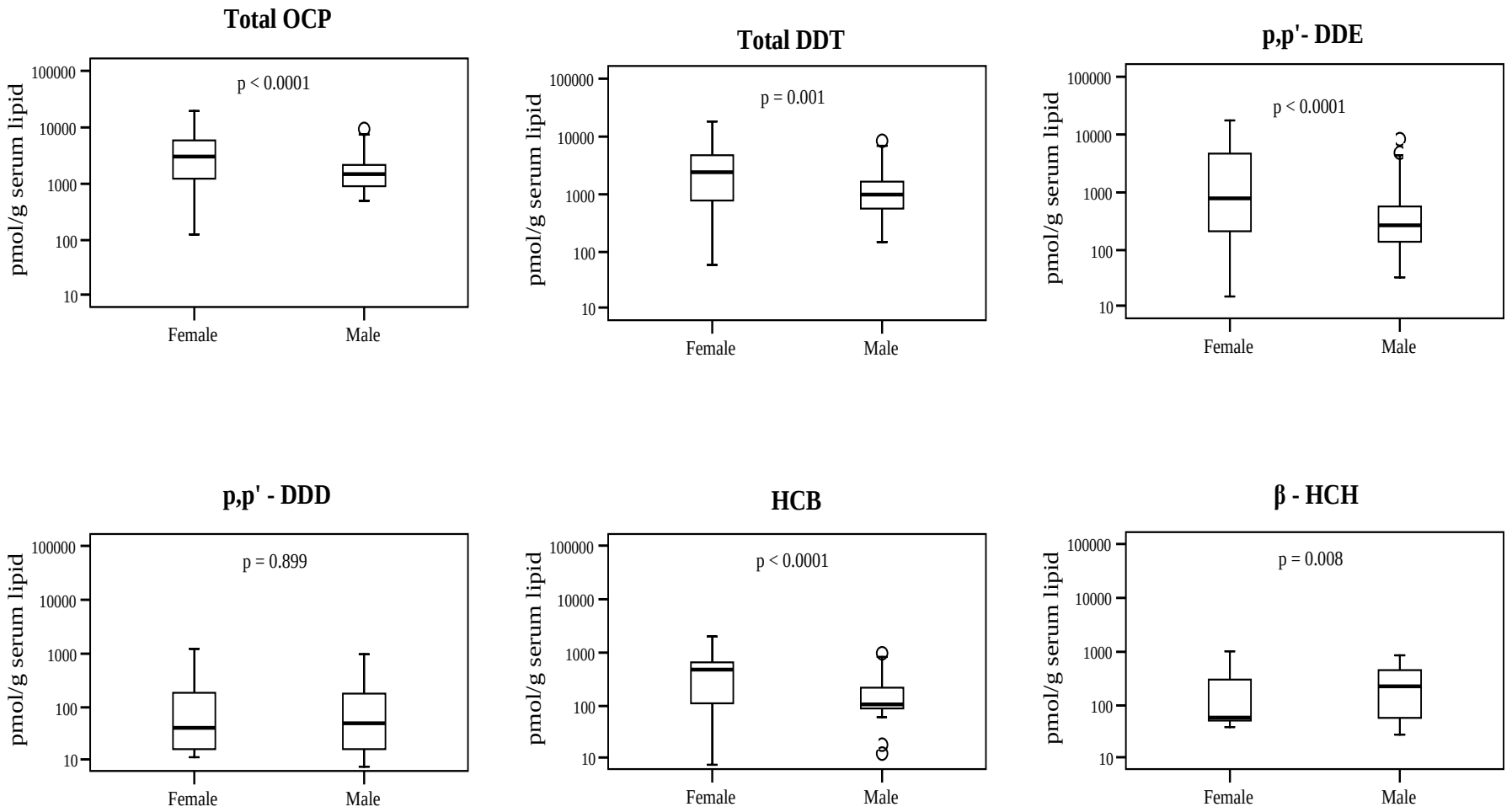


Figure 3.3. Distribution of OCPs concentration expressed in pmol/g serum lipid (logarithmic scale) among the study population stratified by gender. Displayed results are for total OCP, total DDT, *p,p'*-DDE, HCB and β-HCH with significantly different concentrations between males and females (except *p,p'*-DDD).

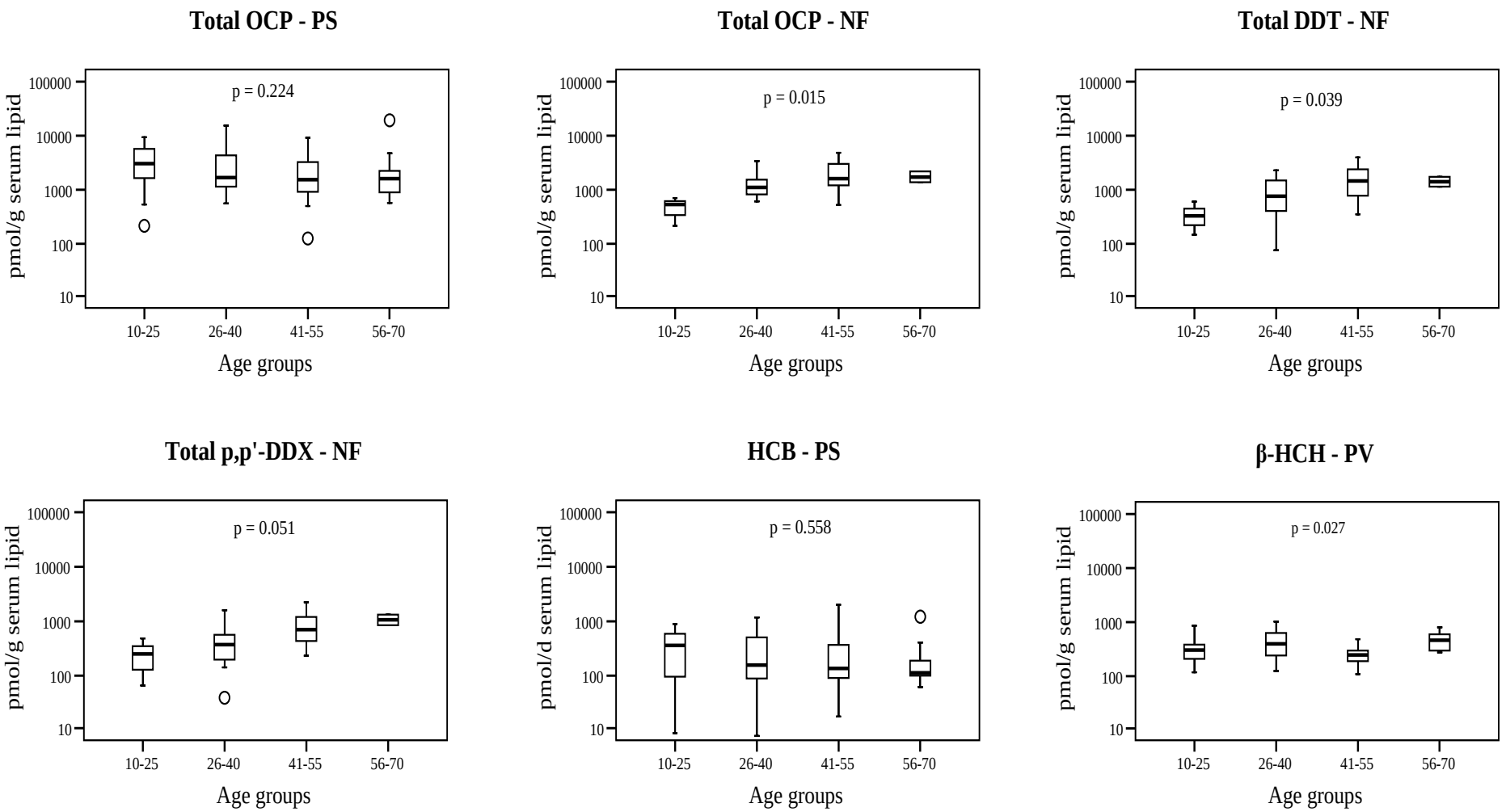


Figure 3.4. Distribution of OCPs expressed in pmol/g serum lipid (logarithmic scale) stratified by age groups. Displayed results are for total OCP, total DDT (overall population sample and Novafeltria samples), HCB (overall population sample), total *p,p'*-DDX (Novafeltria) and β-HCH (Pavia). *Keys*: PS, overall population sample; NF, Novafeltria; PV, Pavia.

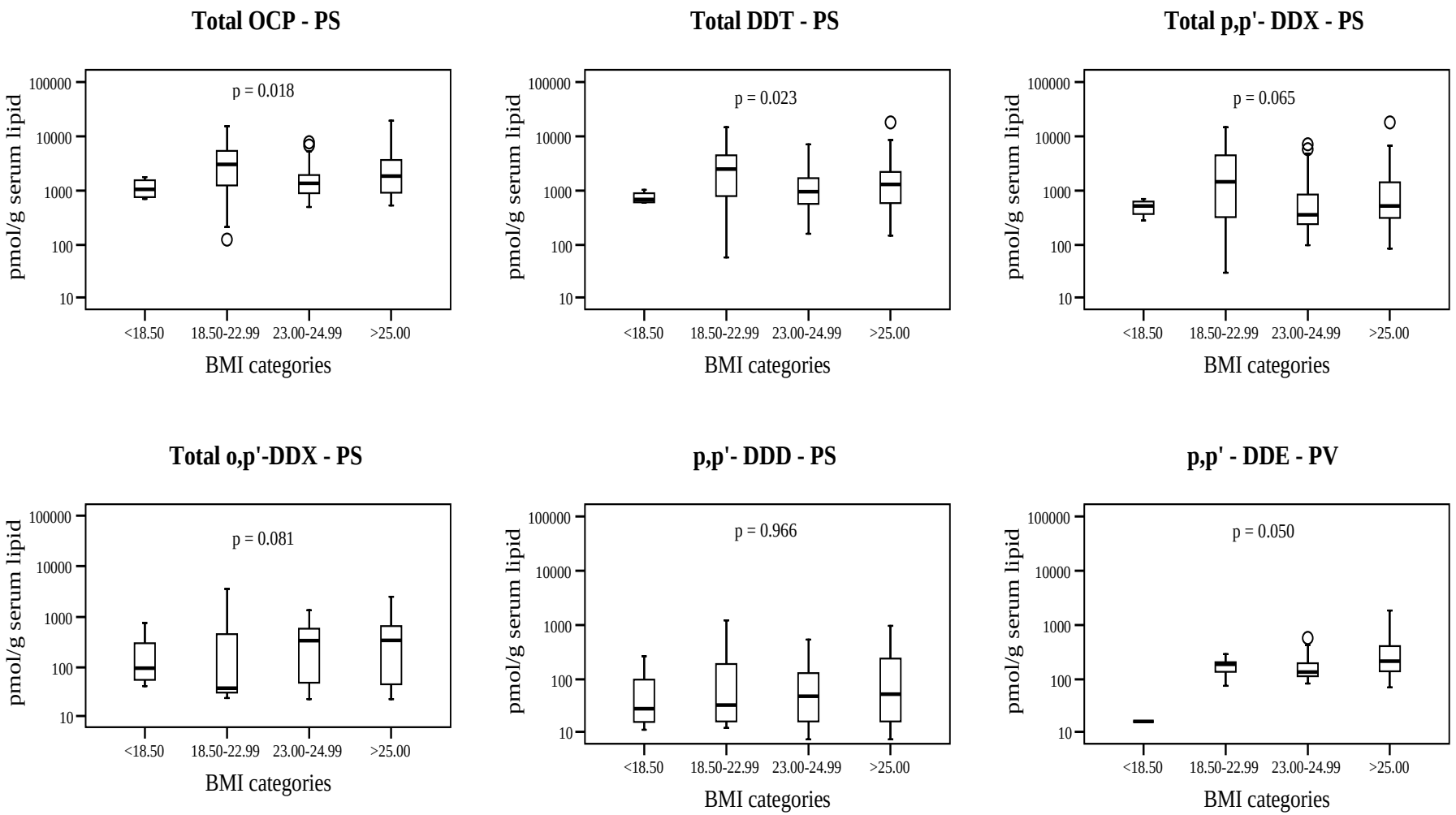


Figure 3.5. Distribution of total OCP, total *p,p'*-DDT (overall population sample) and *p,p'*-DDE (Pavia) where significant difference was evident across BMI categories except for total *o,p'*-DDX, total *p,p'*-DDX and *p,p'*-DDD (overall population sample). The concentrations were expressed in pmol/g serum lipid in logarithmic scale. *Keys*: PS, overall population sample; PV, Pavia.

3.2 Polychlorinated biphenyls study

3.2.1 Study population

The study population is mostly composed of different subjects from that of the OCP study as state earlier. **Table 3.7** displays the main characteristics of the study subjects. Only in Milan the distribution of gender was skewed with females under represented. The mean body weight of the subjects (69.94 kg) and mean BMI (24.36 kg/m^2) are in accordance with those reported by Gallus *et al*. (2006) for a general Italian adult population (69.4 kg and 24.5 kg/m^2, respectively). Our population had individuals who were fairly normal in terms of BMI. In total 372 subjects participated in the study, five subjects who had incomplete information were excluded from the analysis. Thus the final statistical analyses were based on 367 subjects (164 from Pavia, 162 from Novafeltria and 41 from Milan).

Table 3.7. Main characteristics of population study subjects – PCB study.

Characteristic	All Subjects n = 367	Novafeltria n = 162	Pavia n = 164	Milan n = 41
Males, n (%)	182 (50.3)	81 (49.7)	70 (42.2)	31 (75.6)
Females, n (%)	185 (49.5)	82 (50.3)	94 (57.3)	10 (24.4)
Age (years)	40.0 (19–70)	42.5 (19–61)	40.0 (19–70)	33.0 (19–69)
Body weight (kg)	69.0 (42–131)	69.0 (43–110)	70.0 (42–131)	60.0 (45–90)
BMI (kg/m^2)	24.1(15.6–38.5)	24.43(15.6–38.1)	24.32(17.6–38.5)	21.70(18.7–32.0)

Note: Genders are expressed as valid percentages (%) and absolute number (n); Body weight and age are expressed as medians (minima and maxima).

3.2.2 Serum concentrations of PCB congeners

Table 3.8 illustrates the frequency determination of serum PCBs among the study subjects. For better understanding of complex pattern of PCB congeners occurring in samples, PCB congeners were classified in five classes according to the increasing number of chlorine atoms in their structures i.e. tri–, tetra–, penta–, hexa– and hepta–chlorobiphenyls (CBs) and according to toxicological characteristics, according to their insertion into the dioxin–like (DL–PCBs) or non dioxin-like PCBs (NDL–PCBs). Thus, levels of PCBs were computed from the individual results of sample analysis as follows:

- total PCB as sum of 15 PCB congeners (**Σ**28, 31, 52, 77, 101, 105, 118, 126, 138, 153, 156, 167, 169, 180);
- total tri–CBs as the sum of PCBs 28 and 31; total tetra–CBs as the sum of PCBs 52 and 77; total penta–CBs as the sum of 4 PCBs (**Σ**101, 105, 118, 126), total hexa-

CBs as the sum of 5 PCBs (**Σ**128, 138, 153, 156, 169) and total hepta–CBs as the sum of PCB 170 and 180;

- total DL–PCBs as the sum of 6 DL–PCBs (**Σ**77, 105, 118, 126, 156, 169); total NDL–PCB as the sum of 9 NDL–PCBs (**Σ**28, 31, 52, 101, 118, 138, 153, 167, 180).

To account for the undetected congeners, censored data corresponding to half LoDs for each congener were included in the summations (**Table 3.9**).

Table 3.8. Absolute and percentage frequency of PCB congeners as measured in the serum of subjects in the general population of the three Italian population subgroups.

PCB	N°Cl	Absolute Frequency				Percentage Frequency			
		All Samples	Novafeltria	Pavia	Milan	All Samples	Novafeltria	Pavia	Milan
		n = 367	n = 162	n = 164	n = 41	n = 367	n = 162	n = 164	n = 41
Marker PCBs									
28	*3*	151	81	70	***ND***	41.0	50	42.7	***ND***
52	*4*	170	10	150	10	46.2	6.2	91.5	23.8
101	*5*	4	4	***ND***	***ND***	1.1	2.5	***ND***	***ND***
118	*5*	182	146	36	***ND***	49.5	90.1	22	***ND***
138	*6*	366	162	164	40	99.5	100	100	95.2
153	*6*	366	162	164	40	99.5	100	100	95.2
180	*7*	362	162	164	36	98.4	100	100	85.7
DL–PCBs (non-ortho)									
77	*4*	5	3	2	***ND***	1.4	1.9	1.2	***ND***
81	*4*	0	***ND***	*	*	***ND***	***ND***	*	*
126	*5*	2	2	***ND***	***ND***	0.5	1.2	***ND***	***ND***
169	*6*	6	4	2	***ND***	1.6	2.5	1.2	***ND***
DL–PCBs (mono-ortho)									
105	*5*	13	10	3	***ND***	3.5	6.2	1.8	***ND***
118	*5*	182	146	36	***ND***	49.5	90.1	22.0	***ND***
156	*6*	276	138	138	***ND***	75.5	85.2	84.1	***ND***
157	*6*	1	***ND***	1	*	0.3	***ND***	0.6	*
167	*6*	75	17	58	*	20.4	10.5	35.4	*
189	*7*	2	2	*	*	0.5	1.2	*	*
NDL–PCBs									
28	*3*	151	81	70	***ND***	41.0	50.0	42.7	***ND***
31	*3*	139	64	75	***ND***	37.8	39.5	45.7	***ND***
44	*4*	65	***ND***	65	*	17.7	***ND***	39.6	*
47	*4*	292	156	136	*	79.3	96.3	82.9	*
49	*4*	66	***ND***	66	*	17.9	***ND***	40.2	*
52	*4*	170	10	150	10	46.2	6.2	91.5	23.8
61	*4*	29	25	4	*	7.9	15.4	2.4	*
66	*4*	128	26	102	*	34.8	16.0	62.2	*
70	*4*	136	19	117	*	37.0	11.7	71.3	*
74	*4*	307	151	156	*	83.4	93.2	95.1	*
99	*5*	105	102	3	*	28.5	63.0	1.8	*
101	*5*	4	4	***ND***	***ND***	1.1	2.5	***ND***	***ND***
128	*6*	6	3	***ND***	3	1.6	1.9	***ND***	7.1
138	*6*	366	162	164	40	99.5	100	100	95.2
146	*6*	190	73	117	*	51.6	45.1	71.3	*
149	*6*	152	***ND***	152	*	41.3	***ND***	92.7	*
153	*6*	366	162	164	40	99.5	100	100	95.2
170	*7*	332	160	164	8	90.2	98.8	100	19
171	*7*	94	34	60	*	25.5	21.0	36.6	*
172	*7*	94	57	37	*	25.5	35.2	22.6	*
177	*7*	172	86	86	*	46.7	53.1	52.4	*
180	*7*	362	162	164	36	98.4	100	100	85.7
183	*7*	231	134	97	*	62.8	82.7	59.1	*
187	*7*	299	159	140	*	81.3	98.1	85.4	*
190	*7*	115	73	42	*	31.3	45.1	25.6	*
193	*7*	136	84	52	*	37.0	51.9	31.7	*

Keys: N°Cl, number of chlorine atoms; *, the PCB congener not investigated; *ND*, not detected; DL–PCB, dioxin like polychlorinated biphenyls; NDL–PCBs, non dioxin-like polychlorinated biphenyls. *Note*: Thirty six, 34 and 15 individual PCB congeners were investigated in Novafeltria, Pavia and Milan, respectively.

Table 3.9. Results of PCB congeners measurements (in pmol/g serum lipids) in 3 Italian population subgroups.

PCB group	All subjects n = 367			Novafeltria n = 162			Pavia n = 164			Milan n = 41		
	Min.	**Med.**	Max.	Min.	**Med.**	Max.	Min.	**Med.**	Max.	Min.	**Med.**	Max.
∑PCB	199	**1131**	6556	199	**857**	3236	340	**1355**	5416	310	**2062**	6556
∑DL–PCB	9	**96**	1127	14	**110**	858	9	**51**	1127	128	**167**	273
∑NDL–PCB	162	**988**	6221	162	**742**	2454	310	**1278**	4602	814	**1781**	6221
∑Tri–CBs	3	**13**	594	3	**9**	142	4	**10**	594	30	**39**	63
∑Tetra–CBs	3	**24**	884	3	**7**	156	5	**48**	414	52	**72**	884
∑Penta–CBs	2	**61**	1060	9	**79**	763	2	**13**	1060	58	**76**	125
∑Hexa–CBs	107	**615**	4401	112	**494**	1591	155	**721**	2997	107	**1280**	4401
∑Hepta–CBs	21	**303**	1855	38	**261**	1234	64	**347**	1628	21	**423**	1855

3.2.3 Distribution of PCB congeners by places of residence

Novafeltria

The median total PCB in Novafeltria was 857 pmol/g lipid whereas total DL– and NDL–PCB were 110 and 742 pmol/g lipid, respectively. Total hexa–CBs presented the highest median concentration of 493 pmol/g lipid followed by total hepta–CBs (261 pmol/g lipid) (**Table 3.9**). PCB 138, 153, 180, 170, 156 and 118 were detected in between 85–100% of the samples with median levels of 174, 265, 189, 77, 30 and 70 pmol/g lipid, respectively. PCB 77, 126 and 128 were detected in <2% of the samples (**Table 3.8** and **3.10**).

Pavia

The median total PCB, DL– and NDL–PCBs were 1355, 51 and 1278 pmol/g lipid, respectively. Total hexa– and hepta–CBs presented the highest median levels of 721 and 347 pmol/g lipid, respectively (**Table 3.9**). PCB 52, 138, 153, 156, 170 and 180 (median: 44, 294, 394, 33, 88 and 259 pmol/g lipid, respectively) were detected in between 84–100% of the samples. PCB 77, 105 and 169 were detected in <2% of the samples whereas congener 101, 126 and 128 were under LoDs (**Table 3.8** and **3.10**).

Milan

The median total PCB was 2062 pmol/g lipid. All DL–PCBs were not detected in Milan. As for Novafeltria and Pavia, total hexa–CBs presented the highest median (1281 pmol/g lipid) followed by hepta–CBs (423 pmol/g lipid). Total tetra–CBs had the median of 72 pmol/g lipid (**Table 3.9**). Of 15 PCB congeners investigated, six were above the LoDs the most prevalent ones being PCB 138, 153 and 180 with median concentrations of 532, 668 and 403 pmol/g lipid, respectively. PCBs 52, 170 and 128 were the least abundant congeners (**Table 3.8** and **3.10**).

Comparison among places of residence

The distribution of total PCB, total DL–PCB, total tri–, tetra–, penta–, hexa– and hepta–CBs concentrations were significantly different across the sites ($p < 0.0001$). The median levels of these PCB groups follow the order Novafeltria < Pavia < Milan with exception of total DL–PCB and penta–CBs. Based on PCB homologues in overall population, the order is ∑hexa–CBs > ∑hepta–CBs > ∑penta–CBs > ∑tetra–CBs > ∑tri–CBs (**Table 3.11**).

The highly prevalent PCBs were 138, 153, 156, 170 and 180 in overall population and in individual sites with exception of 156 and 170 in Milan which were under LoDs. These congeners differed significantly across the sites ($p < 0.0001$; **Figure 3.6**). The median levels of PCB 138, 153 and 180 follows the order 138 < 180 < 153 for Novafeltria and Pavia with a slight change in Milan (180 < 138 < 153). PCB 153 had the highest median concentrations in each site.

PCB 52 was more prevalent in Pavia (92%). PCB 118 (Novafeltria) and PCB 156 (Novafeltria and Pavia) were detected in >50% samples whereas PCB 28 and 31 were detected in <50% of the samples in each site. PCB 126 was detected in only two samples both from Novafeltria (**Table 3.8**).

Table 3.10. Median, minimum and maximum concentrations of 15 PCB congeners measured in the sera of subjects of the general population recruited in 3 Italian population subgroups (expressed in pmol /g serum lipid).

PCBs	N°Cl	All subjects (n = 367)			Novafeltria (n = 162)			Pavia (n = 164)			Milan (n = 41)			p value
		Min.	**Med.**	Max.	Min.	**Med.**	Max.	Min.	**Med.**	Max.	Min.	**Med.**	Max.	
NLD–PCBs														
28	*3*	2	***nc***	353	2	***nc***	67	2	***nc***	353	15	***nc***	32	0.000
31	*3*	2	***nc***	336	2	***nc***	89	2	***nc***	336	15	***nc***	32	0.000
52	*4*	2	***nc***	830	2	***nc***	153	3	**44**	409	13	***nc***	830	0.000
101	*5*	0.47	***nc***	102	2	***nc***	102	0.47	***nc***	5	12	***nc***	25	0.000
128	*6*	0.42	***nc***	506	1	***nc***	53	0.42	***nc***	5	21	***nc***	506	0.000
138	*6*	13	**236**	1612	49	**174**	685	41	**294**	1223	13	**532**	1612	0.000
153	*6*	13	**342**	2205	27	**265**	884	77	**394**	1704	13	**668**	2205	0.000
170	*7*	3	**75**	1010	3	**772**	279	10	**88**	353	10	***nc***	1010	0.000
180	*7*	10	**223**	1616	35	**190**	955	47	**259**	1275	10	**403**	1616	0.000
DL–PCBs														
77	*4*	1	***nc***	84	2	***nc***	55	1	***nc***	50	39	***nc***	84	0.000
126	*5*	0.47	***nc***	115	2	***nc***	115	0.47	***nc***	5	23	***nc***	50	0.000
169	*6*	0.42	***nc***	328	1	***nc***	328	0.42	***nc***	46	21	***nc***	45	0.000
105	*5*	0.47	***nc***	210	2	***nc***	210	0.47	***nc***	206	12	***nc***	25	0.000
118	*5*	0.47	***nc***	845	2	**70**	547	0.47	***nc***	845	12	***nc***	25	0.000
156	*6*	2	**28**	143	2	**25**	96	2	**33**	143	21	***nc***	45	0.066

Keys: N˚Cl, number of chlorine atoms attached to biphenyl ring; nc, not computed since the congeners were detected in less than 50% of the samples in the respective group.

Table 3.11. Median, minimum and maximum concentrations of total PCBs in three Italian population subgroups (expressed in pmol /g serum lipid).

PCB group	All Subjects (n = 367)			Novafeltria (n = 162)			Pavia (n = 164)			Milan (n = 41)			p value
	Min.	**Med.**	Max.	Min.	**Med.**	Max.	Min.	**Med.**	Max.	Min.	**Med.**	Max.	
∑PCB	199	**1131**	6556	199	**857**	3236	340	**1355**	5416	3104	**2062**	6556	0.000
∑DL–PCBs	9	**96**	1127	14	**110**	857	9	**51**	1127	128	**167**	273	0.000
∑NDL–PCBs	147	**1004**	6317	176	**756**	2473	323	**1283**	4609	147	**1870**	6317	0.000
∑Tris–CBs	3	**13**	593	3	**9**	142	4	**10**	593	30	**39**	63	0.000
∑Tetra–CBs	3	**24**	884	3	**7**	156	5	**48**	414	52	**72**	884	0.000
∑Penta–CBs	2	**61**	1060	9	**79**	763	2	**13**	1060	58	**76**	125	0.000
∑Hexa–CBs	107	**615**	4401	112	**493**	1591	155	**721**	2997	107	**1281**	4401	0.000
∑Hepta–CBs	21	**303**	1855	38	**261**	1234	64	**347**	1628	21	**423**	1855	0.000

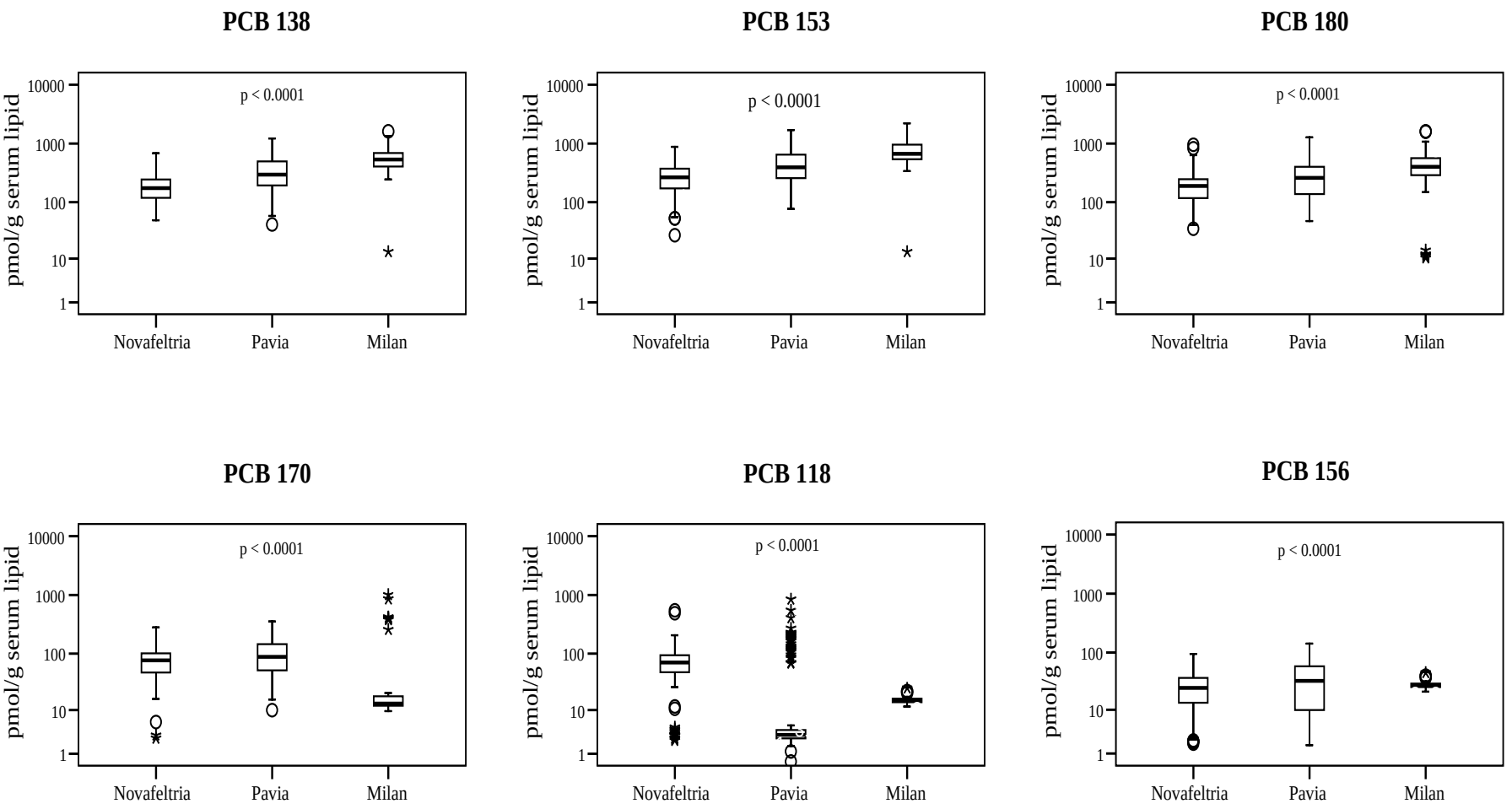


Figure 3.6. Distribution of serum PCB 138, 153, 180, 170, 118 and 156 in pmol/g lipid (logarithmic scale) among the study population stratified by residence. Displayed results had significant different concentrations between the areas of residence. *Keys*: O, mild outliers; *, extreme outliers.

3.2.4 Distribution of PCBs by gender

A total of 182 females and 185 males were investigated. The median total PCB levels were 1142 and 1051 pmol/g lipid, respectively. There was no evidence for the difference in distribution of total PCB levels between genders. However, total DL–PCB levels were significantly higher in females than in males (median 120 and 75 pmol/g lipid, respectively; $p < 0.0001$). Significant difference was also evident for total penta-CBs concentration (median 78 and 17 pmol/g lipid, respectively; $p < 0.0001$, **Figure 3.7**).

In overall population sample, PCB 118 was detected in 102 females (56%) with median concentration of 52 pmol/g lipid while in males it was detected in 80 samples (43%). The distribution of this congener differed significantly by gender ($p < 0.0001$). PCB 128 was least prevalent detected in 1.7 and 1.6% of females and males, respectively.

As observed in overall population, no evidence of variation of total PCB concentrations between genders in individual sites. However, the distribution of total DL–PCB and total penta–CBs differed significantly by gender in Novafeltria and Pavia. As for total tetra–CBs, the distribution differed significantly between genders in only Novafeltria ($p = 0.045$). No evidence of significant difference in distribution of most congeners between genders with exception of PCB 52 and 105 (in Novafeltria) and 118 (Novafeltria and Pavia) (**Table 3.12**).

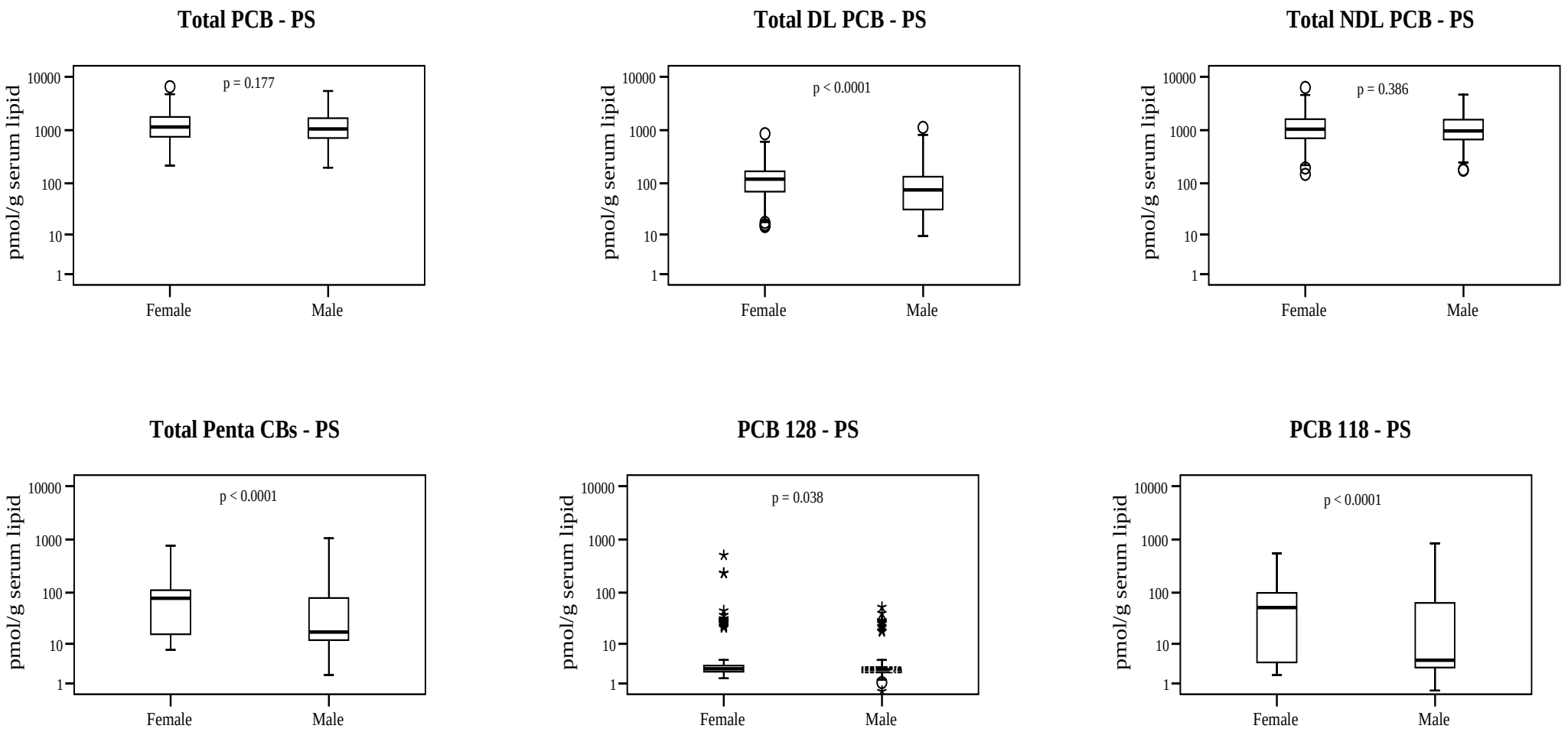


Figure 3.7. Distribution of total -PCB, -DL–PCB, -NDL–PCB, -penta–CBs, PCB 128 and 118 by gender expressed in pmol/g serum lipid (logarithmic scale) in the overall population sample (PS). Displayed results had significant different concentrations between females and males with exception of total -PCB and -NDL–PCB. *Keys*: O, mild outliers; *, extreme outliers.

Table 3.12. Medians, minima and maxima concentrations (in pmol/g serum lipids) for PCB congeners stratified by residence and sex.

PCB	Novafeltria n = 162						p	Pavia n = 164						p	Milan n = 41						p
	Female n = 81			Male n = 81				Female n = 70			Male n = 94				Female n = 31			Male n = 10			
	Min.	**Med.**	Max.	Min.	Med.	Max.	value	Min.	**Med.**	Max.	Min.	**Med.**	Max.	value	Min.	**Med.**	Max.	Min.	**Med.**	Max.	value
NLD–PCBs																					
28	2	**nc**	57	2	**6**	67	0.999	2	**nc**	353	2	**nc**	351	0.203	15	**nc**	32	15	**nc**	28	0.445
31	2	**nc**	63	2	**nc**	89	0.826	2	**nc**	336	2	**nc**	280	0.842	15	**nc**	32	15	**nc**	28	0.445
52	2	**nc**	57	2	**nc**	153	0.037	3	**47**	409	3	**42**	407	0.271	13	**nc**	830	14	**nc**	269	0.964
101	2	**nc**	98	2	**nc**	102	0.061	2	**nc**	5	1	**nc**	5	0.328	12	**nc**	25	12	**nc**	22	0.445
128	2	**nc**	4	1	**nc**	53	0.151	2	**nc**	5	0.4	**nc**	5	0.328	21	**nc**	506	21	**nc**	40	0.200
138	49	**175**	685	51	**173**	607	0.782	92	**316**	889	41	**268**	1223	0.364	13	**572**	1612	374	**492**	635	0.286
153	55	**255**	846	27	**286**	884	0.633	119	**403**	1160	77	**347**	1704	0.458	13	**763**	2205	435	**566**	803	0.143
170	3	**73**	184	3	**81**	279	0.216	10	**94**	292	16	**85**	354	0.934	10	**nc**	855	10	**nc**	1010	0.643
180	42	**178**	830	35	**198**	955	0.332	49	**254**	687	47	**261**	1275	0.813	11	**412**	1616	10	**391**	1587	0.823
DL–PCBs																					
77	2	**nc**	56	2	**nc**	5	0.060	1.8	**nc**	29	0.5	**nc**	50	0.422	39	**nc**	84	405	**nc**	75	0.445
126	2	**nc**	115	2	**nc**	496	0.061	1.7	**nc**	5	0.5	**nc**	5	0.328	23	**nc**	50	24	**nc**	44	0.445
169	2	**nc**	328	1	**nc**	61	0.083	1.5	**nc**	43	0.4	**nc**	46	0.432	21	**nc**	45	21	**nc**	40	0.445
105	2	**nc**	210	2	**nc**	180	0.030	1.7	**nc**	5	0.5	**nc**	206	0.312	12	**nc**	25	12	**nc**	22	0.445
118	3	**81**	547	2	**58**	490	0.000	1.9	**nc**	407	0.5	**nc**	845	0.005	12	**nc**	25	12	**nc**	22	0.445
156	2	**24**	90	2	**28**	96	0.434	2.2	**34**	143	1.8	**29**	140	0.445	21	**nc**	45	21	**nc**	40	0.445

3.2.5 Distribution of PCBs by age

Subjects' age were categorized into 4 groups: 10–25, 26–40, 41–55 and 56–70. Majority of subjects had age between 26–40 and 41–55. Compared to other sites, Milan had fewer subjects in age group 26–40, 41–55 and 56–70 whereas Novafeltria had fewer subjects in group 10–25 (**Table 3.13**).

Table 3.13. Absolute frequency of study subjects in each age category for the overall and individual population subgroups.

Age Class	All Subjects	Novafeltria	Pavia	Milan
10-25	32	7	13	12
26-40	159	66	73	20
41-55	136	73	55	8
56-70	40	16	23	1
Total	367	162	164	41

In the overall population sample, the median concentrations of total PCB, total DL–PCBs, total NDL–PCBs, total hexa– and hepta–CBs increased consistently across the age groups ($p < 0.0001$). The trend was inconsistent for the total tri–, tetra– and penta–CBs. The median concentrations of PCB 138, 153, 180, 156 and 170 presented statistically significant increasing trend across these age groups ($p < 0.0001$) (**Figure 3.8** and **3.9**).

In individual sites, similar trends and significance levels were observed for Novafeltria and Pavia populations. In Milan PCB 153 and 180 levels were shown to increase consistently and significantly ($p = 0.032$ and 0.033, respectively) (**Figure 3.10** and **3.11**)**.** Although PCB 138 had median concentrations which tend to increase across the age groups, there was no evidence of significant difference in its distribution along these age groups.

The trends observed were supported by positive correlations between subjects' age and some PCB congeners. In the overall population sample, PCB 138, 153, 156, 170 and 180 had positive correlations between 0.285 and 0.569 ($p < 0.0001$). Correlations observed in Novafeltria were between 0.404 and 0.670 and in Pavia were between 0.424 and 0.666. PCB 118 correlated significantly with age in Novafeltria ($r = 0.267$, $p = 0.001$). In Milan, positive correlations were observed for PCB 138, 153 and 180 ($r = 0.346$, $p = 0.027$; $r = 0.417$, $p = 0.007$ and $r = 0.329$, $p = 0.035$; respectively). In all sites

age correlated positively with total PCB (r = 0.598, p < 0.0001; r = 0.545, p < 0.0001; r = 0.327, p = 0.037; for Novafeltria, Pavia and Milan, respectively) but at different levels; Novafeltria and Pavia at 0.01 level while Milan at 0.05 level (**Table 3.14**).

Table 3.14. Correlations between age and serum PCBs levels in the 3 Italian population subgroups (Spearman).

PCBs	N˚Cl	Novafeltria		Pavia		Milan	
		r	p–value	r	p–value	r	p–value
138	6	0.573	<0.0001	0.523	<0.0001	0.346	0.027
153	6	0.568	<0.0001	0.571	<0.0001	0.417	0.007
156	6	0.545	<0.0001	0.516	<0.0001	-0.400	0.009
170	7	0.641	<0.0001	0.578	<0.0001	-0.040	0.787
180	7	0.670	<0.0001	0.666	<0.0001	0.329	0.035
∑PCB		0.598	<0.0001	0.545	<0.0001	0.327	0.037
∑DL–PCB		0.404	<0.0001	0.424	<0.0001	-0.404	0.009

3.2.6 Distribution of PCBs by BMI

The minimum and maximum BMI in the study population were 15.6 and 38.5 kg/m^2, respectively. Based on international classification of BMI our population had 5 underweight individuals (1%), 213 normal (58%) and 149 overweight (41%). None of the subjects were severely underweight or severely obese. The influence of BMI in distribution of PCBs was assessed.

Significant difference in distribution across BMI categories was observed for PCB 170 (p = 0.010). Though not significant, consistent increase in median concentrations was observed for PCB 138 across the categories (median: 204, 239, 241 and 243 pmol/g lipid). The median concentrations of PCB 153 and 180 tend to increase across the categories but the trends were inconsistent (**Table 3.15–3.18**). Correlation analyses supported the observed trends. Thus significant correlations with BMI were observed for PCB 170 (overall sample); 138 (Novafeltria); 138, 153, 180 and 170 (Pavia). In Milan this relationship was not observed. All significant correlations observed range between 0.182 and 0.197 (**Table 3.19**).

When analyses were stratified by gender while controlling for places of residence and age we found a weak positive relationship between BMI and PCB 118 concentration in females of overall population (r = 0.032; p = 0.030). Inverse relationship was observed with PCB 28 and 31 in females (r = -0.031, p = 0.01; r = -0.026, p = 0.029, respectively)

whereas in males the relationship was observed with PCB 101, 128, 180 and 156 (r = -0.011, p = 0.02; r = -0.011, p = 0.04; r = 0.010, p = 0.050; r = -0.018, p = 0.044). When analyses were stratified by places of residence and gender while controlling for age a weak positive relationship between BMI and PCB 118 among females of Pavia (r = 0.064; p = 0.038) was observed. Inverse relationship with PCB 180 was observed in female and male subjects of Novafeltria (r = -0.016, p = 0.033; r = -0.013, p = 0.043, respectively) and with PCB 28 in only females of the same population subgroup (r = -0.0.036, p = 0.007).

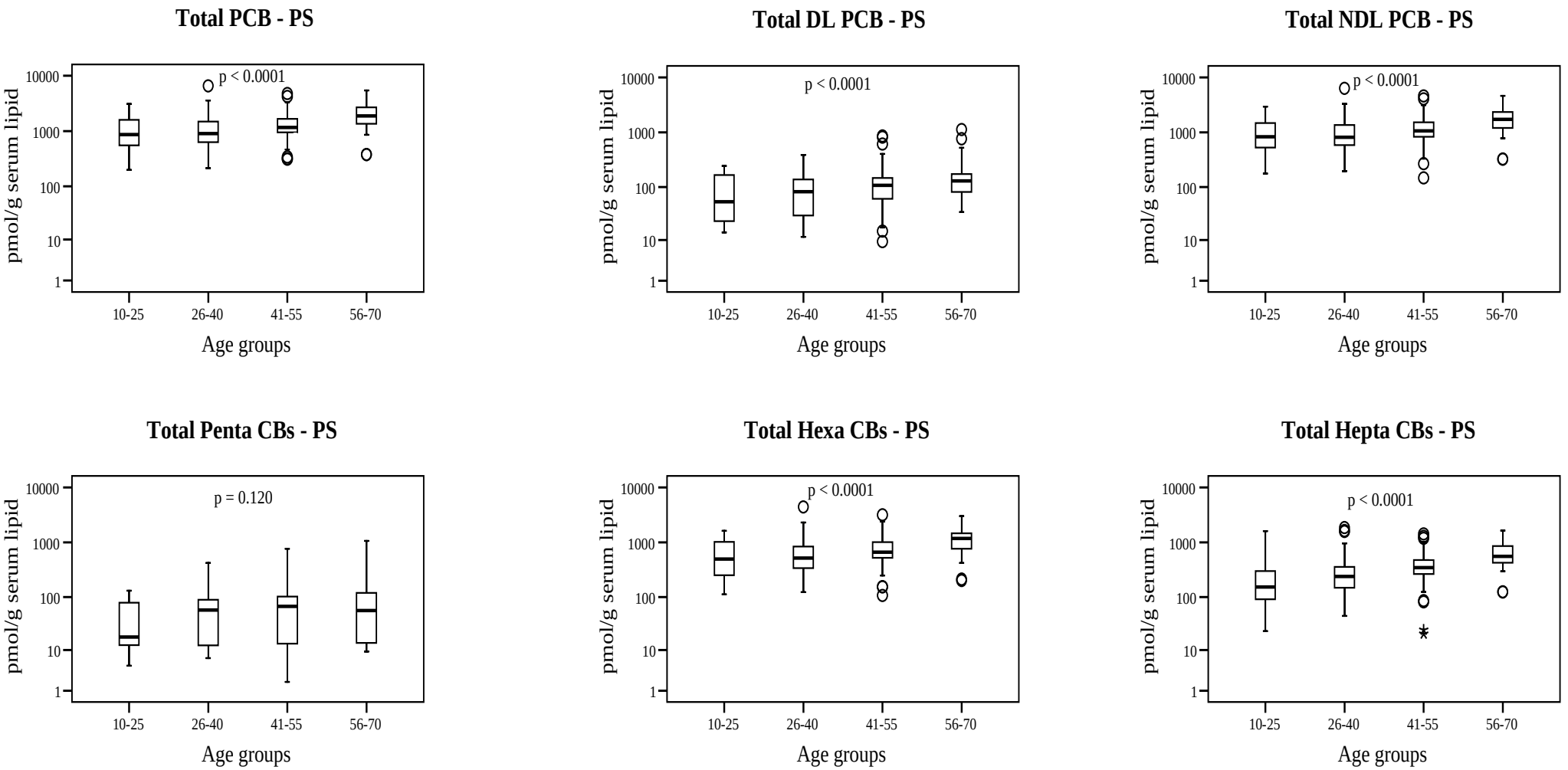


Figure 3.8. Distribution of total -PCB, -DL–PCB, -NDL–PCB, penta–, hexa– and hepta–CBs levels by age group expressed in pmol/g serum lipid (logarithmic scale) in the pooled sample (PS). Displayed results had significant different concentrations across the groups with exception of total penta–CBs. *Keys*: O, mild outliers; *, extreme outliers.

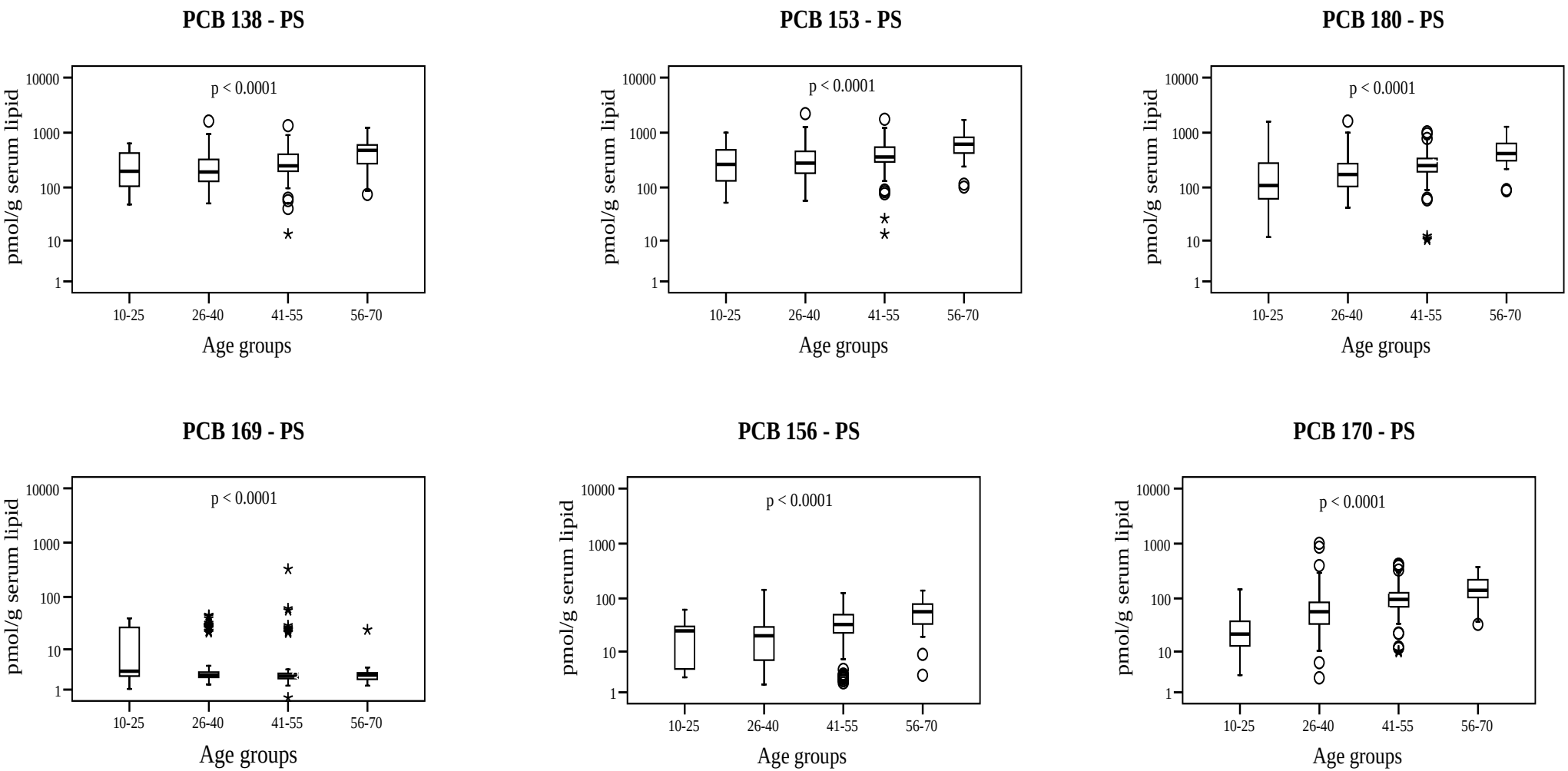


Figure 3.9. Distribution of PCB 153, 138, 180, 169, 156 and 170 concentrations by the age groups expressed in pmol/g serum lipid (logarithmic scale) in the pooled sample (PS). Displayed results had significant different concentrations across the groups. *Keys*: O, mild outliers; *, extreme outliers.

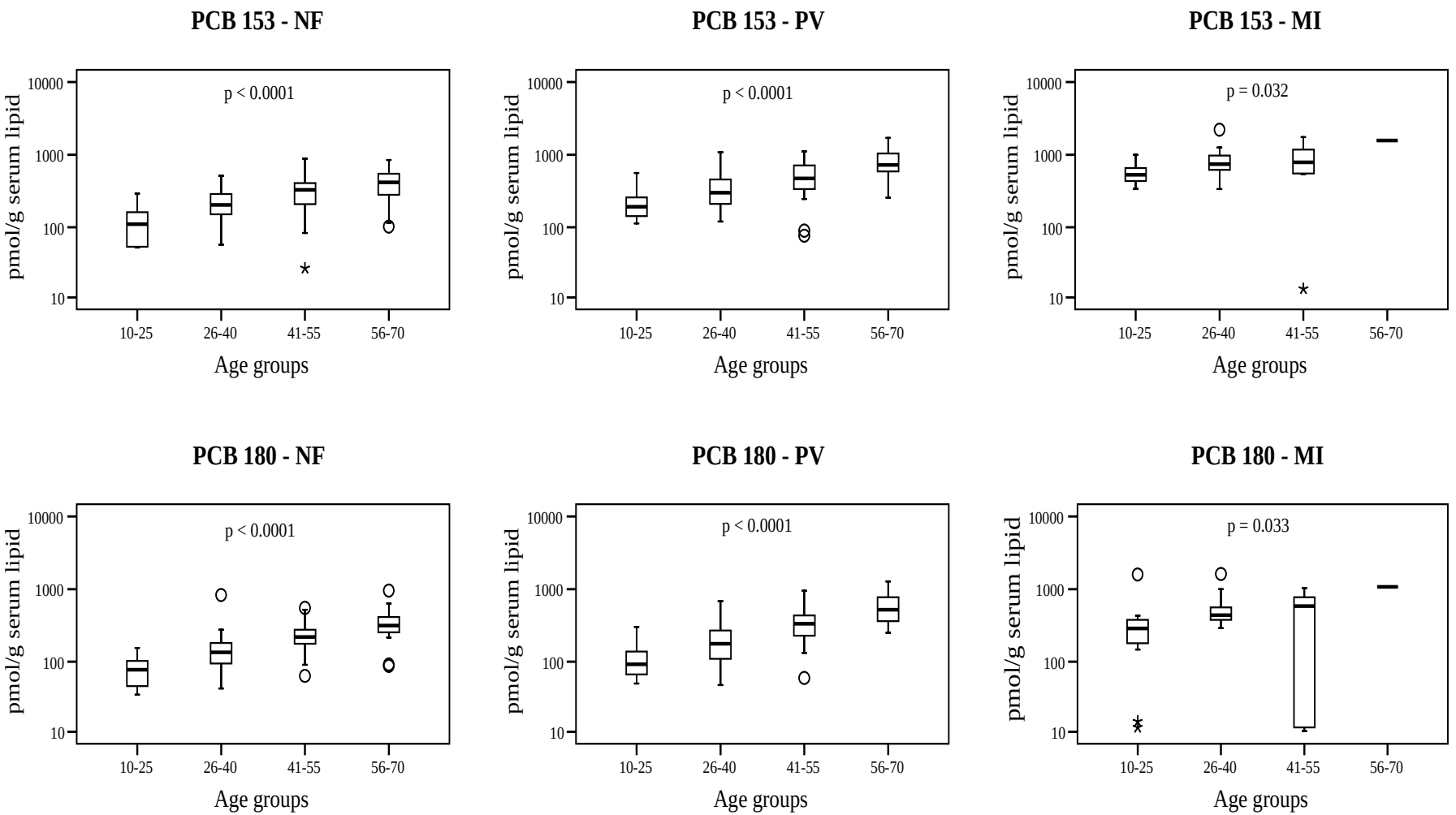


Figure 3.10. Distribution of PCB 153 and 180 concentrations by age groups expressed in pmol/g lipid (logarithmic scale). Displayed results had significant different concentrations across the groups. *Keys*: O, mild outliers; *, extreme outliers; NF, Novafeltria; PV, Pavia; MI, Milan.

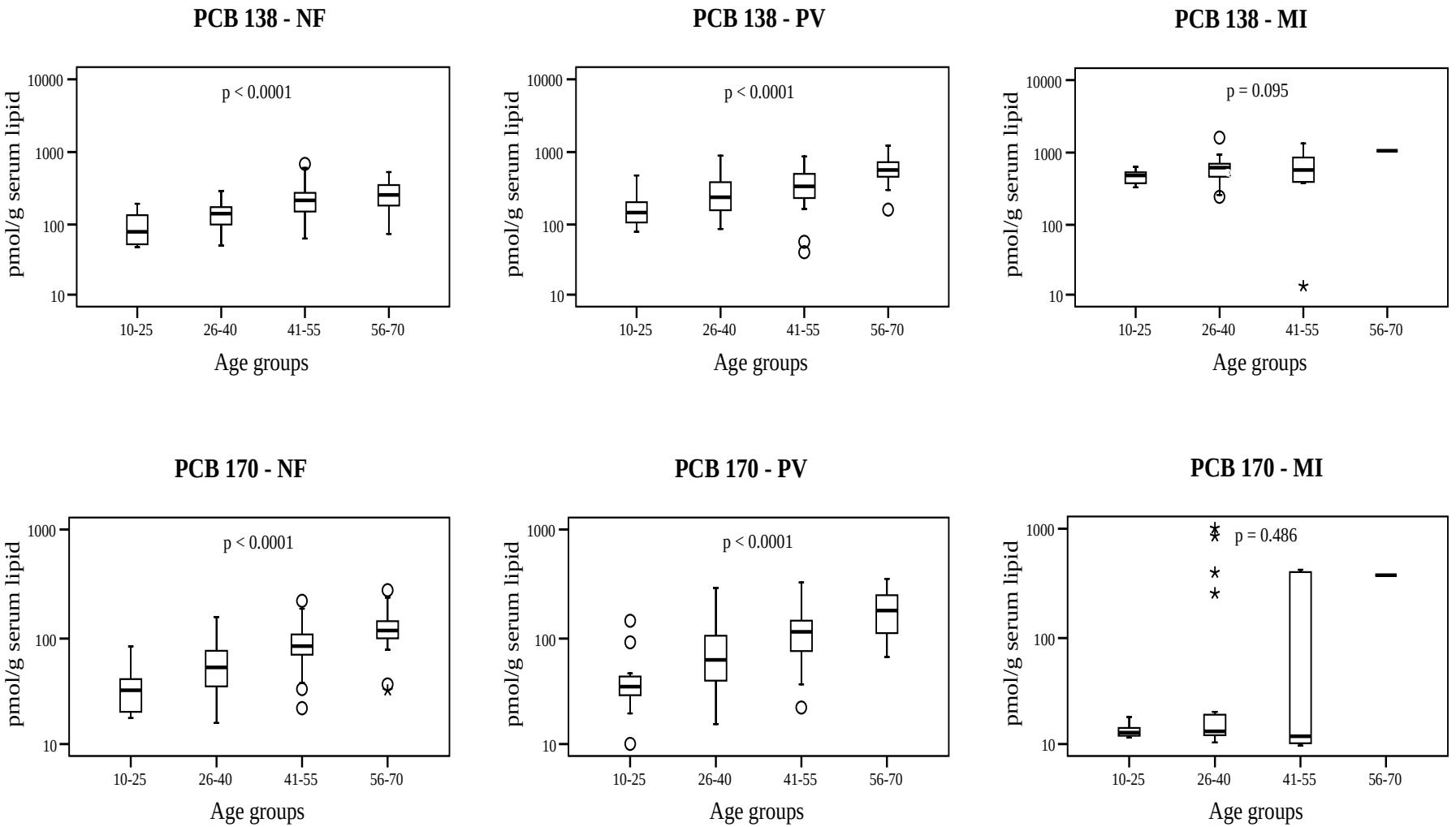


Figure 3.11. Distribution of PCB 138 and 170 concentrations by age groups expressed in pmol/g lipid (logarithmic scale). Displayed results showed significant different concentrations across the groups with exception of PCB 138 and 170 in Milan (MI). *Keys*: O, mild outliers; *, extreme outliers; NF, Novafeltria; PV, Pavia.

Table 3.15. All subjects stratified by BMI categories.

PCBs	BMI Categories												P value	% detection of individual congeners in each BMI group when half are not included			
	<18.50			18.50–22.99			23.00–24.99			>25.00							
	Min.	**Med.**	Max.	Min.	**Med.**	Max.	Min.	**Med.**	Max.	Min.	**Med.**	Max.		<18.50	18.50–22.99	23.00–24.99	>25.00
28	8	**28**	47	2	**nc**	353	3	**nc**	161	2	**nc**	351	0.098	100	40	34	44
31	7	**19**	54	2	**nc**	336	3	**nc**	253	2	**nc**	280	0.054	100	39	29	39
52	3	**nc**	89	2	**nc**	830	2	**nc**	304	2	**nc**	407	0.226	40	48	42	47
101	3	**nc**	4	2	**nc**	98	2	**nc**	102	0.47	**nc**	17	0.001	0	1	3	0
128	2	**nc**	4	1	**nc**	506	2	**nc**	53	0.42	**nc**	233	0.003	0	1	3	2
138	96	**203**	208	13	**230**	1612	51	**241**	871	41	**243**	1223	0.433	100	99	100	100
153	120	**251**	341	13	**339**	2205	70	**357**	1049	27	**339**	1704	0.454	100	99	100	100
170	35	**50**	109	3	**65**	855	12	**83**	257	3	**83**	1010	0.010	100	85	94	95
180	49	**117**	264	10	**225**	1616	58	**220**	651	11	**227**	1070	0.280	100	98	100	99
77	3	**nc**	22	2	**nc**	84	2	**nc**	59	0.52	**nc**	58	0.002	20	1	0	1
126	3	**nc**	115	2	**nc**	50	2	**nc**	49	0.47	**nc**	35	0.001	20	0	2	0
169	2	**nc**	328	1	**nc**	45	2	**nc**	56	0.42	**nc**	61	0.002	20	1	2	1
105	3	**nc**	4	2	**nc**	110	2	**nc**	175	0.47	**nc**	210	0.014	0	1	5	5
118	3	**nc**	87	2	**nc**	270	2	**nc**	549	0.47	**41**	845	0.937	40	47	48	53
156	4	**21**	62	2	**27**	143	2	**27**	97	2	**30**	140	0.560	80	72	75	79
∑PCB	393	**809**	1553	214	**1138**	6556	304	**1147**	3000	199	**1112**	5416	0.589	100	97	100	100
∑DL–PCB	25	**76**	611	14	**108**	371	15	**84**	817	9	**90**	1127	0.613	80	75	77	84
∑NDL–PCB	368	**734**	981	147	**1067**	6317	242	**1037**	2885	176	**976**	4374	0.389	100	97	100	100
∑Tri–CBs	15	**47**	100	3	**nc**	593	5	**nc**	406	4	**nc**	582	0.053	100	44	37	47
∑Tetra–CBs	7	**25**	92	3	**nc**	884	4	**nc**	307	3	**nc**	411	0.089	60	48	42	48
∑Penta–CBs	11	**nc**	204	8	**nc**	332	10	**nc**	732	2	**57**	1060	0.668	40	47	48	53
∑Hexa–CBs	227	**521**	918	107	**613**	4401	154	**652**	2024	114	**611**	2997	0.621	100	97	100	100
∑Hepta–CBs	85	**167**	352	21	**297**	1855	83	**311**	903	29	**310**	1568	0.196	100	100	100	100

Table 3.16. Novafeltria subjects stratified by BMI groups.

PCBs	BMI Categories												p value	% detection of individual congeners in each BMI group when half are not included			
	<18.50			18.50–22.99			23.00–24.99			>25.00							
	Min.	**Med.**	Max.	Min.	**Med.**	Max.	Min.	**Med.**	Max.	Min.	**Med.**	Max.		<18.50	18.50–22.99	23.00–24.99	>25.00
28	8	**28**	41	2	**9**	67	3	**nc**	53	2	**nc**	358	0.035	100	57	41	45
31	74	**19**	23	2	**nc**	63	3	**nc**	89	2	**nc**	43	0.036	100	46	24	387
52	3	**nc**	5	2	**nc**	153	2	**nc**	4	2	**nc**	60	0.058	0	8	0	7
101	3	**nc**	4	2	**nc**	98	2	**nc**	102	2	**nc**	5	0.013	0	3	7	0
128	3	**nc**	4	1	**nc**	4	2	**nc**	53	1	**nc**	19	0.039	0	0	7	1
138	96	**193**	208	49	**151**	607	51	**235**	485	51	**192**	685	0.160	100	100	100	100
153	120	**217**	319	52	**227**	884	70	**355**	770	27	**293**	787	0.430	100	100	100	100
170	35	**50**	109	3	**70**	222	16	**100**	188	3	**79**	279	0.395	100	98	100	99
180	90	**117**	233	40	**177**	830	58	**216**	522	35	**190**	955	0.753	100	100	100	100
77	3	**nc**	22	2	**nc**	55	2	**nc**	4	2	**nc**	5	0.012	33	2	0	1
126	3	**nc**	115	2	**nc**	4	2	**nc**	49	2	**nc**	5	0.008	33	0	3	0
169	3	**nc**	328	1	**nc**	32	2	**nc**	56	1	**nc**	62	0.013	33	2	3	1
105	3	**nc**	4	2	**nc**	110	2	**nc**	133	2	**nc**	210	0.109	0	3	7	9
118	4	**83**	87	3	**70**	205	3	**71**	181	2	**68**	547	0.815	67	92	86	91
156	4	**21**	60	2	**23**	96	2	**34**	83	2	**26**	92	0.476	67	87	93	81
∑PCB	393	**753**	1553	214	**751**	2448	304	**1092**	2255	199	**875**	3236	0.714	100	100	100	100
∑DL–PCB	25	**120**	611	18	**112**	341	16	**118**	402	14	**106**	857	0.946	67	95	97	93
∑NDL–PCB	368	**633**	942	181	**647**	2280	242	**970**	2002	176	**777**	2473	0.515	100	100	100	100
∑Tri–CBs	15	**47**	63	3	**10**	120	5	**nc**	142	4	**8**	78	0.021	100	59	41	51
∑Tetra–CBs	7	**nc**	25	3	**nc**	156	4	**nc**	9	3	**nc**	65	0.036	33	8	0	9
∑Penta–CBs	17	**96**	204	12	**82**	332	11	**79**	419	9	**79**	763	0.781	67	92	86	91
∑Hexa–CBs	227	**436**	918	112	**408**	1591	154	**652**	1345	114	**536**	1457	0.313	100	100	100	100
∑Hepta–CBs	125	**167**	342	45	**239**	923	83	**311**	710	38	**273**	1234	0.671	100	100	100	100

Table 3.17. Pavia subjects stratified by BMI groups.

PCBs	BMI Categories												p value	% detection of individual congeners in each BMI group when half are not included			
	<18.50			18.50–22.99			23.00–24.99			>25.00							
	Min.	**Med.**	Max.	Min.	**Med.**	Max.	Min.	**Med.**	Max.	Min.	**Med.**	Max.		<18.50	18.50–22.99	23.00–24.99	>25.00
28	24	**35**	47	2	**nc**	353	3	**nc**	161	2	**nc**	351	0.742	100	39	32	49
31	10	**32**	54	2	**nc**	336	3	**nc**	253	2	**nc**	280	0.809	100	48	39	46
52	41	**65**	89	3	**46**	409	4	**35**	304	3	**45**	407	0.628	100	95	84	91
101	3	**nc**	3	2	**nc**	4	2	**nc**	5	0.47	**nc**	5	0.036	0	0	0	0
128	2	**nc**	3	1	**nc**	4	2	**nc**	5	0.42	**nc**	4	0.035	0	0	0	0
138	203	**205**	207	79	**285**	889	98	**242**	871	41	**377**	1223	0.227	100	100	100	100
153	251	**296**	341	113	**383**	1360	142	**343**	1049	77	**496**	1704	0.232	100	100	100	100
170	36	**62**	88	10	**85**	353	27	**75**	252	16	**116**	328	0.096	100	100	100	100
180	49	**157**	264	54	**244**	1275	72	**216**	651	47	**334**	999	0.066	100	100	100	100
77	3	**nc**	4	2	**nc**	29	2	**nc**	6	1	**nc**	49	0.056	0	2	0	1
126	3	**nc**	3	2	**nc**	4	2	**nc**	5	0.47	**nc**	5	0.036	0	0.0	0	0
169	2	**nc**	3	1	**nc**	43	2	**nc**	5	0.42	**nc**	46	0.055	0	2	0	1
105	3	**nc**	3	2	**nc**	4	2	**nc**	175	0.47	**nc**	206	0.034	0	0	3	3
118	3	**nc**	3	2	**nc**	270	2	**nc**	549	0.47	**nc**	845	0.137	0	23	19	23
156	18	**40**	62	2	**31**	143	2	**15**	96	2	**39**	140	0.061	100	87	71	87
∑PCB	809	**912**	1015	340	**1242**	4757	440	**1228**	3000	340	**1605**	5416	0.126	100	100	100	100
∑DL–PCB	34	**55**	76	14	**48**	371	15	**36**	817	9	**61**	1127	0.471	67	87	71	87
∑NDL–PCB	734	**857**	981	323	**1209**	4609	421	**1160**	2885	331	**1529**	4289	0.100	100	100	100	100
∑Tri–CBs	33	**67**	100	4	**nc**	593	6	**nc**	406	5	**12**	582	0.912	100	48	39	50
∑Tetra–CBs	45	**68**	92	6	**49**	414	7	**40**	307	5	**48**	411	0.636	100	95	84	91
∑Penta–CBs	11	**12**	13	8	**12**	279	9	**13**	732	2	**12**	1060	0.138	0	23	19	23
∑Hexa–CBs	521	**546**	572	200	**716**	2321	251	**589**	2024	155	**921**	2997	0.211	100	100	100	100
∑Hepta–CBs	85	**219**	352	64	**327**	1628	108	**284**	903	78	**446**	1321	0.068	100	100	100	100

Table 3.18. Milan subjects stratified by BMI groups.

PCBs	BMI Categories									p value	% detection of individual congeners in each BMI group when half are not included			
	18.50–22.99			23.00–24.99			>25.00							
	Min.	**Med.**	Max.	Min.	**Med.**	Max.	Min.	**Med.**	Max.		<18.50	18.50–22.99	23.00–24.99	>25.00
28	15	**nc**	32	15	**nc**	22	15	**nc**	22	0.218	0	0	0	0
31	15	**nc**	32	15	**nc**	22	15	**nc**	22	0.218	0	0	0	0
52	14	**nc**	830	16	**nc**	105	13	**nc**	324	0.232	0	31	20	10
101	12	**nc**	25	12	**nc**	18	12	**nc**	17	0.218	0	0	0	0
128	22	**nc**	506	21	**nc**	32	21	**nc**	233	0.805	0	4	0	20
138	13	**524**	1612	245	**619**	668	376	**597**	1060	0.744	0	96	100	100
153	13	**661**	2205	459	**628**	958	438	**719**	1574	0.665	0	96	100	100
170	10	**nc**	855	12	**nc**	257	10	**nc**	1010	0.958	0	15	20	30
180	10	**402**	1616	290	**345**	584	11	**428**	1070	0.856	0	89	100	80
77	41	**nc**	84	40	**nc**	59	39	**nc**	58	0.218	0	0	0	0
126	24	**nc**	50	24	**nc**	35	23	**nc**	35	0.212	0	0	0	0
169	22	**nc**	45	21	**nc**	32	21	**nc**	31	0.218	0	0	0	0
105	12	**nc**	25	12	**nc**	18	12	**nc**	17	0.218	0	0	0	0
118	12	**nc**	25	12	**nc**	18	12	**nc**	17	0.218	0	0	0	0
156	22	**nc**	45	21	**nc**	32	21	**nc**	31	0.218	0	0	0	0
∑PCB	310	**2120**	6556	1499	**1758**	2483	1144	**2149**	4521	0.664	0	85	100	100
∑DL–PCB	134	**174**	273	129	**163**	193	128	**155**	190	0.218	0	0	0	0
∑NDL–PCB	147	**1902**	6317	1330	**1598**	2353	954	**1978**	4374	0.656	0	85	100	100
∑Tri–CBs	31	**40**	63	30	**38**	45	30	**36**	44	0.218	0	0	0	0
∑Tetra–CBs	55	**72**	884	65	**69**	145	52	**64**	369	0.229	0	31	20	10
∑Penta–CBs	61	**79**	125	59	**74**	88	58	**71**	87	0.218	0	0	0	0
∑Hexa–CBs	107	**1196**	4401	957	**1281**	1707	908	**1363**	2915	0.600	0	85	100	100
∑Hepta–CBs	21	**418**	1855	302	**358**	841	29	**443**	1568	0.741	0	100	100	100

Table 3.19. Correlations between BMI with serum PCBs levels in the 3 Italian population subgroups (Spearman).

PCBs	N°Cl	Novafeltria		Pavia		Milan	
		r	p–value	r	p–value	r	p–value
138	*6*	0.157	0.046	0.186	0.017	-0.053	0.745
153	*6*	0.106	0.181	0.179	0.022	-0.086	0.596
156	*6*	-0.008	0.924	0.136	0.082	-0.306	0.055
170	*7*	0.078	0.327	0.183	0.019	-0.113	0.489
180	*7*	0.039	0.621	0.197	0.011	-0.166	0.307
∑PCB		0.063	0.424	0.182	0.019	-0.189	0.242
∑DL–PCB		-0.028	0.719	0.099	0.207	-0.306	0.055

Table 3.20. Coefficient of determination (R^2) and significance levels (p–values) for the relationship between predictor variables (age, sex, BMI, residence) and dependent variables (total PCBs).

	∑PCB	∑DL–PCB	∑Tri–CBs	∑Tetra–CBs	∑Penta–CBs	∑Hexa–CBs	∑Hepta–CBs
Age	0.000	0.000	0.951	0.728	0.001	0.000	0.000
Gender	0.404	0.021	0.316	0.962	0.013	0.080	0.183
BMI	0.062	0.397	0.891	0.298	0.913	0.271	0.004
Residence	0.000	0.124	0.000	0.000	0.123	0.000	0.000
R^2	0.445	0.082	0.055	0.253	0.061	0.452	0.360

CHAPTER FOUR

DISCUSSION

4.1 Organochlorinated pesticides study

Exposure to ubiquitous pollutants of highly concern such as OCPs (mainly DDT) and chlorinated biphenyls was measured in the Italian general population starting in the early 1960s and more than 50 articles have reported concentrations in biological compartments such as blood serum, adipose tissue and human breast milk. In this article we address only OCPs, whilst polychlorinated biphenyls (PCBs) which were measured in a similar but not coincident cohort are reported and discussed.

The population recruited in our study has, on the overall, a fair gender distribution with reference to that of the general Italian population derived from census (http://www.indexmundi.com/italy/sex_ratio.html). This is the case for the Novafeltria cohort, while those for Pavia and Milan are grossly skewed, with more males in Pavia and more females in Milan. The age distribution of our population does not match that of the Italian population (Gallus *et al.*, 2006) since it lacks the extremes of age (<19 and >75), mainly due to the difficulty of accessing the younger and to ethical considerations on blood withdrawal in the very elderly. The age ranges were 22–59, 20–70 and 19–69 for Novafeltria, Pavia and Milan subjects, respectively. Significant difference in age distribution was observed between these sites (KW test, $p < 0.0001$) with the highest median age (44.5 years) in Novafeltria population (**Table 3.1**). Lack of representativeness of our study sample to the general population of Italy, mainly for age and gender structure was unavoidable due to constraints in the experimental approach (collection of blood samples over time, during routine health surveys of the enrolled subjects). For the same reasons, we could not collect parity and lactation history data of women. This could be better used to explain and understand the difference in OCPs distribution among the gender group since breastfeeding is known to be a major pathway of excretion and transmission of mothers' body burden of OCs to their newborns (Gladen *et al.*, 1999).

Although persistent OCPs such as DDT were banned in Italy since 1978, to date detectable levels are still found in the Italian general population as evident in this study. We have demonstrated a clear pattern of the level of total OCP, total DDTs, *p,p'*-DDE and HCB among the healthy residents in the three investigated locations of Italy. These

levels were significant different across these sites (Milan > Novafeltria > Pavia, $p < 0.0001$) with Milan having the highest median level.

To frame our results within the general picture, a summary of the levels of OCPs measured in various Italian regions between 1972 and 2011 is shown in **Table 4.1**. Data on the body burden of OCPs are not abundant and studies are very heterogeneous in sample size and in the number of target analytes. Limited sample sizes of individual studies and limited collection of data on exposure determinants hamper extrapolation of exposure estimation to the general population for health promotion purposes. The original studies also report OCPs concentrations using different units. To allow comparison of the levels of exposure with those of our study, we converted the results of some literature studies (**Table 4.1**) to pmol/g lipid as described in methodology section (Fängström *et al.*, 2005).

In their study targeted at assessing the levels of several organochlorines in human breast milk collected in 2000–2001 from lactating mothers in Italy, Weiss *et al*. (2003) found high concentration of DDE (*approx.* 27 ng/g fresh weight) from Milan samples. The authors hypothesized that the measured level may be due to the content of OCPs in food imported from countries where DDT is still used.

As for specific sources of exposure to OCPs, a significant association between plasma levels of DDT, DDE and PCBs and fish consumption was demonstrated in the Swedish population (Asplund *et al.*, 1994; Sarcinelli *et al.*, 2003). Also in Italy, intensive harvesting of Manila Clam and fishing in the polluted Venice lagoon caused an increase in the concentration of POPs in mother milk and blood serum of Venetians (Raccanelli *et al.*, 2009). However some studies failed to establish the evidence on the relationship between some POPs levels and consumption of particular type of foods (Abballe *et al.*, 2008).

In our cohort the KW test revealed a significant difference in distribution of β-HCH with places of residence ($p < 0.0001$). Ingelido *et al*. (2009) demonstrated similar trend in β-HCH serum concentration distributions among investigated subjects from northern, central and southern Italy ($p = 0.03$). However, the median level observed in our study (123 pmol/g lipid) is higher than that reported in their study (62 pmol/g lipid), but lower than in Schiavone *et al.* (2010) study (250 pmol/g lipid).

HCB and *p,p'*-DDE are known to have long half-lives in human (To-Figueras *et al.*, 1997; Wolff *et al.*, 2000). This explains their high prevalence (>90%) in our study population. The overall level of HCB observed in Milan population (median = 578, mean = 634 pmol/g lipid) is similar to that found in adipose tissue (mean 534 pmol/g serum lipid) reported elsewhere (Schiavone *et al.*, 2010)). The observed level of *p,p'*-DDE in our study is 177 ng/g lipid (geometric mean; data not shown), much lower than that in the Spanish study (geometric mean 822 ng/g lipid; Jakszyn *et al*., 2009).

Variations by gender in concentrations of some OCPs were observed in our subjects. When the three sites were considered together females had significantly higher levels of *p,p'*-DDE than males ($p < 0.0001$). This difference can be due to inter-gender physiological differences. In consistent with previous studies (Jakszyn *et al*., 2009; Dirtu *et al.*, 2006; Porta *et al.*, 2010; Sala *et al.*, 1999) we also observed higher HCB concentrations in women than in men. In addition to inter-gender physiological differences, our observation could also be related to differences in metabolism. It has been found that metabolism of HCB is higher in men than in women (To-Figueras *et al.*, 1997). As *p,p'*-DDE and HCB were the major contributor of total OCPs we also observed significant higher level of total OCPs in females than in male subjects. Contrary to our study previous studies reported lower level of *p,p'*-DDE in women than in men (Jakszyn *et al*., 2009; Dirtu *et al.*, 2006; Porta *et al.*, 2010; Kang *et al.*, 2008; Kalantzi *et al.*, 2011; Zumbado *et al.* 2005) which can be explained as the consequence of lactation and menstruation (Amodio *et al*., 2012; Mishra *et al*., 2011), the major routes of OCs excretion in females.

In our population serum *p,p'*-DDT was detected in 11 and 14% of female and male subjects, respectively. The maximum concentration of 207 ng/g lipid (data not shown) was found, a level which is comparable to 257 ng/g lipids reported in Jakszyn *et al*. (2009), but almost double of that measured in Sweden (124 ng/g lipid) (Glynn *et al.*, 2000, 2007). In the latter study *p,p'*-DDT was highly prevalent detected in about 80% of females and 90% of males.

The distribution of β-HCH between females and males was statistically significant different (Mann–Whitney U test, $p = 0.008$) with males showing higher median level than females (229 and 59 pmol/g lipids, respectively). While other studies observed the opposite trend for this OC (Kang *et al.*, 2008; Porta *et al.*, 2010), Ingelido *et al.* (2009)

failed to show the evidence for the significant differences in distribution of β-HCH between genders (Mann–Whitney U test, p = 0.81). This was also the case for our study when the analyses were restricted to the places of residence and gender.

The differences of exposure between genders could not be accounted for as we did not have factors associated with our subjects that could provide better explanation. However, this variation is not anticipated in people of the general population (Dirtu *et al.*, 2006).

Age is one of the personal characteristics which plays a role in differentiating the levels of OCPs, since it defines the time frame of accumulation of biologically persistent chemicals. Usually it is expected that accumulation of these contaminants increase with age of the subjects. In this study total OCP and total DDT presented a significant increasing median concentration across the age groups in Novafeltria population. This behaviour was also evident in distribution of total *p,p'*-DDX (Novafeltria) and β-HCH (Pavia) except that the median concentration of age 41–55 was a little higher than that of age group 56–70 for total DDX while for β-HCH the median concentration of age group 41–55 was slightly lower than that of 10–25. However, significant and positive correlations with age were found for total OCP, total DDT, total *p,p'*-DDX, *p,p'*-DDE and HCB (r = 0.479, p = 0.003; r = 0.446, p = 0.006; r = 0.454, p = 0.005; r = 0.468, p = 0.004 and r = 0.468, p = 0.004, respectively) in only Novafeltria group. Generally the oldest part of the population was characterised by the highest median concentrations. The increasing trend of median concentrations along the age groups was demonstrated by Ingelido *et al.* (2009), where 11, 18 and 39 ng/g lipid of β-HCH were found in subjects in the age ranges 20–35, 36–50 and 51–65 years, respectively. This behaviour was also observed in Porta *et al.* (2010) study where higher median concentrations of *p,p'*-DDE and HCB were found among the older subjects who were between 60–74 years of age as compared to the younger ones with age between 18–29 years. Bates *et al.* (2004) also demonstrated increasing mean concentrations of dieldrin and *p,p'*-DDE along the age groups (15–24, 25–34, 35–49, 50–64 and 65+ years).

BMI is a relative measurement of the amount of body fat in different subjects much used in population studies. OCPs preferentially accumulate in body fat and thus a larger body burden is expected in subjects with higher BMI compared to those with lower BMI (**Table 3.6**). It has been shown that individuals with more body fat tend to

eliminate OCs more slowly than those with less body fat (Aylward *et al*., 2005; Furuya *et al.*, 2010; Michalek and Tripathi, 1999). We observed that in overall sample, subjects with progressively higher BMI had higher levels of total OCP ($p = 0.018$) and total DDT ($p = 0.023$). There was no significant positive correlation between most OCPs and BMI with exception of HCB in Novafeltria ($r = 0.435$, $p = 0.008$) and Pavia ($r = 0.268$, $p = 0.040$). Sala *et al.* (1999) and Medehouenou *et al.* (2011) showed the increase in HCB, β-HCH and *p,p'*-DDE levels with BMI.

General comparison of our results with other studies conducted outside Italy showed lower median level of *p,p'*-DDE (125.42 ng/g lipid) than those reported in some Westernized Asian countries such as Japan and Korea (221 and 224 ng/g lipid, respectively) (Kang et *al.*, 2008; Tsukino *et al.*, 2006), in other European countries such as Romania and Sweden (1975 and 497 ng/g lipid, respectively) (Dirtu *et al.*, 2006; Glynn *et al.*, 2003), in New Zealand (919 ng/g lipid; Zumbado *et al.*, 2005) and in the USA (204 ng/g lipid; Meeker *et al.*, 2007). However this level is slightly higher than 100 ng/g lipid reported in the United Kingdom (Thomas *et al.*, 2006). β-HCH had a median concentration of 35.68 ng/g lipid which was higher than those reported in some studies (Bates *et al.*, 2004; Thomas *et al.*, 2006) but lower than in other studies (Dirtu *et al.* 2006; Kang *et al.*, 2008; Tsukino *et al.*, 2006; Glynn *et al.*, 2003). HCB had a median of 43.84 ng/g lipid which is higher than those reported in these studies (Dirtu *et al.*, 2006; Kang *et al.*, 2008; Tsukino *et al.*, 2006; Meeker *et al.*, 2007; Thomas *et al.*, 2006) but lower than that in Glynn *et al.* (2003) (median of 65 ng/g lipid). HCB had a geometric mean of 54.38 ng/g lipid (data not shown) which is lower than 379 ng/g lipid reported in Jakszyn *et al.* (2009) The observed concentrations of HCB among women in Novafeltria and Milan (geometric mean 193 and 564 pmol/g serum lipid, respectively) were higher than those observed among Italian women with endometriosis and those with other gynaecologic conditions (geometric mean 126 and 144 pmol/g lipid, respectively) (**Table 4.1**; Porpora *et al*., 2009).

To give a final picture of the outcome of our study, a further consideration may be useful. In particular, in the subjects who live in Milan and who are younger, many OCPs (β-HCH, *p,p'*-DDT, *o,p'*-DDE, *o,p'*-DDD in all genders and *o,p'*-DDT in females) were not detected. However those which were detected surprisingly presented higher levels than in the older subjects of Novafeltria and Pavia. Milan is an industrial city in Northern Italy where DDT and other chlorinated insecticides were used with much

lower frequency than in other regions, such as rural and Southern Italy. In the same decades when DDT was used, Milan hosted a high immigration of formerly rural workers from Center and Southern Italy, which raised its population by *approx.* 30% from 1950 to 1970. It is thus conceivable that the high level of OCPs observed in the current inhabitants of Milan may be due to several factors, among which: (a) the body burden of people coming from DDT–treated areas, including the contribution of contaminated agricultural food and (b) for the younger subjects, transmission of mothers' body burden of DDT through pregnancy and breastfeeding. On the contrary, Pavia experienced much less and later immigration from remote areas of Italy while Novafeltria never attracted considerable immigration from distant places. Mobility census in Italy was abolished in the early 1970s, demographic data of the examined subjects were not considered when this research was planned and this information is no more retrievable, due to the anonymity of subjects after the questionnaire was filled in, in order to test this hypothetical explanation.

Table 4.1. Concentrations of OCPs (in pmol/g serum lipid) in blood serum and in other body compartments of Italians living in various regions (1972–2011).

Reference	Sample size	Study Population	Location	Sampling Year	Body Compartment	β-HCH	HCB	*p,p′*-DDT	*o,p′*-DDT	*p,p′*-DDE	*o,p′*-DDE	*p,p′*-DDD	*o,p′*-DDD
Cocco *et al.* (2004)	107	Men who sprayed DDT in the 1946-1950 anti-malarial campaign	Sardinia	–	Blood Serum			18120–35384[h]		170174–332310[h]			
Ingelido *et al.* (2009)	116	General Population	Rome Brescia Naples	2008–2009	Blood serum	43[h] 86[h] 77[h]							
Porpora *et al.* (2009)	158	Women who underwent surgery for endometriosis or other gynaecologic conditions	Rome	2004–2005	Blood serum		126[k] 144[k]			1380[k] 953[k]			
Turci *et al.* (2010a)	95	General population	Novafeltria Pavia	–	Blood serum		127–248[h] 70–136[h]		58–113[h] 233–455[h]	294–575[h] 116–227[h]		23–46[h] 75–146[h]	15–29[h] 56–108[h]
Giannandrea *et al.* (2011)	98	Testicular cancer patients and healthy men who underwent andrological examination	Rome	2006–2008	Blood Serum		398–778[j]			516–1007[j]			
This study	137	General population	Novafeltria Pavia Milan	–	Blood serum	340[h] 324[h] –	209[h] 100[h] 566[h]	213[h] 175[h] 395[h]	335[h] 365[h] 1747[h]	450[h] 173[h] 4294[h]	34[h] 59[h] -	49[h] 135[h] 527[h]	27[h] 81[h]
Bergonzi *et al.* (2011)	70	Women in highly polluted urban area	Brescia	2006	Mother serum Cord serum Serum Placenta Adipose tissue		77–150[h] 24–47[h] 68[h] 70[h] 91[h]			413–80[h] 0.79[h] 353[h] 197[h] 635[h]			
Abballe *et al.* (2008)	39	Breast feeding mothers	Venice Rome	1998–2000 2000–2001	Human Breast Milk		133–246[l] 179[m]	27–124[l] 124[m]		660–1604[l] 1384[m]			
Di *et al.* (1990)	93	Breast feeding mothers	Rome Florence	1982–1985	Human Breast Milk			7[h]		34[h]			
Larsen *et al.* (1994)		Breast feeding mothers	4 Italian cities	–	Human Breast Milk	2068–4038[i]	2687–5248[i]	1735–3388[i]		30081–58742[i]			
Franchi and Focardi (1991)	56	Breast-feeding mothers	Certaldo Grosseto		Human Breast Milk		1299[i] 2223[i]	987[i] 841[i]		7157[i] 7138[i]			

Table 4.1. Concentrations of OCPs (in pmol/g serum lipid) in blood serum and in other body compartments of Italians living in various regions (1972–2011).

Reference	Sample size	Study Population	Location	Sampling Year	Body Compartment	β-HCH	HCB	*p,p′*-DDT	*o,p′*-DDT	*p,p′*-DDE	*o,p′*-DDE	*p,p′*-DDD	*o,p′*-DDD
Focardi *et al. (1986)*	26	Patients admitted for general surgery	Siena	1983–1984	Adipose tissue		2260[i]	830[i]		7350[i]			
Schiavone *et al.* (2010)	12	Patients from general surgery unit	Siena	2005–2006	Adipose tissue	250[i]	534[i]	<28[i]	<28[i]	6330[i]	<31[i]	53[i]	<31[i]
Gallelli *et al.* (1995)	28	Deceased males and females	Genua	1989	Adipose tissue	732[i]		181[i]		1242[i]	732[i]		18[i]
Mariottini *et al.* (2002)	18		Central Italy	1997–2000	Adipose tissue		1239[i]						
De Felip *et al.* (2004)	12	Women attending fertility clinic who underwent multiple ovulation and oocyte retrieval	Rome	2000	Follicular fluids		242–256			1981–2610			
Prati *et al.* (1972)	85	General Population between 1–80 years	Ferrara	1966–1969	Autopsy fat			1.6–3.0[n, i]		3.8–7.5[n,i]			
					Biopsy fat			1.0–2.0[n,i]		2.7–5.3[n,i]			
					Liver			2.4–4.7[o, i]		2.4–4.7[o,i]			
					Kidney			1.7–3.2[o, i]		2.4–4.7[o,i]			
					Brain			8.5–16.6[p,i]		1.2–2.3[p,i]			
					Spleen			1.5–3.0[p, i]		4.2–8.3[p,i]			

Keys: h, median; I, mean; j, maximum level; k, geometric mean; l, range; m, individual measurement of the pooled sample; n, concentrations are multiplied by 10^6; o, concentrations are multiplied by 10^5; p, concentrations are multiplied by 10^4.

4.2 Polychlorinated biphenyl study

This study is one of the largest performed in Italy and in Western Europe, as for the number of examined subjects. Samples were taken from the participating subjects within the frame of population-wide surveys. Some pertinent information such as women's reproductive histories, general health status with reference to degenerative illnesses, not to say clinical parameters such as peripheral sex hormone levels were not available, nor requested for. There is no chance whatsoever to retrieve this information retrospectively, since sample anonymization was performed well before samples were delivered to the laboratory for PCBs and OCPs measurements. The four determinants of PCB body burden which can be considered in a causative analysis given the information which could be collected on the subjects of our study are residence, gender, body mass and age.

To understand the magnitude of exposure in our population, we compare our results with previous results of studies (Apostoli *et al.*, 2005; Bergonzi *et al.*, 2009; De Felip *et al.*, 2004, 2008; Donato *et al.*, 2008; Porpora *et al.*, 2006, 2009; Turci *et al.*, 2004, 2006, 2010a; Turrio-Baldassarri *et al.*, 2008) published in the literature and collected in **Table 4.2**. For the epidemiological studies reported, only the data for control groups were considered, to reflect levels of exposure in the general population rather than in specific groups for which specific sources of exposure were known or suspected. The numbers of PCB congeners measured in those studies were between only four and sixty different molecules out of 209 congeners. As a result the total PCB levels and concentrations measured in the different studies also varied according to the number of congeners considered for laboratory analysis. In order to allow comparisons, only 15 congeners were considered and their units were converted to match our unit of pmol/g lipid. In most literature, results are reported in heterogeneous units, such as raw concentration (nanograms/milliliter in serum) without reporting relevant biochemical measurements necessary to define total blood lipid contents of the subjects. We thus used upper and lower limits of cholesterol and triglycerides of the healthy population to compute the corresponding total lipids which were used to adjust the PCB concentrations reported in the literature. Finally the literature results were expressed in toxicologically relevant molecular units as picomoles per gram serum lipid which facilitated comparison of our results.

We observed a significant difference in total PCB among residents of the three sites. The residents from Milan had the highest PCB concentrations followed by Pavia and Novafeltria. These variations may be related with different dietary habits, sample group characteristics (Turci *et al.*, 2010a) and industrial activities. Milan is an urban area with intense past industrial activities with a considerable proportion of the population coming from other regions of Italy, while Pavia is a town with little current and past industrial activity and few immigrants. Novafeltria is a small village, with no industrial activity with a predominantly local population. Our results agree with previous literature studies which show that urban residents have significantly higher levels of PCBs than people from semi-urban or rural areas (Heywood *et al.*, 2006; Wong *et al.*, 2009). What is seldom clear is the exact source of higher levels in the urban population with respect to that of small town and of rural areas. In most industrial countries where studies were performed, the use of PCBs has been discontinued for at least fifteen years. Thus the spread of the PCB pool into the environment through several global cycles should have led to a leveling of differences from localized emission sources to less contaminated areas.

The explanations presented to account for the observed differences in PCB body burden of individuals from the three locations are mostly conjectural, since no reliable retrospective information can be obtained to strongly support any claimed explanation. Starting from the reasonable assumption that for the general population dietary intake of PCBs is estimated to account for >90% of human exposure to these chemicals (Duarte-Davidson *et al.*, 1994), we nevertheless face with the very limited information available on dietary exposure to PCB in Italy. Studies report that, in Italy, dairy products, meat and fish are the principal sources of PCB contamination (Zuccato *et al.*, 1999) while vegetables contribute very little to the daily dietary exposure. Turci *et al*. (2006) reported that the average daily intake of total PCBs for the population subgroup of Pavia is *approx.* 0.26 µg. Since dietary habits vary among individuals even within the population of an individual site, levels of PCBs and main sources may differ in different population subgroups. Donato *et al.* (2006) found high serum PCB levels in humans living in an industrialized town in Italy and related this observation mainly due to consumption of food produced in polluted areas.

It should also be considered that large-scale food distribution through the General Distribution Organization (GDO; supermarkets) has become increasingly popular in Italy even in small towns. This accounts, on the nation-wide average for approximately 50% of supply, expressed as monetary value (Fulponi, 2004) and with a marked North-South gradient (Esposti *et al*., 2008). These store chains get food from the global market at national and supra-national scale so that it is conceivable that the proportion of daily PCB intake which comes from 'supermarket' food is approximately analogous throughout the Italian population.

To account for the differences in PCB body burden among the three Italia populations investigated, we may well propose that the amount of consumed food which originates locally, in presumably less-contaminated areas, and which is marketed outside the GDO, accounts for the lower body burden (proportional to the approximately constant intake) in rural Novafeltria and in small-town Pavia with reference to large urban Milan.

Direct exposure to PCBs happens to be relatively rare in Italy outside well-known specific areas such as in the neighbours of the only production plant in Italy (the Caffaro plant in urban Brescia) and of a few big industrial areas where large electrical appliances and heat exchangers were manufactured or installed. Localized spills from transformers' fires were however common although mostly unreported (except for the recording of local blackouts by maintenance staff with little if any awareness of the hazard of transformer oil), especially in rural areas. One case of spillage of PCB-containing oil occurred in 1988 in Southern Italy during road transportation of a large transformer. This area is well distant from any of the investigated sample areas (De Felip and Di Domenico, 1990; Liberti *et al.*, 1992).

Overall population had the median total PCB level of 1131 pmol/g lipid; males having the median of 1051 pmol/g lipid and females of 1142 pmol/g lipid and thus showing no gender differences. As for congener groups, the only which showed differences were total dioxin–like (tDL)–PCBs (with males having lower levels than females), total penta–CBs (in Novafeltria and in Pavia) and total tetra–CBs (only in Novafeltria), which were also lower in males. Most individual PCB congeners did not differ significantly between genders with exception of PCB 52, 105 and 118, for which the levels were lower in males than in females.

Our measured levels are within the range (0.9 to 56 μg/L) reported in the metanalysis of Mangili *et al.* (2004) and are also consistent with results of previous studies which did not found substantial differences in concentrations of PCBs between males and females (Turci *et al.*, 2010a; Donato *et al.* 2008; Burns *et al.*, 2009; Focardi *et al.*, 1986; Henriquez-Hernandez *et al.*, 2011; Zubero *et al.*, 2009).

Other studies (Bates *et al.* 2004; Park *et al.*, 2007; Cerná *et al.*, 2008; Porta *et al.*, 2010) measured higher PCBs concentrations in males than in females and in particular Park *et al.* (2005) found higher level of PCB 118 in males than in females. Minh *et al.* (2005) found higher concentrations of hepta–CBs in males than in female while tetra– and penta–CBs were lower in males. The authors suggested preferential elimination of more chlorinated biphenyls in females. We found total median hepta–CBs level of 303 and 306; penta–CBs 78 and 17; and tetra–CBs 28 and 19 pmol/g lipid; for females and males, respectively.

In the general population exposure to PCBs of males and females is not expected to be significantly different, the factor which can lead to lower serum concentrations of some congeners in females than in males (Thomas *et al.*, 2006; Dirtu *et al.*, 2006). Differences in the PCBs distribution between genders observed in the examined cohorts may be explained as inter-gender physiological differences, females having a higher proportion of body fat than males (Zubero *et al.*, 2009; Geyer *et al.*, 2002) Alternative explanation can be due to possible differences in exposure, males possibly having additional occupational exposure or their diet may be richer in fatty foods which may be in turn a more abundant source of ubiquitous PCBs. However, there is no clear or demonstrated explanation of the observed differences.

When lower levels of PCBs are measured in women, one explanation may be that body burden has been cleared through breastfeeding. This explanation needs support from data on reproductive history of females such as parity and lactations, which could not be collected from our subjects and is seldom reported in literature studies.

Body Mass Index is a proxy for the proportion of human body fat based on a calculation from individual's weight and height. This is widely used in several fields of clinical medicine and epidemiology as a first-tier approach to understand the effect of obesity on health. It does not actually measure the percentage of body fat, the accurate evaluation of which entails the use of physiological measurements such as total body

impedentiometry which were not available at subjects' recruitment stage. Due to lipophilic character of PCBs, 'fatter' subjects are expected to accumulate more of these chemicals than 'leaner' ones with the same body weight. However, we did not observe significant difference in distribution of most PCB congeners and total PCB across the BMI categories, nor any significant correlation of total PCB concentration with BMI in the overall population. Only for some congeners (PCB 138, 153, 180 and 170) we observed very weak positive correlations.

Few studies, however, observed a positive association of BMI and PCB concentration (Henriquez-Hernandez *et al.* 2011; Fernandez *et al.*, 2008) while in one case an inverse association with PCB 180 was reported (Zubero *et al.*, 2009;). Apostoli *et al.* (2005) warned that spurious positive association between PCB levels and BMI can be obtained when serum lipid adjustment is not performed. However, the role of BMI on the level of organochlorine compounds has not yet been clearly established (Zubero *et al.*, 2009; Moysich *et al.*, 2002). In our study the multivariate analyses confirmed age and residence but not BMI as the only important predictors for total PCB level (**Table 3.20**). Accumulation of persistent chemicals is expected to increase with age due to continued exposure without excretion. We observed consistent increase in median levels of total PCB and of PCB congeners 138, 153, 180, 156 and 170 across the age groups, as highlighted by correlation analyses. Duarte-Davidson and Jones showed a relatively high proportion of the more persistent congeners among older people but did not observe significant differences in the total PCB between gender, residence and the body weight (Duarte-Davidson *et al.*, 1994). The body burden of serum NDL–PCB was shown to increase among two 55+ year age groups living close to incineration plants in Italy (De Felip *et al.*, 2008). Hirai *et al.* (2005) observed significant correlation between total PCB level and age (Spearman $r = 0.62$, $p < 0.01$). Apostoli *et al.* (2005) calculated in an Italian study population an increase of total PCB levels of a 1.7 ng/ml for every 10-year increase in age. The increase in PCBs levels with age can be the consequence of exposure prior to the ban of PCB production (Henriquez-Hernandez *et al*., 2011). The influence of age on the levels of most PCB congeners has also been confirmed by a worldwide review conducted recently by Consonni *et al.* (2012) where strong association of subjects' age with levels of most congeners was found.

Our results reported in **Table 3.9** show a greater accumulation of the more highly chlorinated PCBs. This phenomenon results from preferential clearance of lower

chlorinated congeners by biotransformation and excretion. The global half-life of a chemical with low metabolic clearance depends on the physicochemical properties, especially its lipophilicity (log K_{ow}) and on its bio-transformation rate. As the number of chlorine atoms in the biphenyl nucleus increases, lipophilicity also increases, so that the compound is preferentially stored in body fat, a compartment where biotransformation of xenobiotics is intrinsically low, due to low-to-nil expression of pertinent enzymes by adipocytes. Biotransformation leading to clearance therefore occurs only for the fraction of PCB pool which is at equilibrium in the 'non-fat' body compartment, where lower-chlorine PCB congeners are more abundantly represented. Biotransformation of PCBs mostly occurs by mono-oxygenation through the arene-oxide pathway catalyzed by P450 iso-enzymes. The biotransformation rate of individual PCB congeners thus depends on the availability of positions in the chemical structure where mono-oxygenation can occur. Congeners with a higher number of suitable positions in the molecular structure are therefore more easily biotransformed, the fat to non-fat equilibrium in concentrations is thus perturbed and more of the easily bio-transformed congeners are displaced from the fat to the non-fat compartment, where they are biotransformed. As a quantitative consequence of both phenomena, the more chlorinated congeners, which are both, less abundant in the non-fat, biotransforming compartment and less efficiently biotransformed are preferably left in the body. The less chlorinated congeners, which are both more abundant in the non-fat, biotransforming compartment and more efficiently biotransformed, are preferably cleared. As such they may be measured as circulating hydroxy–PCBs or as conjugated products excreted through the biliary route; on the global they account for a minute fraction of body fat PCBs (Fernandez *et al.*, 2008).

The median concentrations of PCB 138 (Pavia) and 153 (Milan) reported in our study are within the lower and upper median concentrations reported in 2005 by Apostoli *et al.* (2005) and comparable with those reported in Turrio-Baldassarri *et al.* (2008). In Milan PCB138 concentration was higher than those reported in these studies. In Novafeltria and Pavia the median concentrations of PCB 153 and 180 fell within the lower and upper median levels observed in Turci *et al.* (2010a). In all sites the median levels of PCB 180 were lower compared to Apostoli *et al.* (2005), Donato *et al.* (2008) and Turrio-Baldassarri *et al.* (2008), but higher than that observed in Bergonzi *et al.* (2009) particularly in serum matrix (**Table 4.2**). Porpora *et al.* (2006) found significant

higher PCB 153 level in women with endometriosis (416 pmol/g lipid) than the control group (263 pmol/g serum lipid) ($p = 0.0004$). In comparison with our study PCB 153 level (763 pmol/g lipid) found in Milan females was higher than that in endometriosis cases of Porpora *et al.* study while females of Pavia had comparable level (403 pmol/g lipid) with the cases. Novafeltria females had PCB 153 level of 255 pmol/g lipid which was comparable to the control group (**Table 4.2**).

Table 4.2. Concentrations of PCB congeners (in pmol/g serum lipid) in blood/blood serum of Italians living in various regions (2004–2010).

Reference	Sample size	Study population	Location	Sampling year	PCB Congeners represented by IUPAC numbers														
					77	126	169	105	118	156	28	31	52	101	128	138	153	170	180
Turci *et al.* (2004)[h]	162	General population	Novafeltria	–	27.4	81.5	119.2	98.3	83		17.2	13.8	39	76	29.6	197.8	290.1	80.2	202.4
Turci *et al.* (2006)[h]	326	Non occupationally exposed subjects	Novafeltria and Pavia	–	0.5		0.6	2.7	43.5	37.7	43.9	50.9	89.4			354.7	471.1	108	306.1
Turci *et al.* (2010a)[j]	95	Volunteers in the General population	Novafeltria	–				nc	19–37	25–48	nc	nc	18–35	18–33		83–163	212–414	36–71	105–206
			Pavia					nc	nc	nc	nc	nc	49–96	nc		151–296	212–414	41–81	162–317
This study[j]	367	General population	Novafeltria	–	nc	nc	nc	nc	70.05	24.76	nc	nc	nc	nc	nc	173.70	264.92	76.62	189.16
			Pavia		nc	nc	nc	nc	nc	32.59	nc	nc	44.33	nc	nc	293.95	394.03	88.13	258.82
			Milan		nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	532.15	667.5	nc	402.97
De Felip *et al.* (2004)[k]	22	Women suspected of endometriosis and benign adnexal mass	Rome	2000–2001	0.17	0.12	0.04	7.66	33.7	9.7									
					0.17	0.11	0.06	7.96	36.76	11.08									
					0.17	0.12	0.06	9.5	39.82	12.47									
Apostoli *et al.* (2005)[j]	579	General population	Brescia	2001–2003					21–41	19–37						242–473	428–836	131–257	446–871
Donato *et al.* (2008)[j]	527	Random sample of adult residents	Brescia	2001–2002												327.53	576.09	162.91	591.67
Turrio-Baldassarri *et al.* (2008)		Local general population	Brescia	2004–2005	<0.03	0.3	0.5	13.2	95	73.2	<1.2		<1.4	<1.2		299.3	670.6		766.5
De Felip *et al.* (2008)[k]	74	Non-occupationally exposed population in Tuscany, Grosseto	F	2005–2006	<0.14	0.17	0.14	9.50	52.08	33.25									
			FL		<0.17	0.18	0.17	8.88	52.08	30.48									
			FLA		<0.14	0.37	0.24	17.77	91.90	52.65									
			S		<0.21	0.15	0.18	7.66	42.89	33.25									
			SA		<0.14	0.37	0.27	20.22	113.35	63.73									
			V		<0.14	0.23	0.18	19.61	91.90	38.79									
			M1		<0.14	0.24	0.17	12.87	67.40	38.79									
			M2		<0.14	0.17	0.14	9.50	52.08	33.25									
Porpora *et al.* (2006)[h]	154	Women suspected for endometriosis or		2000–2004				30.6	156.2	61.0	21.4		8.6	11.0		235.5	415.7	32.9	164.4
		benign gynaecologic conditions						24.5	73.5	27.7	21.7		10.3	9.5		146.9	263.2	25.3	113.8
Porpora *et al.* (2009)[i]	158	Women with surgery for endometriosis or	Rome	2004–2005				20.8	71.7	23.0	16.7		7.5	6.4		140.5	276.5	22.3	122.9
		other gynaecologic conditions						17.5	46.6	15.8	13.2		5.5	4.9		93.7	171.2	15.4	87.0
Bergonzi *et al.* (2009)[j]	70	Women in highly polluted urban area	Brescia	2006					33.7	13.9	n.d					97	149.6	40.5	124.0

Keys: F, Follonica sample pool of 9 subjects who lived 3-5 kilometres (km) from incinerator; FL, Follonica sample pool of 9 subjects who live >5 km from incinerator; FLA, Follonica sample pool of 10 subjects who live >5 km from incinerator; S; Scarlino pool of 8 subjects; SA, Scarlino pool of 10 subjects; V, Valpiana pool of 8 subjects; M1, Massa Marittima first sample pool 10 subjects; M2, Massa Marittima second sample pool of another 10 subjects; h, mean; i, geometric mean; j, median; k, level of individual pool; nc, not computed.

4.3 Possible Health Effects

The abundant organochlorine compounds (OCs) can be used as indicators in evaluating risk of OCs to human health. In our study the frequently detected POPs were PCB 156, 138, 153, 170, 180, *p,p'*-DDE and HCB in the overall population sample. In consistent with previous studies, PCB 153 strongly correlated with total PCBs ($r = 0.927$, $p < 0.05$) and PCB 118 with total dioxin-like PCBs ($r = 0.914$, $p < 0.05$). Thus the two congeners are considered to be suitable indicators for total PCBs and total dioxin-like PCBs, respectively (Kim *et al*., 2005, Park *et al.*, 2007). *p,p'*-DDE is another relevant biomarker and the major metabolite of DDT with a long biological half-live. In our study it correlated strongly with total DDT ($r = 0.794$, $p < 0.05$).

Various studies have shown association between the PCB congeners and some adverse health effects such as endometriosis (De Felip *et al.* 2004; Porpora *et al*., 2006, 2009), pancreatic cancer (Hardell *et al.*, 2007), cardiovascular diseases (Ha *et al.* 2007, 2009), breast cancer (Aronson *et al.* 2000; Demers *et al*., 2002, Laden *et al.* 2002; Moysich *et al.,* 1999; Recio-Vega *et al.,* 2011; Zhang *et al.,* 2004), non-Hodgkin lymphoma (Bertrand *et al.*, 2010), reproductive abnormalities (Hauser *et al*., 2003; Rignell-Hydbom *et al.*, 2004, 2005) and diabetes (Lee *et al*., 2006). The levels of PCBs reported in our study are similar with some of those found in the studies which have shown to be associated with health effects (**Table 4.3**). Some of these studies are discussed in the subsequent paragraphs.

Moysich *et al*., (1999) reported increased risk of breast cancer associated with the presence of at least one valine allele in women with serum PCB levels above the median of the distribution in the control group (odds ratio, 2.93; 95% confidence interval, 1.17–7.36). Among women with low PCB body burden, no association between *CYP1A1* genotype and breast cancer risk was observed. Subjects in high PCB concentration group had levels ranged from 3.73 to 19.04 ng/g serum lipids (~11–58 pmol/g serum lipid for PCB 118; 10–53 pmol/g serum lipids for PCB 138, PCB 153 and PCB 156; 9–48 pmol/g serum lipid for PCB 170 and PCB 180). Our study recorded the median levels of PCB 118 within the reported range (52 pmol/g serum lipid for the overall women) and above the maximum level of the range (81 pmol/g serum lipid for Novafeltria women). For PCB 156 we found the median levels within the range especially for women of the overall population, Novafeltria and Pavia (28, 24 and 34 pmol/g serum lipid,

respectively). Women of the overall population as well as of individual population subgroups had median levels of PCB 138, 153, 170 and 180 above the maximum level of the reported range.

Aronson *et al*. (2000) demonstrated clear associations of PCBs 118, 170 and 180 with breast cancer risk. ORs were 2.31 (1.11–4.78), 3.27 (1.44–7.44) and 2.43 (1.09–5.43) respectively and concentration range in the tertiles were ≥50, 24–34 and 52–71 µg/kg (≥153, 61–86, 132–180 pmol/g serum lipid), respectively. For PCBs 170 and 180, risks were higher among postmenopausal women in the second tertile while for PCB 118 the risk was above two in the highest concentration categories of the whole sample. For PCB 170 the women of the overall population and those of Novafeltria showed median levels (71 and 73 pmol/g serum lipid, respectively) which are within the reported concentration the category in Aronson *et al*. study whereas Novafeltria women were above the maximum levels (94 pmol/g serum lipid). As for PCB 180 median concentrations for women of the overall population, Pavia and Milan (221, 254 and 412 pmol/g lipid, respectively) were above the range whereas Novafeltria female had median of 178 pmol/g serum lipid which was within the range. For PCB 118 the observed levels were far below the concentration category reported for this congener.

Demers *et al.* (2002) found associations between breast cancer risk and PCB 118 (OR = 1.60, 95% CI: 1.01, 2.53 fourth vs. first quartile) and PCB 156 (OR = 1.80, 95% CI: 1.11, 2.94 fourth vs. first quartile). Subjects in fourth quartiles had PCB 118 concentration >22.1 µg/kg (>67 pmol/g serum lipid) which is relevant for the levels observed in Novafeltria women samples (81 pmol/g serum lipid) whereas for PCB 156 subjects in fourth quartile had concentration >9.8 µg/kg (>27 pmol/g serum lipid) which is relevant for the levels observed in females of our overall population and Pavia sample (28 and 34 pmol/g serum lipid, respectively).

Recio-Vega *et al*. (2011) found risk of breast cancer to be positively associated with PCB 118, 156, 170 and 180 cases having geometric mean of 0.51 ppb (1.56 pmol/g serum lipid), 0.58 ppb (1.61 pmol/g serum lipid), 0.63 and 0.57 ppb (1.60 and 1.44 pmol/g serum lipid), respectively. As compared to this study we found higher geometric means of PCB 118 in our overall women samples (27 pmol/g lipid), Novafeltria women (69 pmol/g lipid), Pavia women (12 pmol/g serum lipid) and Milan women (15 pmol/g serum lipid). For PCB 156 we found higher geometric means in overall population,

Novafeltria and Milan (22, 18, 26 and 28 pmol/g serum lipid, respectively). We found higher geometric means of PCB 170 and PCB 180 in women of overall population, Novafeltria, Pavia and Milan (60, 63, 84 and 26 pmol/g serum lipid, respectively for PCB 170 and 210, 165, 237 and 300 pmol/g serum lipids, respectively for PCB 180).

Porpora *et al*. (2009) reported geometric mean concentrations of PCBs 118, 138, 153, 156, 170 and 180 (72, 141, 277, 23, 25 and 123 pmol/g, respectively) among women who underwent surgery for endometriosis. The reported level of PCB 118 is comparable to the observed level in Novafeltria women in our study (69 pmol/g serum lipid). For PCB 138 our study recorded higher geometric mean concentration for females in overall population (260 pmol/g serum lipid) as well as in individual population subgroups (171, 313 and 518 pmol/g serum lipids respectively for NF, PV and MI). For PCB 153 our study reported higher geometric mean concentration among females of overall population, Pavia and Milan (354, 410 and 680 pmol/g serum lipid, respectively) but lower than that of Novafeltria females (243 pmol/g serum lipid). A geometric mean concentration of PCB 156 is comparable to that of the females of overall population of our study (22 pmol/g serum lipid). As for PCB 170 the reported geometric mean was comparable to that of Milan females (26 pmol/g serum lipid) but lower than of females of overall population, Novafeltria and Pavia (60, 63 and 84 pmol/g serum lipid, respectively).

De Felip *et al.* (2004) reported concentrations of 34 and 37 pmol/g serum lipid (PCB 118) and 10 and 11 pmol/g lipid (PCB 156) in the pools of women samples suspected of endometriosis. Our study found median concentrations of 52 and 80 pmol/g serum lipid (PCB 118) for the overall and Novafeltria women samples, respectively. As for PCB 156, reported median levels of 28, 24 and 34 pmol/g serum lipid for the women of the overall population, Novafeltria and Pavia, respectively which were higher than those in the study of De Felip *et al.* (2004).

Bertrand *et al*. (2010) found positive association between the risk of non-Hodgkin lymphoma with PCBs 118, 138, 153, and 180 among men cohort. The ORs for the highest quintile versus the lowest were 1.7 (1.0–2.9), 2.2 (1.3–3.8), 2.4 (1.4–4.2) and 2.4 (1.4–4.3), respectively and the medians of these highest quintiles were 139 (>105–734), 161 (>122–541), 242 (>188–761) and 154 (>126–528) ng/g serum lipid [~426 (>322–2249); 446 (>338–1499); 671 (>521–2108) and 390 (>319–1336) pmol/g serum lipid].

The median levels of PCB 118 in our study were lower compared to the one reported in this study. As for PCB 138 the median observed in men subjects of our overall population (228 pmol/g lipid) and of individual sites (173, 268 and 492 pmol/g serum lipid for Novafeltria, Pavia and Milan, respectively) were lower than the reported one in this study but they were within the range of the highest quintile for this congener. PCB 153 showed similar trend. Male subjects from Milan had median concentration of PCB 180 (391 pmol/g serum lipid) higher than the reported in Bertrand *et al*. (2010) whereas median level of the overall male population, Novafeltria and Pavia were lower falling below the minimum level of the highest quintile range.

After adjusting for age, sex, race/ethnicity, poverty income ratio, BMI and waist circumference Hardell *et al.* (2007) found significant higher median level of PCB 153 among pancreatic cancer patients [353 ng/g (~978 pmol/g lipid)] as compared with control 156 ng/g (~432 pmol/g lipid). Significant increase OR was observed although no OR could be calculated since the concentration in all cases was greater than the median concentration in control. As compared to our result median levels recorded in all three sites were lower than the reported median in this study.

Strong positive association of Diabetes with PCB153 has been demonstrated (Lee *et al.*, 2006). The ORs for the highest percentile versus the lowest were 2.5 (1.1–6.0), 4.3 (2.2–8.6), 5.9 (3.0–11.9), 5.9 (3.1–11.3) and 6.8 (3.0–15.5). The concentrations were 14.3, 36.7, 60.2, 93.6 and 164 ng/g lipid (~40, 102, 167, 259 and 454 pmol/g lipid) in 25, 25 to <50, 50 to <75, 75 to <90 and $\geq 90^{th}$ percentiles, respectively. All reported concentrations were associated with diabetics and the trends were significantly different (P_{trend} <0.001). Our study presented median concentration of Milan sample which is higher than the concentration in $\geq 90^{th}$ percentile while overall population, Novafeltria and Pavia samples had median levels (342, 265 and 394 pmol/g lipid, respectively) higher than the levels reported in other percentiles.

After adjusting for age, abstinence and smoking, dose-response relationships between PCB 138 and sperm motility (odds ratio per tertile, 1.00, 1.68, 2.35, $p_{trend} = 0.03$) was observed (Hauser *et al*., 2003). The median concentration was 31.2 ng/g (~86 pmol/g lipid) which is by far lower than that observed in male samples of our study (~228, 173, 268, 492 pmol/g lipid, respectively for the overall sample, Novafeltria, Pavia and Milan samples).

In their study to investigate whether exposure to PCB 153 affects semen quantity and reproductive hormones Rignell-Hydbom *et al.* (2004) investigated Swedish fishermen, aged 24–65 years. They found a median PCB 153 serum level of 193 ng/g lipid (534.80 pmol/g lipid). When PCB 153 was categorized into quintiles, the subjects in the quintile with the highest concentration (>328 ng/g lipid = >909 pmol/g lipid), tended to have decreased sperm motility compared with the subjects in the lowest quintile (<113 ng/g lipid = <313 pmol/g lipid). The association between PCB 153 and sperm motility, although not formally significant, is of interest considering the possible endocrine-disrupting effects of polychlorinated biphenyls (PCBs). In our study we found the median level of 566 pmol/g lipids for PCB 153 among male subjects of Milan which was higher than the reported median.

Ha *et al.* (2007) found positive association of dioxin–like PCBs, non dioxin–like PCBs and OC pesticides with the prevalence of cardiovascular diseases only among females. The ORs were 13.4 (1.6–115.0), 10.4 (1.1–94.1), 9.2 (1.0–84.5) for PCB 138, 153 and 170 respectively among the individual in the highest percentile i.e $\geq 75^{th}$. The observed concentrations were 91.0, 127.0, 36.4 ng/g (~252, 352 and 92 pmol/g serum lipid), respectively. Our study found higher median PCB 138 levels for females of overall population, Pavia and Milan (258, 316, 572 pmol/g serum lipid, respectively) whereas in Novafeltria the median level (175 pmol/g lipid) was less than the reported level. For PCB 153 similar trend was observed. For PCB 170 the median level for females of Pavia (94 pmol/g serum lipid) was comparable to the reported level for this congener while in the overall sample and Novafeltria the median level were lower.

McGlynn *et al.* (2008) found significant association between testicular germ cell tumour risk and higher plasma levels of *p,p′*-DDE detected from male subjects. The OR for the for the highest quartile vs lowest quartile was 1.71 (95% CI, 1.23–2.38, *p* for trend = 0.0002) (**Table 1.1R**). The observed concentration in the highest quartile was >0.390 μg/g serum lipid (>1,226 pmol/g serum lipid). As observed in our study male subjects from Milan had median concentration of 3,398 pmol/g serum lipids for this OCP which is in consistent with the level in the highest quartile of the reported study. Pavuk *et al.* (2003) observed higher serum levels of *p,p′*-DDE positively associated with risk of breast cancer in 3^{rd} quartile (OR = 3.04, 95% Cl 0.65–14.3). The concentration in this

quartile was 4,389–19,912 ng/g lipid (~13,801–626,110 pmol/g serum lipid). Our study found median levels which are much far below the lower level of this quartile.

Considering that the levels of some OCs in our study are similar or even higher than the reported levels which showed association with some health effects, adverse health effects for our observed levels are possible. However, our study was not designed to study the cause and effects relationship thus it is difficult to conclude any association with these health effects. This calls for a well designed and detailed studies which will address the causal and effects relationship of the concerning POPs which have shown an indication of health effects to human. In particular, further research should be addressed at endocrine disruption effects such as breast cancer. Recent study has reported the highest increase in the incidence of breast cancer (+28.68% in 2005 *vs* 2000 increase) among Italian women who are less than 45 years old an age group (Piscitelli *et al.*, 2009). Madigan *et al.* (1995) showed that only 45–55% of breast cancer cases can be explained by known risk factors such as later age at first birth, nulliparity, family history of breast cancer, higher socioeconomic status, earlier age at menarche and prior benign breast disease. It will be interesting to explore if environmental factors such as exposure to POPs can explain the observed increase in breast cancer incidence.

Table 4.3. Comparison of the concentrations of selected PCB congeners and OCPs (expressed in pmol/g serum lipid) reported in our study with those of previous studies which suggest possible associations with health effects.

Reference	Sample size	Study population	Location	Sampling year	PCB Congeners (represented by IUPAC numbers) and abundant OCPs							
					118	156	138	153	170	180	*p,p'*-DDE	HCB
This study[j]	367	General population	**PS**		**nc**	**28**	**236**	**342**	**75**	**223**	**395**	**154**
			Female		52	28	258	357	71	221	789	482
			Male		nc	28	228	324	81	223	268	107
			NF sample		**70**	**25**	**174**	**265**	**77**	**189**	**428**	**209**
			Females		81	24	175	255	73	178	538	220
			Male		58	28	173	286	81	198	339	189
			PV sample		**nc**	**33**	**294**	**394**	**88**	**259**	**163**	**100**
			Females		nc	34	316	403	94	254	143	86
			Males		nc	29	268	347	85	261	173	100
			MI sample		**nc**	**nc**	**532**	**668**	**nc**	**403**	**4380**	**571**
			Females		nc	nc	572	763	nc	411	5363	627
			Males		nc	nc	492	566	nc	391	3398	472
De Felip *et al.* (2004)	22	Women suspected of endometriosis and benign adnexal mass	Rome	2000–2001	34 37 40	10 11 12						
Porpora *et al.* (2006)	154	Women suspected for endometriosis or benign gynaecologic conditions	Rome	2000–2004	156 74	61 28	236 147	416 263	33 25	164 114		
Porpora *et al*. (2009)	158	Women with surgery for endometriosis or other gynaecologic conditions	Rome	2004–2005	72 47	23 16	141 94	277 171	22 15	123 87		
Hardell *et al*. (2007)	21	Pancreatic cancer patients	Mid Sweden	1996–1999				978				
	59	Control						432				
Lee *et al*. (2006)	67/401	Diabetic Cases	USA	1999–2000				40, 102, 167, 260, 454				
Rignell-Hydbom *et al.* (2004)	195	Swedish fishermen	Sweden	2001–2002				535				
Hauser *et al*. (2003)	212	Males	USA	2000–2001				86				
Demers *et al.* (2002)	104	Incident case of breast cancer	Quebec, Canada	1994–1997	≥ 67	≥ 27						
	130	Control										
Recio-Vega *et al.* (2011)	70	Breast Cancer women	Mexico		1.56	1.61			1.60	1.44		

Reference	Sample size	Study population	Location	Sampling year	PCB Congeners (represented by IUPAC numbers) and abundant OCPs							
					118	156	138	153	170	180	*p,p'*-DDE	HCB
	70	Control			0.70	0.67			0.63	1.01		
Bertrand *et al.* (2010)	205	Men diagnosed with non-Hodgkin lymphoma	USA	1982–1984	>322–2249		>338–1499	>521–2109		>319–1336		
Ha *et al*. (2009)	17/45	Men samples cases	USA	1999–2002		49						
Medehouenou *et al.* (2010)	391	Men	Nunavik, Québec-Canada	2004				535				
	480	Women						445				
Rignell-Hydbom *et al.* (2005)	176	Fishermen	Sweden	2001-2002				313				
Ha *et al*. (2007)	462	Women	USA	1999–2000 2001–2002			252	352	92	219		
Zhang *et al.* (2004)	374	Breast cancer women	Connecticut, USA	1999–2002	1872–7965	1693–7205	1693–7205	1693–7205	1546	1546		
Moysich *et al.* (1999)	154	Breast Cancer women	Western New York, USA	1986–1991	11–58	10–53	10–53	10–53	9–48	9–48		
Laden *et al.* (2002)	32,826	Breast Cancer women	USA	1989–1990	2053–6096		1857–5514	1857–5514		1695–5034		
Aronson *et al*. (2000)	217	Breast Cancer women	Ontario, Canada	1995–1997	≥153				61–86	132–180		
McGlynn *et al.* (2008)	754	Men with testicular germ cell tumour	USA	2002–2005							>1226	
Pavuk *et al*. (2003)	37	Women with breast cancer	Eastern Slovakia	1997–1999							13801–626110	

Keys: PS, pooled sample from the three sites; NF, Novafeltria; PV, Pavia; MI, Milan.

Conclusion and Recommendations

Our study has shown detectable levels of PCB congeners and OCPs among the residents of Italian general population despite long time ban indicating that they are still circulating among non-occupationally exposed population. These levels are within the range of those observed in people of general population in other studies worldwide.

We have demonstrated a pattern of distribution of the levels of the main PCB congeners and of OCPs in a fairly large population in Italy. PCB 138, 153 and 180 were the most abundant with high concentrations. Difference in PCB concentrations with places of residence was evident; Milan having the highest levels. A reason for these elevated concentrations in the city notwithstanding ban of use and cessation of industrial activities is obscured; more research is needed to understand the cause. Total PCBs and some abundant congeners correlated positively with age. The correlation with age indicates a life-long accumulation, the higher levels in older people being the results of accumulation over a long period of time, especially since the period when the use of these chemicals was more widespread. The only significant difference in concentrations between females and males was observed for total dioxin-like (tDL)–PCBs, with females having higher levels than males. This finding may concern long-term health of women, since this particular class of PCBs shows a weak estrogenic activity and can thus be a factor in determining the incidence of gender-specific cancer.

As for OCPs study, *p,p'*-DDE and HCB were the most abundant and major contributor of total OCP concentration and their concentration differed significantly with places of residence. In particular, the subjects who live in Milan, who are younger, presented higher levels of total OCP, total DDT, *p,p'*-DDE and HCB than in the older subjects of Novafeltria and Pavia as observed in PCB study. It is still not clear what caused such elevated concentrations in this population since in this city there was no history of intensive use of pesticides in the past. Residential history, diet and industrial activities are thus suspected as determinants and this may warrant more detailed investigations on people living in Milan.

Data collected in our study however provide information regarding the levels of PCBs and OCPs exposure of the Italian general population and provides indications for the conduction of further investigations and opens the way to planning more detailed investigations in other similar population studies. Last, since POPs have been shown to have adverse effects even at

low concentrations, regular surveillance and monitoring programs should evaluate their trends and patterns in food and in the general population, to highlight exposure hotspots and counter their release into the environment as a consequence of past use.

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Appendix I: Study Plan.

Activity/Time	Jan – March 2010	April 2010 – April 2011	May – July 2011	July – Sept. 2011	Oct. 2011 – Dec. 2012
Literature search, preparation of tools, proposal development and presentation					
Field work					
Laboratory analysis					
Data entry and analysis					
Preparation and submission of papers					
Compilation and submission of Thesis					

Annex II: Questionnaire.

RESEARCH PROJECT

Exposure to Persistent Organic Pollutant and Health Risks

1. Interviewer ID: _____
2. Surname: _____ Name: _____
3. Date of Interview ☐☐/☐☐/☐☐☐☐
   Day Month Year

Personal Details

4. Date of birth ☐☐/☐☐/☐☐☐☐
   Day Month Year
5. Place of birth Region _____
   District _____
6. Place of Residence Region _____
   District _____
7. Sex ☐ Female = 1 ☐ Male = 2
8. Residence Years ☐☐
9. Are there in the neighbourhood:
   Industries? Yes = 1 No = 0
   Heavy traffic streets/roads? Yes = 1 No = 0
   Power Plants? Yes = 1 No = 0
10. Height ☐☐☐ Centimetres
11. Weight ☐☐☐ Kilograms
12. Level of education
    1 = Literate
    2 = Primary School
    3 = Lower Middle School
    4 = Upper Middle School
    5 = University

Lifestyle

13. Do you smoke? Yes = 1 No = 0
14. How many cigarettes per day? ☐☐
15. Do you chew tobacco? Yes = 1 No = 0
16. How many times per day? ☐☐
17. Are you an ex smoker? Yes = 1 No = 0
18. How many years of smoking? ☐☐

19. Do you do physical exercise e.g. sports? Yes = 1 No = 0

20. How many hours per week? ☐☐

Dietary and drinking habits

21. The following questions are related to eating and drinking habits last year. How often, on average, do you eat/drink the following products?	Never=0	1 time per month =1	2-4 times per month =2	1-2 times per week=3	3-4 times per week=4	1 day =5	2-4 days=6	5-6 days=7	≥6 days =8	
21a. Chicken	☐	☐	☐	☐	☐	☐	☐	☐	☐	Chicken
21b. Cattle	☐	☐	☐	☐	☐	☐	☐	☐	☐	Cattle
21c. Sheep	☐	☐	☐	☐	☐	☐	☐	☐	☐	Sheep
21d. Pork	☐	☐	☐	☐	☐	☐	☐	☐	☐	Pork
21e. Rabbit	☐	☐	☐	☐	☐	☐	☐	☐	☐	Rabbit
21f. Fish	☐	☐	☐	☐	☐	☐	☐	☐	☐	Fish
21g. Fruits	☐	☐	☐	☐	☐	☐	☐	☐	☐	Fruits
21h. Eggs	☐	☐	☐	☐	☐	☐	☐	☐	☐	Eggs
21i. Cheese	☐	☐	☐	☐	☐	☐	☐	☐	☐	Cheese
21j. Vegetables	☐	☐	☐	☐	☐	☐	☐	☐	☐	Vegetables
21k. Coffee	☐	☐	☐	☐	☐	☐	☐	☐	☐	Coffee
21l. Milk	☐	☐	☐	☐	☐	☐	☐	☐	☐	Milk
21m. Wine	☐	☐	☐	☐	☐	☐	☐	☐	☐	Wine
21n. Liquor (Strong alcohol)	☐	☐	☐	☐	☐	☐	☐	☐	☐	Liquor

21o. The source of cooking/drinking water

1 = Bottled Water
2 = Tap water
3 = Well Water
89 = Other sources
Mention_____

Occupation History

22. (a) What is your current job?

_____

_____

22. (b) What is your monthly income?

_____

23. What was your past job?

_____

_____

24. What are/were your main responsibilities/tasks?

_____
_____
_____

Health Status

25. Pathological History

_____
_____
_____
_____
_____

26.Allergy:_____
_____
_____
_____
_____

27.Drugs:_____
_____
_____
_____
_____
_____

28. Maximum blood pressure :_____49. Minimum blood pressure:_____

LABORATORY PARAMETERS (from hospital personal clinical records)

29.	Blood Glucose:	_____	38.	Gamma Glutanin Transpeptidases	_____
30.	Blood Nitrogen:	_____	39.	Alanine transaminase (ALT)	_____
31.	Blood Creatinine:	_____	40.	Erythrocyte sedimentation rate:	_____
32.	Potassium:	_____	41.	White Blood Cells:	_____
33.	Cholesterol:	_____	42.	Red Blood Cells	_____
34.	Creatinine in Urine:	_____	43.	Haemoglobin:	_____
35.	Triglycerides:	_____	44.	Hematocrit:	_____
36.	Aspatite (AST):	_____	45.	Platelets:	_____
37.	Urine Examination:	_____			

Annex III: Curriculum Vitae.

Biodata

Name:	Mr. Ezra J. Mrema
Date of birth:	2nd May 1970
Place of birth:	Moshi, Tanzania
Nationality:	Tanzanian
Languages:	English and Swahili

Affiliation

Muhimbili University of Health and Allied Sciences (MUHAS)
Department of Environmental and Occupational Health
School of Public Health and Social Sciences
P.O. Box 65015 Dar es Salaam, Tanzania
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Academic Qualifications

2010–2012: PhD student in Occupational Medicine and Industrial Hygiene, Università degli Studi di Milano, Italy. Thesis: "**Exposure Assessment of Persitent Organic Pollutants and Associated Health Risks among Italian Population"**. Supervisor: Prof. Claudio Colosio and Dr. Federico Maria Rubino.

1998–2001: Master of Science in Environmental Sciences - University of Dar es Salaam. Tanzania. Dissertation: **"Natural Products from *Monodora angolensis* Welw.** and ***Cleistochlamys kirkii* Benth. (Oliv.) as Potential Insecticides".** Supervisor: Prof. Mayunga H. H. Nkunya and Dr. Cosam C. Joseph.

1993–1997: Bachelor of Science in Education (Hons), University of Dar es Salaam, Tanzania.

Employment Records

Feb 2007 to date:	Assistant Lecturer Department of Environmental and Occupational Health, Muhimbili University of Health and Allied Sciences (MUHAS).
April 2006 – Jan. 2007:	Senior Quality Assurance Officer (Chemicals), Tanzania Bureau of Standards (TBS).
Jan.2002 – March 2006:	Research Scientist, Ifakara Health Research and Development Centre (IHRDC).

Feb – Oct 1998:	Biology Teacher**,** St. Anthony's Secondary School, Dar es Salaam, Tanzania.
Sept – Nov 1997:	Biology Teacher, Shaaban Robert Secondary School, Dar es Salaam, Tanzania.

Research Interests

- Risk and Exposure Assessment of Chemicals and the Health effects
- Environmental Chemistry
- Environmental and Biological Monitoring
- Environmental Impact Assessment

Major Works, Assignments and Consultancies

14 – 15 Sept. 2011: Facilitator in Awareness Workshop on Health Effects of Persistent Organic Pollutants (POPS) to Medical Practitioners organized by Tanzania Vice President's office Division of Environment under SAICM through Quick Start Programme support.

Review WHO documents on DDT Human Exposure and Health Hazards Assessment. The documents were published in 2011 under the title "Environmental health criteria: 241– DDT in indoor residual spraying: human health aspects."

Mar. – Dec. 2009: Consultant: ELISA Training support for Malaria Prevention and Control in Mainland Tanzania and Zanzibar Project, funded by Research Triangle International (RTI, International, USA).

Oct. 2004 – Apr. 2005: Consultant: Establishment of Baseline susceptibility levels of insecticides (deltamethrin, permethrine and DDT) to malaria and non–malaria mosquitoes. The consultancy was funded by National Institute for Medical Research, Tanzania (NIMR–Tanzania).

Aug. – Sept. 2001: Qualitative Interviews with Providers from Outlets Selling Drugs in three districts Kilombero, Ulanga and Rufiji, Tanzania supported by IMPACT project, CDC – Atlanta, USA.

June – July 2001: Qualitative interviews (key informant interviews and focused group discussions) with study subjects participated in the project titled: "The Rapid Qualitative Assessment of Factors that Influence Anti-malarial Drug Use Practice among Adults and School-Aged Children in Tanzania" funded by IMPACT project, CDC – Atlanta, USA.

Feb. – Apr. 2001: A local contact and collaborator of the Project entitled "Paleoclimate Evidence from Freshwater Wetlands in the Eastern African Rift". The project was conducted under Department of Geological Sciences, Rutgers University, USA.

Conferences, Workshops, Seminars and Training Courses

A Seminar on Occupational health and primary health care held in Azienda Ospedale San Paolo – Polo Universitario, Aula Curie – Settore Didattico, Milan – Italy, **3 July 2012**. The seminar was provided by **Dr Ivan Dimov Ivanov** Team Leader, WHO Global Occupational Health Programme.

A Seminar on Musculoskeletal Injury, Risk and Prevention in Agriculture held in Azienda Ospedale San Paolo – Polo Universitario, Aula Curie – Settore Didattico, Milan – Italy, **10 May 2012**. The Seminar was delivered by **John Rosecrance**, Associate Professor, Colorado State University, USA.

A Training Course in Health Risk Assessment (Pesticides, water quality and nanotechnology) given by Faculty of Health Science, University of Witwatersrand, South Africa, **22 Feb. – 1 Mar. 2012**.

The 47th Congress of the European Societies of Toxicology (EUROTOX 2011) held in Paris, France, **28 – 31 Aug. 2011.**

The Conference on **"Malaria: Stato Dell'Arte e Prospettive Future"** held in Centro Ospedaliero di Milano (Ospedale Militare) Via Simone Saint Bon, 7 Milano, Italy, **27 – 28 May 2011**.

A Short Course on Data Analysis by SPSS, Computer Lab, MUHAS, Dar es Salaam, Tanzania **1 – 5 May 2011.**

A 20th Anniversary of International Centre for Pesticides and Health Risk Prevention Risk Assessment of Chemicals, Aula Magna Ospedale Luigi Sacco, Milano, Italy, **11 Nov. 2010.**

The Conference entitled Nuove Sfide per La Medicina del Lavoro: Immigrazione-Promozione Della Salute, Facolta di Medicina e Chirurgia Università degli Studi di Brescia **6 Nov. 2010.**

The Conference entitled Le sfide ambientali: Attività e competenze dell'Università degli Studi di Milano, Sala Napoleonica Via Sant Antonio 12, Milano, Italy, **15 Feb. 2010.**

Convegno Regionale in Linee Guida per la Sorveglianza Sanitaria e la Prevenzione dei Rischi per la Salute e la Sicurezza nel Settore Cerealicolo, Sala Salvini in Fiera Agricolo Zootecnica Italiana, Centro Fiera Via Brescia129-25018 Montichiari (BS), **20 Feb. 2010.**

Attended 44 hours Epidemiology and Statistic Seminars (Elements of Statistics in Occupational Medicine) offered by Department of Occupational Health, Università degli Studi di Milano, Italy with a period from **Feb. – Dec. 2010.** The seminars were delivered by **Prof. Pieralberto Bertazzi** and **Dr. Dario Consonni**.

A Short Course in Introductory Epidemiology and Biostatistics, SOSMED Conference Hall, Dar es Salaam, Tanzania **20 April – 1 May 2009.**

The Training of Trainers Workshop for National Facilitators of Local Government Authority on HIV and AIDS on Community Capacity Enhancement, Bagamoyo, Tanzania **17 – 26 Nov. 2008**.

A Webster Memorial Seminar: Using Biological Monitoring to Reduce Occupational Exposure: Action to Improve Working Conditions, National Institute for Occupational Health (NIOH), Johannesburg, South Africa **30 Oct. 2008**.

Analytical Course given by Analytical Services Laboratory, National Institute for Occupational Health (NIOH) , Johannesburg, South Africa **10 – 30 Oct. 2008**.

International Course on Biological Monitoring: A Valuable Tool for a Healthier Workplace, Johannesburg, South Africa **20 – 21 Oct. 2008**.

A Training Course in Epidemiology and Field Research Methods given by Epidemiology and Public Health Sciences, Department of Public Health and Clinical Medicine, Umeå University, Sweden **2 – 14 June 2008**.

A Short Course on Ethical Issues In African Health Research, SOSMED Conference Center, Dar es Salaam, Tanzania **28 Feb. – 1 Mar. 2008.**

Stakeholders' Workshop on Reviewing Monitoring and Evaluation Tools for Occupational Health Services, NAM Hotel, Dodoma, Tanzania **5 – 9 June 2008.**

Training Course on Data Management, SOSMED Conference Center, Dar es Salaam, Tanzania **15 – 26 Oct. 2007.**

Training Course on Leadership in Health System, Bagamoyo Beach Resort, Coast region, Tanzania **9 – 17 Aug. 2007**

Training of Trainers for Urban Development and Environmental Planning (UDEM) National Anchor Institutions Module 2, Morogoro, Tanzania **2 – 6 July 2007.**

Training of Trainers for Urban Development and Environmental Planning (UDEM) National Anchor Institutions Module 1, Hotel Peakcock, Dar es salaam, Tanzania **28 May – 1 June 2007.**

The Fourth MIM Pan-African Malaria Conference, New Strategies against an Ancient Scourge, Palais des Congress, Youndé Cameroon **13 – 18 Nov. 2005.**

A Workshop on Critical Research Ethics Issues in the Era of HIV in Tanzania Clinical/Programmatic Track April **14 – 15 April 2005.**

AMANET Workshop on Malaria Vaccinology in Developing Countries Paradise Holiday Resort, Bagamoyo, Tanzania **14 – 18 March 2005.**

Basic Operation of Facscallibur, IHRDC, Ifakara – Morogoro **13 – 16 July 2004.**

Simple models of pathogen transmission for biologists and epidemiologists: a beginner's course using malaria as a motivating example, IHRDC, Ifakara **10 July 2004.**

Detection and Monitoring of Insecticide Resistance Among Malaria Vectors in the Context of Insecticide Treated Nets Scaling-Up in Tanzania, Hotel Kola Prieto, Tanga **24 – 26 Nov. 2003.**

Appointment

On 8 June 2010 I was appointed a member of scientific committee of the 3rd International Congress on Rural Health in Mediterranean and Balkan Countries held in Tirana, Albania **22 – 25 Sept. 2010**. I was responsible to prepare the congress programme.

Awards

October 1998 I was among ten recipients of scholarships for Masters Study from The Netherlands Organization for International Cooperation in Higher Organisation (NUFFIC) in its MHO programme through Faculty of Science at University of Dar es Salaam within the Environmental Science (ENVIRONS) project coordinated by **Dr. Felista Urasa.**

A Winner of two years Fellowship for the Collaborative Research following the competition launched on 26 December 2009 by the Rector's Act (n.265480). The award was made available by Italian Ministry of Health through Department of Occupational Medicine, "Clinica del Lavoro L. Devoto" of Università degli Studi di Milano, The research activities were within the programme "Esposizione occupazionale e ambientale a contaminanti organici persistenti e rischio per la salute" under supervision of **Prof. Colosio Claudio.** The award amounted **€**19,367.00 per annum.

A Winner of one year Fellowship for Collaborative Research following the competition launched on 18 January 2012 by the Rector's Act (n.276874). The award was made available by European Union project HEALTH VENT - DG SANCO through Department of Occupational Medicine, "Clinica del Lavoro L. Devoto" of Università degli Studi di Milano. The research activities were within the programme "Valutazione dei rischi in relazione a inguinamento dell'aria indoor" under supervision of **Prof. Paolo Carrer.** The award amounted **€**19,367.00 per annum.

Publications

Mrema EJ, Rubino FM, Brambilla G, Moretto A, Tsatsakis AM, Colosio C. Persistent organochlorinated pesticides and mechanisms of their toxicity. Toxicology 2012. doi:pii: S0300-483X(12)00412-X. 10.1016/j.tox.2012.11.015. [**Impact Factor 3.641**]

EJ Mrema, FM Rubino, S Mandic-Rajcevic, E Sturchio, R Turci, A Osculati, G Brambilla, C Minoia and C Colosio. Exposure to priority organo-chlorine contaminants in the Italian general population. Part 1. Eight priority organochlorinated pesticides (OCPs) in serum (Accepted for publication in Human and Experimental Toxicology) [**Impact Factor 1.772**].

EJ Mrema, FM Rubino, S Mandic-Rajcevic, E Sturchio, R Turci, A Osculati, G Brambilla, C Minoia and C Colosio. Exposure to priority organo-chlorine contaminants in the Italian general population. Part 2. Fifteen priority polychlorinated biphenyl congeners (PCBs) in blood serum (Accepted for publication in Human and Experimental Toxicology) [**Impact Factor 1.772**].

Kulkarni MA, Malima R, Mosha FW, Msangi S, **Mrema E,** Kabula B, Lawrence B, Kinung'hi S, Swilla J, Kisinza W, Rau ME, Miller JE, Schellenberg JA, Maxwell C, Rowland M, Magesa S, Drakeley C. Efficacy of pyrethroid-treated nets against malaria vectors and nuisance-biting mosquitoes in Tanzania in areas with long-term insecticide-treated net use. ***Tropical Medicine and International Health (2007) 12(9):1061–1073.*** **[Impact Factor 2.795].**

C Colosio, **E Mrema**, S Mandic Rajcevic, G Vianello, G Brambilla, FM Rubino. Plant protection products: New tools for exposure and risk assessment in agriculture

[Antiparassitari: Nuovi strumenti per la valutazione dell'esposizione e del rischio in ambito agricolo]. ***Giornale italiano di medicina del lavoro ed ergonomia 2012; 34: 393–397.***

Full Conference Papers

Malisa, A.L., Muggitu K., Ndejembi, M., **Mrema, E.,** Mayagaya, V., Urassa, H. Biotechnology in the Growing Momentum of Globalization: Inseparable Forces Affecting Public Health at a Global Scale. ***21st Annual Scientific Conference of the Tanzania Public Health Association held at Arusha International Conference Centre (AICC), Arusha, Tanzania, 4 – 8 Nov. 2002.***

Abstracts in Conferences

Mrema, E.J., R. Turci, F. Rubino, L. Fugnoli, M. Pitton, S. Mandic-Rajcevic, C. Colosio and C. Minoia. Serum Levels of Polychlorinated biphenyls (PCBs) and Organochorinated Pesticides (OCPs) among individuals of general population in three Italian Geographic Regions. ***Toxicology Letters 205S (2011) S60 – S179***. doi:10.1016/j.toxlet.2011.05.472.

C. Colosio, F. M. Rubino, L. Fugnoli, S. Mandic-Rajcevic, **E. J. Mrema**, G. Vianello, G. Brambilla. Risk assessment of pesticide use in agriculture is easier by integration of traditional monitoring approaches and new computational tools. ***30th International Congress on Occupational Health "Occupational Health for all: from research to practice", Cancun, Mexico, 18 – 23 Mar. 2012.***

Colosio C, Mandic-Rajcevic S, **Mrema E**, Rabozzi G, Rizzo R, Rubino F, Sokooti M, Somaruga C, Vellere F, Tabibi R, Brambilla G. Occupational Health and Safety in Agriculture: Current Situation and Objectives for The Future. ***5th Croatian Congress on Occupational Health with International Participation from 28 Sep. – 2 Oct. 2011.***

EJ Mrema, AL Malisa, SP Kachur, H Mshinda and S Abdulla Molecular Detection of *DHFR* and *DHPS* genes from Sporozoite Infected Mosquitoes. ***Acta Tropica 95S (2005) S1 – S506.***

Chapters

Ezra J. Mrema, Federico Rubino and Claudio Colosio. Chapter 238. Pesticide Residue-Organochlorines. (Accepted for publication to online Elservier Encyclopedia of Food Safety).

Federico M. Rubino, **Ezra. J. Mrema** and Claudio Colosio. Chapter 241. Fungicide Residues – Dithiocarbamates (Accepted for publication to online Elsevier Encyclopedia of Food Safety).

Mrema EJ, Rubino FM and Colosio C. Obsolete Pesticides – A Threat to Environment, Biodiversity and Human Health. *L. Simeonov, F. Macaev and B. Simeonova (Eds.),* Environmental Security Assessment and Management of Obsolete Pesticides in South East Europe, © *Springer Science+Business Media B.V. 2012*.

Membership

- Environment, Human Right Care and Gender Organization (ENVIROCARE)
- Young African Scientist Network Trust (YASNET)

Referees

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Declaration

I, the undersigned certify to the best of my knowledge and belief that the above information is true and correctly describe my qualification, experience and my traits.

Ezra J. Mrema

12 January 2013
Date

Annex IV: Individual personal characteristics of the examined subjects and results of individual PCB congeners' measurements (All PCB measurements are reported in pmol/g serum lipid).

PID	Sex	Age (years)	Weigh (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	PCB 31	PCB 28	PCB 52	PCB 101	PCB 77	PCB 118	PCB 105	PCB 153	PCB 138	PCB 126	PCB 156	PCB 128	PCB 180	PCB 169	PCB 170
1NF	M	44	76	183	22.69	526.9	9.29	6.30	3.25	2.91	3.25	30.38	2.91	256.13	163.10	2.91	22.47	2.63	193.28	2.63	85.26
2NF	M	45	74	180	22.84	488.4	31.40	34.09	3.51	3.14	3.51	58.58	3.14	206.74	104.56	3.14	32.09	2.84	196.61	2.84	38.94
3NF	M	57	78	174	25.76	480.4	4.04	4.04	3.56	3.19	3.56	69.57	3.19	449.70	289.39	3.19	32.24	2.88	414.53	2.88	139.28
4NF	F	52	58	157	23.53	780.8	6.65	6.97	2.19	1.96	2.19	86.70	1.96	352.80	262.04	1.96	31.22	1.77	191.19	1.77	70.86
5NF	M	55	74	168	26.22	513.3	13.69	17.08	3.34	2.98	3.34	78.39	2.98	336.00	222.98	2.98	60.65	2.70	258.21	2.70	111.62
6NF	M	44	101	175	32.98	601.1	7.00	6.01	2.85	2.55	2.85	47.67	2.55	188.85	132.37	2.55	12.36	2.31	117.56	2.31	40.77
7NF	M	47	81	180	25.00	513.8	3.78	8.53	3.33	2.98	3.33	70.03	2.98	292.64	215.56	2.98	26.06	2.70	197.96	2.70	84.51
8NF	M	54	71	168	25.16	594.8	7.76	4.91	2.88	2.58	2.88	64.36	2.58	312.17	205.85	2.58	36.99	2.33	277.17	2.33	97.63
10NF	F	60	65	163	24.46	571.3	3.40	11.08	3.00	2.68	3.00	79.52	2.68	357.04	256.64	2.68	33.61	2.42	278.68	2.43	101.94
11NF	F	54	55	155	22.89	541.5	3.59	4.32	3.16	2.83	3.16	70.06	2.83	308.05	230.13	2.83	22.81	2.56	206.60	2.56	80.94
12NF	M	36	87	177	27.77	586.8	13.70	17.65	2.92	2.61	2.92	64.79	2.61	249.76	171.55	2.61	19.32	2.36	158.07	2.36	70.19
13NF	F	32	58	168	20.55	437.3	21.77	10.37	3.92	3.50	3.92	82.47	3.50	227.43	159.56	3.50	15.04	3.17	119.83	3.17	45.90
14NF	M	40	82	176	26.47	507.2	3.83	3.83	3.38	3.02	3.38	49.43	3.02	285.83	219.13	3.02	27.95	2.73	168.89	2.73	72.07
15NF	F	39	56	165	20.57	515.5	3.77	3.77	3.32	2.97	3.32	41.30	2.97	129.04	110.85	2.97	16.13	2.69	108.80	2.69	54.04
16NF	F	39	80	175	26.12	440.1	4.41	4.41	3.89	3.48	3.89	69.26	3.48	288.06	192.21	3.48	49.92	3.15	135.00	3.15	72.58
17NF	F	31	64	163	24.09	505.8	3.84	3.84	3.39	3.03	3.39	59.81	3.03	172.36	120.90	3.03	12.67	2.74	94.77	2.74	40.26
18NF	M	52	88	170	30.45	857.6	2.26	2.26	2.00	1.79	2.00	65.64	1.79	419.78	289.79	1.79	34.05	1.62	213.84	1.62	86.14
19NF	M	38	80	180	24.69	509.3	88.50	53.33	3.36	102.27	3.36	180.92	133.07	355.36	235.29	3.01	63.04	17.60	210.95	2.72	99.60
20NF	F	39	65	160	25.39	457.0	4.25	4.25	3.75	3.35	3.75	72.99	3.35	273.70	175.15	3.35	23.39	3.03	190.12	3.03	82.70
21NF	F	48	70	170	24.22	411.0	4.72	4.72	4.17	3.73	4.17	174.77	3.73	571.47	392.73	3.73	58.81	3.37	309.94	3.37	128.78
22NF	M	43	73	170	25.26	648.4	8.64	11.17	2.64	2.36	2.64	39.28	2.36	130.68	96.98	2.36	16.88	2.14	131.23	2.14	46.70
23NF	F	57	69	168	24.45	741.3	2.62	2.62	2.31	2.07	2.31	96.65	2.07	629.41	387.13	2.07	57.79	1.87	352.28	1.87	136.74
24NF	F	56	70	168	24.80	654.6	2.97	2.97	2.62	2.34	2.62	108.44	2.34	477.11	318.92	2.34	41.73	2.12	309.37	2.12	113.45
25NF	F	45	50	159	19.78	459.3	4.23	4.23	3.73	3.33	3.73	93.86	3.33	499.06	337.33	3.33	53.11	3.02	329.47	3.02	156.58
26NF	F	53	55	163	20.70	868.9	2.23	7.56	1.97	1.76	1.97	70.12	1.76	302.32	190.88	1.76	36.78	1.59	233.53	1.59	95.53
27NF	F	40	62	159	24.52	521.1	3.73	3.73	3.29	2.94	3.29	70.14	2.94	371.61	240.64	2.94	37.90	2.66	250.79	2.66	92.63
28NF	M	38	97	191	26.59	550.0	3.53	8.75	3.11	2.79	3.11	54.99	2.79	208.23	142.50	2.79	15.93	2.52	166.74	2.52	48.72
29NF	M	40	78	180	24.07	640.8	3.03	16.89	2.67	2.39	2.67	27.96	2.39	102.42	76.65	2.39	13.35	2.16	93.31	2.16	18.51
30NF	F	55	59	157	23.94	430.8	4.51	4.51	3.97	3.56	3.98	136.42	3.56	688.34	464.93	3.56	61.71	3.22	458.71	3.22	183.90
31NF	F	50	75	167	26.89	505.9	3.84	3.84	3.38	3.03	3.38	80.46	3.03	446.01	317.85	3.03	31.58	2.74	296.52	2.74	120.83
32NF	M	45	83	176	26.79	617.0	3.15	3.15	2.78	2.48	2.78	11.71	2.48	180.71	125.51	2.48	2.25	2.25	95.53	2.25	46.85
33NF	F	36	58	163	21.83	465.6	4.17	4.17	3.68	3.29	3.68	56.21	3.29	106.16	81.14	3.29	2.98	2.98	80.53	2.98	5.89
34NF	M	44	78	178	24.62	466.6	4.16	8.27	3.67	47.84	3.67	115.61	95.27	396.35	275.19	48.61	82.71	52.76	277.44	55.78	129.83
35NF	F	26	49	160	19.14	422.9	7.79	15.21	4.05	3.62	4.05	98.09	3.62	138.07	117.67	3.62	15.66	3.28	102.54	3.28	29.66
36NF	F	29	65	170	22.49	515.9	3.76	3.76	3.32	2.97	3.32	86.81	2.97	228.50	162.38	2.97	13.50	2.69	144.32	2.69	47.79
37NF	M	31	77	178	24.30	679.4	2.86	2.86	2.52	2.25	2.52	10.63	2.25	186.62	92.74	2.25	15.43	2.04	75.65	2.04	31.99
38NF	F	39	52	160	20.31	587.5	8.19	22.94	2.91	2.61	2.91	204.98	85.56	511.53	290.74	2.61	42.26	2.36	830.16	2.36	92.38
39NF	F	37	60	168	21.26	600.8	63.13	56.99	56.65	97.80	55.47	121.94	109.53	214.07	143.61	2.55	31.25	2.31	102.16	2.31	45.75
40NF	F	30	65	158	26.04	422.9	4.59	4.59	4.05	3.62	4.05	61.47	3.62	138.20	94.49	3.62	3.28	3.28	87.81	3.28	35.99

PID	Sex	Age (years)	Weigh (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	PCB 31	PCB 28	PCB 52	PCB 101	PCB 77	PCB 118	PCB 105	PCB 153	PCB 138	PCB 126	PCB 156	PCB 128	PCB 180	PCB 169	PCB 170
41NF	F	47	50	168	17.72	500.9	22.74	40.51	3.42	3.06	21.86	83.29	3.06	319.08	208.17	114.66	60.00	2.77	233.20	327.73	109.19
42NF	F	34	64	168	22.68	518.6	3.74	3.74	3.30	2.95	3.30	51.01	2.95	157.81	114.64	2.95	21.51	2.67	132.32	2.67	42.14
43NF	M	50	82	173	27.40	598.7	15.73	14.72	2.86	2.56	2.86	44.04	2.56	146.71	111.52	2.56	15.89	2.31	106.91	2.31	55.57
44NF	F	48	68	157	27.59	598.7	3.24	3.24	2.86	2.56	2.86	58.58	2.56	202.02	168.89	2.56	20.05	2.31	164.60	2.31	60.32
45NF	F	28	63	160	24.61	462.0	4.20	4.20	3.71	3.32	3.71	37.97	3.32	69.87	70.96	3.32	10.55	3.00	57.98	3.00	24.52
46NF	F	43	58	164	21.56	511.9	3.79	3.79	3.35	2.99	3.35	54.88	2.99	208.38	149.78	2.99	17.19	2.71	176.52	2.71	64.18
47NF	F	46	62	155	25.81	616.0	3.15	3.15	11.81	2.49	2.78	48.93	2.49	254.56	178.65	2.49	30.25	2.25	193.80	2.25	73.34
48NF	M	46	92	172	31.10	657.1	2.95	9.15	2.61	2.33	2.61	71.77	2.33	343.80	253.03	2.33	24.57	2.11	203.96	2.11	80.70
49NF	M	21	70	175	22.86	578.1	3.36	3.36	2.96	2.65	2.96	32.15	2.65	52.38	55.55	2.65	3.78	2.40	39.97	2.40	18.00
50NF	F	31	63	168	22.32	386.9	5.02	5.02	23.98	56.03	4.43	58.51	3.96	57.16	54.10	3.96	5.05	3.58	57.97	3.58	20.75
51NF	M	52	80	170	27.68	540.1	17.58	9.92	3.17	2.84	3.17	92.59	2.84	26.63	461.90	2.84	92.05	2.57	276.15	2.57	114.61
52NF	M	48	80	174	26.42	525.0	3.70	3.70	3.26	2.92	3.26	55.43	2.92	418.81	274.36	2.92	30.73	2.64	317.38	2.64	117.98
53NF	M	43	75	172	25.35	1013.4	1.92	1.92	1.69	1.51	1.69	53.69	1.51	239.31	157.02	1.51	18.09	1.37	118.26	1.37	46.88
54NF	M	39	78	177	24.90	589.2	3.29	3.29	2.91	2.60	2.91	33.76	2.60	89.21	51.02	2.60	12.48	2.35	66.28	2.35	25.90
55NF	M	48	110	170	38.06	537.8	3.61	3.61	3.18	2.85	3.18	47.42	2.85	140.18	109.53	2.85	35.85	2.58	110.86	2.58	63.71
56NF	F	40	53	155	22.06	456.0	4.26	4.26	3.75	3.36	3.76	58.17	3.36	81.94	86.51	3.36	4.35	3.04	80.87	3.04	35.38
57NF	M	31	74	183	22.10	452.3	14.55	10.01	3.79	3.39	3.79	58.14	3.39	250.28	174.16	3.39	23.93	3.06	181.91	3.06	70.98
58NF	F	46	59	169	20.66	499.8	3.88	10.52	3.43	3.06	3.43	53.27	3.06	139.38	108.31	3.06	4.18	2.77	90.12	2.77	33.92
59NF	F	48	63	159	24.92	615.0	3.16	24.47	2.78	2.49	2.78	35.94	2.49	83.18	63.71	2.49	2.53	2.25	63.32	2.25	22.37
60NF	F	57	57	156	23.42	606.7	3.20	3.20	2.82	2.52	2.82	32.35	2.52	101.96	86.25	2.52	8.73	2.28	91.27	2.28	33.02
61NF	M	44	77	172	26.03	609.2	3.19	3.19	2.81	2.51	2.81	2.51	2.51	146.56	147.30	2.51	12.62	2.27	129.28	2.27	74.10
62NF	M	44	72	176	23.24	566.5	10.85	11.20	3.02	2.70	3.02	70.53	2.70	369.00	231.07	2.70	53.70	2.45	294.34	2.45	122.93
63NF	F	52	73	163	27.48	642.5	12.80	14.00	2.67	2.38	2.67	101.70	49.04	474.06	356.53	2.38	44.53	2.16	260.77	2.16	98.08
64NF	F	31	60	166	21.77	506.6	3.83	3.83	3.38	3.02	3.38	100.23	3.02	223.38	150.27	3.02	2.73	2.73	178.57	2.73	59.14
65NF	M	55	75	173	25.06	631.6	3.07	11.06	2.71	2.43	2.71	72.44	2.43	391.80	291.33	2.43	41.54	2.19	239.79	2.19	85.03
66NF	F	52	63	156	25.89	929.2	8.63	9.50	1.84	1.65	1.84	79.82	42.30	308.62	205.06	1.65	35.22	1.49	200.44	1.49	84.19
67NF	F	42	50	155	20.81	396.8	4.89	19.53	4.32	3.86	4.32	100.85	3.86	187.43	134.33	3.86	16.74	3.49	178.41	3.49	81.59
68NF	F	40	53	160	20.70	793.4	2.45	2.45	2.16	1.93	2.16	116.87	1.93	327.47	200.18	1.93	23.51	1.75	200.70	1.75	81.94
69NF	F	20	43	160	16.80	521.4	18.81	28.02	3.28	2.94	3.28	87.47	2.94	217.33	193.30	2.94	20.51	2.66	116.69	2.66	50.08
70NF	M	42	69	175	22.53	538.5	3.61	3.61	3.18	2.84	3.18	61.27	2.84	883.58	606.96	2.84	95.65	2.57	551.37	2.57	222.32
71NF	F	44	60	152	25.97	418.5	4.64	4.64	4.09	3.66	4.09	546.59	209.51	675.38	684.58	3.66	90.32	3.31	238.93	3.31	110.45
72NF	F	46	49	150	21.78	566.8	3.43	3.43	3.02	2.70	3.02	87.84	2.70	488.86	292.07	2.70	35.58	2.44	280.63	2.44	106.38
73NF	M	40	85	178	26.83	484.8	4.00	11.21	3.53	3.16	3.53	89.80	38.78	314.46	222.48	3.16	19.35	2.86	199.17	2.86	81.38
74NF	F	51	49	156	20.13	536.1	3.62	18.17	3.19	2.86	3.19	128.51	2.86	574.47	358.78	2.86	49.95	2.58	366.62	2.58	132.13
75NF	M	40	85	180	26.23	575.5	3.37	3.37	2.98	2.66	2.98	48.51	2.66	231.09	149.59	2.66	24.57	2.41	190.32	2.41	84.78
76NF	F	30	52	167	18.65	494.9	3.92	3.92	3.46	3.10	3.46	3.10	3.10	189.49	132.18	3.10	13.73	2.80	133.83	2.80	57.09
77NF	M	35	70	170	24.22	483.5	4.02	4.02	3.54	3.17	3.54	3.17	3.17	243.17	236.18	3.17	68.22	2.87	255.97	2.87	158.04
78NF	F	48	55	170	19.03	506.2	7.98	12.26	3.38	3.03	3.38	107.78	3.03	291.79	191.22	3.03	26.26	2.74	214.04	2.74	89.16
79NF	M	45	86	186	24.86	412.1	4.71	4.71	4.16	3.72	4.16	94.59	3.72	386.07	227.80	3.72	36.57	3.36	263.50	3.36	102.60
80NF	F	41	57	165	20.94	557.8	3.48	3.48	3.07	2.75	3.07	56.60	2.75	275.95	205.36	2.75	24.76	2.48	194.77	2.48	80.32
81NF	M	57	60	175	19.59	430.4	16.10	14.79	3.98	3.56	3.98	63.72	3.56	390.31	200.11	3.56	76.20	3.22	412.25	3.22	145.84
82NF	M	29	98	177	31.28	376.3	27.89	24.53	4.55	4.07	4.55	71.81	4.07	130.77	99.46	4.07	13.55	3.68	84.65	3.68	20.97
83NF	F	42	60	167	21.51	625.1	30.98	14.64	2.74	2.45	2.74	78.31	2.45	202.82	247.99	2.45	44.36	2.22	165.14	2.22	52.15

PID	Sex	Age (years)	Weigh (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	PCB 31	PCB 28	PCB 52	PCB 101	PCB 77	PCB 118	PCB 105	PCB 153	PCB 138	PCB 126	PCB 156	PCB 128	PCB 180	PCB 169	PCB 170
84NF	M	56	69	172	23.32	899.2	9.61	5.74	1.90	1.70	1.90	50.83	1.70	469.37	255.71	1.70	52.59	1.54	322.61	1.54	112.48
85NF	M	59	78	171	26.67	338.6	5.73	20.29	5.06	4.52	5.06	489.50	180.10	668.47	530.80	4.52	79.32	4.09	955.30	4.09	278.76
86NF	F	38	52	162	19.81	639.6	13.61	43.86	2.68	2.39	2.68	83.98	2.39	295.47	204.27	2.39	21.82	2.17	176.31	2.17	70.14
87NF	M	38	60	173	20.05	499.3	16.08	18.37	3.43	3.07	3.43	38.07	3.07	199.61	116.69	3.07	12.68	2.78	202.97	2.78	84.28
88NF	M	46	85	186	24.57	401.3	4.84	19.92	4.27	3.82	4.27	155.06	3.82	769.99	485.10	3.82	83.22	3.45	522.28	3.45	187.97
89NF	M	43	85	180	26.23	950.5	2.04	2.04	11.10	1.61	1.80	26.97	1.61	173.88	118.14	1.61	19.28	1.46	127.97	1.46	55.42
90NF	M	28	71	170	24.57	550.8	18.31	11.32	3.11	2.78	3.11	47.68	2.78	118.74	98.33	2.78	12.78	2.52	72.70	2.52	16.15
91NF	M	48	84	174	27.74	677.5	14.01	14.98	15.37	2.26	2.53	78.26	2.26	436.52	245.32	2.26	47.78	2.05	292.91	2.05	101.36
92NF	F	54	62	156	25.48	566.2	42.81	34.98	30.76	2.71	3.02	106.27	2.71	311.79	206.69	2.71	42.42	2.45	237.44	2.45	106.81
93NF	M	44	90	180	27.78	598.6	3.24	3.24	2.86	2.56	2.86	91.95	2.56	186.75	141.47	2.56	17.82	2.31	107.29	2.31	70.46
94NF	M	41	60	167	21.51	514.1	3.78	3.78	3.33	2.98	3.33	53.23	2.98	203.84	144.43	2.98	20.34	2.69	159.18	2.69	55.47
95NF	F	26	55	168	19.49	601.2	22.22	27.94	20.65	2.55	2.85	91.90	2.55	157.66	116.43	2.55	15.04	2.30	98.77	2.30	27.81
96NF	F	48	52	167	18.65	520.5	3.73	18.45	3.29	2.94	3.29	82.95	2.94	356.14	216.18	2.94	43.21	2.66	297.46	2.66	99.67
97NF	M	48	53	164	19.71	556.5	3.49	66.72	153.09	2.75	3.08	108.22	2.75	323.10	206.15	2.75	71.76	2.49	229.90	2.49	85.48
98NF	M	21	75	180	23.15	409.4	4.74	4.74	4.18	3.74	4.18	3.74	3.74	110.36	79.22	3.74	24.75	3.38	77.50	3.38	23.58
99NF	M	21	92	185	26.88	406.0	11.94	11.58	4.22	3.77	4.22	3.77	3.77	52.36	51.15	3.77	3.41	3.41	34.58	3.41	3.12
100NF	M	28	87	186	25.15	376.7	5.15	5.15	59.99	4.07	4.55	4.07	4.07	113.89	101.64	4.07	3.68	3.68	73.92	3.68	18.93
101NF	F	42	55	164	20.45	614.1	19.89	11.37	7.08	2.49	2.79	81.91	2.49	189.56	125.96	2.49	2.26	2.26	132.66	2.26	56.12
102NF	M	58	92	187	26.31	446.3	29.78	4.35	3.84	3.43	3.84	45.04	3.43	390.68	215.23	3.43	3.10	3.10	385.54	3.10	142.73
103NF	M	19	76	174	25.10	330.6	5.87	5.87	5.18	4.63	5.18	4.63	4.63	293.43	189.85	4.63	4.19	4.19	155.17	4.19	84.61
104NF	F	53	59	158	23.63	525.4	5.90	16.43	3.26	2.92	3.26	82.79	2.92	405.24	298.18	2.92	23.16	2.64	219.63	2.64	83.13
105NF	F	37	48	160	18.75	578.4	6.59	3.36	2.96	2.65	2.96	39.19	2.65	166.49	92.89	2.65	13.33	2.40	148.50	2.40	54.43
106NF	F	28	53	167	19.00	567.9	6.57	11.06	3.02	2.70	3.02	53.12	2.70	161.06	122.27	2.70	19.39	2.44	96.75	2.44	44.36
107NF	M	48	72	168	25.51	806.5	6.46	11.22	2.12	1.90	2.12	63.33	1.90	294.22	169.58	1.90	27.92	1.72	206.37	1.72	82.57
108NF	M	55	65	170	22.49	554.7	5.70	14.30	3.09	2.76	3.09	51.90	2.76	351.62	135.38	2.76	32.95	2.50	229.84	2.50	73.08
109NF	F	29	68	155	28.30	471.5	7.60	30.54	3.63	3.25	3.63	151.42	3.25	327.27	199.68	3.25	6.35	2.94	146.33	2.94	68.16
110NF	F	19	45	170	15.57	362.5	6.74	7.96	4.72	4.23	4.72	4.23	4.23	120.07	95.60	4.23	3.82	3.82	89.60	3.82	35.23
111NF	M	50	84	164	31.23	765.4	3.26	2.54	2.24	2.00	4.73	57.82	40.20	352.26	249.06	2.00	86.96	18.50	299.32	60.81	169.20
112NF	M	54	90	175	29.39	612.6	4.60	3.17	2.80	2.50	2.80	80.92	2.50	372.21	208.76	2.50	34.26	2.26	259.76	2.26	106.05
113NF	F	37	50	162	19.05	529.9	6.33	14.44	3.23	2.89	3.23	46.25	2.89	178.64	113.31	2.89	28.84	2.61	158.19	32.36	81.18
114NF	M	53	83	170	28.72	602.5	4.35	18.83	2.84	2.54	2.84	114.27	2.54	654.45	455.15	2.54	53.47	2.30	430.12	2.30	170.37
115NF	M	35	63	168	22.32	538.3	4.63	5.75	3.18	2.85	3.18	90.00	2.85	316.22	172.40	2.85	23.49	2.57	169.04	2.57	69.78
116NF	F	33	48	160	18.75	527.1	3.68	11.92	3.25	2.91	3.25	68.06	2.91	209.43	141.70	2.91	26.37	2.63	163.35	2.63	70.83
117NF	F	40	50	155	20.81	594.1	4.32	11.22	2.88	2.58	2.88	50.07	2.58	292.51	159.01	2.58	23.88	2.33	225.86	2.33	89.18
118NF	F	34	72	160	28.13	518.2	3.75	3.75	3.30	2.96	3.30	118.29	2.96	157.42	111.90	2.96	12.45	2.67	75.10	2.67	40.65
119NF	M	28	80	173	26.73	596.3	3.26	3.26	2.87	2.57	2.87	43.11	2.57	169.85	94.53	2.57	2.32	2.32	94.27	2.32	44.00
120NF	F	28	57	162	21.72	411.0	17.95	33.51	4.17	3.73	4.17	78.26	3.73	151.43	98.95	3.73	16.17	3.37	104.63	3.37	43.46
121NF	F	59	69	155	28.72	699.7	4.18	2.91	2.45	2.19	2.45	50.84	2.19	326.34	191.59	2.19	27.11	1.98	216.63	1.98	79.01
122NF	M	48	93	178	29.35	504.2	3.85	3.85	3.40	3.04	3.40	41.98	3.04	327.91	200.00	3.04	27.95	2.75	256.76	2.75	96.99
123NF	F	19	51	165	18.73	505.9	6.44	14.43	3.38	3.03	3.38	3.03	3.03	54.92	48.61	3.03	2.74	2.74	52.21	2.74	33.04
124NF	F	44	68	169	23.81	434.6	4.47	4.47	3.94	3.52	3.94	98.18	3.52	382.43	258.49	3.52	27.98	3.19	219.42	3.19	119.33
125NF	M	37	76	175	24.82	520.0	3.73	3.73	3.29	2.95	3.29	98.36	2.95	394.32	234.69	2.95	34.67	2.66	216.40	2.66	108.20
126NF	F	33	63	162	24.01	412.7	4.70	4.70	4.15	3.71	4.15	99.16	3.71	140.07	130.19	3.71	3.36	3.36	88.47	3.36	38.11

PID	Sex	Age (years)	Weigh (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	PCB 31	PCB 28	PCB 52	PCB 101	PCB 77	PCB 118	PCB 105	PCB 153	PCB 138	PCB 126	PCB 156	PCB 128	PCB 180	PCB 169	PCB 170
127NF	M	52	82	177	26.17	762.1	2.55	2.55	2.25	2.01	2.25	89.72	2.01	332.70	204.73	2.01	34.74	1.82	200.83	1.82	76.01
128NF	M	30	88	178	27.77	787.6	10.25	14.24	2.17	1.94	2.17	87.35	1.94	152.63	93.87	1.94	14.51	1.76	76.48	1.76	20.03
129NF	F	61	62	151	27.19	913.8	2.12	2.12	1.87	1.68	1.87	26.02	1.68	115.48	74.33	1.68	19.43	1.52	87.24	1.52	37.58
130NF	F	61	56	167	20.08	635.2	3.06	3.06	2.70	2.41	2.70	75.19	2.41	241.13	134.99	2.41	41.52	2.18	289.32	2.18	108.28
131NF	M	33	100	182	30.19	604.9	3.53	15.98	2.83	2.53	2.83	52.99	2.53	250.77	154.11	2.53	15.74	2.29	135.85	2.29	48.36
132NF	F	42	62	167	22.23	402.1	4.83	4.83	4.26	3.81	4.26	113.38	3.81	411.54	242.68	3.81	31.56	3.45	239.03	3.45	100.20
133NF	F	38	55	165	20.20	537.3	3.61	3.61	3.19	2.85	3.19	79.48	2.85	150.49	112.58	2.85	2.58	2.58	115.24	2.58	55.99
134NF	F	40	48	160	18.75	437.7	5.75	25.87	3.91	3.50	3.91	122.91	3.50	462.87	282.89	3.50	39.10	3.17	276.03	3.17	93.47
135NF	F	40	56	162	21.34	500.7	6.01	11.54	3.42	3.06	3.42	82.57	3.06	326.85	189.02	3.06	31.10	2.77	214.98	2.77	80.26
136NF	M	56	100	185	29.22	495.5	3.92	3.92	3.46	3.09	3.46	96.45	3.09	786.67	496.53	3.09	64.32	2.80	635.17	2.80	237.82
137NF	F	43	66	172	22.31	479.3	4.05	4.05	3.57	3.20	3.57	94.97	3.20	397.74	221.71	3.20	31.32	2.89	234.58	2.89	104.73
138NF	M	51	94	181	28.69	658.8	2.95	2.95	2.60	2.33	2.60	101.93	2.33	468.83	268.95	2.33	32.42	2.10	278.62	2.10	112.37
139NF	M	55	80	172	27.04	423.7	4.58	4.58	4.04	3.61	4.04	67.71	3.61	541.78	358.39	3.61	46.52	3.27	361.88	3.27	133.23
140NF	M	61	55	165	20.20	1001.6	4.25	8.92	1.71	1.53	1.71	105.79	1.53	466.00	297.97	1.53	39.23	1.38	303.61	1.38	124.90
141NF	M	38	83	164	30.86	539.6	9.75	21.13	3.17	2.84	3.17	74.12	2.84	224.69	172.49	2.84	2.57	2.57	130.27	2.57	51.84
142NF	F	38	49	156	20.13	593.0	3.27	3.27	2.89	2.58	2.89	65.04	2.58	293.75	170.08	2.58	23.32	2.34	180.63	2.34	72.71
143NF	M	39	74	166	26.85	525.3	4.69	13.45	3.26	2.92	3.26	95.19	2.92	324.46	170.77	2.92	30.18	2.64	188.19	2.64	69.06
144NF	M	45	76	174	25.10	567.5	3.42	12.81	3.02	2.70	3.02	94.17	2.70	397.15	243.49	2.70	31.49	2.44	228.35	2.44	82.06
145NF	F	38	57	163	21.45	458.9	7.87	21.78	3.73	3.34	3.73	100.69	3.34	408.74	288.09	3.34	51.05	3.02	246.13	3.02	96.22
146NF	F	48	50	154	21.08	878.3	4.24	11.48	1.95	1.74	1.95	52.72	1.74	213.39	150.51	1.74	26.71	1.58	128.46	1.58	50.40
147NF	M	38	88	183	26.28	767.7	2.53	2.53	2.23	2.00	2.23	62.77	2.00	245.45	137.92	2.00	29.55	1.80	174.14	1.80	77.22
148NF	F	37	60	164	22.31	477.5	4.07	4.07	3.59	3.21	3.59	3.21	3.21	77.23	54.90	3.21	2.90	2.90	42.37	2.90	2.65
149NF	F	32	60	170	20.76	484.1	4.01	4.01	3.54	3.16	3.54	106.19	3.16	228.17	158.75	3.16	2.86	2.86	153.15	2.86	70.15
150NF	F	54	65	160	25.39	532.1	3.65	3.65	3.22	2.88	3.22	74.75	2.88	208.76	118.78	2.88	25.64	2.60	151.30	2.60	73.41
151NF	F	61	55	160	21.48	580.9	3.34	3.34	2.95	2.64	2.95	200.23	2.64	846.29	499.95	2.64	78.15	2.39	415.77	2.39	170.28
152NF	M	33	93	181	28.39	638.4	10.12	25.79	2.68	2.40	2.68	47.49	2.40	200.17	156.88	2.40	2.17	2.17	131.68	2.17	47.79
153NF	M	47	78	170	26.99	711.0	2.73	2.73	2.41	2.15	2.41	79.51	2.15	198.85	146.66	2.15	1.95	1.95	124.10	1.95	53.43
154NF	M	35	91	181	27.78	654.2	6.26	17.35	2.62	2.34	2.62	2.34	2.34	157.08	151.84	2.34	2.12	2.12	100.11	2.12	45.07
155NF	M	27	63	172	21.30	453.6	4.28	4.28	3.78	3.38	3.78	3.38	3.38	145.15	58.80	3.38	3.05	3.05	101.99	3.05	18.34
156NF	M	26	54	158	21.63	473.1	8.87	15.82	3.62	3.24	3.62	3.24	3.24	176.87	127.31	3.24	32.17	2.93	125.76	2.93	57.50
157NF	F	53	80	156	32.87	647.1	3.00	3.00	2.65	2.37	2.65	106.94	2.37	406.93	312.95	2.37	40.93	2.14	212.97	2.14	78.12
158NF	M	59	75	173	25.06	786.9	2.47	20.23	2.18	1.95	2.18	40.99	1.95	243.44	173.23	1.95	33.86	1.76	231.93	1.76	99.45
159NF	M	27	74	179	23.10	566.2	8.16	10.65	3.02	2.71	3.02	2.71	2.71	125.41	95.79	2.71	2.45	2.45	71.80	2.45	33.74
160NF	M	35	75	176	24.21	574.0	3.38	3.38	2.98	2.67	2.98	2.67	2.67	327.21	187.28	2.67	47.60	2.41	198.93	2.41	103.85
161NF	F	50	79	160	30.86	466.5	4.16	4.16	3.67	3.28	3.67	147.74	3.28	348.06	253.54	3.28	2.97	2.97	181.95	2.97	80.76
162NF	M	41	82	181	25.03	700.4	2.77	2.77	2.44	2.19	2.44	78.87	2.19	352.78	230.09	2.19	29.66	1.98	233.23	1.98	94.81
163NF	M	53	84	173	28.07	490.5	3.96	3.96	3.49	3.12	3.49	3.12	3.12	315.92	236.71	3.12	2.82	2.82	200.72	2.82	105.29
1PV	M	32	68	175	22.20	417	4.65	4.65	31.09	3.67	4.11	3.67	3.67	405.17	265.35	3.67	5.73	3.32	267.21	3.32	101.22
2PV	M	35	69	169	24.16	468	8.51	4.15	15.31	3.27	3.66	3.27	3.27	273.86	226.75	3.27	8.44	2.96	163.37	2.96	56.73
3PV	F	32	46	152	19.91	740	2.62	2.62	21.92	2.07	2.31	2.07	2.07	169.66	127.26	2.07	7.80	1.87	97.22	1.87	22.55
4PV	F	27	59	155	24.56	487	3.99	3.99	3.52	3.15	3.52	3.15	3.15	163.98	131.32	3.15	2.84	2.84	81.32	2.84	26.80
5PV	M	51	98	179	30.58	586	3.31	3.31	22.57	2.61	2.92	2.61	2.61	473.63	315.45	2.61	34.79	2.36	432.58	2.36	123.61
6PV	M	40	78	174	25.76	498	3.90	3.90	11.76	3.08	3.44	3.08	3.08	629.49	457.51	3.08	39.36	2.78	377.89	2.78	127.62

PID	Sex	Age (years)	Weigh (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	PCB 31	PCB 28	PCB 52	PCB 101	PCB 77	PCB 118	PCB 105	PCB 153	PCB 138	PCB 126	PCB 156	PCB 128	PCB 180	PCB 169	PCB 170
7PV	F	27	45	155	18.73	588	3.30	3.30	9.80	2.61	2.91	2.61	2.61	153.04	131.93	2.61	2.36	2.36	77.70	2.36	18.53
8PV	M	48	73	174	24.11	405	4.79	4.79	17.79	3.78	4.23	549.22	174.94	710.54	644.95	3.78	81.84	3.42	437.48	3.42	127.09
9PV	M	46	75	180	23.15	455	4.26	4.26	29.93	3.36	3.76	3.36	3.36	343.05	223.38	3.36	3.04	3.04	261.75	3.04	101.94
10PV	F	36	48	162	18.29	484	9.85	23.54	41.14	3.17	3.54	3.17	3.17	340.96	207.31	3.17	18.07	2.86	263.91	2.86	88.10
11PV	M	23	68	170	23.53	478	4.06	4.06	25.92	3.20	3.58	3.20	3.20	203.45	155.99	3.20	4.37	2.90	91.61	2.90	29.67
12PV	M	40	104	182	31.40	347	5.60	5.60	24.99	4.42	4.94	4.42	4.42	650.03	504.89	4.42	28.48	3.99	394.34	3.99	123.43
13PV	F	40	54	164	20.00	414	4.69	4.69	29.64	3.70	4.14	3.70	3.70	468.36	322.96	3.70	29.63	3.35	304.18	3.35	106.46
14PV	M	27	66	174	21.80	542	3.58	3.58	25.45	2.82	3.16	2.82	2.82	119.47	87.02	2.82	2.55	2.55	62.20	2.55	16.14
15PV	M	41	131	188	37.06	439	4.42	4.42	24.85	3.49	3.90	3.49	3.49	342.15	259.07	3.49	3.16	3.16	207.81	3.16	70.14
16PV	M	41	65	179	20.29	583	3.33	3.33	25.22	2.63	2.94	2.63	2.63	244.96	173.53	2.63	16.49	2.38	208.43	2.38	57.20
17PV	M	63	79	167	28.33	472	4.11	4.11	28.71	3.24	3.62	3.24	3.24	1009.33	738.82	3.24	70.88	2.93	737.09	2.93	230.61
18PV	M	71	85	176	27.44	346	5.62	66.00	39.76	4.43	4.95	844.94	205.70	1703.82	1222.77	4.43	62.66	4.01	966.42	4.01	276.00
19PV	F	56	74	154	31.20	502	3.87	58.76	36.85	3.05	3.41	406.90	3.05	1159.78	769.68	3.05	102.46	2.76	632.39	2.76	210.93
20PV	M	59	97	172	32.79	509	3.81	3.81	3.36	3.01	3.36	3.01	3.01	474.05	394.64	3.01	38.53	2.72	341.68	2.72	100.87
21PV	M	52	89	176	28.73	559	3.47	3.47	57.16	2.74	3.06	2.74	2.74	659.46	545.61	2.74	42.43	2.48	334.49	2.48	90.50
22PV	M	40	83	182	25.05	535	3.63	3.63	40.06	2.86	3.20	2.86	2.86	330.32	270.20	2.86	2.59	2.59	72.67	2.59	67.03
23PV	M	45	68	165	24.98	608	3.19	3.19	21.16	2.52	2.82	2.52	2.52	735.92	514.58	2.52	2.28	2.28	462.59	2.28	143.90
24PV	M	38	81	172	27.38	509	3.81	3.81	3.36	3.01	3.36	3.01	3.01	792.22	534.62	3.01	46.51	2.72	470.17	2.72	154.48
25PV	M	52	77	168	27.28	475	4.09	4.09	14.93	3.23	3.61	3.23	3.23	777.69	520.28	3.23	42.96	2.92	414.52	2.92	135.00
26PV	M	42	69	182	20.83	514	3.78	3.78	31.86	2.98	3.33	2.98	2.98	317.38	241.27	2.98	36.53	2.70	243.79	2.70	80.72
27PV	F	50	50	155	20.81	484	4.01	4.01	24.63	3.17	3.54	3.17	3.17	455.86	289.91	3.17	40.99	2.87	231.91	2.87	60.30
28PV	F	50	62	150	27.55	576	3.37	3.37	31.71	2.66	2.98	2.66	2.66	354.62	336.03	2.66	35.00	2.41	202.71	2.41	58.43
29PV	M	40	80	173	26.73	542	3.58	3.58	60.65	2.82	3.16	2.82	2.82	313.39	243.39	2.82	29.35	2.56	172.48	2.56	69.90
30PV	M	40	118	175	38.53	649	2.99	2.99	16.52	2.36	2.64	2.36	2.36	241.90	226.74	2.36	2.14	2.14	47.14	2.14	40.10
31PV	M	35	85	185	24.83	524	3.71	3.71	28.84	2.92	3.27	2.92	2.92	292.85	234.13	2.92	2.64	2.64	122.82	2.65	30.33
32PV	M	58	89	175	29.06	422	4.61	4.61	23.64	3.63	4.06	3.63	3.63	503.75	378.47	3.63	61.98	3.29	362.41	3.29	67.54
33PV	M	56	88	178	27.77	502	3.87	3.87	3.41	3.05	3.41	3.05	3.05	725.56	602.04	3.05	72.07	2.76	497.20	2.76	181.01
34PV	M	64	68	170	23.53	412	4.72	4.72	4.16	3.72	4.16	3.72	3.72	579.26	595.16	3.72	94.72	3.36	623.07	3.37	202.67
35PV	M	31	87	187	24.88	466	4.16	4.16	3.67	3.28	3.67	3.28	3.28	232.57	195.21	3.28	2.97	2.97	111.60	2.97	39.35
36PV	M	34	72	175	23.51	485	4.00	4.00	3.53	3.16	3.53	3.16	3.16	265.78	271.63	3.16	20.48	2.85	133.51	2.85	75.46
37PV	M	64	68	165	24.97	557	3.48	3.48	33.97	2.75	3.07	2.75	2.75	604.29	457.72	2.75	56.22	2.49	282.32	2.49	84.88
38PV	M	58	67	164	24.91	451	4.31	4.31	43.84	3.40	3.80	3.40	3.40	649.29	542.29	3.40	33.21	3.07	363.71	3.07	106.62
39PV	F	34	48	154	20.24	615	3.15	3.15	2.78	2.49	2.78	2.49	2.49	290.96	223.41	2.49	10.80	2.25	136.76	2.25	45.50
40PV	F	39	59	156	24.24	306	6.35	6.35	5.60	5.01	5.60	5.01	5.01	456.84	451.46	5.01	33.01	4.53	216.21	4.53	67.57
41PV	F	42	50	155	20.81	494	3.93	3.93	24.21	3.10	3.46	3.10	3.10	417.37	374.27	3.10	14.79	2.80	224.85	2.80	53.37
42PV	M	53	60	167	21.51	507	3.83	3.83	44.20	3.02	3.38	3.02	3.02	536.17	388.64	3.02	64.62	2.73	391.37	2.73	121.53
43PV	M	37	70	175	22.86	531	3.65	3.65	33.18	2.88	3.22	2.88	2.88	175.99	165.44	2.88	3.21	2.61	76.43	2.61	25.18
44PV	M	25	65	173	21.72	581	3.34	3.34	20.39	2.64	2.95	2.64	2.64	161.81	129.64	2.64	13.12	2.39	75.62	2.39	35.04
45PV	M	39	79	168	28.00	788	2.46	2.46	15.34	1.94	2.17	1.94	1.94	202.40	161.46	1.94	1.76	1.76	101.93	1.76	33.70
46PV	M	48	85	163	31.99	612	3.17	3.17	36.02	2.50	2.80	2.50	2.50	760.08	595.10	2.50	61.98	2.26	485.83	2.26	150.11
47PV	F	29	59	166	21.41	627	45.60	31.04	91.12	2.44	2.73	2.44	2.44	200.96	168.83	2.44	2.21	2.21	70.83	2.21	36.59
48PV	F	30	48	154	20.24	518	51.59	48.93	91.42	2.96	3.31	2.96	2.96	207.50	194.09	2.96	2.68	2.68	62.82	2.68	35.35
49PV	F	52	50	163	18.82	416	52.47	61.16	136.30	3.69	4.12	3.69	3.69	355.45	323.01	3.69	30.77	3.33	299.46	3.33	101.43

PID	Sex	Age (years)	Weigh (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	PCB 31	PCB 28	PCB 52	PCB 101	PCB 77	PCB 118	PCB 105	PCB 153	PCB 138	PCB 126	PCB 156	PCB 128	PCB 180	PCB 169	PCB 170
50PV	F	25	48	165	17.63	566	53.61	46.77	89.03	2.71	3.03	2.71	2.71	250.96	202.93	2.71	62.03	2.45	49.41	2.45	35.82
51PV	M	53	70	165	30.80	626	3.10	3.10	25.07	2.45	2.74	2.45	2.45	270.37	193.57	2.45	19.26	2.21	132.98	2.21	37.61
52PV	M	39	78	177	24.89	474	4.09	4.09	30.15	3.23	3.61	3.23	3.23	315.38	237.62	3.23	2.92	2.92	189.47	2.92	52.48
53PV	M	33	89	187	25.45	541	3.59	3.59	27.97	2.83	3.17	2.83	2.83	262.56	128.64	2.83	60.40	2.56	62.30	2.56	15.74
54PV	M	45	64	167	22.94	728	2.67	2.67	42.42	2.11	2.35	2.11	2.11	260.15	233.78	2.11	6.99	1.90	133.46	1.90	54.28
55PV	M	44	100	169	35.00	637	3.05	3.05	2.69	2.41	2.69	2.41	2.41	531.48	442.84	2.41	49.22	2.18	262.73	2.18	73.04
56PV	M	35	72	170	24.91	382	5.09	5.09	35.09	4.01	4.49	4.01	4.01	235.35	227.55	4.01	3.63	3.63	126.46	3.63	47.84
57PV	M	45	65	171	22.23	407	4.78	4.78	53.64	3.77	4.21	3.77	3.77	567.49	424.82	3.77	24.98	3.41	334.86	3.41	145.26
58PV	M	34	87	172	29.41	309	6.29	6.29	61.34	4.96	5.55	4.96	4.96	490.49	531.02	4.96	4.49	4.49	227.31	4.49	116.49
59PV	M	41	69	166	25.04	519	3.74	3.74	56.47	2.95	3.30	2.95	2.95	271.54	214.49	2.95	2.67	2.67	134.78	2.67	46.26
60PV	F	34	65	165	23.87	459	4.23	4.23	64.99	3.33	3.73	3.33	3.33	297.58	282.74	3.33	3.02	3.02	136.47	3.02	60.32
61PV	M	35	77	175	25.14	795	98.28	94.23	155.69	1.93	2.15	1.93	1.93	202.86	121.62	1.93	17.47	1.74	148.64	1.74	57.09
62PV	M	21	78	183	23.29	429	132.03	97.11	175.43	3.57	3.99	3.57	3.57	143.01	106.86	3.57	3.23	3.23	71.78	3.23	42.79
63PV	M	42	78	176	25.18	453	4.28	4.28	61.24	3.38	3.78	3.38	3.38	755.80	486.83	3.38	84.36	3.06	553.93	3.06	225.81
64PV	M	32	83	185	24.25	465	185.59	141.90	280.04	3.29	3.68	3.29	3.29	277.65	190.40	3.29	12.56	2.98	146.84	2.98	63.26
65PV	M	54	80	175	26.12	466	217.09	148.64	284.49	3.29	3.68	3.29	3.29	76.55	322.73	3.29	43.62	2.97	439.32	2.97	133.30
66PV	F	27	60	167	21.51	456	238.10	352.86	373.98	3.36	3.75	3.36	3.36	450.87	296.10	3.36	30.96	3.04	269.86	3.04	98.75
67PV	F	23	52	164	19.33	356	251.16	221.47	409.27	4.31	4.82	4.31	4.31	273.16	208.91	4.31	7.79	3.90	137.48	3.90	44.50
68PV	M	37	87	168	30.82	485	246.12	210.59	327.66	3.16	49.45	183.86	3.16	507.14	388.58	3.16	95.07	2.86	330.11	45.73	165.77
69PV	M	24	64	178	20.20	481	75.86	56.96	67.81	3.18	3.56	3.18	3.18	258.58	146.11	3.18	38.03	2.88	139.05	2.88	40.38
70PV	M	57	83	183	24.78	653	103.38	85.73	138.64	2.35	2.62	2.35	2.35	257.63	160.78	2.35	22.66	2.12	250.70	2.12	84.51
71PV	M	54	94	187	26.88	519	122.18	113.82	235.19	2.95	3.30	2.95	2.95	738.50	472.24	2.95	92.40	2.67	760.23	2.67	261.56
72PV	M	26	104	186	30.06	507	164.12	119.31	222.33	3.02	3.38	3.02	3.02	152.09	115.20	3.02	12.11	2.73	92.16	2.73	32.58
73PV	M	56	79	172	26.70	375	280.27	258.60	406.74	4.09	4.57	4.09	4.09	1111.46	712.12	4.09	125.64	3.70	952.69	3.70	328.18
74PV	F	52	70	160	27.34	567	186.87	150.41	266.22	2.70	3.02	2.70	2.70	246.49	172.03	2.70	36.20	2.44	258.54	2.44	86.74
76PV	M	27	70	180	21.60	680	119.72	94.78	163.59	2.25	2.52	2.25	2.25	212.66	134.10	2.25	7.44	2.04	135.85	2.04	33.75
77PV	M	50	100	171	34.20	862	104.02	73.97	190.90	1.78	1.99	1.78	1.78	292.80	214.63	1.78	28.52	1.61	205.77	1.61	80.73
78PV	M	44	86	187	24.59	451	62.33	52.10	86.37	3.40	3.80	3.40	3.40	350.67	196.77	3.40	44.10	3.07	265.20	3.07	85.90
79PV	M	38	83	169	29.06	691	135.42	158.77	194.32	2.22	2.48	2.22	2.22	224.88	157.77	2.22	23.52	2.01	177.46	2.01	62.44
80PV	M	28	83	186	23.99	516	253.14	152.87	304.05	2.97	3.32	2.97	2.97	188.63	122.77	2.97	14.92	2.69	119.73	2.69	51.42
81PV	M	63	84	174	27.74	506	172.28	225.37	267.69	3.03	3.38	3.03	3.03	1075.66	708.58	3.03	93.29	2.74	808.45	2.74	273.79
82PV	M	40	78	165	28.65	901	95.68	74.61	135.45	1.70	1.90	1.70	1.70	137.06	95.58	1.70	8.40	1.54	102.92	1.54	35.50
83PV	M	30	118	185	34.48	884	129.84	96.58	178.59	1.73	1.94	1.73	1.73	152.97	120.56	1.73	6.96	1.57	108.91	1.57	39.49
84PV	F	32	60	165	22.04	435	207.32	169.54	291.56	3.52	3.93	3.52	3.52	381.47	261.48	3.52	20.08	3.18	272.62	3.18	101.90
85PV	M	37	88	172	29.74	657	231.24	351.12	313.83	2.33	2.61	2.33	2.33	299.84	209.00	2.33	2.11	2.11	244.12	2.11	73.92
86PV	M	64	96	171	32.83	563	176.48	143.04	241.47	2.72	3.04	2.72	2.72	405.28	300.43	2.72	40.56	2.46	303.99	2.46	102.66
87PV	M	53	90	178	28.40	552	148.09	126.04	213.93	2.77	3.10	2.77	2.77	853.20	671.29	2.77	65.65	2.51	660.53	2.51	256.30
88PV	M	21	74	189	20.72	722	120.32	104.25	174.21	2.12	2.37	2.12	2.12	113.22	79.22	2.12	3.37	1.92	57.14	1.92	19.94
89PV	F	46	55	160	21.48	602	154.24	134.23	206.59	2.54	2.84	2.54	2.54	329.95	205.26	2.54	36.84	2.30	288.57	2.30	101.58
90PV	M	67	72	181	21.97	412	264.49	194.39	330.34	3.72	4.16	3.72	3.72	1359.96	824.70	3.72	129.40	3.36	1274.83	3.37	353.48
92PV	F	53	64	163	24.08	485	202.18	160.69	290.75	3.16	3.53	3.16	3.16	565.80	407.11	3.16	43.57	2.86	372.02	2.86	124.13
93PV	F	47	57	162	21.72	498	176.79	149.22	253.31	3.08	3.44	3.08	3.08	717.05	406.75	3.08	93.29	2.78	676.51	2.78	201.10
94PV	M	43	69	164	25.65	3266	31.77	26.02	43.30	0.47	0.52	0.47	0.47	89.55	57.53	0.47	6.95	0.42	59.32	0.42	22.74

PID	Sex	Age (years)	Weigh (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	PCB 31	PCB 28	PCB 52	PCB 101	PCB 77	PCB 118	PCB 105	PCB 153	PCB 138	PCB 126	PCB 156	PCB 128	PCB 180	PCB 169	PCB 170
95PV	F	23	68	179	21.22	750	132.36	103.74	193.56	2.04	2.28	2.04	2.04	186.85	117.48	2.04	13.87	1.85	102.11	1.85	23.75
96PV	F	32	61	165	22.40	777	143.79	128.59	210.70	1.97	2.20	1.97	1.97	366.38	264.46	1.97	25.84	1.78	227.94	1.78	78.88
97PV	M	53	83	172	28.05	761	150.94	153.86	216.80	2.01	2.25	2.01	2.01	680.20	40.92	2.01	56.53	1.82	500.50	1.82	175.88
98PV	M	44	54	164	20.07	742	125.79	113.78	101.49	2.06	2.31	2.06	2.06	255.41	165.14	2.06	26.02	1.87	260.30	1.87	88.15
99PV	M	30	83	181	25.33	686	134.47	117.44	180.43	2.23	2.50	2.23	2.23	210.23	138.64	2.23	30.32	2.02	162.47	2.02	61.16
100PV	M	33	76	181	23.20	524	60.64	61.73	147.92	2.92	3.27	2.92	2.92	142.09	98.18	2.92	5.05	2.64	104.31	2.64	45.72
101PV	F	44	53	158	21.23	704	91.07	134.28	239.14	2.18	2.43	2.18	2.18	233.28	170.60	2.18	10.70	1.97	174.68	1.97	49.66
102PV	M	44	118	188	33.39	774	58.43	41.17	96.92	1.98	2.21	1.98	1.98	315.83	230.40	1.98	27.59	1.79	183.07	1.79	65.93
103PV	M	36	92	172	31.10	655	24.57	20.01	37.28	2.34	2.61	2.34	2.34	508.06	420.21	2.34	55.17	2.11	325.20	2.11	130.31
104PV	M	33	86	174	28.40	499	106.73	81.31	120.96	3.07	3.43	3.07	3.07	165.21	122.62	3.07	2.78	2.78	116.51	2.78	43.40
105PV	M	34	95	174	31.37	741	74.64	58.53	114.44	2.07	2.31	2.07	2.07	167.46	122.09	2.07	12.90	1.87	109.32	1.87	38.89
106PV	F	20	50	162	19.05	466	78.55	59.98	142.18	3.29	3.68	3.29	3.29	118.54	91.97	3.29	2.98	2.98	54.07	2.98	10.02
107PV	M	25	89	188	25.18	1297	3.62	6.90	10.21	1.18	1.32	1.18	1.18	122.30	103.28	1.18	13.78	1.07	66.72	1.07	31.21
108PV	M	69	64	162	24.38	687	5.30	16.06	26.73	2.23	2.49	223.21	2.23	797.87	565.34	2.23	79.75	2.02	592.19	2.02	181.01
109PV	F	43	69	165	25.34	658	2.95	2.95	32.09	2.33	2.60	67.89	2.33	330.70	291.79	2.33	24.06	2.11	200.35	2.11	98.90
110PV	F	41	69	158	27.64	828	10.13	16.59	17.90	1.85	2.07	69.68	1.85	288.75	240.45	1.85	28.06	1.67	186.08	1.67	66.98
111PV	F	49	61	170	21.10	522	23.44	38.40	42.98	2.94	3.28	2.94	2.94	530.06	410.00	2.94	43.93	2.66	337.62	2.66	124.71
112PV	M	33	72	182	21.73	402	4.83	4.83	44.80	3.81	4.26	86.89	3.81	453.57	395.24	3.81	41.39	3.45	276.17	3.45	135.31
113PV	M	60	90	173	30.07	439	4.42	4.42	47.26	3.49	3.90	3.49	3.49	902.84	674.48	3.49	125.59	3.15	999.39	3.15	321.35
114PV	M	56	87	183	25.98	492	3.94	21.43	24.61	3.11	3.48	190.20	3.11	793.90	587.27	3.11	85.24	2.81	579.44	2.81	182.83
115PV	M	27	81	182	24.45	457	4.25	4.25	42.12	3.35	3.75	3.35	3.35	442.94	384.40	3.35	28.79	3.03	223.09	3.03	112.55
116PV	F	54	98	160	38.28	416	4.67	4.67	4.12	3.69	4.12	3.69	3.69	707.61	591.02	3.69	60.10	3.33	422.08	3.33	172.72
117PV	M	58	81	178	25.56	467	8.55	12.83	28.08	3.28	3.67	3.28	3.28	1189.42	759.50	3.28	115.93	2.97	911.50	2.97	316.10
118PV	M	59	93	170	32.18	481	11.85	21.51	25.51	3.19	3.56	3.19	3.19	664.72	527.72	3.19	60.04	2.88	519.24	2.88	191.21
119PV	M	22	82	176	26.47	673	4.75	15.17	16.72	2.28	2.55	2.28	2.28	192.11	159.72	2.28	10.75	2.06	94.04	2.06	47.59
120PV	F	52	77	159	30.45	655	2.96	2.96	19.40	2.34	2.61	2.34	2.34	502.29	390.14	2.34	32.67	2.11	332.93	2.11	115.53
121PV	F	24	60	166	21.77	502	3.87	3.87	46.16	3.05	3.41	3.05	3.05	559.16	476.35	3.05	37.52	2.76	302.13	2.76	146.05
122PV	M	67	98	178	30.93	436	27.29	51.63	48.44	3.52	3.93	3.52	3.52	1316.72	970.87	3.52	139.78	3.18	887.55	3.18	321.65
123PV	F	44	43	150	19.11	389	4.99	4.99	32.65	3.94	4.40	3.94	3.94	530.40	466.01	3.94	62.49	3.56	382.25	3.56	147.21
124PV	M	41	75	173	25.06	423	4.59	4.59	4.05	3.62	4.05	3.62	3.62	339.23	235.41	3.62	3.27	3.27	259.89	3.27	95.35
125PV	F	28	51	158	20.43	479	30.21	4.05	147.65	3.20	3.57	176.44	3.20	624.84	524.65	3.20	76.09	2.89	361.73	2.89	171.91
126PV	F	29	59	167	21.15	588	19.76	3.30	28.81	2.60	2.91	2.60	2.60	386.12	340.72	2.60	32.43	2.36	210.52	2.36	100.15
127PV	F	30	53	164	19.70	426	4.56	4.56	4.02	3.60	4.02	3.60	3.60	412.80	284.88	3.60	3.25	3.25	250.84	3.25	82.52
128PV	F	44	75	176	24.21	403	4.82	4.82	44.46	3.80	4.25	3.80	3.80	1049.30	871.16	3.80	96.17	3.44	650.93	3.44	252.15
129PV	F	33	49	460	19.14	463	32.73	4.20	67.48	3.31	3.70	3.31	3.31	1060.82	820.78	3.31	90.96	3.00	651.29	3.00	291.81
130PV	F	37	66	168	23.38	436	4.45	4.45	54.32	3.51	3.93	3.51	3.51	787.92	689.86	3.51	77.45	3.18	475.50	3.18	197.11
131PV	F	38	63	170	21.80	503	31.65	92.91	58.97	3.05	3.41	182.60	3.05	681.25	586.27	3.05	55.81	2.76	469.01	2.76	185.68
132PV	F	28	77	165	28.28	382	41.54	5.09	48.63	4.01	4.49	161.95	4.01	756.00	597.18	4.01	74.74	3.63	380.64	3.63	176.70
133PV	F	32	42	150	18.66	403	68.47	86.29	107.36	3.80	4.25	210.86	3.80	1085.62	888.57	3.80	142.98	3.44	686.59	3.44	272.04
135PV	M	46	83	179	25.90	469	60.11	102.75	73.25	3.27	3.65	198.54	3.27	1007.64	783.89	3.27	65.70	2.95	665.28	2.95	299.81
136PV	F	38	61	150	27.10	468	4.15	4.15	41.10	3.27	3.66	121.06	3.27	534.19	483.39	3.27	47.81	2.96	351.70	2.96	162.43
137PV	F	28	50	148	22.82	583	3.33	3.33	46.11	2.63	2.94	82.46	2.63	383.07	324.56	2.63	34.48	2.37	213.62	2.37	24.81
138PV	F	21	62	162	23.62	614	3.16	3.16	28.10	2.50	2.79	123.94	2.50	430.77	374.35	2.50	33.98	2.26	222.95	2.26	92.35

PID	Sex	Age (years)	Weigh (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	PCB 31	PCB 28	PCB 52	PCB 101	PCB 77	PCB 118	PCB 105	PCB 153	PCB 138	PCB 126	PCB 156	PCB 128	PCB 180	PCB 169	PCB 170
139PV	M	49	77	175	25.14	383	104.57	125.12	88.09	3.99	4.47	178.88	55.71	441.76	328.90	3.99	113.70	3.61	377.73	3.61	163.39
140PV	F	57	53	154	22.34	771	16.30	17.61	39.08	1.99	2.22	116.75	1.99	601.95	457.20	1.99	55.66	1.80	457.87	1.80	169.32
141PV	M	68	75	167	26.89	660	2.94	2.94	40.39	2.32	2.59	228.52	2.32	672.36	517.35	2.32	37.86	2.10	354.20	2.10	118.87
142PV	M	37	67	179	20.91	571	3.40	3.40	18.78	2.68	3.00	2.68	2.68	478.05	346.62	2.68	19.74	2.43	288.29	2.43	65.42
143PV	F	34	55	167	19.72	471	4.12	4.12	21.02	3.25	3.63	3.25	3.25	230.01	169.28	3.25	2.94	2.94	109.55	2.94	44.69
144PV	F	41	48	153	20.50	585	3.32	3.32	54.79	2.62	2.93	2.62	2.62	737.73	538.52	2.62	58.99	2.37	405.55	2.37	156.70
145PV	F	34	53	155	22.06	551	3.52	3.52	88.53	2.78	3.11	2.78	2.78	889.01	721.33	2.78	62.69	2.51	461.14	2.51	192.07
146PV	F	29	56	153	23.92	630	125.48	3.08	171.56	2.43	2.72	146.32	2.43	230.56	148.26	2.43	3.55	2.20	100.07	2.20	58.12
147PV	F	39	52	165	19.10	578	87.39	77.85	42.28	2.65	2.96	2.65	2.65	347.50	213.95	2.65	2.40	2.40	256.67	2.40	70.26
148PV	F	46	71	154	29.93	738	2.63	2.63	144.72	2.08	2.32	110.37	2.08	342.57	255.67	2.08	17.55	1.88	186.04	1.88	50.58
149PV	M	59	93	173	31.07	826	2.35	2.35	64.93	1.85	2.07	132.35	1.85	570.67	375.68	1.85	33.72	1.68	517.07	1.68	118.04
150PV	M	57	98	177	31.28	608	3.19	3.19	48.93	2.52	2.81	201.72	2.52	737.00	553.65	2.52	72.91	2.28	415.51	2.28	128.10
151PV	F	50	58	160	22.65	614	3.16	3.16	40.74	2.50	2.79	196.51	2.50	773.05	531.54	2.50	84.39	2.26	507.12	2.26	186.72
152PV	F	38	55	163	20.70	634	3.06	3.06	19.64	2.42	2.70	2.42	2.42	401.93	313.46	2.42	29.40	2.19	242.83	2.19	107.59
153PV	F	49	55	160	21.48	726	2.67	2.67	24.96	2.11	2.36	112.65	2.11	514.55	370.76	2.11	54.30	1.91	338.42	1.91	118.22
154PV	F	37	60	165	22.03	930	2.09	2.09	32.95	1.65	1.84	105.33	1.65	375.33	318.56	1.65	25.52	1.49	189.13	1.49	84.93
155PV	F	48	73	161	28.16	585	3.32	3.32	52.58	2.62	2.93	227.06	2.62	803.69	617.63	2.62	54.37	2.37	456.86	2.37	132.54
156PV	F	52	69	161	26.61	676	2.87	2.87	42.15	2.27	2.53	174.26	2.27	679.01	548.40	2.27	57.30	2.05	339.01	2.05	127.78
157PV	F	42	54	160	21.09	601	3.23	3.23	2.85	2.55	2.85	130.71	2.55	496.97	401.77	2.55	51.09	2.31	333.27	2.31	128.47
158PV	F	35	53	160	20.70	533	3.64	3.64	45.35	2.87	3.21	270.34	2.87	856.08	647.75	2.87	66.05	2.60	470.08	2.60	164.42
159PV	F	32	66	170	22.83	614	3.16	3.16	42.81	2.50	2.79	222.09	2.50	552.05	449.97	2.50	34.09	2.26	239.80	2.26	87.64
160PV	F	27	58	173	19.37	671	2.89	2.89	190.56	2.28	2.55	166.48	2.28	203.03	155.75	2.28	25.78	2.06	91.57	2.06	50.90
161PV	F	42	58	158	23.23	644	157.94	103.23	166.39	2.38	2.66	93.89	2.38	366.10	247.39	2.38	9.06	2.15	176.28	2.15	52.26
162PV	F	29	61	172	20.61	703	336.30	257.17	256.95	2.18	29.22	217.51	2.18	323.03	265.30	2.18	76.36	1.97	164.06	43.34	63.72
163PV	F	52	52	155	21.64	758	149.21	100.28	147.13	2.02	2.26	2.02	2.02	332.99	213.29	2.02	32.51	1.83	214.43	1.83	71.12
164PV	F	38	85	183	25.38	822	95.03	47.01	79.31	1.86	2.08	1.86	1.86	190.50	140.55	1.86	18.44	1.69	139.71	1.69	40.74
165PV	F	49	56	164	20.82	542	165.26	3.58	184.69	2.83	3.16	2.83	2.83	291.53	191.52	2.83	36.90	2.56	202.64	2.56	51.27
166PV	F	54	61	160	23.82	677	81.32	53.94	154.52	2.26	2.53	214.56	2.26	403.29	241.94	2.26	38.37	2.05	259.09	2.05	94.63
167PV	F	53	59	165	21.67	575	158.18	3.38	161.76	2.66	2.98	2.66	2.66	451.12	230.65	2.66	32.99	2.41	312.80	2.41	104.81
1MI	M	27	72	165	26.40	476	20.39	20.39	17.99	16.09	53.97	16.09	16.09	675.31	442.45	32.18	29.11	29.11	558.02	29.11	1009.75
2MI	F	27	65	173	21.70	468	20.74	20.74	18.29	16.36	54.89	16.36	16.36	337.49	260.52	32.73	29.60	29.60	378.36	29.61	13.51
3MI	F	34	56	170	19.30	475	20.44	20.44	18.03	16.12	54.08	16.12	16.12	875.05	711.71	32.25	29.17	29.17	548.52	29.17	13.31
4MI	F	25	59	168	20.90	545	17.81	17.81	15.71	14.05	47.13	14.05	14.05	340.66	330.49	28.10	25.42	25.42	287.77	25.42	11.60
5MI	F	24	60	160	23.45	443	21.93	21.93	19.34	17.30	58.03	17.30	17.30	438.22	375.62	34.60	31.30	31.30	14.29	31.30	14.29
6MI	F	21	50	163	19.00	544	17.85	17.85	15.74	14.08	47.23	14.08	14.08	509.54	377.06	28.17	25.48	25.48	11.63	25.48	11.63
7MI	M	42	75	185	21.90	616	15.75	15.75	13.89	12.42	41.67	12.42	12.42	566.37	404.55	24.85	22.48	22.48	10.26	22.48	10.26
8MI	F	34	84	168	29.70	458	21.18	21.18	18.68	16.71	56.05	16.71	16.71	610.74	622.83	33.43	30.23	30.23	402.97	30.24	13.80
9MI	F	26	67	165	24.60	515	18.86	18.86	16.63	14.88	49.90	14.88	14.88	958.22	667.53	29.76	26.92	26.92	422.63	26.92	12.29
10MI	F	54	65	150	29.00	657	14.77	14.77	13.03	11.65	39.08	11.65	11.65	944.29	813.61	23.30	21.08	21.08	781.21	21.08	9.62
11MI	F	36	45	150	20.00	605	16.04	16.04	282.93	12.65	42.44	12.65	12.65	792.08	384.59	25.31	22.89	22.89	564.25	22.89	10.45
12MI	M	32	90	185	23.00	650	14.94	14.94	105.44	11.79	39.54	11.79	11.79	725.14	618.51	23.58	21.33	21.33	584.09	21.33	257.00
13MI	M	40	88	183	26.00	538	18.03	18.03	15.91	14.23	47.72	14.23	14.23	633.19	453.01	28.46	25.74	25.74	390.05	25.74	11.75
15MI	F	41	52	160	20.30	570	17.02	17.02	15.01	13.43	45.04	13.43	13.43	1749.12	1340.99	26.86	24.29	24.29	1029.01	24.29	390.31

PID	Sex	Age (years)	Weigh (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	PCB 31	PCB 28	PCB 52	PCB 101	PCB 77	PCB 118	PCB 105	PCB 153	PCB 138	PCB 126	PCB 156	PCB 128	PCB 180	PCB 169	PCB 170
16MI	F	49	78	172	26.36	572	16.98	16.98	323.59	13.40	44.94	13.40	13.40	1207.27	896.96	26.80	24.24	232.73	11.07	24.24	411.62
17MI	F	32	76	167	27.95	611	15.90	15.90	14.02	12.54	42.07	12.54	12.54	762.45	571.83	25.09	22.69	22.69	290.01	22.69	10.36
18MI	F	38	55	162	20.95	483	20.11	20.11	17.73	15.86	53.20	15.86	15.86	1256.96	941.28	31.73	28.70	28.70	518.71	28.70	398.20
19MI	F	38	86	164	31.96	526	18.46	18.46	16.28	14.56	48.84	14.56	14.56	995.81	642.80	29.12	26.34	26.34	452.12	26.35	12.02
20MI	F	38	46	150	20.40	471	20.63	20.63	829.77	16.28	54.59	16.28	16.28	1219.06	694.93	32.55	29.45	29.45	376.33	29.45	13.44
21MI	F	29	60	174	20.00	526	18.46	18.46	520.95	14.56	48.84	14.56	14.56	1001.08	532.15	29.12	26.34	26.34	432.88	26.35	12.02
22MI	F	26	70	175	23.00	498	19.48	19.48	17.18	15.37	51.56	15.37	15.37	628.48	244.72	30.74	27.81	27.81	345.25	27.81	12.69
23MI	F	46	47	151	20.60	627	15.49	15.49	13.66	12.22	40.98	12.22	12.22	654.29	380.19	24.44	22.10	22.10	746.61	22.11	10.09
24MI	F	34	53	160	20.70	499	19.44	19.44	17.15	15.34	51.45	15.34	15.34	532.86	516.21	30.68	27.75	27.75	354.69	27.75	12.67
25MI	M	43	83	170	28.70	544	17.85	17.85	15.74	14.08	47.23	14.08	14.08	540.09	484.05	28.16	25.48	25.48	460.48	25.48	11.63
26MI	F	36	55	172	18.70	351	27.68	27.68	283.22	21.84	73.25	21.84	21.84	2204.65	1612.00	43.68	39.51	505.73	1615.83	39.51	18.03
27MI	F	34	54	162	20.57	307	31.58	31.58	390.00	24.92	83.57	24.92	24.92	982.71	757.32	49.84	45.08	45.08	411.51	45.08	20.58
28MI	F	69	59	153	25.20	572	16.96	16.96	14.96	13.38	44.88	13.38	13.38	1573.55	1060.33	26.76	24.21	232.40	1069.61	24.21	375.69
29MI	F	34	55	163	20.70	483	20.12	20.12	17.74	15.87	53.23	15.87	15.87	867.15	941.81	31.74	28.71	28.71	629.09	28.72	13.11
30MI	F	44	58	160	22.60	516	18.80	18.80	16.58	14.83	49.75	14.83	14.83	13.42	13.42	29.67	26.84	26.84	12.25	26.84	12.25
31MI	F	33	55	163	20.70	476	20.41	20.41	18.01	16.11	54.02	16.11	16.11	472.01	477.84	32.21	29.14	29.14	542.60	29.14	13.30
32MI	F	21	53	163	19.90	376	25.84	25.84	273.51	20.39	68.38	20.39	20.39	759.80	531.12	40.78	36.88	36.88	148.15	36.89	16.84
33MI	F	44	63	173	21.00	474	20.48	20.48	18.07	16.16	54.20	16.16	16.16	1151.90	689.97	32.32	29.24	29.24	763.30	29.24	421.69
34MI	M	20	57	168	20.20	345	28.14	28.14	24.82	22.20	74.45	22.20	22.20	803.14	602.35	44.39	40.16	40.16	366.58	40.16	18.33
35MI	F	20	65	173	21.70	466	20.85	20.85	18.39	16.45	55.17	16.45	16.45	416.57	380.87	32.90	29.76	29.76	217.30	29.76	13.58
36MI	F	25	62	160	24.00	435	22.31	22.31	19.68	17.60	59.03	17.60	17.60	458.52	471.26	35.20	31.84	31.84	290.68	31.84	14.53
37MI	M	21	78	186	22.50	472	20.59	20.59	18.16	16.24	54.47	16.24	16.24	558.25	499.49	32.48	29.38	29.38	268.22	29.38	13.41
38MI	F	27	60	163	22.60	520	18.66	18.66	16.46	14.72	49.39	14.72	14.72	586.05	479.49	29.45	26.64	26.64	340.45	26.64	12.16
39MI	M	21	69	172	23.30	524	18.54	18.54	16.35	14.63	49.06	14.63	14.63	566.30	635.10	29.26	26.46	26.46	289.88	26.46	12.08
40MI	F	26	59	170	20.40	473	20.51	20.51	18.09	16.18	54.28	16.18	16.18	667.50	667.50	32.37	29.28	29.28	999.54	29.28	855.22
41MI	M	20	85	194	22.60	503	19.29	19.29	102.09	15.22	51.04	15.22	15.22	550.65	374.44	30.44	27.53	27.53	392.09	27.53	12.57
42MI	M	20	62	177	19.80	510	19.03	19.03	268.49	15.01	50.34	15.01	15.01	434.48	537.67	30.02	27.15	27.15	1586.50	27.16	12.39

Abbreviations: PID, personal identification number; NF, Novafeltria; PV, Pavia; MI, Milan; F, female; M, male; BMI, body mass index; TL, total lipids; PCB, polychlorinated biphenyls.

Annex V: Individual personal characteristics of the study subjects and their computed results of PCB groupings measurements (expressed in pmol/g serum lipid).

PID	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	∑Tri–CBs	∑Tetra–CBs	∑Penta–CBs	∑Hexa–CBs	∑Hepta–CBs	∑DL–PCB	∑NDL–PCB	∑PCBs
1NF	M	44	76	183	22.69	526.9	15.60	6.50	39.10	446.96	278.54	64.55	722.15	786.70
2NF	M	45	74	180	22.84	488.4	65.49	7.01	67.99	349.06	235.55	103.28	621.82	725.10
3NF	M	57	78	174	25.76	480.4	8.08	7.13	79.14	777.10	553.81	114.63	1310.62	1425.26
4NF	F	52	58	157	23.53	780.8	13.62	4.39	92.59	649.60	262.05	125.81	896.43	1022.24
5NF	M	55	74	168	26.22	513.3	30.76	6.67	87.34	625.04	369.83	151.04	968.60	1119.65
6NF	M	44	101	175	32.98	601.1	13.01	5.70	55.32	338.19	158.33	70.29	500.26	570.55
7NF	M	47	81	180	25.00	513.8	12.31	6.67	78.97	539.66	282.47	108.08	812.00	920.08
8NF	M	54	71	168	25.16	594.8	12.67	5.76	72.08	559.67	374.79	111.71	913.27	1024.98
10NF	F	60	65	163	24.46	571.3	14.48	5.99	87.56	652.14	380.62	123.91	1016.88	1140.80
11NF	F	54	55	155	22.89	541.5	7.90	6.32	78.54	566.11	287.54	104.25	842.18	946.43
12NF	M	36	87	177	27.77	586.8	31.35	5.84	72.62	445.35	228.25	94.61	688.80	783.41
13NF	F	32	58	168	20.55	437.3	32.14	7.83	92.98	408.37	165.74	111.59	595.46	707.05
14NF	M	40	82	176	26.47	507.2	7.65	6.75	58.49	538.37	240.97	89.52	762.70	852.23
15NF	F	39	56	165	20.57	515.5	7.53	6.64	50.21	261.39	162.83	69.38	419.23	488.61
16NF	F	39	80	175	26.12	440.1	8.82	7.78	79.70	536.48	207.58	133.18	707.18	840.36
17NF	F	31	64	163	24.09	505.8	7.68	6.77	68.90	311.41	135.03	84.66	445.12	529.79
18NF	M	52	88	170	30.45	857.6	4.53	3.99	71.00	746.86	299.97	106.88	1019.47	1126.35
19NF	M	38	80	180	24.69	509.3	141.83	6.72	419.26	674.02	310.55	386.12	1166.26	1552.38
20NF	F	39	65	160	25.39	457.0	8.50	7.49	83.05	478.30	272.83	109.87	740.30	850.17
21NF	F	48	70	170	24.22	411.0	9.45	8.33	185.95	1029.76	438.71	248.57	1423.63	1672.20
22NF	M	43	73	170	25.26	648.4	19.81	5.28	46.37	248.81	177.94	65.66	432.55	498.21
23NF	F	57	69	168	24.45	741.3	5.24	4.62	102.85	1078.07	489.02	162.75	1517.03	1679.79
24NF	F	56	70	168	24.80	654.6	5.93	5.23	115.46	842.00	422.82	159.59	1231.86	1391.44
25NF	F	45	50	159	19.78	459.3	8.45	7.46	103.86	895.53	486.05	160.38	1340.98	1501.36
26NF	F	53	55	163	20.70	868.9	9.80	3.94	75.41	533.17	329.06	113.99	837.39	951.37
27NF	F	40	62	159	24.52	521.1	7.45	6.57	78.96	655.46	343.41	119.86	972.00	1091.86
28NF	M	38	97	191	26.59	550.0	12.28	6.23	63.35	371.70	215.46	82.13	586.90	669.02
29NF	M	40	78	180	24.07	640.8	19.92	5.34	35.13	196.74	111.81	50.93	318.03	368.96
30NF	F	55	59	157	23.94	430.8	9.01	7.95	147.09	1221.41	642.61	212.43	1815.64	2028.07
31NF	F	50	75	167	26.89	505.9	7.68	6.77	89.54	800.93	417.35	124.22	1198.04	1322.26
32NF	M	45	83	176	26.79	617.0	6.29	5.55	19.15	312.96	142.38	23.94	462.39	486.33
33NF	F	36	58	163	21.83	465.6	8.34	7.36	66.07	196.22	86.42	72.41	292.00	364.41
34NF	M	44	78	178	24.62	466.6	12.43	7.34	307.33	862.80	407.27	401.65	1195.52	1597.17
35NF	F	26	49	160	19.14	422.9	23.00	8.10	108.96	277.96	132.20	128.33	421.89	550.21
36NF	F	29	65	170	22.49	515.9	7.53	6.64	95.72	409.75	192.12	112.26	599.50	711.76
37NF	M	31	77	178	24.30	679.4	5.72	5.04	17.39	298.87	107.64	35.13	399.54	434.67
38NF	F	39	52	160	20.31	587.5	31.13	5.83	295.75	849.25	922.53	340.68	1763.82	2104.50
39NF	F	37	60	168	21.26	600.8	120.11	112.12	331.81	393.54	147.91	323.04	782.45	1105.50
40NF	F	30	65	158	26.04	422.9	9.18	8.10	72.33	242.52	123.80	79.31	376.62	455.93

PID	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	∑Tri–CBs	∑Tetra–CBs	∑Penta–CBs	∑Hexa–CBs	∑Hepta–CBs	∑DL–PCB	∑NDL–PCB	∑PCBs
41NF	F	47	50	168	17.72	500.9	63.24	25.28	204.07	917.75	342.39	610.61	942.13	1552.74
42NF	F	34	64	168	22.68	518.6	7.49	6.60	59.87	299.30	174.46	84.40	463.32	547.72
43NF	M	50	82	173	27.40	598.7	30.45	5.72	51.72	278.76	162.48	70.22	458.90	529.13
44NF	F	48	68	157	27.59	598.7	6.49	5.72	66.25	395.58	224.92	88.91	610.04	698.96
45NF	F	28	63	160	24.61	462.0	8.40	7.41	47.91	157.39	82.50	61.85	241.76	303.61
46NF	F	43	58	164	21.56	511.9	7.59	6.69	63.86	380.76	240.70	84.11	615.49	699.60
47NF	F	46	62	155	25.81	616.0	6.30	14.59	56.38	467.95	267.14	89.17	723.20	812.37
48NF	M	46	92	172	31.10	657.1	12.10	5.21	78.77	625.61	284.66	105.72	900.64	1006.36
49NF	M	21	70	175	22.86	578.1	6.72	5.92	40.10	116.50	57.97	46.59	180.63	227.22
50NF	F	31	63	168	22.32	386.9	10.04	28.40	122.45	123.48	78.72	79.48	283.61	363.09
51NF	M	52	80	170	27.68	540.1	27.50	6.34	101.10	585.70	390.76	196.04	915.35	1111.40
52NF	M	48	80	174	26.42	525.0	7.40	6.52	64.18	729.18	435.36	97.89	1144.75	1242.64
53NF	M	43	75	172	25.35	1013.4	3.83	3.38	58.22	417.15	165.14	77.85	569.87	647.73
54NF	M	39	78	177	24.90	589.2	6.59	5.81	41.56	157.42	92.18	56.69	246.86	303.56
55NF	M	48	110	170	38.06	537.8	7.22	6.37	55.96	290.71	174.57	94.72	440.10	534.83
56NF	F	40	53	155	22.06	456.0	8.51	7.51	68.25	178.87	116.26	76.03	303.37	379.40
57NF	M	31	74	183	22.10	452.3	24.56	7.57	68.30	454.49	252.89	95.69	712.13	807.82
58NF	F	46	59	169	20.66	499.8	14.41	6.85	62.46	257.41	124.04	69.78	395.39	465.17
59NF	F	48	63	159	24.92	615.0	27.63	5.57	43.41	153.92	85.69	48.49	267.74	316.23
60NF	F	57	57	156	23.42	606.7	6.40	5.65	39.93	201.50	124.30	51.23	326.54	377.77
61NF	M	44	77	172	26.03	609.2	6.37	5.62	10.06	311.02	203.38	25.24	511.21	536.46
62NF	M	44	72	176	23.24	566.5	22.05	6.05	78.64	658.66	417.28	135.10	1047.57	1182.67
63NF	F	52	73	163	27.48	642.5	26.80	5.33	155.51	879.44	358.85	202.48	1223.46	1425.93
64NF	F	31	60	166	21.77	506.6	7.66	6.76	109.30	381.86	237.71	115.13	628.17	743.30
65NF	M	55	75	173	25.06	631.6	14.13	5.42	79.72	729.06	324.82	123.74	1029.42	1153.16
66NF	F	52	63	156	25.89	929.2	18.13	3.69	125.42	551.89	284.64	162.34	821.44	983.77
67NF	F	42	50	155	20.81	396.8	24.42	8.63	112.43	345.48	260.00	133.12	617.85	750.96
68NF	F	40	53	160	20.70	793.4	4.89	4.32	122.66	554.66	282.64	148.14	821.03	969.17
69NF	F	20	43	160	16.80	521.4	46.83	6.57	96.29	436.47	166.78	119.80	633.13	752.93
70NF	M	42	69	175	22.53	538.5	7.21	6.36	69.80	1591.34	773.69	168.36	2280.04	2448.40
71NF	F	44	60	152	25.97	418.5	9.28	8.18	763.42	1456.90	349.39	857.48	1729.68	2587.16
72NF	F	46	49	150	21.78	566.8	6.85	6.04	95.94	821.40	387.02	134.29	1182.96	1317.25
73NF	M	40	85	178	26.83	484.8	15.21	7.06	134.89	562.01	280.54	157.48	842.24	999.72
74NF	F	51	49	156	20.13	536.1	21.79	6.39	137.08	988.37	498.75	189.96	1462.43	1652.38
75NF	M	40	85	180	26.23	575.5	6.75	5.95	56.50	410.06	275.10	83.79	670.57	754.36
76NF	F	30	52	167	18.65	494.9	7.85	6.92	12.38	341.00	190.92	29.27	529.80	559.07
77NF	M	35	70	170	24.22	483.5	8.03	7.08	12.67	553.30	414.02	84.13	910.98	995.11
78NF	F	48	55	170	19.03	506.2	20.23	6.77	116.86	514.74	303.20	146.22	815.59	961.80
79NF	M	45	86	186	24.86	412.1	9.42	8.31	105.74	657.16	366.10	146.11	1000.62	1146.73
80NF	F	41	57	165	20.94	557.8	6.96	6.14	64.83	511.05	275.08	92.41	771.66	864.07
81NF	M	57	60	175	19.59	430.4	30.89	7.96	74.40	673.05	558.10	154.24	1190.16	1344.40
82NF	M	29	98	177	31.28	376.3	52.41	9.10	84.02	251.15	105.62	101.73	400.57	502.30
83NF	F	42	60	167	21.51	625.1	45.62	5.48	85.66	499.60	217.30	132.52	721.14	853.67

PID	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	∑Tri–CBs	∑Tetra–CBs	∑Penta–CBs	∑Hexa–CBs	∑Hepta–CBs	∑DL–PCB	∑NDL–PCB	∑PCBs
84NF	M	56	69	172	23.32	899.2	15.35	3.81	55.94	780.75	435.09	110.28	1180.67	1290.95
85NF	M	59	78	171	26.67	338.6	26.02	10.11	678.65	1286.78	1234.06	762.60	2473.03	3235.63
86NF	F	38	52	162	19.81	639.6	57.48	5.35	91.17	525.89	246.45	115.44	810.90	926.34
87NF	M	38	60	173	20.05	499.3	34.45	6.86	47.27	334.54	287.24	63.09	647.27	710.36
88NF	M	46	85	186	24.57	401.3	24.76	8.53	166.52	1345.21	710.25	253.63	2001.63	2255.27
89NF	M	43	85	180	26.23	950.5	4.09	12.90	31.81	314.22	183.39	52.73	493.67	546.40
90NF	M	28	71	170	24.57	550.8	29.63	6.22	56.02	234.89	88.84	71.65	343.95	415.60
91NF	M	48	84	174	27.74	677.5	28.99	17.90	85.04	733.71	394.27	135.14	1124.77	1259.91
92NF	F	54	62	156	25.48	566.2	77.79	33.79	114.38	565.80	344.25	159.57	976.44	1136.01
93NF	M	44	90	180	27.78	598.6	6.49	5.72	99.63	350.67	177.75	120.07	520.19	640.26
94NF	M	41	60	167	21.51	514.1	7.55	6.66	62.16	374.00	214.65	85.55	579.48	665.03
95NF	F	26	55	168	19.49	601.2	50.16	23.50	99.54	293.73	126.58	117.18	476.32	593.51
96NF	F	48	52	167	18.65	520.5	22.18	6.58	91.77	620.86	397.13	138.00	1000.52	1138.52
97NF	M	48	53	164	19.71	556.5	70.21	156.17	116.47	605.99	315.38	191.05	1073.17	1264.22
98NF	M	21	75	180	23.15	409.4	9.48	8.37	14.96	221.10	101.08	43.54	311.45	354.99
99NF	M	21	92	185	26.88	406.0	23.53	8.44	15.09	113.75	37.70	22.36	176.14	198.51
100NF	M	28	87	186	25.15	376.7	10.31	64.54	16.26	226.56	92.85	24.10	386.43	410.53
101NF	F	42	55	164	20.45	614.1	31.25	9.86	89.39	322.28	188.78	94.20	547.38	641.57
102NF	M	58	92	187	26.31	446.3	34.13	7.67	55.34	615.22	528.26	61.95	1178.67	1240.62
103NF	M	19	76	174	25.10	330.6	11.75	10.36	18.53	495.85	239.78	27.46	748.80	776.27
104NF	F	53	59	158	23.63	525.4	22.33	6.52	91.53	731.86	302.76	117.68	1037.32	1155.00
105NF	F	37	48	160	18.75	578.4	9.95	5.92	47.14	277.50	202.94	63.17	480.27	543.44
106NF	F	28	53	167	19.00	567.9	17.63	6.03	61.21	307.60	141.11	83.36	450.22	533.58
107NF	M	48	72	168	25.51	806.5	17.69	4.25	69.03	495.15	288.94	98.89	776.16	875.05
108NF	M	55	65	170	22.49	554.7	20.00	6.17	60.18	524.94	302.92	95.95	818.27	914.22
109NF	F	29	68	155	28.30	471.5	38.13	7.26	161.17	539.17	214.50	170.84	789.39	960.23
110NF	F	19	45	170	15.57	362.5	14.70	9.45	16.90	227.13	124.83	25.04	367.97	393.01
111NF	M	50	84	164	31.23	765.4	5.80	6.97	102.03	767.60	468.52	252.53	1098.38	1350.91
112NF	M	54	90	175	29.39	612.6	7.77	5.59	88.42	619.75	365.81	125.24	962.11	1087.35
113NF	F	37	50	162	19.05	529.9	20.77	6.46	54.93	355.77	239.37	116.47	560.83	677.30
114NF	M	53	83	170	28.72	602.5	23.18	5.68	121.90	1167.66	600.49	177.97	1740.94	1918.91
115NF	M	35	63	168	22.32	538.3	10.37	6.36	98.54	517.25	238.82	124.94	746.41	871.35
116NF	F	33	48	160	18.75	527.1	15.60	6.50	76.78	382.76	234.18	106.12	609.69	715.81
117NF	F	40	50	155	20.81	594.1	15.54	5.77	57.81	480.07	315.04	84.33	789.90	874.23
118NF	F	34	72	160	28.13	518.2	7.49	6.61	127.16	287.12	115.76	142.63	401.50	544.14
119NF	M	28	80	173	26.73	596.3	6.51	5.74	50.81	271.34	138.27	55.76	416.92	472.69
120NF	F	28	57	162	21.72	411.0	51.46	8.33	89.44	273.29	148.09	109.41	461.19	570.60
121NF	F	59	69	155	28.72	699.7	7.09	4.89	57.41	548.99	295.63	86.76	827.27	914.02
122NF	M	48	93	178	29.35	504.2	7.70	6.79	51.10	561.36	353.75	82.16	898.55	980.71
123NF	F	19	51	165	18.73	505.9	20.87	6.77	12.11	111.74	85.25	17.94	218.79	236.74
124NF	F	44	68	169	23.81	434.6	8.94	7.88	108.75	675.28	338.75	140.34	999.25	1139.59
125NF	M	37	76	175	24.82	520.0	7.47	6.59	107.20	669.01	324.60	144.88	969.98	1114.86
126NF	F	33	63	162	24.01	412.7	9.41	8.30	110.30	280.33	126.59	117.45	417.47	534.93

PID	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	∑Tri–CBs	∑Tetra–CBs	∑Penta–CBs	∑Hexa–CBs	∑Hepta–CBs	∑DL–PCB	∑NDL–PCB	∑PCBs
127NF	M	52	82	177	26.17	762.1	5.09	4.49	95.75	575.81	276.84	132.55	825.44	957.99
128NF	M	30	88	178	27.77	787.6	24.48	4.35	93.18	264.52	96.52	109.68	373.37	483.05
129NF	F	61	62	151	27.19	913.8	4.25	3.75	31.05	212.28	124.82	52.19	323.96	376.15
130NF	F	61	56	167	20.08	635.2	6.11	5.39	82.42	422.00	397.60	126.40	787.12	913.52
131NF	M	33	100	182	30.19	604.9	19.51	5.66	60.59	425.19	184.21	78.91	616.25	695.16
132NF	F	42	62	167	22.23	402.1	9.66	8.52	124.81	692.66	339.23	160.27	1014.61	1174.88
133NF	F	38	55	165	20.20	537.3	7.23	6.37	88.03	270.81	171.23	93.52	450.15	543.67
134NF	F	40	48	160	18.75	437.7	31.61	7.82	133.41	791.19	369.50	176.08	1157.45	1333.53
135NF	F	40	56	162	21.34	500.7	17.55	6.84	91.75	552.50	295.23	125.98	837.90	963.87
136NF	M	56	100	185	29.22	495.5	7.84	6.91	105.73	1353.11	872.99	173.21	2173.37	2346.57
137NF	F	43	66	172	22.31	479.3	8.10	7.15	104.55	656.55	339.31	139.14	976.52	1115.66
138NF	M	51	94	181	28.69	658.8	5.89	5.20	108.91	774.41	391.00	143.70	1141.70	1285.40
139NF	M	55	80	172	27.04	423.7	9.16	8.08	78.55	953.23	495.11	128.77	1415.36	1544.13
140NF	M	61	55	165	20.20	1001.6	13.17	3.42	110.38	805.97	428.51	151.18	1210.28	1361.45
141NF	M	38	83	164	30.86	539.6	30.89	6.35	82.64	404.89	182.11	88.11	618.76	706.87
142NF	F	38	49	156	20.13	593.0	6.55	5.78	72.79	491.83	253.35	98.75	731.54	830.29
143NF	M	39	74	166	26.85	525.3	18.15	6.52	103.94	530.69	257.25	137.10	779.44	916.54
144NF	M	45	76	174	25.10	567.5	16.23	6.03	102.26	677.01	310.41	136.51	975.43	1111.94
145NF	F	38	57	163	21.45	458.9	29.65	7.46	110.70	753.91	342.36	165.16	1078.92	1244.08
146NF	F	48	50	154	21.08	878.3	15.72	3.90	57.95	393.77	178.85	86.45	563.75	650.20
147NF	M	38	88	183	26.28	767.7	5.06	4.46	68.76	416.53	251.35	100.34	645.82	746.17
148NF	F	37	60	164	22.31	477.5	8.13	7.17	12.83	140.83	45.02	19.01	194.98	213.99
149NF	F	32	60	170	20.76	484.1	8.02	7.07	115.68	395.50	223.31	121.78	627.80	749.58
150NF	F	54	65	160	25.39	532.1	7.30	6.44	83.38	358.39	224.71	111.97	568.25	680.21
151NF	F	61	55	160	21.48	580.9	6.68	5.90	208.14	1429.16	586.05	288.99	1946.94	2235.93
152NF	M	33	93	181	28.39	638.4	35.91	5.36	54.69	363.56	179.48	59.32	579.69	639.01
153NF	M	47	78	170	26.99	711.0	5.46	4.82	85.98	351.36	177.53	90.13	535.02	625.15
154NF	M	35	91	181	27.78	654.2	23.61	5.24	9.37	315.28	145.18	13.88	484.79	498.67
155NF	M	27	63	172	21.30	453.6	8.56	7.55	13.51	213.11	120.33	20.01	343.05	363.06
156NF	M	26	54	158	21.63	473.1	24.68	7.24	12.95	342.21	183.26	48.44	521.91	570.35
157NF	F	53	80	156	32.87	647.1	6.00	5.29	114.05	765.09	291.09	157.40	1024.12	1181.52
158NF	M	59	75	173	25.06	786.9	22.70	4.35	46.83	454.06	331.38	82.68	776.64	859.32
159NF	M	27	74	179	23.10	566.2	18.81	6.05	10.82	228.54	105.54	16.03	353.72	369.76
160NF	M	35	75	176	24.21	574.0	6.76	5.97	10.67	566.92	302.77	61.01	832.09	893.10
161NF	F	50	79	160	30.86	466.5	8.32	7.34	157.59	610.51	262.72	163.91	882.56	1046.47
162NF	M	41	82	181	25.03	700.4	5.54	4.89	85.43	616.48	328.04	117.32	923.06	1040.39
163NF	M	53	84	173	28.07	490.5	7.92	6.98	12.49	561.11	306.01	18.51	876.00	894.50
1PV	M	32	68	175	22.20	417	9.31	35.19	14.69	682.89	368.42	24.17	1086.33	1110.50
2PV	M	35	69	169	24.16	468	12.65	18.97	13.08	514.97	220.10	24.87	754.90	779.77
3PV	F	32	46	152	19.91	740	5.24	24.23	8.27	308.46	119.77	18.19	447.79	465.99
4PV	F	27	59	155	24.56	487	7.97	7.03	12.58	303.83	108.12	18.64	420.90	439.54
5PV	M	51	98	179	30.58	586	6.63	25.49	10.46	828.59	556.19	47.92	1379.44	1427.36
6PV	M	40	78	174	25.76	498	7.80	15.20	12.31	1131.93	505.51	54.82	1617.92	1672.74

PID	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	∑Tri–CBs	∑Tetra–CBs	∑Penta–CBs	∑Hexa–CBs	∑Hepta–CBs	∑DL–PCB	∑NDL–PCB	∑PCBs
7PV	F	27	45	155	18.73	588	6.61	12.71	10.43	292.05	96.23	15.45	402.58	418.03
8PV	M	48	73	174	24.11	405	9.59	22.02	731.73	1444.16	564.57	817.43	1954.64	2772.07
9PV	M	46	75	180	23.15	455	8.53	33.69	13.45	575.55	363.69	19.93	974.97	994.91
10PV	F	36	48	162	18.29	484	33.39	44.68	12.66	572.07	352.01	33.96	980.85	1014.81
11PV	M	23	68	170	23.53	478	8.12	29.50	12.81	369.60	121.28	20.45	520.86	541.30
12PV	M	40	104	182	31.40	347	11.20	29.93	17.67	1191.39	517.78	50.66	1717.29	1767.95
13PV	F	40	54	164	20.00	414	9.38	33.78	14.81	827.64	410.64	48.22	1248.04	1296.25
14PV	M	27	66	174	21.80	542	7.16	28.61	11.29	214.16	78.34	16.74	322.82	339.55
15PV	M	41	131	188	37.06	439	8.85	28.75	13.96	610.69	277.95	20.69	919.51	940.20
16PV	M	41	65	179	20.29	583	6.67	28.16	10.52	439.73	265.63	29.70	721.01	750.71
17PV	M	63	79	167	28.33	472	8.22	32.34	12.97	1824.90	967.71	87.16	2758.97	2846.13
18PV	M	71	85	176	27.44	346	71.62	44.71	1059.50	2997.27	1242.42	1126.69	4288.82	5415.51
19PV	F	56	74	154	31.20	502	62.63	40.27	416.06	2037.45	843.33	521.65	2878.08	3399.73
20PV	M	59	97	172	32.79	509	7.62	6.72	12.03	912.65	442.55	53.63	1327.95	1381.58
21PV	M	52	89	176	28.73	559	6.94	60.22	10.95	1252.46	424.99	56.18	1699.38	1755.56
22PV	M	40	83	182	25.05	535	7.25	43.26	11.45	608.29	139.70	16.96	792.99	809.95
23PV	M	45	68	165	24.98	608	6.39	23.98	10.08	1257.34	606.49	14.93	1889.34	1904.28
24PV	M	38	81	172	27.38	509	7.62	6.72	12.03	1378.78	624.65	61.61	1968.19	2029.80
25PV	M	52	77	168	27.28	475	8.18	18.54	12.91	1346.76	549.52	59.17	1876.74	1935.91
26PV	M	42	69	182	20.83	514	7.56	35.19	11.93	600.57	324.51	51.50	928.26	979.76
27PV	F	50	50	155	20.81	484	8.03	28.17	12.67	792.49	292.21	56.90	1076.67	1133.57
28PV	F	50	62	150	27.55	576	6.75	34.69	10.65	730.47	261.15	48.37	995.32	1043.69
29PV	M	40	80	173	26.73	542	7.16	63.80	11.30	591.24	242.38	43.53	872.35	915.88
30PV	M	40	118	175	38.53	649	5.99	19.16	9.45	475.05	87.23	14.00	582.89	596.89
31PV	M	35	85	185	24.83	524	7.41	32.11	11.70	534.91	153.15	17.33	721.95	739.28
32PV	M	58	89	175	29.06	422	9.21	27.70	14.53	950.78	429.95	80.23	1351.94	1432.17
33PV	M	56	88	178	27.77	502	7.74	6.83	12.21	1405.20	678.21	87.41	2022.78	2110.19
34PV	M	64	68	170	23.53	412	9.43	8.32	14.88	1275.88	825.75	113.40	2020.85	2134.25
35PV	M	31	87	187	24.88	466	8.33	7.34	13.14	436.70	150.95	19.46	596.99	616.46
36PV	M	34	72	175	23.51	485	8.00	7.05	12.62	563.61	208.97	36.33	763.92	800.25
37PV	M	64	68	165	24.97	557	6.97	37.04	10.99	1123.20	367.21	70.02	1475.39	1545.41
38PV	M	58	67	164	24.91	451	8.61	47.64	13.59	1230.93	470.33	50.27	1720.84	1771.11
39PV	F	34	48	154	20.24	615	6.31	5.56	9.95	529.67	182.26	23.30	710.46	733.76
40PV	F	39	59	156	24.24	306	12.71	11.21	20.05	950.37	283.77	58.18	1219.93	1278.11
41PV	F	42	50	155	20.81	494	7.86	27.68	12.40	812.03	278.22	30.35	1107.83	1138.18
42PV	M	53	60	167	21.51	507	7.66	47.58	12.09	994.90	512.91	79.80	1495.33	1575.13
43PV	M	37	70	175	22.86	531	7.31	36.40	11.53	349.85	101.61	17.68	489.02	506.70
44PV	M	25	65	173	21.72	581	6.69	23.34	10.55	309.34	110.67	26.36	434.22	460.58
45PV	M	39	79	168	28.00	788	4.93	17.52	7.78	369.14	135.63	11.52	523.47	534.99
46PV	M	48	85	163	31.99	612	6.34	38.82	10.01	1421.69	635.94	74.55	2038.25	2112.81
47PV	F	29	59	166	21.41	627	76.64	93.85	9.78	376.42	107.42	14.49	649.63	664.11
48PV	F	30	48	154	20.24	518	100.51	94.73	11.83	409.61	98.17	17.53	697.33	714.86
49PV	F	52	50	163	18.82	416	113.63	140.42	14.74	715.90	400.90	49.28	1336.32	1385.59

PID	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	∑Tri–CBs	∑Tetra–CBs	∑Penta–CBs	∑Hexa–CBs	∑Hepta–CBs	∑DL–PCB	∑NDL–PCB	∑PCBs
50PV	F	25	48	165	17.63	566	100.37	92.05	10.83	520.83	85.23	75.63	733.68	809.31
51PV	M	53	70	165	30.80	626	6.20	27.81	9.79	487.63	170.59	31.55	670.47	702.02
52PV	M	39	78	177	24.89	474	8.19	33.76	12.92	561.76	241.95	19.14	839.42	858.56
53PV	M	33	89	187	25.45	541	7.18	31.14	11.33	456.73	78.04	74.62	509.78	584.41
54PV	M	45	64	167	22.94	728	5.34	44.78	8.42	504.73	187.74	17.56	733.45	751.01
55PV	M	44	100	169	35.00	637	6.10	5.38	9.62	1027.89	335.77	61.30	1323.46	1384.75
56PV	M	35	72	170	24.91	382	10.17	39.57	16.05	473.79	174.30	23.78	690.11	713.89
57PV	M	45	65	171	22.23	407	9.55	57.86	15.07	1024.10	480.12	43.90	1542.80	1586.70
58PV	M	34	87	172	29.41	309	12.58	66.89	19.84	1034.98	343.80	29.40	1448.68	1478.09
59PV	M	41	69	166	25.04	519	7.48	59.77	11.80	494.03	181.04	17.48	736.64	754.12
60PV	F	34	65	165	23.87	459	8.45	68.72	13.34	589.37	196.80	19.76	856.91	876.68
61PV	M	35	77	175	25.14	795	192.51	157.84	7.71	345.43	205.73	27.14	882.07	909.22
62PV	M	21	78	183	23.29	429	229.14	179.42	14.27	259.55	114.57	21.15	775.80	796.95
63PV	M	42	78	176	25.18	453	8.57	65.02	13.52	1333.11	779.74	101.34	2098.62	2199.95
64PV	M	32	83	185	24.25	465	327.48	283.73	13.18	486.58	210.10	29.11	1291.96	1321.07
65PV	M	54	80	175	26.12	466	365.73	288.16	13.15	448.85	572.62	60.14	1628.38	1688.52
66PV	F	27	60	167	21.51	456	590.96	377.73	13.43	784.01	368.61	47.82	2086.92	2134.74
67PV	F	23	52	164	19.33	356	472.62	414.09	17.23	497.65	181.98	29.42	1554.16	1583.58
68PV	M	37	87	168	30.82	485	456.71	377.11	193.34	1039.37	495.88	380.42	2181.98	2562.40
69PV	M	24	64	178	20.20	481	132.82	71.37	12.74	448.49	179.42	54.03	790.82	844.85
70PV	M	57	83	183	24.78	653	189.11	141.27	9.39	445.31	335.22	34.45	1085.84	1120.29
71PV	M	54	94	187	26.88	519	236.00	238.49	11.80	1308.48	1021.79	107.22	2709.34	2816.56
72PV	M	26	104	186	30.06	507	283.43	225.71	12.08	284.86	124.74	27.28	903.54	930.82
73PV	M	56	79	172	26.70	375	538.88	411.31	16.35	1956.61	1280.86	146.16	4057.83	4204.00
74PV	F	52	70	160	27.34	567	337.29	269.24	10.80	459.60	345.27	49.76	1372.44	1422.20
76PV	M	27	70	180	21.60	680	214.50	166.11	9.01	358.28	169.59	18.76	898.73	917.49
77PV	M	50	100	171	34.20	862	178.00	192.89	7.11	539.16	286.50	37.44	1166.21	1203.65
78PV	M	44	86	187	24.59	451	114.43	90.16	13.58	597.68	351.10	61.15	1105.80	1166.95
79PV	M	38	83	169	29.06	691	294.19	196.80	8.87	410.18	239.90	34.66	1115.28	1149.94
80PV	M	28	83	186	23.99	516	406.01	307.37	11.87	331.69	171.15	29.83	1198.27	1228.10
81PV	M	63	84	174	27.74	506	397.65	271.07	12.10	1883.00	1082.24	108.49	3537.58	3646.07
82PV	M	40	78	165	28.65	901	170.29	137.35	6.80	244.11	138.43	16.94	680.05	696.99
83PV	M	30	118	185	34.48	884	226.42	180.53	6.93	283.63	148.41	15.67	830.25	845.92
84PV	F	32	60	165	22.04	435	376.86	295.49	14.07	669.40	374.53	37.76	1692.60	1730.35
85PV	M	37	88	172	29.74	657	582.36	316.44	9.33	515.17	318.04	13.82	1727.52	1741.34
86PV	M	64	96	171	32.83	563	319.53	244.51	10.89	751.19	406.65	54.23	1678.53	1732.76
87PV	M	53	90	178	28.40	552	274.13	217.03	11.10	1595.15	916.84	79.58	2934.67	3014.25
88PV	M	21	74	189	20.72	722	224.56	176.59	8.49	199.65	77.08	14.02	672.34	686.36
89PV	F	46	55	160	21.48	602	288.47	209.43	10.18	576.66	390.15	49.62	1425.27	1474.89
90PV	M	67	72	181	21.97	412	458.87	334.49	14.88	2320.79	1628.31	148.09	4609.26	4757.34
92PV	F	53	64	163	24.08	485	362.86	294.28	12.63	1022.19	496.15	59.43	2128.69	2188.12
93PV	F	47	57	162	21.72	498	326.01	256.75	12.30	1222.66	877.61	108.74	2586.60	2695.34
94PV	M	43	69	164	25.65	3266	57.79	43.83	1.88	154.87	82.06	9.31	331.13	340.43

PID	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	∑Tri–CBs	∑Tetra–CBs	∑Penta–CBs	∑Hexa–CBs	∑Hepta–CBs	∑DL–PCB	∑NDL–PCB	∑PCBs
95PV	F	23	68	179	21.22	750	236.09	195.84	8.17	321.90	125.86	24.13	863.75	887.88
96PV	F	32	61	165	22.40	777	272.38	212.90	7.88	660.25	306.82	35.74	1424.49	1460.23
97PV	M	53	83	172	28.05	761	304.80	219.05	8.05	781.29	676.39	66.65	1922.93	1989.58
98PV	M	44	54	164	20.07	742	239.57	103.80	8.26	450.31	348.45	36.39	1114.00	1150.39
99PV	M	30	83	181	25.33	686	251.90	182.92	8.93	383.23	223.62	41.54	1009.08	1050.62
100PV	M	33	76	181	23.20	524	122.37	151.19	11.69	250.60	150.03	19.72	666.16	685.88
101PV	F	44	53	158	21.23	704	225.36	241.58	8.71	418.52	224.33	21.64	1096.86	1118.49
102PV	M	44	118	188	33.39	774	99.60	99.13	7.92	577.41	249.00	37.53	995.52	1033.05
103PV	M	36	92	172	31.10	655	44.58	39.89	9.35	987.67	455.50	66.91	1470.08	1536.99
104PV	M	33	86	174	28.40	499	188.04	124.39	12.28	296.16	159.91	18.19	762.58	780.77
105PV	M	34	95	174	31.37	741	133.17	116.75	8.27	306.19	148.21	23.28	689.31	712.59
106PV	F	20	50	162	19.05	466	138.53	145.85	13.16	219.44	64.09	19.49	561.58	581.07
107PV	M	25	89	188	25.18	1297	10.52	11.53	4.72	241.50	97.93	19.71	346.50	366.22
108PV	M	69	64	162	24.38	687	21.36	29.22	229.90	1446.98	773.19	311.92	2188.73	2500.65
109PV	F	43	69	165	25.34	658	5.90	34.69	74.87	650.77	299.25	101.31	964.16	1065.47
110PV	F	41	69	158	27.64	828	26.72	19.97	75.23	560.61	253.06	105.18	830.41	935.60
111PV	F	49	61	170	21.10	522	61.84	46.26	11.74	989.31	462.33	58.68	1512.81	1571.49
112PV	M	33	72	182	21.73	402	9.67	49.06	98.33	897.11	411.48	143.61	1322.03	1465.64
113PV	M	60	90	173	30.07	439	8.84	51.16	13.95	1709.22	1320.74	143.11	2960.80	3103.91
114PV	M	56	87	183	25.98	492	25.38	28.09	199.54	1472.04	762.27	287.96	2199.35	2487.31
115PV	M	27	81	182	24.45	457	8.49	45.86	13.40	862.19	335.64	45.62	1219.96	1265.58
116PV	F	54	98	160	38.28	416	9.34	8.24	14.74	1365.40	594.80	78.61	1913.91	1992.52
117PV	M	58	81	178	25.56	467	21.39	31.75	13.13	2070.79	1227.61	132.42	3232.25	3364.67
118PV	M	59	93	170	32.18	481	33.36	29.08	12.75	1258.24	710.45	76.05	1967.83	2043.88
119PV	M	22	82	176	26.47	673	19.92	19.27	9.11	366.70	141.62	22.18	534.43	556.61
120PV	F	52	77	159	30.45	655	5.93	22.01	9.35	929.32	448.45	44.41	1370.66	1415.07
121PV	F	24	60	166	21.77	502	7.73	49.57	12.20	1078.55	448.18	52.84	1543.40	1596.24
122PV	M	67	98	178	30.93	436	78.92	52.37	14.06	2433.74	1209.20	157.44	3630.85	3788.29
123PV	F	44	43	150	19.11	389	9.98	37.05	15.75	1066.02	529.46	82.27	1576.00	1658.27
124PV	M	41	75	173	25.06	423	9.17	8.09	14.47	584.45	355.25	21.45	949.99	971.44
125PV	F	28	51	158	20.43	479	34.26	151.23	186.03	1231.36	533.64	265.39	1871.14	2136.53
126PV	F	29	59	167	21.15	588	23.06	31.72	10.42	763.99	310.68	45.51	1094.35	1139.86
127PV	F	30	53	164	19.70	426	9.12	8.04	14.39	707.45	333.35	21.32	1051.03	1072.35
128PV	F	44	75	176	24.21	403	9.64	48.71	15.21	2023.50	903.08	115.26	2884.87	3000.13
129PV	F	33	49	460	19.14	463	36.92	71.18	13.24	1978.54	943.10	107.59	2935.40	3042.98
130PV	F	37	66	168	23.38	436	8.91	58.25	14.06	1561.59	672.60	95.10	2220.30	2315.40
131PV	F	38	63	170	21.80	503	124.56	62.38	191.74	1328.84	654.69	250.67	2111.55	2362.22
132PV	F	28	77	165	28.28	382	46.63	53.11	173.99	1435.18	557.35	252.83	2013.42	2266.25
133PV	F	32	42	150	18.66	403	154.75	111.61	222.26	2124.05	958.64	369.13	3202.17	3571.30
135PV	M	46	83	179	25.90	469	162.86	76.90	208.34	1863.14	965.09	277.37	2998.96	3276.33
136PV	F	38	61	150	27.10	468	8.30	44.76	130.88	1071.31	514.13	182.03	1587.34	1769.37
137PV	F	28	50	148	22.82	583	6.66	49.04	90.34	746.86	238.43	127.50	1003.82	1131.33
138PV	F	21	62	162	23.62	614	6.33	30.89	131.42	843.62	315.30	167.96	1159.60	1327.55

PID	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	∑Tri–CBs	∑Tetra–CBs	∑Penta–CBs	∑Hexa–CBs	∑Hepta–CBs	∑DL–PCB	∑NDL–PCB	∑PCBs
139PV	M	49	77	175	25.14	383	229.69	92.55	242.58	891.59	541.12	360.36	1637.17	1997.53
140PV	F	57	53	154	22.34	771	33.91	41.30	122.72	1118.41	627.19	180.41	1763.11	1943.52
141PV	M	68	75	167	26.89	660	5.88	42.98	235.47	1231.77	473.07	275.71	1713.46	1989.17
142PV	M	37	67	179	20.91	571	6.81	21.78	10.74	849.27	353.72	33.22	1209.09	1242.31
143PV	F	34	55	167	19.72	471	8.24	24.65	13.00	408.10	154.24	19.26	588.96	608.22
144PV	F	41	48	153	20.50	585	6.64	57.72	10.48	1339.98	562.25	72.15	1904.93	1977.08
145PV	F	34	53	155	22.06	551	7.04	91.64	11.12	1678.05	653.21	76.64	2364.42	2441.06
146PV	F	29	56	153	23.92	630	128.56	174.28	153.62	386.76	158.19	159.65	841.76	1001.41
147PV	F	39	52	165	19.10	578	165.25	45.24	10.61	568.64	326.93	15.72	1100.95	1116.66
148PV	F	46	71	154	29.93	738	5.26	147.04	116.60	619.55	236.62	136.27	988.81	1125.07
149PV	M	59	93	173	31.07	826	4.70	67.00	137.91	983.42	635.11	173.53	1654.61	1828.14
150PV	M	57	98	177	31.28	608	6.38	51.74	209.27	1368.11	543.61	284.75	1894.36	2179.11
151PV	F	50	58	160	22.65	614	6.33	43.53	204.00	1393.50	693.84	290.94	2050.25	2341.19
152PV	F	38	55	163	20.70	634	6.12	22.34	9.66	749.15	350.42	41.53	1096.18	1137.71
153PV	F	49	55	160	21.48	726	5.35	27.32	118.98	943.42	456.64	175.44	1376.27	1551.71
154PV	F	37	60	165	22.03	930	4.18	34.80	110.27	722.38	274.06	137.47	1008.22	1145.69
155PV	F	48	73	161	28.16	585	6.64	55.51	234.92	1480.43	589.40	291.96	2074.93	2366.89
156PV	F	52	69	161	26.61	676	5.75	44.68	181.07	1288.80	466.79	240.68	1746.41	1987.09
157PV	F	42	54	160	21.09	601	6.46	5.70	138.35	954.43	461.74	192.05	1374.64	1566.69
158PV	F	35	53	160	20.70	533	7.28	48.56	278.97	1575.08	634.50	347.95	2196.44	2544.39
159PV	F	32	66	170	22.83	614	6.33	45.60	229.58	1040.63	327.44	266.23	1383.35	1649.58
160PV	F	27	58	173	19.37	671	5.79	193.11	173.32	388.70	142.47	201.44	701.95	903.39
161PV	F	42	58	158	23.23	644	261.16	169.05	101.03	626.86	228.55	112.53	1274.12	1386.64
162PV	F	29	61	172	20.61	703	593.47	286.17	224.04	710.00	227.78	370.78	1670.69	2041.47
163PV	F	52	52	155	21.64	758	249.49	149.39	8.09	582.44	285.55	42.67	1232.30	1274.96
164PV	F	38	85	183	25.38	822	142.04	81.39	7.45	352.86	180.45	27.80	736.39	764.20
165PV	F	49	56	164	20.82	542	168.84	187.84	11.30	525.06	253.92	51.09	1095.88	1146.97
166PV	F	54	61	160	23.82	677	135.26	157.05	221.35	687.69	353.72	262.04	1293.03	1555.07
167PV	F	53	59	165	21.67	575	161.56	164.73	10.66	719.58	417.61	46.38	1427.76	1474.14
1MI	M	27	72	165	26.40	476	40.79	71.95	80.45	1205.08	1567.77	176.54	2789.50	2966.04
2MI	F	27	65	173	21.70	468	41.48	73.18	81.82	686.83	391.87	179.56	1095.64	1275.19
3MI	F	34	56	170	19.30	475	40.87	72.10	80.62	1674.27	561.84	176.91	2252.79	2429.70
4MI	F	25	59	168	20.90	545	35.62	62.84	70.26	747.41	299.37	154.19	1061.32	1215.51
5MI	F	24	60	160	23.45	443	43.86	77.37	86.51	907.74	28.57	189.85	954.22	1144.06
6MI	F	21	50	163	19.00	544	35.70	62.98	70.41	963.03	23.26	154.52	1000.86	1155.38
7MI	M	42	75	185	21.90	616	31.49	55.56	62.12	1038.35	20.52	136.31	1071.72	1208.03
8MI	F	34	84	168	29.70	458	42.37	74.74	83.56	1324.27	416.77	183.37	1758.33	1941.70
9MI	F	26	67	165	24.60	515	37.72	66.54	74.39	1706.50	434.91	163.25	2156.81	2320.06
10MI	F	54	65	150	29.00	657	29.54	52.10	58.26	1821.14	790.83	127.84	2624.02	2751.86
11MI	F	36	45	150	20.00	605	32.08	325.38	63.27	1245.35	574.70	138.84	2101.93	2240.77
12MI	M	32	90	185	23.00	650	29.89	144.98	58.95	1407.64	841.09	129.35	2353.18	2482.54
13MI	M	40	88	183	26.00	538	36.07	63.63	71.14	1163.42	401.80	156.11	1579.94	1736.05
15MI	F	41	52	160	20.30	570	34.04	60.05	67.14	3162.99	1419.32	147.34	4596.20	4743.54

PID	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	∑Tri–CBs	∑Tetra–CBs	∑Penta–CBs	∑Hexa–CBs	∑Hepta–CBs	∑DL–PCB	∑NDL–PCB	∑PCBs
16MI	F	49	78	172	26.36	572	33.97	368.53	67.00	2385.44	422.69	147.03	3130.60	3277.63
17MI	F	32	76	167	27.95	611	31.80	56.09	62.72	1402.36	300.37	137.63	1715.70	1853.33
18MI	F	38	55	162	20.95	483	40.21	70.94	79.32	2284.33	916.91	174.05	3217.66	3391.71
19MI	F	38	86	164	31.96	526	36.92	65.12	72.81	1717.64	464.15	159.78	2196.86	2356.64
20MI	F	38	46	150	20.40	471	41.26	884.36	81.38	2002.33	389.77	178.59	3220.51	3399.10
21MI	F	29	60	174	20.00	526	36.92	569.80	72.81	1612.27	444.91	159.78	2576.92	2736.70
22MI	F	26	70	175	23.00	498	38.97	68.74	76.86	956.63	357.95	168.66	1330.48	1499.14
23MI	F	46	47	151	20.60	627	30.97	54.64	61.09	1100.79	756.70	134.06	1870.13	2004.20
24MI	F	34	53	160	20.70	499	38.89	68.60	76.71	1132.33	367.36	168.33	1515.57	1683.89
25MI	M	43	83	170	28.70	544	35.70	62.98	70.41	1100.57	472.11	154.52	1587.25	1741.77
26MI	F	36	55	172	18.70	351	55.36	356.47	109.20	4401.39	1633.87	239.63	6316.67	6556.30
27MI	F	34	54	162	20.57	307	63.17	473.57	124.59	1875.27	432.09	273.41	2695.28	2968.69
28MI	F	69	59	153	25.20	572	33.92	59.84	66.91	2914.69	1445.30	146.83	4373.84	4520.66
29MI	F	34	55	163	20.70	483	40.24	70.98	79.36	1895.11	642.20	174.15	2553.73	2727.88
30MI	F	44	58	160	22.60	516	37.60	66.33	74.17	107.34	24.50	162.76	147.19	309.94
31MI	F	33	55	163	20.70	476	40.83	72.02	80.53	1037.26	555.90	176.71	1609.82	1786.53
32MI	F	21	53	163	19.90	376	51.68	341.89	101.94	1401.57	164.98	223.70	1838.37	2062.08
33MI	F	44	63	173	21.00	474	40.97	72.27	80.80	1929.58	1184.99	177.32	3131.29	3308.61
34MI	M	20	57	168	20.20	345	56.27	99.26	110.99	1525.96	384.91	243.55	1933.84	2177.40
35MI	F	20	65	173	21.70	466	41.69	73.55	82.24	886.71	230.89	180.47	1134.61	1315.08
36MI	F	25	62	160	24.00	435	44.62	78.71	88.01	1025.30	305.21	193.12	1348.72	1541.85
37MI	M	21	78	186	22.50	472	41.17	72.63	81.21	1145.89	281.63	178.20	1444.32	1622.52
38MI	F	27	60	163	22.60	520	37.33	65.85	73.62	1145.46	352.61	161.56	1513.30	1674.86
39MI	M	21	69	172	23.30	524	37.08	65.41	73.14	1280.78	301.96	160.50	1597.88	1758.38
40MI	F	26	59	170	20.40	473	41.02	72.37	80.91	1422.82	1854.76	177.56	3294.32	3471.89
41MI	M	20	85	194	22.60	503	38.58	153.13	76.10	1007.69	404.66	166.99	1513.17	1680.16
42MI	M	20	62	177	19.80	510	38.05	318.84	75.05	1053.61	1598.90	164.70	2919.75	3084.45

Keys: ∑PCB, ∑PCB 28, 31, 52, 77, 101, 105, 118, 126, 138, 153, 156, 167, 169, 180; ∑tri–CBs, ∑PCB 28, 31; ∑tetra–CBs ∑PCB 52, 77; ∑penta–CBs, ∑PCB 101, 105, 118, 126; ∑hexa–CBs, ∑PCB 128, 138, 153, 156, 169) and ∑hepta–CBs, ∑PCB 170,180; ∑DL–PCBs, (∑77, 105, 118, 126, 156, 169); ∑NDL–PCB, ∑PCB 28, 31, 52, 101, 118, 138, 153, 167, 180).

Annex VI: Individual personal characteristics of the examined subjects and results of measurements (All OCP measurements are reported in pmol/g serum lipid).

PID	Sex	Age	Weight (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	β-HCH	HCB	*p,p′*-DDE	*o,p′*-DDD	*p,p′*-DDD	*o,p′*-DDT	∑*p,p′*-DDX	∑*o,p′*-DDX	∑DDT	∑OCP
1NF	F	47	54	164	20.08	597	340	541	727	382	1021	704	1747	1086	2834	3714
2NF	M	45	85	180	26.23	729	35	72	272	266	13	925	285	1191	1475	1583
3NF	M	30	73	171	24.96	393	66	214	72	8	127	998	199	1006	1205	1485
4NF	M	50	76	185	22.21	410	75	231	169	183	198	3378	367	3561	3928	4235
5NF	M	43	61	173	20.38	482	756	153	33	6	920	2997	953	3003	3956	4865
6NF	F	53	75	173	25.06	455	552	1350	650	17	41	1674	691	1691	2382	4284
7NF	F	40	58	162	22.10	465	55	1050	277	34	161	1826	439	1860	2298	3403
8NF	F	44	56	165	20.57	543	47	220	168	12	1231	1086	1399	1097	2497	2764
9NF	M	53	85	173	28.40	908	28	131	339	21	79	1019	419	1040	1458	1618
10NF	M	48	70	170	24.22	575	45	18	405	5	33	20	437	25	462	525
11NF	M	59	70	168	24.80	576	45	189	306	22	542	274	848	296	1144	1378
12NF	M	40	70	170	24.22	454	57	240	332	83	48	112	381	194	575	872
13NF	F	53	80	160	31.25	483	441	2014	1081	26	453	520	1533	546	2079	4534
14NF	F	46	62	167	22.23	399	65	528	1348	63	901	106	2248	169	2417	3009
15NF	F	44	87	175	28.41	452	57	831	744	28	249	94	993	121	1114	2003
16NF	M	48	100	176	32.28	467	55	970	1212	348	120	6	1332	354	1686	2711
17NF	F	53	59	156	24.24	442	54	278	726	49	49	51	775	101	876	1208
18NF	M	49	90	173	30.07	473	80	282	492	7	7	54	499	60	559	921
19NF	F	44	51	155	21.23	552	355	64	177	28	57	608	233	636	870	1288
20NF	F	37	56	168	19.84	520	50	7	538	18	919	16	1457	34	1492	1548
21NF	F	22	47	158	18.83	427	60	8	52	22	15	59	66	81	148	216
22NF	M	34	68	171	23.26	437	59	209	317	21	7	607	324	628	952	1220
23NF	M	49	80	185	23.37	398	65	71	1177	149	31	617	1209	766	1974	2110
24NF	M	22	80	175	26.12	401	120	88	227	31	23	49	251	80	331	539
25NF	F	26	57	168	20.20	440	250	160	121	43	21	16	143	59	201	611
26NF	M	27	67	175	21.88	285	229	12	1556	22	33	25	1589	47	1635	1877
27NF	M	49	80	180	24.69	477	54	228	831	26	20	118	850	144	995	1277
28NF	F	52	52	168	18.42	570	380	209	695	16	11	59	706	76	782	1371
29NF	F	25	45	160	17.58	433	60	41	450	43	36	78	486	121	608	708
30NF	M	57	80	170	27.68	536	48	393	1320	15	15	395	1334	409	1744	2185
31NF	F	32	58	160	22.66	396	65	222	349	20	20	18	369	38	407	693
32NF	M	43	100	191	27.41	495	486	78	305	16	16	14	321	30	351	915
33NF	F	29	51	164	18.96	401	815	114	20	19	19	18	39	37	76	1005
34NF	F	37	52	168	18.42	352	73	140	545	22	22	20	567	42	609	822
35NF	M	45	80	176	25.83	457	56	254	647	17	17	15	664	33	696	1006
36NF	M	49	70	177	22.34	466	243	166	567	17	17	15	584	32	615	1025
1PV	M	32	68	175	22.20	417	511	84	196	349	229	460	425	809	1234	1829
2PV	M	35	69	169	24.16	468	632	105	138	180	130	380	268	560	828	1565
3PV	F	32	46	152	19.91	740	195	64	76	30	93	231	169	260	430	689

PID	Sex	Age	Weight (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	β-HCH	HCB	*p,p′*-DDE	*o,p′*-DDD	*p,p′*-DDD	*o,p′*-DDT	∑*p,p′*-DDX	∑*o,p′*-DDX	∑DDT	∑OCP
4PV	F	27	59	155	24.56	487	413	101	100	80	183	330	283	410	693	1207
5PV	M	51	98	179	30.58	586	288	78	123	53	280	313	403	366	770	1135
6PV	M	40	78	174	25.76	498	839	92	259	78	144	212	403	291	694	1625
7PV	F	27	45	155	18.73	588	304	75	142	138	186	389	328	527	854	1233
8PV	M	48	73	174	24.11	405	471	104	105	108	401	1191	506	1299	1805	2380
9PV	M	46	75	180	23.15	455	264	112	124	72	220	350	344	422	767	1143
10PV	F	36	48	162	18.29	484	661	80	16	107	268	647	284	753	1038	1778
11PV	M	23	68	170	23.53	478	860	107	135	235	252	351	387	586	973	1939
12PV	M	40	104	182	31.40	347	708	187	199	104	329	976	528	1079	1607	2503
13PV	F	40	54	164	20.00	414	801	153	137	155	185	365	322	519	841	1795
14PV	M	27	66	174	21.80	542	676	81	116	95	167	284	283	379	662	1419
15PV	M	41	131	188	37.06	439	454	116	183	100	520	591	702	691	1393	1963
16PV	M	41	65	179	20.29	583	602	78	140	150	13	508	154	658	812	1492
17PV	M	63	79	167	28.33	472	561	100	523	17	278	377	801	393	1194	1855
18PV	M	71	85	176	27.44	346	790	406	1854	154	564	958	2418	1111	3530	4726
19PV	F	56	74	154	31.20	502	647	182	789	106	401	641	1191	746	1937	2766
20PV	M	59	97	172	32.79	509	811	121	507	71	239	490	746	561	1307	2238
21PV	M	52	89	176	28.73	559	154	101	298	87	14	194	312	281	593	847
22PV	M	40	83	182	25.05	535	125	95	94	64	50	134	144	199	342	563
23PV	M	45	68	165	24.98	608	249	72	140	54	59	264	199	318	517	838
24PV	M	38	81	172	27.38	509	331	100	71	15	15	47	86	62	149	580
25PV	M	52	77	168	27.28	475	485	126	420	62	66	330	486	392	878	1489
26PV	M	42	69	182	20.83	514	301	92	205	15	15	14	220	29	249	642
27PV	F	50	50	155	20.81	484	327	112	205	16	126	15	331	31	361	800
28PV	F	50	62	150	27.55	576	167	107	407	81	90	12	496	94	590	864
29PV	M	40	80	173	26.73	542	165	91	128	55	69	289	197	344	540	796
30PV	M	40	118	175	38.53	649	344	84	75	12	29	165	104	177	281	709
31PV	M	35	85	185	24.83	524	472	161	198	15	15	13	213	28	241	875
32PV	M	58	89	175	29.06	422	293	100	123	19	19	17	141	35	177	570
33PV	M	56	88	178	27.77	502	298	101	157	16	16	14	172	30	202	601
34PV	M	64	68	170	23.53	412	467	132	84	99	15	294	99	393	492	1092
35PV	M	31	87	187	24.88	466	454	79	132	80	205	336	336	416	753	1285
36PV	M	34	72	175	23.51	485	262	80	139	16	16	337	155	353	509	851
37PV	M	64	68	165	24.97	557	278	98	581	79	140	251	722	329	1051	1426
38PV	M	58	67	164	24.91	451	469	105	268	17	17	22	286	39	325	899
39PV	F	34	48	154	20.24	615	436	86	212	79	71	328	283	407	690	1212
40PV	F	39	59	156	24.24	306	1023	155	87	26	26	23	113	49	161	1339
57PV	M	45	65	171	22.23	407	190	116	282	123	184	697	466	819	1286	1592
62PV	M	21	78	183	23.29	429	305	106	198	135	127	677	325	812	1137	1548
63PV	M	42	78	176	25.18	453	228	143	163	17	17	243	180	260	440	812
64PV	M	32	83	185	24.25	465	362	143	433	101	91	601	523	701	1225	1731
66PV	F	27	60	167	21.51	456	388	127	293	99	116	560	410	659	1069	1584
67PV	F	23	52	164	19.33	356	386	158	283	149	219	1854	502	2003	2505	3050

PID	Sex	Age	Weight (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	β-HCH	HCB	*p,p′*-DDE	*o,p′*-DDD	*p,p′*-DDD	*o,p′*-DDT	∑*p,p′*-DDX	∑*o,p′*-DDX	∑DDT	∑OCP
68PV	M	37	87	168	30.82	485	135	134	477	64	81	561	557	626	1183	1451
70PV	M	57	83	183	24.78	653	300	62	116	81	12	1274	128	1356	1483	1845
71PV	M	54	94	187	26.88	519	215	101	303	66	178	1343	481	1409	1889	2206
72PV	M	26	104	186	30.06	507	180	100	198	71	250	1174	448	1245	1693	1973
74PV	F	52	70	160	27.34	567	273	84	349	50	110	440	460	490	949	1306
75PV	M	43	75	170	26.01	595	246	91	217	92	53	261	269	353	622	959
76PV	M	27	70	180	21.60	680	243	67	190	69	76	442	265	511	776	1086
80PV	M	28	83	186	23.99	516	203	85	149	82	15	366	164	448	612	901
91PV	M	44	73	173	24.39	776	109	68	128	68	121	9	248	78	326	502
95PV	F	23	68	179	21.22	750	211	70	143	92	73	314	215	406	621	902
138PV	F	21	62	162	23.62	614	118	80	384	81	51	1181	435	1262	1697	1895
140PV	F	57	53	154	22.34	771	323	66	116	45	41	101	157	145	302	691
166PV	F	54	61	160	23.82	677	130	67	114	60	129	325	243	385	628	825
1MI	M	27	72	165	26.40	476	54	479	4380	16	16	2495	4396	2512	6908	7441
2MI	F	27	65	173	21.70	468	55	510	3292	17	17	15	3309	32	3341	3906
3MI	F	34	56	170	19.30	475	54	628	8539	16	16	15	8556	31	8587	9270
4MI	F	25	59	168	20.90	545	47	316	2885	14	14	13	2899	27	2926	3289
5MI	F	24	60	160	23.45	443	58	555	7104	18	18	16	7121	34	7155	7769
6MI	F	21	50	163	19.00	544	47	497	2417	14	14	13	2431	27	2459	3003
7MI	M	42	75	185	21.90	616	42	370	2805	13	13	11	2818	24	2842	3254
8MI	F	34	84	168	29.70	458	56	843	3637	17	17	15	3654	32	3686	4585
9MI	F	26	67	165	24.60	515	50	512	2138	15	15	14	2153	29	2182	2744
10MI	F	54	65	150	29.00	657	39	657	3588	12	12	11	3600	23	3622	4318
11MI	F	36	45	150	20.00	605	43	957	6250	13	13	12	6263	25	6287	7287
12MI	M	32	90	185	23.00	650	40	351	4840	12	12	11	4852	23	4875	5266
13MI	M	40	88	183	26.00	538	48	470	2629	15	15	13	2643	28	2671	3188
14MI																
15MI	F	41	52	160	20.30	570	45	979	6065	14	14	12	6078	26	6104	7128
16MI	F	49	78	172	26.36	572	45	571	6767	14	14	1747	6781	1761	8542	9158
17MI	F	32	76	167	27.95	611	42	713	4614	13	13	12	4627	24	4651	5407
18MI	F	38	55	162	20.95	483	53	560	14654	16	16	15	14670	31	14701	15314
19MI	F	38	86	164	31.96	526	49	888	3288	15	15	13	3303	28	3331	4268
20MI	F	38	46	150	20.40	471	55	746	5446	17	17	15	5463	32	5495	6296
21MI	F	29	60	174	20.00	526	49	774	3217	15	15	13	3231	28	3260	4083
22MI	F	26	70	175	23.00	498	52	627	2524	16	16	14	2540	30	2570	3249
23MI	F	46	47	151	20.60	627	41	482	6110	12	12	11	6123	24	6146	6669
24MI	F	34	53	160	20.70	499	52	225	2708	16	376	14	3084	30	3114	3390
25MI	M	43	83	170	28.70	544	47	323	2891	14	977	13	3868	27	3895	4265
26MI	F	36	55	172	18.70	351	74	1182	7962	22	22	20	7985	42	8027	9282
27MI	F	34	54	162	20.57	307	84	925	5514	25	315	23	5829	48	5878	6887
28MI	F	69	59	153	25.20	572	45	1215	17531	14	546	12	18077	26	18103	19363
29MI	F	34	55	163	20.70	483	53	488	5363	16	732	15	6095	31	6126	6667
30MI	F	44	58	160	22.60	516	50	17	15	15	15	14	30	29	59	126

PID	Sex	Age	Weight (kg)	Height (cm)	BMI (kg/m^2)	TL (mg/dL)	β-HCH	HCB	*p,p′*-DDE	*o,p′*-DDD	*p,p′*-DDD	*o,p′*-DDT	∑*p,p′*-DDX	∑*o,p′*-DDX	∑DDT	∑OCP
31MI	F	33	55	163	20.70	476	54	495	3306	16	493	15	3799	31	3830	4379
32MI	F	21	53	163	19.90	376	69	523	5859	21	624	19	6483	40	6523	7115
33MI	F	44	63	173	21.00	474	54	585	7962	16	527	15	8489	31	8521	9161
34MI	M	20	57	168	20.20	345	75	834	8339	23	23	20	8361	43	8405	9314
35MI	F	20	65	173	21.70	466	55	324	5726	17	17	15	5743	32	5775	6155
36MI	F	25	62	160	24.00	435	59	896	5781	18	18	16	5799	34	5833	6788
37MI	M	21	78	186	22.50	472	55	633	4208	17	17	15	4224	32	4256	4943
38MI	F	27	60	163	22.60	520	50	1060	4691	15	15	14	4706	29	4735	5844
39MI	M	21	69	172	23.30	524	49	637	3904	15	465	216	4369	230	4600	5286
40MI	F	26	59	170	20.40	473	54	660	3654	17	17	15	3671	31	3702	4417
41MI	M	20	85	194	22.60	503	51	474	2499	16	16	14	2515	30	2544	3070
42MI	M	20	62	177	19.80	510	51	406	1233	15	15	14	1248	29	1277	1734

*Abbreviations***:** BMI, body mass index; TL, total lipids; β-HCH, beta-hexacyclohexane; HCB, hexachlorobenzene; *o,p′*-DDE, 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethylene; *p,p′*-DDE, 1,1-dichloro-2,2-bis-(p-chlorophenyl)-ethylene; *o,p′*-DDD, 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane; *p,p′*-DDD, 1,1-dichloro-2, 2-bis (4-chlorophenyl)ethane; *o,p′*-DDT, 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p* chlorophenyl)-ethane; *p,p′*-DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane; ∑*p,p′*-DDX = (*p,p′*-DDE + *p,p′*-DDD); ∑*o,p′*-DDX = (*o,p′*-DDD + *o,p′*-DDT); ∑DDT = (∑*p,p′*-DDX + ∑*o,p′*-DDX); ∑OCP = (β-HCH + HCB + ∑DDT).