Occurrence of Persistent Toxic Substances in Soils, Sediments, Fishes and Human Breast Milk in Southern Vietnam

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ABSTRACT

Global contamination and toxic effects of persistent toxic substances (PTSs) have been an emerging environmental issue and have received considerable attention during the past four decades. The rapid agricultural and industrial growths as well as the expansion of urban areas in Hochiminh City and Mekong River Delta, two of the most densely populated areas in the world with about 26 million peoples, result in the widespread contamination of PTSs in southern Vietnam. Due to adverse effects to human health and environment, more attention has been paid to PTSs in Vietnam since the early 1990s. However, these works mainly focused on PTSs in water, soil and sediment media. PTSs in animals, birds and human (blood, adipose tissue, breast milk, etc.) have drawn less attention due to high cost of research and the need of sophisticated analytical techniques.

With the support of Swiss Agency for Development and Cooperation (SDC) project in the frame of a collaboration between Vietnam and Switzerland, we have carried out the research named "Persistent Toxic Substances in southern Vietnam; Development analytical methods, bioaccumulation and modeling". Our research has established suitable analytical methods, which is able to analyze simultaneously PCBs, PBDEs, OCls pesticides and especially, mirex and toxaphene, which are analyzed for the first time in Vietnam, in various matrices such as soil/sediment, fish and human breast milk samples by HRGC/LRMS.

The sampling sites (including Hochiminh City, Dongnai – Baria Vungtau province and Mekong Delta) have been selected based on previous studies in Vietnam and collected documents from Vietnam Environment Administration and POPs Project of Vietnamese government. Twenty-one PCB congeners, twelve PBDE congeners and twenty-six organochlorine pesticides were chosen for research due to their high toxicities. Soil, sediment, fish tissue, and human breast milk were selected as the matrices to examine the PTSs residue.

The obtained results showed that PTSs levels of soil and sediment samples in southern Vietnam are not so high, e.g. DDTs and PCBs levels are lower than those in previous studies in Vietnam. Endosulfans were found in almost all sites suggesting the widespread contamination due to their large usage in agriculture. Toxaphene was only found in soil close to agrichemical warehouse at high level indicating potential source of toxaphene from the pesticide stockpiles. PBDEs were detected in most of the samples but at the low levels, which shows a possibly atmospheric deposition source. Other PTSs were detected at low levels (< 5 ng/g dry wt.) or below LOD of the analytical method.

PTSs concentrations in wild fish samples from Saigon and Tien Rivers are lower than those determined in previous studies in Vietnam and in the world. However, unlike DDTs, PCBs showed a slow decreasing in fish samples and this suggests a continuous contamination. Besides, due to large usage in agricultural activities, endosulfans were also found in almost all wild fish samples from Saigon and Tien River (23.9 and 8.3 ng/g lipid wt., respectively). Other PTSs were detected at low levels (< 5 ng/g dry wt.) or below LOD of the analytical method.

PTSs levels in human breast milk of HCMC residents are lower than those in previous study (except PCBs and CHLs). The higher PCB residues observed in human breast milk from HCMC suggest continuously high exposure to PCBs via the food chain to human. In this study, PTSs

levels in human breast milk of primiparous mother higher than those in multiparous mothers. This trend well agrees with previous study in Hanoi and HCMC.

By using statistic methods for obtained results (cluster analysis and PCA), we have shown the similarity of PTSs profiles pattern between landfill soil, TN-SG River sediments and Saigon River fish samples. Logically, we might suggest that there is a transfer of PTSs compounds from landfill and TN-SG River to fish samples collected from Saigon river. Our results clearly demonstrate that municipal and industrial waste from landfill and urban activities of HCMC could be considered as PTSs pollution sources.

The results of the present study have contributed to the improvement of PTSs research capability of Vietnam. This research also efficiently supports and serves the projects of monitoring, studying and evaluating impacts of PTSs on South Vietnam ecosystem during the implementation of NIP under Stockholm Convention.

Keywords: PTSs, human breast milk, Vietnam, soil, sediment, fish.

RESUME

La contamination généralisée de l'environnement par les substances toxiques persistantes (PTSs) et les leurs effets toxiques sont un problème préoccupant et a suscité l'attention de la communauté scientifique pendant les dernières quatre décennies. La croissance agricole et industrielle rapide ainsi que l'expansion des zones urbaines dans le delta de fleuve Mékong et à Ho Chi Minh Ville, deux régions parmi le plus peuplé dans le monde avec environ 26 millions d'habitants, ont eu comme conséquence la contamination généralisée du sud Vietnam par les PTSs. En raison de leurs effets nuisibles à la santé humaine et environnementale, une d'attention accrue a été prêtée depuis le début des années 90 aux PTS au Vietnam. Cependant, ces travaux se sont principalement concentré sur des PTSs dans le milieu aquatique (eau et sédiment) et dans les sols. Les PTSs dans les animaux terrestres, dans les oiseaux et chez l'homme (sang, tissu adipeux, lait maternel, etc.) ont attiré moins d'attention en raison des coûts élevés des recherches et au besoin des techniques analytiques sophistiquées.

Avec l'appui de la Direction du Développement et de la Coopération (DDC) de la Suisse et dans le cadre d'un projet de collaboration entre le Vietnam et la Suisse, nous avons effectué cette recherche appelée «Les substances toxiques persistantes au Sud Vietnam: développement de méthodes analytiques, bioaccumulation et modélisation». Notre recherche a établi des méthodes analytiques appropriées, permettant d'analyser simultanément les PCBs, les PBDEs, des pesticides organochlorés (et en particulier, le mirex et le toxaphène, qui ont été recherchés pour la première fois au Vietnam), dans diverses matrices comme le lait maternel humain, des sols, des sédiments et des poissons par HRGC/LRMS.

Les sites de prélèvement (Ho Chi Minh Ville, la province de Dongnai - Baria Vungtau et le delta de Mékong) ont été choisis sur les bases des précédentes études faites au Vietnam et des documents rassemblés par l'Administration Environnemental du Vietnam et par le projet POPs du gouvernement Vietnamien. Vingt et un congénères de PCBs, douze congénères de PBDEs et vingt-six pesticides chlorés ont été choisis pour cette recherche en raison de leurs toxicités élevées. Sols, sédiments, tissus de poisson et le lait maternel humain ont été choisis comme les matrices où rechercher des résidus de PTSs.

Les résultats obtenus ont montré que les niveaux des PTSs dans les échantillons de sol et de sédiment du Sud Vietnam sont plutôt faibles, les niveaux de DDTs et de PCBs, par exemple, sont inférieurs à ceux des précédentes études réalisées dans le pays. Les endosulfans ont été trouvés dans presque tous les lieux, suggérant une contamination généralisée due à leur grande utilisation dans l'agriculture. Le toxaphène a été seulement trouvé dans le sol aux alentours de l'entrepôt des substances agrochimiques représentant ainsi une source potentielle de contamination provenant des anciens stocks de pesticides. Les PBDEs ont été détectés dans la plupart des échantillons mais à des niveaux très bas, ce qui indiquerait une source de contamination probablement d'origine atmosphérique. D'autres PTSs ont été détectés à faibles concentrations (< 5 ng/g poids sec) ou au-dessous des limites de détection de la méthode analytique.

Les concentrations en PTSs dans les échantillons de poissons sauvages provenant des fleuves Saigon et Tien sont inférieurs à ceux qui ont été déterminés dans des études précédant au

Vietnam et dans le monde. Cependant, contrairement aux DDTs, les PCBs n'ont montré qu'une diminution lente dans des échantillons suggérant une contamination continue et toujours actuelle. En outre, en raison de sa grande utilisation dans l'agriculture, l'endosulfan a été trouvés dans pratiquement tous les échantillons de poissons provenant des fleuves Saigon et Tien (23.9 et 8.3 ng/g de poids lipidique, respectivement). Les autres PTSs ont été détectés à des faibles concentrations (< 5 ng/g poids sec) ou au-dessous des limites de détection de la méthode analytique.

Les niveaux de contamination du lait maternel humain des résidants de HCMC par les PTSs sont inférieurs à ceux mesurés dans une étude précédente (excepté PCBs et CHLs). Cependant, les résidus en PCBs observés dans le lait maternel à HCMC suggèrent une exposition continue de l'homme par la chaîne alimentaire. Dans cette étude, les niveaux en PTSs du lait maternel chez les mères primipares est plus élevé que chez les mères multipares. Cette tendance est conforme à une précédente étude faite à Hanoi et HCMC.

En employant des méthodes statistiques pour analyser les résultats obtenus (cluster analysis et PCA), nous avons montré la similitude des profils des PTSs entre le sol de la décharge, les sédiments du fleuve de TN-SG et les poissons du fleuve Saigon. Logiquement, nous pourrions proposer qu'il y a un transfert des PTSs à partir de la décharge et des sédiments du fleuve TN-SG aux poissons du fleuve Saigon. Nos résultats démontrent clairement que les déchets municipaux et industriels de la décharge ainsi que les activités urbaines de HCMC peuvent être considérés comme sources de pollution par les PTSs.

Les résultats de la présente étude ont contribué à l'amélioration des capacités de recherche sur les PTSs au Vietnam. Ce travail de recherche va servir de soutien efficace aux projets de monitoring qui étudieront et évalueront les impacts des PTSs sur les écosystèmes du Sud Vietnam pendant l'exécution du Plan national d'action (NIP) dans le cadre de la Convention de Stockholm.

Mots-clés: PTSs, lait maternel, Vietnam, sol, sédiment, poisson.

Abbreviations

CAS	Chemical Abstracts Service
CEAL	Central Environmental Analysis Laboratory
d.w.	dry weight
DCM	Dichloromethane
EPA	Environmental Protection Agency
EPFL	École Polytechnique Fédérale de Lausanne
Et_2O	diethyl ether
FAO	Food and Agriculture Organization
GC/MS	Gas chromatography/mass spectrometry
HCMC	Hochiminh City - Vietnam
HRGC/LRMS	High resolution gas chromatography/low resolution mass spectrometry
IER	Institute for Environment and Resources – Vietnam
IIE	Institut d'ingénierie d'environnement
IUPAC	International Union of Pure and Applied Chemistry
LOD	limit of detection
MLOD	Detection limit of the method
NIP	National Implementation Plan
OCPs	Organochlorine pesticides
PBDEs	Polybrominated diphenyl ethers
PCA	Principal Component Analysis
PCBs	Polychlorinated biphenyl
POPs	Persistent Organic Pollutants
PTS	Persistent Toxic Substances
UNEP	United Nation Environment Program
USSR	Union of Soviet Socialist Republics
VEA	Vietnam Environment Administration
VNU-HCMC	Vietnam National University of Hochiminh City
WHO	World Health Organization
wt.	weight

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INTRODUCTION

A. Context

Persistent Toxic Substances (PTSs) and Persistent Organic Pollutants (POPs) are a set of chemical substances that persist in the environment, undergo long range transport, bioaccumulation through the food chain, and pose a risk of causing adverse effects to human, wildlife and the environment. There have been more and more evidences that these pollutants, upon exposure of human population and wildlife, may cause serious health effects such as induction of cancers, damage to the nervous system, disruption of the immune and hormonal system, etc. resulting in various abnormalities (*Harrad, 2001; Meeker et al., 2007; Petersen et al., 2007; Todaka et al., 2009; Lignell et al., 2009*).

The PTSs emission sources could come from many natural and socioeconomic activities such as active volcano, forest fire, agricultural cultivations (include use, store and disposal of agricultural pesticides, open burning of biomass for land clearing activities), industrial activities (chemicals, pulp-and-paper, metallurgy, refinery and other industries, etc.), transportation and dumping sites. Besides that, PTSs emission sources are also formed from the burning of industrial, municipal, medical and hazardous waste, diesel fuel, etc (*UNEP, 2002*).

To protect human and environment from PTSs in general and POPs in particular, by 23 May 2001, 92 countries and EU had approved Stockholm Convention on POPs. Convention came into force in May 2004, when it was ratified by 50 nations. The ultimate goal of the Stockholm Convention is to decrease the concentration of POPs in the environment and human. Vietnam ratified the Stockholm convention in 22 July 2002 and is the 14th country in 160 countries which had ratified convention until August 2008. Vietnamese government also approved the National Implementation Plan (NIP) for participating, performing and validating Stockholm convention with decision No. 184/2006/QD-TTg of Prime Minister on the 10th of August 2006. Signatories to Stockholm Convention agree to reduce or eliminate releases from intentional or unintentional production and use of POPs and perform monitoring POPs levels in environment (*Stockholm Convention, 2001*).

According to Stockholm Convention, a list of 12 contaminant species (Table 1) in POPs group was put on priority for global restriction and ban in order to limit their impacts over the global environment. These 12 contaminant groups could be divided into three categories: by-product of other processes (polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans – PCDD/Fs); industrial chemicals (polychlorinated biphenyls - PCBs); and organochlorine pesticides (OCPs)

Table 1: List of POPs and their original sources.

Chemical names	Original sources
Dioxins and Furans (PCDD/Fs)	Formed unintentionally as byproducts in various processes, especially combustion and manufacturing of materials containing chlorine
PCBs	Industrial product used as dielectrics in transformers and large capacitors, as heat exchange fluids, as paint additives etc.
Aldrin	Pesticide used in agriculture to control soil insects such as termites, corn rootworm, etc.
Dieldrin	Pesticide used in agriculture and for control of some insect vectors of diseases
DDT	Pesticide used in agriculture and in control vector born diseases
Endrin	Pesticide used in agriculture to control soil insects, mice and voles
Chlordane	Pesticide used in agriculture and wood industry
Hexaclorobenzene	Organochlorine fungicide used in agriculture
Mirex	Pesticide used in agriculture to kill fire ants and termites
Toxaphene	Pesticide used primarily on cotton, cereal grains fruits
Heptachlor	Pesticide used in agriculture to control soil insect and termites

In March 2003, POPs Global Monitoring Programme (GMP) Workshop at Geneva Switzerland recommended that air, bivalves, biota and humans are to be considered first in a POPs monitoring Programme. There may be cases when countries or regions may choice water, soil sediments, etc. to identify levels of POPs in hot sopts. In a national POPs monitoring programme, the matrices considered were air, water, soils and sediments, wildlife, human foodstuffs and animal feed, and human tissues. These were assessed on the basis of response time to change, homogeneity, ease of sampling, existence of ongoing programmes and networks, and as indicators of source and exposure.

The Intergovernmental Negotiating Committee for a global treaty on POPs developed science-based screening criteria for identifying additional substances as POPs candidates for future. According to Stockholm Convention, the following parameters have generally been identified to be considered primarily for setting criteria to identify POPs: persistence, bio-accumulation, toxicity and potential for long-range transport.

In the International Conference held in 4 May 2009 in Geneva, Switzerland, nine new chemicals were listed under the Stockholm Convention. Many of these are still widely used today as pesticides, flame retardants and in a number of other commercial uses. These are Alpha hexachlorocyclohexane; Beta hexachlorocyclohexane; Hexabromodiphenyl ether and heptabromodiphenyl ether; Tetrabromodiphenyl ether and pentabromodiphenyl ether;

Chlordecone; Hexabromobiphenyl; Lindane; Pentachlorobenzene; Perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride (*Stockholm Convention, 2009*).

B. Overview on PTSs research in the world

Because of adverse effects to human health and environment, great attention has been paid to PTSs all over the world in generally and in Vietnam in particularly recently.

According to result of these researches, PTSs were found everywhere even in the regions where they have never been used. PTSs are ubiquitous in the environment. They have been measured on every continent, at sites representing every major climatic zone and geographic sector throughout the world (*Wagrowski and Hites, 2000; Breivik et al., 2004*). These include remote regions such as the open oceans, the desert, the Arctic and the Antarctic, where no significant local sources exist and the only reasonable explanation for their presence is long - range transport from other parts of the globe (*Macdonald et al., 2000; Scheringer et al., 2004; Hung et al., 2005*).

One of the most important properties of PTSs is their semi-volatility. This property confers a degree of mobility through the atmosphere that is sufficient to allow relative great amounts to enter the atmosphere and be transported over long distances. Thus, these substances may volatilize from the hot regions and will condense and tend to remain in colder areas (Fig. 1)

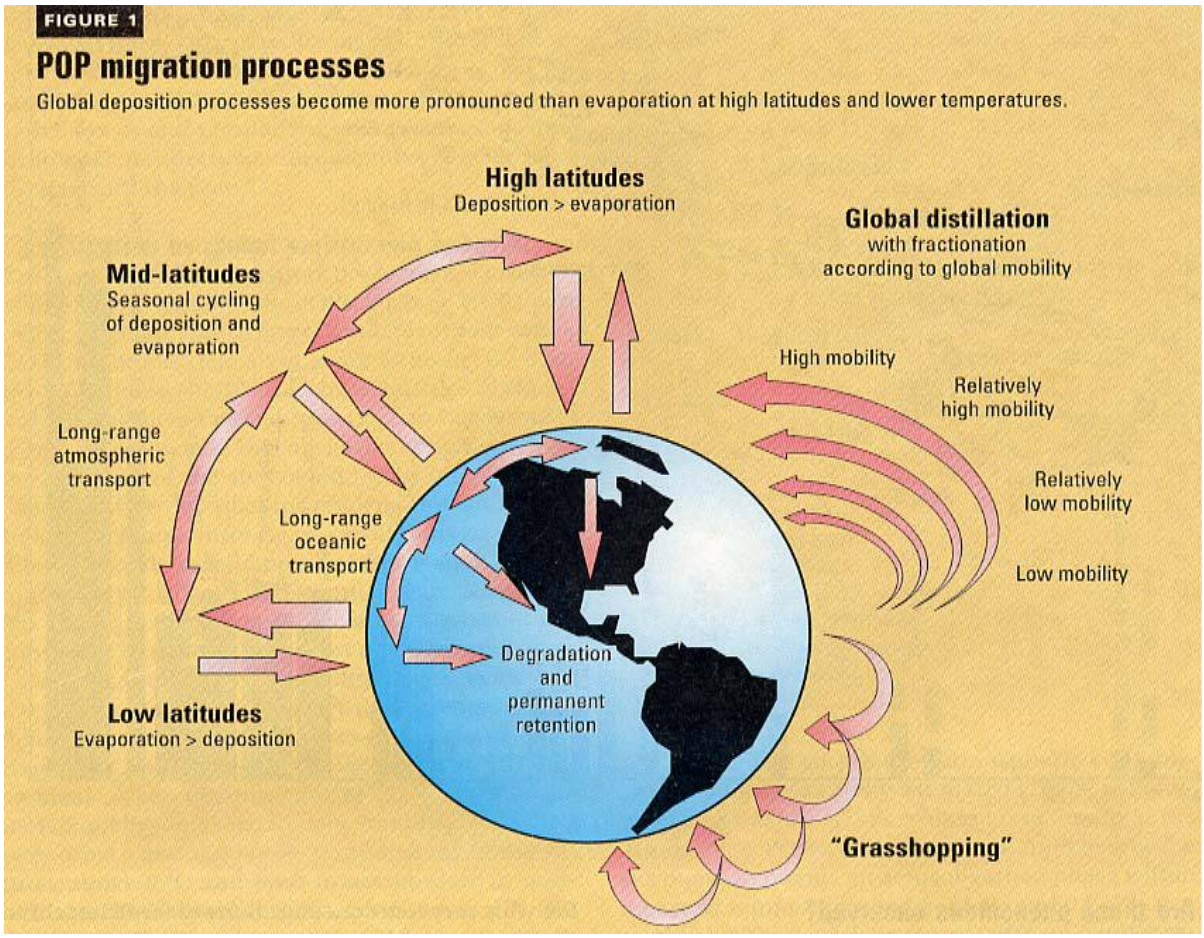

Figure 1: Schematic representation of the global fractionation hypothesis and the revolatilization on grasshopper effect (*Wania and Mackay, 1996*)

The characteristics of the polar ecosystem intensify the problem of contamination by PTSs because the colder climate reduced biological activities and relative small incidence of sunlight would be expected to increase the persistence of PTSs .

Another property of PTSs is accumulation capacity into organisms especially in adipose tissues. This property is lipophilicity. High lipophilicity of PTSs results in the substance bioconcentrating from the surrounding medium into the organism such as from water or sediment to fish (*Gobas et al., 1999*). Combined with environmental persistence and a resistance to biological degradation, lipophilicity of PTSs also results in biomagnification through the food chain. Biomagnification results in much greater exposures in organisms at the top of the food chain such as marine mammals (Fig. 2) (*Ross and Birnbaum, 2003*).

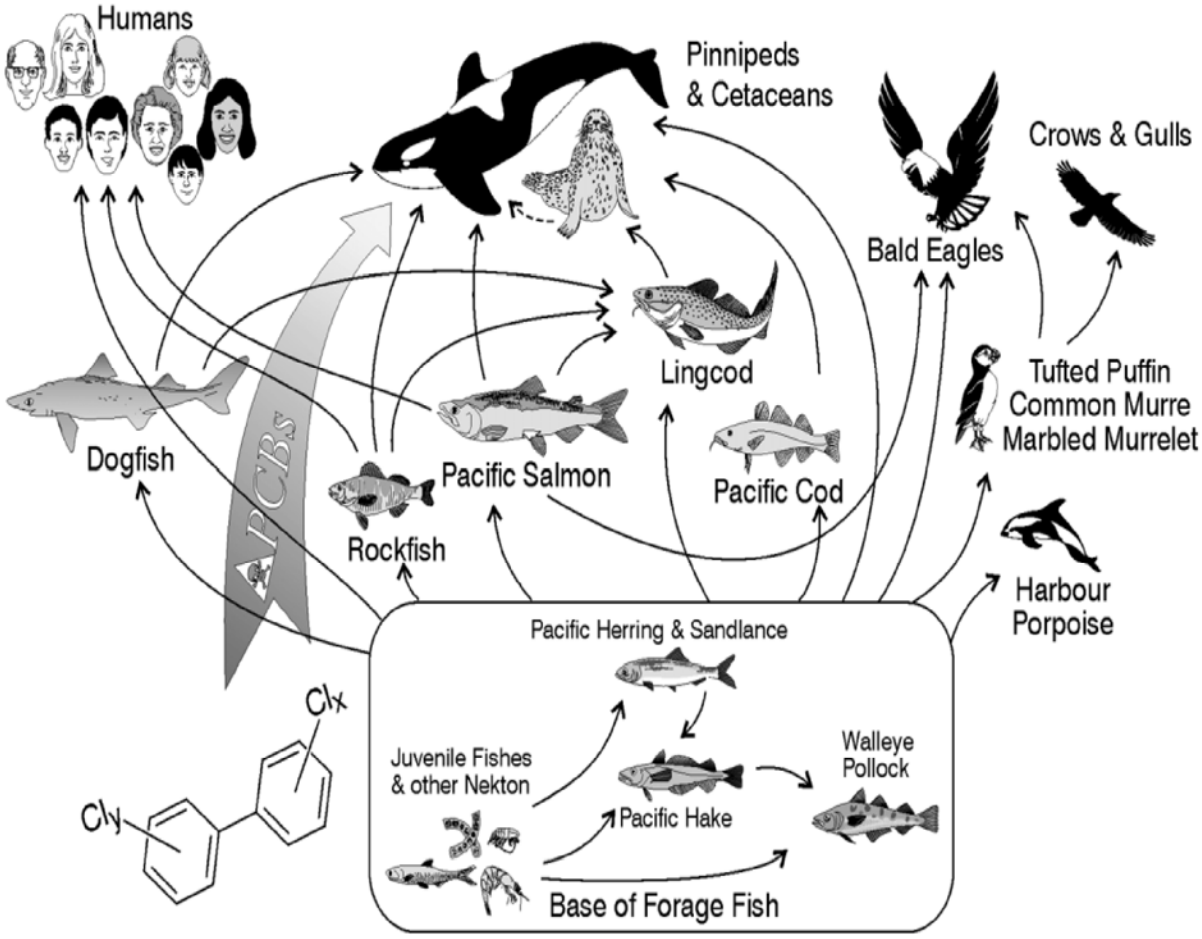

Figure 2. Biomagnifications of POPs through aquatic food chains, resulting high contamination levels in organisms at high trophic levels including humans (*Ross and Birnbaum, 2003*).

It is rather difficult to establish causality of illness or disease that is directly attributable to exposure to a specific persistent toxic substance in the environment. This difficulty is further underscored by the fact that PTSs rarely occur as single compounds and individual field studies are frequently insufficient to provide compelling evidence of cause and effect in their own way (*Vos et al., 2000*). However, it is the fact that the significant lipophilicity of these compounds means that PTSs are likely to accumulate, persist and bioconcentrate and thus they could achieve toxicologically relevant concentrations even though discrete exposure may appear limited (*Tanabe et al., 1994*).

Regarding the long-term toxicity of environmental contaminants to wildlife, greatest concerns are on carcinogenicity, teratogenicity, immunologic dysfunction and reproductive abnormalities. Indeed, some of these symptoms are associated with particular enzyme like cytochrome P-450 monooxygenase. These enzyme systems are known to modify some of these contaminants into active toxic intermediates, which in tern may disrupt the critical balance of endocrine systems. Some PTSs are believed to act in the same way to initiate toxic and biologic effects since they are potential inducers of P-450 enzyme systems (*Safe, 1990; Peakall, 1992*). Figure 3 demonstrated positive correlation between PCB residues and EROD/PROD enzyme activities in seals (*Tanabe, 2002*).

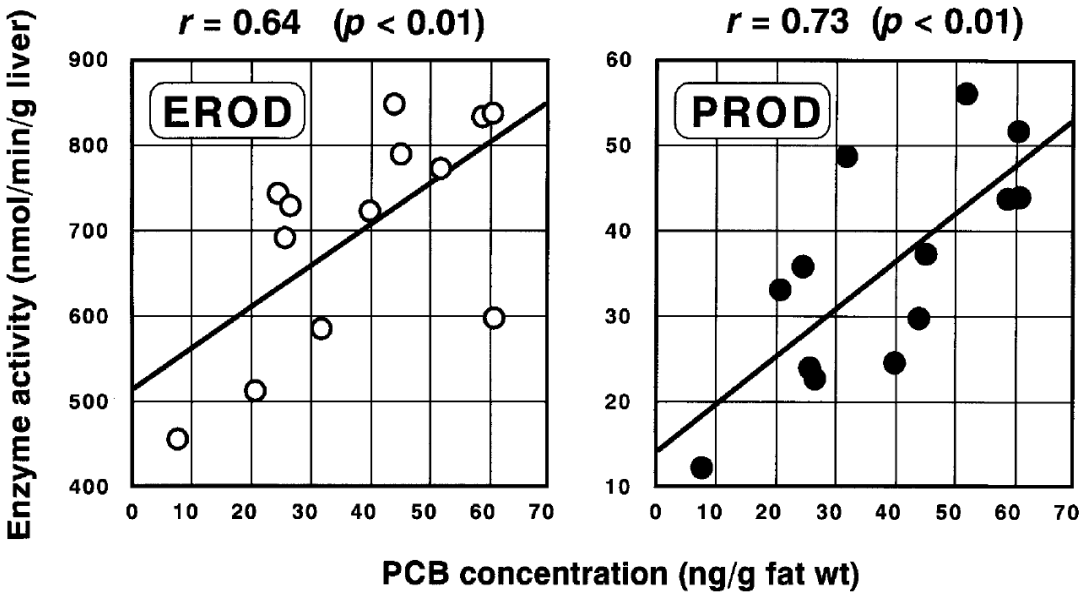

Figure 3: Relationship between enzyme activities in the liver microsome and PCB concentrations in the blood of northern fur seals from the North Pacific (*Tanabe, 2002*)

The effects of PTSs on enzyme activities have been also demonstrated to occur in other species of marine mammals (*Brouwer et al., 1989; Boon et al., 1992*). These results imply that enzyme induction and endocrine disruption by some PTSs may have taken place in nature ecosystems at the environmental levels (*Tanabe, 2002*). More discussion on effects of PTSs will be presented in the next chapter.

C. Situation of PTSs in Vietnam

Hochiminh city (HCMC) and Mekong delta are one of the biggest city and largest agricultural land in the Southeast Asia. They play a very important role for industry, agriculture and fisheries in South Vietnam. HCMC is located 1,725 km south of Hanoi and 50 km west of the South China Sea. HCMC has the area of 2,056 square km and population of 8 million persons. It is a big political, economical and industrial center of Vietnam. The city is also a cluster of hundreds of small river and channels watering the Mekong Delta.

Mekong River basin includes China, Myamar, Thailand, Lao, Cambodia and Vietnam. Its area is nearly of 800 thousands square km. Mekong river delta in South Vietnam, is inhabited by about 20 million persons. It is also one of the most highly productive agricultural lands in the world. Rice production is major economical sector in Mekong Delta contributing half of the rice production in Vietnam. On the other hand, development of agriculture in Mekong Delta raised some concern on environmental quality and disturbance on ecosystem. For example, intensive

use of organochlorine (OC) insecticides such as DDTs, chlordanes, HCHs may lead to considerable residues in the agriculture land. Moreover, relative persistence of such chemicals together with natural processes like evaporation and run-off, might enhance their ubiquitous distribution in environment, food chains and eventually bio-accumulate in humans.

In Vietnam, despite official ban on the usage of OCs on 1995, there have been evidences of recent uses of such chemicals, particularly DDT, throughout the country (*Minh et al., 2004, Nhan et al., 1998, Minh et al. 2002*). It can be anticipated that similar situation may occur in Mekong River delta due to high population density and intensive agriculture activities in this region.

Beside these sources from agricultural activities as described above, PTSs are polluting by-products and could came from many industries such chemical, pulp-and-paper, metallurgical and others. They are also formed from the burning of industrial and municipal waste, leaded gasoline, diesel fuel, etc. Vietnam is being in innovating process and there are many new export processing zones, industrial zones and factories, etc. In addition, with the introduction of market mechanisms over the last 10 years, agricultural production in Vietnam has sharply increased and the country is now a significant exporter of rice and coffee. All of them are the additional source of PTSs and should be considered when doing the research on this subject.

On 20 May 2004, Vietnam Ministry of Natural Resources and Environment (MONRE) sponsored a workshop in Hanoi to start the POPs project (POPs EA – VIE/01/G31/A/1G/99). Vietnam Environment Protection Agency (VEPA) and United Nations Developing Program (UNDP) will develop and promulgate National Implementation Plan (NIP) for participating, performing and validating Stockholm convention. In addition, a training workshop was organized by MONRE and UNDP in Hanoi and Hochiminh city in April 2005 on "**Environmental Permits, Emergency Response and POPs Treatment Technologies"**.

According to the conferences documents, pesticides in general have been used in Vietnam since 1940s. Before 1993, Vietnam imported about 7,500 – 8,000 tons/year. Since 1997, Vietnam has imported 36,000 tons/year including carbamate, organophosphorus, pyrethroid and endosulfan, etc. DDT pesticide had been imported since 1957 and the volume imported each year was around 1000 tons/year. Today, all organochlorine pesticides are prohibited in Vietnam and the country has not a special authorisation to continue using them, even for vector – born disease eradication programs (see Table 2). However, there is large stockpile of confiscated agricultural chemical waste in Viet Nam. It is estimated that 100 storage sites of banned or obsolete pesticides exist in the country. The amount of stocked agrochemicals was approximately 37'000 tons, in which POPs pesticides are about 13'245.7 Kg in powder form and 42 L in liquid form.

Table 2: Regulatory controls on organochlorine pesticides in Vietnam

Aldrin	Chlor-dane	DDT	Diel-drin	Endo-sulfan	Endrin	Hepta-chlor	HCB	Mirex	Toxa-phen
Banned in 1992	Banned in 1992	Banned in 1992	Banned in 1992	Banned in 2005[a]	Banned in 1992	Banned in 1992	Banned in 1992	Banned -	Banned in 1995

(*Source: UNEP, 2002*); [a]: MARD, 2005; -: data not available

PCBs were imported into Vietnam from former Soviet Union, China, and Romania with uncontrollable volume. So far, only the relating statistic from the electricity industry has been

found. This statistic shows that there are 73'647 litres and 5'296'702 kg oil probably containing PCBs in Vietnam. So far, PCBs has not had systematic statistic.

Almost all research on PTSs in Vietnam focused only on the contamination caused by DDTs, PCBs, lindane in environmental samples (soil, sediment, water, etc) as well as in bio-materials (mussel, fish) and human tissue (fat, serum, breast milk, etc). Lower attention was done to other PTSs substances such as chlordane, mirex, toxaphene, PBDEs, etc. Particularly mirex and toxaphene have not ever been analyzed in Vietnam's laboratories. Furthermore, there was still not a comprehensive research on all PTSs, especially on PTSs' source, distribution in natural environment and the transfer of PTSs from their emission sources or polluted compartments into the food chain in Vietnam. In other hand, there are many difficulties to perform a research relating to PTSs subject due to the complexity of the matrices which requires costly analytical equipments and chemicals, etc. Therefore, we would like to carry out the research "**Occurrence of Persistent Toxic Substances in Soils, Sediments, Fishes and Human Breast Milk in Southern Vietnam**"

With the obtained results, we hope to show clearly the relationships between the PTSs sources and their transfer into environment and food chain. They are useful basic to orient the monitoring programs for PTSs in Vietnam in the future.

Moreover, the result of this thesis could be very useful for IER-VNU (Institute for Environment and Resources – Vietnam National University of Hochiminh City) to develop its own skill in scientific research to participate on POPs monitoring network in Southern Vietnam in the frame of Stockholm Convention on POPs.

D Objectives and structure of the thesis

This thesis was set up for the following main objectives :

1. Develop common analytical methods, which adapt to research and analytical conditions in IER lab - Vietnam, for persistent toxic substances (PTSs) including PCBs, PBDEs and organochlorine pesticides

2. Clearly identify the different sources, types, contamination levels and distribution of PTSs in the environment of southern Vietnam (The primary sources could be riverine inflow, municipal and industrial waste water outlets, and secondary sources could be contaminated soil or sediments and dumping sites, etc.).

3. Balance the importance of these different sources according to socioeconomic activities. (for instance: agriculture in/out areas affected by industry, urban areas with and without industrial activities, etc)

4. Finding statistical relationships between emission sources or polluted compartments, fish and human.

Generally, these objectives correspond to the NIP on POPs of Vietnamese government (as a result of POPs Project of VEPA) and they satisfy the purpose of the collaboration project between EPFL-IIE and IER. This purpose was to help IER to develop themselves their capacity in scientific research (familiarize with the new modern analytical methods and techniques – GC/MS and integrate and assess the more complex problem such as PTSs contamination). It also help IER to perform these scientific studies with a quality assurance/quality control (QA/QC) system to ensure that all data generated by IER' lab are of the highest quality possible. In there, key elements in QA/QC are the use of reference materials and quality charts, participation in

inter-laboratory studies and the use of guidelines for sampling and analysis (*UNEP chemical, 2004*).

The thesis is divided into 9 main chapters. Introduction presents the context related to the PTSs pollution in Vietnam and aims of our work. Chapter I presents general properties of selected PTSs and their original sources as well as toxic effects on environment and human health that could be interesting for our analytical procedure and also for interpreting obtained results. Chapter II showed a summary of previous studies on PTSs contamination in Vietnam. Chapter III gives us an overview about the analytical methods based on it we chosen the analytical procedure for our research and alternative choosing of analytical equipments in PTSs analysis in accordance with conditions in IER laboratory. Chapter IV presents the analytical procedures for all types of samples, including soil, sediment and biological sample. The result of methods validation by CRM and SRM is also showed in this chapter. Chapter V, VI, and VII present the analyzed result (PTSs concentration) in all selected types of sample and their comparison with guideline values as well as with results of other studies. Chapter VIII shows the modeling of PTSs in our samples by using Cluster and Principal Component Analysis techniques and Excel, SPSS and SIMCA-P statistical softwares. Based on PTSs relation between the matrices, we defined the sources of PTSs contamination in selected areas as well as the transfer of PTSs from these sources or polluted compartments into the food chain to man. Finally, some conclusions and suggestions will be presented at the last section. The experimental protocols and other supportive information for this work could be find in Appendix.

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Chapter 1: PROPERTIES, FATE AND EFFECTS OF PERSISTENT TOXIC SUBSTANCES

This chapter provides an overview of nomenclature, physical and chemical properties, source and fate, toxicity and health aspects of PTSs.

1.1. Nomenclature

Persistent Toxic Substance (PTSs) is any toxic substance that bioaccumulates, or any toxic chemical that has a half-life greater than eight weeks in any medium as water, air, sediment, soil, or living things (*IJC report, 1998*).

There was no simple wording to define Persistent Toxic Substances and the term "PTS" does not imply any particular level of risk but rather is a broad consideration for organic (including organometallic) substances that are lipophilic, persist in the environment, are found in areas far removed from sources, accumulate in biota and display some level of toxicity (*UNEP, 2003*).

The PTSs considered under the frame of this thesis include three groups: organochlorine pesticides, polychlorinated biphenyl and polybrominated diphenyl ether.

1.1.1 Organochlorine pesticides

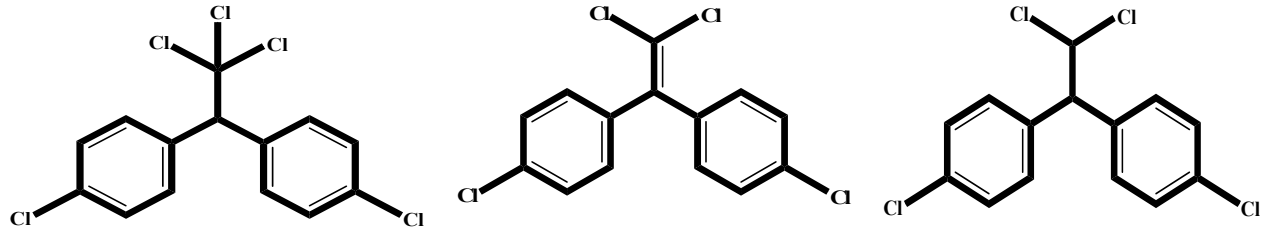
Organochlorine pesticides are ring organic compounds containing chlorine atoms can be divided in some major categories as follow: DDT and its analogues; HCHs isomers (e.g. lindane); cyclodien compounds (e.g. aldrin, dieldrin, endrins, heptachlor, chlordane, endosulfan, and mirex); hexachlorobenzen and toxaphene.

1.1.1.1 DDT (Dichloro Diphenyl Trichloroethane)

The term DDT is generally understood throughout the world and refers to p,p' -DDT (1,1 -[2,2,2-trichloroethylidine]-bis[4-chloro-benzene]). The compound's structure permits several different isomeric forms, such as *o,p'* -DDT (1-chloro-2-[2,2,2-trichloro-1-(4-chloro-phenyl) ethyl] benzene). The term DDT is also applied to commercial products consisting predominantly of p,p'-DDT with smaller amounts of other compounds. A typical example of technical DDT had the following constituents: p,p' -DDT, 85 %; o,p' -DDT, 15 %; p,p' -DDE, 4%; o,p' -DDE, 0.1%; and unidentified products, 3.5% (*ATSDR, 2002a; IPCS-EHC-83, 1989*).

DDE (Dichloro Diphenyl Dichloroethylene) and DDD (Dichloro Diphenyl Dichloroethane) are also the major metabolites and breakdown products of DDT in the environment (Fig.1.1). The term "**total DDT**" is often used to refer to the sum of all DDT related compounds (p,p'-DDT, o,p'-DDT, DDE, and DDD) in a sample.

The scientific (IUPAC) name of DDT is: 4,4'-(2,2,2-trichloroethane-1,1-diyl) bis (chlorobenzene).



p,p'-DDT	p,p'-DDE	p,p'-DDD
CAS number: 50-29-3	CAS number: 72-55-9	CAS number: 72-54-8
$C_{14}H_9Cl_5$ - MW.: 354.49	$C_{14}H_8Cl_4$ - MW.: 318.03	$C_{14}H_{10}Cl_4$ - MW.: 320.05

Figure 1.1: Structure and molecular formula of DDT and its metabolites

1.1.1.2 Lindane

Lindane is the gamma isomer of hexachlorocyclohexane (γ-HCH). Technical-grade hexachlorocyclohexane (HCH) consists of 65-70% alpha-HCH, 7-10% beta-HCH, 14-15% gamma-HCH, and approximately 10% of other isomers and compounds (Fig. 1.2). Lindane contains more than 99% gamma-HCH (*IPCS-EHC-124, 1991*).

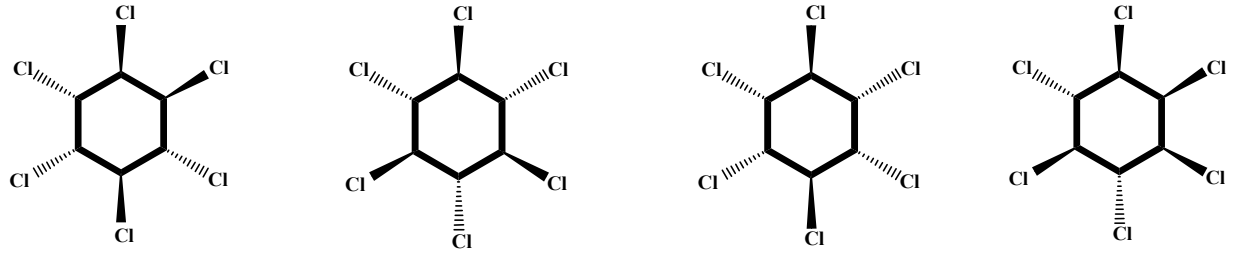
The scientific (IUPAC) name of lindane is 1, 2, 3, 4, 5, 6-hexachlorocyclohexane.

According to IUPAC rules, the designation 'benzene hexachloride' is incorrect; nevertheless, it is still widely used, especially in the form of its abbreviation, BHC. This is therefore another common name approved by the ISO. The compound is called gamma-HCH by the WHO, but gamma-BHC by the FAO. The synonym hexachlorocyclohexane (gamma isomer) is used by the Environmental Protection Agency and the American Conference of Governmental Industrial Hygienists in the USA. The definitions of these different appellations are given in Table 1.1

Table 1.1: Definitions of appellations of lindane

Name	Definition	Remarks
Lindane	Product containing not less than 99% gamma-HCH	ISO-AFNOR name for a product (not yet recognized by BSI)
Lindane	= gamma-HCH	Common name used for gamma-HCH in the USSR only
Gamma-HCH	Gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane	ISO-AFNOR common name
Gamma-BCH	Gamma isomer of 1,2,3,4,5,6- benzen hexachloride	ISO BSI common name in English-speaking countries (recognized by ISO as synonym of gamma-HCH)

(*Source: IPCS-EHC-121, 1991*)



alpha-HCH	**beta-HCH**	**gamma-HCH**	**delta-HCH**
CAS No: 3198-84-6	CAS No: 319-85-7	CAS No: 58-89-9	CAS No: 319-86-8
$C_6H_6Cl_6$	$C_6H_6Cl_6$	$C_6H_6Cl_6$	$C_6H_6Cl_6$
MW.: 290.83	MW.: 290.83	MW.: 290.83	MW.: 290.83

Figure 1.2: Structure and molecular formula of hexachlorocyclohexane isomers

1.1.1.3 Aldrin

Aldrin (Fig. 1.3) is closely related to dieldrin and readily metabolized to dieldrin in plants and animals (*IPCS-EHC-91, 1989*).

The scientific (IUPAC) name of aldrin is: 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-exo-1,4-endo-5,8-dimethanonapthalene. The abbreviation for the scientific name of aldrin is HHDN.

The trade names used for aldrin include *aldrec, aldrex, drinox, octalene, seedrin*, and *compound 118*.

CAS No: 309-00-2

$C_{12}H_8Cl_6$ - MW.: 364.91

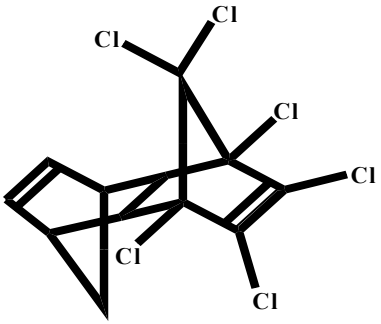


Figure 1.3: Structure and molecular formula of Aldrin

1.1.1.4 Endrin

Endrin (Fig. 1.4) is a cyclodiene insecticide. It is also a *endo* stereoisomer of dielrin compound and is structurally similar to aldrin and heptachor epoxide (*IPCS-EHC-130, 1992*).

CAS No: 72-20-8

$C_{12}H_8Cl_6O$ - MW.: 380.93

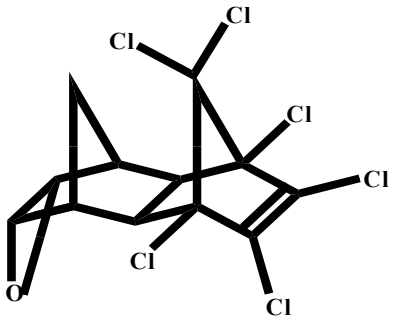


Figure 1.4: Structure and molecular formula of Endrin

The scientific (IUPAC) name of endrin is 1*R,* 4*S,* 4a*S,* 5*S,* 6*S,* 7*R,* 8*R,* 8a*R)*-1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-6, 7-epoxy-1, 4, 5, 8- dimethano – naphthalene

The trade names used for endrin include *Endrex, Experimental Insecticide 269, Hexadrin, Nendrin, NCI-COO157, ENT17251, OMS 197, and Mendrin*.

1.1.1.5 Dieldrin

Dieldrin (Fig. 1.5) is also closely related to its metabolic precursor aldrin which itself breaks down to form dieldrin. Aldrin is not toxic to insect, it is oxidised in the insect to form dieldrin which is the active and toxic compound (*IPCS-EHC-91, 1989*).

The scientific (IUPAC) name of dieldrin is: 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo-1,4-exo-5,8-dimethanonapthalene. The abbreviation for the scientific name of dieldrin is HEOD.

The trade names used for dieldrin include *alvit, dieldrix, octalox, quintox*, and *red shield*.

CAS No: 60-57-1

$C_{12}H_8Cl_6O$ - MW.: 380.93

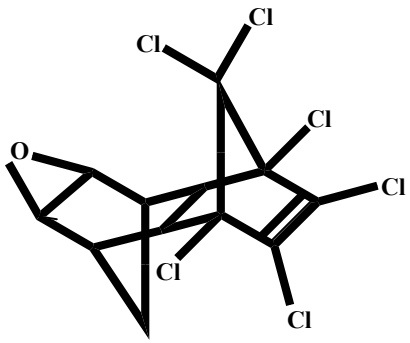


Figure 1.5: Structure and molecular formula of Dieldrin

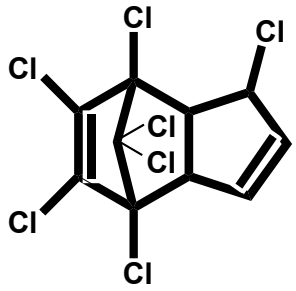
1.1.1.6 Heptachlor

The scientific (IUPAC) name of heptachlor is 1, 4, 5, 6, 7, 8, 8-heptachloro-3a, 4, 7, 7a-tetrahydro-4, 7-methanoindene) (*IPCS-EHC-38, 1984*).

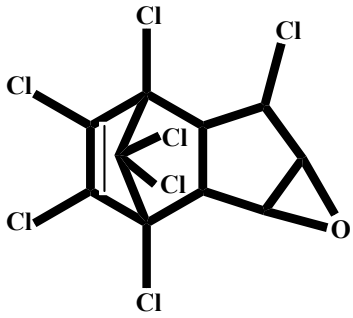
The trade names used for heptachlor include *Aahepta, Agroceres, Basaklor, Drinox, E 3314, GPKh, Heptachlorane, Heptagran, Heptagranox, Heptamak, Heptamul, Heptasol, Heptox, Rhodiachlor, Soleptax, Velsicol 104.*

Heptachlor is stable in daylight, air, moisture and moderate heat (160 ^{0}C) but is oxidized biologically to heptachlor epoxide (*IPCS-EHC-384, 1984*) (Fig. 1.6).

(a)



(b)



CAS No: 76-44-8

$C_{10}H_5Cl_7$ - MW.: 373.32

CAS No: 124-57-3

$C_{10}H_5Cl_7O$ – MW.: 389.32

Figure 1.6: Structure and molecular formula of heptachlor (a) and heptachlor epoxide (b)

1.1.1.7 Chlordane

The scientific (IUPAC) name of chlordane is 1, 2, 4, 5, 6, 7, 8, 8-octachloro-2, 3, 3a, 4, 7, 7a-hexahydro-4, 7-methanoindene (*IPCS-EHC-34, 1984*) (Fig 1.7).

The trade names used for chlordane include *Aspon, Belt, CD 68, Chlorindan, Chlorkil, Chlordane, Corodan, Cortilan-neu, Dowchlor, HCS 3260, Kypchlor, M140, Niran, Octachlor, Octaterr, Ortho-Klor, Synklor, Tat Chlor 4, Topichlor, Toxichlor, Velsicol-1068*.

CAS No: 57-74-9

$C_{10}H_6Cl_8$ - MW.: 409.76

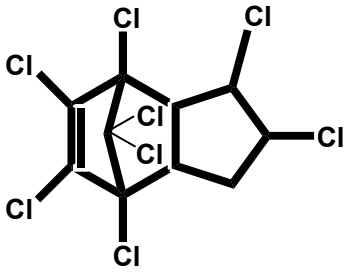


Figure 1.7: Structure and molecular formula of Chlordane

1.1.1.8 Endosulfan

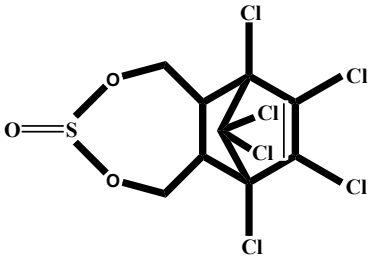
Endosulfan (Fig. 1.8-a) is a neurotoxic organochlorinated insecticide of the cyclodiene family of pesticides. Technical-grade endosulfan contains at least 94% of two pure isomers, α- and β-endosulfan. The α- and β-isomers of technical endosulfan are present in the approximate ratio of 7:3, respectively (*IPCS-EHC-40, 1984; ATSDR, 2000a*).

The scientific (IUPAC) name of endosulfan is 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepine 3-oxide

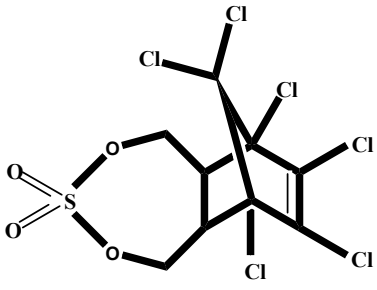
The trade names used for endosulfan include *Benzoepin, Beosit, Chlorthiepin, Cyclodan, FMC 5462, Insectophene, Kop-thiodan, HOE 2671, Thionate Malix, Malix, NCI-C00566, NIA 5462, Thifor, Thimul, Thiodan, Thiofor, Thiomul, Thionex, Thiosulfan, Tionel, Tiovel, Endocide, Endosulphan*.

Endosulfan sulfate (Fig. 1.8-b) is a reaction product found in technical endosulfan; it is also found in the environment due to photolysis and in organisms as a result of oxidation by biotransformation (*ATSDR, 2000a*).

(a)



(b)



CAS No: 115-29-7

$C_9H_6Cl_6O_3S$ - MW.: 406.9

CAS No: 1031-07-8

$C_9H_6Cl_6O_4S$-MW.: 422.9

Figure 1.8: Structure and molecular formula of Endosulfan (a) and endosulfan sulfate (b)

1.1.1.9 Hexachlorobenzene

Hexachlorobenzene (Fig. 1.9) is a chlorinated aromatic hydrocarbon. The its scientific (IUPAC) name is hexachlorobenzen (abbreviated as HCB) (*IPCS-EHC-195, 1997*).

The trade names used for HCB include *Amatin, Anticarie, Bunt Cure, Bunt-No-More, Co-op Hexa, Granox NM, Julin's Carbon Chloride, No Bunt, No Bunt 40, No Bunt 80, No Bunt Liquid, Sanocide, Smut-Go, Snieciotox, HexaCB, Perchlorobenzene, Ceku C.B*.

CAS No: 118-74-1

C_6Cl_6 - MW.: 284.79

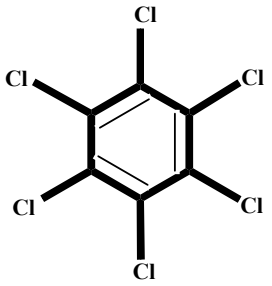


Figure 1.9: Structure and molecular formula of Hexachlorobenzene

1.1.1.10 Toxaphene

Toxaphene (Fig. 1.10) is a complex mixture of poly-chlorinated bicyclic terpenes with chlorinated camphenes predominating (*IPCS-EHC-45, 1984*).

The scientific (IUPAC) name is toxaphene, common synonyms are camphechlor, chlorinated camphene, polychlorocamphene, chlorocamphene, octachlorocamphene,

The trade names used for toxaphene include *Altox, Chem-Phene M5055, Chlor Chem T-590, Crestoxo, Estonox, Fasco- Terpene, Geniphene, Gy-Phene, Hercules 3956, Huilex, Penphene, Phenacide, Phenatox, Polychlor-camphen, Strobane-T, Toxakil, Toxaphene, Toxon 63, Mellipax*.

CAS No: 8001-35-2

$C_{10}H_{10}Cl_8$ (approximately)

MW.: 413.8 (average)

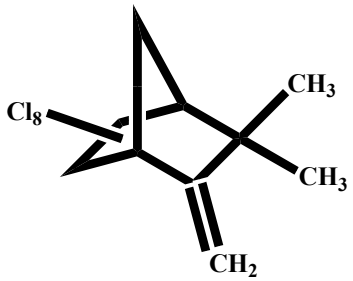


Figure 1.10: Structure and molecular formula of toxaphene

1.1.1.11 Mirex

The scientific (IUPAC) name of mirex (Fig. 1.11) is dodecachloropentacyclo [$5.3.0.0^{2,6}.0^{3,9}.0^{4,8}$] decane. The trade names used for mirex include *Dechlorane, Ferriamicide, GC 1283* (*IPCS-EHC-44, 1984*).

CAS No: 2385-85-5

$C_{10}Cl_{12}$ - MW.: 545.55

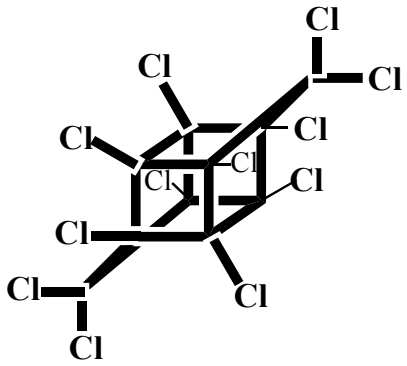


Figure 1.11: Structure and molecular formula of Mirex

1.1.2 Polychorinated Biphenyl

Polychlorinated biphenyl (PCBs) are a class of chemical compounds in which 1–10 chlorine atoms are attached to the biphenyl molecule. Monochlorinated biphenyls (i.e., one chlorine atom attached to the biphenyl molecule) are often included when describing PCBs. The chemical formula can be presented as $C_{12}H_{10-n}Cl_n$, where n, the number of chlorine atoms in the molecule, can range from 1 to 10 (*IPCS-EHC-140, 1992*). The general chemical structure of chlorinated biphenyls is shown in Fig. 1.12 below.

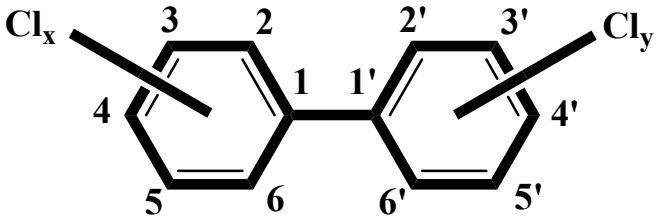


Figure 1.12: Structure formula of PCBs

It can be seen from the structure that a large number of chlorinated compounds are possible. The 209 possible compounds are called congeners. PCBs can also be categorized by degree of chlorination. The term "**homolog**" is used to refer to all PCBs with the same number of chlorines (e.g., trichlorobiphenyls). Homologs with different substitution patterns are referred to as isomers. For example, the dichlorophenyl homolog contains 12 isomers.

The numbering system for the PCBs is also shown above. Table 1.2 presents the number of PCB isomers. Positions 2, 2', 6, and 6' are called ortho positions, positions 3, 3', 5, and 5' are called meta positions, and positions 4 and 4' are called para positions. The benzene rings can rotate around the bond connecting them; the two extreme configurations are planar (the two benzene rings in the same plane) and the nonplanar in which the benzene rings are at a 90 degrees angle to each other. The degree of planarity is largely determined by the number of substitutions in the ortho positions. The replacement of hydrogen atoms in the ortho positions with larger chlorine atoms forces the benzene rings to rotate out of the planar configuration. The benzene rings of non-*ortho* substituted PCBs, as well as mono-*ortho* substituted PCBs, may assume a planar configuration and are referred to as planar or coplanar congeners; the benzene rings of other congeners cannot assume a planar or coplanar configuration and are referred to as non-planar congeners (*ATSDR, Nov. 2000b*).

Table 1.2: Number of PCB isomers of 209 PCB congeners (*ATSDR, 2000b*)

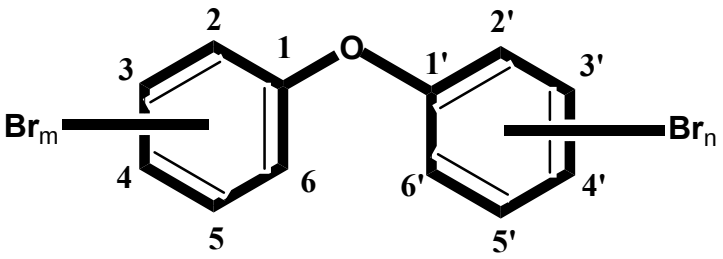
Number of chlorine	CAS number	Number of isomers
1	27323-18-8	3
2	25512-42-9	12
3	25323-68-6	24
4	26914-33-0	42
5	25429-29-2	46
6	26601-64-9	42
7	28655-71-2	24
8	31472-83-0	12
9	53742-07-7	3
10	2051-24-3	1
Total		**209**

Monsanto Corporation, the major U.S. producer of PCBs from 1930 to 1977, marketed mixtures of PCBs under the trade name Aroclor. The Aroclors are identified by a four-digit numbering code in which the first two digits indicate the type of mixture and the last two digits indicate the approximate chlorine content by weight percent. Thus, Aroclor 1242 is a chlorinated biphenyl mixture of varying amounts of mono- through heptachlorinated homologs with an average chlorine content of 42%. The exception to this code is Aroclor 1016, which contains mono- through hexachlorinated homologs with an average chlorine content of 41% (*Hutzinger et al. 1979*).

The trade names of some commercial PCB mixtures manufactured in other countries are Aroclor (U.S.A. and Great Britain), Clophen (Germany), Fenclor (Italy), Kanechlor and Santotherm (Japan), and Phenoclor and Pyralene (France) (*Hutzinger et al. 1979*).

1.1.3 Polybrominated diphenyl ether

Polybrominated diphenyl ethers (PBDEs) are a class of structurally similar brominated hydrocarbons, in which 2–10 bromine atoms are attached to the diphenyl ether molecule. Monobrominated structures (i.e., one bromine atom attached to the molecule) are often included when describing PBDEs. The general chemical structure of PBDEs is shown below:



(Where m + n = 1 – 10)

Figure 1.13: General structure formula of PBDEs

It can be seen from the structure that a large number of brominated compounds are possible. The 209 possible compounds for PBDEs are called “congeners”. However, the number of PBDE congeners that actually exist in commercial PBDE mixtures are much less compared to PCBs. PBDEs can also be categorized by degree of bromination. The term “homolog” is used to refer to all PBDEs with the same number of bromines (e.g., tribromodiphenyl ether refers to PBDEs containing only three bromine atoms). Based on the number of bromine substituents, there are 10 homologous groups of PBDEs (monobrominated through decabrominated). Each homologous group contains one or more congeners (Table 1.3). Homologs with different substitution patterns are referred to as isomers. For example, the group of dibromodiphenyl ether homologs contains 12 isomers. The numbering system for PBDEs is also shown in Fig. 1.13above. Only products based on penta-, octa-, and decabromodiphenyl ethers are of commercial interest (*IPCS-EHC-162, 1994*).

Table 1.3: Number of PBDE isomers of 209 PBDE congeners (*ATSDR, Sept. 2004*)

Number of bromine	CAS number	Number of isomers
1	101-55-3	3
2	2050-47-7	12
3	49690-94-0	24
4	40088-47-9	42
5	32534-81-9	46
6	36483-60-0	42
7	68928-80-3	24
8	32536-52-0	12
9	63936-56-1	3
10	1163-19-5	1
Total		**209**

Like PCBs, the 209 congeners for PBDEs are arranged in ascending numerical order using a numbering system developed by Ballschmiter and Zell that follow the IUPAC rules of substituent characterization in biphenyls. The resulting numbers assigned by Ballschmiter and Zell (which are also referred to as congener, IUPAC, or BZ numbers) are widely used for identifying individual congeners of PBDEs. For example, the PBDE congener, 2,2',4,4'-tetrabromodiphenyl ether may be referred to as BDE 47 in this document (*ATSDR, 2004*).

In the United States, Albemarle Corporation and Great Lakes Chemical Corporation market mixtures of PBDEs under trade names (e.g., DE-60F, DE-61, DE-62, and DE-71 for pentaBDE mixtures; DE-79 for octaBDE mixtures; and DE 83R, Saytex 102E for decaBDE mixtures). There are also several trade names used by producers from Europe and Japan for the BDE mixtures.

1.2 Physical and chemical properties

The physical and chemical properties of PTSs compounds influence their availability, toxicity, fate and also the analytical methods. Knowledge of physical and chemical properties is essential to determining the transportation, distribution, transformation and fate of PTSs in the

environment (*Öberg, 2001*). This knowledge is very useful in the beginning of research to orient our investigation plan as well as analytical method establishment.

There we present the most important properties of PTSs, including water solubility (WS), vapor pressure (VP), Henry's law constant, octanol/water partition coefficient (K_{OW}) and organic carbon partition coefficient (K_{OC}).

1.2.1 Solubility of PTSs

PTSs, particularly the higher halogenated, are poorly soluble in water. They are capable to adsorb strongly to particles and deposit into sediment layer in aquatic ecosystem – sink for PTSs. Thus, PTSs can hardly be identified in water. Tables 1.4, 1.5 and 1.6 present the solubility of some PTSs in water.

Table 1.4: Water solubility of OCs pesticides

No	Chemical	Water solubility (mg/L) at 25°C
1	DDTs[a]	
	pp'-DDT	0.025
	pp'-DDE	0.12
	pp'-DDD	0.09
2	Lindane[b]	17
3	Aldrin[c]	0.011 (20°C)
4	Endrin[d]	0.2
5	Dieldrin[c]	0.110 (20°C)
6	Heptachlor[e]	0.05
7	Chlordane[f]	0.056 (cis:trans - 75:25)
8	Endosulfan[g]	0.28
9	Hexachlorbenzene[h]	0.006
10	Toxaphene[i]	3.0
11	Mirex[j]	0.6

(*Source: [a]ATSDR, 2002a; [b]ATSDR, 2005; [c]ATSDR, 2002b; [d]ATSDR, 1996 ; [e]ATSDR, 2007; [f]ATSDR, 1994; [g]ATSDR, 2000, [h]ATSDR, 2002c, [i]ATSDR, 1996, [j]ATSDR, 1995*).

Table 1.5: Water solubility of PCBs (*ATSDR, 2000b)*.

No	Congener	Water solubility (mg/L)
1	PCB-77	0.00055
2	PCB-138	0.0159
3	PCB-153	0.00086
4	PCB-169	0,000036
5	PCB-180	0,00023

Table 1.6: Water solubility of PBDEs (*ATSDR, 2004*).

No	Congener	Water solubility (mg/L)	No	Congener	Water solubility (mg/L)
1	BDE-15	0.13	8	BDE-99	0.009
2	BDE-17	-	9	BDE-100	0.04
3	BDE-28	0.07	10	BDE-138	-
4	BDE-47	0.015	11	BDE-153	0.001
5	BDE-66	0.018	12	BDE-154	0.001
6	BDE-77	0.006	13	BDE-183	0.002
7	BDE-85	0.006	14	BDE-190	-

-: No data reported

1.2.2 Vapor pressure of PTSs

Vapor pressures are important parameters for the modeling of the environmental fate and incineration behavior of PTSs. Very few measured vapor pressure values are available in the literature for the PTSs.

Vapor pressure can be classified as solid vapor pressure (PS) and (subcooled) liquid vapor pressure (PL). PL is of particular importance since it can well characterize the behavior of pollutants in the real environment.

Table 1.7: OCs pesticides vapor pressure at 25^{o}C

No	Chemical	Vapor pressure (mmHg) at 25^{o}C
1	DDTs[a]	
	pp'-DDT	$1.60x10^{-7}$ torr (20^{o}C)
	pp'-DDE	$6.0x10^{-6}$ torr
	pp'-DDD	$1.35x10^{-6}$ torr
2	Lindane[b]	$4.2x10^{-5}$ (20^{o}C)
3	Aldrin[c]	$1.2x10^{-4}$
4	Endrin[d]	$2.0x10^{-7}$
5	Dieldrin[c]	$5.89x10^{-6}$
6	Heptachlor[e]	$3x10^{-4}$
7	Chlordane[f]	$3.0x10^{-6}$ (crystal, cis); $3.9x10^{-6}$ (crystal, trans)
8	Endosulfan[g]	$1x10^{-5}$
9	Hexachlorbenzen[h]	$1.09x10^{-5}$ (20^{o}C)
10	Toxaphene[i]	0.4
11	Mirex[j]	$3x10^{-7}$

(*Source: [a]ATSDR, 2002; [b]ATSDR, 2005 ; [c]ATSDR, 2002; [d]ATSDR, 1996; [e]ATSDR, 2007; [f]ATSDR, 1994; [g]ATSDR, 2000, [h]ATSDR, 2002, [i]ATSDR, 1996, [j]ATSDR, 1995*).

The boiling point and vapor pressure of the PCBs congeners vary not only with the degree of chlorination, but also with the position of substitution. Vapor pressure can be extrapolated from

the HRGC retention indices (RIs). The effect of ortho substitution was found to increase vapor pressure (i.e., the non- and mono-ortho-CBs will have a lower vapor pressure than other isomers) (*Erickson, 1997*).

Table 1.8: Some PCBs vapor pressure at 25^{o}C (*ATSDR, 2000b)*

No	Congener	Vapor pressure (mmHg)
1	PCB-77	$4.4x10^{-7}$
2	PCB-138	$4.0x10^{-6}$
3	PCB-153	$3.80x10^{-7}$
4	PCB-169	$4.02x10^{-7}$
5	PCB-180	-

-: No data reported

Table 1.9: PBDEs vapor pressure at 25 ^{o}C (*ATSDR, 2004*)

No	Congener	Vapor pressure (mm Hg)	No	Congener	Vapor pressure (mmHg)
1	BDE-15	1.30×10^{-4}	8	BDE-99	1.32×10^{-7}
2	BDE-17	-	9	BDE-100	2.15×10^{-7}
3	BDE-28	1.64×10^{-5}	10	BDE-138	1.19×10^{-8}
4	BDE-47	1.40×10^{-6}	11	BDE-153	1.57×10^{-8}
5	BDE-66	9.15×10^{-7}	12	BDE-154	2.85×10^{-8}
6	BDE-77	5.09×10^{-7}	13	BDE-183	3.51×10^{-9}
7	BDE-85	7.40×10^{-8}	14	BDE-190	2.12×10^{-9}

-: No data reported

1.2.3 Henry's Law Constant

Henry's Law constants are used to estimate the volatilization of the PTSs from soil. They characterize the equilibrium distribution of dilute concentrations of volatile, soluble chemicals between gas and liquid. They are also utilized in estimating the vapor-phase bio-concentration factor from air to plant leaves. The calculated Henry law constants for some PTSs are listed in the table 1.10, table 1.11, table 1.12 shown below.

Table 1.10: Calculated Henry's law constants of OCs pesticides at 25^{o}C

No	Chemical	Henry's law constants ($atm.m^3.mol^{-1}$)
1	DDTs[a]	
	Pp'-DDT	$8.3x10^{-6}$
	Pp'-DDE	$2.1x10^{-5}$
	Pp'-DDD	$4.0x10^{-6}$
2	Lindane[b]	$3.5x10^{-6}$
3	Aldrin[c]	$4.9x10^{-5}$
4	Endrin[d]	$4.0x10^{-7}$
5	Dieldrin[c]	$5.2x10^{-6}$

6	Heptachlor[e]	$2.94x10^{-4}$
7	Chlordane[f]	$4.8x10^{-5}$
8	Endosulfan[g]	$1.91x10^{-5}$
9	Hexachlorbenzen[h]	$5.8x10^{-4}$
10	Toxaphene[i]	0.21
11	Mirex[j]	$5.16x10^{-4}$ (22^0C)

(*Source: [a]ATSDR, 2002; [b]ATSDR, 2005; [c]ATSDR, 2002; [d]ATSDR, 1996; [e]ATSDR, 2007; [f]ATSDR, May 1994; [g]ATSDR, 2000a; [h]ATSDR, 2002, [i]ATSDR, 1996, [j]ATSDR, 1995*).

Table 1.11: Calculated Henry's law constants of PCBs at 25^0C (*ATSDR, 2000b)*

No	Congener	Henry's law constants ($atm.m^3.mol^{-1}$)
1	PCB-77	$0.43x10^{-4}$
2	PCB-138	$1.07x10^{-4}$
3	PCB-153	2.78 (10^4)
4	PCB-169	$0.59x10^{-4}$
5	PCB-180	$1.07x10^{-4}$

Table 1.12: Calculated Henry's law constants of PBDEs ($atm.m^3.mol^{-1}$) (*ATSDR, 2004*)

No	Congener	Henry's law constants	No	Congener	Henry's law constants
1	BDE-15	2.07254×10^{-4}	8	BDE-99	$2.26992x10^{-6}$
2	BDE-17	-	9	BDE-100	$6.80977x10^{-7}$
3	BDE-28	$5.03331x10^{-5}$	10	BDE-138	-
4	BDE-47	$1.48038x10^{-5}$	11	BDE-153	$6.61238x10^{-7}$
5	BDE-66	$4.93461x10^{-6}$	12	BDE-154	$2.36862x10^{-6}$
6	BDE-77	$1.18431x10^{-5}$	13	BDE-183	$7.30323x10^{-8}$
7	BDE-85	$1.08562x10^{-6}$	14	BDE-190	-

-: No data reported

1.2.4 Octanol/Water Partition Coefficient

The octanol/water partition coefficient (K_{OW}) is the ratio of the concentration of a chemical in octanol and in water at equilibrium and at a specified temperature.

$$K_{OW} = \frac{\text{Concentration in octanol phase}}{\text{Concentration in aqueous phase}}$$

Values of K_{OW} are unitless and usually measured at room temperature, with a low solute concentration so the solute itself does not affect the distribution.

It is used in several exposure estimation such: to estimate log K_{OC} when measured data are not available, and it is utilized in estimating the root concentration factor (RCF). RCF is used to

estimate the uptake of contaminants by plant roots. Log K_{OW} is also used to estimate the vapor-phase bio-concentration factor from air to plant leaves.

It is also the most widely used parameter for predicting the bioaccumulation potential of POPs. It is generally assumed that POPs accumulate in lipids and that equilibrium partitioning of these chemicals between organism lipid and water can be estimated by using partitioning to octanol as a measure of their hydrophobicity. However, log–log correlations between lipid-normalized bioaccumulation factors (BAF_{lip}) and K_{ow} are often confounded by a high degree of uncertainty (*Thomas and Deborah, 2005*).

Table 1.13: Calculated K_{OW} constants of OCs pesticides

No	Chemical	Log K_{OW}
1	DDTs[a]	
	Pp'-DDT	6.91
	Pp'-DDE	6.51
	Pp'-DDD	6.02
2	Lindane[b]	3.72
3	Aldrin[c]	6.50
4	Endrin[d]	5.6
5	Dieldrin[c]	6.2
6	Heptachlor[e]	6.10
7	Chlordane[f]	5.54
8	Endosulfan[g]	3.52
9	Hexachlorbenzen[h]	5.73
10	Toxaphene[i]	3.3
11	Mirex[j]	5.28

(*Source: [a]ATSDR, 2002; [b]ATSDR, 2005; [c]ATSDR, 2002; [d]ATSDR, 1996; [e]ATSDR, 2007; [f]ATSDR, 1994; [g]ATSDR, 2000a; [h]ATSDR, 2002, [i]ATSDR, 1996, [j]ATSDR, 1995*).

Table 1.14: Calculated K_{OW} constants of PCBs (*ATSDR, 2000b)*

No	Congener	Log K_{OW}
1	PCB-77	6.04–6.63
2	PCB-138	6.50–7.44
3	PCB-153	6.72
4	PCB-169	7.41
6	PCB-180	6.70–7.21

Table 1.15: Calculated K_{OW} constants of PBDEs (*Braekvelt et al. 2003*)

No	Congener	Log K_{OW}	No	Congener	Log K_{OW}
1	BDE-15	-	8	BDE-99	7.32
2	BDE-17	5.74	9	BDE-100	7.24
3	BDE-28	5.94	10	BDE-138	-
4	BDE-47	6.81	11	BDE-153	7.90
5	BDE-66	-	12	BDE-154	7.82
6	BDE-77	-	13	BDE-183	8.27
7	BDE-85	-	14	BDE-190	-

-: No data reported

The octanol–water partition coefficient (K_{OW}) is an important property in determining the environmental fate of hydrophobic organic chemicals, particularly in biota. Octanol acts as a lipid surrogate: chemicals of high K_{OW} tend to be highly lipophilic and bioaccumulate to a great extent.

1.2.5 Organic Carbon Partition Coefficient

The organic carbon partition coefficient (K_{OC}) is used in several exposure estimations: K_{OC} is used in the estimation of the adsorption partition coefficient, which describes the partitioning of contaminants between suspended sediment and the water column; K_{OC} is also used in estimating the concentration of contaminants in below ground vegetables grown in contaminated soil.

Table 1.16: Log K_{OC} of OCs pesticides

No	Chemical	Log K_{OC}
1	DDTs[a]	
	Pp'-DDT	5.18
	Pp'-DDE	4.70
	Pp'-DDD	5.18
2	Lindane[b]	3.57
3	Aldrin[c]	7.67
4	Endrin[d]	4.53
5	Dieldrin[c]	6.67
6	Heptachlor[e]	4.34
7	Chlordane[f]	6.3 (trans, suspended solids)
8	Endosulfan[g]	No data
9	Hexachlorbenzen[h]	6.08
10	Toxaphene[i]	2.47
11	Mirex[j]	3.763

(*Source: [a]ATSDR, 2002; [b]ATSDR, 2005; [c]ATSDR, 2002; [d]ATSDR, 1996; [e]ATSDR, 2007; [f]ATSDR, 1994; [g]ATSDR, 2000; [h]ATSDR, 2002, [i]ATSDR, 1996, [j]ATSDR, 1995*).

Table 1.17: Log K_{OC} of PCBs (*ATSDR, 2000)*

No	Congener	Log K_{OC}
1	PCB-77	4.41–5.75
2	PCB-138	5.21–7.3
3	PCB-153	4.75–7.68
4	PCB-169	6.60
6	PCB-180	5.78–6.9

1.3 Source and fate of PTSs

In the frame of this thesis, we were interested in 10 chemicals listed in the Stockholm Convention and also endosulfan, lindane and PBDEs considered as POP candidates in the future. The sources of these 13 chemicals to the environment have been from direct applications for plant protection or disease vector control, but also from direct and indirect emissions from urban areas.

While the importation of PCBs has been banned all over the world, many countries do not have or maintain national inventories of PCB-contaminated equipment (e.g. transformers an capacitors). Knowledge on the sources of PBDEs is generally lacking especially in the South East Asia (*UNEP, 2002*)

There may be many sources of the PTSs chemicals. The text box 1.1 presents the major source categories of potential releases of PTSs to the environment.

Text box 1.1: Major source categories of potential releases of PTSs to the environment

1. Manufacturing

This sector includes chemical manufacturing of PTS, as well as the manufacturing of products that involves the use of materials that may be contaminated with PTS – for example: textile manufacturing, chlorinated chemical production, Cl_2-production using graphite electrodes, oil refining and catalyst regeneration, pulp and paper (elemental chlorine bleaching), pesticide production and formulation.

2. Thermal Processes

Processes that involve high temperatures and usually combustion can lead to the formation and release of a suite of complex PTSs.

Thermal Manufacturing Processes

Metallurgical processes, primary processes, mainly copper, steel and aluminium, also zinc recovery from steel and other scrap recovery processes including aluminium, steel, copper, zinc, magnesium, lead and others (i.e. cable burning), coke production and carbo-chemical processes (especially using brown coal/lignite), mineral processing (especially cement kilns), asphalt mixing, production of lime, ceramic, glass, brick and other similar processes carried out at small-scale

Controlled Combustion Processes

Municipal (non-hazardous) waste incineration, industrial waste combustion, including treated wood waste combustion, hazardous waste incineration, medical/clinical waste, sludge (non-hazardous) incineration, coal combustion (large volumes), oil combustion (large quantities), wood/biomass (large and small scale quantities) combustion, landfill gas/biogas, crematoria and animal carcass burning

Uncontrolled Combustion

Biomass such as forest, bush, agricultural harvest residues (eg straw and sugar cane leaves), accidental

fires, eg houses, industrial complexes, landfill fires, unintentional and intentional, combustion of other wastes, i.e. flaring of drilling mud, landfill gas etc., building waste and construction debris, domestic (backyard) waste burning, plastic container/barrel burning, hazardous waste/contraband CDs and DVDs/tires/rubber/cable, e-waste - end-of-life electronic products, general open burning

3. Product application and use

Agricultural applications of pesticides (see Table 1.18), application of pesticides outside agricultural lands, for disease vector control, locust control, vegetation control and others, preservatives for wood, leather, textiles, textile and leather dying, industrial bleaching processes, especially using chlorine, transformers and electrical equipment, solvent use and all processes which involve solvents, i.e. dry-cleaning, de-greasing etc. PCB-paint use, PCP-paint, storage of products containing PTS, such as e-waste and contaminated feed.

4. Transport

During the transport of PTS products and products containing PTS, accidents and spillages can occur.

5. Recycling Processes (excluding thermal),

Metals (incl. vehicle) recycling by-products such as shredder (mainly PCB), waste oil, scrap yards with stockpiles, refrigerator recycling, electronic scrap and circuit board recycling *etc*., paper recycling, especially de-inking sludges, sewage sludge (including paper sludge) and effluent applications *i.e.* on land as agricultural fertilizer for composting, solvent recovery processes and especially residue sludge from it, waste oil recovery, plastics recycling including extrusion, metal flyash recycling.

6. Waste Disposal (non-thermal waste disposal)

Landfills (controlled and uncontrolled) of various waste types (municipal, hazardous *etc*.), contaminated incinerator ash, sludge, metal ash, also leaching from those landfills, storage/stocks of transformers containing PCB-oil, ocean dumping of solid/sludge/liquid wastes, dumps of obsolete pesticides, but likely to contain non Stockholm POP pesticides as well, donations of pesticides to developing countries can result in toxic waste dumps.

7. Reservoirs (potential for re-release subsequent to initial accumulation)

Reservoirs (potential for re-release subsequent to initial accumulation) such: soil and sediments, waste and obsolete stockpiles, PCP-treated wood i.e. telephone poles, railroad ties.

(*Source: UNEP, 2003*)

Pesticides constitute an important PTS use category where chemicals are applied, in most cases, directly and intentionally to the environment. A summary of main sources of PTS pesticides to the various environmental compartments is shown in Table 1.18.

Table 1.18: Some sources of pesticides to the environmental compartments (*UNEP, 2003*)

Air	Soil	Freshwater	Marine water
Agricultural usage	Stockpiles	Agricultural usage	Agricultural runoff
Spraying/land	Production and waste (DDT and Dicofol)	Runoff from agricultural use	Major rivers and coastal drains
Production	Misuse	Production (DDT/Dicofol)	

There may be many sources of the PTS chemicals. A generalized flow sheet is shown in Fig. 1.14 that outlines the possible ways that releases may occur. Releases of PTS can occur from and/or

into five major environmental compartments and/or media, namely air, water, land, waste, and product. This section describes all the relevant information that should be obtained to allow source quantification and the assembly of a regional PTS emission inventory.

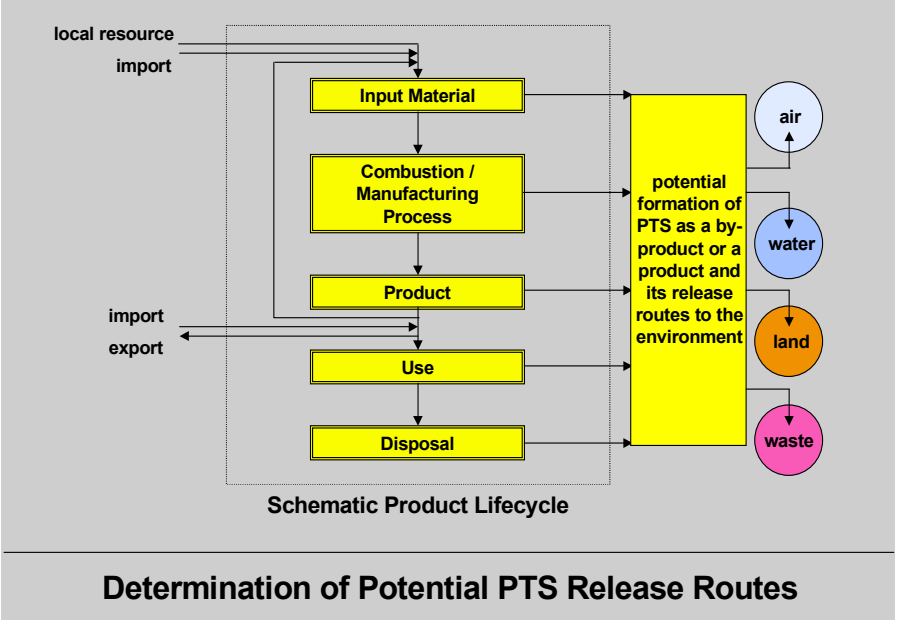


Figure 1.14: Generalized flow-charts of PTS sources and release pathways (*UNEP, August 2000*)

When PTSs are released into the environment they do not normally spread uniformly but tend to accumulate in certain parts of the ecosystem that scientists refer to as "environmental compartments" (Figure 1.2). These may include the air, water (fresh and salt), bottom sediments, soil and tissues of living organisms, but are often somewhat arbitrary. The key point is that the proportion of a chemical compound that accumulates in the different compartments (scientists refer to the process as "partitioning") depends on the particular chemical and its properties. PTSs compounds are only poorly soluble in water but dissolve readily in lipids (fats and oils). Many marine organisms contain large amounts of lipids, so it is not surprising that POPs tend to accumulate preferentially in their tissues (*Percy, 2006*).

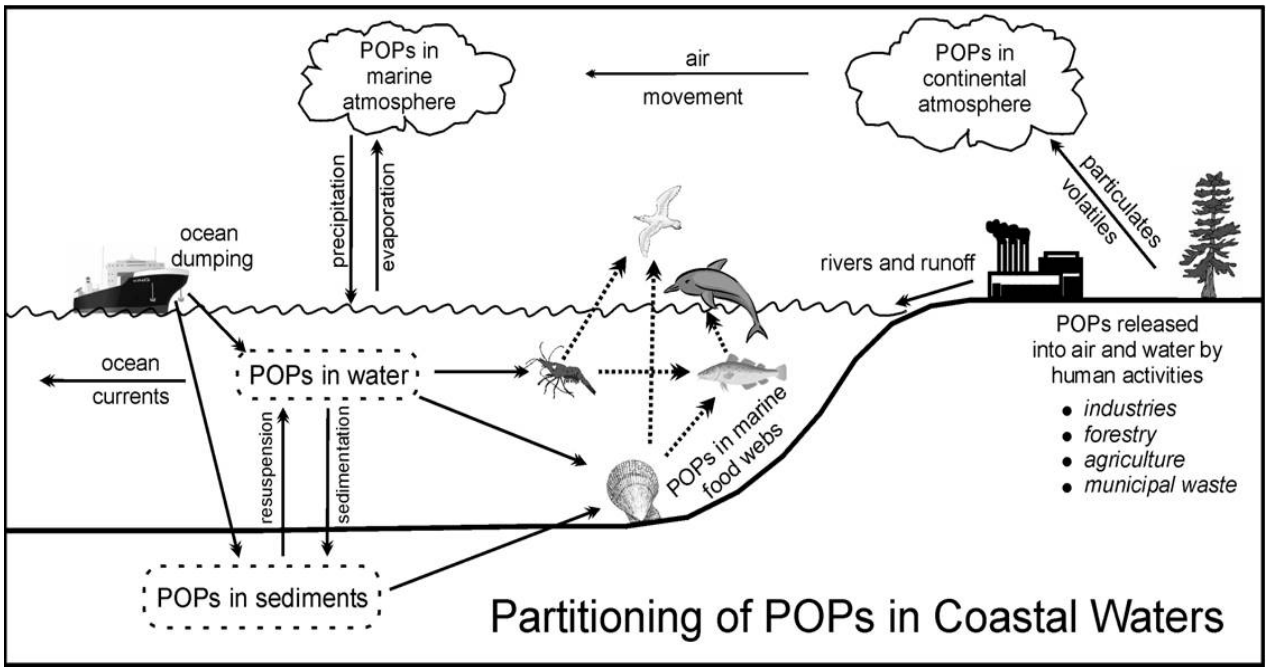


Figure 1.15: Partitioning of POPs in coastal waters (*Percy, 2006*)

1.4 Toxicity and health aspects

1.4.1 Toxicity and mechanism of toxicity of PTSs

PTSs are highly lipid soluble and are stored in adipose tissue; they can also accumulate in breast milk. Figure 1.3 is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation

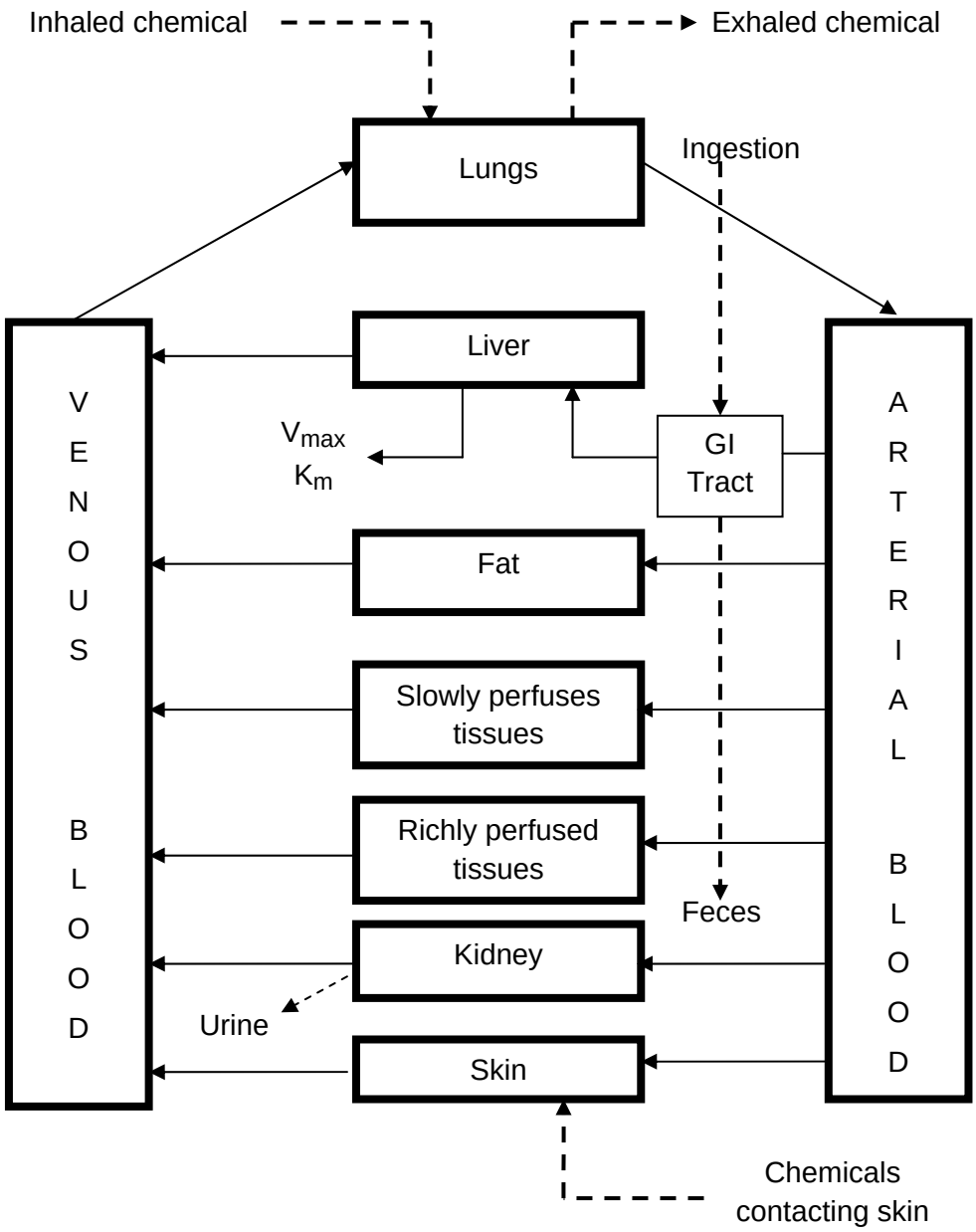


Figure 1-16. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance *(ATSDR, 2007)*

The mechanism of absorption of PCBs by the inhalation and dermal routes of exposure is not known. PCBs are well absorbed from the gastrointestinal tract. Diet is the main source of background human exposures to persistent lipophilic organic pollutants, such as PCBs (*ATSDR, 2000*).

Cyclodien insecticides interfere with the functioning of the chloride channel by blocking its activation by a-aminobutyric acid (GABAA). The available data suggest that the developing nervous system is the most sensitive target of heptachlor toxicity. Impaired spatial memory was observed in rats exposed to 0.03 mg/kg/day heptachlor during gestation and from postnatal day 7–42 (*Moser et al. 2001*). The cause of these alterions is not known. Moser et al. (2001) noted that heptachlor and other cyclodiene insecticides have a high affinity for GABAA (gamma-amino

butyric acid) receptors and can alter the expression of the GABAA receptor during development. In the Moser et al. (2001) study, alterations in GABAA binding sites were observed in the brainstem of female rats, but not in the cortex. However, no alterations in the functional response of the GABA receptor binding were observed.

DeJongh and Blaauboer (1997) simulated the toxicokinetics of γ-HCH in rats with a PBPK model. A five-compartment model for the rat as presented in Figure 1-3 was constructed, including (1) the liver, serving as the metabolizing organ; (2) blood; (3) fat; (4) brain; and (5) a lumped compartment representing all other tissues, consisting mainly of muscle tissue. The structure of the PBPK model is presented in (Fig. 1.17). The model was calibrated on a dataset from the literature on the disposition of γ-HCH from blood *in vivo* after single oral dosage and first-order biotransformation and gastrointestinal absorption constants for γ-HCH were obtained.

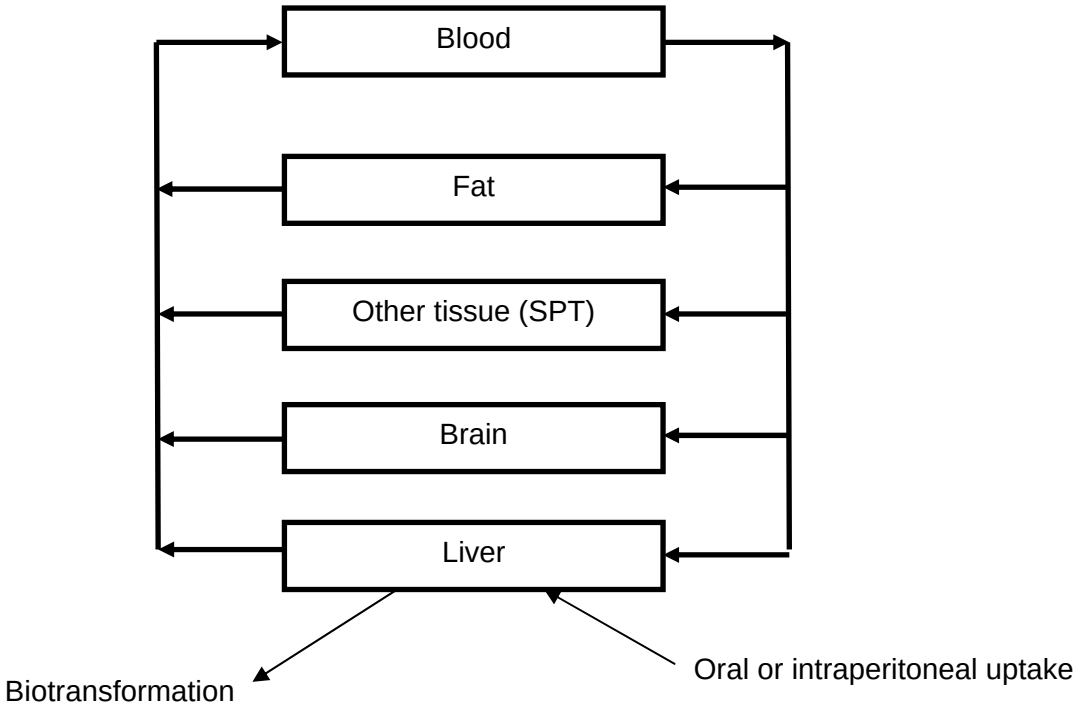


Figure 1.17. Structure of the PBPK model for g-HCH *(DeJongh and Blaauboer, 1997)*

Currently, the Agency of Toxic Substances and Disease Registry (ATSDR) is assessing the feasibility of using tools such as PBPK modeling and pharmacodynamic modeling to extrapolate data across routes or durations of exposure. Such extrapolation may be done on a substance-by-substance basis after adequate toxicokinetic information has been collected (*ATSDR, 2007*).

1.4.2 Toxicity effects of PTSs on environment and human health

Magnified concentrations of PTSs in marine mammals have received particular attention due to their susceptibility to the impacts of PTSs. In a comprehensive review on ecological impacts of persistent organoclorines on marine mammals, Tanabe and coworkers (1994) pointed out three major factors, which might cause particularly high levels of such compounds in marine mammals. First, marine mammals have a large pool of PTSs in their body due to their thick subcutaneous blubber. Secondly, transfer of PTSs from mothers to newborns via milk is very high (e.g. transfer of PCBs may reach to 60% of mother body burden of PCBs). The third factor relates to relatively low metabolic capacity of these mammals to degrade such toxic

contaminants, leading to low excretion of the contaminants. In particular, it is demonstrated that marine mammals have low capacity to metabolize a group of PCB congeners with adjacent non-chlorinated *meta*- and *para*-carbon in biphenyl rings (*Tanabe et al., 1988*). The comparative approach of PCB compositions also suggested that drug-metabolizing enzyme systems in marine mammals have a small function of MC (3-methylcholanthrene)-type enzymes but not PB (phenobarbital)-type enzymes (Fig. 1.18). It is therefore believed that the third factor regarding metabolic capacity is the most convincing evidence why marine mammals retain a wide variety of POPs at very high levels (*Tanabe et al., 1994*).

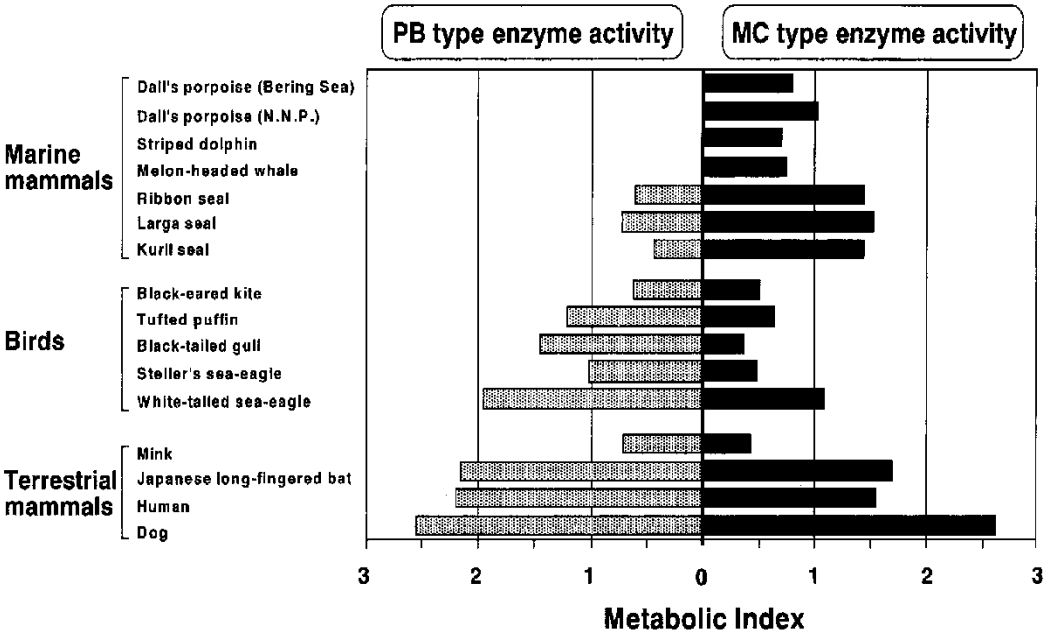


Figure 1.18. Estimated metabolic capacity of PB- and MC-type enzymes in marine and terrestrial higher animals (*Tanabe et al., 1994*)

Adverse health effects associated with exposure to PTSs have been observed in high trophic wildlife and humans. The connection between human and wildlife on PTSs toxicological studies is prompted by the fact that evidences of adverse effects in high trophic wildlife would somehow reflect the risk of adverse health effects in humans. Similar to toxicological studies in wildlife, it is still difficult to definitely establish the link between exposure to POPs at the environmental levels and adverse health outcomes in humans. However, the accumulating "weight of evidence" strongly implicates POPs, especially dioxin-like compounds, in incidents of endocrine and immune dysfunction, reproductive impairment, developmental abnormalities, and neurological retardation in humans as well as other vertebrate species (*Ross et al., 2003*).

Lethal effect

The oral lethal dose LD50, LD1 and lowest dose to kill of some PTSs for rats are presented in the Table 1.19 below

Table 1.19 Oral toxicity to rats (*Fiedler, 2003*)

	LD50 (mg/kg)		LD1 (mg/kg)		Lowest dose to kill (mg/kg)	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Aldrin	39	60	18	27	25	40
pp'-DDT	113	118	52	80	75	100
pp'-DDE	880	1240	360	460	750	500
Dieldrin	46	46	25	25	30	30
Endrin	18	8	5	5	10	6
Mirex	740	600	200	270	400	500

As can be seen from Table 1.19 of acute toxicity of rats, the toxicity of aldrin, dieldrin and endrin is considerably higher than that of DDT. The toxicity of mirex approximates that of DDT.

Half - life

The half-live ($T_{1/2}$) of PTSs in environment and in organisms including humans is an important criterion in hazard assessment. This value provides a convenient measure for the persistence of PTSs in environment, living aquatic and terrestrial organisms.

The estimated half-lives ($T_{1/2}$) of some PTSs in environment are presented in the Table 1.20

Table 1.20: Estimated half-lives ($t_{1/2}$) of some PTSs in environment (*Fiedler, 2003*)

Chemical name	$T_{1/2}$Soil (h)	$T_{1/2}$Water (h)	$T_{1/2}$Air(h)
Aldrin	$3.72x10^3$	$2.68x10^3$	2.86
Chlordane	$1.71x10^4$	$1.95x10^4$	$1.64x10^1$
Pp'-DDT	$3.36x10^4$	$4.62x10^3$	$5.60x10^1$
Pp'-DDE	$3.89x10^4$	$8.76x10^3$	$5.60x10^1$
Dieldrin	$1.49x10^4$	$1.04x10^4$	$1.27x10^1$
Heptachlor	$5.06x10^3$	$1.00x10^2$	$3.13x10^1$
Hept.Epoxide	$3.24x10^3$	$2.07x10^3$	$2.35x10^1$
HCB	$3.41x10^4$	$3.41x10^4$	$1.19x10^4$
a-HCH	$1.03x10^3$	$1.03x10^3$	$3.61x10^1$
g-HCH	$5.00x10^3$	$5.27x10^2$	$6.24x10^1$
PCB 28	$5.50x10^4$	$5.50x10^4$	$2.06x10^2$
PCB 101	$5.50x10^4$	$5.50x10^4$	$5.50x10^2$
PCB 153	$5.50x10^4$	$5.50x10^4$	$1.10x10^3$

Chloracne

PCBs: Chloracne is the most easily recognized effect of exposure to PCBs and structurally-related chlorinated organic chemicals (*Rice and Cohen 1996*). Chloracne is a high-dose response in

animals and humans; and its presence in humans indicates exposure to PCBs and/or other chlorinated organic compounds, but its absence does not preclude such exposure. Furthermore, the variability of the response in more highly exposed individuals suggests that susceptibility varies greatly among individuals. Chloracne can first occur on the face, particularly under the eyes and behind the ears. With increasing exposure, the rest of the face and neck, upper arms, chest, back, abdomen, outer thighs, and genitalia may be affected. When severe, chloracne can cover the entire body. Clinically, changes vary from an eruption of comedones to the occurrence of papules and pustules. Histologically, the lesions consist of keratinous cysts caused by squamous metaplasia of sebaceous glands. The acute stage is followed by vermiculite skin atrophy.

Immunological effects

DDTs: Limited information was located regarding immunological effects in humans after oral exposure to DDT, DDE, or DDD. In a study in which humans were challenged with an injection of *Salmonella typhimurium* vaccine, serum agglutinin titers were significantly higher in three volunteers given capsules containing 5 mg DDT/day (0.07 mg/kg) for 20 days when compared to volunteers who received only the bacterial antigen; immunoglobulin levels were unaffected by treatment with DDT. The volunteers exhibited no apparent symptoms of DDT exposure (*ATSDR, 2002)*.

Hematological effects

DDTs: In a chronic study, exposure of rats to DDT at 20 mg/kg/day for 27 months resulted in alterations in the spleen, which consisted of congestion and hemolysis exceeding that observed in untreated control rats (*Deichmann et al. 1967*). In addition, squirrel monkeys exposed orally to doses of 0.05–50 mg DDT/kg/day for up to 6 months exhibited no hematological changes; however, all monkeys in the highest dose group (six animals) died by week 14 (*Cranmer et al. 1972*); the cause of death was not determined, but before death, the monkeys had apparently recovered from severe neurotoxic symptoms.

Overall, the existing information does not suggest that hematological parameters are sensitive targets for DDT toxicity. The extent to which this is due to limitations in the available studies is uncertain.

In general, hematological effects have not been observed in humans occupationally exposed to PCBs. Capacitor plant workers (152 males, 43 females) exposed to Aroclors 1254, 1242, and 1016 for an average duration of 17 years showed slightly decreased numbers of polymorphonuclear neutrophil (PMN) white cells and slightly increased lymphocyte, monocyte, and eosinophil counts when compared to normal values (*Lawton et al. 1985*). Limited exposure characterization, consisting of monitoring in one area of the plant several months prior to hematological evaluation, showed a geometric mean PCB concentration of 0.69 mg/m3. Values for other white cells, erythrocytes, hemoglobin, and hematocrit were within normal ranges

Neurological effects

DDTs: The nervous system appears to be one of the primary target systems for DDT toxicity in humans after acute, high exposures. A number of investigators conducted experimental studies on humans in the 1940s and 1950s at controlled doses that produced effects. Other data come from accidental poisonings where dose levels were crudely estimated. Persons exposed to 6 mg DDT/kg administered orally by capsule generally exhibited no illness, but perspiration, headache, and nausea have been reported (*ATSDR, Sept. 2002*)

Reproductive effects

DDTs: Concentration of p,p'-DDE in human milk was inversely related to duration of lactation in women of Tlahualilo, Mexico, who had lactated previously, but not among women having their first lactation (*Gladen and Rogan 1995*). Median lactation duration declined from 7.5 months among women with lipid-adjusted DDE concentration of 0–2.5 ppm, to 3 months among women with 12.5 ppm in their milk; the difference was statistically significant. Significantly elevated crude hazard ratios (defined by the authors as estimated ratios of the hazard of weaning relative to the 0–2.5 ppm group; hazard was defined as the instantaneous probability of weaning) were observed in groups with milk DDE levels 7.5 ppm; hazard ratios adjusted for various determinant factors were elevated above unity in the group with milk DDE 12.5 ppm. A similar inverse relationship between milk DDE and lactation duration was seen in women in the United States (*Rogan et al. 1987*). In contrast, no correlation was seen between DDE concentration in maternal milk fat and birth weights, head circumference, or neonatal jaundice, but the authors indicated that higher levels of DDE (>4 ppm) in maternal milk fat were associated with hyporeflexia in infants (*Rogan et al. 1986*). The effects of DDE on lactation duration are likely due to the ability of DDE to disrupt the normal endocrine regulation of lactation, as estrogen is a potent inhibitor of milk secretion (*Guyton and Hall, 2000*).

DDT intake, particularly during sexual differentiation, can adversely affect the reproductive system of male animals. Such effects have been attributed to DDT and related compounds acting in any of the following manners or in any combination of them: (1) mimicking endogenous hormones, (2) antagonizing endogenous androgenic hormones, (3) altering the pattern of synthesis or metabolism of hormones, and (4) modifying hormone receptor levels. DDT is primarily suspected of influencing reproduction and development through its interaction with steroid hormones receptors for estrogens and androgens (*ATSDR, Sept. 2002)*

Endocrine effects

DDTs: Exposure to DDT and DDT-related compounds, particularly during development, can adversely affect the development and function of the reproductive system of both female and male animals. This is due primarily to the ability of some of these compounds to disrupt the action of natural steroids and bind to receptors for estrogens and androgens. Hormonal effects of DDT and residues that lead to altered reproduction and/or development are discussed in Sections Reproductive Effects (*ATSDR, Sept. 2002)*

PCBs: Total thyroxine (T4) and free T4 (T4 index) were significantly lower (approximately 10%) in a group of 55 transformer maintenance workers compared to a comparison control group of workers (*Emmett et al. 1988b*), even though thyroid hormone levels were in the normal range for adults in both groups. The transformer workers were primarily exposed to Aroclor 1260 at levels ranging from 0.00001 to 0.012 mg/m3; the mean length of exposure was approximately 4 years. Although there was a statistically significant increase in thyroxine levels in the PCB-exposed cohort, there was no correlation between PCB levels in serum or adipose tissue and serum T4 concentrations (adjusted for age, smoking, and alcohol consumption)

PBDEs: There is suggestive evidence of hypothyroidism in a small group of workers who were occupationally exposed to decaBDE (*Bahn et al. 1980*), as summarized in the preceding subsection on endocrine effects of PBBs. In another study, plasma levels of thyroid hormones (T3 and free T4) and eight PBDE congeners (tetra- to heptaBDEs) were monitored for 198–221 days in three electronic dismantling workers (*Pettersson et al. 2002*). The hormones remained within normal ranges and there were no correlations between levels of hormones and congeners.

PBDEs: Plasma levels of the congener 2,2',4,4'-tetraBDE (BDE 47) and various other persistent organohalogen compounds (non-PBDEs), as well as hormone levels (free and total T3 and T4, thyroid stimulating hormone [TSH], free testosterone, follicle-stimulating hormone, lutenizing hormone, and prolactin), were analyzed in 110 men who consumed varying amounts of fatty fish (0–32 meals per month) from the Baltic Sea (*Hagmar et al. 2001*). There was a weak negative correlation between BDE 47 and plasma TSH after age adjustment, but the congener could not explain more than 10% of the variance in TSH ($r2=0.10$, $p<0.001$). The fact that BDE 47 could only explain 10% of the variance in TSH is not surprising due to the occurrence of PCBs and other likely similarly acting compounds in the Baltic fisherman.

Cancer

DDTs: Occupational exposure to DDT was associated with increased lung cancer in a case control study of the Uruguayan work force (*De Stefani et al. 1996*). Elevated, but not statistically significant, odds ratios (OR) for any type of lung cancer were observed in 34 workers who had been exposed for 1–20 years (OR=1.6; 95% confidence interval [CI]=0.9–4.6), in 16 workers who had been exposed for greater than 20 years (OR=2.0; 95% CI=0.9–4.7), and in 50 workers who had ever been exposed to DDT (OR=1.7; 95% CI=1.0–2.8). Significantly elevated odds ratios were reported in a subset of 33 DDT-exposed lung cancer patients with small cell cancer (OR=3.6; 95% CI=1.5–8.9) or in 57 with adenocarcinoma (OR=2.3; 95% CI=1.2–4.7). Analyses were adjusted for age, residence, education, tobacco smoking, and alcohol consumption

PBDEs: There was no clear association between risk of non-Hodgkin's lymphoma (NHL) and exposure to 2,2'4,4'-tetraBDE in a case-control study of 77 Swedish men and women who were recruited in 1995–1997 and ranged in age from 28 to 85 years (*Hardell et al. 1998; Lindstrom et al. 1998*). Adipose tissue levels of 2,2',4,4'-tetraBDE (BDE 47) (used as a marker for total PBDE exposure) were compared in 19 patients with NHL, 23 patients with malignant melanoma, 8 patients with other cancers or *in situ* changes, and 27 persons with no cancer diagnosis. The highest concentrations were seen in the patients with NHL. The mean concentration of BDE 47 was 13.0 ng/g (ppb) lipid (range 1.0–98.2 ppb) in the 19 NHL patients and 5.1 ppb (range 0.6–27.5 ppb) in the 27 persons without known malignancies. Logistic regression, adjusted for age, gender, sum of PCBs, and sum of chlordanes, was performed on cases and controls in three concentration groups (<2.05, 2.05– <5.43, and ≥5.43 ppb). A nonsignificantly elevated risk with a suggestive dose-response was found for NHL in the two highest concentration groups compared with the lowest group; the ORs and 95% Cis were 1.9 (0.3–14) and 3.8 (0.7–26) in the middle and high groups, respectively. Although the risk was highest in the group with the highest concentration of 2,2'4,4'-tetraBDE ($p=0.09$ for trend), there was no significant difference between cases and controls ($p=0.14$). The results for patients with malignant melanoma did not differ from controls.

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Chapter 2: STUDIES ON PTSs CONTAMINATION IN VIETNAM

2.1. Introduction

Because of adverse effects to human health and environment, more attention has been paid to PTSs in Vietnam and a large number of studies have been performed to better know exposition and effects on the environment. However, these researches mainly focused on PTSs in water, soil and sediment media. PTSs in animals, birds and human (blood, adipose tissue, breast milk, etc.) have drawn less attention due to high cost of research and the sophistication of analytical techniques needed. Moreover, taking these types of samples faces many obstacles. Sampling at landfills, plants, dockyards, ports or industrial zones must be approved by the authorized organisations. Given the bureaucratic nature of these proceses, it takes much time to gain the approval. This is even more time consuming when the recognition on the PTSs polution issue of the authorized organisations is not up to date. To other type of sampling, breast milk, in addition to the process of attaining the approval from hospital management board, persuading potential donors to donate their breast milk is very complicated. In cultural aspect, Vietnamese women who is in breast - feeding are conservative and superstitious to some extent. They tent to avoid having social contact during first months of the new-born child, especially giving their breast milk. For these reasons, taking samples from these sources requires much time, persuation and extra technical means to ensure the quality of taken samples.

Vietnam ratified the Stockholm convention in 22 July 2002 and Vietnamese government also approved the National Implementation Plan (NIP) for participating, performing and validating Stockholm convention with decision No. 184/2006/QD-TTg of Prime Minister on the 10th of August 2006. All members of Convention agree to reduce or eliminate releases from intentional or unintentional production and use of POPs as well as to perform monitoring POPs levels in environment (*Stockholm Convention, 2001*). Therefore, capacity building in research on PTSs is essential.

The effectiveness of the Stockholm Convention on POPs shall be evaluated within four years of entry into force of the Convention. In order to perform a scientifically sound and meaningful evaluation based on comparable monitoring data, all available data from existing national, regional and global monitoring programmes should be considered. UNEP Chemicals has initiated an activity that aims at linking together existing national, regional and global activities on POPs monitoring. The capacity and capability to fully participate in such program is not sufficient in many countries and also in Vietnam. Therefore transferring technology, knowledge and building capacity in these areas is essential to close the gap.

Some International Conferences and Projects on PTSs had been performed in Vietnam as follow:

- Workshop on the Management of Persistent Organic Pollutants (POPs), United Nations Environmental Program, 16 – 19, March 1999, Hanoi, Vietnam.

- Workshop on Management, Use and Assessment of Environmental Pollution of Pesticides, 28 – 29, September 2000, Hanoi, Vietnam.

- UNU International Symposium on Tracing Pollutants from Agrochemical Use: Focus on EDC Pollution, 15 – 16, April, 2002, Hanoi, Vietnam

- 2nd Asian Pacific International Conference on Pollutants Analysis and Control, 1 – 3 December 2003, Hochiminh City, Vietnam.

- Training workshop on "Stockholm Convention Implementation and International Practices", 04 – 08 April 2005, Hanoi, Vietnam.

- Training workshop on "Environmental Permits, Emergency Response and POPs Treatment Technologies", 18 – 20 April 2005, Hochiminh City, Vietnam.

- 1st International Conference on Environment and Natural Resources, 17 – 18, March, 2008, Hochiminh City, Vietnam.

2.2. Use of PTSs in Vietnam

Vietnam is an agricultural country where 80% of the population works in the agricultural sector. Progress on improved intensive farming system, expanding of cultivated area, diversification of high value crops have contributed to the tremendous increase of agricultural production output. Most of the crops cultivated areas is for rice. In 2007, the rice cultivation areas account for 87,6% total areas of crops cultivation (*VGSO, 2009a*). Vietnam rice production output and the rice export volume have rapidly increased. In 1999, Vietnam produced 24,96 million tons, exported 1,98 million tons of rice and in 2007 these numbers were 35,86 millions tons and 3,96 million tons, respectively (*VGSO, 2009b*). With much effort in rice cultivation and trade, Vietnam has exported 3,86 million tons in first half 2009 and intended to raise the volume of rice export to 6 million tons in 2009 (*VET, 2009*). However, with the great achievements in many aspects, agricultural activities in Vietnam have considerable effects on natural resources and the environment. In reality, the enhancement of short-term varieties with high yield requires the addition utilization of chemical fertilizers and pesticides.

Pesticides are still being used widely anywhere with any crop. The farmers master themselves their whole agricultural production process. However, due to lack of knowledge on pest control and poor management of pesticides, famers have used improperly pesticides (*Viet and Quyen, 2000*). The kinds of pesticides used in Vietnam vary greatly with about 250 kinds of active ingredient and 760 trademarks (*Lam, 2005*).

The frequency of pesticides utilization is different according to cultivation regions (rice, vegetable, cash crop). In vegetable growing regions, pesticide was used many times more than they were in rice cultivation regions. For instance, in vegetable growing regions of Hanoi, Bacninh, Haiduong, Haiphong, Danang, Dalat, Hochiminh City (HCMC), pesticides were sprayed 28 – 30 times/season. In rice fields of the Red river delta, the Mekong delta and along Coastal of Central Vietnam, pesticides were applied 1 – 3 times/season. In case which pest outbreak occurs, pesticides spraying has been increased up to 4 – 5 times/season, lasting 1 – 2 days per time (*Anh et al., 2000*).

According to the report of the United Nations Environment Programs (UNEP) on POPs monitoring, pesticides such as DDT and HCB were imported into Vietnam from former Soviet Union and some socialistic countries with a quantity of 6,500 – 9,000 tons/year. The statistical data showed that the total amount of DDT imported to Vietnam for malaria control from 1957-1979 was 14,847 tons (DDT 30%) and from 1976 – 1990 was approximately 9,195 tons (DDT 75%) (*UNEP, 2002*). The quantities of DDT and pesticides imported and used in Vietnam are presented in table 2.1, 2.2, 2.3 and 2.4 below.

Table 2.1: Imports of DDT in Vietnam

Years	Quantity (tons)	Types of DDT
1957 – 1979	14,847	DDT 30%
1976 – 1990	9,195	DDT 75%

(*Source: UNEP, 2002*)

Table 2.2: DDT usage in Vietnam

Years	Quantity (tons)
1992	237.748
1993	33.935
1994	151.675

(*Source: UNEP, 2002*)

Table 2.3: Quantity of imported pesticides into Vietnam from 1991 - 1997

Year	Total (tons)	Monetary value (million USD)	Insecticides	
			Quantity (tons)	(%)
1991	23,300	22.50	16,900	83.30
1992	23,100	24.10	18,000	75.40
1993	24,800	33.40	18,000	72.70
1994	20,380	58.90	15,226	68.30
1995	25,666	100.40	16,451	64.10
1996	32,751	124.30	17,352	53.00
1997	30,406	-	15,351	50.46

-: Data not collected

(*Source: Bui, 2002*)

Table 2.4: Quantity of imported pesticides (formulation) into Southern Vietnam from 1991 - 1997 (*Bui, 2002*)

Year	Total (tons)	(%)			
		Insecticides	Fungicides	Herbicides	Other
1991	8,569	85.0	-	-	-
1993	3,877	-	-	-	-
1994	4,800	41.0	28.0	31.0	-
1995	10,536	51.0	25.5	21.5	2.0
1996	18,489	52.0	32.5	12.0	3.2
1997	25,876	50.0	26.5	21.3	1.8

-: Data not collected

PCBs: Regarding usage of PCBs in Vietnam, a report revealed that about 27,000 – 30,000 tons of oils containing PCBs were imported from former USSR, China and Romania (*Sinh et al., 1999*). In addition, electrical equipments containing PCBs, such as transformers and capacitors, were also imported from Australia until the mid-1980s (*Kannan et al., 1995*).

Nowadays the major sources of PCBs in Vietnam could be from old electric equipment and hydraulic fluids. The majority of PCBs contaminated waste oil is from old or damaged electrical equipment freely traded in market without PCBs examination of authorized organizations. This is one of significant PCBs emission source to environment. It has also been speculated that widespread and serious environmental contamination by PCBs would exist in Vietnam due to intensive use of weapon during the 1961 - 1971 Indochina war (*Nhan et al., 1999*).

2.3 Contamination status of PTSs in Vietnam

2.3.1 PTSs contamination in soil and sediment

Vietnam is a developing country located in the tropical region. This is an agriculture – based country, where a large number of pesticides including organochlorinated pesticides were used for a long period of time (*Thao et al., 1993b*).

Viet et al. (2000) reported the concentration of some OCs pesticides and PCBs in soil and sediment samples. These samples were collected from three areas in Northern Vietnam: Hanoi, Viettri and Halong Bay. Greater residues in sediment from Halong Bay than other locations indicate a more serious organochlorine contamination in the marine environment, and probably are due to source discharges from the river and cities along the river. The study results were compared with other locations and other Asian countries in table 2.5 below.

Nhan et al., (1999) reported one of the first surveys on PTSs, i.e. chlorinated pesticides and PCBs, carried out on the coast of the north of Vietnam. The study results suggest a widespread use of DDT along the entire coastline of north Vietnam. Concentrations of PCBs (under the sum of Aroclor 1254 and 1260) in sediments were generally low (table 2.5). PCBs were quantified base on levels of six congeners (CB 28, 52, 101, 153, 138 and 180). The total PCBs level was calculated by multiplying the sum of levels of six PCB with the factor corresponding to their theoretical distribution in Aroclor 1254 and 1260. The higher concentrations, with a maximum of 34.3 and 28 ng/g, were recorded in the samples taken near large cities such as Mongcai (Station 1) and Haiphong (Station 4), whereas the lowest values correspond to the samples taken in rural areas. These results confirm that Aroclors were originated from industrial wastes of these cities, and the possible sources may be from transformer fluids and hydraulic additives. Several pesticide formulations for household insect control available in the local markets are likely to contain DDT (*Nhan. et al., 1999*).

In another survey, Minh et al. (2004a) show that DDT levels in Hau River's sediment are about 3 to 4 times lower than those from coastal areas of the North in the mid 1990s or from mangroves of the South in the early 1990s (table 2.5), suggesting lesser inputs of DDTs in recent years. Strandberg et al., (1998) suggested that DDT/ DDE ratios lower than 0.33 could be the result of the aged mixtures in environment, while those > 0.5 might indicate recent use of DDT. According to the research of Minh et al., (2004a) some sediment samples such as in Can Tho city (CC-1, CC-4, CC-7, NK-SE) or close to Long Xuyen town (Hau-5, Hau-6) had the DDT/DDE ratio higher than 0.5 and thus indicating possible recent input of DDT to the Hau River (Fig. 2.1). However, the question regarding degree of recent use of DDTs compared to other sources such as soil run-off is still not clear because the magnitude of contamination has actually decreased over the last decade and became relatively low at the present.

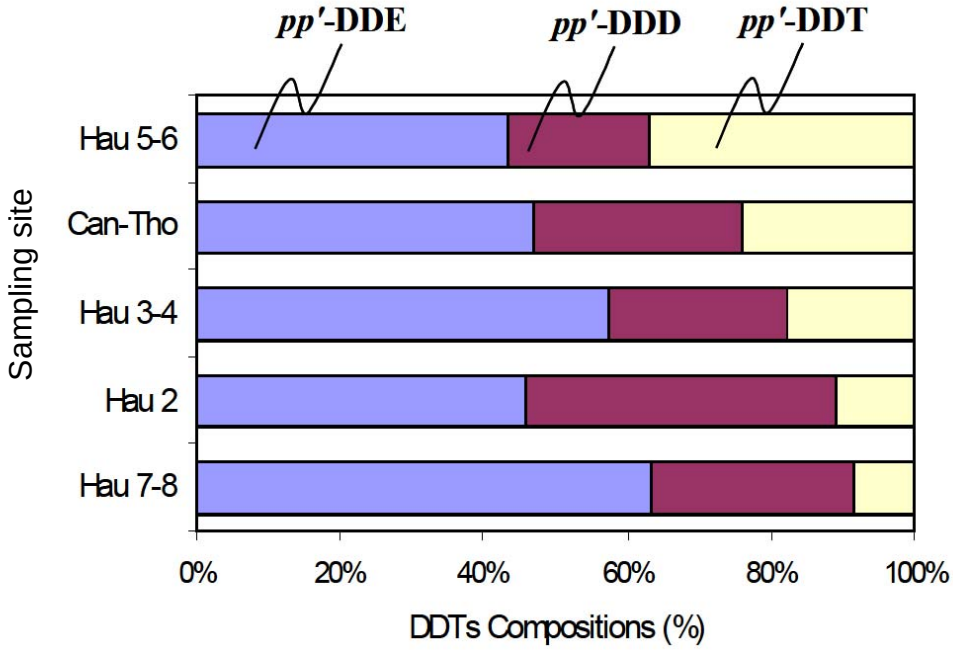

Figure 2.1: DDTs composition in sediment in Hau River, South Vietnam

According to another research in HCMC canals and Saigon-Dong Nai (SG-DN) River (*Minh et al., 2007*), PCB and DDTs residue levels are about 3 to 6 times lower compared to previous results reported in the early 1990s (*Phuong et al. 1998*; *Iwata et al. 1994*). This finding demonstrates decreasing levels of PCBs and DDTs in the aquatic environment of the HCMC canals and SG-DN River system (table 2.5).

Levels of PTSs in sediments from Vietnam were compared to those from other locations in the world (table 2.5). Data for other countries were cited from previous studies (since 1990) to provide a more realistic comparison. Levels of PCBs and DDTs in Vietnam are lower than those in areas such as Macao harbor (China). These results highlight elevated contamination by PTSs in the aquatic environment of Vietnam.

Table 2.5: Comparison of OCs residue levels (mean concentration in $ng.g^{-1}$ dry wt.) in sediment from Vietnam with those from other Asian countries

Location	Year	n	PCBs	∑DDTs	HCHs	Reference
Vietnam						
Halong Bay	2003-2004	16	1.6[g]	22.52	0.22	Hong et al., 2008
Haiphong Bay	2003-2004	15	4.5[g]	11.17	0.31	Hong et al., 2008
Balat Estuary	2003-2004	10	0.1[g]	0.91	0.11	Hong et al., 2008
Hochiminh	1990	9	120[h]	110	1.1 [b]	Iwata et al., 1994
Hochiminh (canal)	1996	10	220[d]	79.9	-	Phuong et al., 1998
Hochiminh (canal)	2004	6	81	37	-	Minh et al., 2007
Mangroves (South of VN)	1990	9	5.2 [h]	8.5	0.97	Iwata et al., 1994
CanTho (south)	2003	10	0.9	1.9	0.08	Minh et al., 2004a
Halong Bay	1998	6	37[i]	28	6.06 [b]	Viet et al., 2000
Hanoi	1998	17	45[i]	5.0	0.35 [b]	Viet et al., 2000
Viettri	1998	9	2.3[i]	5.2	0.68 [b]	Viet et al., 2000
Hanoi (urban)	1997	12	11[a]	30	0.8	Nhan et al., 2001
Hanoi (Outskirt)	1995-1996	2	5.5[d]	10	-	Nhan et al., 1998
North coast	1995-1996	4	1.7[d]	5.5	-	Nhan et al., 1998

Station-1 (Mongcai)	1997	1[c]	34.3[a]	10.41	33.74	Nhan et al., 1999
Station-2 (Mongduong)	1997	1[c]	5.7[a]	8.08	4.11	Nhan et al., 1999
Station-3 (Halong Bay)	1997	1[c]	16.3[a]	7.24	1.84	Nhan et al., 1999
Station-4 (Haiphong)	1997	1[c]	28[a]	6.70	1.73	Nhan et al., 1999
Station-5 (Balat Estuary)	1997	1[c]	0.7[a]	6.25	1.23	Nhan et al., 1999
Binhtrithien	1990	1	0.4[h]	34	0.29[b]	Iwata et al., 1994
Other countries						
Coastal Region (Singapore)	2003	13	73.9[j]	6.7	18.1	Wurl and Obbard, 2005
Gao-ping River (Taiwan)	2000	12	1.43[k]	0.78	2.49	Doong et al., 2008
Hugli Estuary (India)	2003	10	0.8[l]	0.96[e]	0.24	Guzzella et al., 2005
Korea (Masan Bay)	1997	20	15[m]	13.6	-	Hong et al., 2003
Hong Kong	2004	6	4.2	5.6[e]	0.17[f]	Wei et al., 2008
Japan	1990	3	150[h]	6.4	0.56	Iwata et al., 1994
China (Minjiang River)	1999	9	35[n]	6.7	-	Zhang et al. 2003
China (Macao Harbour)	1997	1	340	1630	-	Kang et al., 2000
Korea (Ulsan Bay)	2001	32	-	3.3	-	Khim et al., 2001
Taiwan (Wu-Shi River)	1997-1999	19	-	2.5	3.8	Doong et al., 2002

- : Data not available; [a]: sum of mix Aroclor1254 + Aroclor1260; [b]: g-HCH; [c]: composite sample; [d]: as Aroclor 1254; ∑DDTs = pp'-DDE + pp'-DDD + pp'-DDT; [e]: Sum of op'-DDE, pp'-DDE, op'-DDD, pp'-DDD, op'-DDT and pp'-DDT; [f]: sum of a-HCH and g-HCH; [g]: sum of 22 congeners; [h]: mixture KC300-KC600; [i]: mixture of Kanechlor preparations (KC300, KC400, KC500, KC600); [j]: sum of 36 congeners; [k]: sum of 42 congeners; [l]: sum of 13 congeners; [m]: sum of 22 congeners; [n]: sum of 21 congeners;

As for soil samples, an extensive survey in northern and southern Vietnam indicated higher concentrations of DDTs from paddy field sites than from upland areas (*Thao et al. 1993a,b*). This result clearly reflects the status of DDT use as an insecticide in the past in Vietnam. In some specific sites in Tay Ninh, southern Vietnam, where a U.S. Army base was located, elevated PCB levels were recorded (*Thao et al. 1993a,b*). A recent survey in the open dumping sites for municipal wastes in Hanoi and Hochiminh City, the two largest cities in Vietnam, revealed that concentrations of DDTs and PCBs in soils collected from dumping sites were much higher than those in paddy fields far from the dumping sites (Fig. 2.2). PCBs and OC insecticides likely originated from continuous loadings of municipal waste containing residues of these compounds. The open dumping sites therefore, may act as reservoir sources of PCBs and OC insecticides (*Minh et al., 2006b*).

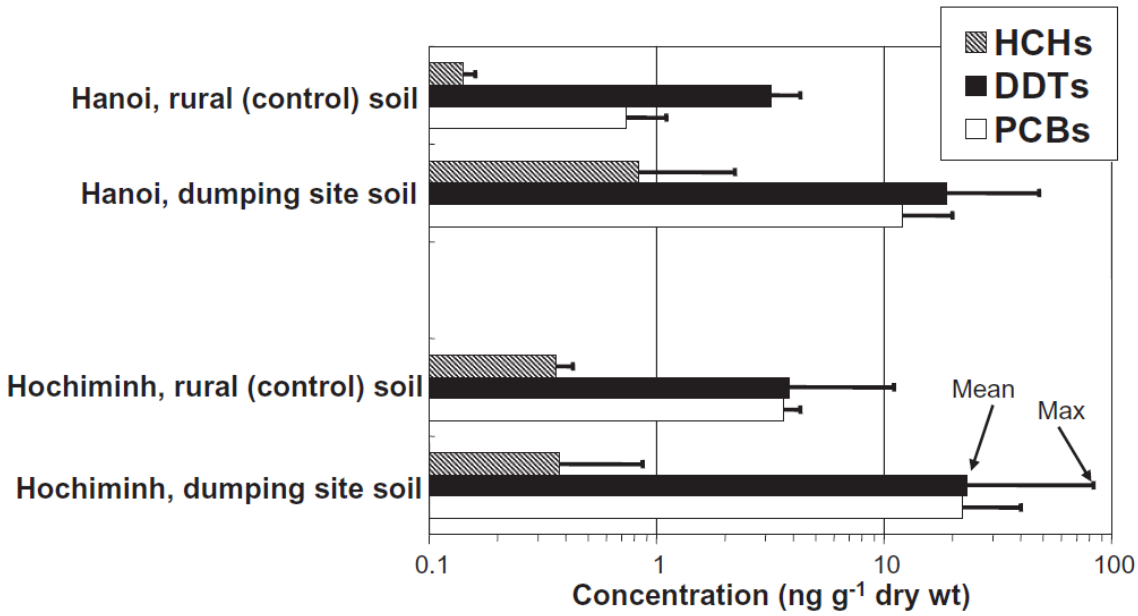

Figure 2.2: Residue concentrations of PTSs in soils from open dumping sites and rural areas (control sites) in Hanoi and Hochiminh City (*Minh et al., 2006b*).

2.3.2 PTSs contamination in biological samples

Surveys examined POP residue levels in various biological samples such as fish, bivalves, and birds from the Asia-Pacific region, including Vietnam, have made clear the status of contamination, distribution, and source allocation. An extensive study on PTSs contamination in fish from Asia and Oceania including Vietnam was carried out by Kannan et al. (1995). In Vietnam, residues of DDTs were relatively high in the surveys conducted in 1990 and 1997 (Table 2.6).

Monirith et al. (2003) reported high contamination of DDTs in mussels collected from Vietnam, China, Hong Kong and Far East Russia (Fig. 2.3). Mussels from Vietnam and these countries showed higher proportion of pp'-DDT residues than pp'-DDE (Fig. 2.4). Clams and fish from Hong Kong, China and Vietnam (*Kannan et al., 1995; Nhan et al., 1999, 2000; Tanabe, 2000*) also showed high proportion of p,p'-DDT. Considerably higher ratio of pp'-DDT in total DDTs may again indicate the presence of current emission sources of DDTs in China, Hong Kong and Vietnam. The compositions and residue levels of DDTs in mussels in this study indicate current usage of DDT for agriculture and public health purposes in Vietnam and some Asian developing countries.

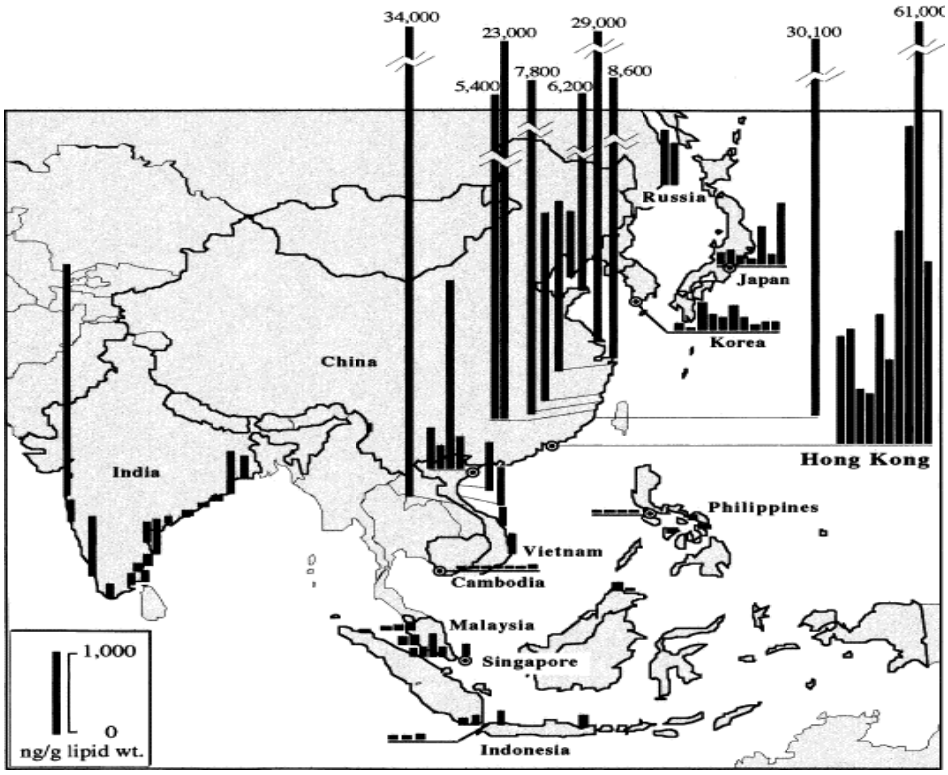

Figure 2.3 Distribution of DDTs levels in mussels collected from Asian countries *(Source: Monirith et al., 2003)*

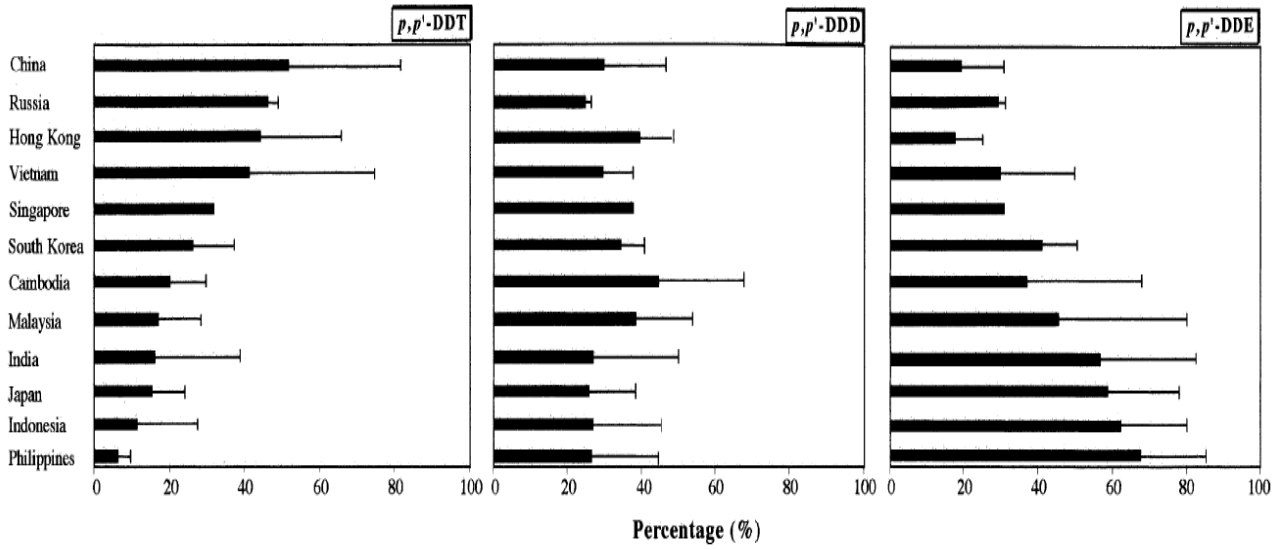

Figure 2.4 Composition of DDT compounds in mussels collected from coastal waters of Vietnam and some Asian countries *(Source: Monirith et al., 2003).*

In general, lower DDTs levels were observed in mussels from Phillipines, Cambodia, Indonesia and Malaysia (Fig. 2.3), which indicates less usage of DDTs in these countries.

Fig. 2.5 presented HCHs levels in mussels collected from Vietnam and other Asian countries. According to the results of research, the levels of HCHs, measured in mussels collected from Vietnam are generally much lower than HCHs levels in mussels from the other Asian countries such as Indian, Chin and Russia. Extremely higher levels of HCHs were observed in mussels from India (20–430 ng/g lipid wt.). Relatively high concentrations were also found in China

(2.1–110 ng/g lipid wt.). Higher concentrations of HCHs in mussels from India, China and Russia might be partly caused by continued usage of this chemical in these countries (*Monirith et al., 2003*).

The PCBs concentrations distribution in mussels collected from Asian coastal waters are shown in Fig. 2.6. Higher concentrations of PCBs were found in mussels from Japan (up to 12,000 ng/g lipid wt.) and Russia (up to 3700 ng/g lipid wt.). Contrarily, low concentrations PCBs were found in mussels from Vietnam (160 ng/g lipid wt.) and other Asian countries such as Cambodia (35 ng/g lipid wt.), Indonesia (87 ng/g lipid wt.), Malaysia (56 ng/g lipid wt.), India (340 ng/g lipid wt.) and China (120 ng/g lipid wt.), which indicate fewer local sources (*Monirith et al., 2003*).

Table 2.6: PTSs concentrations (mean value in ng/g lipid wt.) in biological samples from Vietnam

Location	Sample description	Year	n	Lipid content (%)	PCBs	DDTs	HCHs	CHLs	Reference
Vietnam									
Hanoi	Fish	1990	7	1.9	580	1,900	120	7.9	Kannan et al., 1995
Phu Da, Hue	Fish	1990	6	1.9	630	1,100	48	8.9	Kannan et al., 1995
Hochiminh City	Fish	1990	6	1.9	950	1,100	110	3.2	Kannan et al., 1995
Conlu island (north VN)	Fish	1997	10	3.2	110[a]	4,200	350	110	Minh et al., 2002
TraCo (Mong Cai)	Clam	1997	1 pooled sample	-	900	1,200	2,400	-	Nhan et al., 1999
Mongduong (North VN)	Clam	1997	1 pooled sample	-	480	590	400	-	Nhan et al., 1999
Halong (Quang Ninh)	Clam	1997	1 pooled sample	-	470	660	93	-	Nhan et al., 1999
Nhue River (Hanoi)	Freshwater snails	1997	1 pooled sample	-	720	23,000	29	14	Nhan et al., 2001
Mekong River	Fish, aquaculture	2004	20	3.8	7.2	59	0.47	0.62	Minh et al., 2006a
Cantho (South VN)	Fish from a pond near dumping site	2004	5	3.6	50	390	2.2	5.7	Minh et al., 2006a
Conlu island (North VN)	Resident birds	1997	16	1.9-16	780[a]	6,200	150	100	Minh et al., 2002
Conlu island (North VN)	Migratory birds	1997	84	4.1-33	530[a]	2,900	330	22	Minh et al., 2002
Other countries									
India (wild fish)	Several species	1993	48	2.4	150	630	1200	100	Kannan et al., 1995
Switzerland (wild fish)	Trout fish	2003	7 pooled sample	2.3	880	550[c]	2.17[b]	-	Schmid et al., 2007
Yangtze, China (wild fish)	Several species	2004	7 pooled sample	-	13.2[d]	0.02[d]	0.07[d]	-	Hu et al., 2009
China (wild fish)	Several species	2000	3	-	180	1000	68	160	Nakata et al., 2005
Korea (wild fish)	Several species	1997-2001	14	1.8	1440	560[c]	58	68[e]	Yim et al., 2005

-: data not available; DDTs: Sum of pp'-DDE, pp'-DDD and pp'-DDT; HCHs: sum of a-HCH, b-HCH and g-HCH; CHLs: sum of cis-chlordane, trans-chlordane, cis-nonachlor, trans-nonachlor and oxy-chlordane; [a]: PCBs quantified by an equivalent mixture of Kanechlor preparations (KC-300, KC-400, KC-500 and KC-600); [b]: lindane; [c]: sum of op'-DDD, pp'-DDD, op'-DDE, pp'-DDE, op'-DDT and pp'-DDT ; [d]: ng/g wet weight ; [e] : sum of CHLs and heptachor and heptachlor epoxide

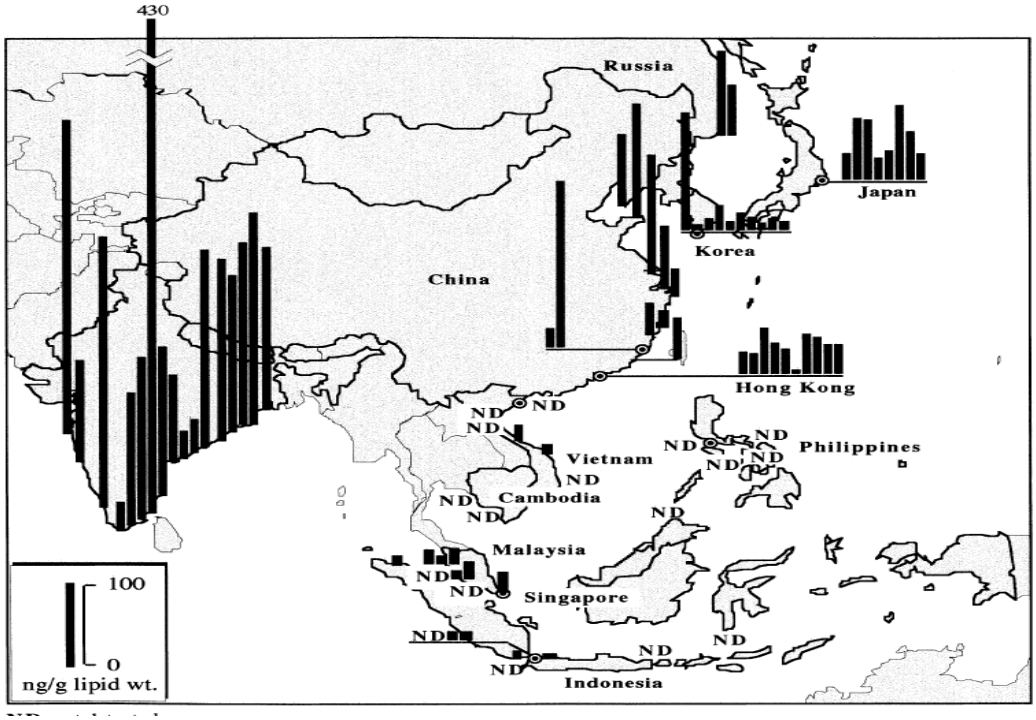

Figure 2.5: Distribution of concentrations of HCHs in mussels collected from coastal waters of Vietnam and some Asian countries *(Monirith et al., 2003)*

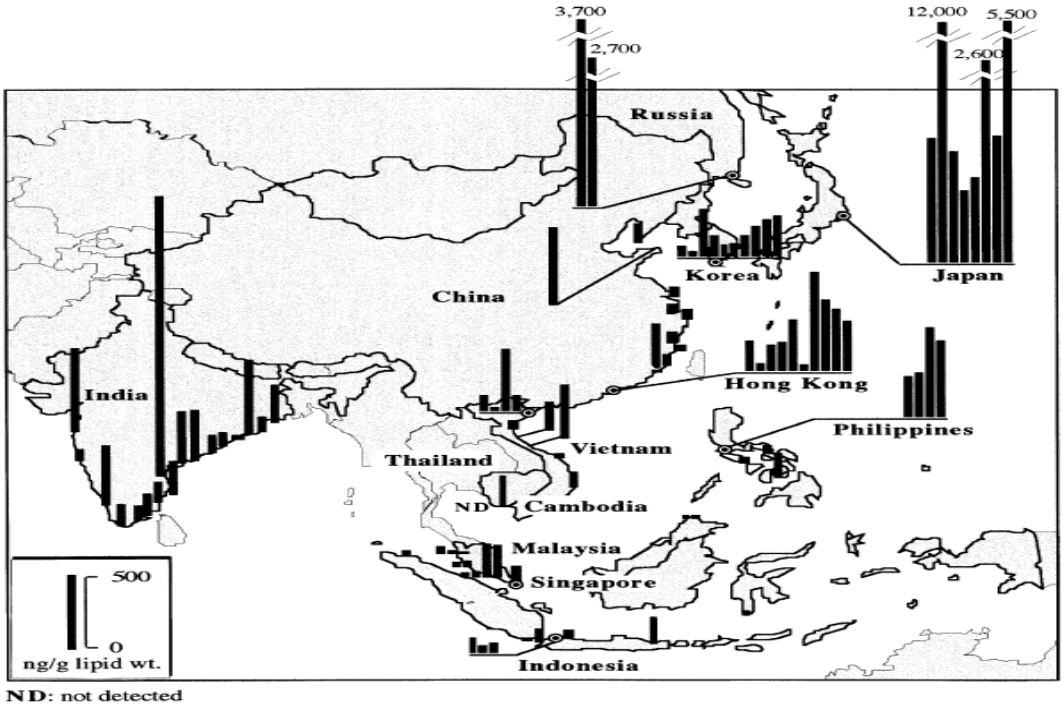

Figure 2.6: Distribution of concentrations of PCBs in mussels collected from coastal waters of Vietnam and some Asian countries *(Monirith et al., 2003)*

PBDEs preliminary survey to examine the levels of PBDEs and other PTSs in common aquaculture catfish and in catfish from a pond located near a municipal waste site from Can Tho, in southern Vietnam (*Minh et al., 2006a*). Interestingly, concentrations of PBDEs and other PTSs such as PCBs, DDTs, CHLs, and HCHs in fish collected close to dumping site were significantly

higher than those in common aquaculture fish (table 2.6), suggesting an additional exposure of the dumpsite catfish to PBDEs (Fig. 2.7). In the dumping site, municipal wastes including household goods and small electric appliances, that may contain PBDEs as flame retardants, were dumped. Under ambient conditions, PBDEs may be emitted from such materials and consequently contaminate the dumping site soil. Therefore, it is anticipated that runoff and leaching water from the dumping site during flood and rains may have carried PBDEs to other vicinities, causing higher contamination in the catfish.

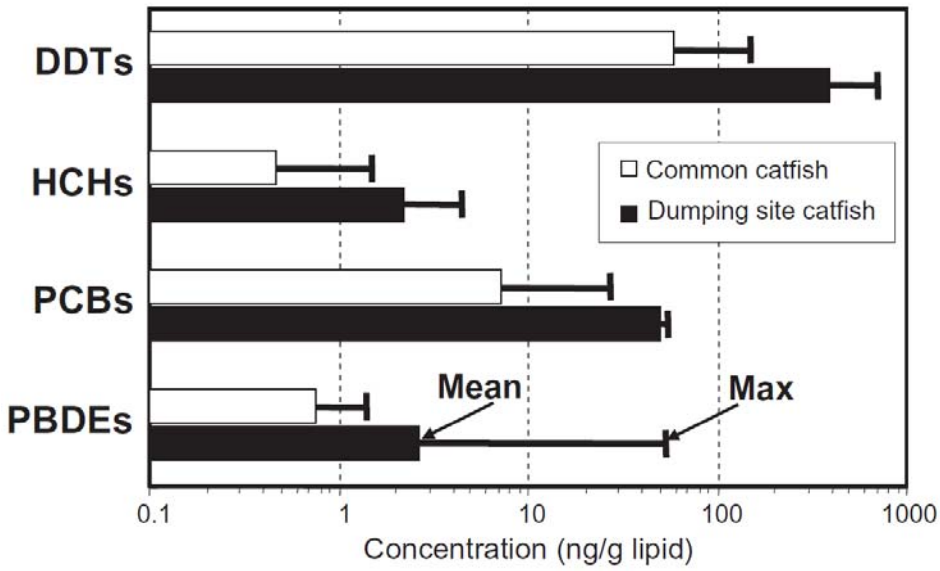

Figure 2.7: Comparison of PBDE and PTSs residues in catfish from open dumping sites and in common aquaculture from Can Tho City southern Vietnam (*Minh et al. 2006a*)

So far, to our knowledge, there are very few studies on PTSs contamination in higher trophic animals from Vietnam. A survey conducted in 1997 determined PTSs concentrations in resident and migratory birds from North Vietnam (*Minh et al., 2002*). Resident birds contained higher concentrations of DDTs than those in migrants (Fig. 2.8), this indicates recent exposure to DDTs in resident birds from North Vietnam, where elevated DDT contamination is very common, as discussed in fish, bivalve, freshwater snail above (table 2.6). Interestingly, accumulation of HCHs revealed a contrasting pattern, showing apparently greater concentration in migratory birds (Fig. 2.8), which could be the result of accumulation in stopover sites during migration in some polluted areas such as India, southern China, and Japan. The role of these countries as potential sources of HCH accumulation in wintering migratory birds breeding in Lake Baikal, Russia, has also been suggested in a recent study (*Kunisue et al. 2002*). Concentrations of PCBs were similar in residents and migratory species and levels were relatively low, indicating a smaller source of PCBs in North Vietnam in recent years. Thus, accumulation pattern of PTSs in birds from North Vietnam according to their migratory behavior reflects the status of each contaminant. This phenomenon also suggests the suitability of using birds as bioindicators for monitoring POPs in the environment.

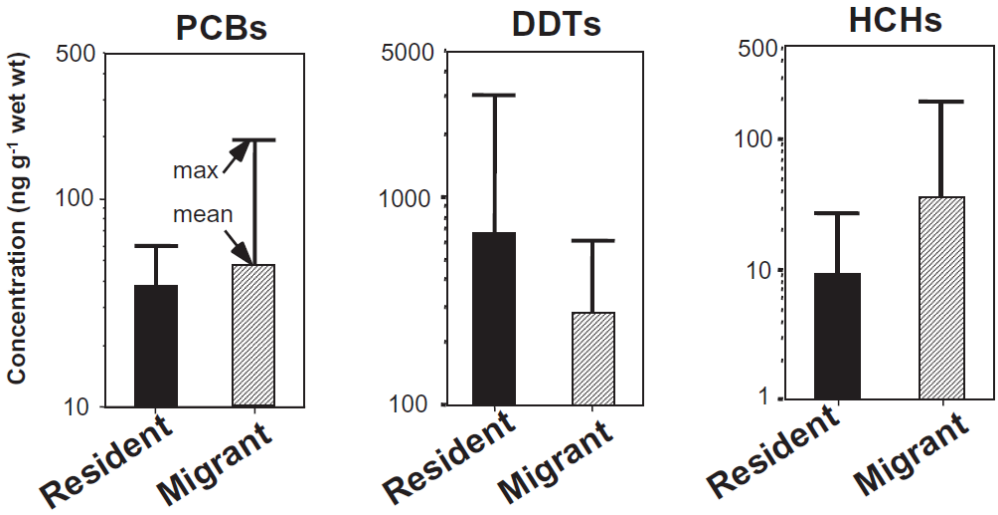

Figure 2.8: Accumulation of some PTSs in birds from Vietnam according to migratory behavior (*Minh et al., 2002*)

2.3.3 PTSs contamination in humans

As mentioned above, PTSs are highly lipophilic and persistent, therefore human chronic exposure via food chain has led to the accumulation of both parent compounds and their metabolites in lipid rich tissues such as adipose tissues and human breast milk. Among human tissues, breast milk is a convenient sampling matrix for measuring residue concentrations of persistent PTSs. The samples are easy to collect and highly suitable for estimating body burdens of PTSs, and thus may provide useful information about their accumulation kinetics in humans. In addition, the PTSs residue concentrations in human breast milk are a key factor for evaluating the toxic potential of contaminants in infants (Fig. 2.9). Available data on human exposure to PTSs in Vietnamese residents are limited.

A preliminary survey by Schecter et al. (1989) reported very high concentrations of DDTs and HCHs in human breast milk from some locations in and around Hochiminh. DDTs levels were higher in both rural and urban areas, ranging from 10,500 to 12,000 ng/g lipid wt.. PCB levels, however, were relatively low compared to other countries. A subsequent study conducted in 2000–2001 provided more extensive data on human exposure and insights into the accumulation kinetics of PCBs and OCls insecticides such as DDTs, HCHs, CHLs, and HCB, a newly detected environmental contaminant that exhibits weak endocrine-disrupting properties.

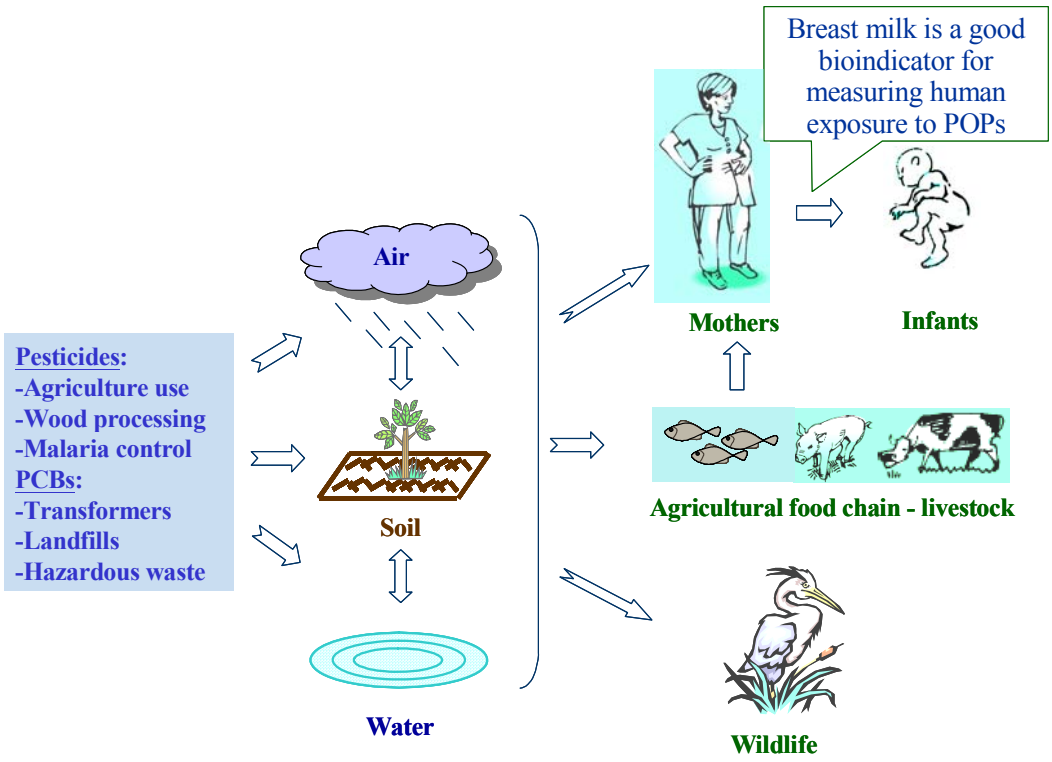

Figure 2.9 Biomagnification of POPs through food chains to mothers and subsequently POPs transfer in both pre- and sub-natal states to children (*Minh et al., 2004b*)

Breast milk from 96 nursing women living near the dumping sites of municipal wastes in Hanoi and Hochiminh, the two largest metropolitan cities in Vietnam, was analyzed for these contaminants (table 2.8). In addition, accumulation kinetics of these PTSs in human was studied and potential risk for breast-fed infants due to PTSs exposure was also evaluated. In general, similar degrees of exposure to DDTs, CHLs, HCB, and PCBs were observed among samples from Hanoi and Hochiminh. The residue pattern of OCs in human breast milk in Vietnam followed the order of DDTs > PCBs > HCHs > CHLs ≈ HCB. Significant differences in OC levels between two cities were found only for HCHs. HCHs residues in breast milk of women from Hanoi were higher than those in Hochiminh, suggesting recent high background levels of HCHs in the northern compared to the southern region. Consistent results where also observed in the survey on sediment and bivalves from various locations along the northern coast of Vietnam, which demonstrated relatively higher residues in sites near the China border (*Nhan et al. 1998, 1999*). Earlier reports also pointed out similar spatial distribution in different kinds of environmental samples, showing higher levels of HCHs in Hanoi compared to Hochiminh (*Thao et al. 1993a; Iwata et al. 1994; Kannan et al. 1995*). In addition to the influence of the possible transport from China, the world leading HCH user, differences in climate between Hanoi and Hochiminh could be an alternative explanation. The Mekong River delta in southern Vietnam is characterized by the typical tropical climate with high temperatures and heavy rainfall. Rapid volatilization of highly volatile HCH isomers may therefore be enhanced in southern Vietnam, resulting in lower residues in various environmental and human samples.

Table 2.7: Concentrations of organochlorines in human breast milk (ng/g lipid wt.) in Hanoi (n = 42) and Hochiminh City (n = 44), Vietnam (*Minh et al., 2004b*)

	Age (years)	Fat content	PCBs	DDT compounds				CHL compounds				b-HCH	HCB
				pp'-DDE	pp'-DDD	pp'-DDT	DDTs	oxy	t-nona	c-nona	CHLs		
Hanoi													
Primiparas	27	2.5	76	2200	13	190	2400	0.88	2.2	1.9	2.5	69	4.2
Multiparas	31	2.1	72	1500	8.9	150	1700	0.92	0.86	0.3	1.4	46	3.5
Overall[a]	29	2.3	74	1850	11	170	2100	0.90	1.53	1.1	2.0	58	3.9
Range	20-44	0.7-5.7	26-210	420-6300	3-50	34-960	480-6900	0.25-1.6	<0.21-8.1	<0.21-3.2	<0.72-13	11-160	0.62-9.5
Hochiminh													
Primiparas	24	3.3	88	2700	8.3	310	3020	2.7	4.2	0.8	7.8	14	2.8
Multiparas	29	2.9	70	1300	5.7	220	1500	2.1	3.2	0.8	6.0	13	2.1
Overall	27	3.1	79	2000	7.0	265	2300	2.4	3.7	0.8	6.9	13.5	2.5
Range	18-37	1.5-6.9	29-200	340-16000	2.7-18	100-1000	440-17000	<0.25-9.3	1.3-15	<0.21-2.1	1.3-26	4.1-35	1.3-10

CHLs: chlordane compounds, oxy: oxychlordane, t-nona: tran-nonachlor, c-nona: cis-nonachlor; DDTs = pp'-DDE + pp'-DDD + pp'-DDT; CHLs = oxy + t-nona + c-nona; [a]: Average concentration from primiparas and multiparas

Table 2.8: Comparison of organochlorine residues in human breast milk from various countries (ng/g lipid wt.)

Country	Year	n	PCBs	∑DDTs	CHLs	HCHs	HCB	PBDEs	Reference
Industrialized countries									
Bavaria (Germany)	2005	39	252	180	-	-	27	1.9	Raab et al., 2008
Japan	2001-2004	93	116	275	58[a]	92[b]	12.8	-	Kunisue et al., 2006
Poland	2004	22	153	868[c]	-	14.1[d]	32.2	2.5	Jaraczewska et al., 2006
Norway	2000-2002	29	172	110	14[f]	14[g]	18	3.8	Polder et al., 2008a
Finland	1997-2000	43	-	82	4	13	8	-	Shen et al., 2007
Sweden	2000-2004	94	113	90	9.9	10[h]	12	3.4	Lignell et al., 2006
Russia (Murmansk)	2000	14	346	900	22	235	65	1.2	Polder et al., 2008b
Poland (Wielkopolska)	200-2001	14	115	1195	-	20	23	-	Szyrwinska & Lulek, 2007
Russia (Buryatiya)	2003-2004	17	240	660	19	810	100	1.0	Tsydenova et al., 2007
Developing countries									
Cambodia	2000	28	42	1600	1.8	5.5	1.7		Kunisue, et al., 2002
Tunisia	2003-2005	237	196	1931	-	65[d]	85	-	Ennaceur et al., 2008
China (Zhejiang)	2003-2005	21	248	1173.5[i]	-	259.5	36.6	-	Zhao et al., 2007
Indonesia	2001	55	33	640	2.0	14	2.2	-	Sudaryanto et al., 2006
India	2000	8	30	420	0.9	650	1.0	-	Kunisue, et al., 2002b
Philippines	2000	10	72	190	15	4.7	-	-	Kunisue, et al., 2002b
Vietnam (North)	2000	42	74	2100	2.0	58	3.9	-	Minh et al., 2004b
Vietnam ((South)	2001	54	79	2300	6.9	14	2.5	-	Minh et al., 2004b
Brazil	1992	40	150	1700	-	276	12	-	Paumgartten, et al., 2000
Mexico	1998	60	-	4100	-	60	30	-	Waliszewski, et al., 2001

- Data not available; [a]: sum of o-chlordane, cis-nonachlor and trans-nonachlor; [b]: sum of a-HCH and b-HCH; [c]: sum of pp'-DDE and pp'-DDT; [d]: sum of b-HCH and g-HCH; [e]: b-HCH; [f]: sum of oxy-chlordane, cis-chlordane and trans-nonachlor; [g]: sum of a-HCH, b-HCH and g-HCH; [h]: b-HCH; [i]: pp'-DDE

The global comparison of PTSs residues in human breast milk is given in table 2.8. Although the cited data may differ between laboratories, it is possible to draw some relevant comparison to understand the magnitude of contamination. In comparison to other developing countries like Cambodia, India and the Philippines, residue levels of PCBs in human breast milk from Vietnam are slightly higher. However, these PCBs levels are still below those reported for developed countries (Table 2.8). Recent global inventory of PCBs production and consumption has indicated that common applications of PCBs (i.e. for industrial purposes) in Vietnam during the past years were not higher than those in China, Hong Kong, India and the Philippines (*Breivik et al., 2002*). Hence, the higher PCB residues observed in human breast milk from Vietnam suggest additional sources of PCBs besides industrial sources like transformers, capacitors, etc. A likely source of PCBs in Vietnam could be the release from different kinds of military weapons used extensively during the Vietnam War as suggested earlier (*Thao et al., 1993*).

Similar to those observed in environmental samples, human exposures to DDTs in Vietnam were among the highest in the developing as well as developed nations. As discussed earlier, high DDT contamination in Vietnam has been apparent in many environmental samples. This is also the case for humans, which raises concern over the possible impacts on human health. These results suggest that Vietnam is a potential source of DDTs in the South Asian region.

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Chapter 3: REVIEW OF METHODS FOR PTSs RESIDUE ANALYSIS

3.1. Introduction

In the past few years, the analytical methods for PTSs residues in various matrices have been developed very rapidly and the detection limit is lower as well as the sample size required for analysis is reduced by the time. Analytical methods for the determination of PTSs in environmental samples and biological tissues vary depending upon the matrix and required limit of detection. Normally the PTSs residues are bound inside the structure of complex matrices such soil, sediment, biomaterial, vegetable, etc. In general, the analytical procedures for PTSs are composed of the following four steps:

1. Sample collection and extraction
2. Clean – up using partition and chromatographic fractionation
3. Separation on gas chromatography (GC)
4. Identification and quantification with selective and sensitive detectors.

Since the early 1960s, PTSs have been determined using gas chromatography (GC) techniques with electron capture detection (ECD), initially using packed columns. More advanced methods, such as capillary GC-ECD and GC coupled with mass spectrometry (GC-MS) have been used in more recent studies to identify the individual congeners, to improve the comparability of the analytical data from different sources and to establish a basis for the understanding of geochemical cycles and toxicological implications (*UNEP, 2004*).

Nowadays with the development of analytical methods and equipments we could find very low concentration such microgram per kilogram (ppb) levels and lower. These levels are sufficient to account for most of our body burden based on the existing standards.

Due to the extremely high toxicity and biological activity of PTSs as shown in chapter I, we should consider choosing very sensitive, selective and specific analytical techniques to limit the risks of exposure to PTSs.

This chapter presents all basic steps for PTSs analysis, the required equipment and conditions to apply it. We also consider the advantages and also disadvantages of analytical methods to find an analytical procedure which corresponds to our laboratory conditions.

3.2 Sampling, sample storing and handling

Sampling procedures, locations, equipment, and sample preservation as well as handling requirements are to be specified in a sampling plan. The procedures describe how the sampling operations are actually performed in the field should be specified.

Contamination during field collection can occur, e.g. PCBs in electrical equipment, or due to the ongoing use of OCPs. “Clean” techniques need to be adopted, such as the use of special clothing and disposable gloves for sampling, sealed shipping containers, and field blanks. Clear polyethylene bags, and polypropylene jars, are also appropriate for temporary storage but may not be suitable for long-term storage because of the possibility of migration of plasticizers (such as phthalates) into the tissue, especially for samples with high lipid contents.

The sample handling is very important if we should analyze a wide range of sample media (in our case). In this case, initial sample treatment may have to be carried out in separate laboratories.In

addition, to avoid the cross contamination from solvents, reagents, glassware and equipments we must follow strictly some criteria such as: using ultra trace level/pestigrade level solvent; glassware used for analysis must be washed with detergents and rinsed with suitable solvent before using; contacting with plastic materials should be limited; adsorbents should be solvent-washed; gases used for analysis should be purified (e.g. Nitrogen 99.9995%) (*US.EPA, 1996a, 1996b, 2000a*).

A basic requirement for analytical laboratories involved in the measurement of PTSs is the availability of freezer and refrigerator capacity for sample storage and archiving. Soil and sediment samples could be stored in low temperature (-10 ^{0}C) and in sun-light absence; biological samples such fish, adipose tissue, and milk should be stored in the fridge (-20 ^{0}C). De Boer and Smedes (1997) found no significant changes in the concentrations of PCBs and DDT compounds in fish tissue stored at -20 to -70 ^{0}C in the dark for up to 24 months. In case of necessary, biological samples should be stored at -70 ^{0}C to minimize any effects for long term storage (> 2 years) (*Kiriluk et al., 1996; de Boer and Smedes, 1997*).

Wells et al (1997) found that freeze-drying samples reduced recovery of PTSs may be due to tighter binding and occlusion of residues in the dry material. Volatilization losses might also occur during freeze-drying of volatile compounds such as HCB, and there is a greater potential for contamination in the lab (*Söderström et al., 2005*). Thus, in general, maintaining our biological samples in their original wet state and drying soil/sediment samples in ambient air temperature is regarded as the appropriate method for preparing samples for PTSs analysis.

Sample size depends on the availability of the medium and the detection threshold required. Two points should be considered: first is the possibility to do the sampling, especially for the biological samples such as breast milk, blood, adipose tissues; and the second is the representation of the sample. For example: to analysis of PCBs and OCls pesticides concentrations in blood serum or breast milk, normally the analytical procedure requires a volume about 50-100mL of blood/breast milk, it is not always possible. Thus, for blood serum, we often do sampling on a group of people, and then make the composite sample. Before beginning our analysis, we should to have as much as possible information about our samples – contamination potential.

3.3 Extraction techniques

Extraction is the first step to isolate PTSs from initial materials. Common extraction techniques for solid matrices include Soxhlet extraction, sonication extraction, supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and Pressurized liquid extraction - PLE (or accelerated- solvent extraction- ASE). Others, such as the shake method, are also in use, but they work well mainly for very porous matrices with fewer analytes to be extracted. Table 3.1 summarizes the most common techniques for solid matrices, and Table 3. 2 present their advantages and disadvantages.

Table 3.1: Extraction techniques used for solid environmental samples (*Avila, 1999*)

Technique	Overview	Method reference
Conventional soxhlet	Sample and desiccant mixture in glass or paper thimble is leached with warm (condensed) solvent for 16 – 24hrs. Solvent are e.g., Diethyl ether, DCM, hexane, acetone.	US.EPA., 1996d
Automated soxhlet (e.g., Soxtec)	Extraction thimble is immersed in boiling solvent (30–60 min) then raised for Soxhlet extraction. Solvent can also be evaporated.	US.EPA., 1994

Supercritical fluid extraction (SFE)	Sample (usually +desiccant) placed in high-pressure cartridge and carbon dioxide at 150–450 atm. at temp of 40–150 ^{0}C passed through. After depressurization, analytes are collected in solvent trap	US.EPA., 1996c US.EPA., 1998
Sonication-assisted extraction	Sample in open or closed vessel immersed in solvent and heated with ultrasonic radiation using ultrasonic bath or probe	US.EPA., 1996e
Microwave-assisted extraction (MAE)	Sample in open or closed vessel immersed in solvent and heated with microwave energy	US.EPA., 2000b
Pressurized liquid extraction (PLE)	Sample (usually +desiccant) placed in extraction cartridge and solvent (heated, pressurized) passed through then dispensed in extraction vial	US.EPA., 1996f

Table 3.2: Sample preparation for solid matrices-Advantages and disadvantages (*Avila, 1999*)

Technique	Advantages	Disadvantages
Soxhlet extraction	- Standard method - Filtration not required - Not matrix dependent - Unattended operation - Low cost	- Long extraction time, up to 28–48 hrs. - Large amount of sample (10–30 g) and solvent (300-500 ml) - Evaporation of extract is mandatory
Supercritical fluid extraction (SFE)	- Fast (30-60 min) - Carbon dioxide is nontoxic, nonflammable, environmentally-friendly - Selectivity can be archived by varying pressure, temperature, and modifier - Small amount of solvent (5-10 ml) - Filtration not required - No solvent exposure - Automated	- Limited sample size (< 10 g) - Matrix dependent - Modifier addition to improve efficiency - High cost
Sonication-assisted extraction	- Fast (30-60 min) - Not matrix dependent - Low cost	- Large amount of sample (10-30 g) and solvent (300-500 ml) - Labor intensive - Filtration required - Exposure to solvent vapor
Microwave-assisted extraction (MAE)	- Fast (20-30 min) - Small amount of solvent compared to soxhlet (30ml vs. 300-500 ml) - Full control of extraction parameters (time, power, temperature) - Stirring possible - Higher temperatures - No drying agents are needed	- Extracts must be filtered - Polar solvent needed - Everything gets extracted (clean-up needed) - Moderate cost
Pressurized liquid extraction (PLE or ASE)	- Fast (15 min) - Minimal solvent usage (15-40 ml) - No filtration needed - Automated (allow sequential extraction of up to 24 samples)	- High capital cost - Matrix dependent

	- Easy to use	

The newer extraction techniques such as SFE, MAE, and ASE are very attractive because they are a lot faster, use much smaller amounts of solvents, and are environmentally friendly techniques.

PLE or ASE technique (Fig. 3.1) is increasingly being used to replace soxhlet and column extraction methods (*Koester et al., 2003*). PLE uses much less solvent than soxhlet. However, it suffers the disadvantage of initial high cost and the need for a stable power supply to avoid premature instrument shutdown. Also, operation of an automated PLE requires regular scheduled maintenance by trained service personnel. A US EPA method (3545) using PLE for solid waste extraction is available (*US.EPA., 1995*). This method recommends acetone/hexane (1:1, v /v) or acetone/dichloromethane (DCM) for extraction of PTSs from solid waste, and these solvent systems appear to be the most commonly used (*Björklund et al., 2000*).

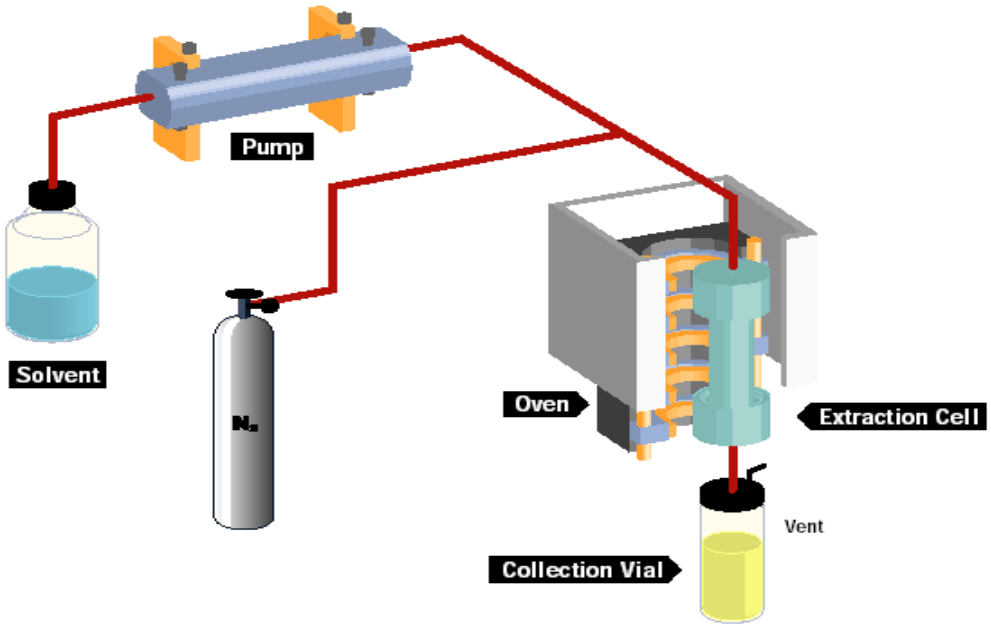

Figure 3.1: ASE Dionex ™ system scheme *(Ferrary, 2003)*

There has been a recent trend toward not using chlorinated (potentially toxic) solvents such as chloroform and DCM, due to concerns over exposure to lab personnel. Binary mixtures such as hexane/acetone (1:1) are regarded as equally effective for the extraction of PTSs from solid samples. In general, extraction with a polar:polar binary mixture has been found to be more efficient for recovering PTSs from fish tissues of low lipid content than an apolar solvent (*de Boer, 1988*).

The use of microwave (Fig 3.2), sonication, supercritical fluids (Fig. 3.3), or elevated temperatures and pressure (as in PLE) increases the rates of diffusion and desorption and thus speeds up extraction (*Koester et at., 2003, Avila, 1999*). Pressurized hot water extraction has even been used to extract PTSs from sediment and soil. Under pressure, the dielectric constant of the water can be manipulated to facilitate the extraction of nonpolar analytes (*Ramos et al., 2002*).

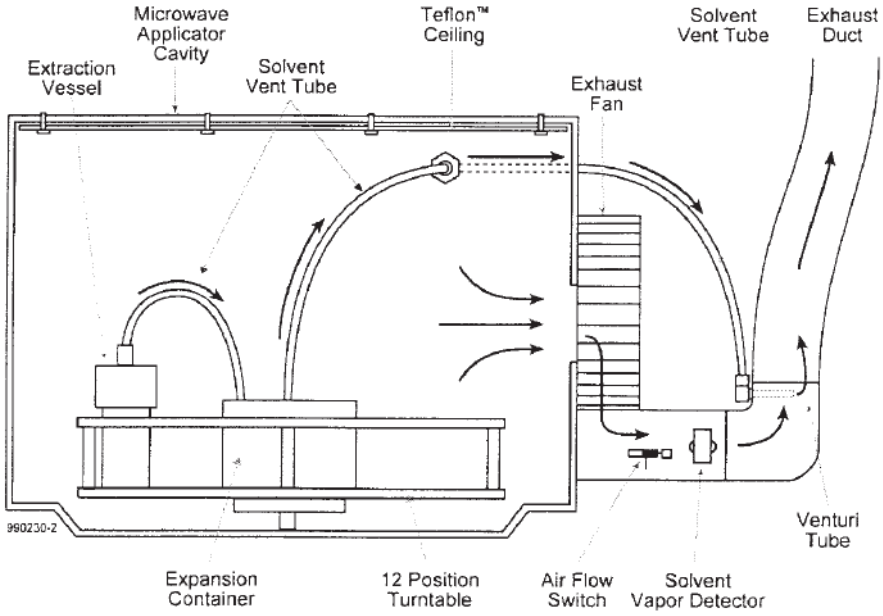

Figure 3.2: Schematic diagram of closed-vessel MAE system (*Avila, 1999*).

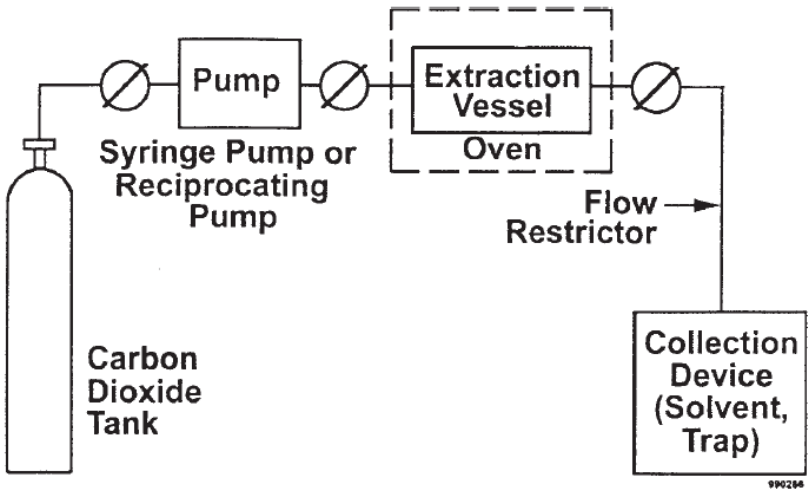

Figure 3.3: Basic components of the SFE system (*Avila, 1999*).

Sonication, microwave-assisted extraction (MAE) and supercritical fluid (SFE) extraction have all been successfully employed to recover PCBs and OCPs from solid samples. From the table 3.2 we can see that, Soxhlet is regarded as more reliable than sonication, SFE, or shake flask-type methods and equivalent to (but not as rapid as) MAE and PLE for a wide range of environmental matrices. Based on advantages of soxhlet presented above and real conditions at IER lab and CELA lab, we decided to use soxhlet technique for soil/sediment and biological samples extractions.

3.3.1 Soil and sediment extraction

For extractions of soil/sediment samples, Soxhlet (Fig. 3.4) is widely accepted as a robust liquid–solid extraction technique. Typical solvents in Soxhlet extraction of PTSs from soil and sediments have been toluene, hexane and hexane/acetone mixtures. The extraction time has varied in the range 12–24 h. Soxhlet extraction has been considered as a reference technique in many works (usually used to compare the effectiveness with other methods). One evident advantage of this technique is its very simple and easy to use. In addition with regard to the primary investment, it is relatively cheap.

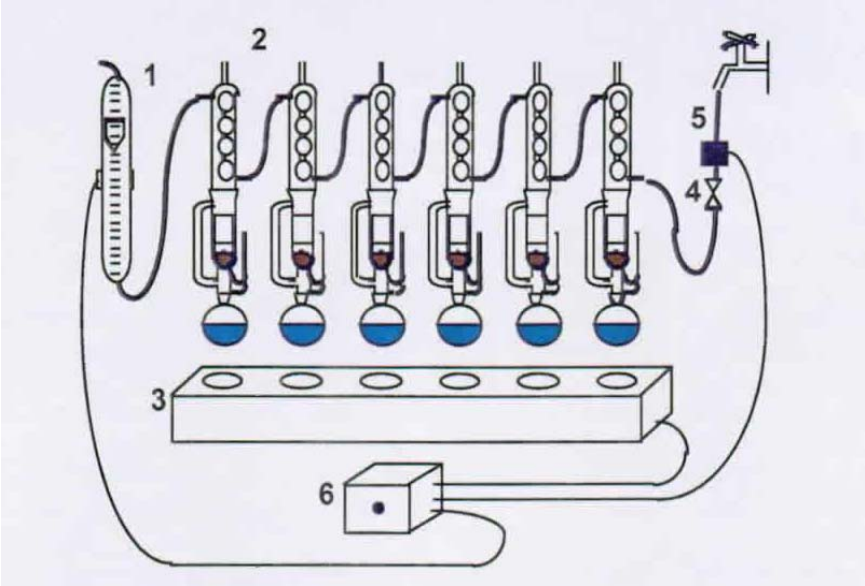

Figure 3.4: Soxhlet extraction system scheme

1. Flow meter/security system
2. Soxhlet system
3. Heating plate
4. Valve
5. Electric valve
6. Power source

3.3.2 Biological sample extraction

Biological samples such fish, bivalve and human adipose tissue should grounded with anhydrous sodium sulfate to powder form before extraction. Extraction techniques for such samples are similar to the soils and sediments samples, but the time of extraction and the ratio of solvent mixtures used are different. The common solvents used for extraction are dichloromethane (DCM), n-hexane : acetone (1:1, v/v).

3.4 Sample purification

Purification of the extracts is carried out to remove co-extracted compounds which would prevent further concentration and interfere in the final analysis. There are two aspects to this phase of PTSs analysis. The analytes must be separated from nonvolatile materials which affect the performance of GC columns, such as pigments, inorganic sulfur, and triglycerides. Also, there is a need to separate the OCPs, PCBs and PBDEs from each other as much as possible prior to GC analysis in order to limit coelution problems. These purification steps are also useful, and

sometimes essential, for the application of bioanalytical methods to PCBs/OCPs and dioxin-like activity.

3.4.1 Adsorption clean-up column

In this technique, chromatographic procedures are most often used. Isolation steps can be relatively straightforward for low lipid samples such as air, soils, sediments and vegetation. Generally small Silica gel or Florisil columns (either prepared in the lab (Fig. 3.5) or pre-purchased) will suffice. The purpose of this step is to remove co-extractive pigments and to separate non-polar PCB (plus p,p'-DDE) from more polar PTSs (HCH, most chlordanes, dieldrin/endrin). This is achieved by applying the extract in a small volume of non-polar solvent and fractionating by eluting with hexane followed by one or two other elutions of increasing polarity. Alumina is not recommended because of possible dehydrochlorination of some POPs, e.g. 4, 4'-DDT (*UNEP, 2004*).

Figure 3.5: Florisil clean-up column system

3.4.2 Sulfur removal

Sulfur is coextracted with PTSs and presents a particular problem for GC–ECD analysis of sediment extracts because of its strong response in this detector. Sulfur can be removed by GPC but can also be removed using activated copper turnings (washed with concentrated HCl and held in an apolar solvent) or using mercury. The latter method removes sulfur more efficiently but is not recommended due to potential for contamination of the lab and lab effluent. Shaking with tetrabutylammonium sulfide has also been used to remove sulfur (*Jensen et al., 1977*).

3.4.3 Gel permeation chromatography columns

For high lipid samples, such as fish tissue and marine mammal blubber, a lipid removal step must be included. This can be achieved using size exclusion or gel permeation chromatography (GPC) either in automated systems, using high pressure liquid chromatography (HPLC) columns or by gravity flow columns. Basically, GPC (Fig. 3.6) is a technique which separates components of a

sample based on their molecular size. Since lipids are very large molecules compared to the target compounds in these methods, they are effectively removed from the extract prior to analysis. This improves method performance and extends GC column life, leading to more efficient analyses. The advantage of GPC is that it is non-destructive while the disadvantage is a requirement for large volumes of solvent (low pressure or gravity systems) or expensive columns (HPLC). Lipid removal using sulfuric acid washing or sulfuric acid – silica columns is also effective but does result in loss of some analytes such as dieldrin and endosulfan (*UNEP, 2004; Minh et al., 2004*).

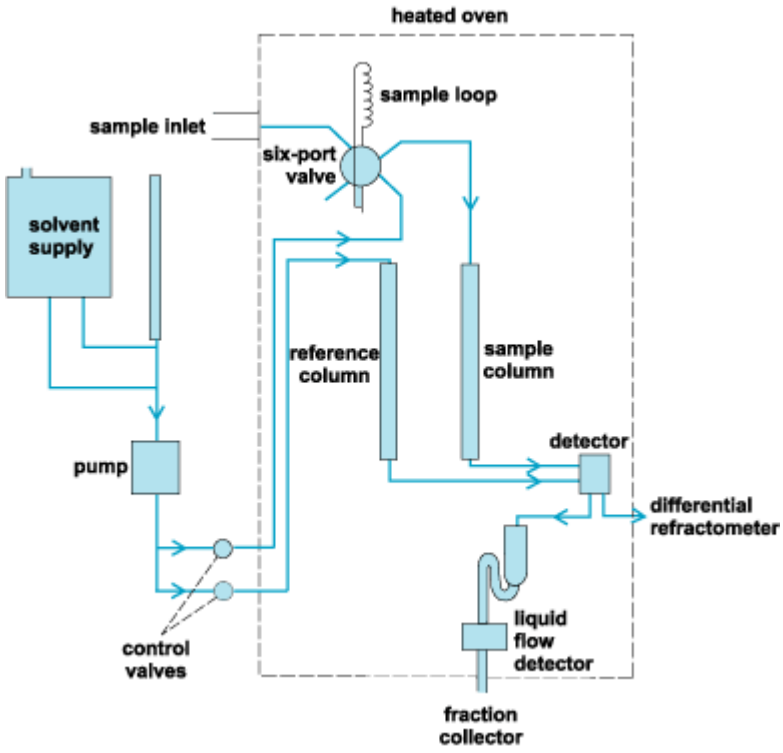

Figure 3.6: Gel permeation chromatography system scheme

3.5 Concentration for final analysis

Before analysis, the extract containing interested compounds should be concentrated to sufficiently small volumes (as low as a few microlitres, µL – usually 50 µL). By this way the concentration of PTSs in our extract is multiplied to 100 – 1.000 times and reached the detection limit of analytical equipment used for analyses (ppb or ppt.). Large volume of solvent is concentrated in a rotary evaporator at a temperature according to the solvent type used (e.g. 40^0C for n-hexane). The smaller volume of solvent is concentrated by a stream of purified nitrogen at room temperature to volume lower than 40 µL. Then extract will be refilled to 50 µL by a 50 µL syringe.

3.6 Identification and quantification of PTSs by GC/MS

Following fractionation on silica or Florisil, final extracts are prepared in GC vials for analysis. Careful evaporation is required at this step, and only high-purity compressed gas (usually nitrogen 99.9995%) should be used. Addition of an internal standard to check response factor of ECD/MS detector is recommended at this stage. The final purified extract is analyzed by GC/ECD or GC/MS equipment: GC – gas chromatography is being used for homologue and

isomer separation; and ECD (electron capture detector) and MS (mass spectrometry) for the selective detection, identification and quantification of PTSs. Gas chromatography (GC) and ECD or MS make an effective combination for chemical analysis.

3.6.1 Gas chromatography equipment

GC analysis is a common analytical technique. GC analysis separates the components in a sample and provides a representative spectral output. The sample is injected into the injection port of the GC device. The GC instrument (Fig. 3.7) vaporizes the sample and then separates and analyzes the various components. Each component ideally produces a specific spectral peak that may be recorded. The time elapsed between injection and elution is called the "retention time - t_R." The retention time can help to differentiate between interested compounds. The size of the peaks is proportional to the quantity of the corresponding substances in the specimen analyzed.

Cochran and Frame (1999) noted that separation requires attention to (1) proper injection to minimize analyte band-broadening; (2) choice of carrier gas; (3) optimized carrier gas velocity; (4) GC oven programming; (5) column dimensions such as length, inside diameter, film thickness, number of plates; and (6) the type of column stationary phase.

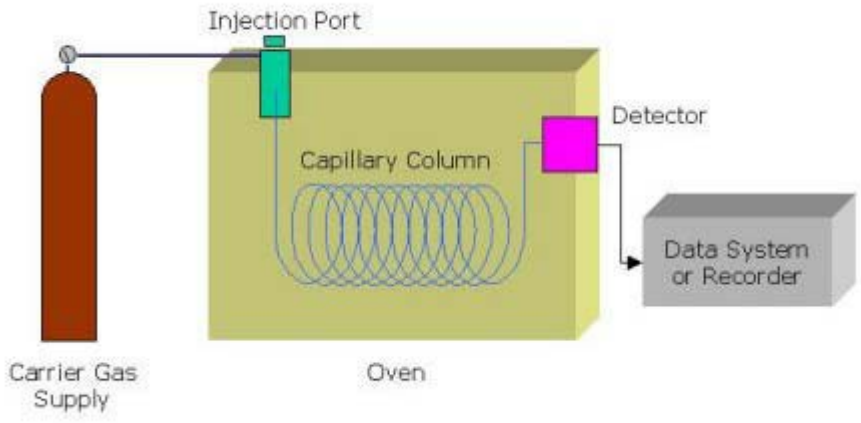

Figure 3.7: Diagram of GC system

Resolution in GC analysis

The plate model supposes that the chromatographic column contains a large number of separate layers, called theoretical plates. Separate equilibrations of the sample between the stationary and mobile phase occur in these "plates". The analyte moves down the column by transfer of equilibrated mobile phase from one plate to the next. It is important to remember that the plates do not really exist; they are a figment of the imagination that helps us understand the processes at work in the column. For measuring column efficiency we can use the number of theoretical plates (N) and Height Equivalent to a Theoretical Plate (HETP):

$$\mathrm{HETP} = L/N$$

Where L - length of the column; N – number of theoretical plates

The number of theoretical plates that a real column possesses can be found by examining a chromatographic peak after elution:

$$N = \frac{5.55 t_R^2}{w_{1/2}^2}$$

Where $w_{1/2}$ is the peak width at half-height and tR is retention time

As can be seen from this equation, columns behave as if they have different numbers of plates for different solutes in a mixture.

The resolution of two species, A and B, is defined as

$$R = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$$

Where: (t_R) is the retention time ; W is the peak width.

$W = 4\sigma$

$W_{1/2} = 2.354\sigma$ => $W = 4\times(W_{1/2}/2.354)$

Baseline resolution is achieved when $R = 1.5$

It is useful to relate the resolution to the number of plates in the column, the selectivity factor and the retention factors of the two solutes.

GC injection ports

Optimization of injection conditions is critical to proper GC analysis (*Lang, 1992*). In the analysis of POPs and OCPs, problems often occur with nonvolatile coextractives such as triglycerides and pigments that, despite various isolation procedures, are still present in final extracts. Most GC applications for PCB and OCP analysis have employed split/splitless injection systems, although on-column injection has also been used. Although on-column injection avoids artifacts associated with heated split/splitless systems (i.e., degradation of labile compounds), it requires highly clean extracts to avoid matrix effects (*Lang, 1992*). Retention gaps consisting of an empty capillary column have been used to reduce these effects and permit larger on-column injection volumes. Pressure programming allows improved control over carrier gas flow, resulting in improved separation of PCB and OCPs.

GC columns

Fused silica open tubular capillary columns, generally coated with nonpolar or medium-polarity chemically bonded liquid phases are almost universally used for GC separation of PCBs and OCPs (*Lang, 1992*). The use of capillary columns revolutionized PCB analysis, allowing identification of the individual congeners. This improved the comparability of the analytical data from different sources and helped to establish a basis for the understanding of geochemical cycles and toxicological implications. The basic technology for separation of PCB congeners described by Mullins et al. (1984) has not changed greatly over the years. Improved routine separations of PCBs have been achieved using 60 m×0.25 mm i.d. columns with hydrogen carrier gas. PCBs within a homolog group elute according to their number of ortho chlorines: 4<3<2<1<0. Coelution of congeners remains a problem for routine analysis by GC–ECD. However, several modified polydimethylsiloxane phases with n-octyl or n-octyldecyl substituents, e.g., DBXLB (J&W Scientific, Folsom, CA, USA) and HT 8 (SGE Inc., Austin, TX, USA) can resolve all but four pairs of significant congeners and five pairs of minor congeners (*Frame, 1999*) using mass spectrometry detection. The coelution of PCB congeners on the more commonly used 30 m DB-5

columns is outlined in Table 3.3. Coelutions are thus important even for routinely monitored congeners such as CB 28, 31, 105 and 153. However, separation of these congeners can be routinely achieved on 60 m 5% phenylmethyl silicone phase capillary columns using H_2 carrier gas and on n-octyl phases as well (*Frame, 1999*). Alternatively, confirmation of peak identity in a subset of samples, using a second column of different polarity, can be done instead.

Table 3.3: Significant PCB congener co-elution on 5% phenyl phases (*Cochran and Frame, 1999*)

Classification[a]	Coeluting PCBs	Number of chlorines
A	4, 10	1, 2
A	9, 7	2, 2
A	12, 13	2, 2
A	17, 15	2, 3
A	27, 24	3, 3
A	32, 16	3, 3
A	28, 31	3, 3
A	33, 20, 53	3, 3, 4
A	43, 49	4, 4
A	47, 75, 48	4, 4, 4
A	44, 59	4, 4
A	37, 42	4, 4
A	71, 41, 64	4, 4, 4
A	66, 95	4, 5
A	56, 60	4, 4
A	84, 89, 101, 90	5, 5, 5, 5
A	117, 87, 115	5, 5, 5
A	77, 110	4, 5
A	135, 144, 124	6, 6, 5
A	147, 109	6, 5
A	123, 139, 149, 118	5, 5, 6, 5
A	114, 133	5, 6
A	131, 122	6, 5
A	153, 132, 105	6, 6, 5
A	176, 130	7, 6
A	164, 163, 138	6, 6, 6
A	158, 129	6, 6
A	175, 166	7, 6
A	173, 157, 201	7, 6, 8
A	170, 190	7, 7
A	198, 199	8, 8
A	203, 196	8, 8

[a] Capital A indicates a major Aroclor congener

Coelution of major OCPs with each other or with PCBs is also a problem in GC–ECD analysis, despite preseparation by adsorption chromatography. For example, p,p′-DDD can coelute with cis-nonachlor, CB99 with a trans-nonachlor isomer (*Muir et al., 1988*), p,p′-DDE with dieldrin and CB85, and toxaphene congener Parlar 50 with CB128. Recently, the coelution of CB180 and brominated diphenyl ether congener 47 has also been noted (*Alaee et al., 2001*).

3.6.2 Electron capture detector

Since the 1960s, PTSs have been determined by GC with electron capture detection (ECD), initially using packed columns. Capillary GC–ECD began to be routinely applied by the early 1980s. Use of GC–ECD is recommended for routine analysis of OCPs, except for toxaphene, as well as for ortho-PCBs but not for non-ortho-PCBs (*Reiner et al., 2006*). This instrumentation is widely available at relatively low cost from at least four instrument manufacturers. A substantial knowledge base exists on the use of this 40-year-old technology. GC–ECD is capable of determining PTSs at low ng/g levels or higher in environmental matrices. Although at one time tritium-based ECDs were once available, the ^{63}Ni detector is now universally used. ECDs are normally operated with N_2 gas, which combines with the flow from the GC column (N_2, He or H_2 carrier gas). Gases used for GC–ECD must be ultrapure to protect both the GC column (which can be oxidized by trace oxygen or siloxanes hydrolyzed by trace water) and the ECD itself. Recent refinements in ECD technology include the use of microcells which have greater linear range than older detector cells (*Klee et al., 1999*) and can provide greater sensitivity. ECD suffers from the potential for false positives due to interferences such as those from sulfur, phthalate esters, and negative peaks generated by hydrocarbons as well as high potential for misidentification of some PTSs due to coeluting peaks.

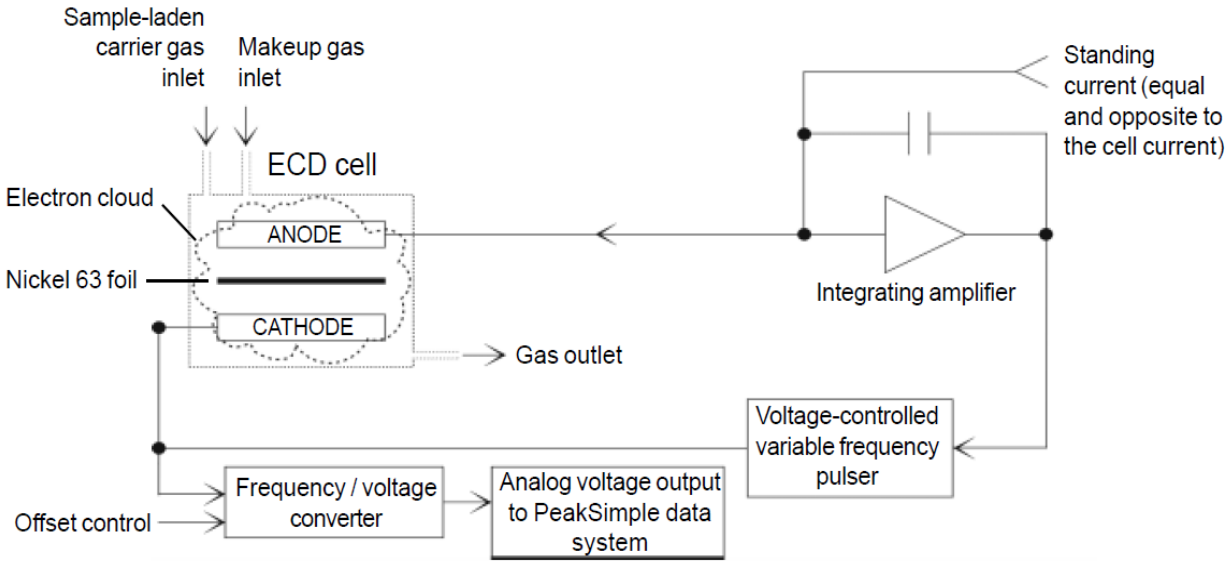

Figure 3.8: ECD detector operational diagram

3.6.3 Mass spectrometry

Capillary GC coupled with mass spectrometry (GC–MS) revolutionized environmental organic analysis in the 1980s (*Richardson, 2001*), particularly with the advent of bench-top instruments. Current GC–low-resolution (quadrupole) mass spectrometry (LRMS) instrumentation (Fig. 3.9) is capable of determining most PCB/OCPs/PBDEs at low pg concentrations using electron ionization (EI) in selected ion mode. Electron capture negative ion ionization (ECNI) is capable of detection of low femtogram amounts of highly chlorinated OCPs such as chlordane and toxaphene congeners.

Mass spectrometers use the difference in mass-to-charge ratio (m/z) of ionized atoms or molecules to separate them from each other. Mass spectrometry is therefore useful for quantification of atoms or molecules and also for determining chemical and structural

information about molecules. Molecules have distinctive fragmentation patterns that provide structural information to identify structural components.

The general operation of a mass spectrometer is:

- Ionization
- separate the ions in space or time based on their mass-to-charge ratio (m/z)
- measure the quantity of ions of each mass-to-charge ratio

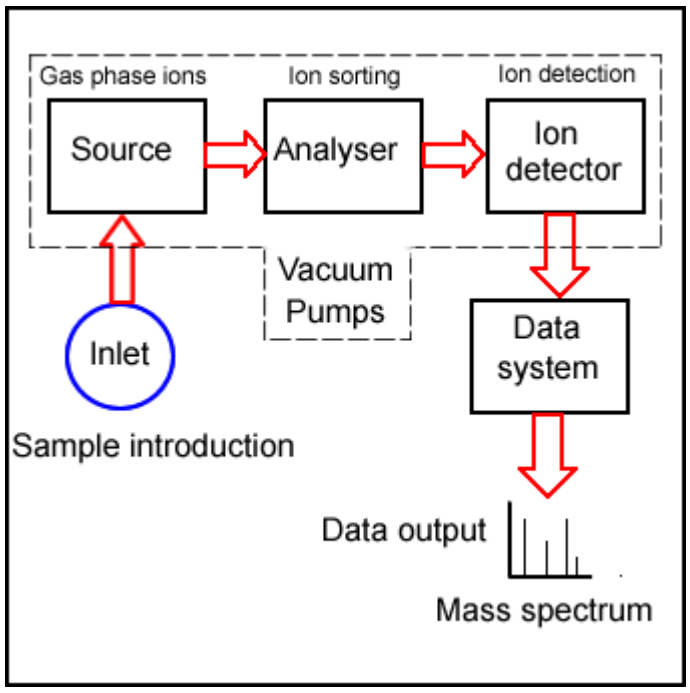

Figure 3.9: Mass spectrometer block diagram

3.6.3.1 Ionization types for GC/MS

a/ Electron impact (EI)

Electron impact ionization is the classical and the most common ionization technique in mass spectrometry. It uses a heated filament to produce electrons. The filament is usually made of rhenium or tungsten. Once the electrons are produced they are accelerated through a potential difference of around 70V this gives electrons with 70eV of energy. The sample to be analysed is then introduced into the ion source.

In the ion source (10^{-7} - 10^{-5} mbar, 200^0C – 250^0C), the gaseous sample is bombarded with 70 eV electrons. Because the pressure is kept that low, ion-molecule reactions do not occur, e.g. a $[M^+H]^+$ signal due to proton transfer is not observed.

$$M + e\ (70eV) \text{ -----> } M^+ + 2e$$

The application of EI is restricted to thermally stable samples with low molecular masses (< ca. 2000 Da). Since the ion source temperature and the bombarding electron's energy are kept constant, the number and amount of fragments are constant for every mass spectrometer, too. The number and amount of ionic fragments ('daughter ions') and the amount of the M^+ are characteristic for each substance. Therefore, most mass spectra libraries are only available for EI

- ionization. There is an 8000 EI mass spectra library available on-line. (Nist Chemistry WebBook)

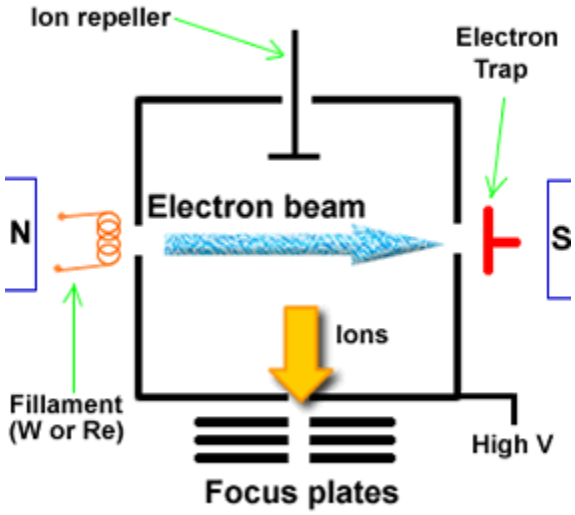

Figure 3.10: Diagram of EI system

EI characteristics in summary:

- Can be used for GC/MS systems and direct inlet techniques.
- Produces "classical" compound spectra that are library searchable and/or interpretable.
- Useful for positive compound identification and/or structure elucidation.
- EI spectra are relatively easy to obtain.
- Comparatively rugged and sensitive ionization technique.
- Can be employed for analyzing air- and moisture-sensitive compounds.
- Analytes have to be vaporized - problems with thermal degradation.

b/ Chemical ionization (CI)

Chemical ionisation uses an ionisation source that is filled with a reagent gas e.g. methane. This gas is at a pressure of about 1 mbar. “Energetic electrons (100-200eV) convert CH_4 to a variety of reactive products”:

There are two types of chemical ionization: positive and negative (PCI and NCI). Chemical Ionization is an ionization technique similar to the classical EI but the knowledge and results of ion-molecule reactions are exploited. In CI a similar ion source is used like in EI. One notable exception: The CI ion source is almost closed, i. e. much smaller holes as the EI source, leading to high pressures (ca. 10^{-3} to 1 mbar!).

The reagent ions are produced by introducing a large excess of methane (relative to the analyte) into an electron impact (EI) ion source. Electron collisions produce CH_4^+ and CH_3^+ which further react with methane to form CH_5^+ and $C_2H_5^+$:

$$CH_4^{+\bullet} + CH_4 \text{ --> } CH_5^+ + CH_3^{\bullet} \text{ (reagent ion)}$$

$$CH_3^+ + CH_4 \text{ --> } C_2H_5^+ + H_2$$

PCI uses a reagent ion to react with the analyte molecules to form ions by either a proton or hydride transfer:

$$MH + C_2H_5^+ \text{ --> } MH_2^+ + C_2H_4$$

$$MH + C_2H_5^+ \text{ --> } M^+ + C_2H_6$$

Many important compounds of environmental or biological interest can produce negative ions under the right conditions. Negative ions can be produced by a number of processes. The electron energy is very low, and the specific energy required for electron capture depends on the molecular structure of the analyte.

Benefits of NCI are efficient ionization, higher sensitivity and less fragmentation than positive-ion EI and CI. There is also a greater selectivity for certain environmentally or biologically important compounds. The limitations are that not all volatile compounds produce negative ions and a poor reproducibility of the measurements.

$$e^- + CH4 \text{ --> } e^-_{th}$$

$$M+ e_{th} \text{ --> } M^-$$

CI characteristics in summary:

- Provides molecular weight information.
- Quantification is almost impossible without internal standards.
- Monomeric or covalently-bound dimeric constitutes show no differences.
- CI can be used as ionization methods in GC/MS.

Common CI Reagent Gases:

Methane:

- good for most organic compounds
- usually produces $[M+H]^+$, $[M+CH_3]^+$ adducts
- adducts are not always abundant
- extensive fragmentation

Isobutane:

- usually produces $[M+H]^+$, $[M+C_4H_9]^+$ adducts and some fragmentation
- adducts are relatively more abundant than for methane CI
- not as universal as methane

Ammonia:

- fragmentation virtually absent
- polar compounds produce [M+NH4]+ adducts
- basic compounds produce [M+H]+ adducts
- non-polar and non-basic compounds are not ionized

3.6.3.2 Resolution in MS analysis

Several different definitions of resolution are used in mass spectrometry. It is useful to understand the distinctions between the different definitions to understand the characteristics of different mass spectrometers:

Unit resolution: means that you can separate each mass from the next integer mass. That is, you can distinguish mass 50 from mass 51, and you can distinguish mass 1000 from mass 1001. This definition is commonly used when discussing resolution on quadrupole and ion trap mass spectrometers.

Magnetic sector mass spectrometers define resolving power as:

$$R = m/\Delta m$$

Where m is the ion mass and Δm is the difference in mass between two resolvable peaks in a mass spectrum. E.g., a mass spectrometer with a resolution of 1000 can resolve an ion with a m/z of 100.0 from an ion with an m/z of 100.1.

In magnetic sector mass spectrometers, peaks are usually defined to be separated down to a 10% valley, that is, a point that is 1/10 of the height of the higher of the two peaks. If you only have one peak, then you can estimate the resolving power by using the peak width at the 5% level divided by the mass of the observed peak. The resolving power value as defined above is constant across the mass range. The 10% valley definition is usually considered adequate for resolving small isotope peaks

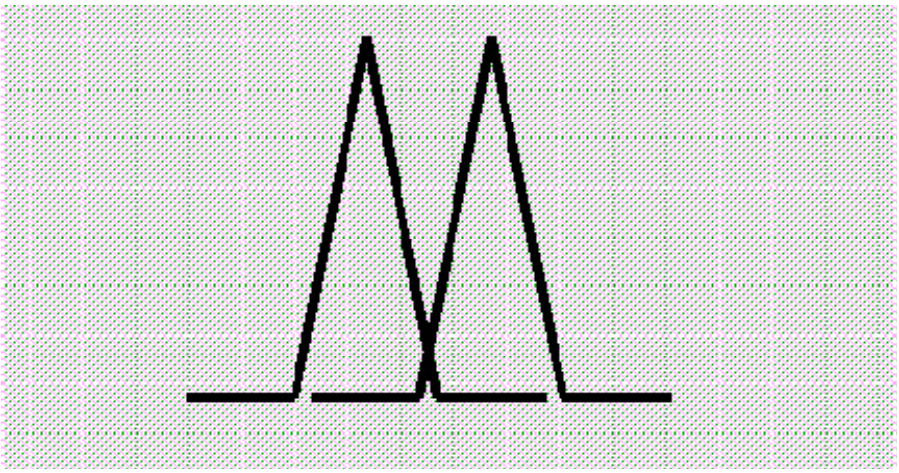

Figure 3.11: Two peaks resolved to 10% valley

Consider the difference between the definition of unit resolution and resolving power as defined in a magnetic sector mass spectrometer. If we have 5000 resolving power on a magnetic sector mass spectrometer, we can separate m/z 50.000 from m/z 50.010, or separate m/z 100.000 from m/z 100.020, or separate m/z 1000.000 from m/z 1000.200 (all down to a 10% valley between the two peaks).

3.6.3.3 Mass analyzers

The mass analyser is the next part of the instrument. This is used to sort ions in the source according to their mass-to-charge ratios and focus them on the detector. Various types of mass

analyser are magnetic sectors, quadrupole mass filters, quadrupole ion traps, Fourier transform ion cyclotron resonance spectrometers, and time-of-flight mass analyzers.

All commonly used mass analyzers use electric and magnetic fields to apply a force on charged particles (ions). From Newton's second law, it is apparent that the force causes an acceleration that is mass dependent, and the Lorentz force law tells us that the applied force is also dependent on the ionic charge. Therefore, it should be understood that mass spectrometers separate ions according to their mass-to-charge ratio (m/z) rather than by their mass alone.

a/ Quadrupole mass spectrometers (low – resolution MS)

The quadrupole mass analyzer is a "mass filter". Combined DC and RF potentials on the quadrupole rods can be set to pass only a selected mass-to-charge ratio. All other ions do not have a stable trajectory through the quadrupole mass analyzer and will collide with the quadrupole rods, never reaching the detector.

The rods shown in the diagram (Fig. 3.12) are coupled together in diagonally opposite pairs. The rods should be machined to a hyperbolic shape but due to the cost of engineering them like this round ones are used as they will still give good performance at lower manufacturing cost. One pair of rods has a positive DC potential applied and on the other pair a negative potential is applied to give mass separation. A 180^0 out of phase radio frequency AC voltage is superimposed on the DC. The peak of this voltage is greater than the DC voltage so the 'positive pairs' are sometimes negative and vice versa. Ions with roughly 20 eV of energy are introduced and follow a spiral path through the analyser due to the oscillating field. They travel between the rods along the z-axis.

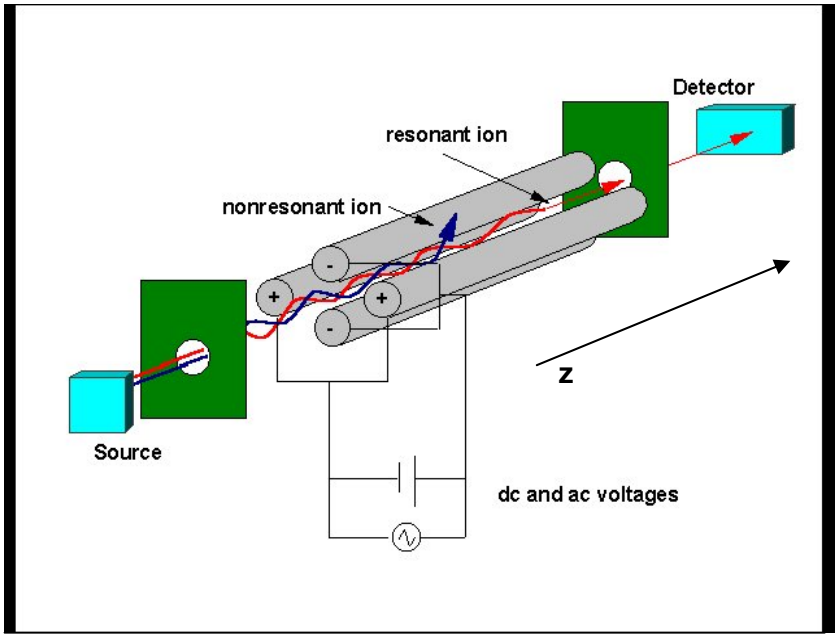

Figure 3.12: Schematic of a quadrupole mass filter

Changing the slope of the scan line will change the resolution. Good resolution also depends on the quality of the machining for the quadrupole rods. Quadrupole rods can have other functions besides their use as a mass filter. An RF-only quadrupole will act as an ion guide for ions within a broad mass range. For example, the collision region of a triple quadrupole mass spectrometer

uses an RF ion guide. A DC-only quadrupole is used as a lens element in some ion optic designs (such as JEOL's magnetic sector mass spectrometers).

Benefits

- Classical mass spectra
- Good reproducibility
- Relatively small and low-cost systems
- Low-energy collision-induced dissociation (CID) MS/MS spectra in triple quadrupole and hybrid mass spectrometers have efficient conversion of precursor to product

Limitations

- Limited resolution
- Peak heights variable as a function of mass (mass discrimination). Peak height vs. mass response must be 'tuned'.
- Not well suited for pulsed ionization methods
- Low-energy collision-induced dissociation (CID) MS/MS spectra in triple quadrupole and hybrid mass spectrometers depend strongly on energy, collision gas, pressure, and other factors.

Applications

- Majority of benchtop GC/MS and LC/MS systems
- Triple quadrupole MS/MS systems
- Sector / quadrupole hybrid MS/MS systems

b/ Magnetic sector mass spectrometers (high resolution MS)

The analogy between scanning mass spectrometry and scanning optical spectroscopy is most apparent for magnetic sector mass spectrometers. In a magnetic deflection mass spectrometer, ions leaving the ion source are accelerated to a high velocity. The ions then pass through a magnetic sector in which the magnetic field is applied in a direction perpendicular to the direction of ion motion. From physics, we know that when acceleration is applied perpendicular to the direction of motion of an object, the object's velocity remains constant, but the object travels in a circular path. Therefore, the magnetic sector follows an arc; the radius and angle of the arc vary with different ion optical designs.

A magnetic sector alone will separate ions according to their mass-to-charge ratio. However, the resolution will be limited by the fact that ions leaving the ion source do not all have exactly the same energy and therefore do not have exactly the same velocity. This is analogous to the chromatic aberration in optical spectroscopy. To achieve better resolution, it is necessary to add an electric sector that focuses ions according to their kinetic energy. Like the magnetic sector, the electric sector applies a force perpendicular to the direction of ion motion, and therefore has the form of an arc.

A schematic representation of the magnetic sector mass spectrometer is shown below.

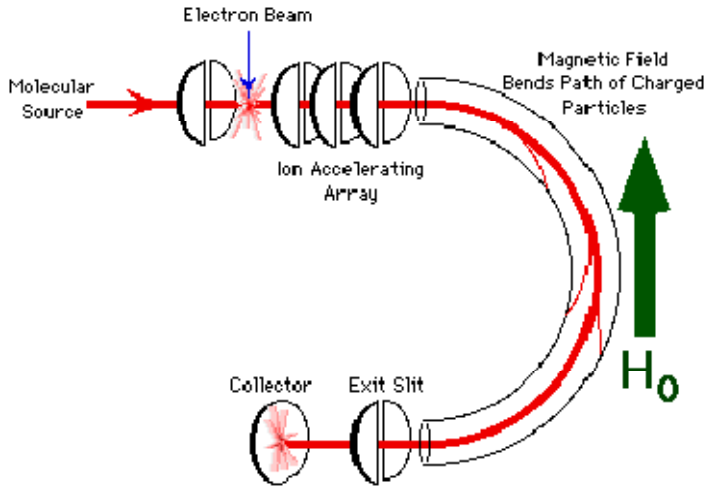

Figure 3.13: Schematic of magnetic sector mass spectrometer (IVV, 2000)

The simplest mode of operation of a magnetic sector mass spectrometer keeps the accelerating potential and the electric sector at a constant potential and varies the magnetic field. Ions that have a constant kinetic energy, but different mass-to-charge ratio are brought into focus at the detector slit (called the 'collector slit") at different magnetic field strengths.

As mentioned previously, the electric sector is usually held constant at a value which passes only ions having the specific kinetic energy. Therefore the parameter that is most commonly varied is B, the magnetic field strength. The magnetic field is usually scanned exponentially or linearly to obtain the mass spectrum. A magnetic field scan can be used to cover a wide range of mass-to-charge ratios with a sensitivity that is essentially independent of the mass-to-charge ratio.

An alternative is to hold B constant and scan V. The electric sector potential tracks the accelerating voltage. This has the advantage that the electric field is not subject to hysteresis, so the relationship between mass-to-charge ratio and accelerating voltage is a simple linear relationship. The disadvantage of an accelerating voltage (electric field) scan is that the sensitivity is roughly proportional to the mass-to-charge ratio.

The maximum ion transmission and sensitivity occur at the maximum working accelerating voltage for a given magnetic sector mass spectrometer. The effective mass range of the mass spectrometer can be increased by decreasing the accelerating voltage, with a sensitivity that is roughly proportional to the accelerating voltage.

Focal-plane (array) detectors can detect a range of masses simultaneously. This provides a multichannel advantage that can improve the sensitivity for magnetic sectors, and detection limits can be improved if the analysis is limited by the analyte ion current instead of the chemical background level. This is the case for experiments such as MS/MS, electrospray ionization, and field desorption. Array detectors can be used with pulsed ionization methods, but the array detectors for commercial magnetic sector mass spectrometers can only detect a portion of the entire mass range at any given instant.

The resolving power of a magnetic sector mass spectrometer is determined by the slit widths. Higher resolution is obtained by decreasing the slit widths, and thereby decreasing the number of ions that reach the detector.

Benefits

- Double focusing magnetic sector mass analyzers are the "classical" model against which other mass analyzers are compared.
- Classical mass spectra
- Very high reproducibility
- Best quantitative performance of all mass spectrometer analyzers
- High resolution
- High sensitivity
- High dynamic range
- Linked scan MS/MS does not require another analyzer
- High-energy CID MS/MS spectra are very reproducible

Limitations

- Not well-suited for pulsed ionization methods (e.g. MALDI)
- Usually larger and higher cost than other mass analyzers
- Linked scan MS/MS gives either limited precursor selectivity with unit product-ion resolution, or unit precursor selection with poor product-ion resolution

Applications

- All organic MS analysis methods
- Accurate mass measurements
- Quantification
- Isotope ratio measurements

In general, GC–ion trap MS (ITMS) in MS/MS mode offers an equivalent sensitivity to quadrupole ITMS in selected ion mode and improved specificity by examining product ions of major mass fragments. ITMS has been successfully applied to detect mono-ortho-PCBs at subpicogram levels in a range of environmental samples (*Leonards et al., 1996*) and to determine pg levels of toxaphene congeners (*Chan et al., 1998*). In addition, both quadrupole and ion trap LRMS can suffer from false positives due to unit mass resolution. Thus experienced analysts are needed to interpret results using confirmatory information such as full-scan analyses, fragmentation patterns and ion ratios. GC coupled to HRMS provides much higher specificity for individual PCB/OCPs/PBDEs due to its ability to providemillimass resolution and can also be used for the determination of all ortho-substituted PCBs (e.g., EPA Method 1668-US.EPA, 1999), PBDEs and OCPs too, and provides a very high level of confidence in the results compared to GC–ECD and LRMS. The use of GC–MS allows for the application of isotopedilution techniques and these have increasingly come into routine use for PCB/OCPs/PBDEs analysis in environmental samples due to the sensitivities of the latest generation of quadrupole and ion-trap MS systems (*Chu et al., 2003; Carlson and Hites, 2005*). A wide range of PCBs, PBDEs and OCPs are available as ^{13}C-labeled compounds from several chemical supply companies, which, when added at the extraction step, increase precision and reproducibility for the native analytes.

3.6.4 Retention time in PTSs analysis

Retention time is used as a parameter for PTSs identification (for identification with MS, we should use both data: retention time and MS data). Retention time for various PTSs compounds show definite pattern related to molecular structure. Individual PTSs compounds can be identified by retention time matching with known standards and nowadays almost PTSs compounds pure standards could be found in the market.

Retention time depend on the GC capillary column used for the analysis and also on the operating conditions of the analytical instruments (GC/ECD and GC/MS).

3.6.5 GC column temperature program and injection mode in PTSs analysis

Generally we should use the temperature program for GC column to enhance its separation efficiency. Temperature programming is an essential feature of all GC column ovens and is necessary to handle a sufficiently wide molecular and polarity range of samples. Linear programming is the most common although other functions of time are often available. The temperature program will influence on the analyzing time that normally takes from 20 to 100 min depending on the types of columns used. GC ovens usually require an operating range from about 5°C to about 400°C although the majority of GC analyses are carried out between temperatures of 75°C and 200°C.

Initial column temperatures are primarily determined by the boiling-point (b.p.) of the solvent and are generally about 20-30^{o}C below the solvent b.p. to make use of the solvent-effect: e.g. for isooctane with the b.p of 99.2^{o}C, the column initial temperature is set at 80^{o}C. After injection, the columns are kept for a interval (1-2 min) at the initial temperature, then programmed at 20 – 40^{o}C/min to an intermediate temperature defined by the most volatile components of interest. The rest of PTSs compounds are then eluted by gradual increasing of column temperature. Depend on the methods, columns and analytical equipments used we could define the final temperature of the elution, and then we should make the tests to find the optimum temperature program for GC column to have the best of peak resolution with reasonable time. Generally the final elution temperature not exceed the temperature limit of used column (always keep a interval of 10-20^{o}C below the temperature limit to safe the column). The temperature rate is typically 4-10^{o}C/min (for short column, 15-30m) and 2-4^{o}C (for long column, 50-60m), resulting in analysis times of 20-25min and 40-80min, respectively.

PTSs compounds are eluted out of the GC column by a carrier gas. Typical gas for GC/MS equipment is helium (He). The hydrogen can use as carrier gas with a shorter time of analysis, but the use of hydrogen is very limited due to its easy explosion by reaction with oxygen. For PTSs analysis by GC/MS, the column flow constant mode is usually employed. Table 3.5 below presents the recommended velocities and flow for helium and hydrogen

Table 3.4 Recommended Linear Velocities and Flow Rates

Column i.d.	*Linear Velocity (cm/sec)*		*Flow rate (mL/min)*	
	Helium	Hydrogen	Helium	Hydrogen
0.18 mm	30 - 45	45 - 60	0.5 – 0.7	0.7 – 0.9
0.25 mm	30 – 45	45 – 60	0.9 – 1.3	1.3 – 1.8

0.32 mm	30 – 45	45 – 60	1.4 – 2.2	2.2 – 2.9
0.53 mm	30 - 45	45 - 60	4.0 – 6.0	6.0 – 7.9

(*Source: IARC, 1991; Kitson et al., 2002*)

There are two common types of injection: split/splitless and on-column injection. In splitless injection, the splitter vent is closed so that the entire sample flows onto the head of the column. After a specific time called the purge activation time, the splitter vent is opened to purge solvent from the injector and low-boiling components of the sample that are not adsorbed by the column. Splitless injection, therefore, concentrates the sample onto the head of the cool column and purges most of the volatile solvent. With on-column injection, the sample is injected directly onto the column using a small syringe needle; the column is kept below the b.p. of the solvent. Solvent and sample components are trapped near the head of the column, then slowly evaporated and carried through the column.

Techniques are widely used in PTSs analysis, with split/splitless injection we can inject a larger volume of sample (generally 1.5 to 2 µl) and we can use the solvent with relative high b.p such as dodecane or tetradecane for our standard solutions. The use of such solvents will minimize the loss by evaporation. Split/splitless injection transfers 90-95% of compound with low and medium b.p. into column, but for the compounds with very high b.p only 50% (*IARC, 1991*). On-column injection always injects only a small volume of sample (1 µl) and uses the solvents with medium b.p such toluene or isooctane. This technique is expected to be superior for accurate quantitative analyses; it results in larger sample transfer and less discrimination against less volatile components.

To protect the GC column form undesired contaminants, a deactivated, uncoated column is used – called pre-column. One end of this pre-column is connected with injector and the other end connected with column by a glass connector. The length of pre-column normally from 1 to 5m and its diameter is bigger than GC-column (e.g. for GC column with i.d. of 0.25mm, a pre-column with i.d. of 0.32mm usually employed). The pre-column serves one hand as a guard column, other hand as a retention gap. Pre-column is conveniently cut or replaced time to time. By this wy we could lengthen the life time of GC column used.

3.6.6 Detection, quantification and confirmation by MS technique

The MS techniques include low-resolution MS (LRMS), commonly carried out with quadrupole instruments, and HRMS, done with magnetic sector (double focusing) instruments. Electron-impact (EI) and negative chemical ionization (NCI) ionization are most commonly used for PTSs.

3.6.6.1 EI-MS

A mass spectrum will usually be presented as a vertical bar graph, in which each bar represents an ion having a specific mass-to-charge ratio (m/z) and the length of the bar indicates the relative abundance of the ion. The most intense ion is assigned an abundance of 100, and it is referred to as the **base peak**. Most of the ions formed in a mass spectrometer have a single charge, so the m/z value is equivalent to mass itself. Modern mass spectrometers easily distinguish (resolve) ions differing by only a single atomic mass unit (amu), and thus provide completely accurate values for the molecular mass of a compound. The highest-mass ion in a spectrum is normally considered to be the molecular ion, and lower-mass ions are fragments from the molecular ion, assuming the sample is a single pure compound.

EI-MS usually allows reliable identification of PTSs in the presence of other chlorinated compounds. EI mass spectra of PTSs show the following characteristic features: (a) intense, generally base peak, molecular ions (M^+ and satelliters) with characteristic ion clustering due to the Cl isotopes; (b) typical fragmentation via consecutive losses of CO and halogen; and (c) doubly charged molecular and fragment ions of some intensity (*IARC, 1991*).

Accurate mass

In assigning mass values to atoms and molecules, we have assumed integral values for isotopic masses. However, accurate measurements show that this is not strictly true. Because the strong nuclear forces that bind the components of an atomic nucleus together vary, the actual mass of a given isotope deviates from its nominal integer by a small but characteristic amount (remember $E = mc^2$).

Carbon is present as a mixture of ^{12}C and ^{13}C isotopes. Present in the proportions of 98.9% to 1.1%, respectively. By definition ^{12}C is given the mass of 12.00000 and all other isotope masses are referred to this standard: ^{13}C then has a mass of 13.00335; ^{1}H is 1.00783; ^{16}O is 15.99491; ^{35}Cl is 34.98665; ^{37}Cl is 36.96590; etc.

Full scan analysis and Selected Ion Monitoring (SIM)

The complexity of fragmentation patterns (full scan) has led to mass spectra being used as "fingerprints" for identifying compounds. Generally we choose a interval of mass to record the mass spectrum, e.g for PTSs analysis a mass interval from 50 to 550 is chosen for full scan analysis. Then we compare the obtained mass spectrum with mass spectrum pattern in available library to identify the compounds of interest. This method is very good for screening test of unknown compounds.

With full scan analysis, the recorded signal (peak) is usually very small due to the big noise of base line and the interference of other compounds presented in our sample extract (because many compounds could give the fragmentation ions in interested chosen mass interval). Since, for PTSs analysis the SIM is predominated. With SIM, the mass spectrometers record only the mass spectrum of some fragmentation ions giving the biggest relative abundance rather than the entire mass spectrum. By this way, the recorded signal is multiplied a lot (10 to 1000 or more depend on the compounds). To do the SIM, we should know or predict the retention time of interested compounds to set up an appropriate SIM scan methods. The SIM give a more clearly mass spectrum than full scan, however for this method we cannot use the available library such Nist or Wiley to identify the compounds (not enough data to compare). Since for PTSs analysis by SIM we should follow some criteria for confirmation that we will discuss later. The SIM technique can be used for both LRMS and HRMS.

3.6.6.2 NCI-MS

Even if EI-MS is the most common technique for PTSs analysis, the use of NCI-MS can improve instrumental sensitivity because less molecular fragmentation occurs, with the resulting ion current concentrated in fewer ions compared to EI as reported by many authors (*Crespin et al., 1999; Nakagawa et al., 2001; Lacorte and Guillamon, 2008*). NCI is very selective for those compounds that tend to capture electrons and form negative ions. A number of specific ion-molecule reaction are know in NCI-MS and involve charge transfer, proton or hydride transfer, oxygen exchange reaction with halogens and anion-molecule adduct formation.

NCI-MS technique gives high selectivity for particular classes of compounds and, in certain cases, very high sensitivity attained in comparison with EI-MS. Since NCI-MS is a suitable

technique for PTSs analysis, especially in the cases of trace-levels (nanogram/kg) and the use of LRMS. By using of this technique, we can reach the lower detection limit even if we have only LRMS equipment – this is a big advantage for us. As we presented above, CH_4 pure gas is commonly used as reagent gas. The sensitivity is very good for higher chlorinated PTSs.

The sensitivities and the relative intensities of molecular and fragment ions in the mass spectra obtained with NCI are much more dependent on ion source conditions (temperature, pressure, oxygen content, residence time of ions) than in case with EI. (*IARC, 1991*).

3.6.6.3 Two-dimensional gas chromatography (GC×GC)

Recently, a new technique called GC×GC is developed and it is seemed to be a good effective and competitive tool in comparison with traditional GC techniques for PTSs analysis. This technique has been reported by many authors as an alternative method for PTSs analysis (*Lee et al, 2000; Korytar et al., 2002; Dimandja, 2003; Paníc and Górecki, 2006*).

Comprehensive two-dimensional gas chromatography (GC×GC) is a relatively new analytical technique that combines the advantages of selective (heartcut) 2-D GC and high-speed GC (HSGC) to produce a system in which every portion of the first-dimension eluent is subjected to a separation in the second dimension. A block diagram of a typical GC×GC set up is illustrated in Fig. 3.14. In GC×GC, two columns are serially connected through an on-column injector (called a modulator) at the junction between the two columns. The modulator collects sample components emerging from the first column and transfers them as sharp pulses into the second column to generate a series of high-speed chromatograms. The array of high-speed secondary chromatograms forms the two-dimensional chromatogram. Thus, every portion of the first-dimension chromatogram is heart-cut, and the result is a high-peak-capacity system that provides enhanced separation power without a time penalty because of the high-speed operation of the second dimension.

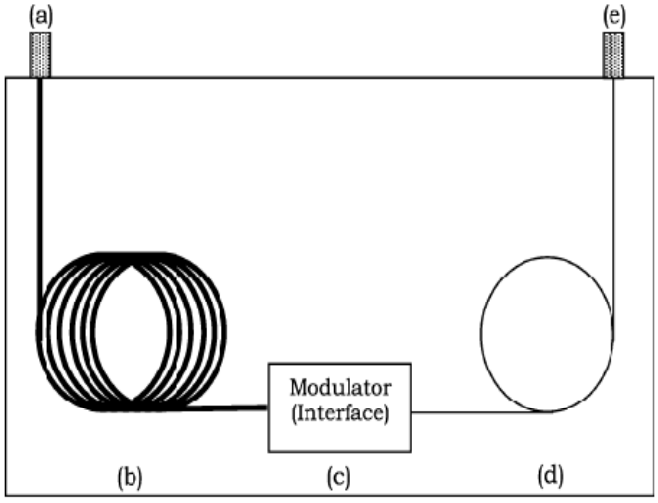

Figure 3.14: A block diagram of a GC×GC system (a: injector; b: primary column; c: modulator or interface; d: secondary column; and e: detector)

Since the second dimension operates under fast GC conditions, detector choices in GC×GC are limited to those capable of fast data acquisition rates. Examples of detectors that were found suitable for GC×GC include a flame ionization detector (FID), an electron capture detector (ECD), an atomic emission detector (AED), a sulfur chemiluminescence detector (SCD), a nitrogen chemiluminescence detector (NCD), and a time-of-flight mass spectrometer (TOF MS).

Numerous reviews dealing with GC×GC instrumentation have been published, and readers interested in this topic should consult them (*Dallüge et al., 2003; Górecki et al., 2006*).

The implementation of GC×GC offers the following advantages over 1D separation methods: enhanced separation power; improved mass sensitivity (observed only with thermal modulators due to the focusing effect); and structured, or highly ordered, chromatograms. In environmental analysis, GC×GC has the potential to improve separation of the toxic compounds from the coeluting analytes and matrix components, to increase the detection limits of such chemicals, and to provide structured twodimensional chromatograms ideal for monitoring applications. Consequently, this can lead to minimized sample preparation procedures, and hence decreased analysis time.

GC×GC offers an alternative and advantageous approach to the analysis of PCBs in complex matrices. In one of their early experiments, Haglund et al. used a liquid crystal primary column (separation based on planarity) and a nonpolar secondary column (separation based on vapor pressure) to separate mono - and non-ortho PCB congeners from a technical mixture (*Haglund et al., 2001*). The group was successful in baseline separation of the 12 marker PCBs and the seven EU indicator PCBs from a technical mixture in 15 minutes; Korytár et al. (2002) applied GC×GC linked to microelectron-capture detection (μECD) for the determination of toxic PCBs, in cod liver samples. The results of the analysis illustrated full separation and identification of all 12 priority PCB congeners from liver samples spiked with 90 PCBs. Additionally, when compared to standard sample preparation procedures, the liver sample pretreatment was nonselective and minimized. It consisted of cell lysis, centrifugation and fractionation followed by direct injection into the GC×GC system.

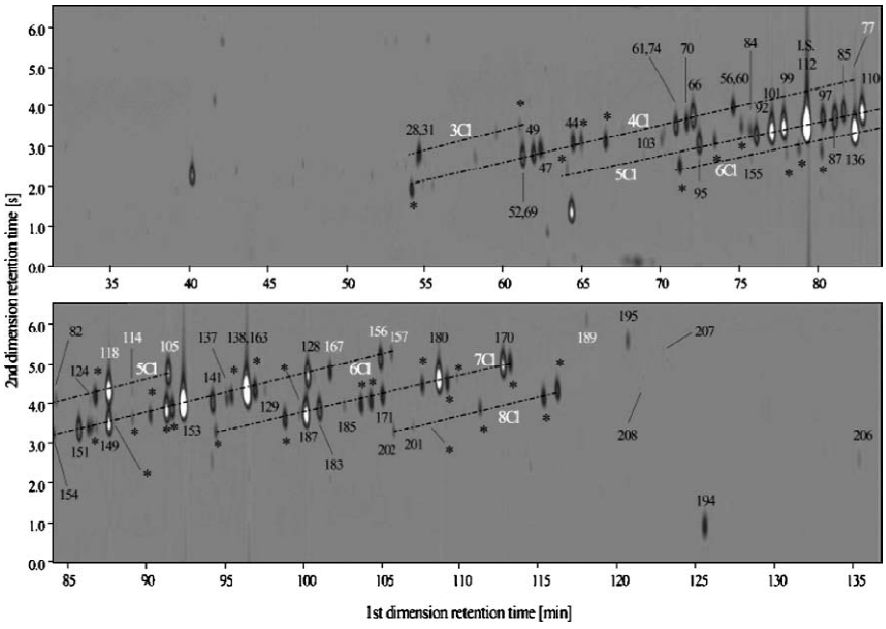

Figure 3.15: GC×GC–ECD chromatogram of a cod liver sample spiked with 90 PCBs (*Korytár et al. 2002*)

Recently, Korytár et al. (2005) evaluated five different GC×GC column combinations for the group separation of 12 halogenated compound classes, consisting of PCBs, PCDDs, PCDFs, polychlorinated diphenyl esters (PCDEs), polychlorinated naphthalenes (PCNs), polychlorinated dibenzothiophenes (PCDTs), polychlorinated terphenyls (PCTs), polychlorinated alkanes

(PCAs), toxaphene, polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs).

GC×GC has quickly achieved the status of being one of the most powerful tools for the analysis of volatile organic compounds. It has established itself as a technology that is perfectly suitable for PTSs analysis in complex samples. In the area of environmental analysis, this is evidenced by the numerous examples of analysis of common environmental pollutants—including PCBs, PBDEs, and OCls pesticides in complex environmental matrices. Additionally, GC×GC has the potential to simplify the sample preparation procedures (or even eliminate them entirely), while simultaneously generating high-resolution chromatograms in a shorter overall analysis time.

3.6.6.4. Confirmation of PTSs analysis by MS techniques

Generally, both false-positive and false-negative results of PTSs analysis are the matters of potential concern. There are some important points that we should follow (*IARC, 1991*):

1. Sample processing to remove the bulk of potential interfering substances;
2. Gas chromatographic separation of extract components on a column which has been determined to be adequate with respect to the separation of the analyte from possible interfering compounds;
3. Mass spectrometric detection, with m/z values chosen to be characteristic of the analyte(s) of concern.

To control the effectiveness of the sample processing, we should to use the fortified (spiked) samples as well as blank samples, so that we have the acceptable recoveries of the analytes at the concentration levels of interest. Chemicals (sovents, reagents, adsorbants) and glassware used for PTSs analysis should be free of contaminants. The use of surrogate and internal standards (isotope labelled ^{13}C) is indispensable for PTSs analysis. Surrogate standards are added before extraction and cleanup procedure to determine recoveries and to find the loss during sample processing. Internal standards are added to the final extract before injection to control the analytical equipment.

Theoretically the presence of PTSs could be confirmed by full scan mass spectra in which appropriate M+ (M-) ions and characteristic isotope and fragmentation patterns may be observed. However the full scan mode of MS is not sensitive enough to detect the low concentrations of PTSs presented in some samples. The GC×GC technique presented above is very useful and serves as official and recognized technique for PTSs analysis. LRMS-SIM (EI and NCI) could be sufficient for PTSs analysis with the acceptable low detection limit depend on the methods and equipments used for the analysis (*we will discuss in more details in next chapter*). If LRMS is used, some criteria should be followed:

1. Correct retention time with respect to reference PTSs isomer on HRGC columns, preferably on both non-polar DB-5 MS and polar other equivalents;
2. Correct retention time with respect to stable-isotope-labelled internal standards, where appropriate;
3. Correct isotope ratio (less than 10% deviation from theoretical values) for at least two ions (e.g., M+/M++2);
4. Correct retention time by GC/MS multiple ion monitoring of native and labelled compounds;
5. Observed signal (peak) intensities greater than three times noise.

3.6.7 Safety in PTSs analysis

As presented in Chapter I, PTSs could cause many adverse effects on human health so we should take a great attention when we work with them. For PTSs analysis we use the standards and surrogates that could be a source of PTSs exposure for laboratorians. The safety procedures in PTs analysis are available in many literatures. In addition, each laboratory has own regulation that the laboratorians/researchers should follow when working in.

There are five factors that we should take into account:

1. The samples and standards are well contained in sealed small containers (alum box, glass bottles, etc.);
2. Use the autosampler for sample injection to minimize the exposure to laboratorians;
3. Use very small volume of sample extracts and standards for injection, as well as use the pure solvents to wash the syringe (not use the samples/standards);
4. The splitless injection vent and the outputs from the forepumps of GC/MS are all vented (passed through a tube containing activated carbon or silica).

The final sample vial, used standards and solvents are the most concerned waste from ASL. Normally, these wastes are collected separately: used solvents are collected in two containers (non-chlorinated and chlorinated), final vials and standards are put in labelled glass bottles. Then all wastes are given to a specific company to treat it.

In addition, only good-trained persons (having experiences for laboratory work and knowledge about toxicity of PTSs as well as solvents used for analysis) have permit to work alone.

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Chapter 4: SET – UP THE ANALYTICAL METHODS

4.1 Introduction

According to the research plan of this thesis, the development of a proper analytical method for PTSs is one of the main objectives for the research. PTSs are measured in several matrices (such as soil/sediment, fish/bivalve and human milk). Methodology is available for PTSs as a result of a vast amount of environmental analytical chemistry research and development over the past 30 to 40 years. However, the establishment of an analytical laboratory and the application of this methodology at currently acceptable international standards is a relatively expensive undertaking. Furthermore, for environmental purposes it is necessary that the methods applied to these matrices are comparable and making use of the same principles and instrumentation. Hence, it can happen that the same method may be good for one case, but not for the other. The selected method is always related to the type and complexity of the matrix. Each method must be validated individually for the determination of an specific analyte in a specific type of matrix.

The utilization of Standard Reference Materials (SRM) play a very important role in QA/QC procedure. In fact, there are many methods available for PTSs analysis, however each method has own advantages and also disadvantages that we should consider when apply it for our research. In addition, there are some limitations of real conditions (available equipments, finance, time, etc.) that can be limiting factors for our selection.

Today, using a GC/MS has some additional advantages over the ECD. The mass spectrometer is more specific and selective ion monitoring can eliminate interfering compounds. An electron capture detector detects all compounds containing halogens and thus compounds co-eluting on the capillary column in the GC cannot be distinguished. Another advantage using GC/MS is that chemically identical ^{13}C labeled stable isotopes can be used as internal and recovery standards. This way the samples can be manipulated during sample pre-treatment and clean-up without the loss of quantitative information (*Bavel et al., 2000*).

Negative Chemical Ionization-Mass Spectrometry (NCI-MS) is similar to both Chemical Ionization (CI-MS) and the Electron Capture Detector (ECD). The basic difference between negative and positive CI is the polarity of the various voltage potentials on the spectrometer and the detector. NCI-MS is particularly suitable for compounds containing electron withdrawing groups (e.g., halogen, nitro, etc.), which can resonance stabilize negative charges. The major reasons for use of NCI-MS for PTSs analysis are its sensitive and selectivity. NCI-MS with electron capture techniques (using methane as a reagent gas) can be 100 times more sensitive than PCI-MS (*Grob and Barry, 2004*). This technique can be used to replace the customary GC/ECD in analysis of PTS because the ability to discriminate PTSs from interferences and the wealth of data available for computerized pattern recognition analysis (*Erickson, 1997*). Therefore, due to the potential for increased sensitivity and selectivity to PTSs compounds and the transparency to many otherwise interfering compounds, NCI-MS technique is the important tool for analysis of environmental contaminants and their metabolites.

Besides the above reasons, we chose the GC/LRMS due to following facilities:

- The LRMS is good for multipurpose analysis. In addition, we can detect the low enough concentration of PTSs with the NCI-SIM mode, adapting to objectives of our research.

- This equipment was available in both CEAL Lab and IER Lab, so we can do the analysis in both laboratories with the same level of MS resolution. It is more convenient in comparison of the analytical results between both labs as well as performing the training work for utilization.

- The investment cost is adapted to our budget for second phase of our collaboration project between CEAL (former CECOTOX-EPFL) and IER.

4.2 Extraction and clean – up for soil and sediment sample

The analytical procedure is set up based on the certified methods (*US.EPA, 1996a, 1996b, 1996c, 2000, 2003*), as well as modified from tested methods proposed by many authors (*Doong et al., 2008; Minh et al., 2007a, 2007b; Laiguo et al., 2005; Fatin et al., 2006; Anh et al., 2003*) and from analytical protocol of CEAL - EPFL.

a) Sampling

Sampling is considered a crucial step in the analysis of PTSs. Soil and sediment samples were taken with soil borer tool and Ekman dredge, respectively. Samples were stored in clean aluminium can and transported to our laboratory in the boxes with dry ice. All glassware and materials must to be clean and rinsed with acetone and n-hexane before using.

b) Sample treatment before analysis

After sampling, samples ware dried at room temperature for about 4-7 days depending on their water content. Dried soil and sediment were ground with a ceramic mortar then sifted through a 1mm$\times$1mm stainless steel sieve to remove the stones, roots, etc. Finally, samples were labeled and stored in a brown glass bottle at -20^{0}C until analysis.

c) Determination of the organic matter and water content in dry soil and sediment

The organic matter and water content in dry soil and sediment samples are controlled with the steps presented below:

- Prepare an aluminum cup with aluminum foil. Heat this cup to 550^{0}C for two hours. Let cool it in a desiccator and record its weight in gram (X_0).
- Weight precisely about two grams of the dry soil or sediment sample in the aluminum cup and record its weight (X_1). Put it in oven at 105^{0}C during six hours. After cooling in desiccator, the weight is recorded in gram (X_2).
- The sample in then put in a muffle furnace at 550^{0}C during three hours. After cooling in desiccators until constant weight, the weight is recorded in gram (X_3)
- Determine the organic and water content (%) as follows:

$$\text{Water content (\%)} = [(X_1-X_2)/(X_1-X_0)] \times 100$$

$$\text{Organic matter content (\%)} = [(X_2-X_3)/(X_1-X_0)] \times 100$$

d) Extraction

Though the time for soxhlet extraction is relatively long (16 to 18 hrs depending on the matrix type), this extraction method is always chosen as a basic, traditional technique due to two advantages as follows:

- Very effective when comparison with other techniques;

- Very easy to use and not requires any special equipments (minimize the investment cost).

The Accelerate Solvent Extraction (ASE) is also an effective method, but it requires costly system, so we consider it as an alternative selection for the future investment when IER lab participate in the POPs monitoring network in southern Vietnam in the frame of Stockholm Convention.

The preparation procedure for PTSs analysis in soil and sediment is presented in Fig. 4.1

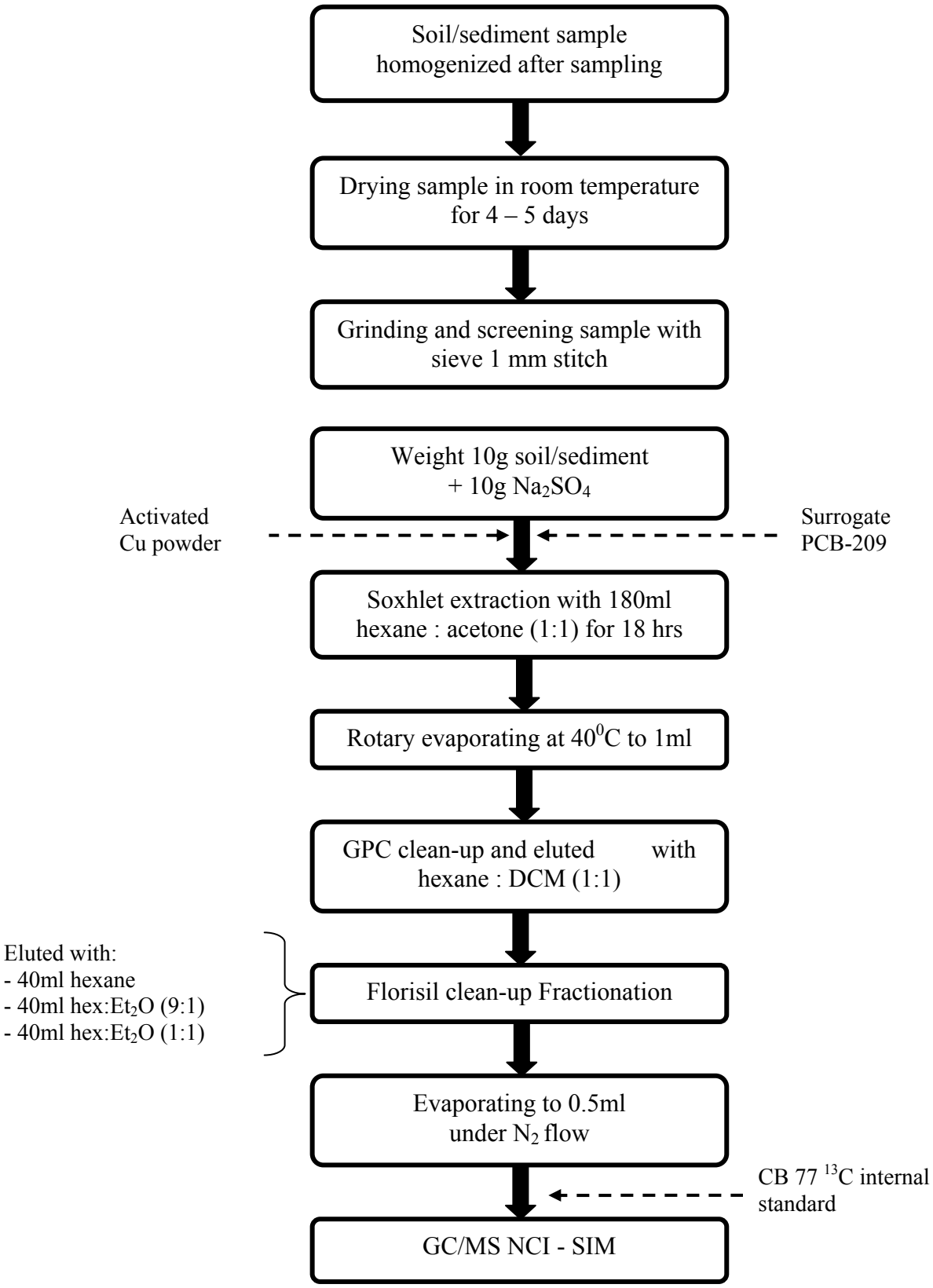


Figure 4.1: Analytical procedure for PTS in soil/sediment samples

Figure 4.2: Soxhlet extraction system used for PTSs analysis

Hexane-acetone (1:1) used as an extraction solvent for PTSs analysis in some environmental matrices may be more effective than other solvent mixture. The use of hexane-acetone mixture generally reduces the amount of interferences that are extracted and improves the signal-to-noise ratio (*US.EPA, 2000*).

The choice of extraction solvent will depend on the analytes of interest. No single solvent is universally applicable to all analyte groups. The analyst must demonstrate adequate performance for the analytes of interest, at the levels of interest, for any solvent system employed, including those specifically listed in this method. Each new sample type must be spiked with the surrogate compounds (in our research is PCB-209) of interest to determine the percent recovery.

There is possibility that PTSs could be stuck or strongly bind with soil/sediment matrices. This may result in the imprecise calculation of soxhlet extraction procedure recovery. In principle, the efficiency of soxhlet extraction can be checked by continuously extracting in 48 hours more the previously 18-hour extracted soil/sediment matrices with another solvent mixture. The first soxhlet extraction (for 18 hours) is considered sufficient when PTSs are not found after extra 48 extracting hours

Weigh exactly 10g soil/sediment sample in thimble. Anhydrous sodium sulfate (10g) is added above and below the sample to remove the water content during the extraction process. The active Cu powder was introduced into the thimble using for removing sulphur compounds. The PCB-209 surrogate was spiked directly on the sample keeping in the thimble just before extraction. The soxhlet extraction was maintained within 18 hrs for finishing the extraction.

e) Clean - up

Generally, there are many compounds co–extracting together with PTSs. Clean – up the extract solution with H_2SO_4 96% is one of the most popular method due to it is easy to use. However, we cannot use H_2SO_4 96% because it will destroy some analytes in OCs pesticides group such as aldrin, dieldrin, endrin, endosulfan (I and II) and endosulfan sulphate (*US.EPA, 1996d*). Clean – up with alumina column is also not recommended because of possible dehydrochlorination of some PTSs, e.g. pp'-DDT (*UNEP, 2004; Tadeo, 2008*)

The soxhlet extract was concentrated at 40 ^{0}C by rotary evaporator to a volume of 1ml before clean – up. Do not let dry. The concentrated extract containing interesting compounds is again cleaned-up by several steps described below

Gel permeation chromatography (GPC) column: A glass column (600mm x 25mm) was packed with about 100g of pre-swelled absorbent Bio Bead SX-3 (200 – 400 mesh) and flushed with n-hexane/methylene chloride (1:1, v/v) for an extended period at a flow rate of 5ml/min. To determine the elution profile of the GPC column, a calibration solution was prepared in n-hexane/methylene chloride containing all interesting PTSs. The calibration solution was injected after solvent flow and column pressure were established and stabilised. Based on the CEAL-EPFL protocol and reference literature, column eluate collection was started just from 38th min. (before any PTSs elution) till 80th min. (after all PTSs elution). All GPC system parameters are presented as follows:

Column:	Glass column (600 x 25 mm ID), 100 g Bio-Beads SX-3
Eluent:	Hexane/DCM (1:1, v/v)
Flow:	5 ml/min
Pressure:	1 - 10 bar
Sample Volume:	5 ml
Collection time:	38th – 80th min

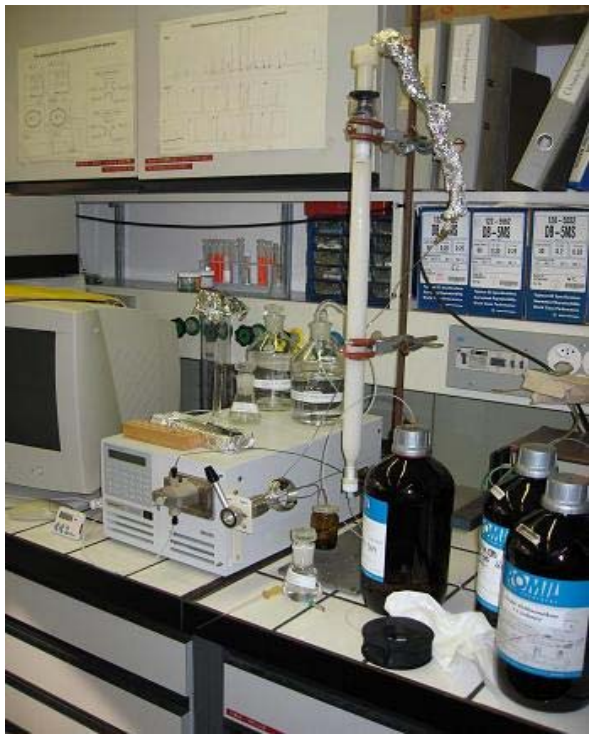


Figure 4.3: GPC column system

We had to check the recovery of all PTSs after clean - up with GPC column and the result are showed in table 4.1.

Table 4.1: Recovery of PTSs after clean-up with GPC column (n = 3)

Compound	Recovery (%)	STDEV	Compound	Recovery (%)	
Organochlorinated pesticides					
Lindane	76	11	Endrin	92	4
HCB	85	5	Endosulfan-II	93	6
Heptachlor	92	8	pp'-DDD	95	3
Aldrin	71	8	Endrin aldehyde	94	3
oxy-chlordane	77	5	Endosulfan sulfate	93	6
Heptachlor epoxide	86	6	pp'-DDT	112	2
g-Chlordane	87	5	Mirex	82	4
a-chlordane	89	4	Toxaphene-26	77	8
Endosulfan - I	88	2	Toxaphene-32	78	7
Transnonachlor	85	9	Toxaphene-50	69	9
pp'-DDE	89	2	Toxaphene-62	75	9
Dieldrin	87	5	Toxaphene-69	81	6
Polychlorinated biphenyl					
PCB 28	104	4	PCB 123	101	7
PCB 52	106	3	PCB 118	109	5
PCB 101	110	4	PCB 114	104	2
PCB 153	110	2	PCB 105	102	6
PCB 138	105	3	PCB 126	97	8
PCB 180	107	3	PCB 167	106	4
PCB 170	106	5	PCB 156	105	3
PCB 149	113	2	PCB 157	108	5
PCB 128	104	6	PCB 169	106	5
PCB 81	98	7	PCB 189	112	5
PCB 77	102	4	PCB 209	107	4
Polybrominated diphenylether					
PBDE 28	114	7	PBDE 99	103	5
PBDE 49	131	8	PBDE 85	109	2
PBDE 47	104	8	PBDE 154	102	3
PBDE 66	108	6	PBDE 153	106	3
PBDE 100	94	3	PBDE 138	112	6
PBDE 119	102	4	PBDE 183	102	7

Eluate collection was stopped after all PTSs elution. The fraction collected were carefully evaporated by a rotary evaporator up to 1 ml. The next step is clean-up with florisil column. The low recovery of some OCls pesticides may result from several reasons, such as the discrimination between PTSs compounds inside the injector of GC or the lost of OCls during the preparation process.

Fractionation on florisil column: A glass column (400mm, 10mm ID) was packed with 10g of florisil (100 – 200 mesh) deactivated to 2% water and covered on the top with 2g of Na_2SO_4 anhydrous. The extract was eluted with mix solvent as follows:

i) 40ml n-hexane (fraction containing majority of PCBs, PBDEs and some OCs pesticides such HCB, heptachlor, aldrin, pp'-DDE)

ii) 40mL n-hexane/Et_2O (9:1)(fraction containing most of the organochlorinated pesticides and some PCBs congeners as PCB 126, PCB 128, PCB 169)

iii) 40mL n-hexane/Et_2O (diethyl ether) (1:1) (fraction containing endosulfan sulfate)

The adsorbent preparation and column filling is described in detail in appendices, here we want to comment about something related to clean-up with florisil column. As we know processing the MS data is very complicated, especially in case of simultaneous analysis of all PTSs groups. One of the main utilities of florisil column is separation of OCs pesticides from the PCBs group, in three fractions: (i) contain PCBs congeners and fraction (ii + iii) contains OCs pesticides. Hence, we can get more convenient in set – up method for data acquisition and data processing in analysis of PTSs by GC/MS.

The cleaned extracts (including three fractions) now can be analyzed using GC/MS after spiking internal standard ^{13}C isotope of CB 77 to check response factor of MS detector to specific matrix of sample.

4.3 Extraction and clean – up for fish sample

a) Sample treatment before analysis: Normally biological samples in general and fish in particular are in the heterogeneous form. Besides, it is hard for the solvent to penetrate evenly into the matrix structure. Those problems results in the inaccuracy of analysis results. To improve the accuracy of fish samples analysis results as well as their representativeness, these samples must be totally homogenized before analyzing. Fish fillet sample should be homogenized until thoroughly mixed by Büchi Mixer B-400 automated homogenizer.

b) Extraction: Approximately 20g of homogenized fish sample was ground with about 60 – 80g of anhydrous Na_2SO_4 to fine powder by the IKA grinder and was extracted with soxhlet extractor. Before extraction, the sample was spiked with surrogate PCB 209 to control the recovery of the whole procedure. For Soxhlet extraction, a 7:3 mixture of hexane and acetone was applied. The extraction time is about 18 – 20hrs as proposed by certified methods (*US.EPA, 2003; Darko et al., 2008; Brambilla et al., 2007; Peng et al., 2007; Munshi et al., 2004*). The entire extraction procedure is presented in Fig. 4.4 below.

The soxhlet extract was concentrated to a volume of 1ml by rotary evaporator before clean – up. Do not let dry. The concentrated extract containing interesting compounds is again cleaned-up and fractionated by two steps as described below.

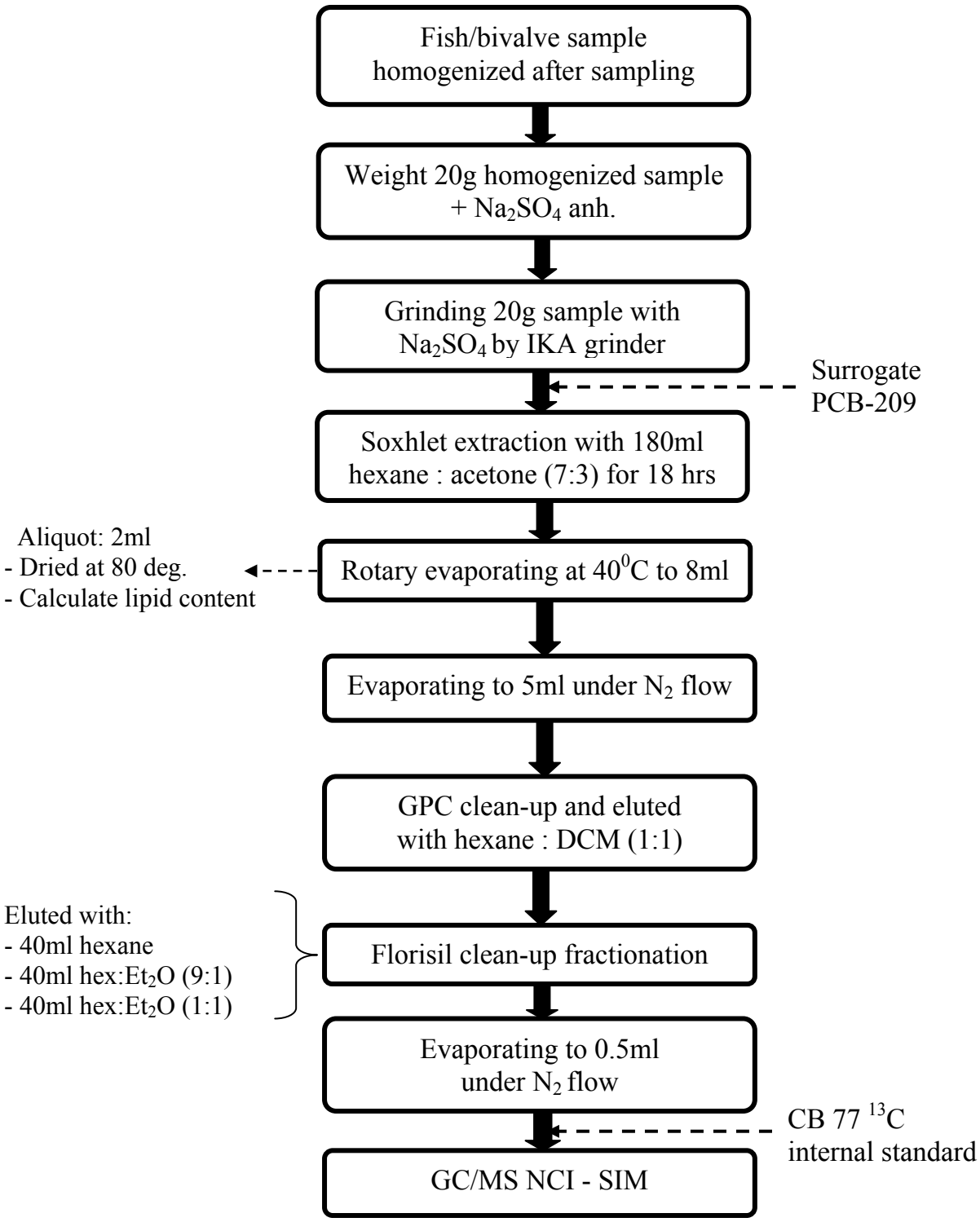


Figure 4.4: Analytical procedure for PTS in biological samples

As soil/sediment case, the efficiency of soxhlet extraction in case of fish matrix was examined by continuous extracting the previously extracted fish samples for 48 hours with another solvent mixture.

c) Determination of lipid content: After extract evaporation, gravimetric lipid determination was performed. A portion of the extract was used for lipid content measurement by evaporating the solvent until a constant weight was obtained. The entire procedure is presented below:

- Dry a small aluminum cup at 105^0C during two hours, let cool in a desiccators

- Weight exactly the aluminum cup (X_0)

- Pour 2ml extract (taken from 8ml extract after concentrating by rotary evaporator, see Fig. 4.4) into aluminum cup with pasteur pipette.

- Let the solvent evaporate under a hood during a night (12 hours) and dry the cup at 80^0C during four hours. Let cool in a desiccators and record exactly the final weight (X_1)

Calculate the lipid content as follows:

$$percent\ lipid\ (\%) = \left[\frac{(X_1 - X_0) \times 4}{\text{Initial weight of tissue}}\right] \times 100$$

Where:

- $X_1 – X_0$: weight of extracted fat after solvent evaporation.
- Initial weight of tissue: weight of fish sample extracted with soxhlet extractor

d) Purification and fractionation:

The next steps in purification procedure are the same with the clean-up steps applied in analysis of PTSs in soil/sediment samples including GPC and florisil clean – up.

4.4 Extraction and clean – up for human milk sample

a) Extraction: Extraction of PTS in human milk followed the available procedure previously reported (*Minh et al., 2004b; Wang et al., 2008; Tsydenova et al., 2007; Raab et al., 2008; Kunisue et al., 2002)*. In details, a portion of 20 grams of milk samples was applied to extraction column (60cm, 2.5cm ID) packed with 10g of pre-cleaned diatomite earth (Merck, Damstadt, Germany). The samples were then kept in the columns for 30 minutes allowing maximum absorption of the samples onto the material before they were eluted by 200 ml of diethyl ether at a flow rate of 1ml/min. The eluates were dried by anhydrous Na_2SO_4 and concentrated to 8 ml. An aliquot of 2 ml was used for fat content determination by gravimetric method. This procedure is the same with the section **4.3.c** in lipid determination for fish sample.

b) Purification: The remaining volume was evaporated under gentle nitrogen stream down to 5 ml, which was then mixed with 5 ml of dichloromethane (DCM) to obtain 10 ml extract in hexane/DCM (1:1). The extract was then subjected to gel permeation chromatography (GPC) for fat removal and a same mixture of hexane/DCM (1:1) was used as eluting solvent in GPC system at a flow rate of 5 ml/min.

The elution procedure and the next clean – up step (with florisil column) are the same with clean – up process applied in the section of soil and fish sample preparation.

A procedural blank was run for every batch of ten samples to verify cross-contamination. The extraction procedure is presented in Fig. 4.5 below.

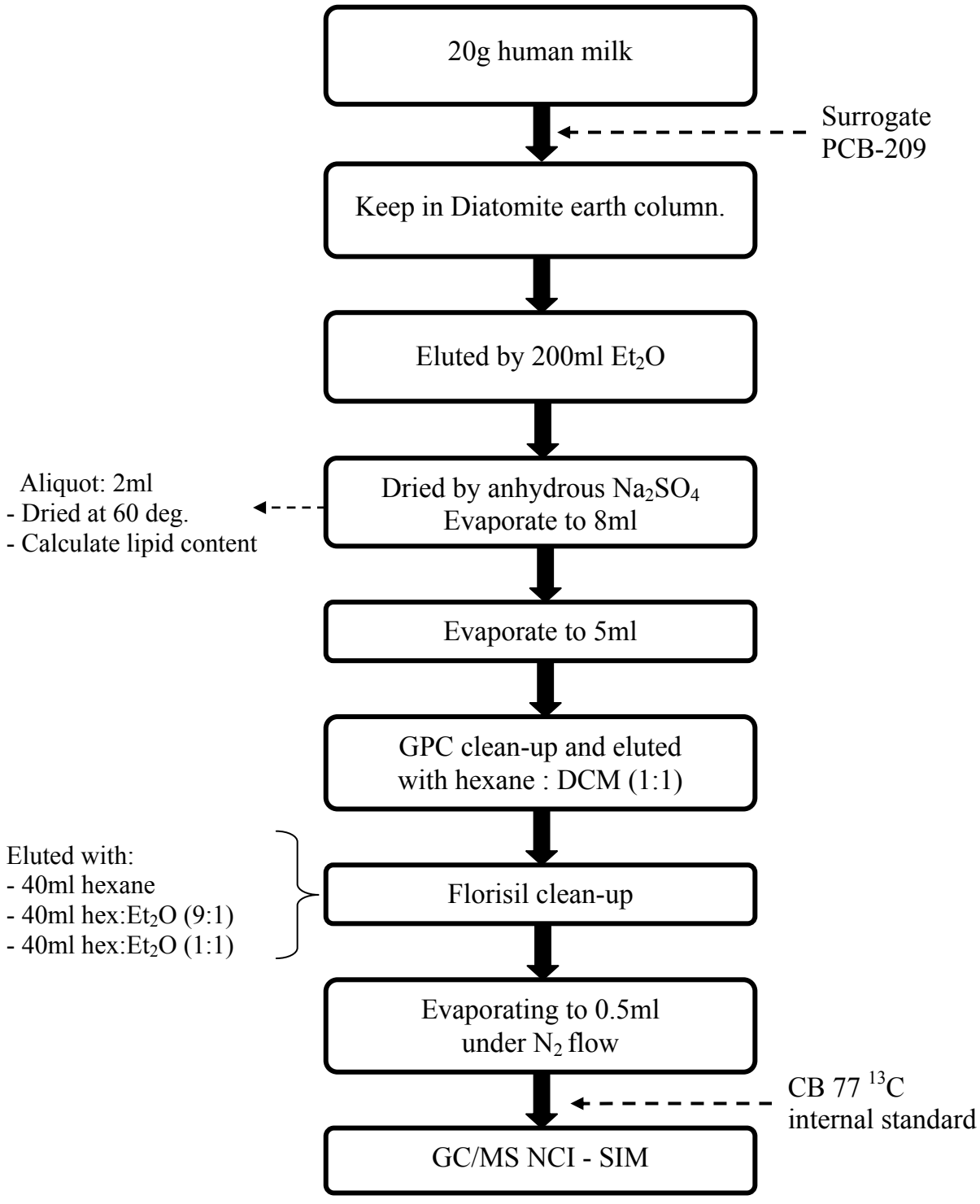


Figure 4.5: Analytical procedure for PTS in human milk samples

4.5 Identification - quantification

4.5.1 GC separation

Samples were identified and quantified by GC-MS in both IER lab (Vietnam) and in CEAL lab (ISTE – EPFL, Switzerland).

a) **IER lab** is equipped with the GC/MS (QP2010) from Shimadzu – Japan. The weak – point of this instrument is we cannot change ion source from EI mode to NCI mode without stop working

of GC/MS. This spent a lot of time in the beginning of the research when we want to survey the selection as well as sensitivity of MS between EI and NCI mode for analysis of PTSs. The injection is done by auto-sampler (Shimadzu – AOC-20i+s) with a syringe of 10 µl.

- Injector is split/splitless injector, with glass liner, i.d. 0.5mm
- The capillary column (Zebron) was used for identification and quantification of PTSs: ZB-5ms 60m×0.25mm×0.25µm, temp. max. = 320^{0}C. Guard column was used to protect the column from interference in injector: 5m×0.32mm (Restek Siltek Guard column 0.32mm ID)

The guard column is connected to the GC capillary column by glass connector. Generally, we had to change the liner and cut a part of 20cm of guard column (in the end connected to injector) after a series of analysis (40 - 50 samples) depending on type of the matrix.

- The GC working conditions:
- Injection mode : Splitless - Carrier gas: Helium
- Injection volume : 1 µL - Pressure: 134.2 kPa
- Injector temperature : 250^{o}C - Constant flow: 0.74 mL/min
- High pressure injection: 300 kPa - Linear velocity: 23.1cm/sec
- Temperature program : see table 4.1 and 4.2

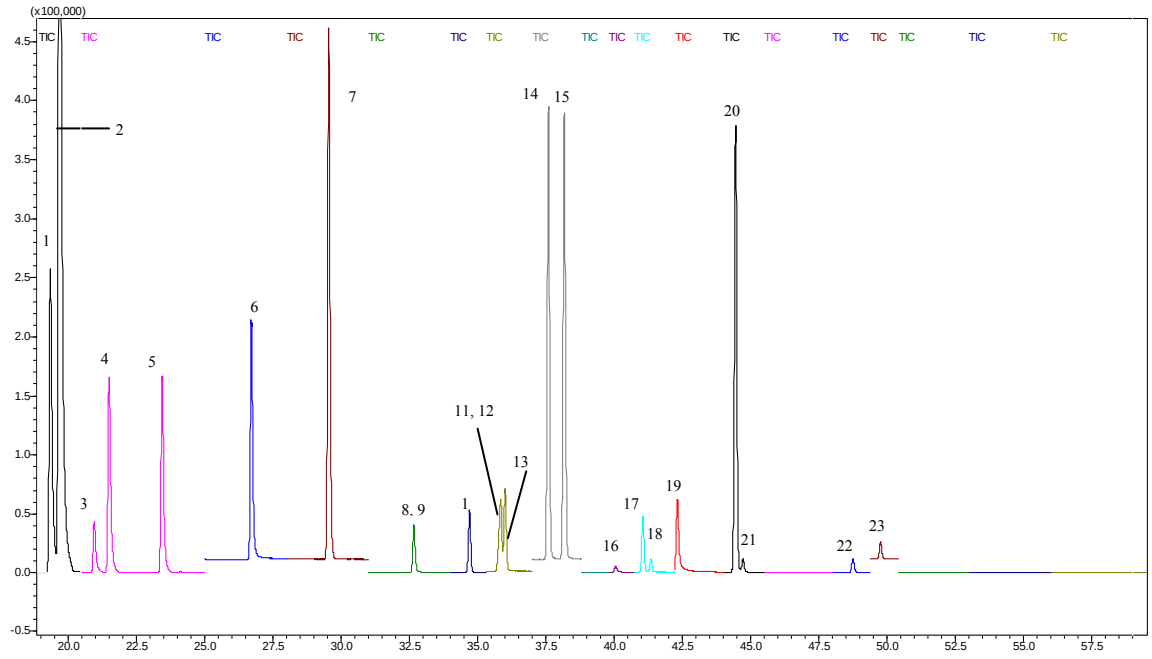
Table 4.2: GC column temperature for OCs pesticides and PCBs analysis

Temp. (^{0}C)	Rate (^{0}C/min)	Hold (min)	Total (min)
150	0.0	3.0	3.0
200	4	3.0	18.5
285	2	9.0	70.0

Table 4.3: GC column temperature for PBDEs analysis

Temp. (^{0}C)	Rate (^{0}C/min)	Hold (min)	Total (min)
150	0.0	3.0	3.0
200	4	3.0	18.5
285	2	9.0	85.0

The retention time of every PTSs has been checked by injection of individual PTSs standard solutions with Scan mode. Based on these retention times of individual standard we can set-up exactly the time of acquisition for SIM mode (Fig. 4.6, 4.7, 4.8).



1,3,4,5: HCHs	9: Heptachlor epoxide	14: pp'-DDE	19: Endrin aldehyde
2: HCB	10: γ-chlordane	15: Dieldrin	20: Endosulfan sulfate
6: Heptachlor	11: α-chlordane,	16: Endrin	21: pp'-DDT
7: Aldrin	12: Endosulfan-I	17: Endosulfan-II	22: Endrin ketone
8: O-chlordane	13: Trans-nonachlor	18: pp'-DDD	23: Methoxychlor

Figure 4.6: Chromatogram of organochlorine pesticides with NCI-SIM mode

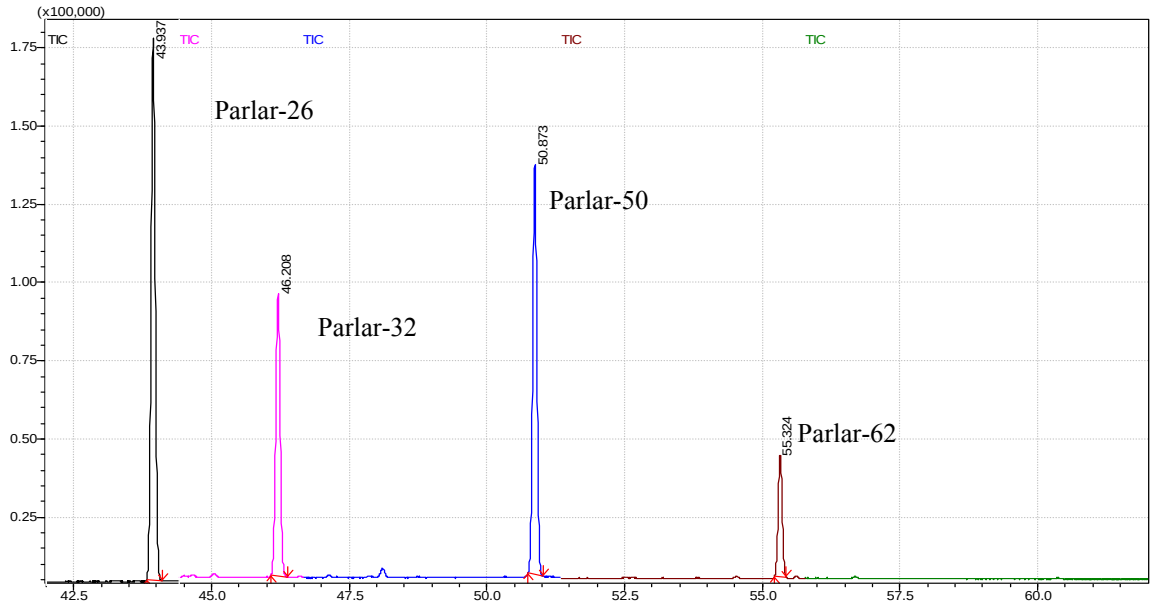


Figure 4.7: Chromatogram of Toxaphene pesticides with NCI-SIM mode

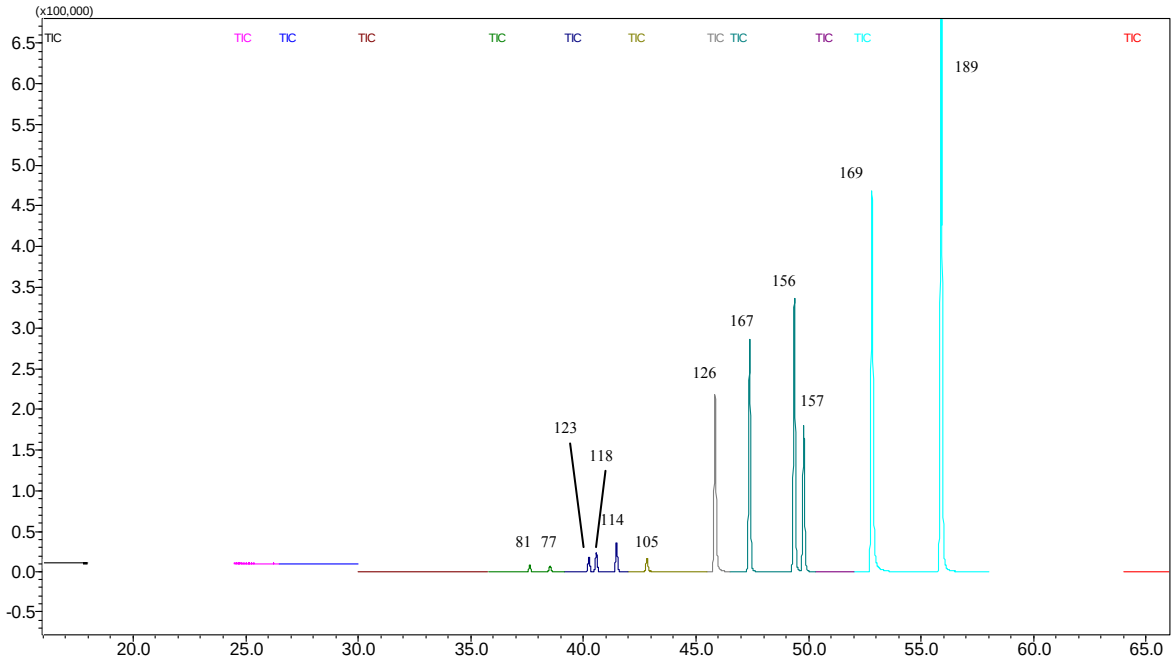


Figure 4.8: Chromatogram of dioxin - like PCBs congeners with NCI-SIM mode

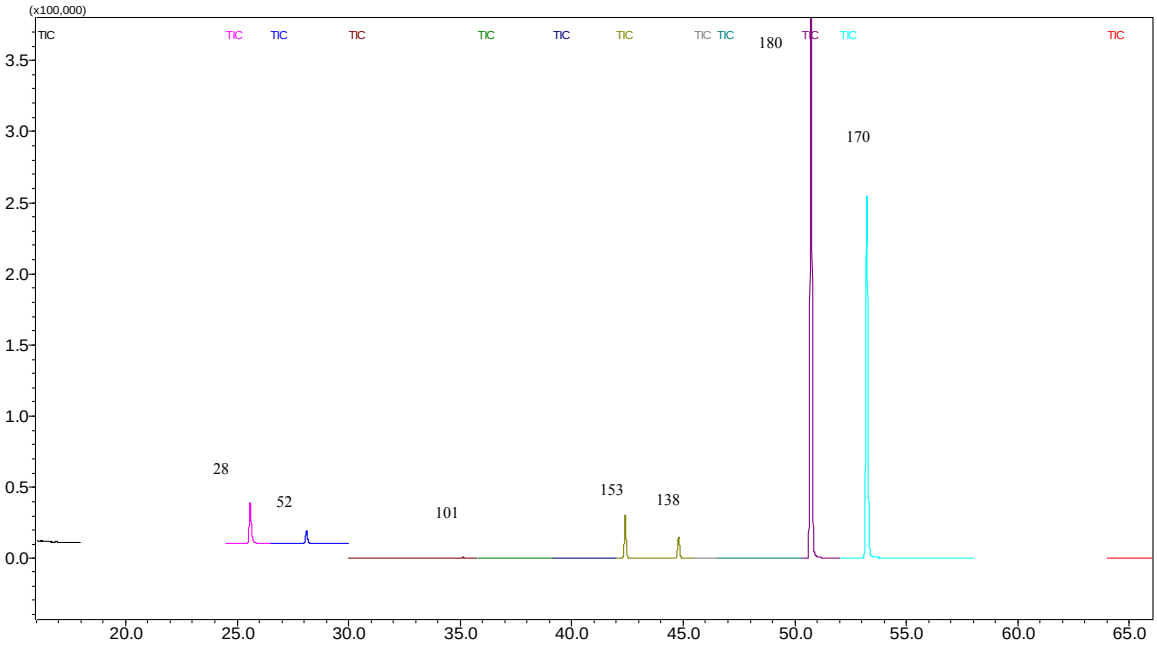


Figure 4.9: Chromatogram of indicator PCBs congeners with NCI-SIM mode

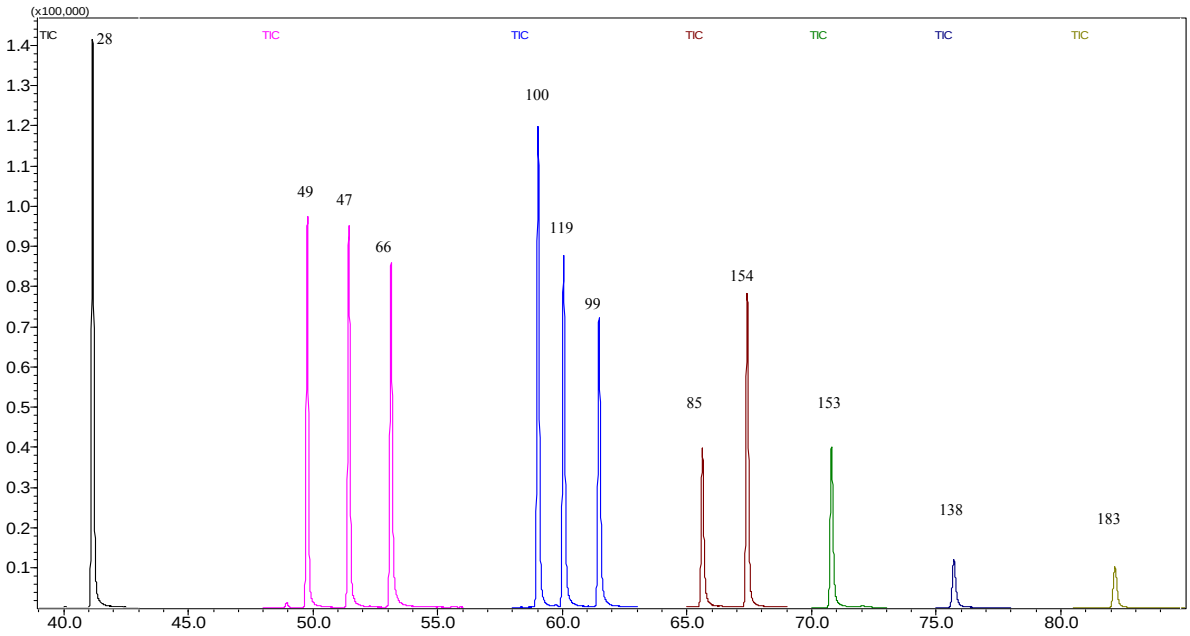


Figure 4.10: Chromatogram of PBDEs congeners with NCI-SIM mode

We had seen problem in the GC separation process from the analysis of OCs pesticides. The ZB-5ms capillary column cannot properly separate o-chlordane from heptachlor epoxide and endosulfan I from a-chlordane. After determining all specific mass of these four OCs compounds, we can see separately these peaks in the chromatograph by MS, therefore qualitative and quantitative analysis of these four OCs compounds can be performed more precisely (Fig. 4.11 – 4.12).

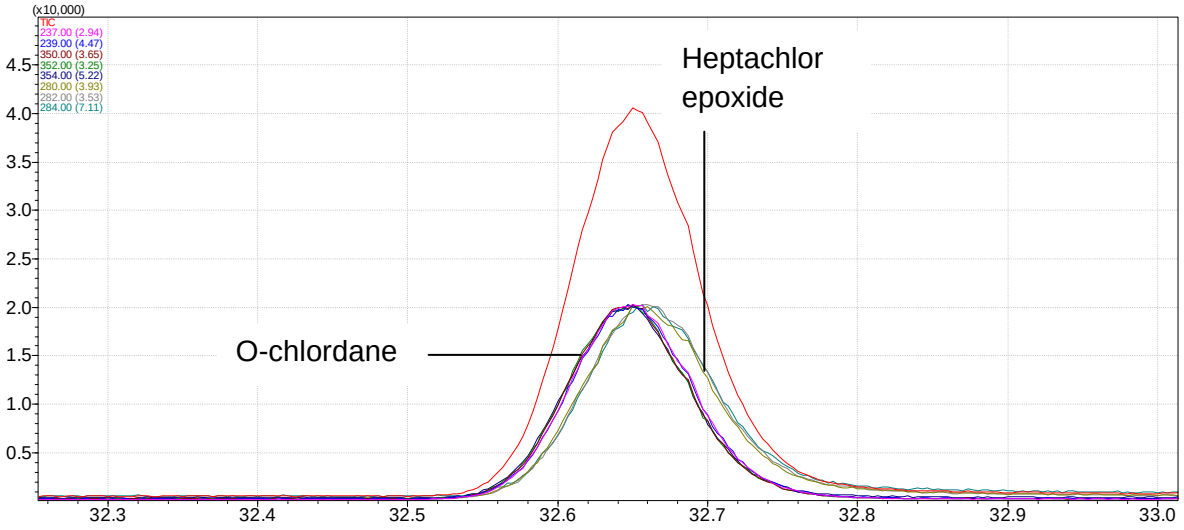


Figure 4.11: Chromatogram of o-chlordane and heptachlor epoxide

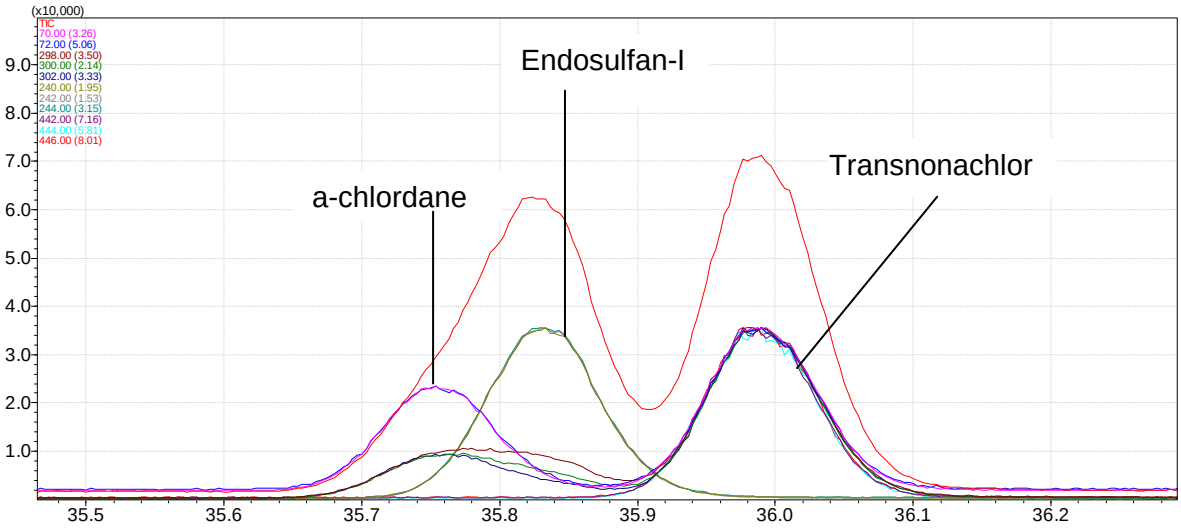


Figure 4.12: Chromatogram of a-chlordane and endosulfan-I

b) In the **CEAL lab** (ISTE – EPFL, Switzerland), we used a GC Varian CP-3800 connected to a Varian 1200L quadrupole MS. The Varian MS comes standard with a vacuum interlock, allowing easy access to the ion volumes, without breaking vacuum. The injection is done by autosampler (Varian CP-8400) with a syringe of 10µL.

- Injector is on-column injector, with glass liner, i.d. 0.5mm (Restek SPI liner 0.5mm ID for Varian GC's)
- The capillary column (J&W) was used for identification and quantification of PTSs: DB-5MS 60m×0.25mm×0.25µm, temp. max. = 325^0C. Guard column was used to protect the column from interference in injector: 5m×0.32mm (Restek Siltek Guard column 0.32mm ID)

The guard column is connected to the GC capillary column by glass connector. Generally, we had to change the liner and cut a part of 20cm of guard column (in the end connected to injector) after a series of analysis (40 - 50 samples) depending on type of the matrix.

- The GC working conditions:

- Carrier gas : Helium
- Injection mode : on-column
- Constant column flow: 1mL/min
- Injection volume : 1 µL
- Injector temperature : 250^oC (85^0C/(0.2min) to 250^0C/(100^0C/min) 58.15min)
- Temperature program : see table 4.4 – 4.5

Table 4.4: GC column temperature for OCs pesticides and PCBs analysis

Temp. (^0C)	Rate (^0C/min)	Hold (min)	Total (min)
80	0.0	0.50	0.00
150	50	1.00	2.90
285	2.5	3.10	60.0

Table 4.5: GC column temperature for PBDEs analysis

Temp. (^0C)	Rate (^0C/min)	Hold (min)	Total (min)
150	0.0	3.00	3.00
200	4	3.00	18.50
285	2.5	0.00	56.90
300	10.0	28.50	86.90

4.5.2 MS identification/quantification

In the GC/MS analysis, electronic impact ionization method (EI) is used most frequently. In this ionization method electrons from the filament are directly irradiated to sample molecules and cut the weak portions of chemical bond of the molecules. So this is called hard ionization method. On the other hand, the NCI method is called a soft ionization method because of providing us mass spectrum with less fragmentation. This ionization method is carried by introducing reagent gas (e.g. methane) to the ion source box. The electrons from filament lose energy by collision with the reagent gas at first, and then the slow electrons are captured by sample molecules to produce negative ions. The tendency to negative ion is closely related to the electron affinity of the sample molecule. The substance having large electron affinity, such as halogenated compounds, is easy to detect selectively with high sensitivity in NCI mode (*Christopher et al, 2003*).

As presented in the chapter one, all PTSs analyzed in the frame of our research are halogenated compounds such as organochlorinated pesticides, PCBs and PBDEs. We decide to use NCI mode for analysis of these PTSs due to selection as well as sensitivity of NCI mode with these PTSs are very high.

a) The MS operation conditions with NCI – SIM mode

- In IER lab we used GC/MS QP-2010 from Shimadzu:

- Ionization mode: NCI – SIM
- Electron energy: -70eV
- Detector: 1200 Volt

- Filament current: 150 µA (typical)
- Ion source temperature: 200^0C
- Transfer line temperature: 250^0C
- Solvent cut time: 10 min (typical)

▪ In CEAL lab (ISTE – EPFL) we used GC/MS 1200L from Varian:

- Ionization mode: NCI – SIM
- Electron energy: -70eV
- Detector: 1200 Volt
- Filament current: 150 µA (typical)
- Manifold temperature: 40^0C (typical)
- Ion source temperature: 200^0C
- Transfer line temperature: 250^0C
- Solvent cut time: 10 min (typical)
- SIM width: 0.500s

All the selected PTSs' mass (m/z) used in qualitative and quantitative analysis by GC/MS with NCI – SIM mode were established according to:

- Reported certified methods (*Schulz, 2004; US.EPA, 1996b, Fulton et al. 1996*).
- Data of the injections of individual PTSs standard on GC/MS with NCI - Scan mode.

Base on these factors, we perform injection and selection m/z of PTS with individual standards as well as the time group for acquisition data with mixture standard by GC/MS analysis with NCI – SIM mode till archiving the best sensitivities of all PTSs peak.

All selected mass are presented in the Table 4.6, 4.7, 4.8 below.

Table 4.6: Mass selection for OCs pesticides analysis with NCI – SIM mode

Compound	m/z		Compound	m/z	
	Target	Reference		Target	Reference
Lindane	71	73, 253, 255, 257	Endrin	272	270, 274
HCB	284	282, 286	Endosulfan-II	406	404, 408
Heptachlor	35	37, 264, 266, 268	pp'-DDD	71	73, 248, 250
Aldrin	35	37, 235, 237, 239	Endrin aldehyde	272	270, 274
O-chlordane	237	239, 350, 352, 354	Endosulfan sulfate	97	384, 386, 388
Heptachlor epoxide	282	280, 284	pp'-DDT	71	73, 262, 264
g-Chlordane	266	408, 410, 412	Mirex	368	370, 372
a-chlordane	410	408, 412	Toxaphene-26	377	375, 379
Endosulfan - I	242	240, 244	Toxaphene-32	343	341, 345
Transnonachlor	444	442, 446	Toxaphene-50	413	411, 415
pp'-DDE	35	37, 316, 318, 320	Toxaphene-62	377	70, 71, 375, 379
Dieldrin	35	37, 277, 279, 281	Toxaphene-69	446	444, 448

Table 4.7: Mass selection for PCBs analysis with NCI – SIM mode

Congener	m/z		Congener	m/z	
	Target	Reference		Target	Reference
PCB 28	35	37, 256, 258, 260	PCB 118	326	324, 328
PCB 52	35	37, 290, 292, 294	PCB 114	326	324, 328
PCB 101	326	324, 328	PCB 105	326	324, 328
PCB 153	360	358, 362	PCB 126	326	324, 328
PCB 138	360	358, 362	PCB 167	360	358, 362
PCB 180	394	392, 396	PCB 156	360	358, 362
PCB 170	394	392, 396	PCB 157	360	358, 362
PCB 149	360	358, 362	PCB 169	360	358, 362
PCB 128	360	358, 362	PCB 189	394	392, 396
PCB 81	292	290, 294	PCB 209	498	496, 500
PCB 77	292	290, 294	PCB 77 (^{13}C)	304	302, 306
PCB 123	326	324, 328			

Table 4.8: Mass selection for PBDEs analysis with NCI – SIM mode

Congener	m/z	Congener	m/z
PBDE 28	79, 81	PBDE 99	79, 81
PBDE 49	79, 81	PBDE 85	79, 81
PBDE 47	79, 81	PBDE 154	79, 81
PBDE 66	79, 81	PBDE 153	79, 81
PBDE 100	79, 81	PBDE 138	79, 81
PBDE 119	79, 81	PBDE 183	79, 81

b) Identification and quantification procedure

PTSs are identified based on some criteria as follow:

1. The retention time is correct in comparison with the retention time of native compound in external standards. The relative retention time (RRT) of the sample component is within ± 0.06 RRT units of the RRT of the standard component (*US.EPA, 1996c*).
2. The relative intensities of the characteristic ions agree within 30% of the relative intensities of these ions in the reference spectrum (*US.EPA, 1996b*). (Example: For an ion with an abundance of 50% in the reference spectrum, the corresponding abundance in a sample spectrum can range between 20% and 80%). In case of PCBs analysis, correct theoretical ion abundance ratios and their control limits as presented in the table 4.9 below.

Table 4.9: Theoretical ion abundance ratios and QC limit in PCBs analysis (*US.EPA, 2003*)

Chlorine atoms	m/z forming ratio	Theoretical ratio	Lower QC limit	Upper QC limit
3	M/(M+2)	1.04	0.88	1.20
4	M/(M+2)	0.77	0.65	0.89
5	(M+2)/(M+4)	1.55	1.32	1.78
6	(M+2)/(M+4)	1.24	1.05	1.43
7	(M+2)/(M+4)	1.05	0.89	1.21
8	(M+2)/(M+4)	0.89	0.76	1.02
9	(M+2)/(M+4)	0.77	0.65	0.89
10	(M+4)/(M+6)	1.16	0.99	1.33

3. Good separation between compounds, especially between *o-chlordane and heptachlor epoxide*, and between *a-chlordane and endosulfan-I*.
4. The signal-to-noise ratio (S/N) for the GC peak at each exact m/z must be greater than or equal to 2.5 for each compound detected in a sample extract, and greater than or equal to 10 for all PTSs in the calibration and verification standards.

The PTSs quantitative analysis is based on the method called external standard method which uses the calibration curve and the concentration of all interested PTSs is calculated based on this curve and the response factor of these native compounds to correlative ^{13}C-isotope. In our research, we use ^{13}C-isotope CB 77 to calculate the response factor (RF). The RF is calculated as follows:

$$RF = \frac{A_s \times C_{is}}{A_{is} \times C_s}$$

Where:

- A_s = Peak area (or height) of the analyte or surrogate
- C_s = Concentration of the analyte or surrogate
- A_{is} = Peak area (or height) of the internal standard (^{13}C-CB 77)
- C_{is} = Concentration of the internal standard (^{13}C-CB 77)

In general, the manufacturers supply the quantification program and depending on working schedule we used the MS Work Station software (in CEAL lab - EPFL) or GC/MS Solution software (in IER lab - Vietnam) to process the analysis data and quantify our result. The total amount of PTS in the sample is:

$$M_i = \frac{\text{Amount}_s \times A_i \times RF}{A_s}$$

Where:

- Mi = Amount of analyte in sample
- Amount_s = Amount of the external standard
- A_s = Area of the external standard
- A_i = Area of the analyte in the extract

The recovery of the analysis process will be calculated base on the ratio of remaining surrogate/initial surrogate (added before soxhlet extraction) as follows:

$$Recovery\ (\%) = \frac{\text{Concentration found (ng/g)} \times 100}{\text{Concentration spiked (ng/g)}}$$

The calculation program of MS analysis will checks retention time, isotope ratio and signal-to-noise ratio are within the range required by the quality control.

c) Limit of detection

Instrument Detection Limit (IDL) is the concentration equivalent to a signal, due to the analyte of interest, which is the smallest signal that can be distinguished from background noise by a particular instrument. The IDL should always be below the **method detection limit**, and is not used for compliance data reporting, but may be used for statistical data analysis and comparing the attributes of different instruments (*WDNRL, 1996*).

Detection limit of the MS instrument is calculated based on the standard solution with known concentration:

$$LOD_i = 3 \times C_{std} \times \frac{A_{noise}}{A_i}$$

Where:

- C_{std}: Concentration of standard
- A_{noise}: Area of the noise next to the i peak
- A_i: Area of the i peak

The calculation program of MS software will calculate the area of the peak and surrounding noise area. LOD of all PTSs analyzed by LRMS are presented in the table 4.10.

Table 4.10: Calculated limit of detection of PTSs compounds

No	Compound	LOD (ng/g)	Note
Organochlorinated pesticide			
1	Lindane	0.11	NCI - SIM
2	HCB	0.02	NCI - SIM
3	Heptachlor	0.66	NCI - SIM
4	Aldrin	0.14	NCI – SIM
5	O-chlordane	0.51	NCI – SIM
6	Heptachlor epoxide	0.51	NCI – SIM
7	g-Chlordane	0.10	NCI – SIM
8	a-chlordane	0.41	NCI – SIM
9	Endosulfan - I	0.41	NCI – SIM
10	Transnonachlor	0.43	NCI – SIM
11	pp'-DDE	0.15	NCI – SIM
12	Dieldrin	0.19	NCI – SIM
13	Endrin	1.12	NCI – SIM
14	Endosulfan-II	0.57	NCI – SIM
15	pp'-DDD	3.19	NCI – SIM
16	Endrin aldehyde	0.10	NCI – SIM
17	Endosulfan sulfate	0.03	NCI – SIM
18	pp'-DDT	3.67	NCI – SIM
19	Mirex	0.10	NCI – SIM
20	Toxaphen palar-26	5.01	NCI – SIM
21	Toxaphene palar-32	6.30	NCI – SIM
22	Toxaphene palar-50	4.63	NCI – SIM
23	Toxaphene palar-62	11.91	NCI – SIM
Polychlorinated Biphenyl			
1	PCB 28	1.79	NCI – SIM
2	PCB 52	4.05	NCI – SIM
3	PCB 101	6.03	NCI – SIM
4	PCB 153	0.40	NCI – SIM
5	PCB 138	0.79	NCI – SIM
6	PCB 180	0.02	NCI – SIM
7	PCB 170	0.03	NCI – SIM
8	PCB 81	3.15	NCI – SIM
9	PCB 77	3.12	NCI – SIM
10	PCB 123	1.50	NCI – SIM
11	PCB 118	1.35	NCI – SIM
12	PCB 114	0.90	NCI – SIM
13	PCB 105	1.82	NCI – SIM

14	PCB 126	0.02	NCI – SIM
15	PCB 167	0.01	NCI – SIM
16	PCB 156	0.01	NCI – SIM
17	PCB 157	0.02	NCI – SIM
18	PCB 169	0.02	NCI – SIM
19	PCB 189	0.01	NCI – SIM
Polybrominated Diphenylether			
1	PBDE 28	0.02	NCI – SIM
2	PBDE 49	0.03	NCI – SIM
3	PBDE 47	0.09	NCI – SIM
4	PBDE 66	0.05	NCI – SIM
5	PBDE 100	0.03	NCI – SIM
6	PBDE 119	0.04	NCI – SIM
7	PBDE 99	0.04	NCI – SIM
8	PBDE 85	0.47	NCI – SIM
9	PBDE 154	0.12	NCI – SIM
10	PBDE 153	0.48	NCI – SIM
11	PBDE 138	3.35	NCI – SIM
12	PBDE 183	8.95	NCI – SIM

Base on LOD values in table 4.10, we can calculate LOD for each real matrix type as follows:

$$LOD_{matrix} = \frac{\mathrm{LOD_i} \times \mathrm{m_{final}}}{\mathrm{m_{matrix}}}$$

Where:

- LOD_i: Limit of detection for real matrix type
- m_{final}: final weight of extract before injecting (g)
- m_{matrix}: Initial weight of matrix analyzed (g)

The calculated limit of detection for real matrix types are presented in the table 4.11 below.

Table 4.11: Calculated limit of detection of PTSs for real matrices (NCI-SIM)

No	Compound	LOD $_{matrix}$		
		Soil/sediment (pg/g)	**Fish (pg/g lipid)**	**Human milk (pg/g lipid)**
Organochlorine pesticide				
1	Lindane	3.85	6.09	6.42
2	HCB	0.70	1.11	1.17
3	Heptachlor	23.10	36.54	38.50
4	Aldrin	4.90	7.75	8.17
5	O-chlordane	17.85	28.23	29.75

6	Heptachlor epoxide	17.85	28.23	29.75
7	g-Chlordane	3.50	5.54	5.83
8	a-chlordane	14.35	22.7	23.92
9	Endosulfan - I	14.35	22.7	23.92
10	Transnonachlor	15.05	23.80	25.08
11	pp'-DDE	5.25	8.30	8.75
12	Dieldrin	6.65	10.52	11.08
13	Endrin	39.20	62.00	65.33
14	Endosulfan-II	19.95	31.56	33.25
15	pp'-DDD	111.65	176.60	186.08
16	Endrin aldehyde	3.50	5.54	5.83
17	Endosulfan sulfate	1.05	1.66	1.75
18	pp'-DDT	128.45	203.17	214.08
19	Mirex	3.50	5.54	5.83
20	Toxaphen palar-26	175.28	277.24	292.13
21	Toxaphene palar-32	220.43	348.66	367.38
22	Toxaphene palar-50	162.09	256.37	270.14
23	Toxaphene palar-62	416.85	659.34	694.75
Polychlorinated Biphenyl				
1	PCB 28	62.65	99.09	104.42
2	PCB 52	141.75	224.21	236.25
3	PCB 101	211.05	333.82	351.75
4	PCB 153	14.00	22.14	23.33
5	PCB 138	27.65	43.73	46.08
6	PCB 180	0.70	1.11	1.17
7	PCB 170	1.05	1.66	1.75
8	PCB 81	110.25	174.38	183.75
9	PCB 77	109.20	172.72	182.00
10	PCB 123	52.50	83.04	87.50
11	PCB 118	47.25	74.74	78.75
12	PCB 114	31.50	49.82	52.50
13	PCB 105	63.70	100.76	106.17
14	PCB 126	0.70	1.11	1.17
15	PCB 167	0.35	0.55	0.58
16	PCB 156	0.35	0.55	0.58
17	PCB 157	0.70	1.11	1.17
18	PCB 169	0.70	1.11	1.17
19	PCB 189	0.35	0.55	0.58
Polybrominated diphenyl ether				
1	PBDE 28	0.70	1.11	1.17

2	PBDE 49	1.05	1.66	1.75
3	PBDE 47	3.15	4.98	5.25
4	PBDE 66	1.75	2.77	2.92
5	PBDE 100	1.05	1.66	1.75
6	PBDE 119	1.40	2.21	2.33
7	PBDE 99	1.40	2.21	2.33
8	PBDE 85	16.45	26.02	27.42
9	PBDE 154	4.20	6.64	7.00
10	PBDE 153	16.80	26.57	28.00
11	PBDE 138	117.25	185.46	195.42
12	PBDE 183	313.25	495.47	522.08

From the LOD values of PCBs group in table 4.11 we can see that NCI – SIM mode is very sensitive for high-chlorinated PCBs (from hexachlor to octachlor).

d) Linearity range

The linearity range for each PTSs compound was investigated in the range of 11.79–1280 ng/g (OCls pesticides), 7–354 ng/g (6 indicator PCB congeners), 1.4–664 ng/g (12 dioxin-like PCB congeners) and 20–490 ng/g (toxaphene).

Calibration curves were obtained with five calibration levels of PTSs standards, prepared as a mixture in isooctane (see annex for all calibration curve of PTSs compounds). From these linearity ranges of PTSs, we can be sure that the PTSs' abundances are linear with their concentrations in these concentration intervals. In our case, all concentrations range investigated had relatively good linearity. Figure 4.13 shown one example for linearity of pp'-DDD using range of 11.8 ng/g–1200 ng/g calibration standards. The calibration curve has linearity very high with the value of R2 = 0.998061

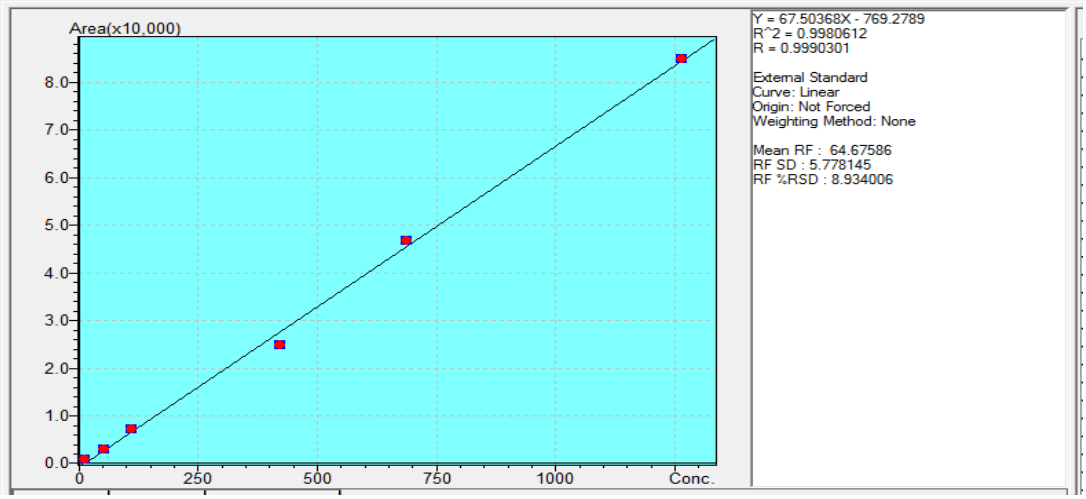


Figure 4.13: Linearity of pp'-DDD with calibration standards (11.8 – 1280 ng/g)

e) Recovery of the analysis method

Recovery is the term used to indicate the yield of an analyte in an analytical method. It is calculated by analyzing a sample with known concentrations (e.g., a control sample) and

comparing the measured value with the true value as spiked with the matrix. Because this accuracy assessment measures the effectiveness of sample preparation, care should be taken to mimic the actual sample preparation as closely as possible. The expected recovery depends on the sample matrix, the sample processing procedure and the analyte concentration.

We have analyzed spiked samples with the added PTSs standards of approximately 50ng. Recovery was calculated for three types of matrices, which are soil/sediment, fish and human breast milk samples. The recoveries for every matrix are presented in table 4.12 below.

Table 4.12: Recovery of PTSs analytical method applied for soil/sediment, fish and human breast milk samples

No	Compound	Recovery (n = 3 for every type of matrix)					
		Sediment / Soil (%)	STDEV	Fish (%)	STDEV	Human milk (%)	STDEV
Organochlorinated pesticide							
1	a-HCH	95	**4**	94	**4**	82	**10**
2	HCB	82	**8**	89	**5**	83	**4**
3	b-HCH	103	**10**	88	**5**	91	**9**
4	g-HCH	99	**6**	91	**14**	83	**6**
5	d-HCH	108	**7**	85	**7**	83	**2**
6	Heptachlor	97	**7**	95	**8**	91	**7**
7	Aldrin	84	**2**	105	**9**	82	**4**
8	O-chlordane	83	**4**	84	**3**	88	**5**
9	Hept. epoxide	90	**3**	100	**7**	91	**4**
10	g-Chlordane	82	**4**	96	**6**	90	**8**
11	a-chlordane	86	**7**	92	**4**	83	**8**
12	Endosulfan - I	86	**5**	99	**9**	96	**15**
13	Transnonachlor	85	**5**	89	**13**	91	**5**
14	pp'-DDE	91	**2**	95	**3**	99	**5**
15	Dieldrin	107	**4**	88	**5**	91	**13**
16	Toxaphene 26	88	**3**	87	**3**	89	**4**
17	Endrin	90	**3**	97	**12**	83	**3**
18	Endosulfan-II	81	**2**	88	**5**	93	**6**
19	pp'-DDD	83	**7**	92	**3**	93	**7**
20	Toxaphene 32	86	**6**	97	**6**	86	**7**
21	Endrin aldehyde	83	**4**	93	**4**	84	**7**
22	Endosulfan sulfate	92	**6**	86	**3**	89	**4**
23	pp'-DDT	91	**8**	94	**6**	91	**10**
24	Toxaphene 50	90	**7**	94	**14**	86	**7**
25	Toxaphene 62	86	**5**	89	**8**	91	**5**
26	Mirex	91	**6**	85	**8**	87	**10**
Polychlorinated Biphenyl							
1	PCB 28	80	**3**	86	**5**	88	**6**

2	PCB 52	81	**15**	86	**4**	89	**4**
3	PCB 101	102	**11**	95	**10**	100	**5**
4	PCB 153	101	**12**	90	**7**	97	**7**
5	PCB 138	108	**17**	98	**7**	97	**10**
6	PCB 180	87	**4**	94	**8**	98	**7**
7	PCB 170	88	**6**	95	**4**	102	**7**
8	PCB 149	89	**9**	89	**8**	87	**3**
9	PCB 128	89	**7**	109	**9**	93	**5**
10	PCB 81	103	**12**	91	**4**	86	**10**
11	PCB 77	105	**13**	93	**8**	84	**3**
12	PCB 123	101	**13**	87	**4**	94	**10**
13	PCB 118	111	**6**	100	**6**	100	**5**
14	PCB 114	95	**3**	91	**6**	93	**12**
15	PCB 105	102	**7**	106	**17**	93	**9**
16	PCB 126	86	**4**	98	**16**	93	**7**
17	PCB 167	91	**5**	100	**7**	100	**7**
18	PCB 156	88	**6**	92	**6**	97	**8**
19	PCB 157	91	**1**	92	**5**	97	**12**
20	PCB 169	80	**3**	103	**14**	95	**8**
21	PCB 189	80	**4**	92	**6**	91	**9**
Polybrominated diphenyl ether							
1	PBDE 28	91	**8**	83	**3**	89	**5**
2	PBDE 49	93	**4**	81	**1**	85	**8**
3	PBDE 47	91	**2**	89	**5**	89	**8**
4	PBDE 66	88	**5**	88	**5**	92	**8**
5	PBDE 100	92	**9**	89	**8**	88	**6**
6	PBDE 119	103	**1**	86	**4**	94	**10**
7	PBDE 99	104	**2**	98	**9**	96	**5**
8	PBDE 85	85	**8**	97	**13**	95	**4**
9	PBDE 154	83	**2**	95	**2**	88	**7**
10	PBDE 153	82	**2**	97	**16**	89	**10**
11	PBDE 138	86	**6**	78	**8**	81	**3**
12	PBDE 183	81	**7**	80	**9**	82	**17**

From the table 4.12 we can see that the recovery values of the analytical methods (for three types of the matrices are soil/sediment, fish and human milk) are good, these values situate in the range of 80 – 110% with the standard deviation values are below 10. That means method's repeatability is relatively stable. The next step is performing validation method with SRMs/CRMs samples.

4.6 Validation of selected analytical methods

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

One method for validating analytical procedures is by analyzing well-characterized reference materials. Firstly, the validation of selected analytical methods is carried out with spiked samples (e.g: for testing the analytical method for soil/sediment analysis we used Merck quartz (clean sand) spiked with PTSs standard solutions). In case the recovery obtained is good, we continued to test the analytical method with Standard Reference Material/Certificate Reference Material (SRMs/CRMs). In our research, we used SRM 1941a (from NIST) for testing analytical method for soil/sediment and CRM EDF-2525 (from CIL) for biological sample case.

4.6.1 Testing analytical method for soil and sediment samples

In recent years the National Institute of Standards and Technology (NIST) has developed several Standard Reference Materials (SRMs) to assist in validating measurements of PTSs compounds such as SRM 1588, 1939, 1974 and 1941. Among them, SRMs 1941a (marine sediment) has been prepared for use as a Performance Evaluation for PTSs analysis of soils/sediment. This reference material is designed to be useful for low resolution GC/MS analysis of PTSs compounds.

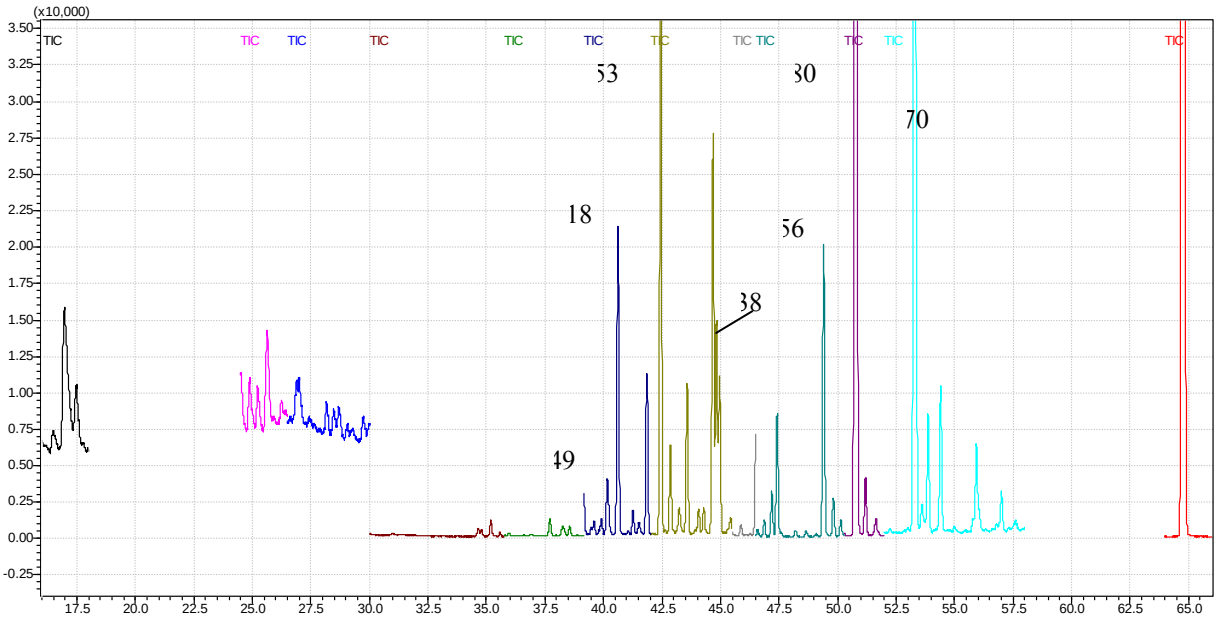


Figure 4.14: Chromatogram of SRM-1941a extract for PCBs determination (NCI-SIM)

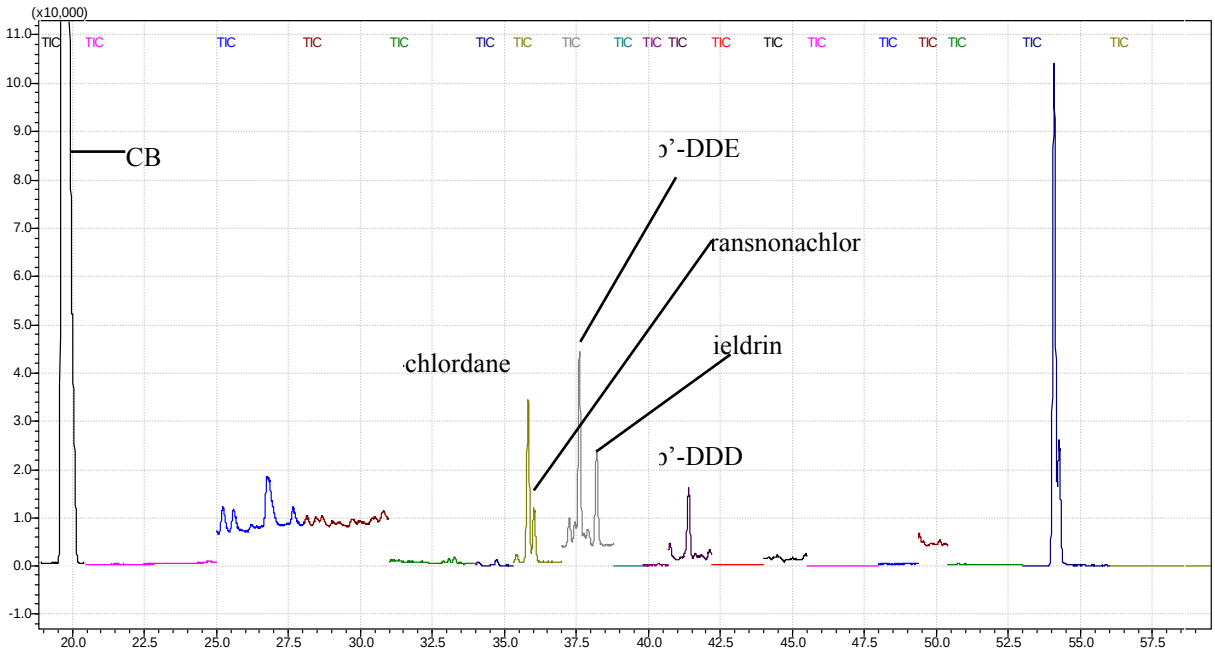


Figure 4.15: Chromatogram of SRM-1941a extract for OCPs determination (NCI-SIM)

The testing result of SRM-1941a is presented in the table 4.13. Generally, the analyzed PCBs and OCls pesticides concentrations for SRM-1941a samples were relatively low in comparison with certified values. The reason may be come from a little bit difference between the PTSs standard concentrations used to quantify or the lost of PTSs during the preparation procedure. However, the PTSs analyzed concentrations were acceptable in comparison with the certified values done by producer. This means the selected analytical method was effective for such contaminated soil/sediment types.

Table 4.13: Result of SRM-1941a test (n = 2)

PTSs compound		Analyzed Conc. (ng/g)	Certified Conc. (ng/g)
Organochlorinated pesticide			
1	HCB	49.81 – 52.39	70 ± 25
2	a-chlordane	1.96 - 2.08	2.33 ± 0.56
3	pp'-DDE	6.22 – 6.42	6.59 ± 0.56
4	Dieldrin	1.64 – 1.68	1.26 ± 0.37
5	pp'-DDD	6.82 – 7.33	5.06 ± 0.58
6	pp'-DDT	0.90 – 1.02	1.25 ± 0.10
Polychlorinated Biphenyl			
1	PCB 52	4.52 – 5.80	6.89 ± 0.56
2	PCB 101	8.30 – 10.85	11.0 ± 1.6
3	PCB 153	10.13 – 13.99	17.6 ± 1.9
4	PCB 138	7.42 – 12.27	13.38 ± 0.97
5	PCB 180	4.23 – 5.00	5.83 ± 0.58
6	PCB 170	2.39 – 3.02	3.00 ± 0.46

7	PCB 118	6.71 – 7.67	10.0 ± 1.1
8	PCB 105	2.56 – 3.22	3.65 ± 0.27
9	PCB 156	0.54 – 0.67	0.93 ± 0.14

Based on the obtained results, we can see that our selected analytical method is acceptable for soil/sediment matrix. This SRM sample did not include PBDEs and toxaphene compounds because we could not afford more SRM sample for all PTS surveyed. However, in spiked samples, the analytical method presented the acceptable recovery values of PBDEs and toxaphenes (Table 4.12).

4.6.2 Testing analytical method for biological samples

In case of biological samples, we used CRM EDF-2525 from Cerilliant Corporation (CIL) for test our analytical method. CRM EDF-2525 is a sample of homogeneous fish matrix from Lake Ontario lake trout, a fresh water fish species found in Canada. This sample is intended for use in evaluating the performance of an analytical method for PTSs compounds such as PCDD/Fs, PCBs, PBDEs and OCls pesticides in complex lipophilic matrices.

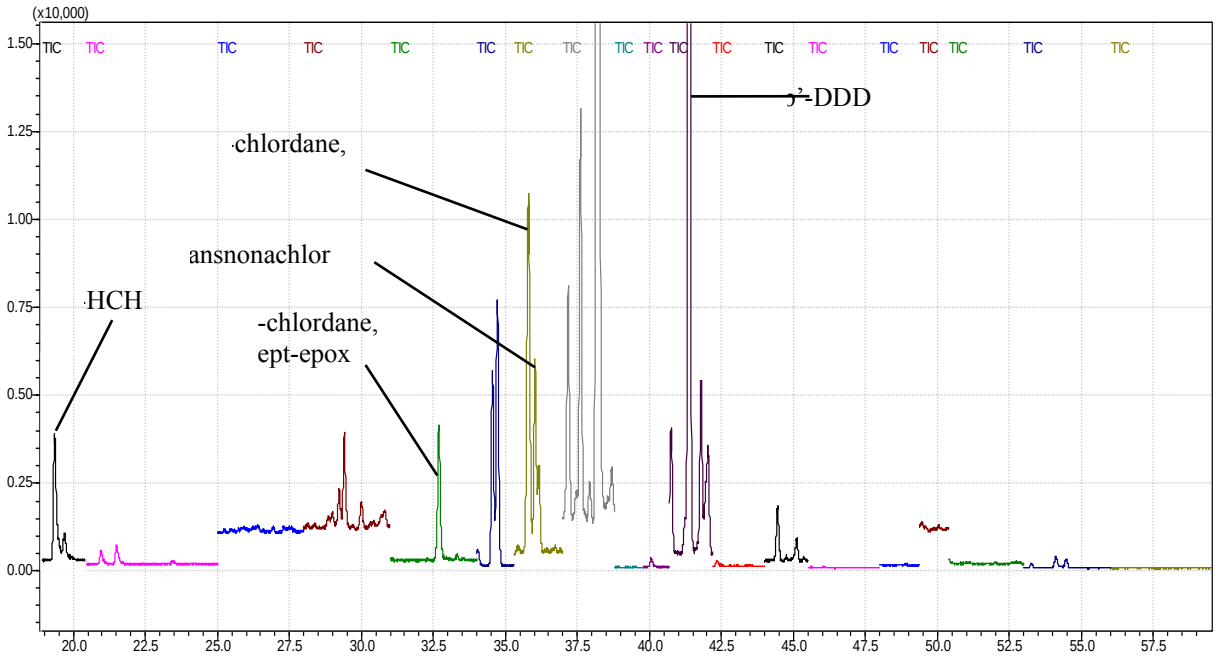


Figure 4.16: Chromatogram of CRM-EDF-2525 extract (fraction 2) for OCPs determination (NCI-SIM)

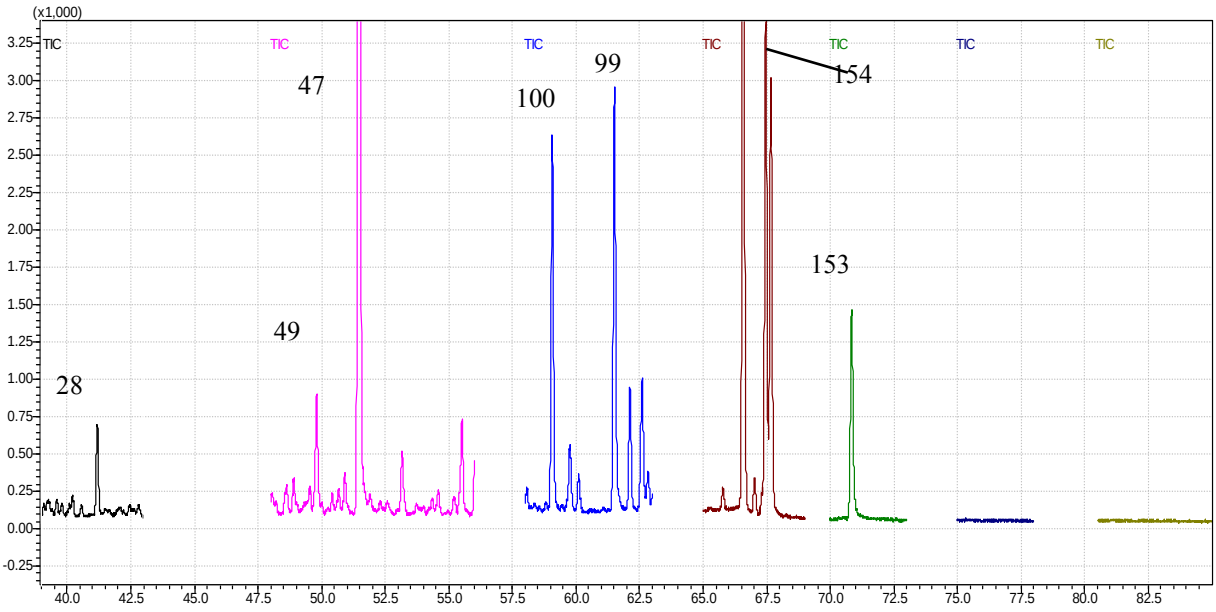


Figure 4.17: Chromatogram of CRM-EDF-2525 extract (fraction 1) for PBDEs determination (NCI-SIM)

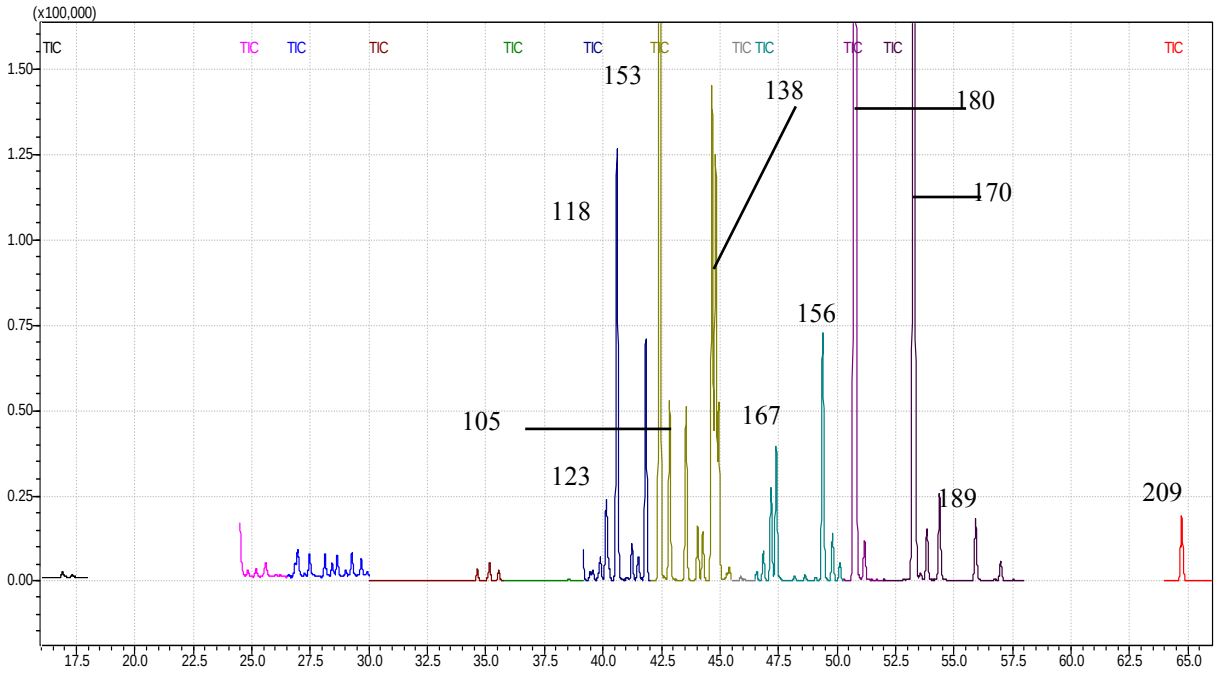


Figure 4.18: Chromatogram of CRM-EDF-2525 extract (fraction 1) for PCBs determination (NCI-SIM)

From the Fig. 4.16, 4.17 and 4.18 we can see the chromatogram of PTSs compounds with the sharp peaks as well as properly separated

The testing result of CRM-1941a is presented in the table 4.14. PBDE 183 was non-detected because their concentration is lower than LOD of our LRMS (*see Table 4.11, section 4.5.2.c,*

chapter 4). Concentrations of other PTSs were acceptable in comparison with the certified values done by producer as shown in table 4.14.

Table: 4.14: Result of CRM EDF-2525 test (n = 1)

PTSs compound		Analyzed Conc. (ng/g)	Certified Conc. (ng/g)
Organochlorinated pesticide			
1	a-chlordane	26.9	35.6 ± 28.7
2	pp'-DDD	74.9	93.4 ± 71.8
3	pp'-DDE	471.9	572 ± 158
4	Dieldrin	46.4	51.6 ± 16.1
5	a-HCH	1.49	2.29 ± 1.41
6	b-HCH	0.953	0.795 ± 0.171
7	g-HCH	0.465	0.443 ± 0.161
8	Heptachlor epoxide	12.87	11.1 ± 10.4
9	HCB	12.0	16.4 ± 6.37
10	Mirex	103	115 ± 95.2
11	Cis-nonachlor	-	37.0 ± 15.1
12	Trans-nonachlor	30.6	46.8 ± 98.0
13	Oxychlordane	12.7	16 ± 4.28
Polychlorinated Biphenyl			
1	PCB 28	6.81	7.98 ± 1.31
2	PCB 52	16.0	28 ± 13.7
3	PCB 101	73.6	88 ± 42.3
4	PCB 153	178.9	244 ± 35.7
5	PCB 138	157.2	200 ± 98.6
6	PCB 180	78.6	109 ± 12.3
7	PCB 170	45.1	36.8 ± 3.41
8	PCB 149	60.6	67.7 ± 17.5
9	PCB 128	20.3	31.8 ± 11.1
10	PCB 81	0.353	0.179 ± 0.035
11	PCB 77	2.31	1.98 ± 0.659
12	PCB 123	5.23	8.41 ± 14.7
13	PCB 118	141.9	112 ± 32.0
14	PCB 114	4.34	3.39 ± 1.01
15	PCB 105	63.6	46.8 ± 18.0
16	PCB 126	0.922	0.647 ± 0.211
17	PCB 167	9.65	7.32 ± 2.01
18	PCB 156	14.9	12.6 ± 2.56
19	PCB 157	4.117	3.29 ± 0.619
20	PCB 169	0.103	0.056 ± 0.012

21	PCB 189	1.774	1.48 ± 0.287
Polybrominated dipheny ether			
1	PBDE 28	1.8	1.5 ± 2.25
2	PBDE 47	9.79	8.17 ± 10.4
3	PBDE 99	1.39	1.91 ± 3.04
4	PBDE 100	0.89	1.36 ± 2.05
5	PBDE 153	0.55	1.98 ± 1.51
6	PBDE 154	1.78	2.06 ± 2.18
7	PBDE 183	nd	0.146 ± 0.075

-: data not available; nd: not detectable (Table 4.11, section 4.5.2.c, chapter 4)

4.7 Conclusions

We have some conclusions from establishment and validation analytical method procedure for PTSs compounds as follow:

- The analytical methods were established for PTSs measurement with various matrices as soil/sediment, fish and human breast milk. Method can simultaneously measure PTSs like PCBs, OCPs and PBDEs with GC/MS and NCI-SIM mode.
- Sensitivity and selectivity to PTSs compounds of NCI-MS technique are good enough and satisfied for PTSs analysis in trace levels through LOD of the method presented in table 4.11.
- The method recovery is good, range in 80 – 110% in three types of matrices investigated and had good repeatability through standard deviation values, major of them are lower ten value (table 4.12).
- Finally, the selected analytical methods are effective and present good results when applied for SRM 1941a and CRM EDF-2525 samples (table 4.13 and 4.14) in validation method procedure and they are ready to serve our research now.

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Chapter 5: PTSs CONTAMINATION IN SOIL AND SEDIMENT

5.1 Introduction

As we presented in our research plan, soil and sediment were the first matrices selected for our research as they act as a sink for PTSs compounds. Furthermore, the rapid industrial and agricultural growth in Hochiminh city and Mekong Delta result in to the widespread contamination of PTSs in soil and sediment (*Anh et al., 2003; Phuong et al., 1998; Minh et al., 2004, 2006, 2007a, 2007b*).

Due to PTSs' hydrophobic property, they are easily adsorbed on suspended particles in water and air, then deposit to the soil and sediment. Soil and sediment are usually PTSs' primary receiver when these compounds penetrate the environment. Therefore, soil and sediment can be sensitive indicators for both spatial and temporal trends when we attempt to assess PTSs contamination in the ecosystem.

Based on the previous studies on PTSs in Vietnam and usage of pesticides (particularly organochlorine insecticides) and PCBs in the past, we selected the areas for our research on PTSs in soil and sediment matrices as follow:

- Dongthanh Landfill – Hochiminh city
- Agrichemical store – Longan province
- Thinghe canal – Saigon river area – Hochiminh city
- Thivai river basin – Dongnai province
- Tien river basin – Tiengiang province

All sampling locations was presented in Fig. 5.1 below.

5.1.1 DongThanh Landfill – Hochiminh city

DongThanh landfill is situated in DongThanh village, HocMon district, on the north of Hochiminh city (HCMC). It had been a spontaneous dumping site in the 1980s and is one of the old dumping sites in HCMC. This 30 ha dumping site officially became the landfill for HCMC urban area in 1990. From 2005, this landfill has received only constructional waste of HCMC (*DONRE, 2007*).

The sampling was done in the foot of the dump. The entire waste hill was covered with a layer of soil which was taken from another place. We could take only the soil sample in the depth of 1 meter with the soil borer tool. This landfill is located close to populated areas, and previously, a large number of people scavenged in this landfill for collecting recycling wastes.

The investigation and soil sampling in DongThanh landfill - HCMC were done in the end 2006. We collected 4 composite soil samples described in table 5.1 below.

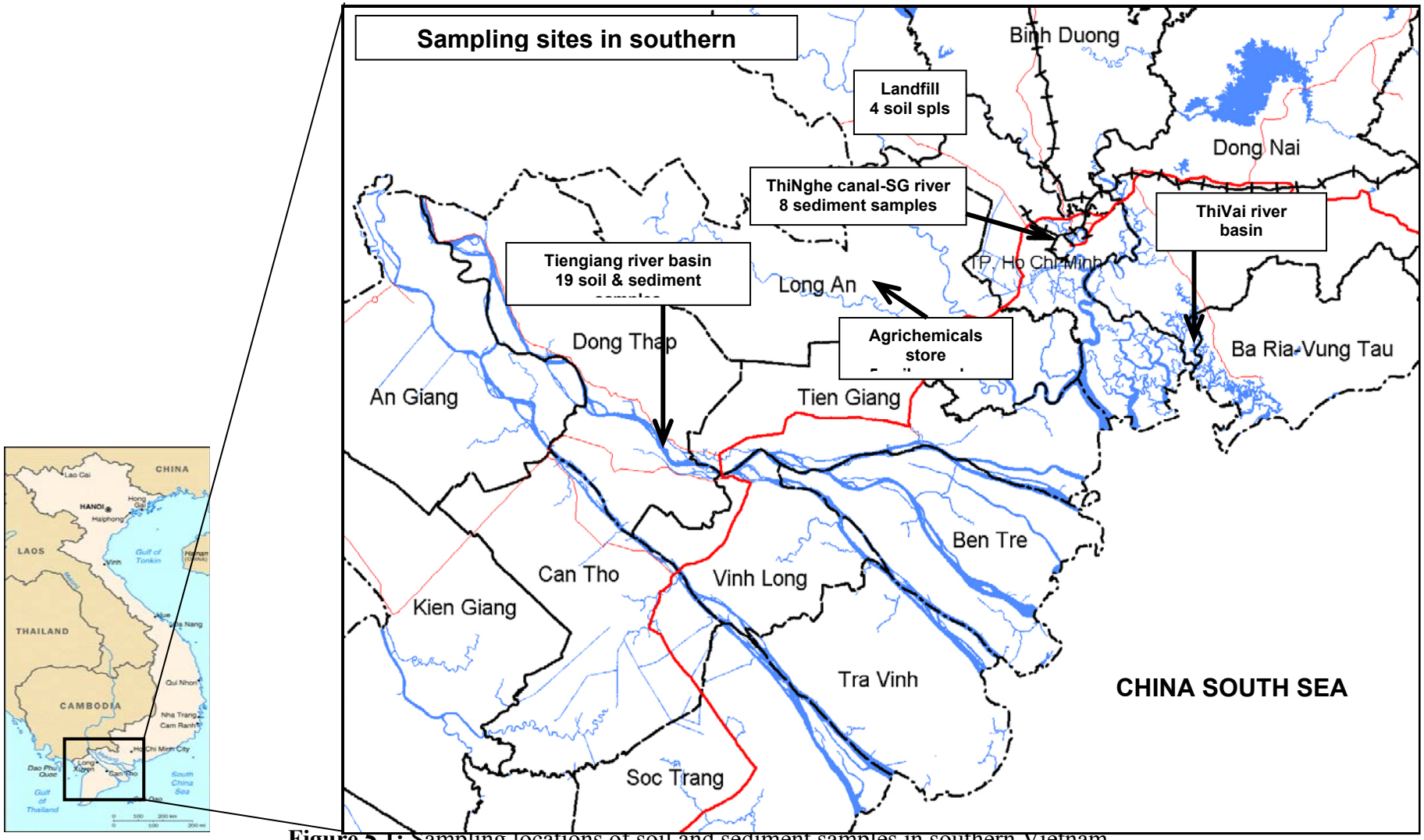


Figure 5.1: Sampling locations of soil and sediment samples in southern Vietnam

Table 5.1: Soil sampling positions in DongThanh landfill - HCMC

No	Sample code	GPS positions	Note
1	DT-1	N: $10^0$54'35.6" E: $106^0$38'37.0"	All soil samples were taken in the foot of the rubbish hill.
2	DT-2	N: $10^0$54'31.6" E: $106^0$38'34.9"	
3	DT-3	N: $10^0$54'30.3" E: $106^0$38'31.9"	
5	DT-4	N: $10^0$54'25.9" E: $106^0$38'25.2"	

5.1.2 Agrichemical warehouse site – Longan province

This site is located at ThanhXuan district, Ward 5, TanAn town, LongAn province. This place has been the agrichemical warehouse of LongAn Branch of Plant Protection (LABPP) – LongAn Department of Agriculture and Rural Development (LADARD) since the 1980s. According to the warehouse manager, only expired, spoilt, and confiscated pesticides have been kept in this warehouse since 2006. We took five composite soil samples around an agrichemical warehouse with introduction of a person from Department of Natural Resources and Environment of LongAn. Five soil samples were code from LA-1 to LA-5 and GPS position of sampling site is N: $10^0$32'27.8"- E: $106^0$25'10.2". The sampling site is shown in Fig. 5.2 below.

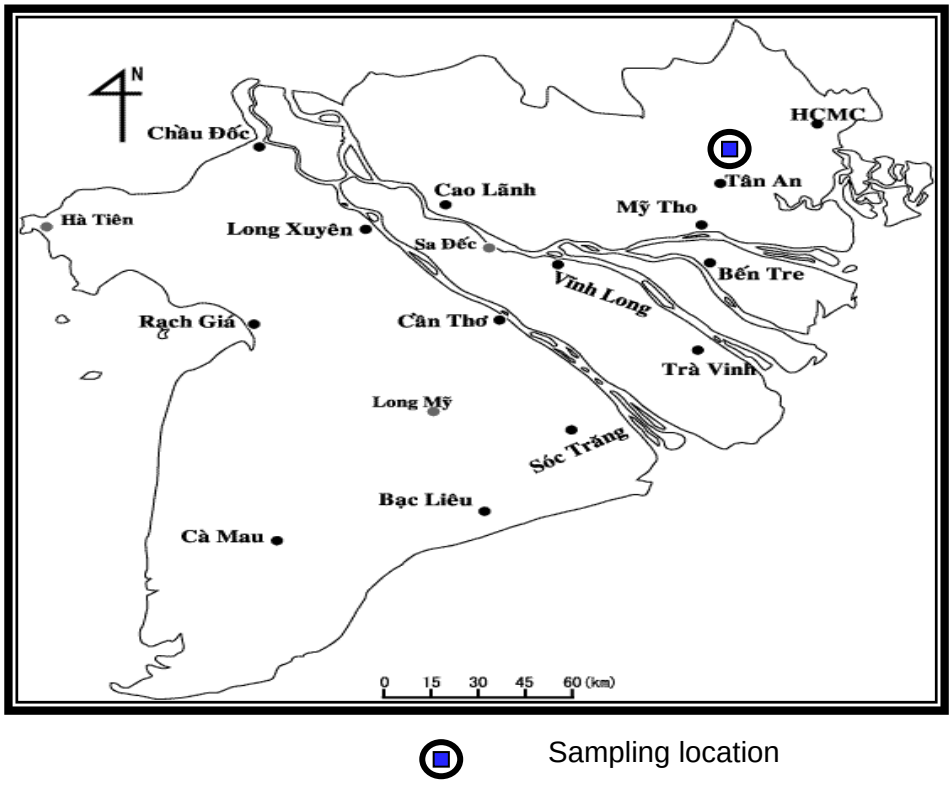


Figure 5.2: Sampling sites of soil samples in agrichemical warehouse LongAn province

5.1.3 Thinghe canal – Saigon river confluence

Hochiminh city (HCMC) is the biggest city in the Southeast Asia. It plays a very important role for industrial, economic, cultural and social development in South of Vietnam. HCMC is located 1,725 km south of Hanoi and 50 km west of the South China Sea. It has the area of 2,056 square km and population of 8 millions. It is a big political, economical and industrial center of Vietnam. The city is also a cluster of hundreds of small river and channels watering the Mekong Delta. Rapid development in this region, however, has raised concerns about the local environment and ecological integrity (*Anh et al., 2003*).

Large amounts of untreated municipal and industrial wastewater as well as accidental spills are released directly into the canal systems of the river. Besides, municipal solid wastes are dumped in open areas with poor management and, therefore, runoff from flood and rain events carry various toxic contaminants from these sites to the surface waters (*Minh et al., 2007a*). The aquatic urban ecosystem is strongly influenced by long term discharge of untreated domestic and industrial wastewaters, stormwater runoff, accidental spills and direct solid waste dumping (*Phuong et al., 1998*).

ThiNghe canal is one of the seriously polluted canal systems in HCMC. The investigation and sampling in ThiNghe canal basin was done in November 2006. The sediment samples were taken from 8 positions along the downstream of ThiNghe canal and ThiNghe canal – Saigon river confluence (TN-SG). The sampling sites are shown in Fig. 5.3 and described in the table 5.2 below.

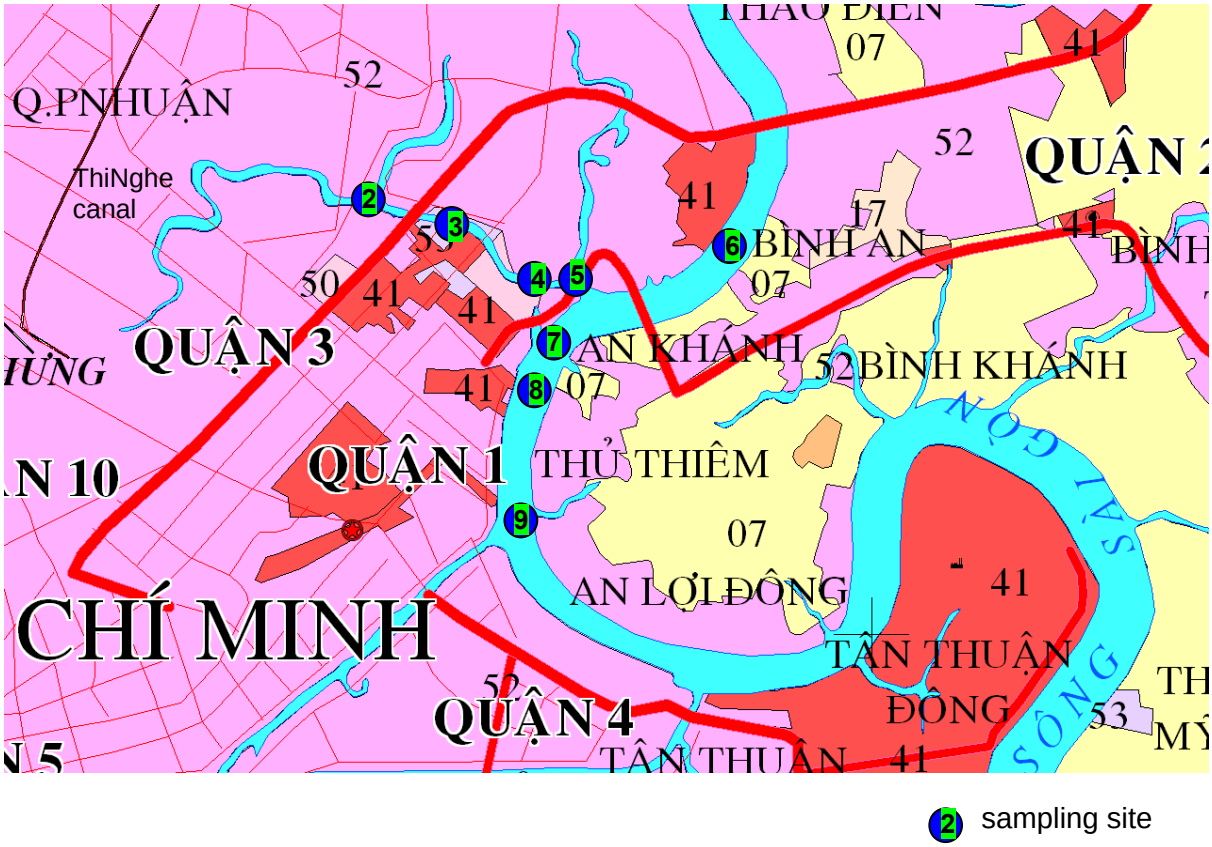


Figure 5.3: Sampling sites of sediment samples in ThiNghe canal – Saigon River

Table 5.2: Description of ThiNghe canal-Saigon river sediment samples

No	Sample code	GPS positions	Note
1	BS-2	N: $10^0$47'36.5" E: $106^0$41'45.9"	Upstream, 100m from Bong bridge
2	BS-3	N: $10^0$47'35.6" E: $106^0$42'2.22"	Downstream, 100m from DienBienPhu bridge
3	BS-4	N: $10^0$47'14.6" E: $106^0$42'34.0"	Downstream, 50m from NguyenHuuCanh bridge
4	BS-5	N: $10^0$47'15.7" E: $106^0$42'47.8"	Downstream, 50m from Sat bridge
5	BS-6	N: $10^0$47'20.3" E: $106^0$43'35.5"	Upstream, 1000m from ThiNghe canal-Saigon river confluence
6	BS-7	N: $10^0$46'48.7" E: $106^0$42'28.3"	Bason Dockyard, Saigon river
7	BS-8	N: $10^0$46'24.6" E: $106^0$42'24.4"	End of Bason Dockyard, saigon river
8	BS-9	N: $10^0$46'4.6" E: $106^0$42'22.3"	BenNghe canal – Saigon river confluence

5.1.4 Thivai river basin – Dongnai and Baria Vungtau province

Thivai river which has area of 120 square km rises from LongThanh district, Dongnai province and flows a distance of 70 km through BaRia – VungTau province. It joins GoGia river near the end of Caimep industrial zone and goes to East Sea at GanhRai gulf (*SEO., 2008*).

There are many industrial zones located along Thivai river basin such as: NhonTrach industrial zones (NhonTrach I, II, III, IV, V, VI), Vedal – GoDau industrial zone, MyXuan industrial zone, Holcim cement factory, PhuMy industrial zone, CaiMep industrial zone. In addition, there are some river port as Vedan port, GoDau port, PhuMy port and Caimep port. All these industrial zones and river ports have been the main polluted sources to Thivai river basin since the 1990s.

We performed a survey and sampling campaign in Thivai river basin by June and November 2006. At each sampling site, sediment samples were taken in both the banks of river and then a composite sediment sample was prepared by mixing the sediment samples taken in both riversides. Eighteen sediment samples were taken along the river from the upstream to downstream. The sampling sites were selected near the places affected by industrial zones. In IER lab, the samples were dried, treated and kept in brown glass bottle at low temperature freezer (about -20^0C) until analysis. Eighteen sampling sites are described in Fig. 5.4 and table 5.3 below.

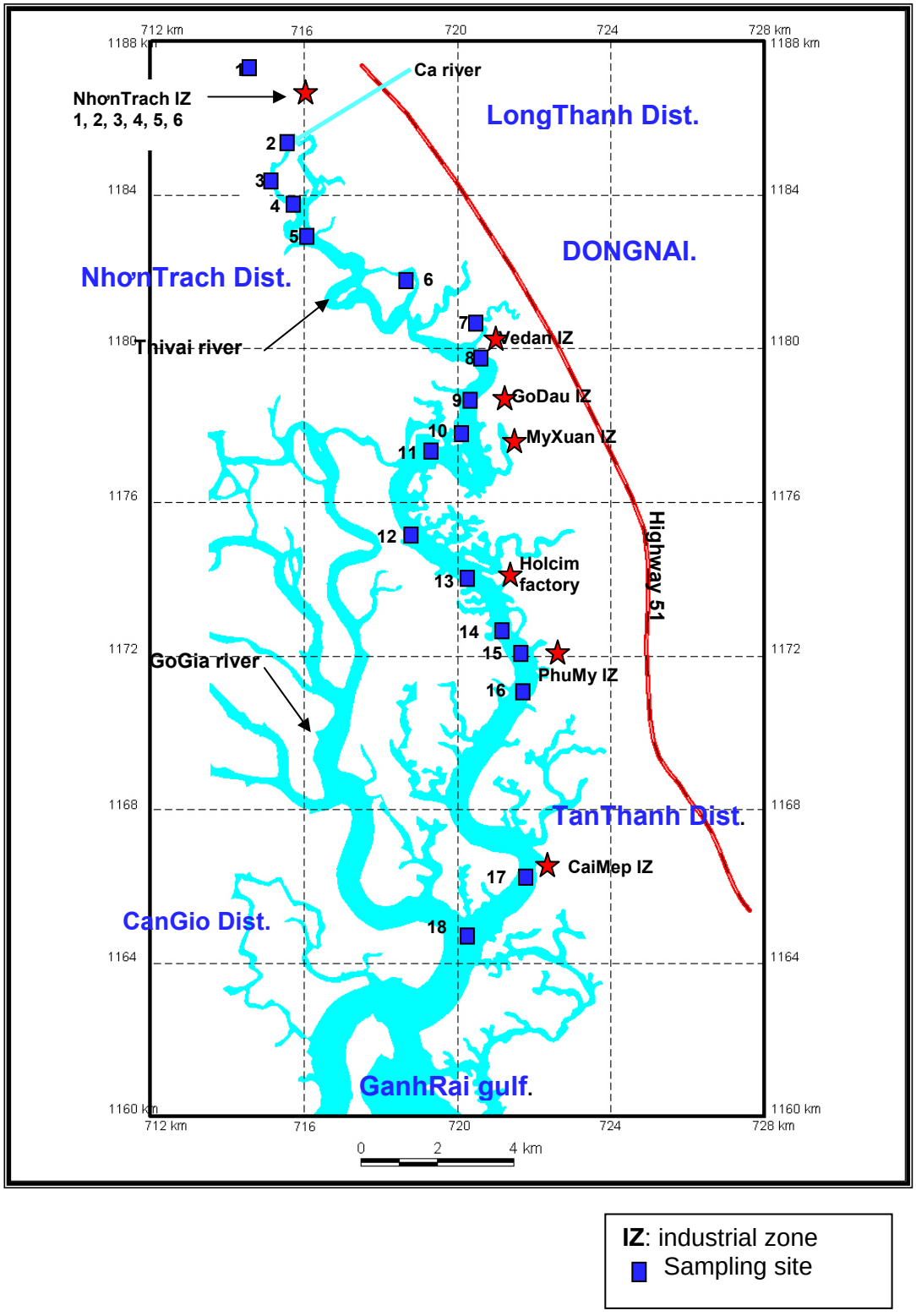


Figure 5.4: Sampling sites of sediment samples in Thivai river basin

Table 5.3: Description on sampling sites of sediment samples in Thivai river basin

No	Sample code	GPS positions	Note
1	TV-1	N: $10^0$42'55.2" E: $106^0$57'36.6"	Loren bridge, small canal, upstream of Thivai river
2	TV-2	N: $10^0$41'51.1" E: $106^0$58'11.2"	Upstream, 1000m from LongTho guard post
3	TV-3	N: $10^0$41'38.9" E: $106^0$58'22.3"	Upstream, 400m from LongTho guard post
4	TV-4	N: $10^0$41'30.5" E: $106^0$58'29.9"	LongTho guard post, Thivai river
5	TV-5	N: $10^0$41'00.2" E: $106^0$59'06.3"	MatTroi canal – Thivai river confluence. Downstream, 2000m from LongTho guard post
6	TV-6	N: $10^0$40'45.1" E: $106^0$59'42.4"	BaBip canal, 500m from BaBip-Thivai confluence
7	TV-7	N: $10^0$40'02.5" E: $107^0$00'57.6"	Vedan sewer, location in NuocLon canal, 300m from NuocLon-Thivai confluence
8	TV-8	N: $10^0$39'39.8" E: $107^0$01'01.3"	Vedan port, Thivai river
9	TV-9	N: $10^0$39'23.8" E: $107^0$01'09.6"	Godau port, Thivai river
10	TV-10	N: $10^0$38'34.9" E: $107^0$01'41.1"	Myxuan industrial zone, Thivai river
11	TV-11	N: $10^0$38'10.4" E: $107^0$00'10.2"	TacCaiChung, light buoy 25, Thivai river
12	TV-12	N: $10^0$36'48.9" E: $107^0$00'29.2"	Upstream, 800m from Holcim factory
13	TV-13	N: $10^0$36'16.5" E: $107^0$01'06.8"	Holcim factory, Thivai river
14	TV-14	N: $10^0$35'41.8" E: $107^0$01'30.5"	Sewer of Phumy Thermal Power Plant
15	TV-15	N: $10^0$35'02.9" E: $107^0$01'36.8"	PhuMy port, Thivai river
16	TV-16	N: $10^0$34'32.7" E: $107^0$01'32.4"	Downstream, 200m from PhuMy port
17	TV-17	N: $10^0$32'03.5" E: $107^0$01'30.0"	CaiMep port, Thivai river
18	TV-18	N: $10^0$31'15.5" E: $107^0$00'36.9"	Thivai – GoGia confluence

5.1.5 Tien river basin – Mekong Delta

The Mekong River is the longest river in southeastern Asia, which rises from the Tibetan plateau and flows a distance of almost 4800 km through 6 countries: China, Myanmar, Laos, Thailand, Cambodia and Vietnam. The Mekong River basin with an area of 795,000 thousand square kilometers is an important habitat for approximately 60 million people (*MRC, 2008*).

From Phnom Penh, Cambodia, Mekong river water flows into Vietnam through the Tien river (Mekong) and Hau river (Bassac). After My Thuan Bridge position at highway 1A, Vietnam, Tien river divaricate into many effluents such as Co Chien river, Ham Luong river, Cua Dai river and CuaTieu river and these rivers flow into the East Sea follow the South - East direction. Tien river plays a very important role for the social and economic development of the five provinces of Mekong Delta (DongThap, TienGiang, BenTre, VinhLong and AnGiang province). This river is one of two main source of water supply for agriculture and fisheries in southern Vietnam. Rice production is a major agronomic activity in this area (*Minh et al., 2007b*). The new urban, resident, industrial zones and vast rice field established along Tien river basin have been the main sources of PTSs pollution.

The sampling campaigns were performed in the end of 2007. Nineteen soil and sediment samples were taken along Tien River basin from upstream to downstream. There are five soil samples taken from fruit-tree gardens and paddy fields besides fourteenth sediment samples. The description of sampling sites is presented in the table 5.4 and Fig. 5.5 below.

Table 5.4: Description of Tien River soil and sediment samples

No	Code	GPS positions	Sample type	Location
1	TG-1	N: $10^0$46’18.3” E: $105^0$21’57.4”	Sediment	AnHoa hamlet, AnBinh village, HongNgu dist. 800m from MuongLon bridge
2	TG-2	N: $10^0$41’56.4” E: $105^0$22’56.1”	Sediment	AnThinh hamlet, AnLong village, TamNong dist. 500m from AnLong bridge
3	TG-3	N: $10^0$39’58.9” E: $105^0$23’34.9”	Sediment	Hamlet 2, PhuNinh village, TamNong dist.
4	TG-4	N: $10^0$32’42.9” E: $105^0$32’05.7”	Sediment	BinhTrung hamlet, BinhThanh village, ThanhBinh dist.
5	TG-5	N: $10^0$31’03.7” E: $105^0$33’32.1”	Sediment	Hamlet 1, PhongMy village, CaoLanh dist.
6	TG-6	N: $10^0$19’04.3” E: $106^0$04’06.2”	Sediment	Hamlet 7, BinhThanh village, CaiLay dist.
7	TG-7	N: $10^0$19’04.3” E: $106^0$04’06.2”	Paddy field soil	Hamlet 7, BinhThanh village, CaiLay dist.
8	TG-8	N: $10^0$18’33.7” E: $106^0$09’02.3”	Sediment	1000m from TamBinh bridge, TamBinh village, CaiLay dist.
9	TG-9	N: $10^0$18’51.8” E: $106^0$09’53.5”	Paddy field soil	TamBinh village, CaiLaydist.
10	TG-10	N: $10^0$19’25.4” E: $106^0$12’35.0”	Sediment	600m from PhuPhong bridge, PhuPhong village, ChauThanh dist.
11	TG-11	N: $10^0$20’17.2” E: $106^0$17’7.3”	Sediment	400m from Boat repairing workshop, KinhXang bridge, SongThuan village, ChauThanh dist.
12	TG-12	N: $10^0$20’58.4” E: $106^0$21’04.9”	Sediment	RạchMieu Ferry Landing, MyTho city
13	TG-13	N: $10^0$20’32.9” E: $106^0$25’50.8”	Sediment	KyHon river, 3000m from highway 53

14	TG-14	N: $10^0$21'14.3" E: $106^0$27'57.5"	Sediment	ChoGao canal, 500m from highway 54, zone 1 – ChoGao town
15	TG-15	N: $10^0$21'28.4" E: $106^0$28'17.6"	Paddy field soil	1000m from ChoGao canal, TanThanh hamlet, BinhPhan village, ChoGao dist.
16	TG-16	N: $10^0$21'20.3" E: $106^0$29'06.1"	Sediment	50m from BinhPhan canal, BinhThoTrung hamlet, BinhPhan village, ChoGao dist.
17	TG-17	N: $10^0$21'30.5" E: $106^0$28'22.9"	Paddy field soil	TanThanh hamlet, BinhPhan village, ChoGao dist.
18	TG-18	N: $10^0$18'36.7" E: $106^0$32'44.2"	Paddy field soil	HoaMy hamlet, BinhNinh village, ChoGao dist.
19	TG-19	N: $10^0$18'02.8" E: $106^0$32'54.9"	Sediment	VamGiong Water Inlet Sluice, Tieu river, ThanhThoi hamlet, VinhHuu village, GoCongTay dist.

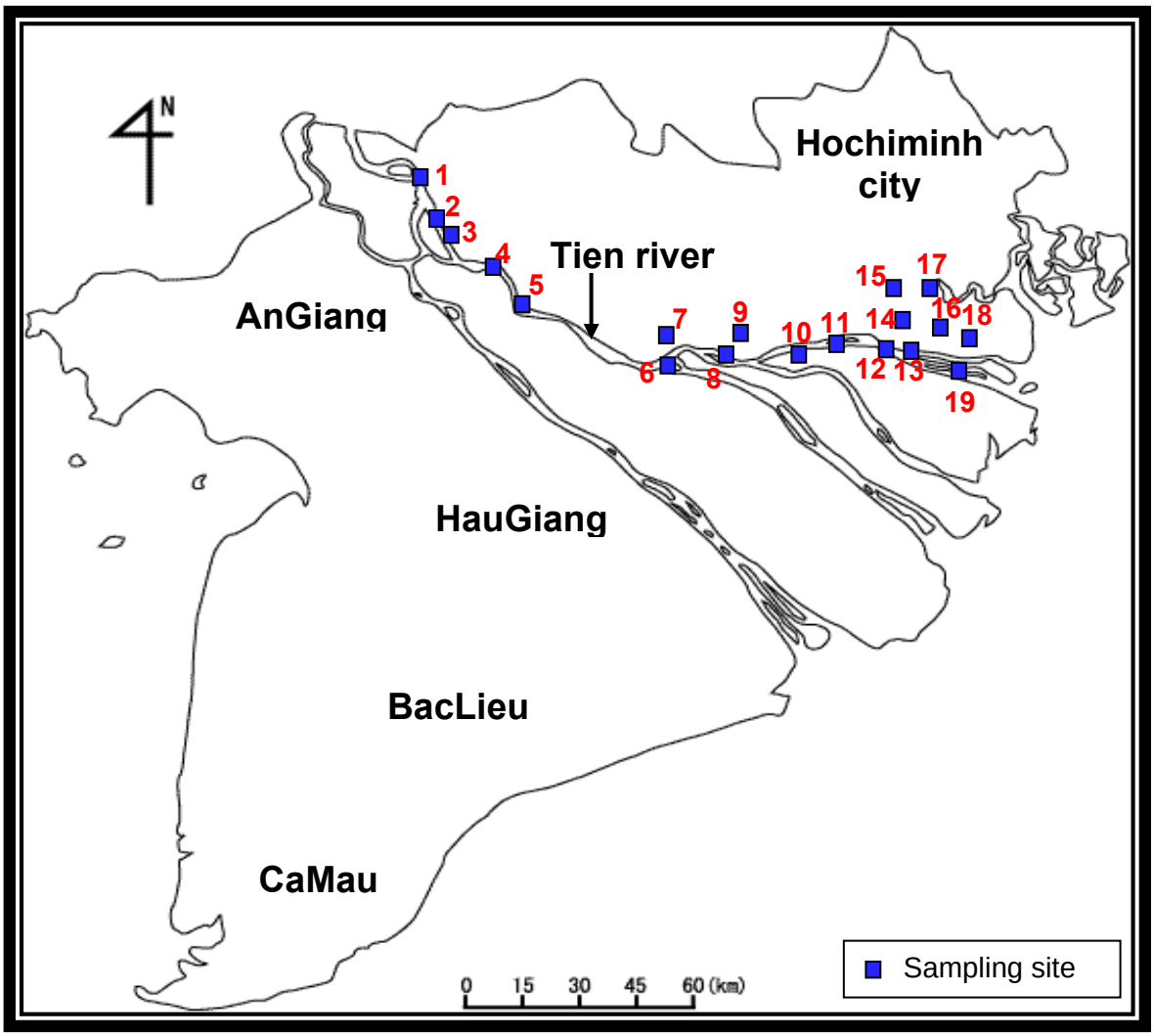


Figure 5.5: Sampling sites of soil – sediment samples in Tien river basin

In other hand, two composite soil samples were collected at the top of Langbiang Mountain. This mountain is situated at 2169 m above sea level and about 20 km from Dalat city - Lamdong province. These soils will be considered as reference samples (where not affected

by industrial, agricultural activities and municipal areas) in comparison with other soil and sediment samples in our research.

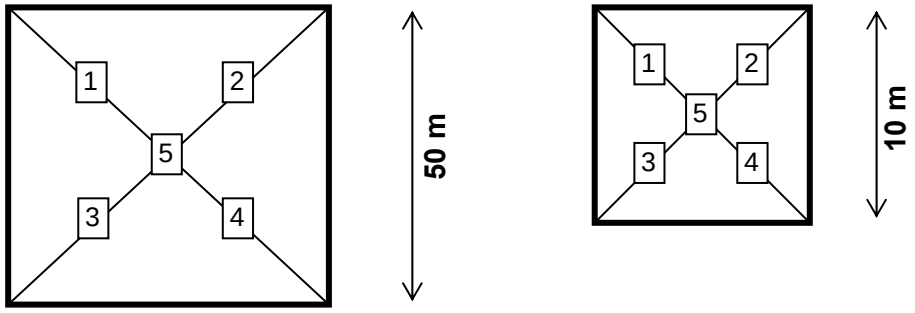
5.2 Material and method

Soil samples were collected in the end of 2006 (DongThanh landfill, LongAn Agrochemical store) and end of 2007 (Tien river basin). In DongThanh landfill in Hochiminh city, we collected soil samples at depth of 100 cm at five points with an area of approximately 2500 square meters. These five samples were combined together and considered as a composite sample.

Figure 5.6: Soil sampling in DongThanh landfill – Hochiminh city

Figure 5.7: Sediment sampling in Thivai river basin

Rice field soil and agrichemical warehouse soil were collected at depth of 10 – 20 cm with the tool named soil hand-borer. The composite samples consisted of at least five samples collected from the same cell (diagonal rule) as described in the Fig. 5.8



Cell size for soil sampling in paddy field and landfill

Cell size for soil sampling in agrichemical warehouse

Figure 5.8: Diagonal rule sampling pattern for soil samples

The composite soil was mixed thoroughly in a tray with a spoon (both stainless steel made), then a portion about 300 g was put in a clean aluminum can and transported to our laboratory. Normally, 10 – 20 cm surface soil is an important sampling medium for the investigation of the PTSs contamination due to its high organic content in comparison with other layer. PTSs adsorbed strongly to soil particles with typical high organic concentrations - normally the first layer (surface soil) in the first few centimeters. In our research we chose the depth soil layer of 10 – 20cm.

Figure 5.9: Soil sampling in Agrichemical store – LongAn province

Sediment samples were collected in November 2006 (Thivai river basin, ThiNghe – Saigon river confluence) and end of 2007 (Tien river basin). At each site, a grab of 5 – 10 cm surface sediment was collected at both river banks by using Eckman dredge. Then, both sediment samples were well mixed and a portion about 300 g was put in a clean aluminum box and transported to our laboratory.

Figure 5.10: Drying sediment samples in IER laboratory

In the laboratory, soil and sediment samples were dried in room temperature for about 5-7 days on the aluminum foil. Dried soil and sediment were crushed by mean of a ceramic mortar than sifted through a 1×1mm stainless steel sieve. Finally, samples were labeled and stored in a brown glass flask at -20^{0}C until analysis.

The soil and sediment samples were analyzed with the analytical method described in chapter IV. All soil and sediment samples were extracted and cleaned-up in both CEAL – EPFL/IER – VNU and identified/quantified with GC/MS Shimadzu QP-2010 in IER lab. Calculation of concentrations has been based on dry weight of soil samples (the weight after drying in the oven at 105^{0}C for 12 hours).

5.3 Results and discussion

5.3.1 PTSs contamination level in soil

The measured PTSs levels of landfill soils, agrichemical store soils, paddy field soils and reference soil are presented in the table 5.5, 5.6, 5.7 and 5.8 respectively below.

Table 5.5: PTSs levels in DongThanh landfill soil samples (ng/g dry wt.)

Compound	DT-1	DT-2	DT-3	DT-4	Min.	Max.	Mean
PCBs	27.15	19.89	7.21	14.62	7.21	27.15	17.22
PBDEs	0.62	1.53	0.06	0.19	0.06	1.53	0.60
HCB	0.26	0.36	0.07	0.46	0.07	0.46	0.29
HCHs	0.04	0.10	0.05	0.08	0.04	0.09	0.06
Heptachlor	nd	0.07	nd	0.08	nd	0.08	0.04
Hept Epox	nd	nd	nd	nd	-	-	-
Aldrin	nd	nd	nd	nd	-	-	-
Dieldrin	1.47	1.36	0.50	4.59	0.50	4.59	1.98
Endrin	nd	nd	nd	nd	-	-	-
Endrin ald	nd	nd	nd	nd	-	-	-
Chlordanes	nd	0.09	0.16	0.59	nd	0.59	0.21
Endosulfans	0.07	0.23	0.07	0.05	0.05	0.23	0.11

pp'-DDE	1.53	2.50	1.78	26.94	1.53	26.94	8.19
pp'-DDD	0.08	1.02	0.55	14.92	0.08	14.92	4.14
pp'-DDT	0.10	0.24	0.13	6.04	0.10	6.04	1.63
DDTs	1.70	3.76	2.45	47.90	1.70	47.90	13.95
Mirex	nd	nd	nd	nd	-	-	-
Toxaphenes	nd	nd	0.56	nd	nd	0.56	0.14

nd: not detectable (Table 4.11, section 4.5.2.c, chapter 4); HCHs: sum of α-, β-, γ-, and δ-HCH; Chlordanes: sum of o-, a- and g-chlordane; Endosulfans: sum of Endosulfan I, II and Endosulfansulfate; DDTs: sum of pp'-DDE, pp'-DDD and pp'-DDT; PCBs: sum of 21 congeners.

Table 5.6: PTSs levels in agrichemical warehouse soil samples (ng/g dry wt.)

	LA-1	**LA-2**	**LA-3**	**LA-4**	**LA-5**	**Min.**	**Max.**	**Mean**
PCBs	0.52	0.38	0.04	0.18	0.52	0.04	0.52	0.33
PBDEs	0.06	0.03	0.03	0.02	0.01	0.01	0.06	0.03
HCB	0.04	0.17	0.05	0.06	0.08	0.04	0.17	0.08
HCHs	0.06	0.10	0.12	0.22	0.11	0.06	0.22	0.12
Heptachlor	nd	nd	0.05	nd	nd	nd	0.05	0.01
Hept. Epox.	nd	nd	nd	nd	0.09	nd	0.09	0.02
Aldrin	nd	nd	0.06	nd	nd	nd	0.06	0.01
Dieldrin	0.15	0.77	0.12	0.25	0.25	0.12	0.77	0.31
Endrin	nd	nd	nd	nd	nd	-	-	-
Endrin ald.	nd	nd	nd	nd	nd	-	-	-
Chlordanes	2.14	0.67	0.39	0.67	6.49	0.39	6.49	2.07
Endosulfans	0.19	0.06	0.47	0.17	0.73	0.06	0.73	0.32
pp'-DDE	2.52	43.53	1.99	1.54	1.64	1.54	43.53	10.24
pp'-DDD	0.67	10.17	0.20	3.29	6.13	0.20	10.17	4.09
pp'-DDT	1.68	29.92	1.14	2.88	1.41	1.14	29.92	7.41
DDTs	1.87	83.63	0.33	7.71	9.18	3.33	83.63	21.74
Mirex	nd	nd	nd	nd	nd	-	-	-
Toxaphenes	51.73	14.07	4.51	7.45	123.95	4.51	123.95	40.34

nd: not detectable (Table 4.11, section 4.5.2.c, chapter 4); HCHs: sum of α-, β-, γ-, and δ-HCH; Chlordanes: sum of o-, a- and g-chlordane; Endosulfans: sum of Endosulfan –I, II and Endosulfansulfate: DDTs: sum of pp'-DDE, pp'-DDD and pp'-DDT; PCBs: sum of 21 congeners

Table 5.7: PTSs levels in Tien River soil samples (ng/g dry wt.)

	TG-7	TG-9	TG-15	TG-17	TG-18	Min.	Max.	Mean
PCBs	4.90	3.69	10.19	5.94	7.14	3.69	10.19	6.37
PBDEs	0.05	0.07	0.131	0.141	0.107	0.045	0.141	0.10
HCB	0.043	0.034	0.024	0.036	0.061	0.024	0.061	0.040
HCHs	0.022	0.073	0.057	0.142	0.048	0.022	0.142	0.068
Heptachlor	0.024	nd	nd	nd	nd	nd	0.024	0.005
Hept. Epox.	nd	nd	nd	nd	nd	-	-	-
Aldrin	nd	nd	nd	nd	nd	-	-	-
Dieldrin	0.284	0.750	0.377	0.413	0.420	0.284	0.750	0.449
Endrin	nd	nd	nd	nd	nd	-	-	-
Endrin ald.	nd	nd	nd	nd	nd	-	-	-
Chlordanes	nd	0.045	nd	0.218	0.161	nd	0.218	0.085
Endosulfans	0.079	0.187	0.547	1.760	2.435	0.079	2.435	1.002
pp'-DDE	0.27	12.66	1.94	2.87	0.82	0.27	12.664	3.710
pp'-DDD	0.02	7.70	0.73	0.95	1.32	0.02	7.700	2.140
pp'-DDT	nd	0.96	0.16	0.10	0.09	nd	0.962	0.263
DDTs	0.28	21.32	2.83	3.91	2.23	0.28	21.32	6.12
Mirex	nd	0.041	nd	nd	nd	nd	0.041	0.008
Toxaphenes	nd	nd	0.076	nd	0.239	nd	0.239	0.063

nd: not detectable (Table 4.11, section 4.5.2.c, chapter 4); HCHs: sum of α-, β-, γ-, and δ-HCH; Chlordanes: sum of o-, a- and g-chlordane; Endosulfans: sum of Endosulfan –I, II and Endosulfansulfate: DDTs: sum of pp'-DDE, pp'-DDD and pp'-DDT; PCBs: sum of 21 congeners

Table 5.8: PTSs levels in Langbiang moutain soil samples (Ref.site) (ng/g dry wt.)

Compound	Ref-1	Ref-2	Mean
PCBs	0.99	0.78	0.89
PBDEs	nd	nd	-
HCB	nd	nd	-
HCHs	nd	nd	-
Heptachlor	nd	nd	-
Heptachlor epoxide	nd	nd	-
Aldrin	nd	nd	-
Dieldrin	nd	nd	-
Endrin	nd	nd	-
Endrin aldehyde	nd	nd	-
Chlordanes	nd	nd	-
Endosulfans	nd	nd	-
pp'-DDE	0.47	0.27	0.37
pp'-DDD	0.11	0.09	0.10
pp'-DDT	nd	nd	-

DDTs	0.58	0.36	0.47
Mirex	nd	nd	-
Toxaphenes	nd	nd	-

nd: not detectable (Table 4.11, section 4.5.2.c, chapter 4); HCHs: sum of α-, β-, γ-, and δ-HCH; Chlordanes: sum of o-, a- and g-chlordane; Endosulfans: sum of Endosulfan-I, II and Endosulfansulfate; DDTs: sum of pp'-DDE, pp'-DDD and pp'-DDT; PCBs: sum of 21 congeners

Results presented in table 5.5, 5.6 and 5.7 showed that most of the PTSs especially DDTs and its degradation products, PCBs, HCB, HCHs and chlordanes (CHLs) have been detected in all soil samples from landfill, agrichemical store and paddy fields. Contamination levels of PTSs in DongThanh landfill followed the order of PCBs > DDTs > HCB > CHLs > HCHs. This is similar to the order of PTSs pattern in paddy field but different from those in agrichemical warehouse (DDT > CHLs > PCBs > HCHs > HCB) where stores pesticides only (table 5.9).

However, the order of PTSs pattern in landfill soil does not agrees well with those in other study on Hanoi and Hochiminh city dumping sites (DDTs > PCBs > HCHs > CHLs > HCB) (*Minh et al., 2006*). The difference in such patterns maybe indicates the loss of the substances from the system (e.g. through offsite transport) relative to new inputs (*Minh et al., 2007b*).

In Vietnam, DDT has been used extensively for both agriculture and vector control during the last few decades (*Nhan et al., 2001; Minh et al. 2006*). Although its application in agriculture is now officially prohibited, the use for vector control and sanitary purposes is continuous in recent years (*UNEP, 2001; Minh et al., 2006*). This may have led to the presence of DDTs in domestic waste and accumulating these wastes in small dumping sites and landfill might have amplified the DDTs levels in soil. This could be a reasonable explanation for relatively higher DDTs levels in landfill and urban compared with rural areas and paddy field (*Minh et al. 2001, 2006; Nhan et al. 2001*) (table 5.9).

Vietnam has only the criteria of Pesticide Residue Limits for Soil Quality (*TCVN 5941, 1995; 100 ng/g*). Which one is applied to control and evaluate of DDTs and lindane contaminant level in soil. So this was used for assessing DDTs and lindane levels in our soil samples. However, from table 5.5, 5.6 and 5.7 we can see that HCHs concentrations (including lindane) in our soils are much lower than those in TCVN 5941-1995 (100 ng/g). One reason is that HCH isomers exhibited very short half-lives in soil under tropical climate condition, varying between 10 and 30 days. Another reason is that approximately 90% of HCHs residues evaporated into the atmosphere (*Ramesh et al., 1991*). Fig. 5.11 and 5.12 shown that all sites in landfill and agrichemical warehouse have DDTs values lower than TCVN5941-1995.

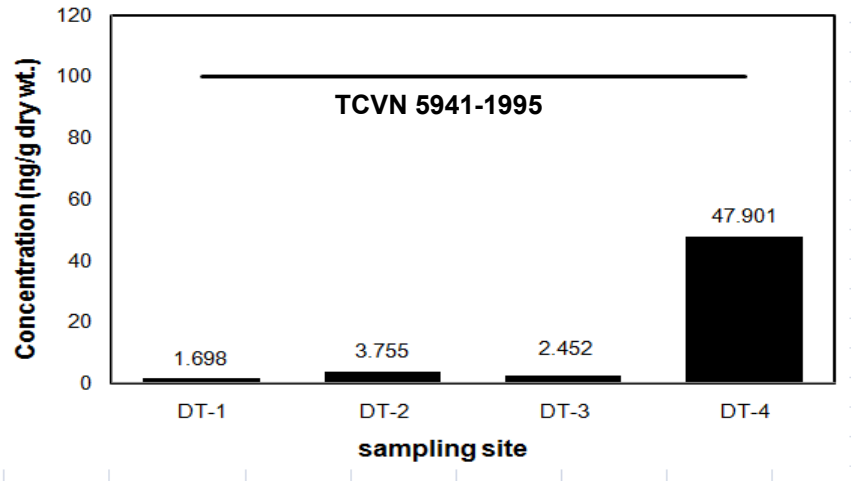


Figure 5.11: DDTs level in DongThanh landfill soil in comparison with TCVN5941

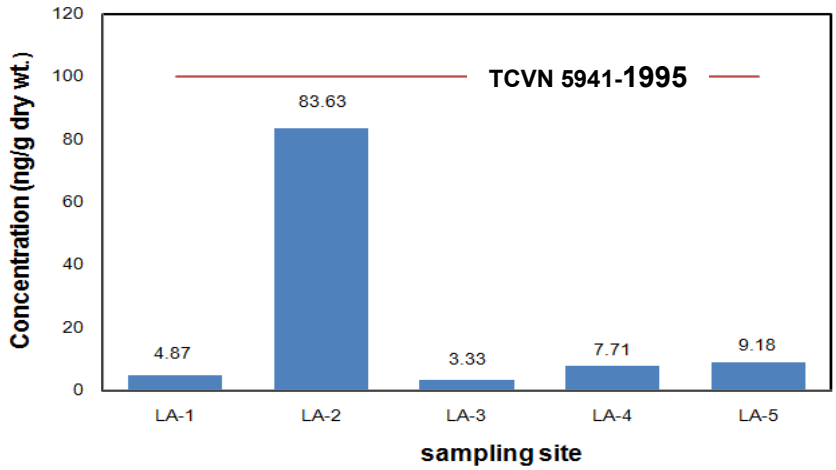


Figure 5.12: DDTs levels in agrichemical warehouse soil in comparison with TCVN5941

It is well known that pp'-DDT is metabolized to pp'-DDD and pp'-DDE. The ratios of pp'-DDT and its major degradation product as pp'-DDE can be used to research and understand the chronology of DDT residues input into the environment. Strandberg et al. (1998) suggested that pp'-DDT/pp'-DDE ratios lower than 0.3 could be the result of the aged mixtures in environment, and those higher than 0.5 might indicate recent use of DDT. The value of 0.3 was established from a research on organochlorine chemicals in the frame of National Pesticide Monitoring Program in United States (*Schmitt et al., 1990*). In this research, Schmitt monitored pp'-DDE in fish from 1980 – 1984 and found that it constituted 73% of total DDTs, up from 70% in 1974. In another research on bioavailable organochlorine pesticides, the ratio (1:2) in the same site was found following the summer exposure (90 days) and DDTs recent use was suggested (*Petty et al., 1995*). From the results of this research, the value of 0.5 was established

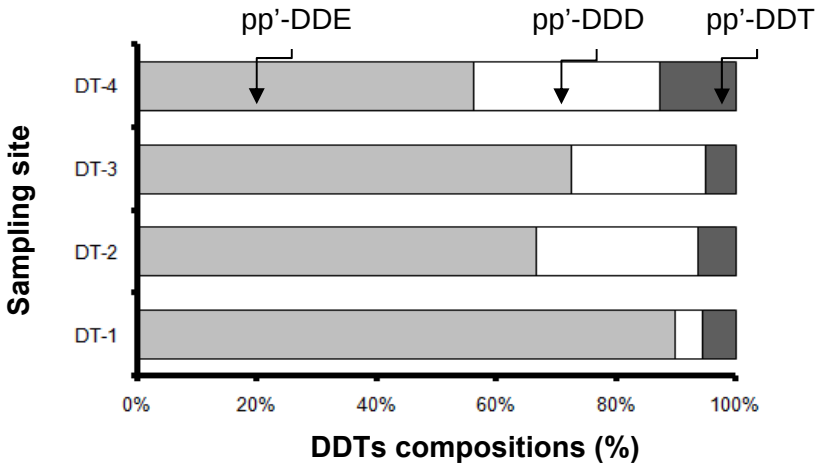


Figure 5.13: Composition of DDTs compounds in landfill soil samples

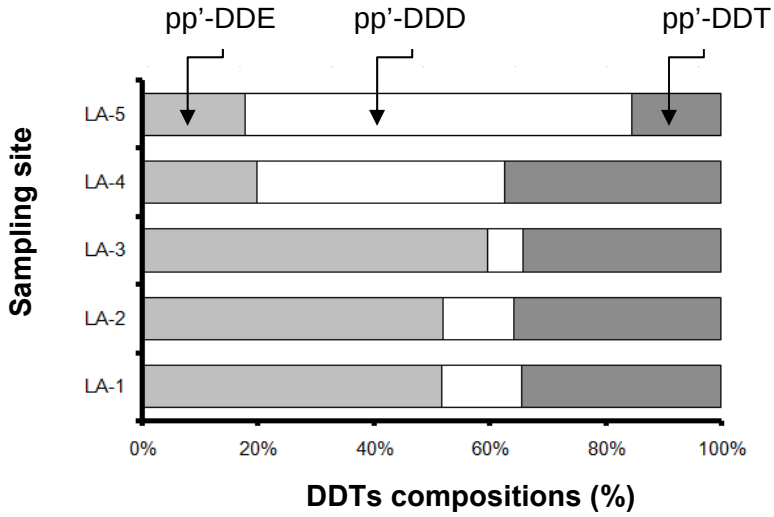


Figure 5.14: Composition of DDT compounds in agrichemical warehouse soils

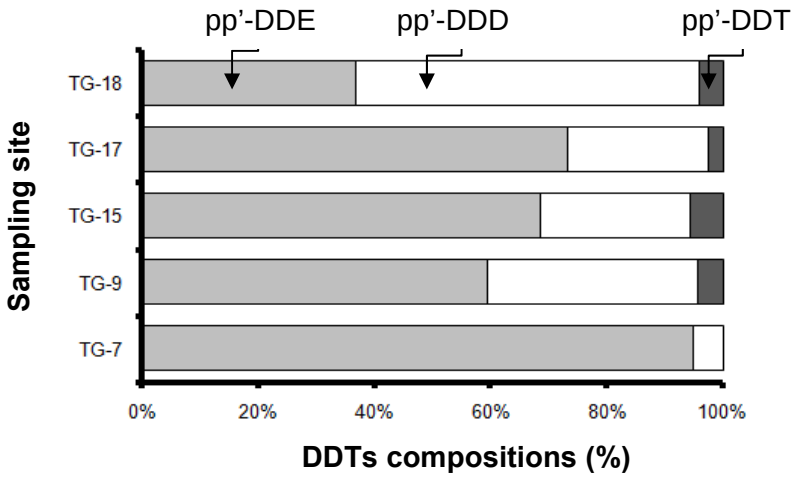


Figure 5.15: Composition of DDTs compounds in paddy field soils

Fig. 5.13, 5.14 and 5.15 show the comparison of DDT composition in landfill soils, in agrichemical warehouse soils and paddy field soils each other, respectively. We can see that the proportion of pp'-DDT and the ratio of pp'-DDT/pp'-DDE are higher in agrichemical warehouse compared with those in landfill and paddy field. These results may strengthen the recent findings that DDT was perhaps no longer used in agriculture.

DDTs level in waste dumping site soils collected from HCMC in 2000 (*Minh et al., 2006*) were higher than present levels (table 5.9). In addition, the proportion of pp'-DDT in dumping site soils collected in 2000 was near four times those in 2006 (30% and 8%, respectively). This observation probably indicates decreasing application of DDT in HCMC area.

In general, DDTs were found in all agrichemical warehouse soils and landfill soils especially in LA-2 (83.6 ng/g dry wt.) and DT-4 (47.9 ng/g dry wt.). This proves that their re-emission to the environment from DDTs-contaminated soils (e.g. agrichemical warehouse soil in LongAn) still remains a significant source of DDTs although they are officially no longer used in agriculture since 1995 (*Sinh et al., 1999*).

For toxaphene, interestingly, we found toxaphene in all five agrichemical warehouse soil samples with relatively high concentration (ranged from 4.509 – 123.946 ng/g dry wt.). According to the agrichemical warehouse supervisor, there were a large amount of toxaphene kept in this place by the 1990s and this agrees well with our analytical results. This is the only place where toxaphene was detected in soil and sediment matrices during our research.

CHLs and HCB were found in almost our soil samples except the reference soil samples though these substances' major application in Vietnam are unclear (*Iwata et al. 1994*). Besides, CHLs were detected in mussels from highly populated areas, industrial areas, fishing harbors (*Monirith et al. 2003*) as well as in soils from urban and dumping site (*Minh et al., 2006*). The researchers therefore suggested that using CHLs to control termites could be a source of CHLs to environments. In Vietnam, HCB was used as fungicide in the past (*Minh et al., 2006*). In addition, it should be noted that some of the HCB in the environment would be formed as a by-product, often in minute quantities, from many industrial chemical processes involving chlorine as well as from the use of several agrochemicals and industrial substances (*Macdonald et al. 2000; Monirith et al. 2003; Öberg & Bergström, 2009*).

Endosulfans were detected in all our soil samples even in common levels. This suggests the widespread endosulfans contamination in the environment due to their large usage in Vietnam agricultural activities. The import of these substances has just been prohibited since 2005 and endosulfans using in agricultural activities has also been banned since 2006 (*MARD, 2005*).

Table 5.9: Comparison of some PTSs residue levels between our soil samples and various soil samples (mean concentration in ng/g dry wt.)

Country	Year	n	PCBs	DDTs	CHLs	HCHs	HCB	Reference
Vietnam								
DongThanh (Landfill)	**2006**	**4**	**17.2**	**13.9**	**0.21**	**0.06**	**0.29**	Present study
LongAn (Agrichemical)	**2006**	**5**	**0.33**	**21.7**	**2.07**	**0.12**	**0.08**	Present study
Tien river basin (paddy field)	**2007**	**5**	**6.37**	**6.12**	**0.09**	**0.07**	**0.04**	Present study
Langbiang mountain (Dalat)	**2008**	**2**	**0.89**	**0.21**	**nd**	**nd**	**nd**	Present study
Hanoi (dumping site)	2000	7	12	19	0.45	0.83	0.45	Minh et al., 2006
Hanoi (rural soil)	2000	3	0.73	3.2	0.15	0.14	0.01	
HCMC (dumping site)	2001	6	22	23	1.30	0.37	0.09	
HCMC (rural soil)	2001	3	3.6	3.8	0.20	0.36	0.13	
Hanoi (various soils)	1990	25	12	35[d]	2.1	-	-	Thao et al., 1993
World								
India (dumping site)	1999-2000	6	210	26	5.6	31	0.31	Minh et al., 2006
Cambodia (dumping site)	1999-2000	8	140	350	1.1	1.7	0.19	Minh et al., 2006
Lithuania	2006	5	42.2	36.3	-	7.8	-	Milukaite et al., 2008
Beijing suburb (farmland)	2003	11	-	8.4	-	2.91	-	Chen et al., 2005
Victoria Land (Antarctica)	1999	4	0.5	0.08[a]	-	0.02[b]	0.11	Borghini et al., 2005
Tree land (Taihu lake), China	2004	5	-	90.25[c]	-	20.94	5.13	Wang et al., 2007
Paddy field (Taihu lake)	2004	9	-	50.23	-	28.53	3.76	Wang et al., 2007
Urban park soil, china	2003	25	-	162	-	10.54	-	Li et al., 2008
Antartica soil	1998	11	34.6	5.65	-	8.48	2.92	Negoita et al., 2003
Urban soil (Ireland)	1992		2.97	1.8	-	1.14	-	Grath et al., 1995
General soil (United State)	1998	-	5.9	-	-	-	-	Meijer et al., 2003

nd: not detectable (Table 4.11, section 4.5.2.c, chapter 4); -: data not available; [a]: sum of pp'-DDE and pp'-DDT; [b]: a-HCH; [c]: sum of pp'-DDE, op'-DDE, pp'-DDD, pp'-DDT and op'-DDT; [d]: sum of pp'-DDE, pp'-DDD, op'-DDT and pp'-DDT;

Others PTSs in our soil samples such as heptachlor, aldrin, endrin, endrin aldehyde and mirex were lower than LOD of the analytical method or small value (< 5 ng/g dry wt.). PBDEs were found in common levels with the higher level of PBDEs in landfill soil (0.6 ng/g dry wt.) compared with those in agrichemical warehouse soils (0.03 ng/g dry wt.) and in paddy filed soils (0.1 ng/g dry wt.). Discharge of municipal sewages, e-waste and atmospheric deposition of the fine particles could be the transport pathway of PBDEs to the landfill (*Minh et al., 2007c; Petreas et Oros, 2009*). PBDEs found in agrichemical warehouse and paddy soil samples might be transported via irrigated canals systems and atmospheric deposition (*Moon et al., 2007*).

PCBs were found in almost all soil samples and PCBs level is distinctively high for landfill soil samples (ranged 7.2 – 27.1 ng/g dry wt.). Interestingly, levels of PCBs of all the landfill soil samples were higher than those of the agrichemical warehouse and paddy field soil samples. This implicates that landfill is considered as a pollution source of PCBs to our environment. Vietnam has not issued Guidelines for PCBs contamination level in soil. Therefore, we compare PCBs level in our soil samples with those in previous studies on PCBs as well as PTSs levels in dumping site soil, urban soil and general soil worldwide (table 5.9), including industrial countries, which have higher background levels of PCBs (*Breivik et al. 2002)*. In global comparison, PCB levels in our soil samples were considerably lower than those in the dumping site soil samples of India and Cambodia and slightly lower than those of Vietnam in previous studies (table 5.9).

PTSs levels in our soil samples are lower than those of samples in previous studies in Vietnam. This demonstrates a decreasing trend of PTSs in environment (table 5.9).

PCBs levels in particular and PTSs levels in general show a minor difference between our result and previous study in Vietnam (*Minh et al., 2006*). This fact again highlights the significance of the landfills and dumping sites as well as agrichemical warehouse as the pollution sources of PTSs in Vietnam. As a consequence of insufficient management and lack of effective treatment, PTSs may again from these sites contaminate the local environment and pose health risks to local communities.

5.3.2 PTSs contaminants level in sediment

The PTSs levels in sediment samples are presented in the table 5.10, 5.11 and 5.12, respectively below.

Table 5.10: PTSs concentration in ThiNghe-Saigon river confluence sediment samples (ng/g dry weight)

PTSs	Bason-2	Bason-3	Bason-4	Bason-5	Bason-6	Bason-7	Bason-8	Bason-9	Min.	Max.	Mean	TEL	PEL
PCBs	21.94	9.94	7.38	25.00	5.31	9.80	5.66	9.09	5.32	25.00	11.76	34.1	277
PBDEs	1.13	0.11	0.31	1.00	0.25	0.17	0.98	0.06	0.06	1.13	0.50		
HCB	15.91	0.23	2.46	41.86	1.42	13.12	0.99	0.13	0.13	41.86	9.51		
HCHs	0.05	0.05	0.08	0.11	0.07	0.07	0.06	0.04	0.04	0.11	0.07	0.9	1.4
Heptachlor	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-		
Hept Epox	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-	0.60	2.74
Aldrin	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-		
Dieldrin	1.80	0.62	1.63	6.50	1.00	1.29	1.07	0.87	0.62	6.50	1.85	2.85	6.67
Endrin	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-	2.67	62.4
Endrin ald	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-		
Chlordanes	1.64	0.28	0.76	2.14	0.51	0.36	0.54	0.04	0.04	2.14	0.79	4.5	8.9
Endosulfans	0.07	0.32	0.10	0.15	0.12	0.07	0.08	0.05	0.05	0.32	0.12		
pp'-DDE	8.81	2.26	6.38	27.20	4.53	3.97	5.22	3.41	2.26	27.20	7.72	1.42	6.75
pp'-DDD	8.72	1.03	6.17	22.64	3.49	3.07	3.95	2.91	1.03	22.64	6.50	3.54	8.51
pp'-DDT	3.16	0.12	5.01	11.55	1.71	0.65	2.06	3.01	0.12	11.54	3.41	1.19	4.77
∑DDTs	20.69	3.41	17.56	61.38	9.73	7.69	11.24	9.33	3.41	61.38	17.63		
Mirex	nd	nd	0.05	2.35	nd	nd	nd	nd	nd	2.347	0.300		
Toxaphenes	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-		

nd: not detectable (Table 4.11, section 4.5.2.c, chapter 4); HCHs: sum of α-, β-, γ-, and δ-HCH; Chlordanes: sum of o-, a- and g-chlordane; Endosulfans: sum of endosulfan –I, II and endosulfansulfate; DDTs: sum of pp'-DDE, pp'-DDD and pp'-DDT; TEL: Threshold Effect Level; PEL: Probable Effect Level (*Sherri et al., 1996; CCME, 1999; 2002*)

Ta ble

5.11: PTSs concentration in Thivai river basin sediment samples (ng/g dry weight)

PTSs	TV-1	TV-2	TV-3	TV-4	TV-5	TV-6	TV-7	TV-8	TV-9	TV-10	TV-11	TV-12	TV-13	TV-14	TV-15	TV-16	TV-17	TV-18	Min.	Max.	Mean	TEL	PEL
PCBs	0.02	0.85	0.82	1.16	0.13	0.15	0.14	0.22	1.23	0.11	1.05	0.29	0.07	0.09	1.27	0.09	0.06	0.05	0.02	1.27	0.43	34.1	277
PBDEs	0.02	0.11	0.08	0.09	0.13	0.06	0.07	0.23	0.08	0.02	0.07	0.03	0.01	0.02	0.10	0.03	0.02	0.02	0.01	0.23	0.07		
HCB	0.02	0.78	0.40	0.07	0.10	0.09	0.35	0.54	0.11	0.08	0.21	0.07	0.04	0.06	0.05	0.05	0.05	0.06	0.02	0.78	0.17		
HCHs	nd	0.07	0.07	0.02	0.25	0.16	0.08	0.21	0.04	0.03	0.02	0.02	0.02	0.03	nd	0.02	0.02	0.02	nd	0.25	0.06	0.94	1.38
Heptachlor	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-		
Hept Epox	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-	0.6	2.74
Aldrin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-		
Dieldrin	0.35	0.47	0.88	0.06	0.08	0.08	0.06	0.06	0.21	0.05	0.04	0.02	0.07	0.02	nd	0.03	0.04	nd	nd	0.88	0.14	2.85	6.67
Endrin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-	2.67	62.4
Endrin Ald	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-		
Chlordanes	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-	4.5	8.9
Endosulfans	nd	0.03	0.04	nd	0.09	0.08	0.13	0.13	0.11	0.18	nd	0.13	0.07	0.14	0.13	0.07	0.29	0.13	nd	0.29	0.10		
pp'-DDE	nd	0.82	0.87	0.80	0.45	0.68	0.96	0.70	0.69	0.62	0.34	0.21	0.31	0.19	0.10	0.17	0.18	0.14	nd	0.96	0.46	1.42	6.75
pp'-DDD	nd	0.20	0.27	0.25	0.14	0.24	0.41	0.25	0.55	0.27	nd	0.13	0.40	0.14	nd	0.13	0.18	0.20	nd	0.55	0.21	3.54	8.51
pp'-DDT	nd	0.09	0.11	0.09	0.10	0.08	0.30	nd	0.48	0.16	nd	0.08	0.16	0.08	nd	0.05	0.14	0.10	nd	0.48	0.11	1.19	4.77
∑DDTs	nd	1.11	1.25	1.13	0.69	1.00	1.67	0.95	1.73	1.04	0.34	0.41	0.82	0.41	0.10	0.35	0.50	0.44	nd	1.73	0.77		
Mirex	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-		
Toxaphenes	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-		

nd: not detectable (Table 4.11, section 4.5.2.c, chapter 4); HCHs: sum of α-, β-, γ-, and δ-HCH; Chlordanes: sum of o-, a- and g-chlordane Endosulfans: sum of Endosulfan –I, II and Endosulfansulfate; DDTs: sum of pp'-DDE, pp'-DDD and pp'-DDT; TEL, PEL *(Sherri et al., 1996; CCME, 2002)*

Table 5.12: PTSs concentration in Tien river basin sediment samples (ng/g dry weight)

	TG1	TG2	TG3	TG4	TG5	TG6	TG8	TG10	TG11	TG12	TG13	TG14	TG16	TG19	Min.	Max.	Mean	TEL	PEL
PCBs	5.84	4.51	4.10	1.96	3.19	4.82	4.18	6.18	4.66	3.73	9.17	6.25	3.99	20.57	1.96	20.57	5.94	34.1	277
PBDEs	0.20	0.03	0.01	0.07	0.09	0.16	0.58	0.06	0.03	0.02	0.05	0.05	0.04	0.04	0.01	0.58	0.10		
HCB	0.04	0.04	0.02	0.02	0.03	0.04	0.07	0.03	0.03	0.04	0.05	0.05	0.10	0.10	0.02	0.10	0.05		
HCHs	0.03	nd	nd	nd	nd	0.16	0.06	0.05	0.03	0.02	0.05	nd	0.04	0.05	nd	0.16	0.04	0.94	1.38
Heptachlor	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-		
Heptachlor Epoxide	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-	0.6	2.74
Aldrin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-		
Dieldrin	0.24	0.19	0.14	0.05	0.09	0.40	0.37	0.44	0.35	0.35	0.45	0.31	0.33	0.76	0.05	0.76	0.32	2.85	6.67
Endrin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-	2.67	62.4
Endrin aldehyde	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-		
Chlordanes	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-	4.5	8.9
Endosulfans	1.44	0.18	0.12	0.09	0.04	0.12	0.07	0.11	0.09	nd	0.21	0.05	0.08	0.16	nd	1.44	0.20		
pp'-DDE	0.30	0.31	0.11	0.20	0.28	1.45	0.98	0.64	0.52	1.26	0.36	0.31	0.61	0.36	0.11	1.45	0.55	1.42	6.75
pp'-DDD	0.17	0.19	nd	0.18	0.27	0.65	0.45	0.38	0.16	1.11	0.31	0.12	0.20	0.20	nd	1.11	0.31	3.54	8.51
pp'-DDT	nd	nd	nd	0.24	nd	nd	nd	0.09	nd	1.39	0.31	nd	nd	nd	nd	1.39	0.15	1.19	4.77
DDTs	0.47	0.49	0.11	0.61	0.55	2.10	1.43	1.11	0.68	3.75	0.98	0.42	0.81	0.56	0.11	3.75	1.01		
Mirex	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-		
Toxaphenes	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-		

nd: not detectable (Table 4.11, section 4.5.2.c, chapter 4); HCHs: sum of α-, β-, γ-, and δ-HCH; Chlordanes: sum of o-, a- and g-chlordane
Endosulfans: sum of Endosulfan –I, II and Endosulfansulfate; DDTs: sum of pp'-DDE, pp'-DDD and pp'-DDT; TEL, PEL *(Sherri et al., 1996; CCME, 2002)*

In general, the residue levels of PTSs in the sediment from ThiNghe canal – Saigon River (TN-SG) and Thivai River follow the order DDTs > PCBs > HCB ≥ CHLs ≥ HCHs and DDTs > PCBs > HCB > HCHs > CHLs, respectively. For Tien River, the order is quite different: PCBs > DDTs > HCB > HCHs > CHLs.

The pattern of PTSs found in TN-SG sediment samples was similar to those in Thivai River and previous study (*Phuong et al., 1998*). However, the order of PTSs levels found in Tien River sediment samples is not similar to those in another study, DDTs > PCBs > CHLs ≥ HCHs ≥ HCB (*Minh et al., 2007b*). It should be noted that these orders are influenced not only by the transformation kinetic but also by the loss of the substances from the system relative to new inputs (e.g. through offsite transport) (*Minh et al., 2007b).*

PCBs and DDTs are the most abundant pollutants in our sediment samples. The abundance of DDTs and PCBs in Vietnam may be due to their larger usage as well as higher persistency over the other PTSs. Until 1985, nearly 30,000 tons of PCB – containing industrial oils had been imported to Vietnam (*Sinh et al., 1999*). In addition, electrical equipments like transformer and capacitors containing PCB-contaminated oil had been also imported to Vietnam until the mid 1980s (*Kannan et al., 1995*). Those materials are parts of PCBs sources to the environment, besides releases from heavy weapons used during the Indochina War (*Thao et al., 1993*).

PCBs residue levels in sediment samples varied from 5.3 – 25.0 ng/g dry wt. in TN-SG River, from 0.02 – 1.27 ng/g dry wt. in Thivai River and from 1.96 – 20.57 ng/g dry wt. in Tien River. Spatial distribution of PCBs demonstrates significantly higher concentrations of PCBs in TN-SG River, compared to Thivai River and Tien River. These levels are much lower than those from previous studies (*Iwata et al., 1994; Phuong et al., 1998; Minh et al., 2007a,b*) (table 5.13). This finding suggests decreasing levels of PCBs in the aquatic environment of HCMC canals and Saigon River.

Like PCBs, DDTs is the most abundant OC contaminant found in our sediments samples and levels of TN-SG River sediment samples is distinctively high (varied 3.41 – 61.38 ng/g dry wt.) (table 5.10, 5.11 and 5.12). From our results, we have found that DDTs levels are higher with TN-SG sediment (urban sites) and lower with Tien River sediment (agricultural sites). This suggests the sources such as the usage for vector control and hygienic purposes (*Nhan et al., 2001; Monirith et al., 2003; Minh et al., 2007a*). This hypothesis may be strengthened by the result of our study. In our study, DDTs levels in TN-SG River (HCMC area) are about 7 – 22 times higher than those in Thivai and Tien River (agricultural area) (table 5.13).

Similar to soil samples, ratios of DDT to its degradation products, as for example ratio of DDT/DDE could be used for evaluating degradation features of the parent compound in sediment. Strandberg et al. (1998) suggested that DDT/ DDE ratio lower than 0.3 could be the result of the aged mixtures in environment, and those higher than 0.5 might indicate recent use of DDT.

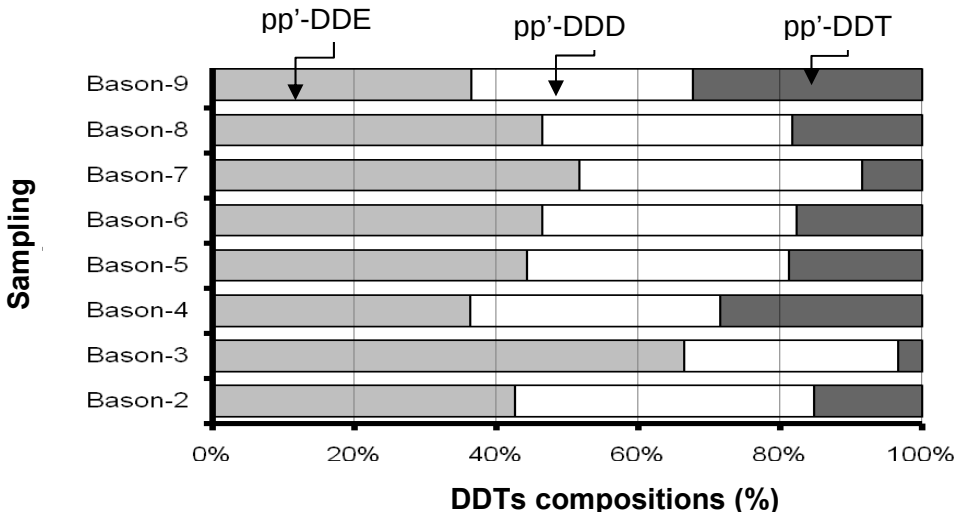


Figure 5.16: DDTs composition in ThiNghe-Saigon river sediment samples

Fig. 5.16 shows DDTs composition of TN-SG River sediment samples. From the table 5.10, Bason-4, Bason-5, Bason-8 and Bason-9 have the ratios of pp'-DDT/pp'-DDE near or higher than 0.5. This suggests possible recent input of DDT to the TN-SG River. In addition, the ratios of pp'-DDT/pp'-DDE in Thivai and Tien River sediment samples (0.2 and 0.3, respectively) were lower than those in TN-SG River sediment (0.5) (Fig.5.17).

The recent inputs of DDTs into the environment might have come from illegal usage of DDT as well as from dicofol, the pesticides that contains 3.5 - 10.8% DDT as byproducts (*Xu et al., 2004*). Commercial dicofol contains op'-DDT, op'-DDE, and pp'-DDT as major impurities with a relatively small proportion of pp'-DDT (*Qiu et al. 2005*). Dicofol commonly used for termite control and this using may contribute as an additional proportion of pp'-DDT. In Vietnam, dicofol is on the list of insecticides (*Pesticides Index 2002*) but no information is available regarding the quantity of it recent use in Vietnam (*Minh et al., 2006*).

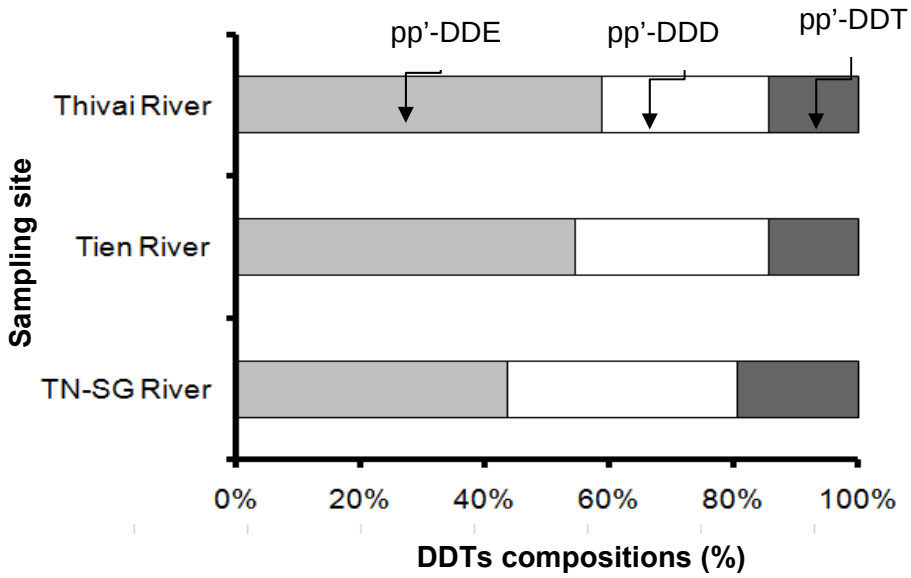


Figure 5.17: Comparison of DDTs composition between TN-SG River, Thivai River and Tien River sediment samples

These results indicate more recent DDTs input in urban sediments, further strengthening our hypothesis that urban areas are pollution sources of DDTs compound in specific and other PTSs in general to our environment.

Vietnam has not issued Guidelines for PTSs levels assessment in freshwater sediment. Therefore, we should use two sediment quality assessment values like Threshold Effect Level (TEL) and Probable Effect Level (PEL) (*Sherri et al., 1996; CCME, 2002; Anh et al., 2003*) in order to assess our analytical results. TEL and PEL are two values used to evaluate the potential impacts of sediment – associated chemicals on various resources uses (e.g., aquatic life or wildlife consumers of aquatic life).

The TEL was calculated as the square root of the product (i.e., the geometric mean) of the lower 15^{th} percentile concentration of the effect data set and the 50^{th} percentile concentration of the no-effect data set. The PEL was calculated as the square root of the product (i.e., the geometric mean) of the 50^{th} percentile concentration of the effect data set and the 85^{th} percentile concentration of the no-effect data set. The TEL represents the upper limit of the range of sediment chemical concentrations that is dominated by no-effect data entries. Within this range concentrations of sediment-associated chemicals are not considered to represent significant hazards to aquatic organisms. The PEL represents the lower limit of the range of chemical concentrations that is usually or always associated with adverse biological effects. The geometric mean is used to account for the uncertainty in the distribution of the data sets (*CCME, 1999*).

Fig 5.18 and 5.19 show the comparison between DDTs levels in TN-SG River sediment samples and TEL, PEL values. Concentration of pp'-DDT and metabolites exceeded TEL value in most of the sites, especially at Bason-2, Bason-4 and Bason-5. However, DDTs levels are above PEL value only in Bason-2 and Bason-5. High DDTs contamination in both sites might result from the large domestic waste water outlet (near Bason-2 and Bason-5 in upstream) and from other small canals. Fig. 5.3 showed Bason-2 and Bason-5 located near the confluences of TN canal and two other small canals.

We can see in the table 5.11 that DDTs levels in all sites of Thivai River are much lower than TEL and PEL values. For Tien River samples, there are only one site (TG-6) has pp'-DEE level and another site (TG-12) which has pp'-DDT level higher than TEL value, respectively.

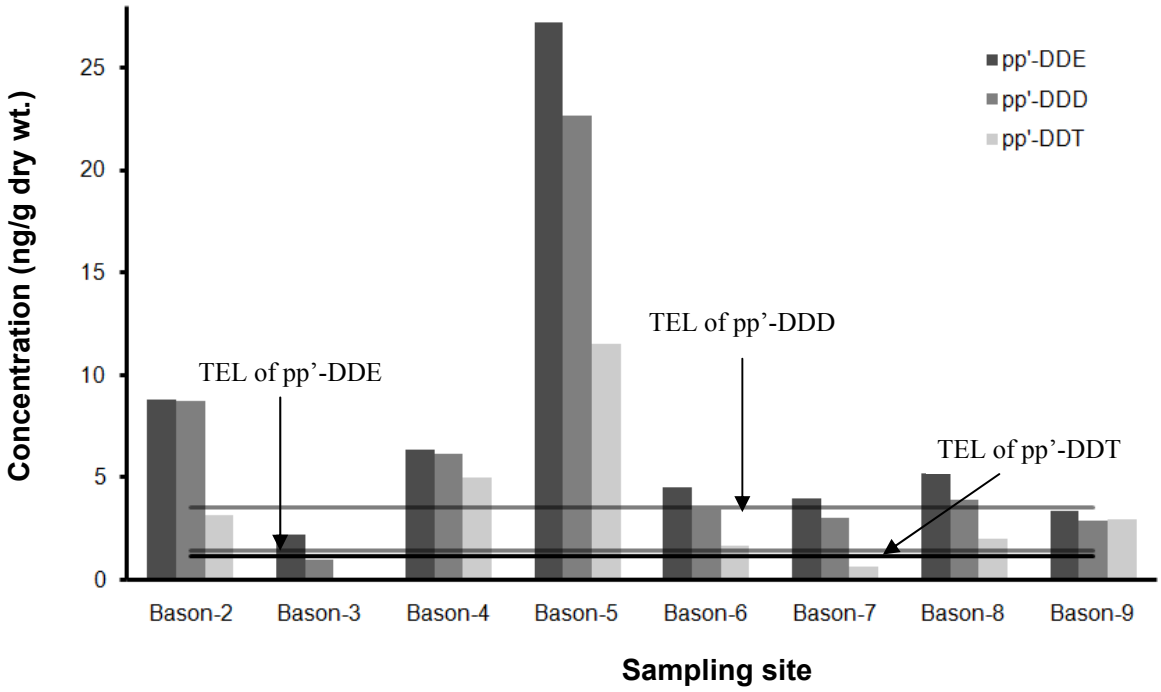


Figure 5.18: Comparison between degradation products of DDT in TN-SG River sediment samples and TEL values respectively

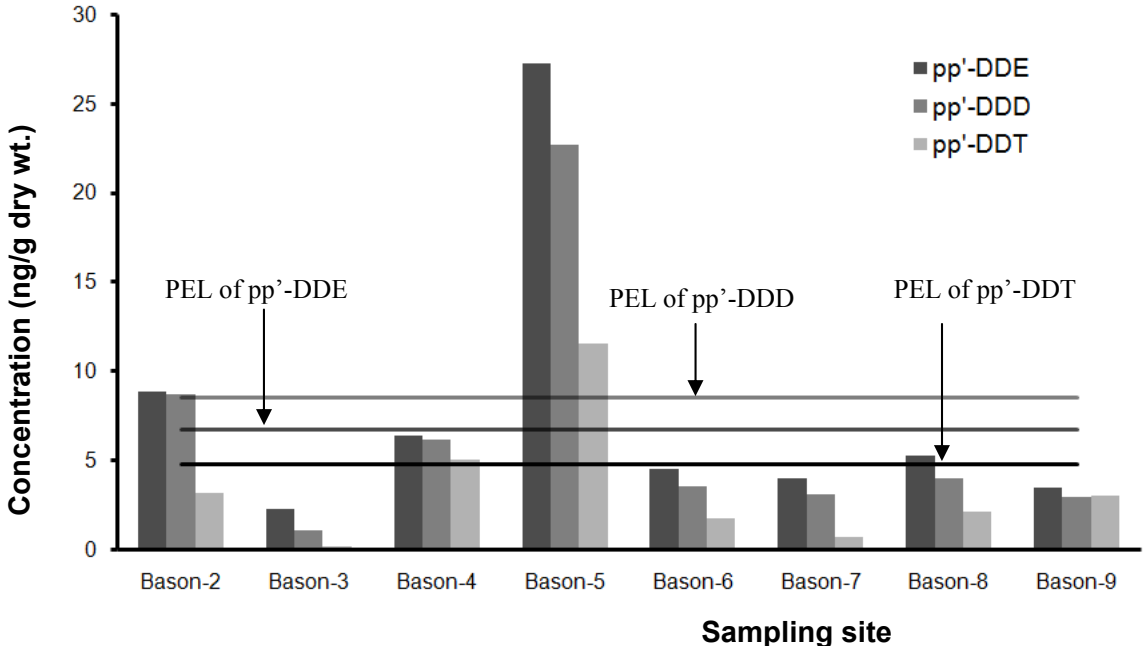


Figure 5.19: Comparison between DDT metabolites in TN-SG River sediment samples and PEL values respectively

Fig. 5.20 and 5.21 show that HCHs and PCBs levels in most of the sites are lower than TEL and PEL values, respectively. PCBs levels in Bason-2 and Bason-5 are close to TEL value.

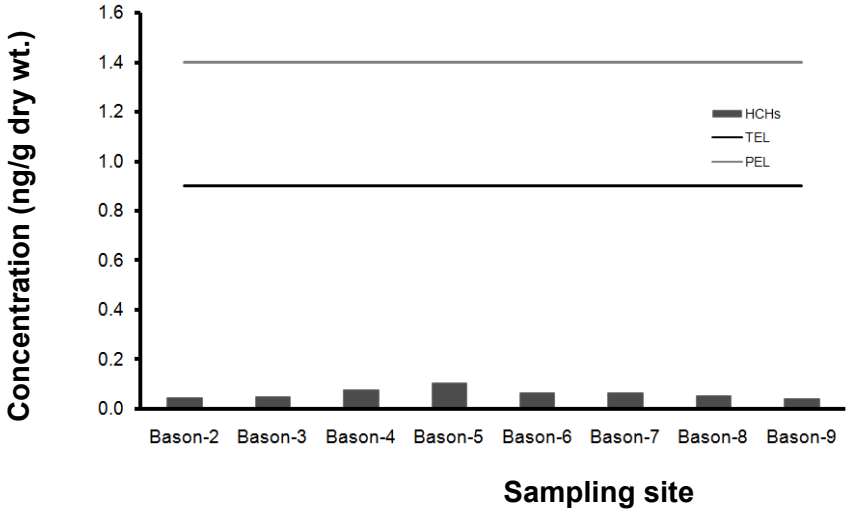


Figure 5.20: Comparison between HCHs concentrations in TN-SG River sediment samples and TEL, PEL values

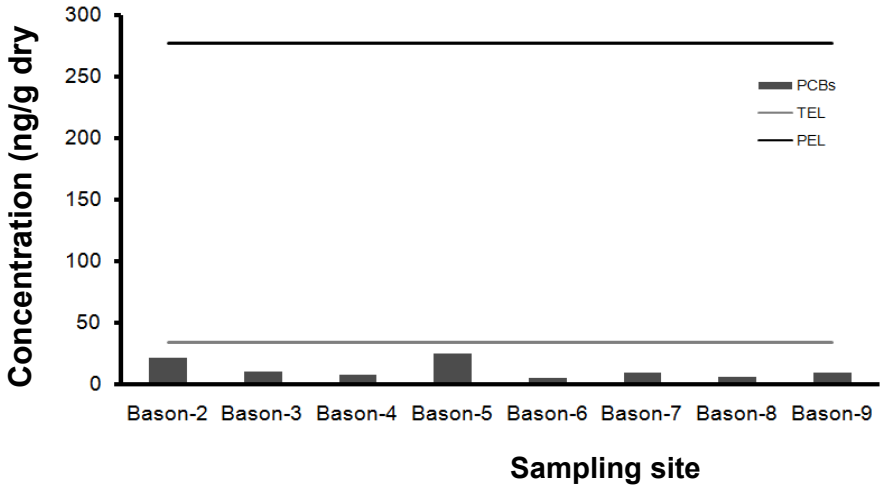


Figure 5.21: Comparison between PCBs concentrations in TN-SG River sediment samples and TEL, PEL values

Dieldrin was found in almost all sediment samples and dieldrin level of TN-SG River sediment samples is distinctively high (ranged 0.623 – 6.502 ng/g dry wt.). In the comparison to TEL and PEL values, dieldrin levels in TN-SG River samples are close to TEL value. Especially, Bason-5 dieldrin level (6.50 ng/g dry wt.) approximates PEL value (6.67 ng/g dry wt.) (Fig. 5.22). However, dieldrin levels in Thivai and Tien River sediments are much lower than those in TEL and PEL values (table 5.11 and 5.12).

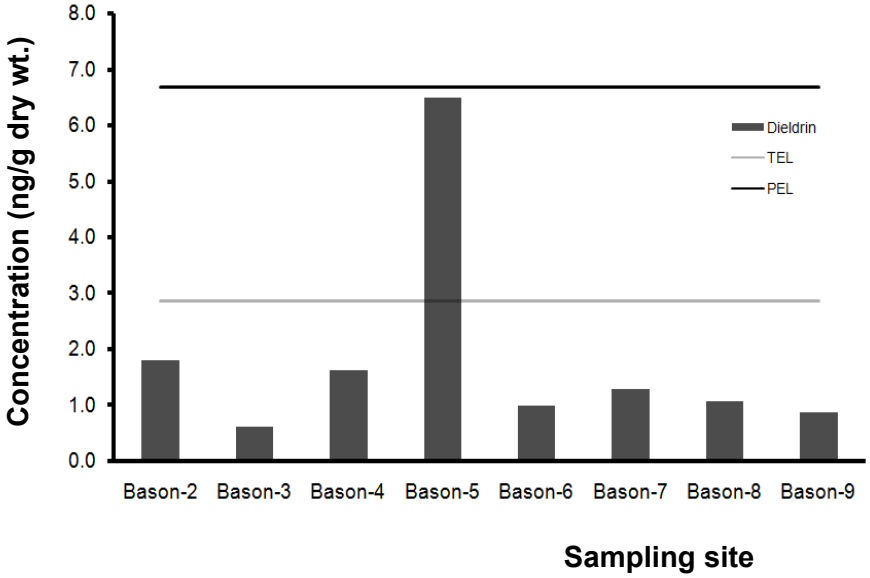


Figure 5.22: Comparison between Dieldrin concentrations in TN-SG River sediment samples and TEL, PEL values

Similar to soil samples, endosulfans were detected in all sediment samples in common levels due to their large usage in Vietnam agricultural activities. This substance import has just been prohibited since 2005 and endosulfans using in agricultural activities has also been banned since 2006 (*MARD, 2005*).

Endosulfans contamination levels in sediment sample follow the order: Tien River > TN-SG River > Thivai River (Fig. 5.23). The highest endosulfans level was found in Tien River sediment samples might due to the widespread usage of endosulfan in agriculture and from the discharge of irrigational systems served for paddy fields and orchards along both river sides. For TN-SG River sediment, endosulfans can come from the leachated water of landfill, municipal waste water of residential areas and solid waste of the pesticide factories in HCMC area.

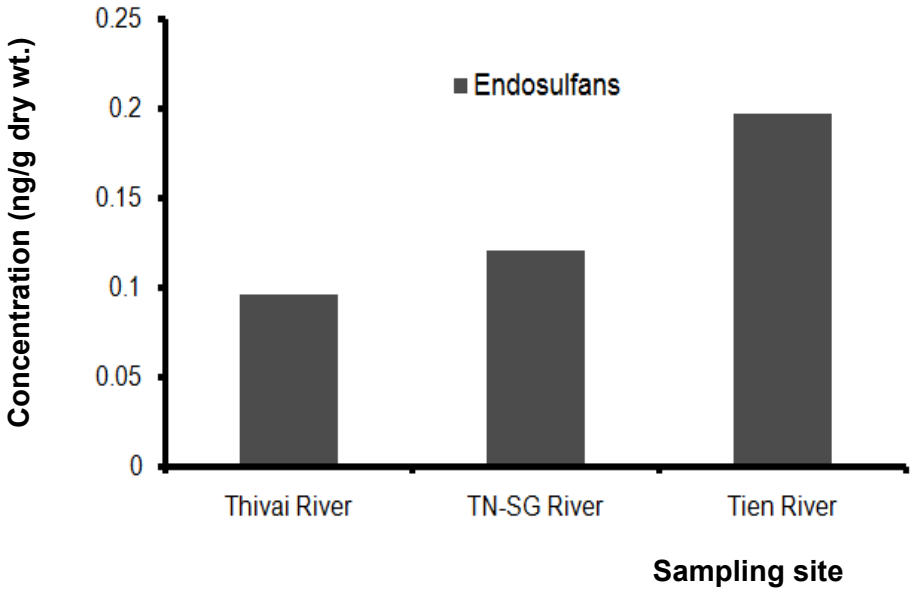


Figure 5.23: Endosulfans concentrations in Rivers sediment samples

Chlordane was detected in all TN-SG sediment samples with levels ranged 0.04 – 2.14 ng/g dry wt. (Table 5.10). In contrast, chlordane levels in Thivai and Tien River sediment samples were lower than LOD of the analytical method or very small values. Interestingly, similar spatial distribution showing higher CHL levels in urban areas than in suburban and rural areas is observed in previous studies (*Iwata et al. 1994; Monirith et al. 2003; Minh et al., 200a*). This fact suggests that the major usage and emission of CHLs are in urban areas of Vietnam.

Contamination by CHLs in Vietnam was scarcely investigated compared to PCBs and DDTs. CHLs might have been used for termite control, wood preservation (*Monirith et al. 2003*), and protective treatment for underground cables (*ATSDR 1994*).

PBDEs residue levels in sediment samples varied from 0.06 – 1.13 ng/g dry wt. in TN-SG River, from 0.01 – 0.23 ng/g dry wt. in Thivai River, and from 0.01 – 0.58 ng/g dry wt. in Tien River (table 5.10, 5.11 and 5.12). The transport pathway of PBDEs to the aquatic environment could be discharge of municipal sewages and atmospheric deposition of fine particles (*Minh et al., 2007c*).

Others PTSs in sediment samples such as heptachlor, heptachlor epoxide, aldrin, endrin, endrin aldehyde and mirex were lower than LOD of the analytical method or very small value.

Comparison of PTSs levels in sediment samples with those in previous studies in Vietnam demonstrated a decreasing trend (table 5.13). In general, DDTs and PCBs levels in TN-SG River sediment samples were lower than those in other studies in Vietnam (*Iwata et al., 1994; Phuong et al., 1998; Minh et al., 2007a; Kishida et al., 2007*). Tien river sediment samples have DDTs, and other PTSs level (except PCBs) several times lower than those in sediment samples collected from Mangroves, southern Vietnam (*Iwata et al., 1994*) and from Hau River (*Minh et al., 2007b*) (table 5.13). These facts suggest consistent decreasing input of PTSs in the environment of southern Vietnam.

The table 5.13 show PCBs and DDTs levels in our study were lower than those in Singapore, Korea and China but higher than those in Taiwan, Hong Kong and India. HCHs levels in our sediment samples were much lower than those in various locations in the world.

Table 5.13: Comparison of PTSs levels between our sediment samples and various locations in the world (mean value in ng/g dry weight)

Location	Year	n	PCBs	DDTs	CHLs	HCHs	HCB	Reference
Vietnam								
Thivai river	**2006**	**18**	**0.43**	**0.77**	**nd**	**0.06**	**0.17**	Present study
Thinghe-Saigon River	**2007**	**8**	**11.76**	**17.6**	**0.79**	**0.07**	**9.51**	Present study
Tiengiang (Tien river)	**2007**	**19**	**6.05**	**2.4**	**0.02**	**0.05**	**0.05**	Present study
Cantho (Mekong river)	2003-2004	22	0.89	6.5	0.93	0.10	0.02	Minh et al., 2007b
HCMC canals	2004	6	81	37	2.0	nd	6.60	Minh et al., 2007a
Saigon-DongNai River	2004	9	6.80	5.6	0.28	0.01	0.24	Minh et al., 2007a
Saigon-DongNai River-Estuary	2004	7	0.90	1.2	0.03	0.01	0.03	Minh et al., 2007a
HCMC canal	2002	2	77	84	5.05	0.13	12.45	Kishida et al., 2007
HCMC canal	1996	10	223.2[a]	79.9	-	-	-	Phuong et al., 1998
Hochiminh (Urban areas)	1990	4	460	425[b]	16.95[c]	7.68[d]	-	Iwata et al., 1994
Hanoi (Urban areas)	1997	12	11	30	0.55[e]	0.80	0.03	Nhan et al., 2001
World								
Coastal Region (Singapore)	2003	13	73.9	6.7		18.1		Wurl and Obbard, 2005
Gao-ping River (Taiwan)	2000	12	1.43	0.78		2.49		Doong et al., 2008
Hong Kong	2004	6	4.2	5.6[f]		0.17[g]		Wei et al., 2008
Hugli Estuary (India)	2003	10	0.8	0.96[e]		0.24		Guzzella et al., 2005
Korea (Masan Bay)	1997	20	15	13.6	-	-	-	Hong et al., 2003
China (Macao Harbour)	1997	1	340	1630	-	-	-	Kang et al., 2000

-: data not available; nd: not detectable (Table 4.11, section 4.5.2.c, chapter 4) [a]: As aroclor 1254 mixture; [b]: sum of pp'-DDE, pp'-DDD, pp'-DDT and op'-DDT; [c]: sum of t-chlordane, c-chlordane, t-nonachlor and c-nonachlor; [d]: sum of a-HCH, b-HCH and g-HCH; [e]: sum of t-chlordane, c-chlordane and t-nonachlor; [f]: Sum of op'-DDE, pp'-DDE, op'-DDD, pp'-DDD, op'-DDT and pp'-DDT; [g]: sum of a-HCH and g-HCH

In global comparison, PBDEs levels in our sediment samples are much lower than those in China, Netherland and other developed countries (Table 5.14). Unlike PCBs and DDTs, contamination by PBDEs in Vietnam was scarcely investigated.

Table 5.14: Comparison of sum of PBDE residue levels between our sediment samples and various locations in the world (mean value in ng/g dry wt.)

Country	Year	n	PBDEs	Reference
Vietnam				
TN canal-SG Rivver	2006	8	0.50	Present study
Thivai River	2006	18	0.07	Present study
Tien River	2007	19	0.10	Present study
World				
Coastal area - Korea	2004	25	0.31[a]	Moon et al., 2007
Tokyo Bay – Japan	2002	6	0.26[b]	Minh et al., 2007c
Lake Maggiore (Italy- Switzerland)	2005	6	1.27[c]	Guzzella et al., 2008
China (sewage sludge)	2005	31	25.6[d]	Wang et al., 2007
Sediment – Netherlands	1999	22	2.40[e]	De Boer et al., 2003

[a]: 20 PBDE congeners; [b]: 10 PBDE congeners; [c]: 7 PBDE congeners; [d]: 12 PBDE congeners; [e]: 5 PBDE congeners.

5.4 Conclusion

Overall, PTSs contamination in the soil and sediment samples is still one of the important environment issues. Comparison PTSs levels in our soil and sediments samples with those in the previous studies in Vietnam, we might suggest a decreasing trend of PTSs levels in the aquatic environment.

PTSs levels in Vietnam soil and sediment samples showed that PCBs and DDTs are prevailing organochlorine contaminants in the environment. Although DDTs was officially phased out in Vietnam, our results provided evidence for the recent inputs of DDTs into the river sediment (Thinghe canal-Saigon river – Fig.5.16).

Toxaphene have been found only in agrichemical warehouse. However, this can be a potential pollution source, especially under insufficient of ineffective pollution management and prevention.

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Chapter 6: PTSs CONTAMINATION IN FISH SAMPLES

6.1 Introduction

One of aims of thesis is to elucidate contamination status and sources of PTSs in fish and follow the transfer of PTSs from contaminated sites into the food chain as well as to assess their potential risk to aquatic biota and human. Based on the results of PTSs contamination in sediment (chapter 5), Saigon River and Tien river basin were chosen as study sites. Thivai River have been so contaminated by waste water from industrial zones along the river that we could not find any aquatic biota or fish in this river basin. Fish was only found in the estuary near Thivai – Gogia river confluence.

Figure 6.1: Saigon River

We have selected three kinds of fish for our research: catfish (*Clarias macrocephalus* and *Mystus nemurus*) and snake-head (*Ophiocephalus striatus*) – They are carnivorous fish and catfish lives in the bottom while snakehead lives in middle water layer. In addition, these kinds of fish are consumed by local residents.

6.2 Material and method

The wild fish samples were collected from the fishing boats along the Saigon and Tien rivers. It was difficult to collect all fish samples from the same species and the same size. We collected 30 fish samples as follow:

- Saigon River: Five catfishes (*Clarias macrocephalus* - coded CSG) and three snake-heads (*Ophiocephalus striatus* - coded SSG). The sampling time was December/2006.

- Tien river: Seven catfishes (*Clarias macrocephalus* – coded CTG), five catfishes (*Mystus nemurus* – coded MTG) and ten snakehead (*Ophiocephalus striatus* – coded STG). The sampling time was October - December/2007.

On the other hand, we have analyzed three catfish, coded RC samples collected from a small pool in family scale in Longkhanh town, Dongnai province. These fishes were fed with formulated diets and considered as Reference samples in comparison with wild fishes. The sampling time was February 2008. All the fish samples were kept in polyethylene bags and preserved with ice during transport to our laboratory, where they were stored at low temperature (-20^{o}C) until chemical analysis.

Before sample treatment, all fishes were measured and weighted. The description of fish samples is presented in the table 6.1 below.

Table 6.1: Description of fish samples

No	Code	Length (cm)	Weight (g)	No	Code	Length (cm)	Weight (g)
Saigon river							
1	CSG1	31	284	5	CSG5	29	222
2	CSG2	26	182	6	SSG1	23	95
3	CSG3	27	207	7	SSG3	21	86
4	CSG4	35	351	8	SSG4	18	51
Tien river							
10	CTG1	29	182	21	MTG5	29	187
11	CTG2	32	256	22	STG1	28	196
12	CTG3	35	326	23	STG2	24	73
13	CTG4	30	301	24	STG3	25	85
14	CTG5	26	198	25	STG4	27	117
15	CTG6	26	147	26	STG5	23	111
16	CTG7	28	176	27	STG6	24	119
17	MTG1	28	221	28	STG7	32	253
18	MTG2	42	396	29	STG8	29	230
19	MTG3	36	285	30	STG9	30	232
20	MTG4	32	261	31	STG10	33	260
Fish – Reference samples							
1	RC1	21	86	3	RC3	25	124
2	RC2	22	112				

Sample treatment: all skinned muscle (fillet) of the fish were cut and homogenized by a meat-grinder with glass grinding chamber and inox cutting blade (Büchi Mixer B-400). After homogenizing, the sample was mixed carefully again with a spoon, then a part of sample (about 80 - 100g) was kept in a glass brown flask with screw cap. The flask containing sample was labelled and stored in fridge at low temperature (-20^{o}C) until chemical analysis. The fish samples were extracted and analyzed in IER lab and CEAL lab with the analytical method described in chapter 4.

Figure 6.2a: Catfish of Saigon river (*Clarias macrocephalus*)

Figure 6.2b: Snakehead of Saigon river (*Ophiocephalus striatus*)

Figure 6.3a: Catfish of Tien river (*Mystus nemurus*)

Figure 6.3b: Homogenize fish samples with Büchi Mixer B-400

Briefly, fish samples (skinned muscle) were ground with Na_2SO_4 and extracted using a soxhlet apparatus with a mixture of n-hexane and acetone (7:3). The aliquot of extract was used for determination of fat content using a gravimetric method, which was described in chapter IV. The PTSs concentration is expressed in ng compound to lipid content. A procedural blank was run for every batch of ten fish samples to verify cross - contamination. Blanks should not contain traces of analytes or their levels were so low in comparison with levels in analyzed extracts that amounts from blanks were not subtracted from samples.

Three catfish were purchased from Longkhanh town, Dongnai province – where considered as a place not affected by PTSs to compare the obtained PTSs contamination level.

6.3 Results and discussion

The lipid contents and PTS concentrations in fish tissue from Saigon River, Tien River and reference fish are presented in the table 6.2, 6.3 and 6.4 below.

Results presented in table 6.2 and 6.3 show that PCBs, DDTs, PBDEs, HCB, and endosulfans were detected in all the fish samples from Saigon River, Tien Rivers, and even from reference fish samples. Contamination pattern was consistent as follows: DDTs > PCBs > Endosulfans > HCB > CHLs > HCHs in Saigon river, and PCBs > DDTs > endosulfans > HCB > HCHs > CHLs in Tien river. However, contrasting to PTSs concentration in fish samples from Tien River, those of Saigon River were much higher (Fig. 6.4)

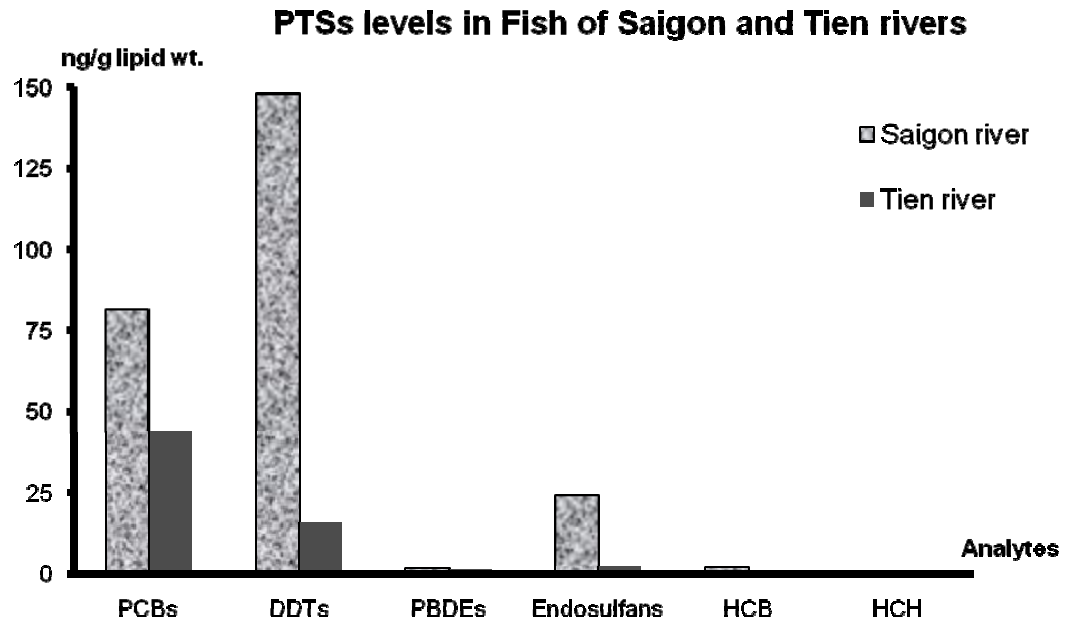

Figure 6.4: Mean concentrations of PTSs in fish from Saigon and Tien River

Table 6.2: PTSs concentrations in fish tissue of Saigon River (ng/g lipid wt.)

PTSs	CSG1	CSG2	CSG3	CSG4	CSG5	SSG1	SSG3	SSG4	Min.	Max	Mean
Lipid content (%)	2.7	3.4	2.0	3.6	2.6	2.1	1.8	2.3	1.8	3.6	2.6
PCBs	139.87	107.78	130.16	18.55	48.48	68.37	97.21	40.93	18.55	139.87	81.42
PBDEs	0.81	4.61	3.04	1.94	0.12	4.57	nd	nd	nd	4.61	1.89
HCB	1.35	3.38	4.25	0.05	0.11	3.26	0.18	0.22	0.05	4.25	1.60
HCHs	nd	nd	1.55	nd	0.14	2.55	nd	nd	0.14	2.55	0.53
Heptachlor	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-
Heptachlor Epoxide	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-
Aldrin	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-
Dieldrin	3.17	8.37	22.51	nd	0.14	8.47	1.82	0.80	0.14	22.51	6.47
Endrin	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-
Endrin aldehyde	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-
Chlordanes	nd	nd	4.330	nd	1.664	nd	nd	nd	nd	4.330	0.749
Endosulfans	21.76	24.28	58.62	18.45	nd	66.76	1.23	nd	nd	66.76	23.89
pp'-DDE	26.53	273.15	258.90	22.74	2.17	73.06	31.29	120.65	2.17	273.15	101.06
pp'-DDD	15.96	192.35	68.19	12.77	1.37	15.24	15.40	12.27	1.37	192.35	41.69
pp'-DDT	3.62	9.60	6.21	3.94	0.46	10.96	3.26	4.36	0.46	10.96	5.30
DDTs	46.12	475.10	333.30	39.44	4.00	99.25	49.95	137.28	4.00	475.10	148.05
Mirex	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-
Toxaphenes	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-

nd: not detectable (Table 4.11, section 4.5.2.c, chapter 4); HCHs: sum of α-, β-, γ-, and δ-HCH; chlordanes: sum of o-, a- and g-chlordane; endosulfans: sum of endosulfan –I, II and endosulfan sulfate; DDTs: sum of pp'-DDE, pp'-DDD and pp'-DDT; PCBs: sum of 21 congeners

Table 6.3: PTSs concentration in fish tissue of Tien River (ng/g lipid wt.)

	CTG1	CTG2	CTG3	CTG4	CTG5	CTG6	CTG7	MTG1	MTG2	MTG3	MTG4	MTG5	STG1	STG2	STG3	STG4	STG5	STG6	STG7	STG8	STG9	STG10
Lipid content (%)	**3.4**	**3.6**	**4.1**	**4.8**	**3.4**	**3.2**	**3.5**	**2.4**	**4.7**	**2.9**	**1.8**	**3.9**	**1.9**	**3.0**	**1.6**	**1.5**	**1.5**	**1.6**	**1.3**	**2.1**	**1.9**	**1.3**
PCBs	32.58	27.23	25.45	16.13	38.04	62.31	71.55	18.74	22.85	58.10	78.14	56.01	3.28	28.02	32.04	34.12	23.74	20.29	125.12	38.84	75.52	70.54
PBDEs	1.49	0.33	0.18	0.08	0.43	0.57	0.50	0.16	0.22	0.41	0.53	nd	2.05	0.20	1.59	2.40	1.31	1.60	7.31	0.11	0.61	5.36
HCB	0.12	0.06	0.03	0.05	0.16	0.14	0.13	0.06	0.04	0.14	0.21	0.15	1.00	0.10	0.15	0.23	0.14	0.24	1.88	1.65	0.13	1.34
HCHs	0.25	0.08	0.10	0.04	0.16	0.17	0.39	nd	0.18	0.12	nd	0.06	0.06	nd	0.10	0.10	0.25	0.13	0.71	0.68	nd	0.58
Heptachlor	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.58	nd	nd	nd	nd	nd	1.77	2.47	nd	0.34
Hept. epoxide	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Aldrin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Dieldrin	0.54	0.23	nd	0.21	2.40	4.17	nd	0.86	2.25	1.84	1.49	2.33	nd	nd	nd	nd	5.57	nd	nd	nd	0.25	nd
Endrin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
End. aldehyde	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Chlordanes	nd	nd	nd	nd	1.07	nd	0.91	nd	0.80	nd	nd	0.75	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Endosulfans	6.13	0.53	0.14	0.41	0.99	2.94	5.35	0.87	2.07	1.35	1.59	2.09	nd	0.57	nd	2.30	5.15	6.94	5.24	nd	1.33	8.33
pp'-DDE	3.30	1.25	1.00	1.38	12.84	3.85	3.41	2.16	2.41	16.58	40.09	15.80	4.85	0.70	1.74	2.71	1.04	1.57	97.51	9.27	11.52	10.79
pp'-DDD	1.77	0.67	0.79	0.81	5.38	2.24	2.86	1.09	1.97	4.61	13.94	6.82	nd	0.25	nd	nd	nd	nd	6.69	nd	4.02	nd
pp'-DDT	nd	nd	nd	0.20	0.40	nd	nd	nd	0.24	2.16	nd	2.90	2.80	0.25	nd	nd	nd	nd	28.62	4.92	nd	1.64
DDTs	5.07	1.92	1.78	2.38	18.63	6.09	6.27	3.25	4.62	23.34	54.03	25.52	7.65	1.20	1.74	2.71	1.04	1.57	132.82	14.19	15.54	12.43
Mirex	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.754	nd	nd	nd	nd	nd	nd	1.025	nd	2.108	nd	nd	nd
Toxaphenes	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd: not detectable (Table 4.11, section 4.5.2.c, chapter 4); HCHs: sum of α-, β-, γ-, and δ-HCH; chlordanes: sum of o-, a- and g-chlordane; endosulfans: sum of endosulfan –I, II and endosulfan sulfate; DDTs: sum of pp'-DDE, pp'-DDD and pp'-DDT; PCBs: sum of 21 congeners

Table 6.3 (cont.)**:** PTSs concentration in fish tissue of Tien river (ng/g lipid wt.)

PTSs	Min.	Max.	Mean
Lipid content (%)	1.3	4.8	2.7
PCBs	3.28	125.12	43.57
PBDDEs	0.08	7.31	1.25
HCB	0.03	1.88	0.37
HCHs	nd	0.71	0.19
Heptachlor	nd	2.47	0.20
Hept. Epox.	-	-	-
Aldrin	-	-	-
Dieldrin	nd	5.57	1.01
Endrin	-	-	-
Endrin ald.	-	-	-
Chlordanes	nd	1.07	0.16
Endosulfans	0.14	8.33	2.86
pp'-DDE	0.70	97.51	11.17
pp'-DDD	0.25	13.94	3.60
pp'-DDT	0.20	28.62	4.41
DDTs	1.04	132.82	15.63
Mirex	nd	2.11	0.18
Toxaphenes	-	-	-

Table 6.4: PTSs levels in catfish tissue of Longkhanh (Reference sample) (ng/g lipid wt.)

PTSs	RS1	RS2	RS3	Min.	Max.	Mean
Lipid content (%)	2.6	2.1	2.3	2.1	2.6	2.3
PCBs	5.06	3.96	3.38	3.38	5.06	4.13
PBDDEs	nd	nd	nd	-	-	-
HCB	0.04	0.04	nd	nd	0.04	0.03
HCHs	nd	nd	nd	-	-	-
Heptachlor	nd	nd	nd	-	-	-
Hept. Epox.	nd	nd	nd	-	-	-
Aldrin	nd	nd	nd	-	-	-
Dieldrin	nd	nd	nd	-	-	-
Endrin	nd	nd	nd	-	-	-
Endrin ald.	nd	nd	nd	-	-	-
Chlordanes	nd	nd	nd	-	-	-
Endosulfans	nd	0.11	nd	nd	0.11	0.04
pp'-DDE	1.25	0.22	3.45	0.22	3.45	1.64
pp'-DDD	0.97	nd	2.23	nd	2.23	1.07
pp'-DDT	nd	nd	0.95	nd	0.95	0.32
DDTs	1.25	0.22	5.70	0.22	6.63	3.03
Mirex	nd	nd	nd	-	-	-
Toxaphenes	nd	nd	nd	-	-	-

nd: not detectable (Table 4.11, section 4.5.2.c, chapter 4), HCHs: sum of α-, β-, γ-, and δ-HCH; chlordanes: sum of o-, a- and g-chlordane; endosulfans: sum of endo–I, II and endosulfan sulfate; DDTs: sum of pp'-DDE, pp'-DDD and pp'-DDT; PCBs: sum of 21 congeners

The PTSs levels in our research clearly demonstrate DDTs and PCBs as two abundant contamination groups in the environment. This remark agrees well with those in previous studies of water, sediment, mussels, fishes, birds and human milk collected from Vietnam (*Nhan et al., 2001, Minh et al., 2002, Minh et al., 2004, 2006a,b*).

Like previous studies, we report the total DDTs level as the sum of pp'-DDE, pp'-DDD and pp'-DDT (*Minh et at., 2002, 2006a, 2007a,b*). The fish levels of DDTs varied from 4 to 475 ng/g lipid wt. in Saigon River fishes and from 1 to 133 ng/g lipid wt. in Tien River fishes. The mean concentration of DDTs from Saigon River fish samples (148 ng/g lipid wt.) was much higher than those in Tien River fish samples (15.6 ng/g lipid wt.) and Reference fish samples (3.0 ng/g lipid wt.), indicating the significant DDTs contamination to the aquatic environment by urban area of Hochiminh City (*Phuong et al., 1998; Minh et al., 2006a, b; Kishida et al., 2007*). Patterns of DDTs in the fishes from Saigon and Tien River are shown in Figure 6.5.

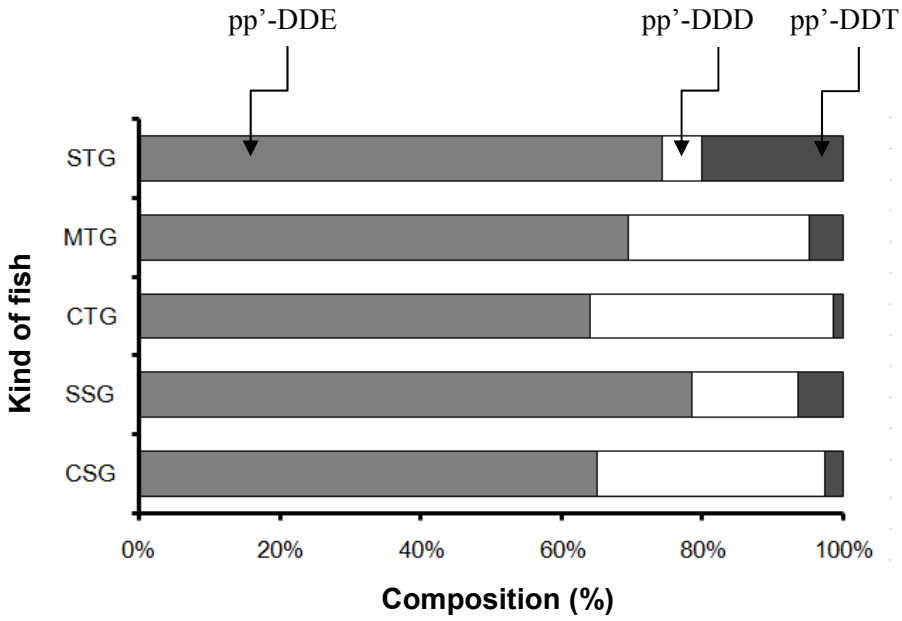

Figure 6.5: DDTs composition in fish samples from Saigon River (CSG-Catfish, SSG-Snakehead) and Tien river (CTG and MTG-catfish, DTG-snakehead)

The composition of DDTs in catfish appears to be slightly different from those in snakehead and proportion of p,p'-DDT is slightly higher in the snakehead fish than those in catfish from both rivers. The ratios of pp'-DDT/pp'-DDE in our fish samples agree well with the ratios of those in landfill soil, Tien River sediment and paddy soil samples presented in chapter 5 (Fig. 6.6). However these ratios are not similar to those in catfish (proportion of pp'-DDT: 20 – 22%) from Hau river in previous study (*Minh et al., 2006a*). This result perhaps indicates less recent DDTs input in Tien River basin compared to Hau River basin.

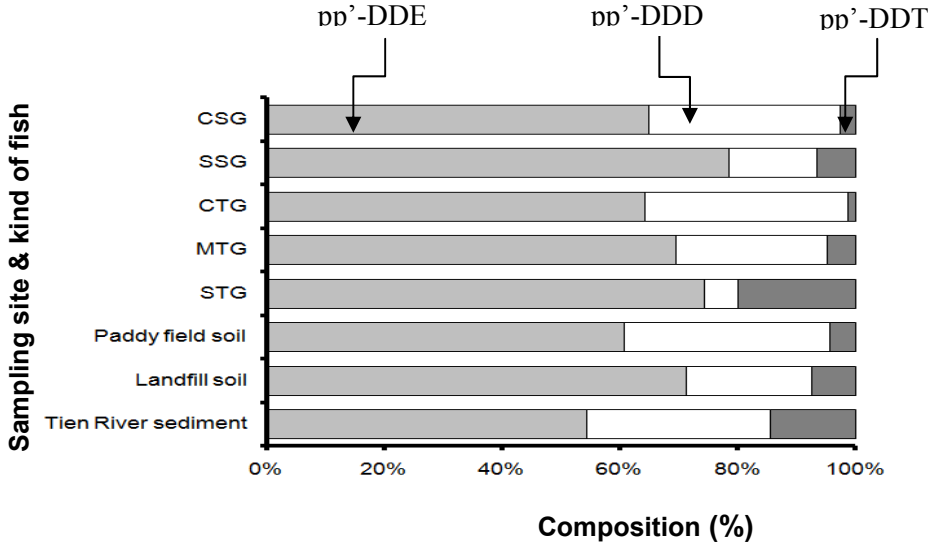

Figure 6.6: DDTs composition in fish samples from Saigon River and Tien river compared with those in paddy soil, landfill soil and Tien River sediment

Similar to DDTs, PCBs was detected in all our fish samples. Fish levels of PCBs varied from 18.6 to 139.9 ng/g lipid wt. in fish samples from Saigon River, from 3.3 to 125.1 ng/g lipid wt. in

fish samples from Tien River, and from 3.4 to 5.1 ng/g lipid wt. in Reference fish samples (table 6.2, 6.3 and 6.4).

Fig. 6.7 demonstrates 21 congener profiles of PCBs in catfish and snake-head fish from Saigon and Tien Rivers. We can see that the congeners profile of five categories of fish from both rivers were very similar to each other. Generally, in our fish samples, PCB 101 was the most abundant congener (17%), followed by congeners PCB 118, 149, 153 and 138. The relative low abundance of tri and tetrachlorinated biphenyls in our fish samples may due to a stronger bio-accumulative ability of higher-chlorinated congeners, such as PCB 149, 138, 153 (*Moon et al., 2006, Minh et al., 2006a*).

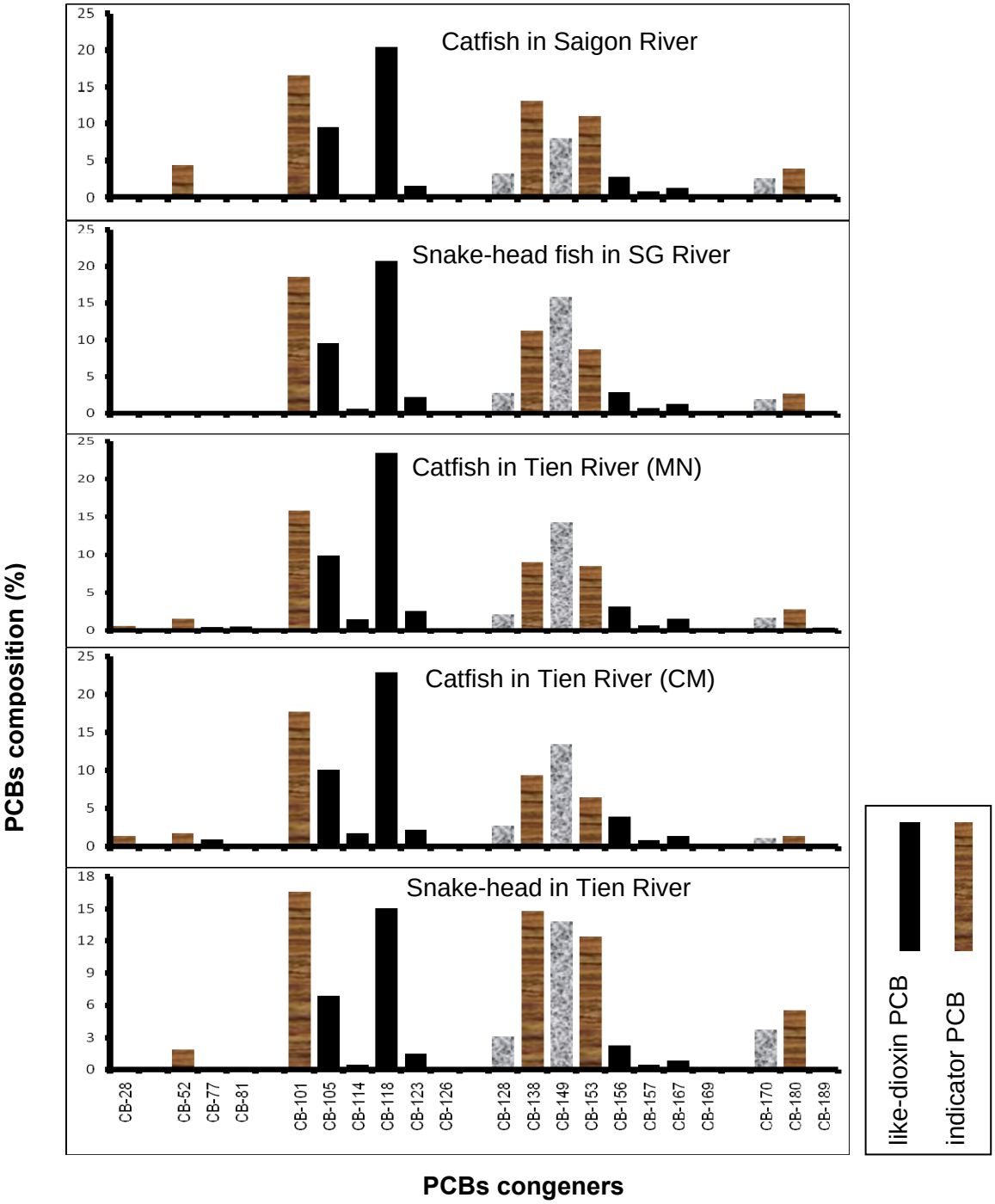

Figure 6.7: PCBs congeners' profiles in wild fish collected from SG and Tien River

In addition, Fig. 6.8 shows that, high chlorinated – PCB congeners were found in fish from Saigon River, soil in landfill and sediment from TN-SG River. Whereas, only low chlorinated – PCB congeners were found in landfill soil and TN-SG River sediment. The relative lower abundance of low chlorinated – PCBs (CB 28, 52) in the fishes may due to stronger bioaccumulative ability of higher chlorinated - PCBs (*Minh et al., 2006a*).

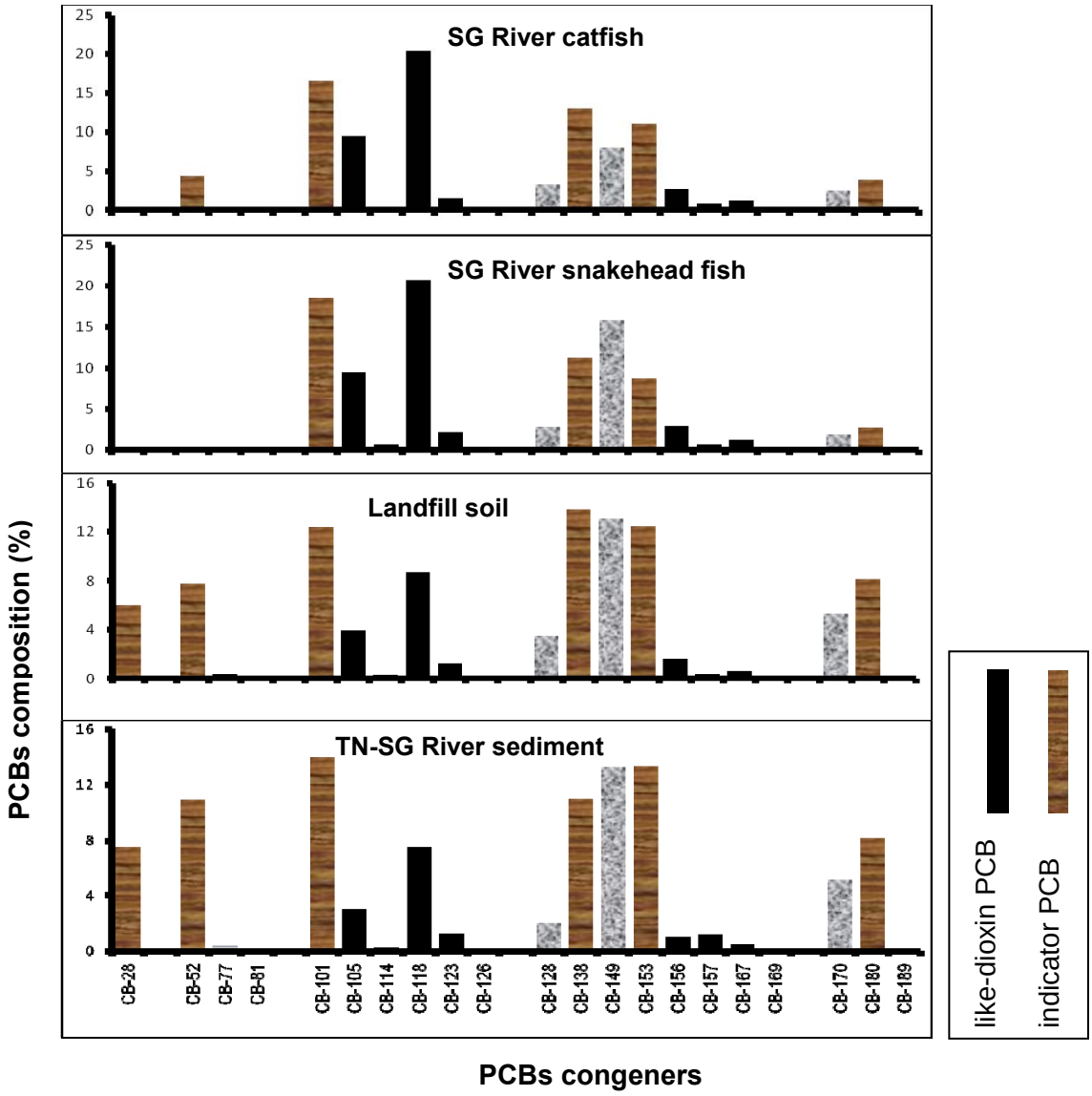

Figure 6.8: Comparison of PCBs congeners' profiles between SG River wild fishes, TN-SG River sediment and landfill soil samples.

Polybrominated diphenyl ethers were found in most of our fish samples except Reference fish samples, suggesting their widespread contamination in the aquatic environment. Mean concentration of PBDEs in fish samples from Saigon River and Tien River were 1.89 ng/g lipid wt. and 1.25 ng/g lipid wt., respectively. Concentrations of PBDEs in the fishes from Saigon River were statistically higher compared to those in the fishes from Tien River, suggesting additional exposure of the fishes from Saigon River to PBDEs.

Fig. 6.9 presents PBDEs congeners profiles found in 5 categories of fishes samples. In general, in fish samples, BDE 47 was the most abundant congener, accounting for 60%, followed by BDE 99 and 100.

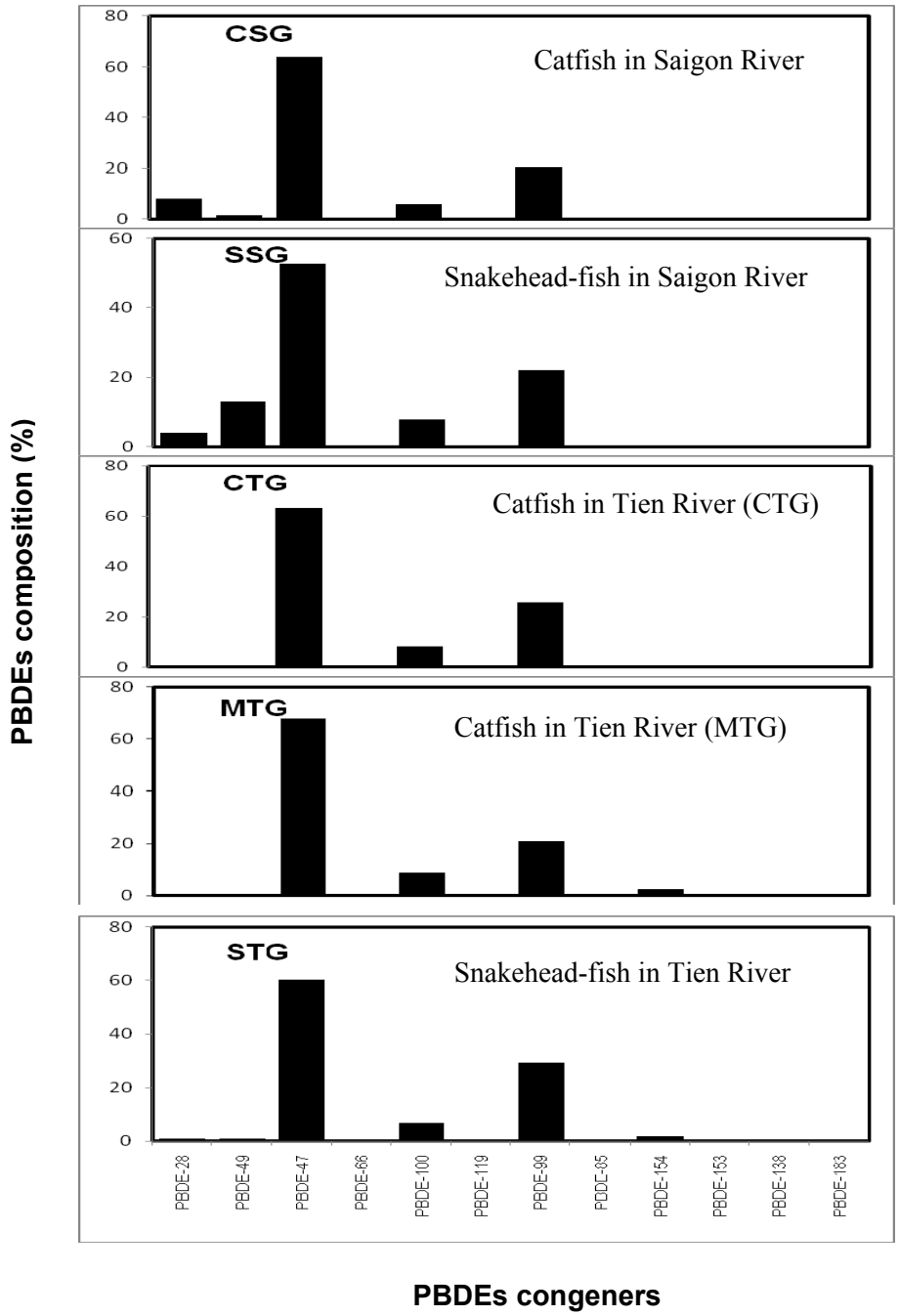

Figure 6.9: PPDEs congeners' profiles in wild fish from Saigon and Tien River

To clarify the usage pattern of PBDEs in Southern Vietnam, the composition of PBDEs in our fishes should be compared with those in in commercial products, such as penta-, octa, deca-BDE products. Our result shows the presence of some representative congeners for penta-product (BDE 99 and 100) (*Hites et al., 2004*), and these congeners also were found in fishes from one previous study (*Minh et al., 2006a*). This fact suggests the using of PBDEs in Vietnam. This hypothesis was supported by the similarity between PBDEs congener's profile of fish samples and those in landfill soil and sediment of TN-SG River and Tien River (Fig 6.10).

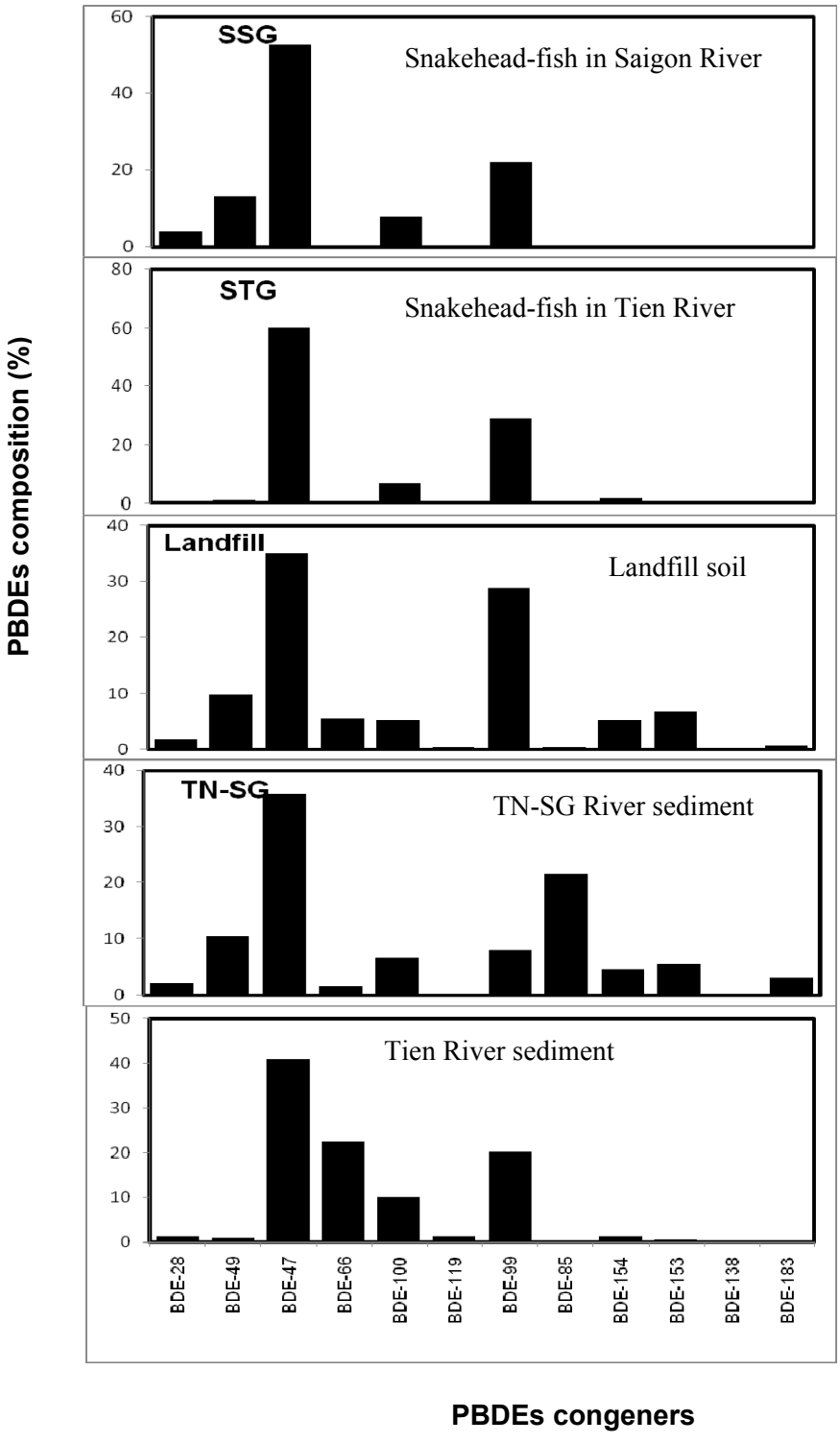

Figure 6.11: PPDEs congeners' profiles in wild fish from Saigon and Tien River and in landfill soil, TN-SG River sediment and Tien River sediment

We could not measure PBDE 209 in our fish samples as well as other environmental samples due to many reasons. However, according to Minh et al. (2006a), there was no PBDE 209 found in fish samples collected from Hau River. Furthermore, PBDE 209 often is not found in biological samples due to its low bioaccumulative ability (*Shaw et al., 2008; Law et al., 2006*).

Concentrations of HCHs, HCB, CHLs and dieldrin in our fish samples were lower than 7 ng/g lipid wt. (table 6.2 and 6.3), suggesting that they are not significant contaminants in fish samples from Saigon and Tien River.

As in soil and sediment samples, endosulfans were detected in almost our fish samples with relatively high levels, 23.89 ng/g lipid wt. in Saigon River and 8.33 ng/g lipid wt. in Tien River (table 6.2 and 6.3). Once again, this suggests the widespread endosulfan contamination in the environment due to their large usage in Vietnam agricultural activities.

Others PTSs in our fish samples such as heptachlor, aldrin, endrin, endrin aldehyde, mirex and toxaphene were lower than LOD of the analytical method or at small value (< 5 ng/g lipid wt.). PTSs levels in our Reference fish samples generally were much lower than those in fish samples from Saigon and Tien River. These levels may be considered as the background of PTSs levels.

In general, concentrations of DDTs and PCBs in our wild fish samples from Saigon and Tien river were lower than those in fish collected during the early 1990s from the coast of Vietnam (*Kannan et al., 1995*) and in 1997 from the Red River Delta in northern Vietnam (*Minh et al., 2002*) (table 6.5). This supports the previous assumption that input of DDTs and PCBs to the environment of Vietnam has consistently decreased over the last decade (*Minh et al., 2004*). Geographical comparisons of PCBs, DDTs, HCB, CHLs and HCHs levels in fishes suggest that their levels in Saigon River fish are in the middle range and those in Tien River fish are low (table 6.5).

It is noteworthy that in this comparison, many fish samples collected during the 1990s, when DDTs was still in used, had levels of DDTs and PCBs comparable to those in the fishes from Saigon river. This may indicate recent exposure of the fishes from Saigon River to the pollutants. From the high residue levels of PTSs in landfill and Thinghe canal in HCMC (in chapter 5) and OCs levels reported in previous studies (*Phuong et al., 1998, Minh et al., 2006b*), we can suggest that Hochiminh and other urban areas might be important sources of PTSs for aquatic environment.

Table 6.5: Geographical comparison of PTSs levels (mean value in ng/g lipid wt.) in fish species

Location	Species	Year	n	Tissue	PCBs	DDTs	CHLs	HCHs	HCB	Reference
Vietnam										
Saigon river	Wild fish	2007	8	Muscle	81	148	0.75	0.53	1.60	Present study
Tien river	Wild fish	2007	22	Muscle	43	15.6	0.16	0.19	0.37	Present study
Longkhanh	Farmed catfish	2008	3	Muscle	4.1	3.0	<0.005	<0.006	0.03	Present study
Hau River	Farmed catfish	2004	20	Muscle	7.2	59	0.62	0.47	0.73	Minh et al., 2006a
Vietnam	Dumpsite catfish	2004	5	Muscle	50	390	5.7	2.2	2.60	Minh et al., 2006a
Vietnam (wild fish)	Several species	1997	-	Whole body	110	4200	110	120	-	Minh et al., 2002
Vietnam (Wild fish)	Several species	1989-1993	19	Muscle	530	1400	5.79[a]	95	2.63	Kannan et al., 1995
Other countries										
Ghana (wild fish)[b]	Tilapia fish	2004-2005	50	Muscle	-	8.88	-	0.13[c]	-	Darko et al., 2008
Switzerland (wild fish)	Trout fish	2003	7[d]	Muscle	880	550[e]	-	2.17[c]	15.43	Schmid et al., 2007
Yangtze, China (wild fish)	Several species	2004-2005	7[d]	Muscle	13.2[f]	0.02[f]	-	0.07[f]	-	Hu et al., 2009
Tibetan, China (wild fish)	Several species	2005	7[d]	Muscle	-	547[g]	-	87	86.60	Yang et al., 2007
India (wild fish)	Several species	1993	48	Muscle	150	630	100[a]	1200	2.92	Kannan et al., 1995
China (Shanghai)-Wild fish	Several species	2000	3	Whole body	180	1000	160[a]	68	22	Nakata et al., 2005
Korea (wild fish)	Several species	1997-2001	14	Muscle	1440	560[e]	68[h]	58	20	Yim et al., 2005
Cambodia (wild fish)	Several species	1998	22	Whole body	10	290	3.0	1.5	2.20	Monirith et al., 1999
Japan (Lake Biwa)-wild fish	Several species	1993		Whole body	3700	1900	-	240	-	Guruge et al., 1997

-: data not available; [a]: sum of t-chlordane, c-chlordane, o-chlordane, t-nonachlor and c-nonachlor; [b]: ng/g wet weight; [c]: Lindane; [d]: pool fish samples; [e]: sum of op'-DDD, pp'-DDD, op'-DDE, pp'-DDE, op'-DDT and pp'-DDT; [f]: ng/g wet weight; [g]: sum of pp'-DDE, pp'-DDD, op'-DDT and pp'-DDT; [h]: sum of [a] and heptachor and heptachlor epoxide

6.4 Conclusion

This chapter demonstrated DDTs and PCBs as two major groups of organochlorine compounds in wild fish samples from Saigon River and Tien River. Besides, endosulfans with relative high levels in fish (23.9 ng/g lipid wt. in Saigon River and 8.3 ng/g lipid wt. in Tien River) show the widespread contamination of endosulfan due to extensively agricultural activities.

PCBs and PBDEs congeners' profiles in fish samples are similar to those found in soil from landfill and in sediment from Thinghe canal-Saigon River. This shows a possible relationship between landfill - HCMC canals - Saigon River fishes in PCBs, PBDEs and other PTSs contamination (Fig. 6.8).

Finally, the comparison between our results and other studies in Vietnam as well as in the world shows that the PTSs levels in fish samples are relatively low (table 6.5), even lower than those in previous studies in Vietnam (*Kannan et al., 1995, Minh et al., 2002, Minh et al., 2006a*). The obtained results suggest a substantial decrease in the PTSs in aquatic environment of southern Vietnam during recent years.

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Chapter 7: PTSs CONTAMINATION IN HUMAN MILK

7.1 Introduction

As we presented in chapter 1, PTSs are highly lipophilic and persistent compounds, so human chronic exposure via food chain will lead to the accumulation of both parent compound and their metabolites in lipid rich tissue such as adipose tissues and human breast milk. Among human tissues, breast milk is a convenient sampling matrix for measuring residue concentrations of PTSs. The samples are easy to collect and highly suitable for estimating body burdens of persistent OCs, and thus may provide useful information about their accumulation kinetics in humans. In addition, the PTSs residue concentrations in human breast milk are a key factor for evaluating the toxic potential of contaminants in infants (*Minh et al. 2004*).

Human milk has been used for monitoring of human body burdens of PTSs particularly PCB and DDTs for several decades (*UNEP, 2004*). The idea behind most studies on PTSs contamination of human milk has been to discover the infant burden of the chemicals from nursing. An important idea of human milk studies is also that this matrix reflects the contamination at a high trophic level. Thus, human milk samples reflect the intake in different areas: the extent of contamination and different consumption habits.

Actually, to assess the PTSs accumulation and transfer through the food chain, the better way is to carry out the investigation on all the components of the same food chain: that means we should to collect and analyze the PTSs at different levels of the same food chain (e.g.: sediment → fish → carnivorous fish → eating-fish bird → .... → human milk). That will be a perfect strategy for sampling, but it depends on many factors such as the limits of time and budget, the difficulties to obtain the completed formalities for sampling permit (with biomaterial sampling: e.g. collecting human breast milk from hospitals in Vietnam).

We received the great supports from the steering committee of Hungvuong Maternity Hospital and Hocmon General Hospital to carry out breast milk collection in these hospitals. The campaign was performed from September to December 2007.

7.2 Material and method

Human breast milk samples were collected from donors live in one urban area (45 samples) and two suburban areas (23 samples in Hocmon district, Suburban-1, and 19 samples in Binhchanh district, Suburban-2) of Hochiminh city in Vietnam during September to December of 2007 (Fig. 7.1). These three areas have different environmental and living conditions, which may results in the differentiation of PTSs level in human milk. In reality, the urban donors live in the city area with busy traffic. Meanwhile, donors in Suburban-1 group live near Dongthanh landfill and donors in Suburban-2 live around industrial and export processing zones

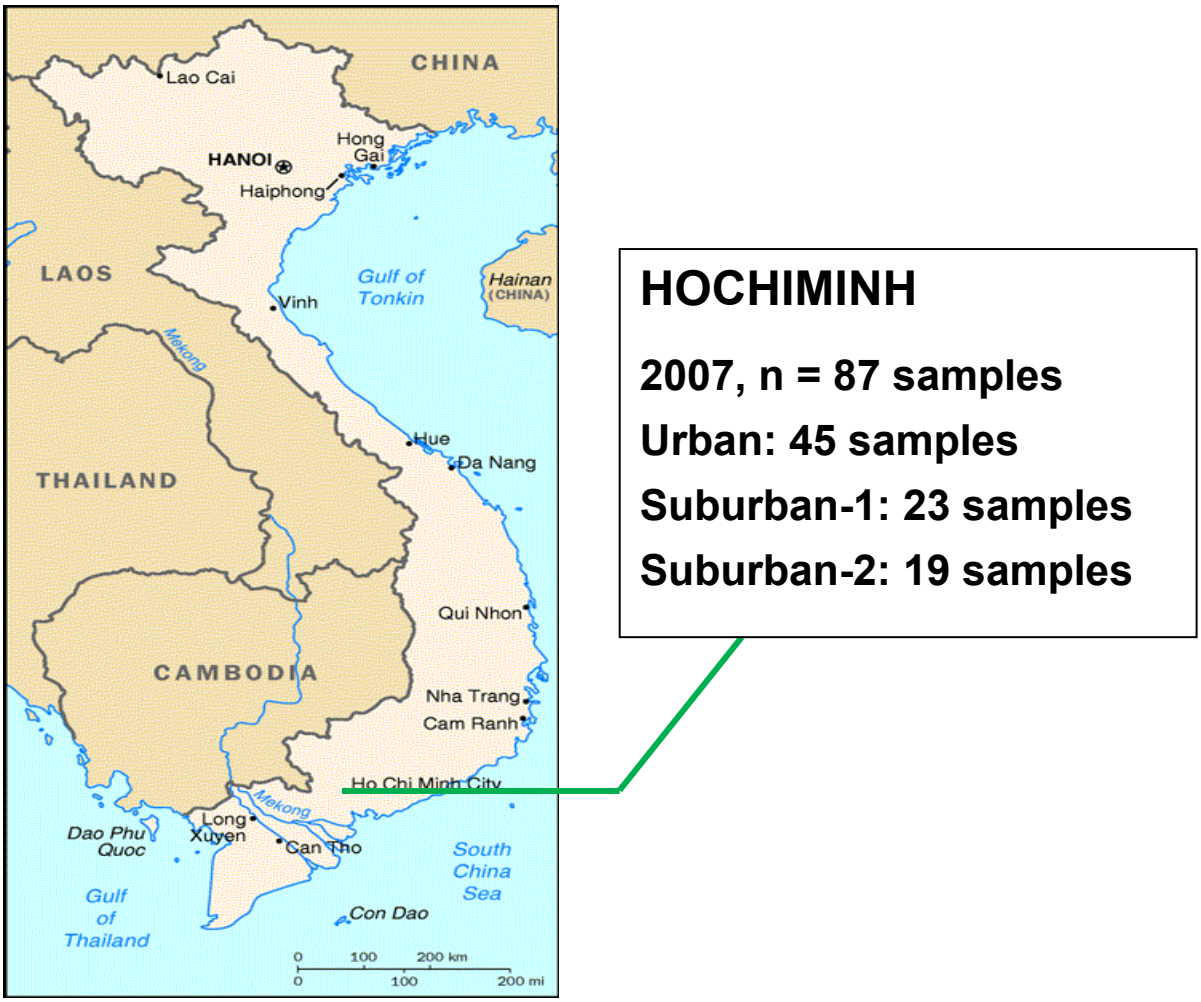

Figure 7.1: Sampling location of human breast milk in Hochiminh city, Vietnam

These milk samples were collected by manual pression of the breast or by a hand pump and transferred into specially cleaned PP tubes. Often 20 to 40 ml milk is collected. They were kept in ice immediately after collection, transported to our laboratory in IER in the same day and preserved there at -20^{0}C until analysis. We randomly selected the breast milk donors from one community in urban area (district 10) and two communities in suburban areas of Hochiminh city (surburban-1: Hocmon district and surburban-2: Binhchanh district). Data on the biological characteristics of the donors and relevant information of sampling sites are given in Table 7.1. The biological characteristics show similarity between such cohorts. The informed consents were obtained from all the donors. Questionnaires on dietary aspects were recorded.

Years of residence of the donors in their area varied widely from 1 to 37 years, indicating that the cohorts actually consist of women from various places and not entirely represent only resident communities. However, the migrations were believed as regular and also within the regions of each city.

All human breast milk samples were extracted and analyzed in IER and CEAL lab with the analytical method described in chapter 4.

Table 7.1: Related information of breast milk donors from Hochiminh city

Location	Age	Weight (kg)	Height (cm)	Number of children	Number of pregnancy	Occupation
Urban (2007, n = 45)						
Mean Range	28.4 18 - 33	47.6 37 - 65	154.5 144 - 168	1.6 1 - 4	1.6 1 - 4	Housewife: 46.6% Other: 53.4%
Surburban-1 (2007, n = 23)						
Mean Range	26.9 18 - 37	49 38 - 68	154.2 142 - 165	1.4 1 - 3	1.4 1 – 3	Housewife: 56.5% Other: 43.5%
Surburban-2 (2007, n = 19)						
Mean Range	27.3 22 - 37	49.9 35 - 68	155.2 146 - 163	1.5 1 - 3	1.5 1 – 3	Housewife: 31.6% Other: 68.4%

7.3 Results and discussion

The PTSs concentrations in human breast milk samples are presented in table 7.2 (*see the results of 87 breast milk samples in annex*). Lipid-normalized concentrations of OCs in human breast milk from Hochiminh city, Vietnam are given in table 7.2. We considered the accumulation of PTSs in 3 groups: urban (district 10) and suburban-1 (Hocmon district) and suburban-2 (Binhchanh district). In general, the concentrations of PTSs residue in human breast milk in Hochiminh city followed the order DDTs > PCBs > Chlordanes > HCHs > HCB. This order of PTSs is different to that found in Vietnamese foodstuff analyzed in the early of 1990s (*Kannan et al., 1992*) or in human breast milk from Hochinh city (*Schecter et al., 1989*), which showed higher level of HCHs compared to PCBs. However, the present order of PTSs in our study agrees well with those in previous study (*Minh et al., 2004*), in birds collected from Red river estuary (*Minh et al., 2002*), mollusks from Hanoi region (*Nhan et al., 2001*), and in biotic samples from Red river delta (*Nhan et al., 1998*)

Mean concentrations of DDTs in human breast milk in suburban-1, suburban-2 and urban of Hochiminh city were 73.2 ng/g, 70.8 ng/g and 150.8 ng/g lipid wt., respectively. The DDTs levels in urban area group were higher than those in suburban areas (table 7.2). Further examination of the DDTs composition revealed that *p,p'*-DDE is the predominant compound accounting for 70 to 90 % of the total DDT concentrations in human breast milk.

Elevated concentrations of DDTs in various environmental compartments in Vietnam have also been reported in a number of recent investigations. Results of the Asia-Pacific Mussel Watch Program indicated that DDT concentrations in mussels and fish from Vietnamese coastal waters are among the highest values reported for the countries in this region (*Kannan et al., 1995; Monirith et al., 2000; Minh et al., 2002*). Interestingly, Phuong et al. (1998) and Minh et al. (2007) reported higher levels of DDTs in sediments from populated locations in Hochiminh city as compared to those from paddy fields in Mekong river delta. This evidence indicates recent applications of DDTs for other purposes such as sanitary and malaria control rather than for agriculture.

The comparison of PTSs levels in urban and suburban areas leads to understand the influence of urbanization on the human contamination by these compounds (Fig. 7.2). The comparison of PCBs, DDTs, HCHs, HCB and endosulfans residues detected in human breast milk collected from urban and rural areas shows that almost of PTSs concentrations are significantly higher in urban areas than those in suburban areas. This indicates important exposure to PTSs in urban locations may through the food chain to man, e.g. fish, meat and dairy products.

From the donor survey results, we found that there are differences in living conditions and dietary habit among the groups of donors, urban and suburban. They show that nursing mothers in urban area consume fish and dairy products approximately 1,5 times, and meat about 1,4 times more than nursing mothers in suburban areas do. This could be one of the reasons why PTSs levels in breast milk from nursing mothers in urban area are higher than those from the others two suburban areas.

Table 7.2: Concentration of PTSs levels in human breast milk (ng/g lipid wt.) in urban and suburban of Hochiminh city, Vietnam

	Urban (n = 45)				Surburban-1 (n = 23)				Surburban-2 (n = 19)				Hochiminh city			
PTSs	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**
Lipid (%)	0.70	5.30	2.53	2.20	0.50	7.30	2.75	2.40	1.00	3.30	1.92	1.90	0.50	7.30	2.40	2.20
PCBs	10.00	337.83	127.96	99.52	27.32	194.06	68.51	63.40	12.13	420.88	82.05	45.08	10.00	420.88	92.84	63.40
PBDEs	0.02	20.02	1.27	0.28	nd	5.17	0.63	0.34	nd	1.98	0.28	0.11	nd	20.02	0.73	0.28
HCB	0.11	3.50	1.33	1.08	0.03	1.67	0.49	0.21	nd	0.56	0.09	0.04	nd	3.50	0.64	0.21
HCHs	1.76	47.23	13.99	10.01	0.37	34.42	6.52	4.21	0.35	18.97	4.30	2.12	0.35	47.23	8.27	4.21
Heptachlor	nd	50.95	7.27	2.74	0.07	63.89	10.28	3.14	nd	16.72	2.09	0.05	nd	63.89	6.54	2.74
Hept Epox	0.21	45.53	7.58	1.78	0.16	87.19	11.70	1.30	0.04	52.49	6.48	1.84	0.04	87.19	8.59	1.78
Aldrin	nd	56.93	6.08	0.90	0.20	30.85	5.55	4.21	0.24	20.92	4.02	2.02	nd	56.93	5.22	2.02
Dieldrin	0.80	39.04	7.69	5.63	0.09	24.22	5.02	2.74	nd	32.61	6.87	4.31	nd	39.04	6.53	4.31
Endrin	nd	19.74	2.09	0.97	nd	24.59	3.73	0.41	nd	33.88	2.49	0.00	nd	33.88	2.77	0.41
EndAld	nd	54.68	7.41	1.97	0.43	177.10	30.21	13.01	0.09	37.14	10.25	7.88	nd	177.10	15.96	7.88
Chlordanes	1.84	49.96	12.83	9.82	1.05	147.77	38.39	29.32	2.83	74.62	25.57	24.14	1.05	147.77	25.60	24.14
Endosulfans	nd	63.62	14.57	7.98	nd	39.61	14.88	10.74	4.24	34.02	16.80	16.33	nd	63.62	15.42	10.74
pp'-DDE	13.91	353.18	104.20	68.50	10.31	188.89	57.46	37.41	12.23	103.53	45.62	40.02	10.31	353.18	69.10	40.02
pp'-DDD	1.31	19.65	7.45	5.61	1.30	30.14	6.86	5.30	1.38	41.95	11.39	5.83	1.30	41.95	8.57	5.61
pp'-DDT	1.64	230.42	39.13	18.37	2.27	36.38	8.92	6.63	5.19	32.02	13.74	12.66	1.64	230.42	20.60	12.66
DDTs	19.25	524.51	150.78	86.05	15.93	227.43	73.24	49.04	20.04	167.32	70.76	62.10	15.93	524.51	98.26	62.10
Mirex	nd	18.35	2.84	0.88	nd	13.58	2.15	0.89	0.20	13.23	4.26	4.11	nd	18.35	3.08	0.89
Toxaphenes	0.21	12.08	3.25	2.44	0.08	11.21	2.80	1.78	0.15	6.83	1.66	1.01	0.08	12.08	2.57	1.78

nd: not detectable (Table 4.11, section 4.5.2.c, chapter 4); PCBs: sum of 21 congeners; PBDEs: sum of 12 congeners; HCHs: sum of α-, β-, γ-, and δ-HCH; Chlordanes: sum of o-, a and g-chlordane; endosulfans: sum of endo–I, II and endosulfan sulfate; DDTs: sum of pp'-DDE, pp'-DDD and pp'-DDT.

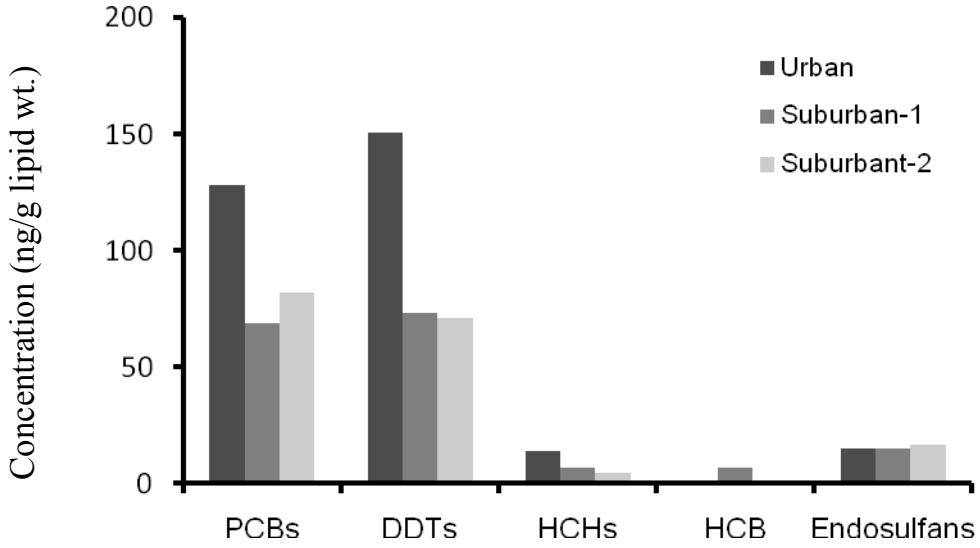

Figure 7.2: PTSs levels in human breast milk from Hochiminh City

It is known that adult female excrete lipophilic contaminants such as PTSs via lactation and thus reducing the body burden of such contaminants. In this study, we observed higher concentrations of OCs in human breast milk of primiparas mothers as compared to multiparas (Table 7.3). This may be due to elimination of PTSs in multiparas via the past lactation. Similar results were also reported in the previous investigations from Vietnam and other countries (*Minh et al., 2004; Nakagawa et al., 1999; Kunisue et al., 2004; Tanabe and Kunisue, 2007*).

To understand the magnitude of DDT contamination in Vietnamese population, residue levels of DDTs in human breast milk in different countries were compiled (Table 7.4). DDTs concentrations in Vietnamese human breast milk were among the lowest values reported for the countries surveyed. This contrast to previous study results of Minh et al. (2004).

Table 7.3: Concentration of PTSs in human breast milk (mean value in ng/g lipid wt.) in Hochiminh City, Vietnam

	Age (Year)	Lipid	PCBs	PBDEs	DDT Compounds				HCB	HCHs	Chlordanes	Endosulfans
					pp'-DDE	pp'-DDD	pp'-DDT	DDTs				
Urban												
Primiparas	27.1	2.7	133.1	1.18	142.6	8.8	61.56	213.03	1.42	16.42	10.11	14.65
Multiparas	29.5	2.4	123.4	1.35	70.5	6.2	19.50	96.31	1.26	11.87	15.22	14.49
Overall	28.3	2.5	128.3	1.27	106.6	7.5	40.53	154.67	1.34	14.14	12.66	14.57
Range	18 - 38	0.7 - 5.3	10.0-337.8	0.02-20.02	13.9-353.2	1.3-19.7	1.64-230.42	19.3-524.51	0.11-3.50	1.76-47.23	1.84-49.96	nd-63.62
Suburban 1												
Primiparas	27.8	2.3	69.9	1.07	68.4	10.20	11.67	90.33	0.69	10.20	41.59	15.90
Multiparas	26.4	3.1	67.5	0.34	50.4	4.71	7.15	62.25	0.37	4.15	36.33	14.22
Overall	27.1	2.7	68.7	0.71	59.4	7.46	9.41	76.29	0.53	7.18	38.96	15.06
Range	18-37	0.5-7.3	27.3-194.0	0.00-5.17	10.3-188.9	1.30-30.14	2.27-36.38	15.9-227.43	0.03-1.67	0.37-34.42	1.05-147.77	nd-39.61
Suburban 2												
Primiparas	28.1	2.0	122.6	0.40	57.3	15.17	16.47	88.97	0.13	6.23	29.97	20.82
Multiparas	26.5	1.8	45.5	0.17	35.1	7.99	11.29	54.37	0.06	2.56	21.61	13.19
Overall	27.3	1.9	84.1	0.28	46.2	11.58	13.88	71.67	0.09	4.39	25.79	17.01
Range	22-37	1.0 - 3.3	12.1-420.8	0.00-1.98	12.2-103.5	1.38-41.95	5.19-32.02	20.0-167.3	nd-0.56	0.35-18.97	2.83-74.62	4.24-34.02

nd: not detectable (Table 4.11, section 4.5.2.c, chapter 4); PCBs: sum of 21 congeners; PBDEs: sum of 12 congeners; HCHs: sum of α-, β-, γ-, and δ-HCH; Chlordanes: sum of o-, a and g-chlordane; endosulfans: sum of endo–I, II and endosulfan sulfate; DDTs: sum of pp'-DDE, pp'-DDD and pp'-DDT.

The global comparison of PTSs residues in human breast milk is given in Table 7.4. We cited data from other studies which used high resolution gas chromatography for quantification of individual PCB congeners. Although the cited data may differ between laboratories, it is possible to draw some relevant comparison to understand the magnitude of contamination. In comparison to other developing countries like China, Cambodia and the Philippines, PCBs levels in breast milk from Vietnam are slightly higher. However, these PCBs levels are still below those reported for developed countries (table 7.3).

Previous global inventory of PCBs production and consumption has indicated that common applications of PCBs (i.e. for industrial purposes) in Vietnam during the past years were not higher than those in China, Hong Kong, India and the Philippines (*Breivik et al., 2002*). Hence, the higher PCB residues observed in human breast milk from Vietnam suggest continuous exposure to PCBs through the food chain to man and an additional sources of PCBs besides industrial sources like transformers, capacitors, etc. A likely source of PCBs in Vietnam could be the release from different kinds of military weapons used extensively during the Vietnam War as suggested earlier (*Thao et al., 1993*).

PCBs, HCHs and HCB concentrations in our study are somewhat in agreement with those reported in earlier study (*Minh et al., 2004*). In the global comparison, human breast milk from South Vietnam showed levels of HCHs, which are lower than those in Hong Kong and Japan but higher than those in other Asian developing countries like Cambodia and the Philippines (Table 7.4). Similarly, levels of HCB in human breast milk were rather low when compared to global levels, which indicate minimal exposure of the general population to this chemical throughout the case-study.

Total chlordane concentrations in suburban-1, suburban-2 and urban of Hochiminh city were 38.39 ng/g, 25.57 ng/g and 12.83 ng/g lipid wt., respectively. The most abundant compound was O-chlordane, accounting for about 50 percent of the total CHLs. In general, chlordane residues in Vietnamese human breast milk were considerably lower than those in industrialized countries such as Japan and Russia, etc. (Table 7.4).

Bioaccumulation is an important character of toxaphene. Without effective management and prevention solutions, this substance is able to penetrate into human being and other creatures via food chains, especially from the places like agrichemical warehouses. From analysis results, we have not found toxaphene in environment samples because their levels are lower than analytical method's LOD. However, toxaphene was detected in almost our human breast milk samples. This suggests a high possibility of through - food chain accumulation

Table 7.4: Comparison of PTSs residues in human breast milk from various countries (mean value in ng/g lipid wt.)

Country	Year of sampling	n	PCBs	DDTs	CHLs	HCHs	HCB	Reference
Vietnam								
Hochiminh (urban-VN)	**2007**	**45**	**128**	**150**	**12.8**	**14**	**1.3**	Present study
Hochiminh (suburban1-VN	**2007**	**19**	**68**	**73**	**38.4**	**6**	**0.5**	Present study
Hochiminh (suburban2-VN)	**2007**	**23**	**82**	**71**	**25.6**	**4**	**0.1**	Present study
Vietnam (North)	2000	42	74	2100	2.0	58	3.9	Minh et al., 2004
Vietnam (South)	2001	54	79	2300	6.9	14	2.5	Minh et al., 2004
Other countries								
Nour (Iran)	2006	23	1818	2680	-	3005	629	Behrooz et al., 2009
Noushahr (Iran)	2006	10	1306	3563	-	5742	1078	Behrooz et al., 2009
Cambodia	2000	28	42	1600	1.8	5.5	1.7	Kunisue et al., 2002
Philippines	2000	10	72	190	15	4.7	-	Kunisue et al., 2002
Hongkong (China)	1999	132	42	2870	-	950	-	Wong et al., 2002
Wielkopolska (Poland)	2000 - 2001	27	108.3	1160	-	18.5	20.95	Katarzyna and Janina, 2007
Russian	1996 – 1997	140	380	1040	37	280	91	Polder et al., 2003
Sweden	1997	40	324	143	-	-	12	Noren et al., 2000
Ukraina	1993 – 1994	197	594	2700	38	730	168	Gladen et al., 1999
UK	1997 – 1998	168	-	470	-	103	43	Harris, 1999
Penang - Malaysia	2003	17	80	1600	23	230[a]	11	Sudaryanto et al., 2005
Japan	1998	49	200	290	85	210	14	Konishi et al., 2001

[a]: sum of a-HCH, b-HCH and g-HCH;

In Vietnam, no data have been published on PBDEs levels in human breast milk. In the global comparison, PBDEs levels in breast milk from Hochiminh city in our research (0.73 ng/g lipid wt.) were lower than those in German breast milk, which ranged from 1.9 to 7.2 ng/g lipid wt. (*Raab et al., 2008*).

7.4 Conclusion

In this chapter, PCBs and DDTs are going to be described as two major groups of organochlorine compounds found in human breast milk samples in Hochiminh City. In comparison with DDTs level in human breast milk in previous studies in Vietnam (*Schecter et al., 1989, Minh et al., 2004*), the obtained results show clearly a decrease of DDTs level in our breast milk samples. In contrast with DDTs levels, the lower decreasing of PCBs suggests that the donors is continuously exposure to PCBs through the food chain

The other PTSs in human breast milk have been found at relatively low levels. However, their appearance proves the widespread contamination of PTSs in environment. It is suggested that more research on PTSs in human breast milk should be carried out to confirm DDTs trend as well as to monitor other PTSs in the future.

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Chapter 8: DATA TREATMENT, PATTERN AND SOURCES DETERMINATION OF PTSs BY STATISTIC METHODS

8.1 Introduction

Identify the origin and pattern of PTSs for the South of Vietnam is one of this thesis's objectives. In our research, we tried to apply the statistic method called multivariate analysis to interpret the obtained results. Multivariate analysis is a very useful tool to assess the data with many variables such as our analytical data. Multivariate analysis may be defined as the analysis of data with three or more variables, that is, where there are a minimum of three measures for each individual under consideration. There are a variety of techniques available to the empirical researcher. These techniques include factor analysis, discriminant analysis, multivariate analysis of variance (MANOVA), canonical correlation analysis, covariance structure analysis and cluster analysis. Essentially, the various techniques may be classified as either hierarchical, (linear composite) where one variable affects another as in Factor Analysis; or clustering, where we attempt to predict group membership based on similar measures, as in Cluster Analysis.

In the case of PTSs, Cluster Analysis and Principal Component Analysis (PCA) techniques have been rarely applied to discover the pattern and sources of these compounds, especially PBDEs group (*Loffler and Bavel, 2000, Enkel et al., 2005, Scrimshaw and Lester, 2001*). However, these techniques have been frequently applied for the case of PCDD/Fs. This has been reported by many authors (*Lindstrom et al., 1989; Fielder et al., 1996; Gotz et al., 1998a, 1998b), 1998; Oberg, 2004, Mai,2006*). At present time, there are many softwares to process automatically all data and quickly produce the calculated results.

In this chapter, the software named Statistical Package For Social Sciences (SPSS) and SIMCA-P+ are used to carry out the Cluster Analysis and PCA analysis and identify the pattern of PTSs as well as the correlation between the types of environmental samples. However, Cluster Analysis and PCA analysis with SPSS should be carried out when we have clearly understood the algorithm and the process to treatment the data before using their models to interpret the result after calculating. The algorithm of Cluster analysis and PCA are presented below.

8.2 Cluster analysis

Cluster Analysis is a way of grouping observations or objects based on the similarity of responses to several PTSs. The goal is to find an optimal grouping for which the observations or objects within each cluster are similar.

The term Cluster Analysis (first used by Tryon, 1939) encompasses a number of different algorithms and methods for grouping objects of similar kind into respective categories. A general question facing researchers in many areas of inquiry is how to organize observed data into meaningful structures, that is, to develop taxonomies. In other words cluster analysis is an exploratory data analysis tool which aims at sorting different objects into groups in a way that the degree of association between two objects is maximal if they belong to the same group and minimal otherwise.

Generally, there are essentially two types of clustering methods: partitioning algorithms and hierarchical algorithms. The main difference between the two clustering techniques is that in

hierarchical clustering once groups are found and elements are assigned to the groups, this assignment cannot be changed. In partitioning technique , the assignment of objects into groups may change during the algorithm application (*Wolfgang and Léopold, 2007*).

1. Partitioning Algorithms. The partitioning algorithms start from a given group definition and proceed by exchanging elements between groups until a certain score is optimized. They describe a method that divides the data set into k clusters, where the integer k needs to specify. Typically, the user runs the algorithm for a range of k-values. For each k, the algorithm carries out the clustering and also yields a "quality index", which allows the user to select the best value of k afterwards. There are four functions used for partitioning algorithms are *k-means, pam, clara*, and f*anny* (*Anja et al., 2009*).

2. Hierarchical Algorithms. The hierarchical algorithms describe a method yielding an entire hierarchy of clusterings for the given data set. The hierarchical algorithms can be divided into agglomerative and splitting procedures. The first type of hierarchical clustering starts from the finest partition possible (each observation forms a cluster) and groups them. The functions *agnes, mclust*, and *hclust* used for agglomerative methods.

The second type starts with the coarsest partition possible: one cluster contains all of the observations. It proceeds by splitting the single cluster up into smaller sized clusters. There are two functions used for splitting method are *diana* and *mona* (*Anja et al., 2009*).

The clustering functions *daisy, pam, clara, fanny, agnes, diana,* and *mona* make up the cluster library, which implements the algorithms described in Kaufman & Rousseeuw (1990). The functions *kmeans, mclust, and hclust* are not part of the cluster library. They have a slightly different syntax than the cluster library functions.

Agglomerative hierarchical: The function *agnes* is an agglomerative hierarchical clustering method, it yields a sequence of clustering. In the first clustering each of the n objects forms its own separate cluster. In subsequent steps clusters are merged, until (after n-1 steps) only one large cluster remains.

The algorithm is based on dissimilarities only. If a data matrix is input, the function starts by computing the dissimilarity matrix. Initially (at step 0), each object is considered as a separate cluster. The rest of the computation consists of iteration of the following steps:

- *Merge the two clusters with smallest between-cluster dissimilarity;*
- *Compute the dissimilarity between the new cluster and all remaining clusters.*

The hierarchy obtained from *agnes* can be graphically displayed in two ways, by means of a clustering tree or by a banner.

- *Clustering tree*. This is a tree in which the leaves represent objects. The vertical coordinate of the place where two branches join equals the dissimilarity between the corresponding clusters.
- *Banner*. The banner shows the successive mergers from left to right. Imagine the ragged flag parts at the left, and the flagstaff at the right; the objects are listed from top to bottom. The mergers, which commence at the between-cluster dissimilarity, are represented by horizontal

bars of the correct length. The banner thus contains the same information as the clustering tree.

In our research, agglomerative hierarchical technique was used for discovering the pattern and sources of PTSs in southern Vietnam.

8.3 Principal Component Analysis (PCA)

Principal component analysis is a statistical technique that linearly transforms an original set of variables into a substantially smaller set of uncorrelated variables that represents most of the information in the original set of variables. Its goal is to reduce the dimensionality of the original data set. A small set of uncorrelated variables is much easier to understand and use further analyses than a large set of correlated variables. It will be better in case the reduction can be done with minimal information loss.

In principal component analysis, we seek to maximize the variance of a linear combination of the variables. The first principal component is the linear combination with maximal variance, we are essentially searching for a dimension along which the observations are maximally separated or spread out. The second principal component is the linear combination with maximal variance in a direction orthogonal to the first principal component, and so on. In general, the principal components define different dimensions from those defined by discriminant functions or canonical variates.

In some applications, the principal components are an end in themselves and may be amenable to interpretation. More often they are obtained for use as input to another analysis. For example, two situation in regression where principal components maybe useful are (1) if the number of independent variables is large relative to the number of observations, a test maybe ineffective or even impossible, and (2) if independent variables are highly correlated, the estimates of repression coefficients maybe unstable. In such cases, the independent variables can be reduced to a smaller number of principal components that will yield a better test or more stable estimates of the regression coefficients (*Alvin, 2002*).

Principal components analysis finds a set of standardized linear combination (SLC), called the principal components, which are orthogonal and taken together explain all the variance of the original data. The principal components are defined as follows (*Mardia, Kent, & Bibby, 1979*):

If x is a random vector with mean μ and covariance matrix Σ, then the principal component transformation is the transformation

$$x \rightarrow y = \Gamma' (x - \mu)$$

where Γ is orthogonal, $\Gamma'\Sigma\Gamma = \Lambda$ is diagonal, and $\lambda 1 \geq \lambda 2 \geq ... \geq 0$. The i^{th} principal component of x may be defined as the ith element of the vector y , namely, as

$$yi = \gamma' i(x-\mu)$$

Here γi is the i^{th} column of Γ and may be called the i^{th} vector of *principal component loadings*.

8.4 Applying Cluster analysis and PCA for our data treatment

There are different algorithms to perform a cluster analysis. Hierarchical algorithms are the most commonly used. For our purposes we chose the WARD method to form groups (= clusters) of samples for which the variance (deviation) is minimized (*Fiedler et al., 1996*). The WARD algorithm tends to form clusters of similar size (number of samples) and therefore seemed to be appropriate to analyze large data sets such as ours, especially with human breast milk samples.

The purpose of PCA is to get an overview of complex data, *e.g.* samples (observations) characterized by several measurements (variables, k). Geometrically, an observation can be presented as points in a k-dimensional space with the variables as k-coordinate" axes. For example, each PCBs analysis (observation) can be presented as a point in the 21-dimensional space.

Using agglomerative hierarchical cluster analysis and PCA analysis, we have investigated and compared the PTSs data in soil, sediment samples between Hochiminh city, Thivai River and Tien river basin as well as PTSs patterns in soil, sediment, fish and human breast milk each other in Hochiminh city area. In order to evaluate the PTSs sources in the soil and sediment we have assumed the DongThanh landfill as a primary source and ThiNghe canal as a secondary source of PTSs in Hochiminh city.

To perform the cluster analysis and PCA procedure, all PTSs concentration data have to be normalized to be comparable. There are some strategies to normalize the variables (*Ding et al., 1989; Fiedler et al., 1996; Sakurai et al., 2002*). While homologue profiles may undergo various transformation reactions in the environment (e.g. dechlorination), the ratio of homologues can change considerably between source and sample. In contrast, congeners of the same degree of chlorination (that is isomers within one homologue) should be affected in the same way. Thus, the congener pattern is more stable than the homologue profile and should be better preserved in the sample.

In this case, we separated the PTSs compounds into three groups: PCBs, organochlorine pesticides and PBDEs group. The standardization method of PTSs concentrations were performed as below:

a) Relative concentration of PCBs congeners

The concentration of each PCB congener is divided by the total sum of PCBs found in every sample, i.e. PCB81/ΣPCBs, PCB170/ΣPCBs. Thus, a single sample is characterized by 21 ratios – PCBs relative profiles.

b) Relative concentration of Organochlorine pesticides

The concentration of each compound in this group is divided by the total sum of OCls pesticides found in every sample, i.e.

$$\frac{HCB}{\sum HCHs + HCB + \cdots + Mirex} \quad \cdots \quad \frac{gHCH}{\sum HCHs + HCB + \cdots + Mirex} \quad \cdots \quad \frac{pp'\_DDT}{\sum HCHs + HCB + \cdots + Mirex}$$

Thus, a single sample is characterized by 26 ratios.

c) Relative concentration of PBDEs

The concentration of each PBDEs congener is divided by the total sum of PBDEs found in every sample, i.e. PBDE28/ΣPCBs, PBDE49/ΣPBDEs, …, PBDE183/ΣPBDEs. Thus, a single sample is characterized by 12 ratios – PBDEs relative profiles.

For all normalizing methods, concentrations below the detection limit were treated as zero.

8.4.1 Comparison of soil and sediment samples

a/ PCBs: For cluster analysis, we use SPSS 16.0 for Window and Euclidean distance measure. The dendogram of the cluster analysis showed in the Fig. 8.1 below.

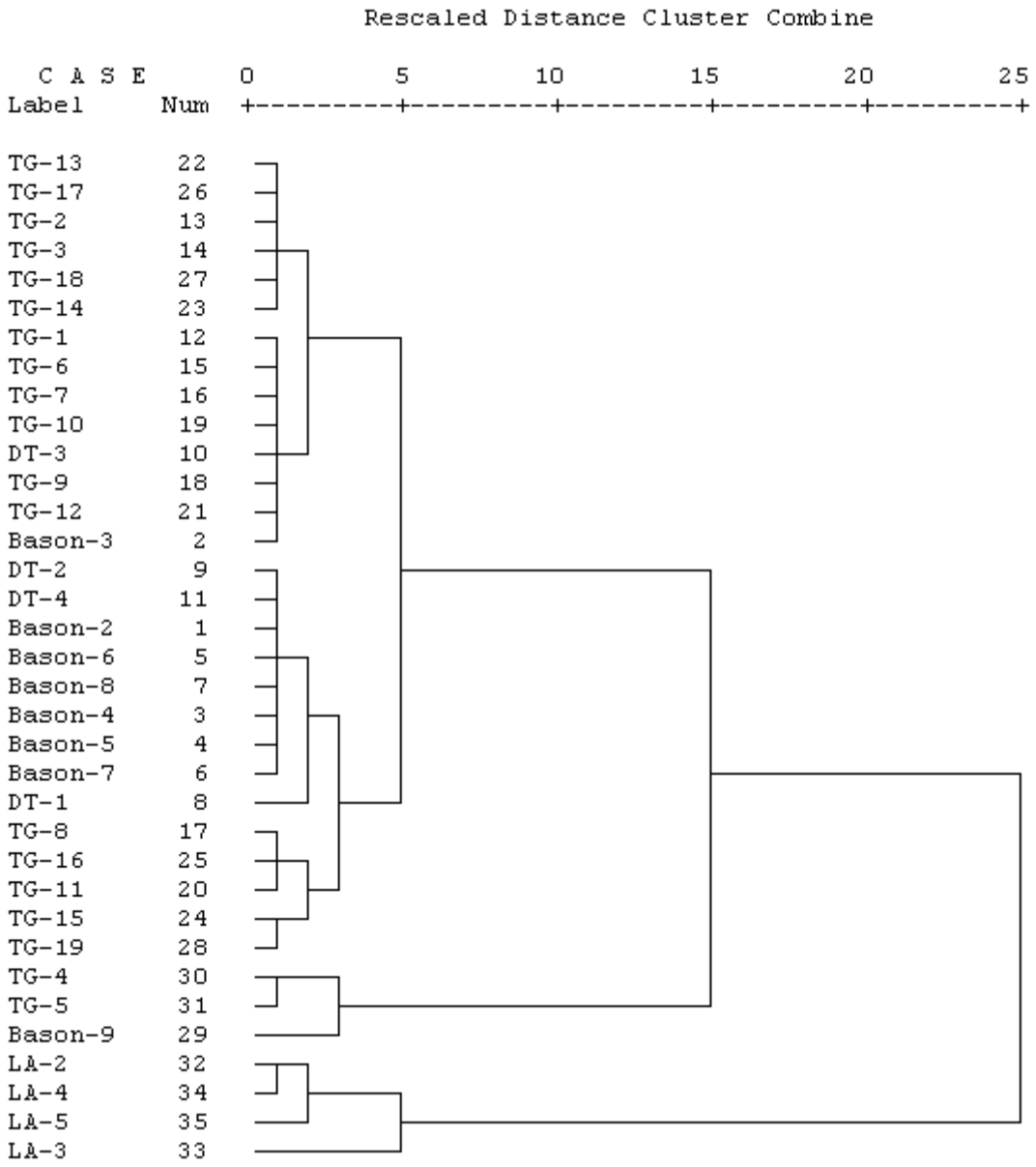


Figure 8.1 Cluster analysis based on PCBs relative concentrations, 36 samples analyzed

From the Fig. 8.1, our soil and sediment samples can be divided into three groups (from left to right): group1 (TG-4, TG-5, and Bason-9), group2 (LA-2, LA-3, LA-4, and LA-5) and group3

(the remaining samples). Actually, it can be seen that it is not possible to group all sediment samples in Tiengiang river basin into one cluster. Soil samples from agrichemical store (LA-2 to LA-5) are group in one cluster

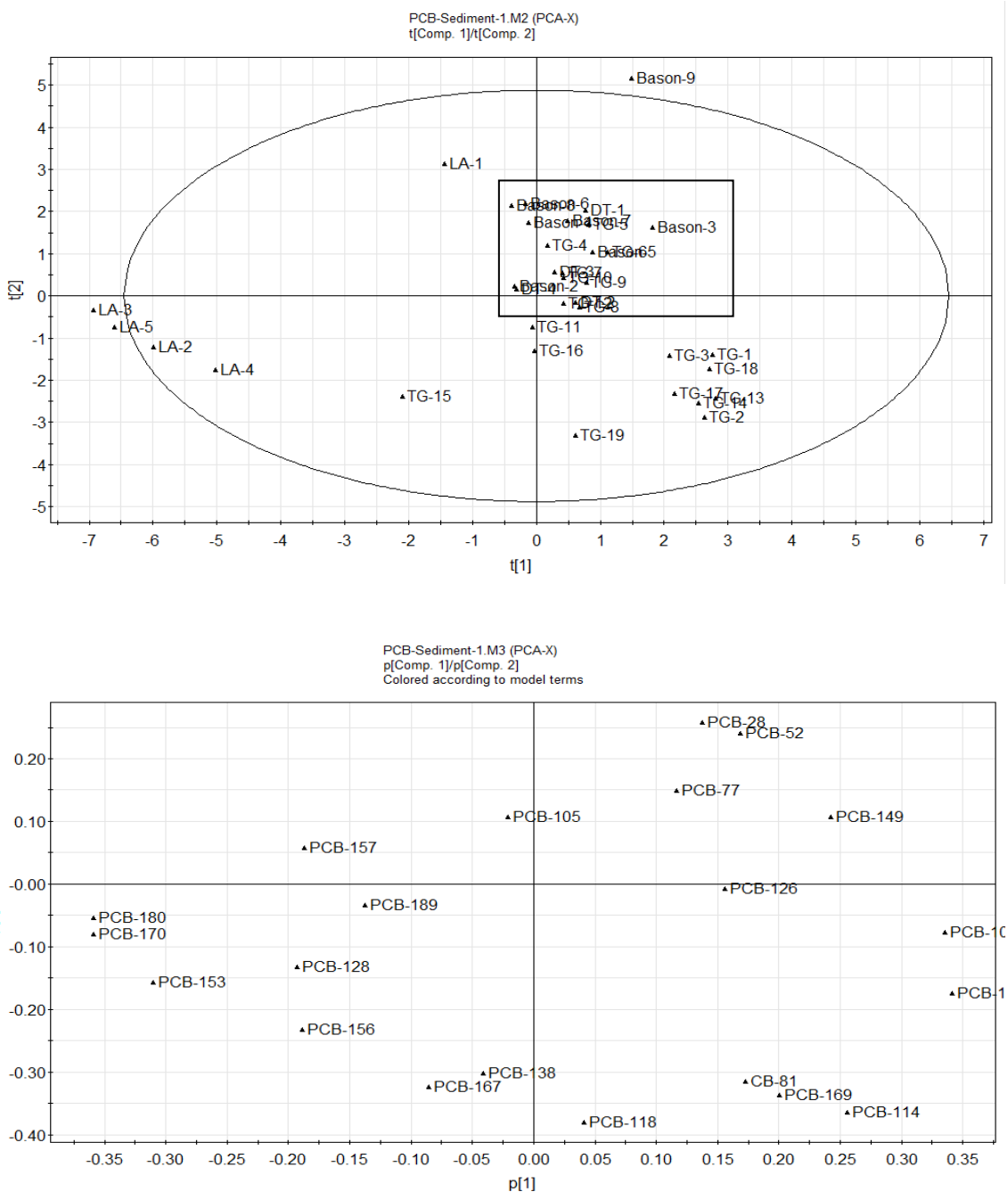


Figure 8.2 PCA analysis based on PCBs relative concentrations, 36 samples analyzed

PCA analysis was performed with SIMCA-P+ Ver. 12.0.1 (Umetric AB) and Fig. 8.2 above showed the better classification of soil and sediment samples, the ellipse represents the Hottelling T2 with 95% confidence. The PCBs profiles are treated by PCA and done in two new variables with 54.4% of the variation. We can see the correlation between the landfill (DT1 to DT4) and Thinghe canal – Saigon River (Bason-2 to Bason-8). In general, we divided sediment and soil samples into three groups: lower-left group (LA-2, LA-3, LA-4, and LA-5), lower-right group

(TG-1, TG-2, TG-3, TG-13, TG-14, TG-17, TG-18, and TG-19) and the remaining group including landfill soils and TN-SG River confluence sediments .

b/ OCls pesticides: The dendogram of the cluster analysis with the OCl pesticides relative concentrations presented in the Fig. 8.3 below.

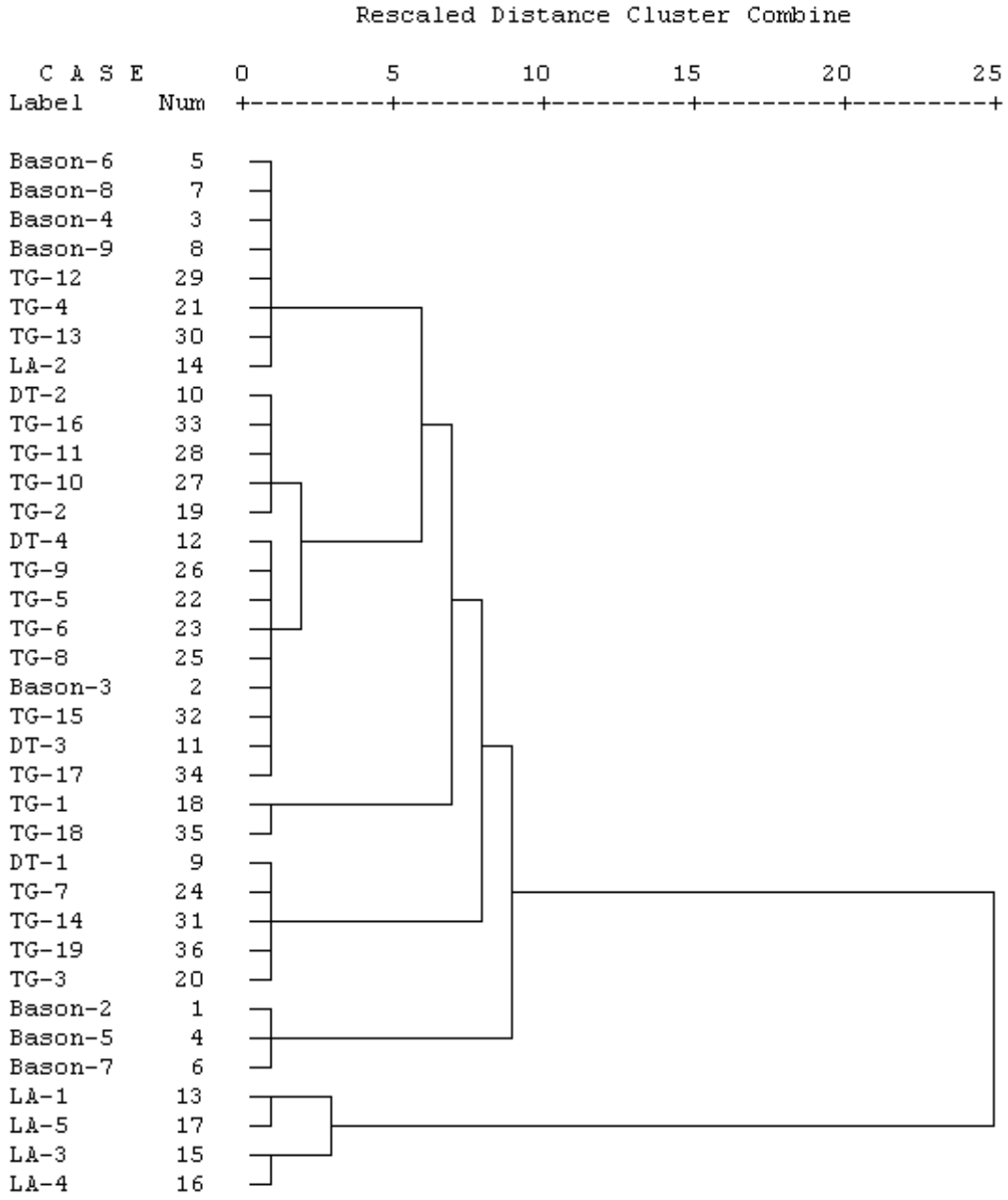


Figure 8.3 Cluster analysis based on OCPs relative concentrations, 36 samples analyzed

From cluster analysis (Fig.8.3), our samples can be divided into three groups. Group1 (including LA-1, LA-3, LA-4, and LA-5): this group contains almost soil samples collected form agrichemical warehouse in LongAn (suburban of Hochiminh city). Group2 included Bason-2, Bason-5, and Bason-7 and group3 was the remaining samples.

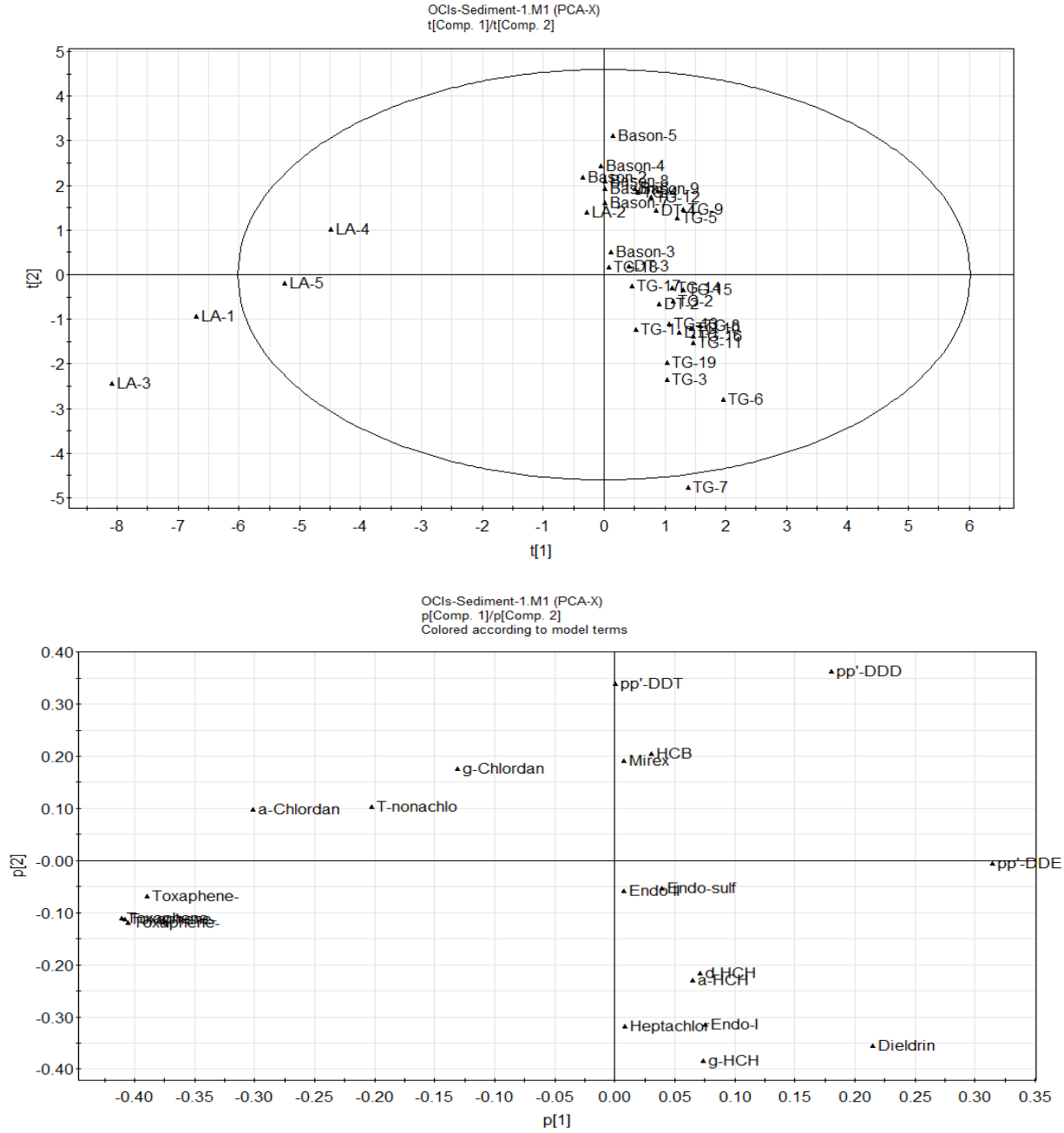


Figure 8.4 PCA analysis based on OCPs relative concentrations, 36 samples analyzed

For PCA analysis, we have the result as presented in Fig 8.4 (variables of 61.2%). The result confirms the grouping with clustering dendogram above. There are three groups: lower-left group includes all samples taken at sampling sites in agrichemical store with interaction of toxaphenes; upper-right group includes almost samples taken at sampling sites in landfill and Thinghe canal – Saigon river (Bason-2,…,DT-3, DT-4) with interaction of pp'-DDT, pp'-DDD, HCB; and lower-right includes samples taken at sampling sites in Tien river basin with interaction of pp'-DDE, g-HCH and Endosulfans.

c/ PBDEs: The dendogram of the cluster analysis with the PBDEs relative concentrations showed in the Fig. 8.5 below:

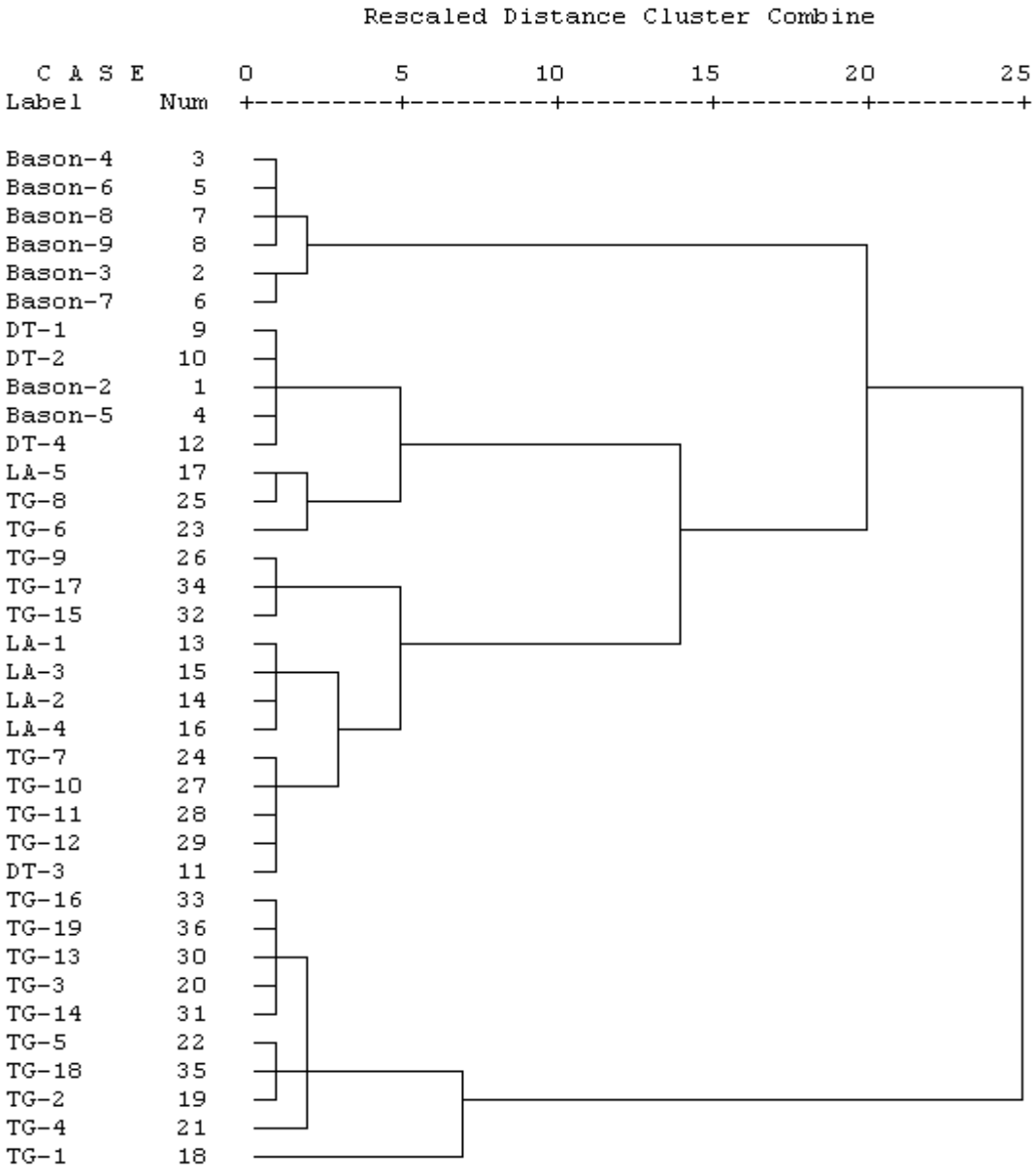


Figure 8.5 Cluster analysis based on PBDEs relative concentrations, 36 samples analyzed

In this case, we can see that it is impossible to group the soil samples in landfill (from DT-1 to DT-4) and sediment samples from Thinghe canal-Saigon River into one group base on relative concentrations of PBDEs congeners.

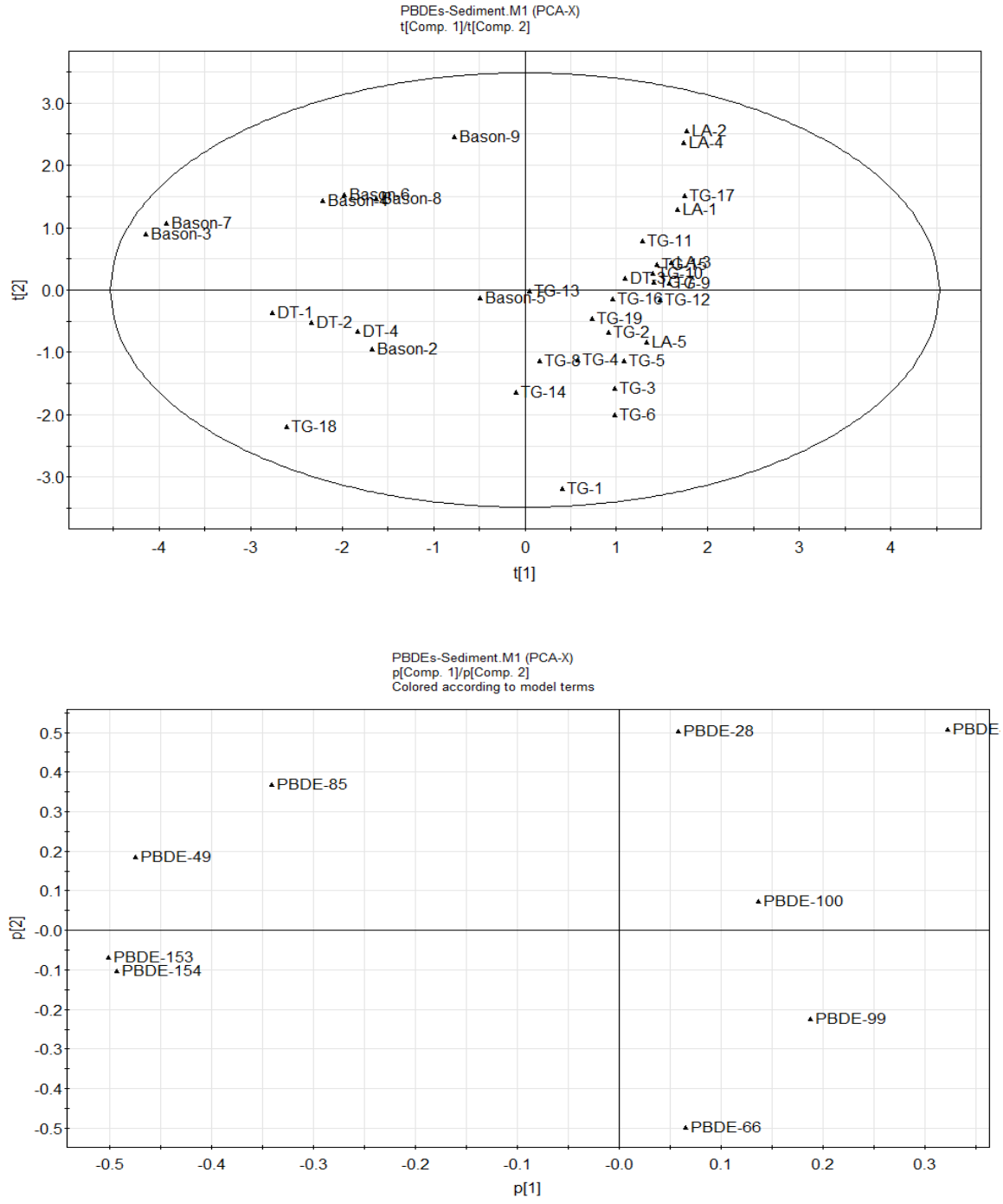


Figure 8.6 PCA analysis based on PBDEs relative concentrations, 36 samples analyzed

The plot obtained from the PCA is shown in Fig. 8.6 (variables of 24.5%). From this result we can divide our samples into three groups: upper-left group (almost Thinghe canal-Saigon river sites) with interaction of PBDE-49 and 85; lower-left group (landfill sites) is mostly interacted by PBDE-153 and 154; and right group (agrichemical store site and Tien river sites) with interaction of PBDE-100, 47, and 99 as shown in Fig. 8.6.

The result of cluster analysis and PCA in Figures 8.1, 8.2, 8.3 and 8.4 showed that there is a similarity between pattern of soil samples (landfill) and sediment samples (Thinghe canal-Saigon river). Their similarity mainly affected by pp'-DDT, pp'-DDD, HCB (OCls pesticides group) and

PCB28, 52, 101, and 149 (PCBs group). These multivariate analysis results can explain about the hypothesis that the PTSs contamination in Hochiminh canals' sediment is related to the landfill.

8.4.2 Comparison of fish tissue samples

a/ PCBs: The plot obtained from the cluster and PCA analysis are shown in Fig. 8.7 and 8.8 below.

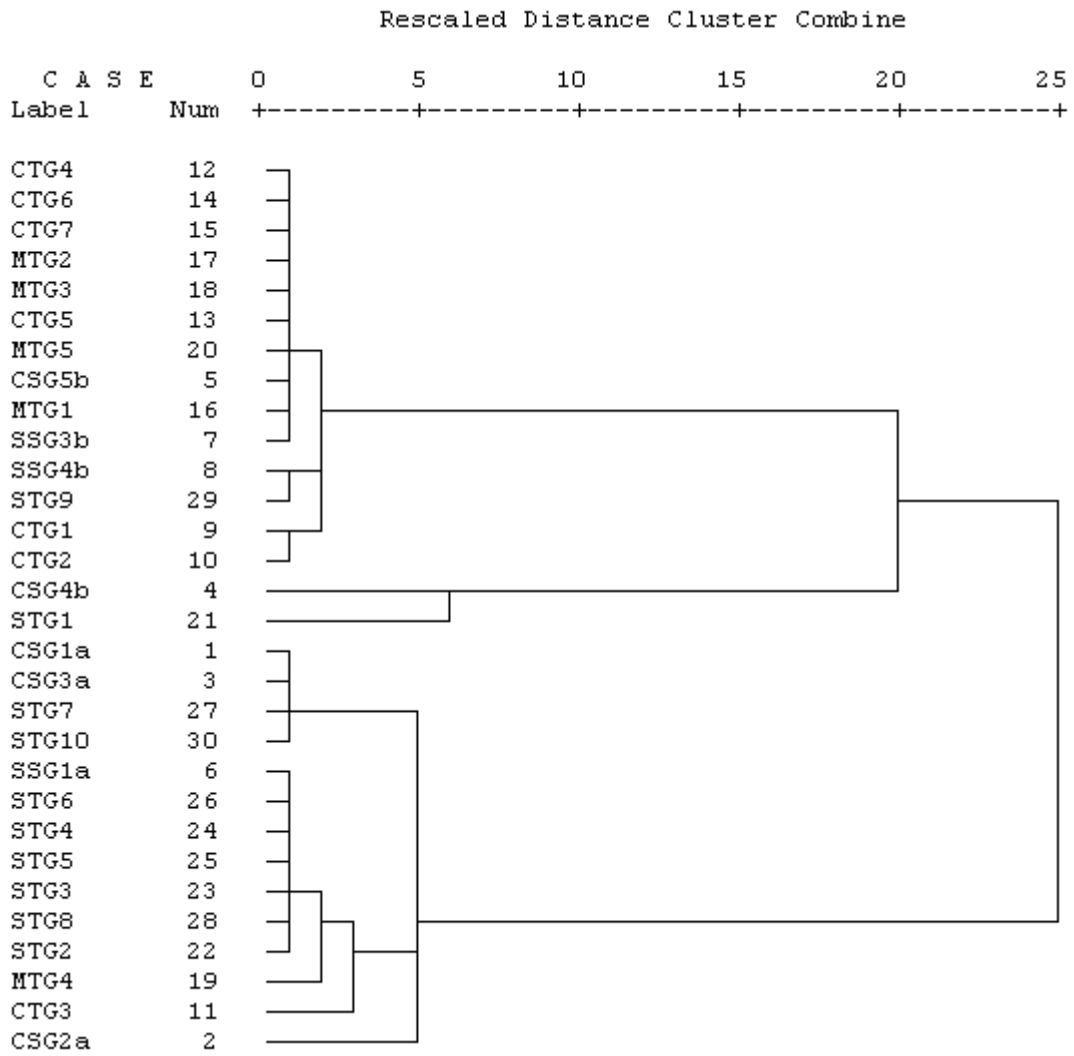


Figure 8.7 Cluster analysis based on PCBs relative concentrations, 30 fish samples analyzed

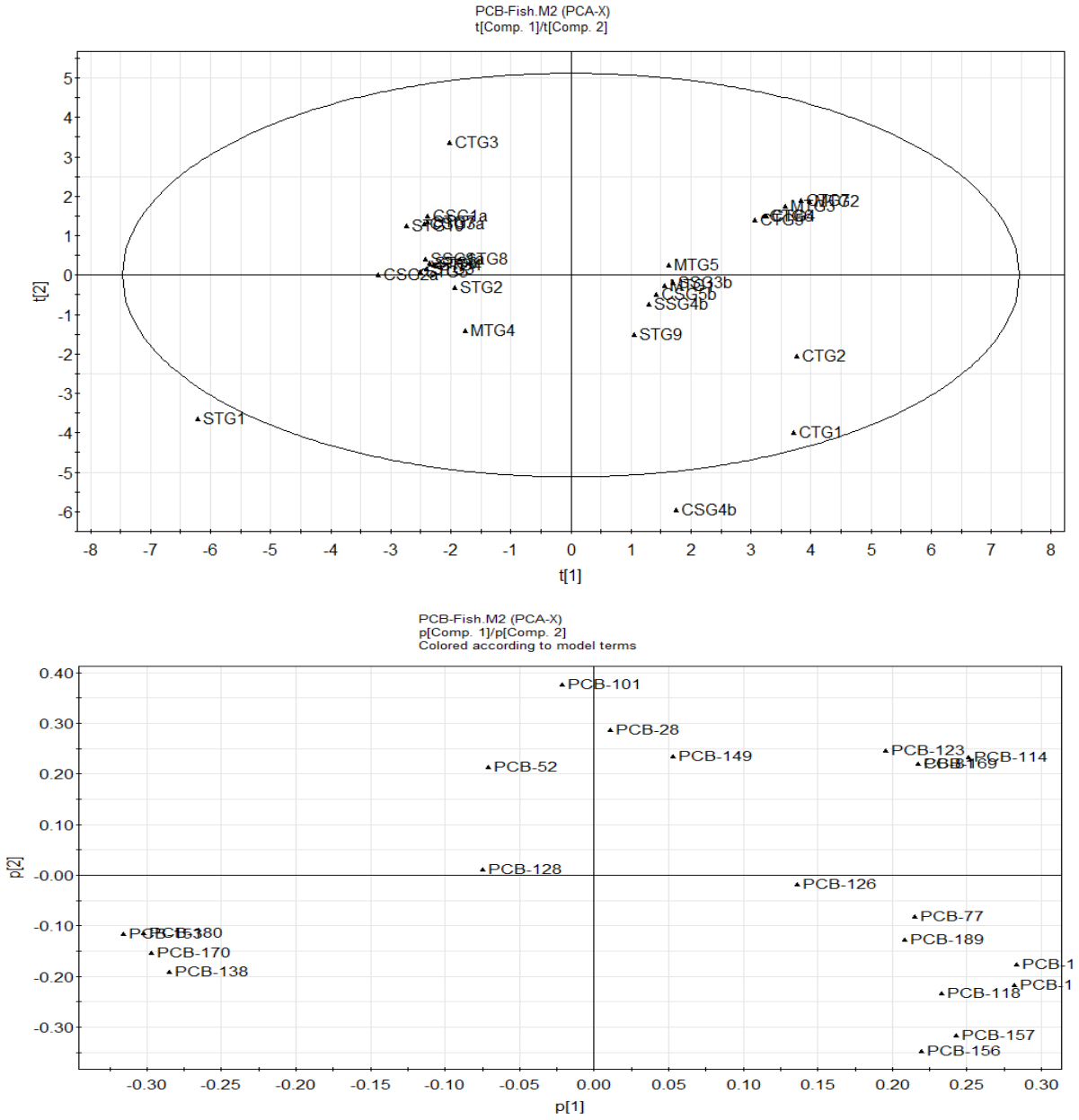


Figure 8.8 PCA analysis based on PCBs relative concentrations, 30 fish samples

From the multivariate analysis results (variables of 42.8%) we can divide 30 fish samples (8 fish samples from Saigon river and 22 fish samples from Tien river) into three groups: upper-right group with the interaction of PCB123, PCB114 and PCB 189; lower-right group with the interaction of PCB77, 118, 156 and 157; left group is mainly interacted by PCB180, 170, 138 and 128.

b/ OCls pesticides: The plot obtained from the cluster and PCA analysis are shown in Fig. 8.9 and 8.10

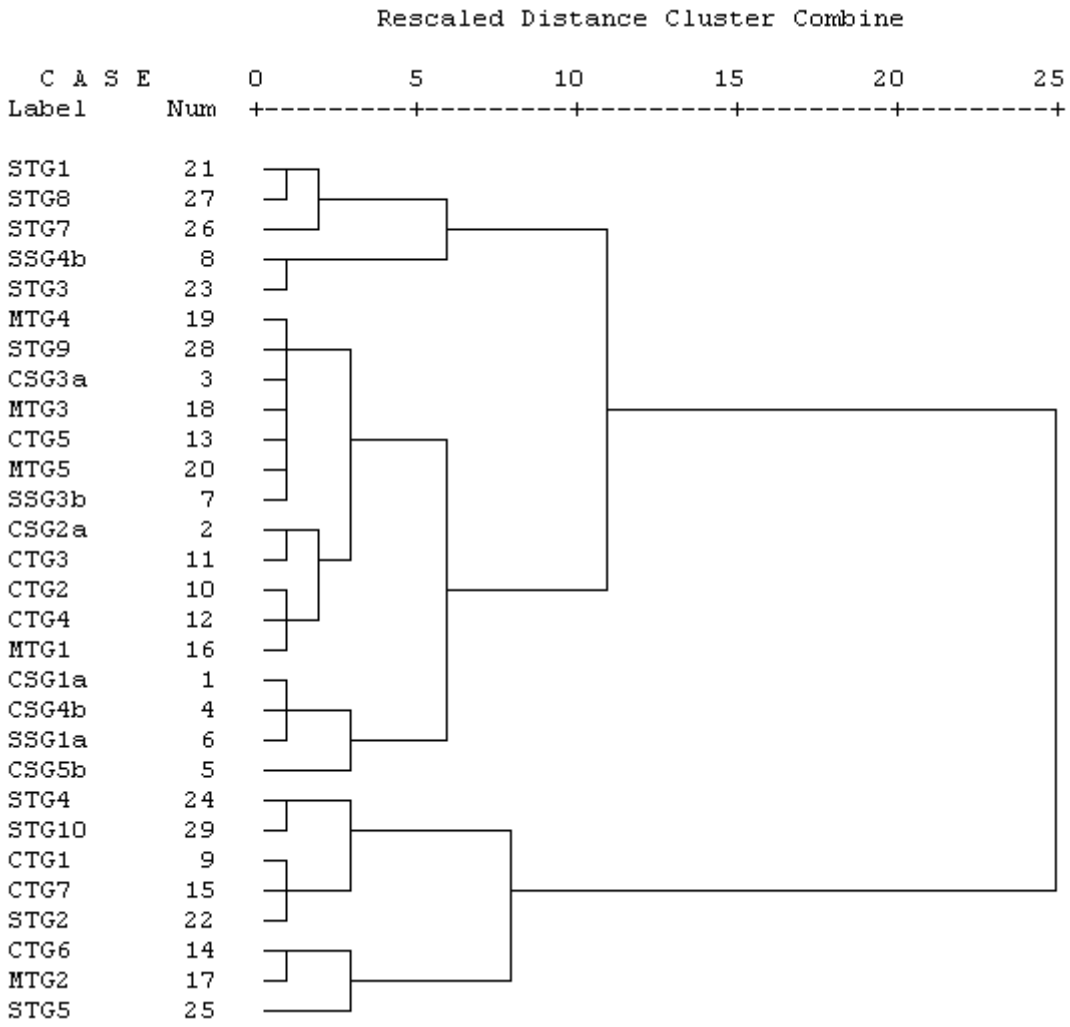


Figure 8.9 Cluster analysis based on OCPs relative concentrations, 30 fish sample analyzed

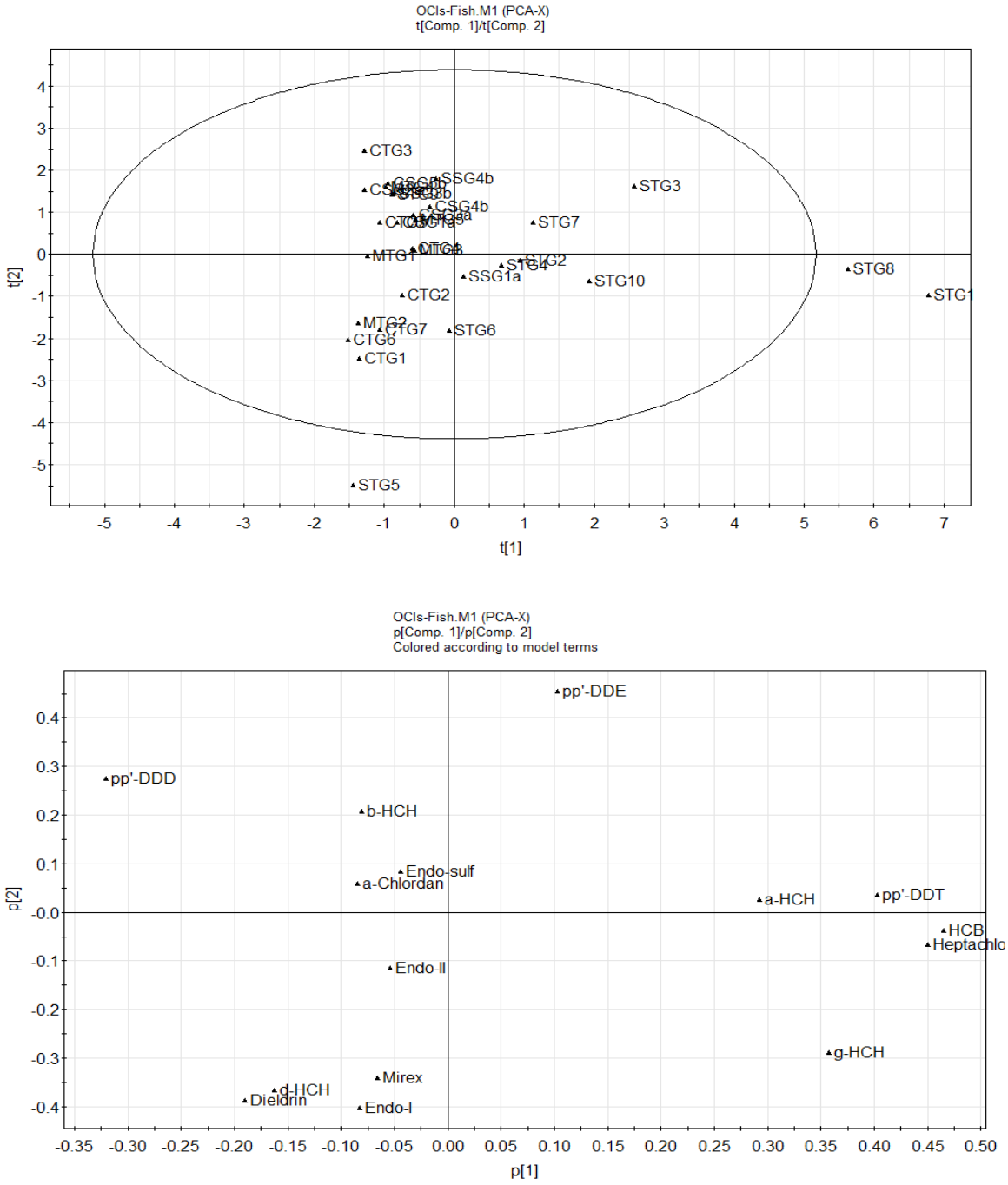


Figure 8.10: PCA analysis based on OCPs relative concentrations, 30 fish samples analyzed

Based on Fig. 8.9 and 8.10 (variables of 39.4%), we can see that there are three groups: upper-left group with the interaction of pp'-DDD, endosulfan sulfate and b-HCH; lower-left group with the interaction of endo I, endo II and mirex; right group with dispersive similarity values and it is interacted by pp'-DDT, HCB, pp'-DDT and g-HCH.

d/ PBDEs: The plot obtained from the cluster and PCA analysis are shown in Fig. 8.11 and 8.12

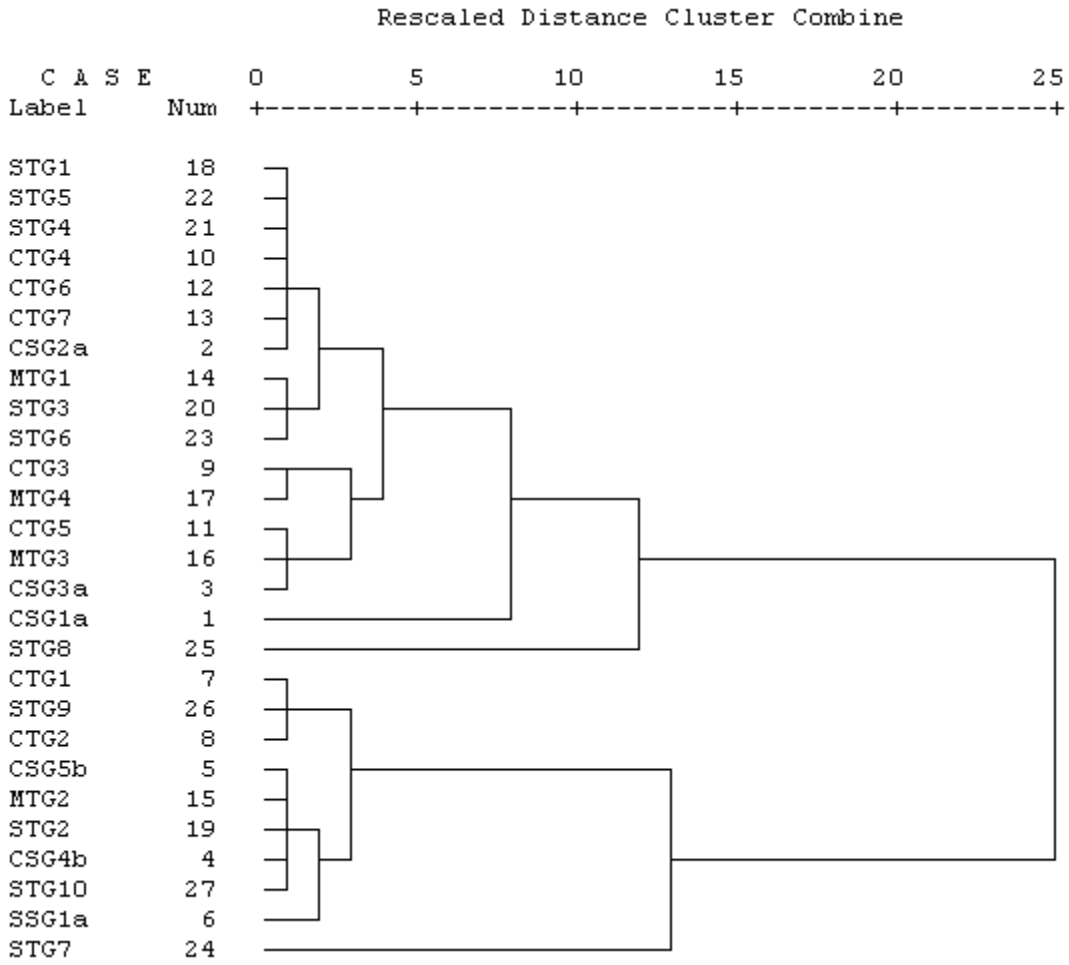


Figure 8.11: Cluster analysis based on PBDEs relative concentrations, 30 fish sample analyzed

From the Fig. 8.11 and 8.12, we see that our fish samples can be divided into two groups: left group with very concentrated similarity values and mostly interacted by PBDE47; the right group is interacted by PBDE49, 100 and 99.

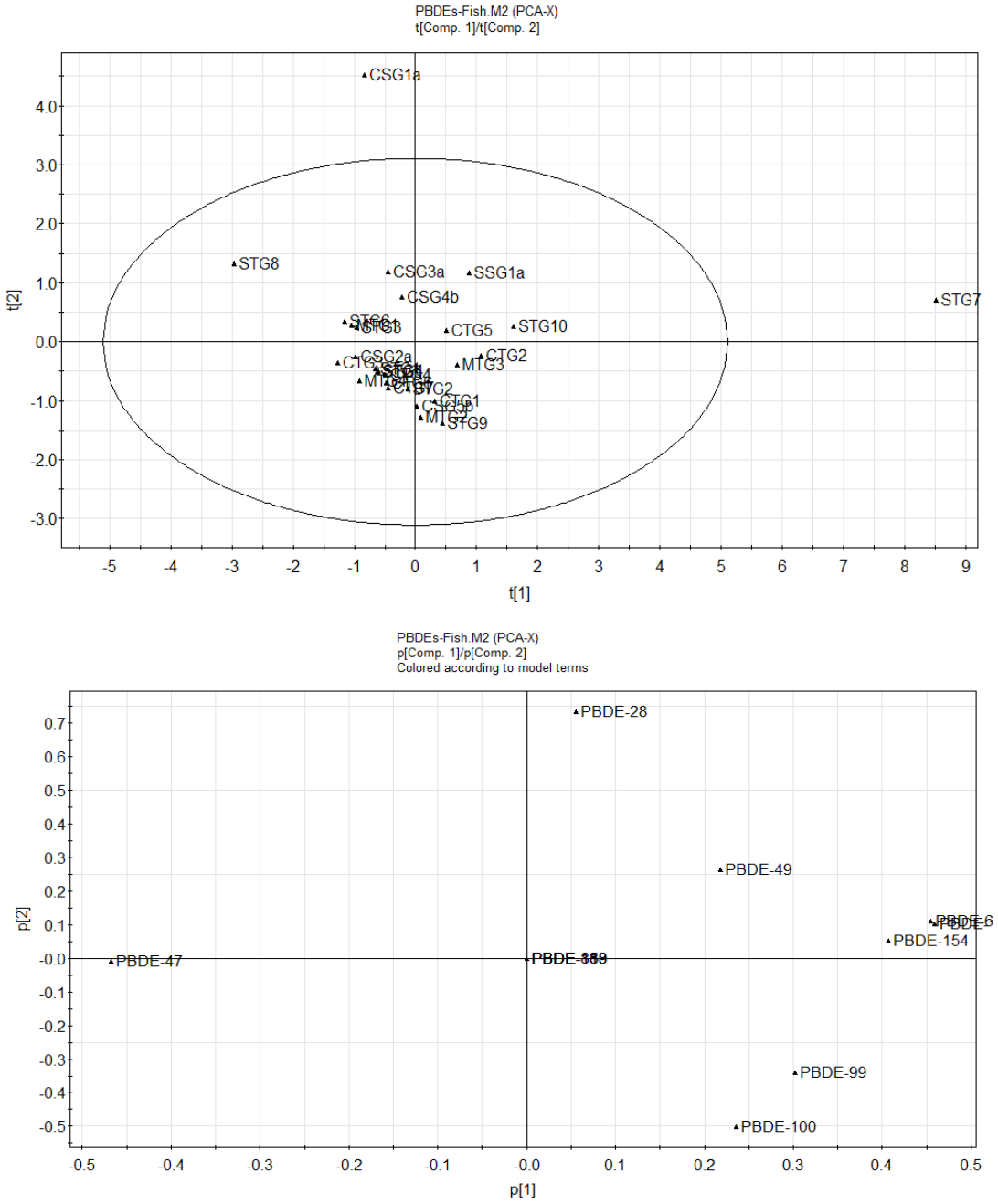


Figure 8.12 PCA analysis based on PBDEs relative concentrations, 30 fish samples analyzed

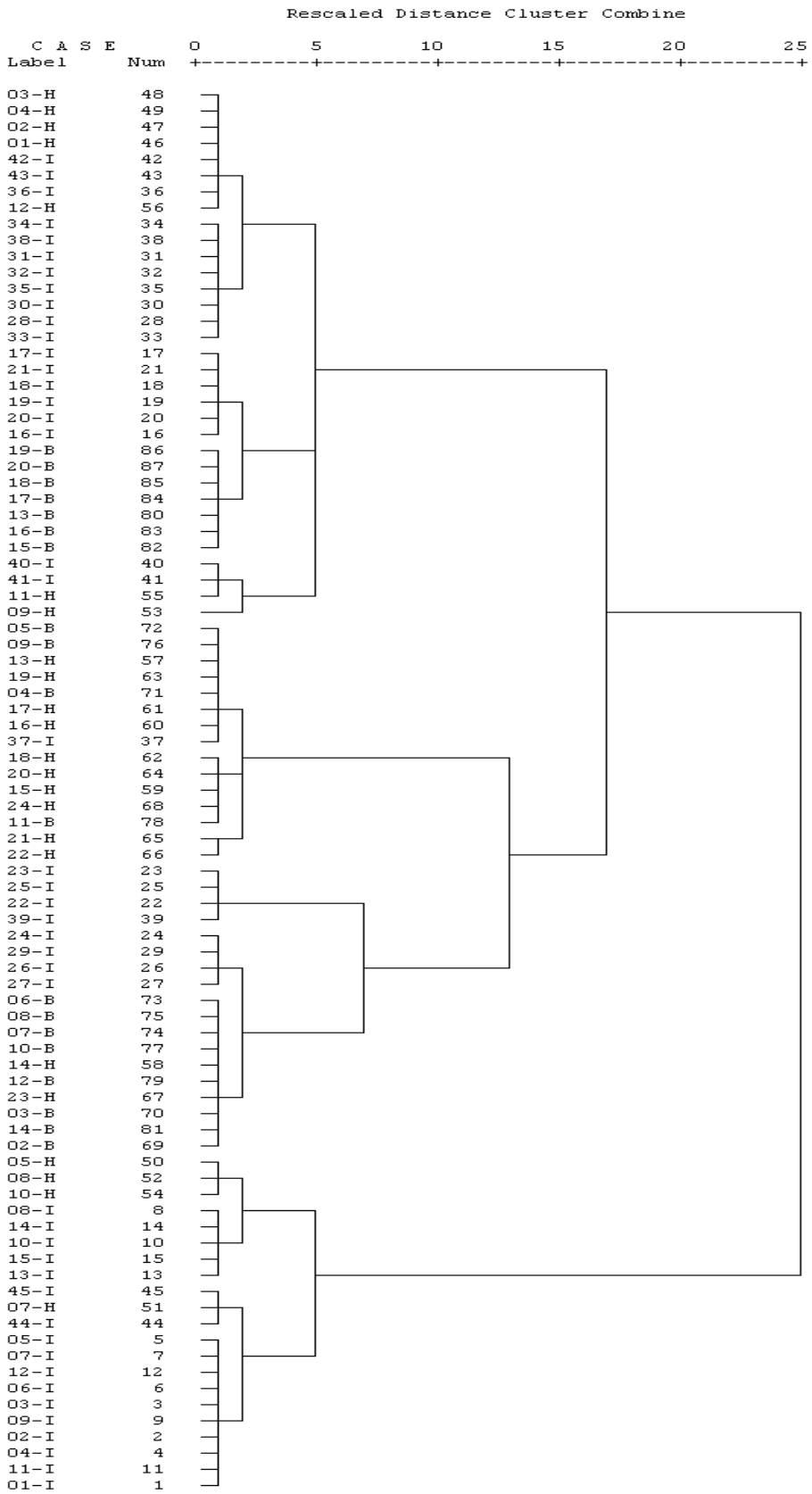
From all multivariate analysis results (variables of 55.8%) of PTSs in our fish samples, it is impossible to group all fish samples each other and we cannot admit that the fish samples from Thinghe canal-Saigon River have the same PTSs pattern with the fish samples from Tien river.

8.4.3 Comparison of human breast milk samples

a/ PCBs: The plot obtained from the cluster and PCA are shown in Fig. 8.13 and 8.14

Figure 8.13:

Cluster analysis based on PCBs relative concentrations, 87 human breast milk samples analyzed



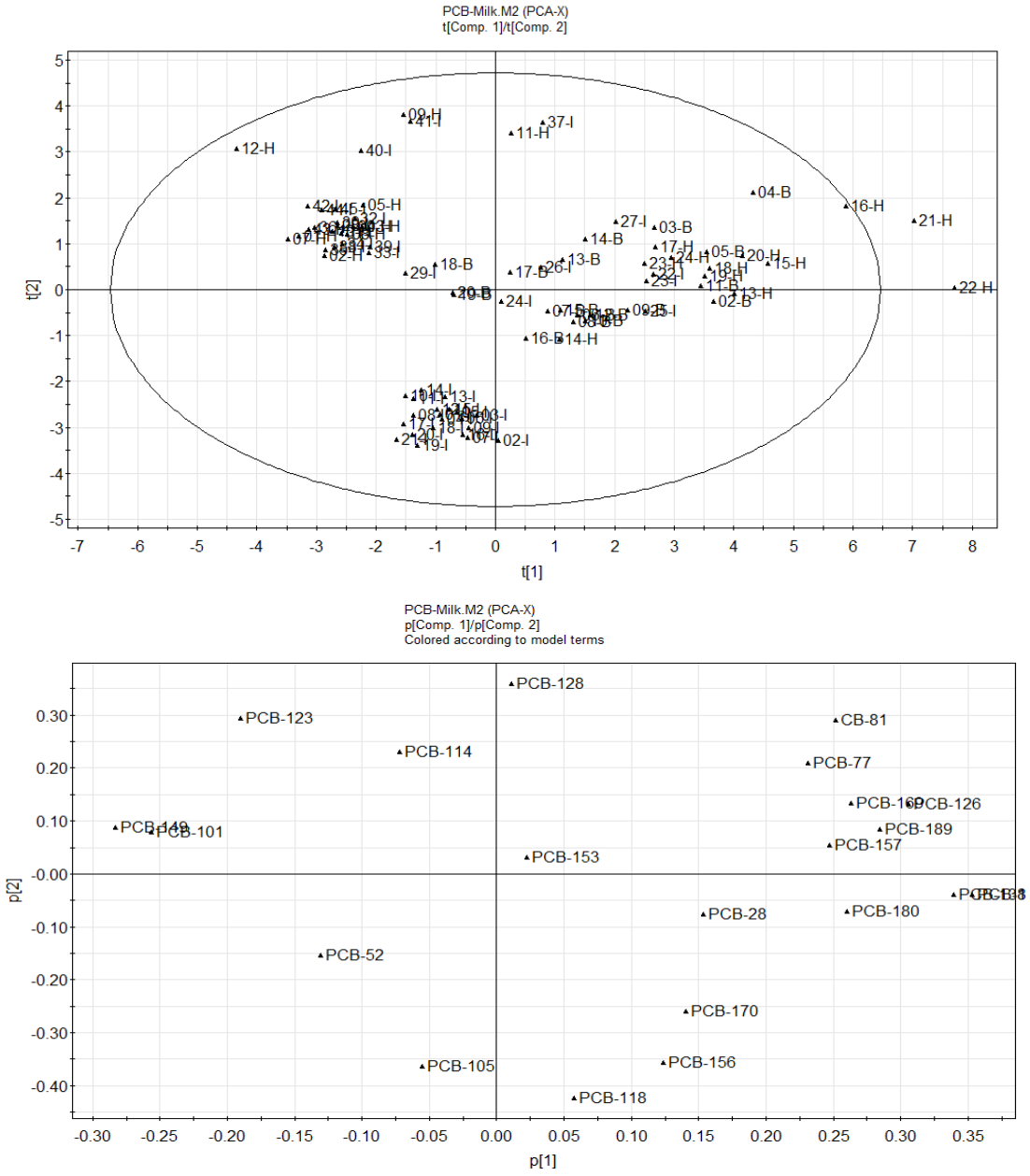


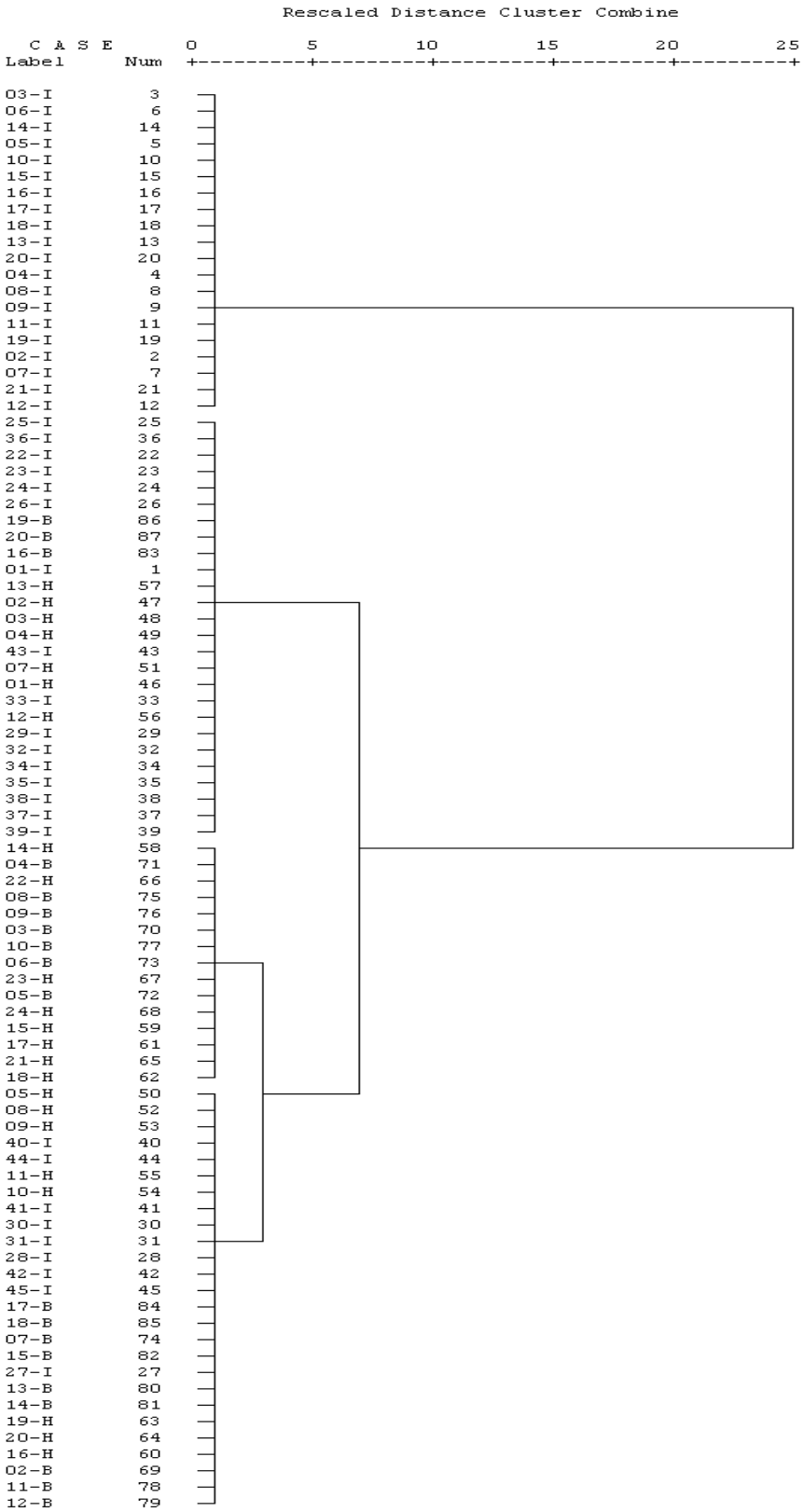
Figure 8.14: PCA analysis based on PCBs relative levels, 87 human milk samples analyzed

From Fig. 8.13 and 8.14 of the cluster and PCA analysis, we divide all milk samples into three groups: upper-left group with the interaction of PCB101, 123, and 114; lower-left group with the interaction of PCB101, 149 and 52; and the right group is relatively dispersive values and interacted by mainly dioxin-like PCBs congeners.

b/ OCls pesticides:

Figure 8.15:

Cluster analysis based on OCls pesticides relative concentrations, 87 human milk samples analyzed



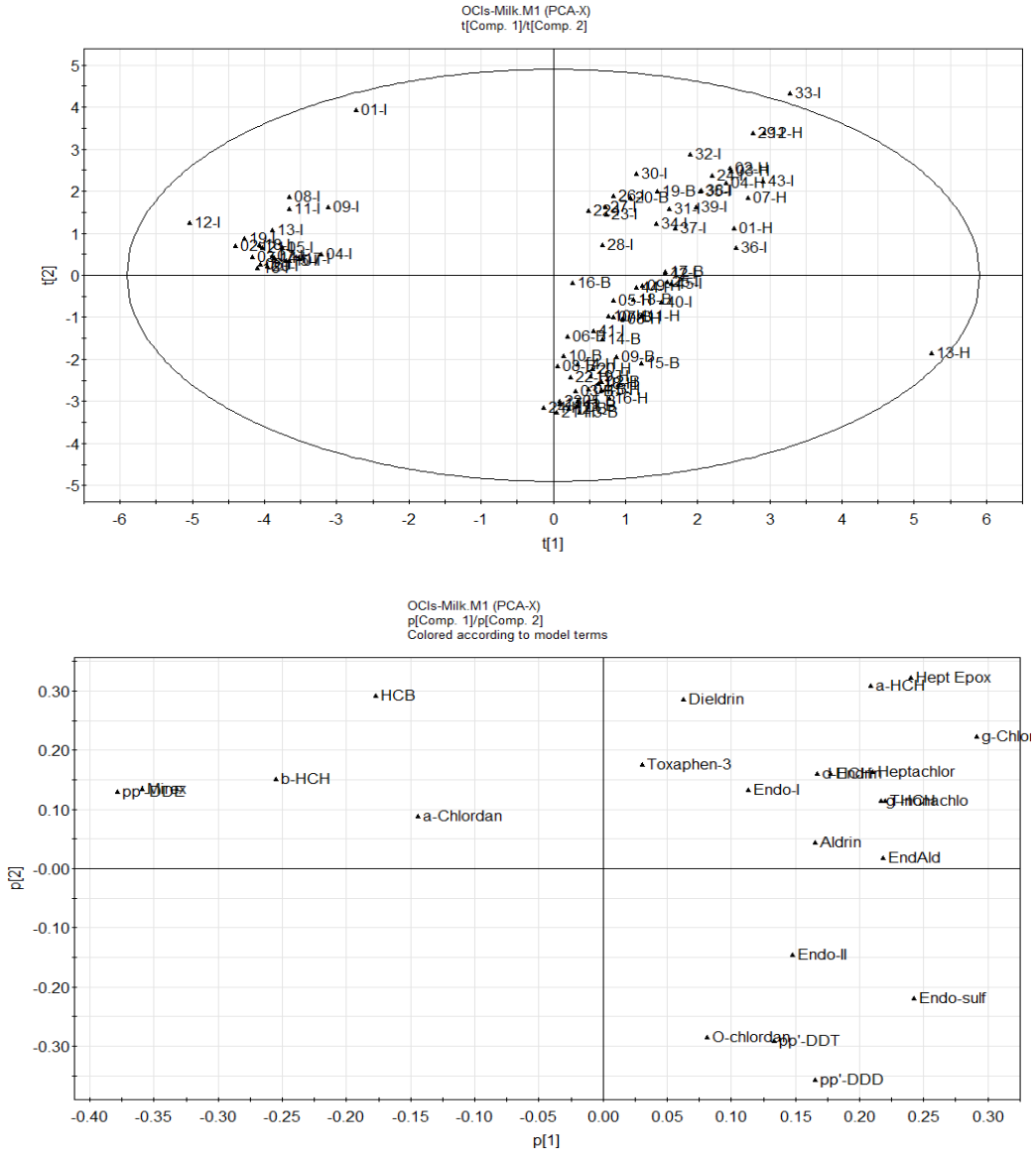


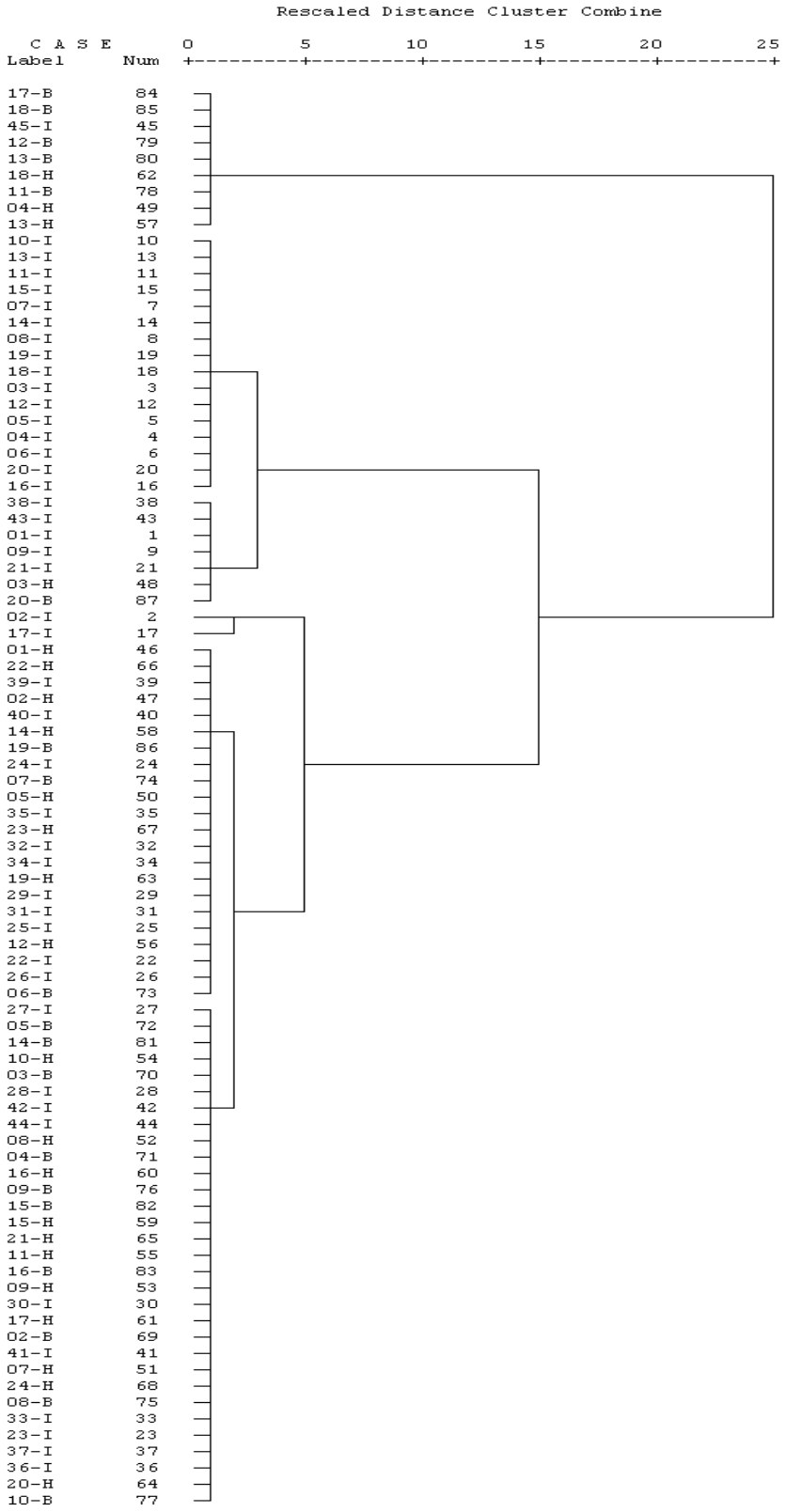
Figure 8.16: PCA analysis based on OCPs relative levels, 87 human milk samples analyzed

Using cluster and PCA analysis for OCPs relative concentration we obtained the results shown in Fig. 8.15 and 8.16. In general, we can divide our milk samples into two groups: upper-left group (half of the urban milk samples, 01-I,…, 21-I) with the interaction of pp'-DDE, HCB; right group includes half of the urban milk samples (milk sample collected in center of HCMC) and all suburban milk samples (milk samples collected from two suburban districts – BinhChanh and Hocmon district) interacted by pp'-DDD, pp'-DDT, g-HCH and endosulfans

c/ PBDEs: The plot obtained from the cluster and PCA analysis are shown in Fig. 8.17 and 8.18

Figure 8.17:

Cluster analysis based on PBDEs relative concentrations, 87 human milk samples analyzed



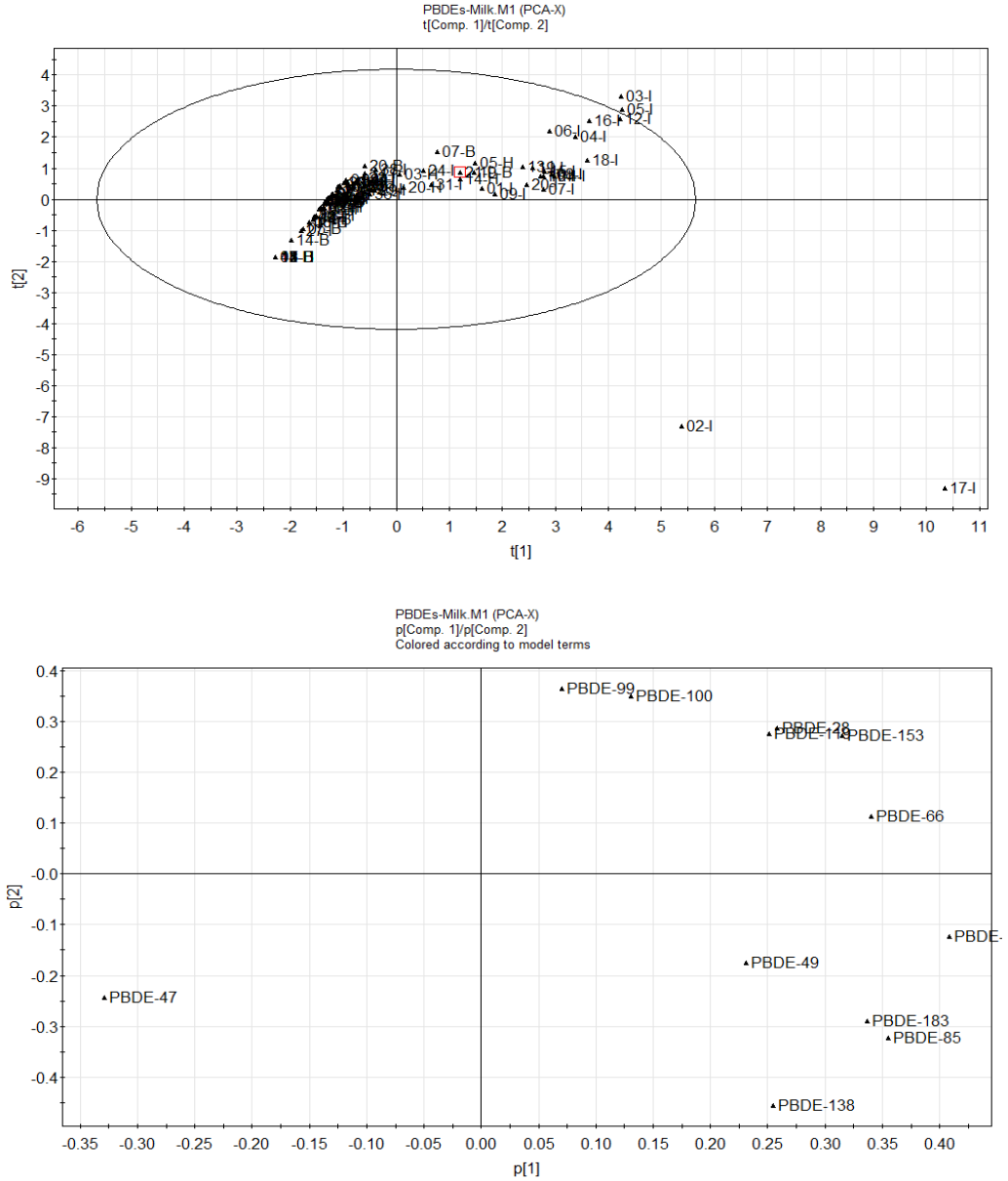


Figure 8.18: PCA analysis based on PBDEs relative levels, 87 human milk samples analyzed

From the cluster and PCA analysis, all milk samples can be divided into two groups: right group includes most urban milk samples interacted by PBDE 99, 100, and 154; left group (including suburban milk samples) mainly interacted by PBDE47.

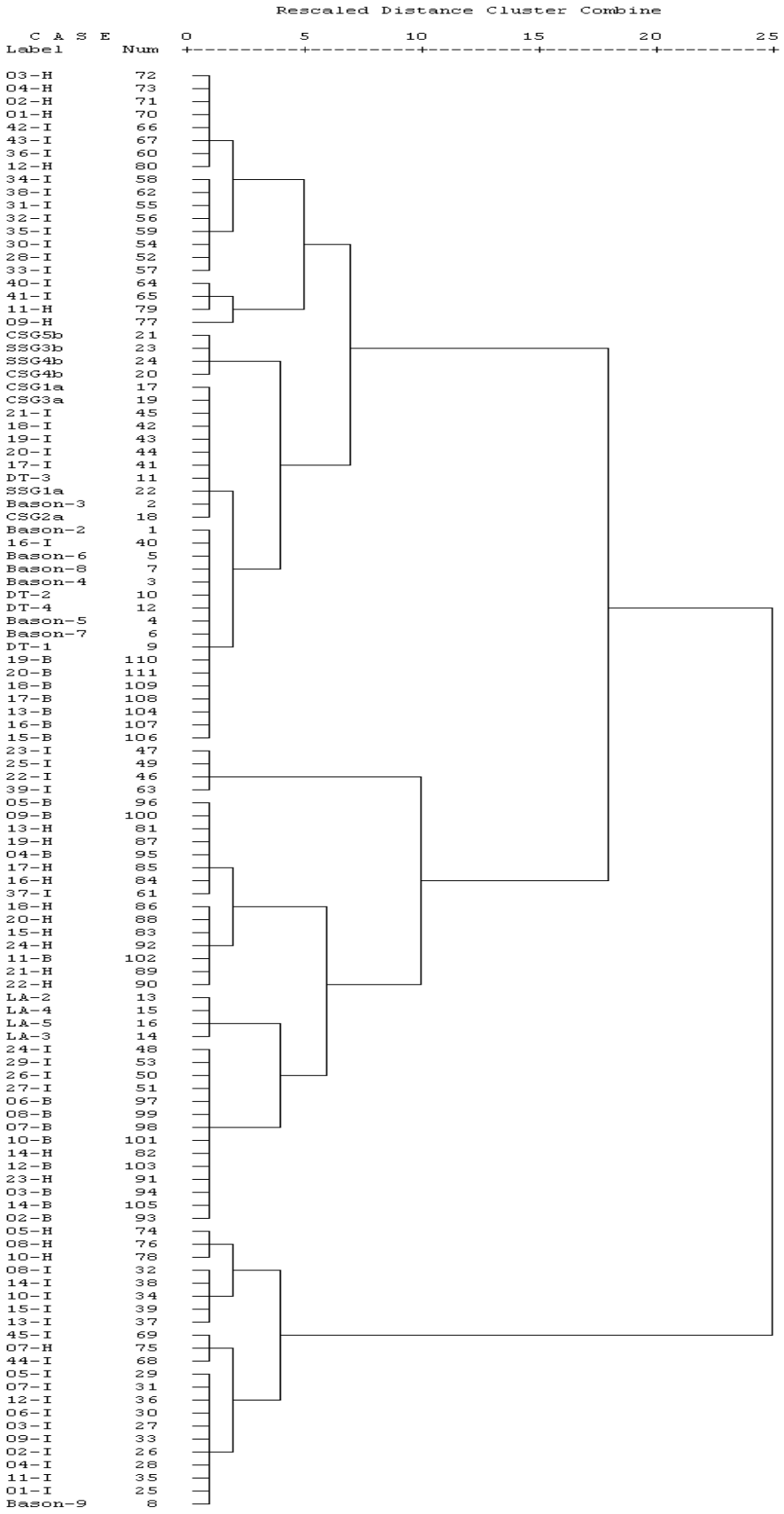
Using multivariate analysis for three groups of Hochiminh's milk samples (45 urban samples, 23 suburban-1 samples and 19 suburban-2 samples), we can see that the urban group is interacted mainly by PCB52, 101, (PCBs), pp'-DDT, HCB (OCPs), PBDE99, 100, 153 (PBDEs group) and two suburban groups is interacted by dioxin-like PCB congeners, pp'-DDD, pp'DDT, g-HCH, endosulfans and particularly PBDE47.

8.4.4 Comparison of soil, sediment, fish tissue and human breast milk samples in Hochiminh City.

a/ PCBs:

Figure: 8.19:

Cluster analysis based on PCBs relative levels of 8 sediment, 9 soil, 8 fish and 87 human milk samples in Hochiminh City area.



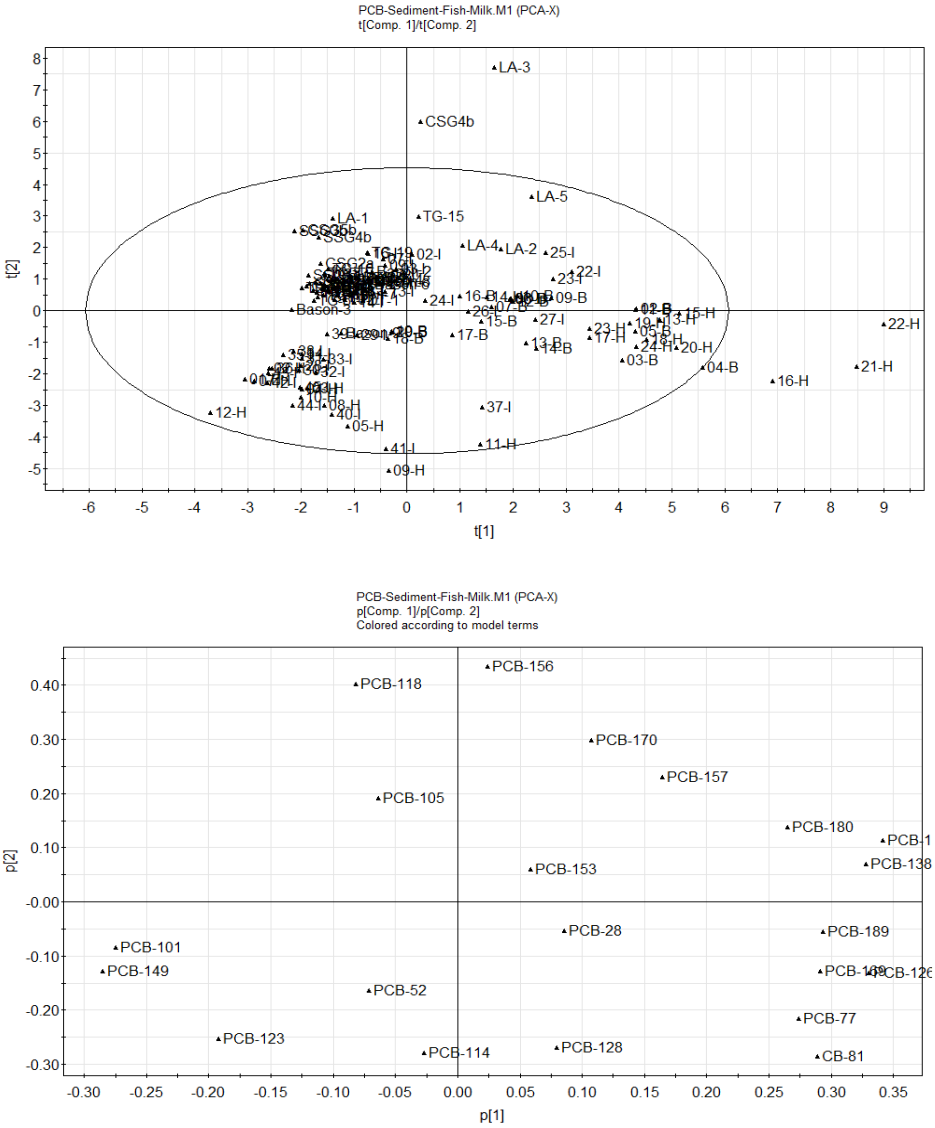


Figure 8.20: PCA analysis based on PCBs relative concentrations, 8 sediment, 9 soil, 8 fish and 87 human milk samples analyzed

By cluster in Fig. 8.19, we can divide samples into three groups:

- Group1: from 03-H to 15-B (from up to down) including most of the soil and sediment in HCMC, all fish samples in Saigon River and haft urban milk samples.
- Group2: from 23-I to 02-B
- Group3: the remaining samples

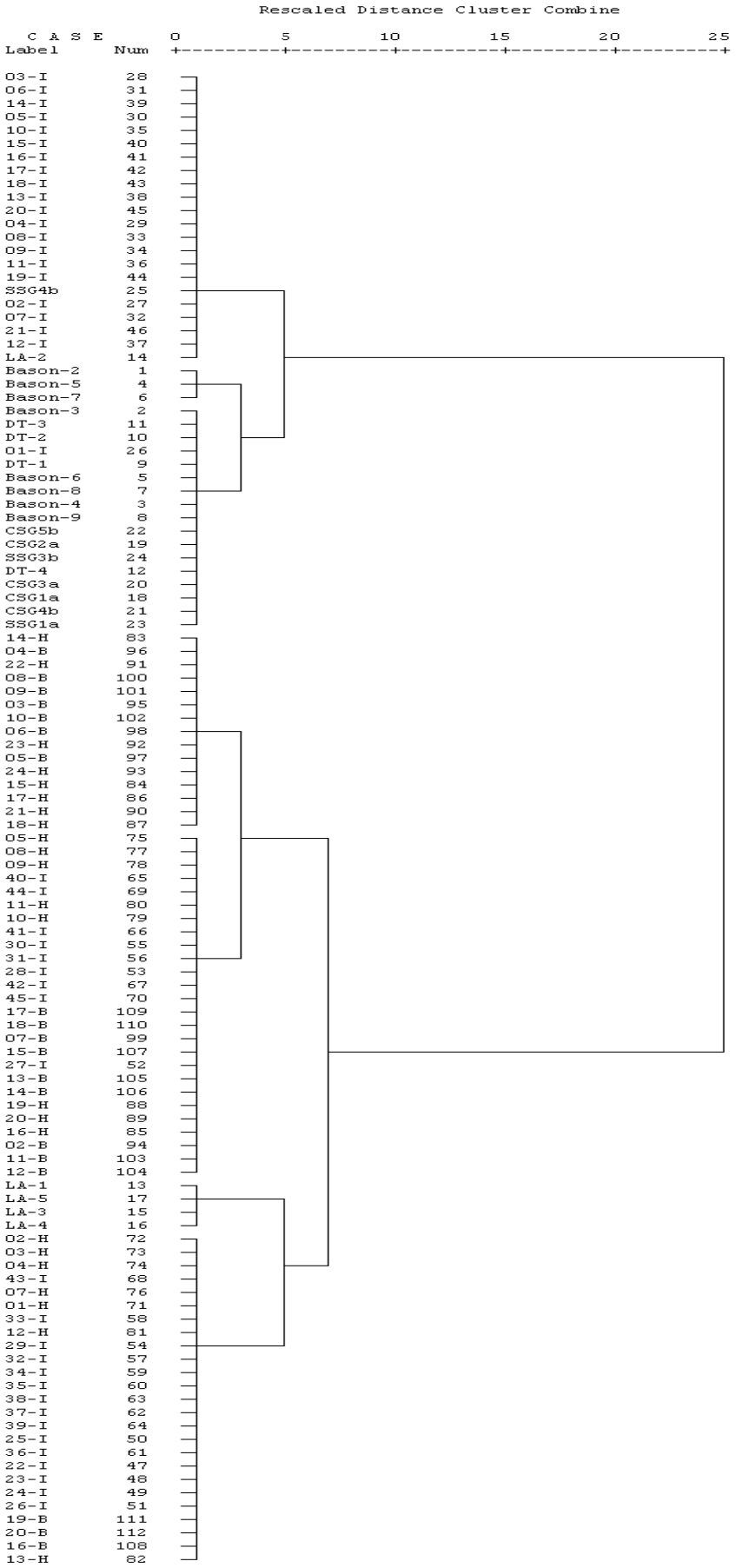
Result of PCA is presented in Fig. 8.20 (variables of 32.4%), and we have two groups:

- Group1 (left) contains most of the soil and sediment samples in HCM, fish samples and urban milk samples mainly interacted by PCB52, PCB101, PCB149
- Group2 (right) contains remaining milk samples from majority of the suburban milk samples and one part of urban milk samples interacted by CB 153, 157, 180 and 170

b/ OCls pesticides:

Figure 8.21

Cluster analysis based on OCls pesticides relative levels of 8 sediment, 9 soil, 8 fish and 87 human milk sample in HCMC analyzed



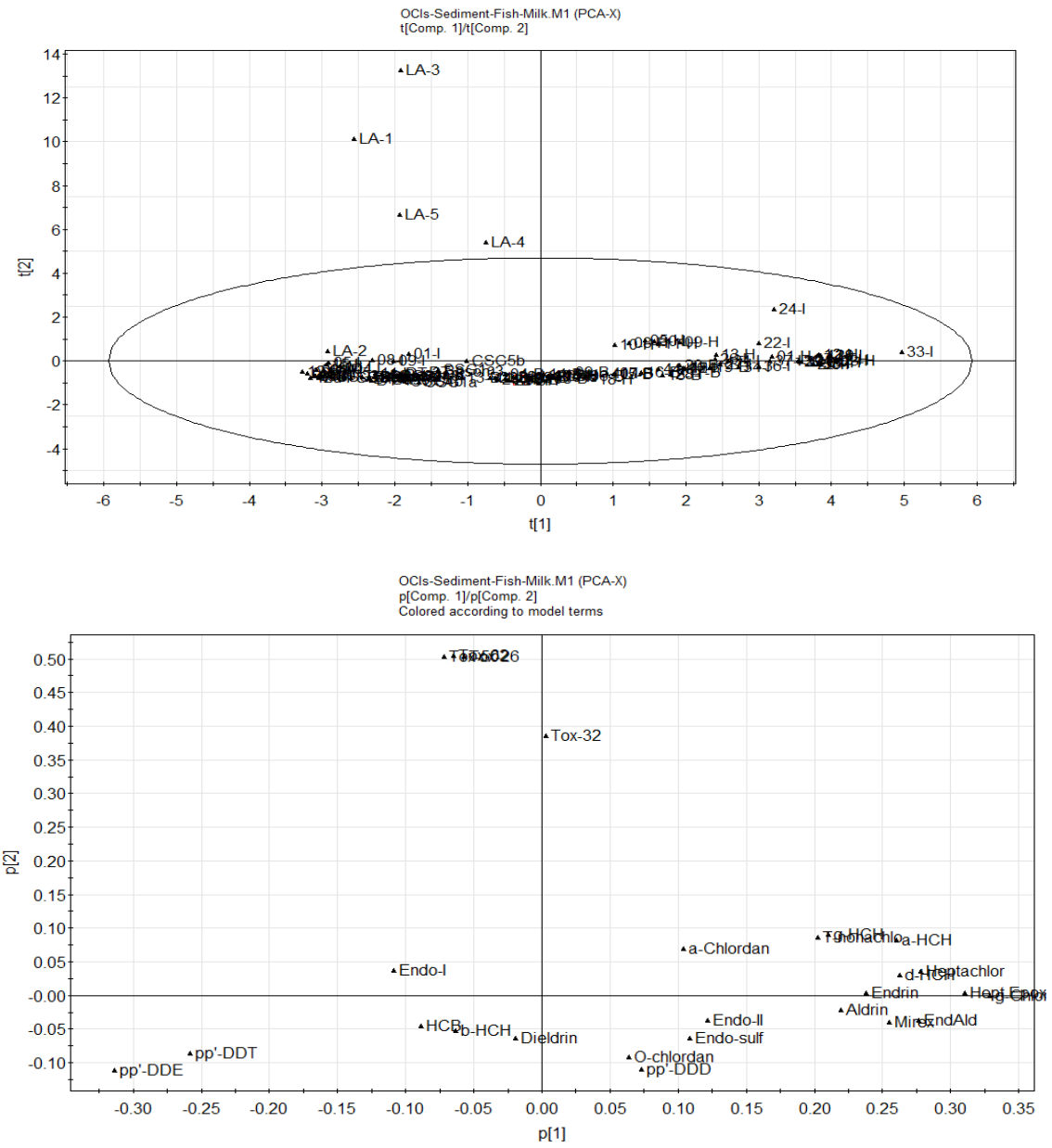


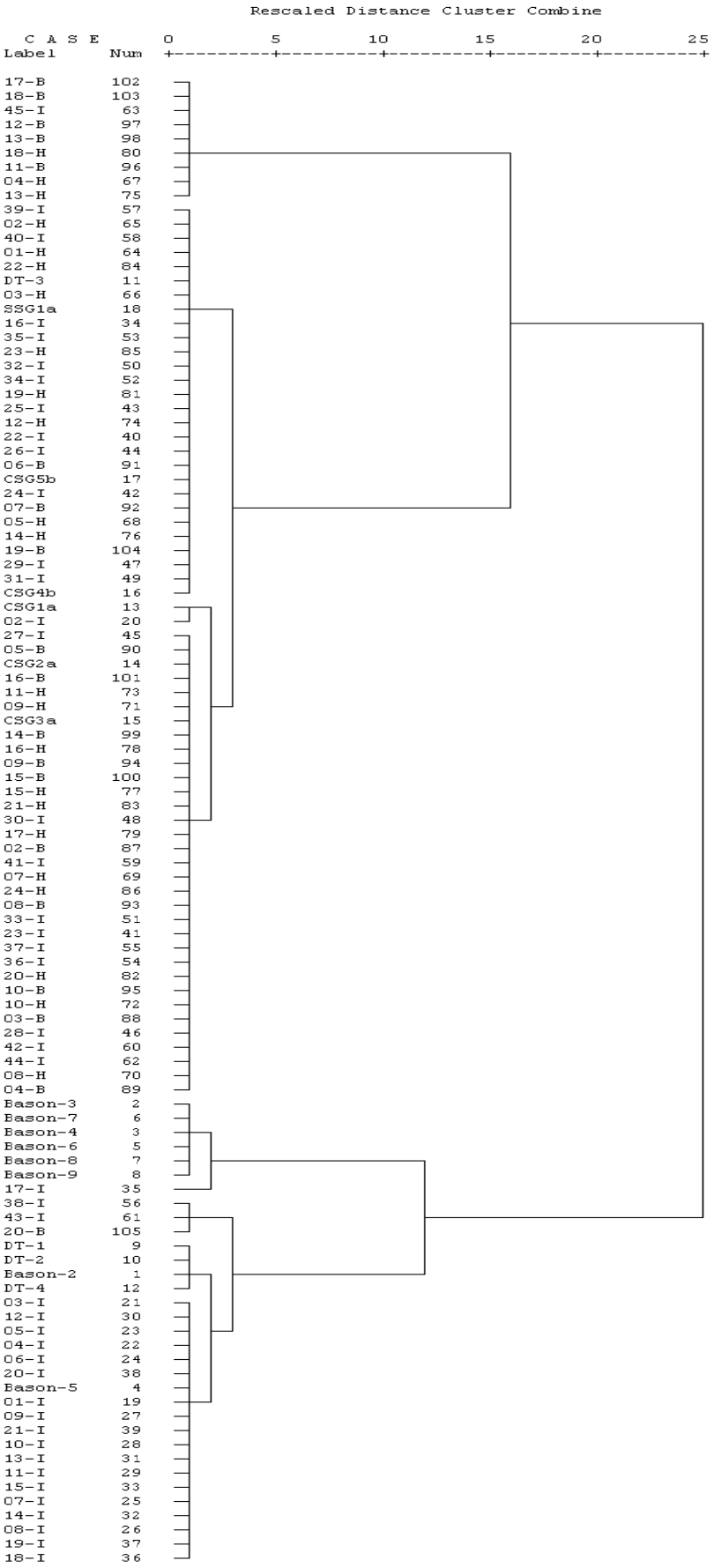
Figure 8.22: PCA analysis based on OCls pesticides relative concentrations, 8 sediments, 9 soils, 8 fish and 87 human milk sample analyzed

In OCls pesticides case, from the Fig 8.22 (variables of 48.7%) we can see clearly that there are two groups: group1 (left) includes most of the soil, sediment, fish and urban milk samples interacted mainly by pp'-DDE, pp'-DDT and HCB. Group 2 (right) includes most of the suburban milk samples and one part of the urban milk samples interacted by pp'-DDD, d-HCH, endosulfanII.

c/ PBDEs:

Figure 8.23

Cluster analysis based on PBDEs relative concentrations of 8 sediment, 9 soil, 8 fish and 87 human milk sample analyzed



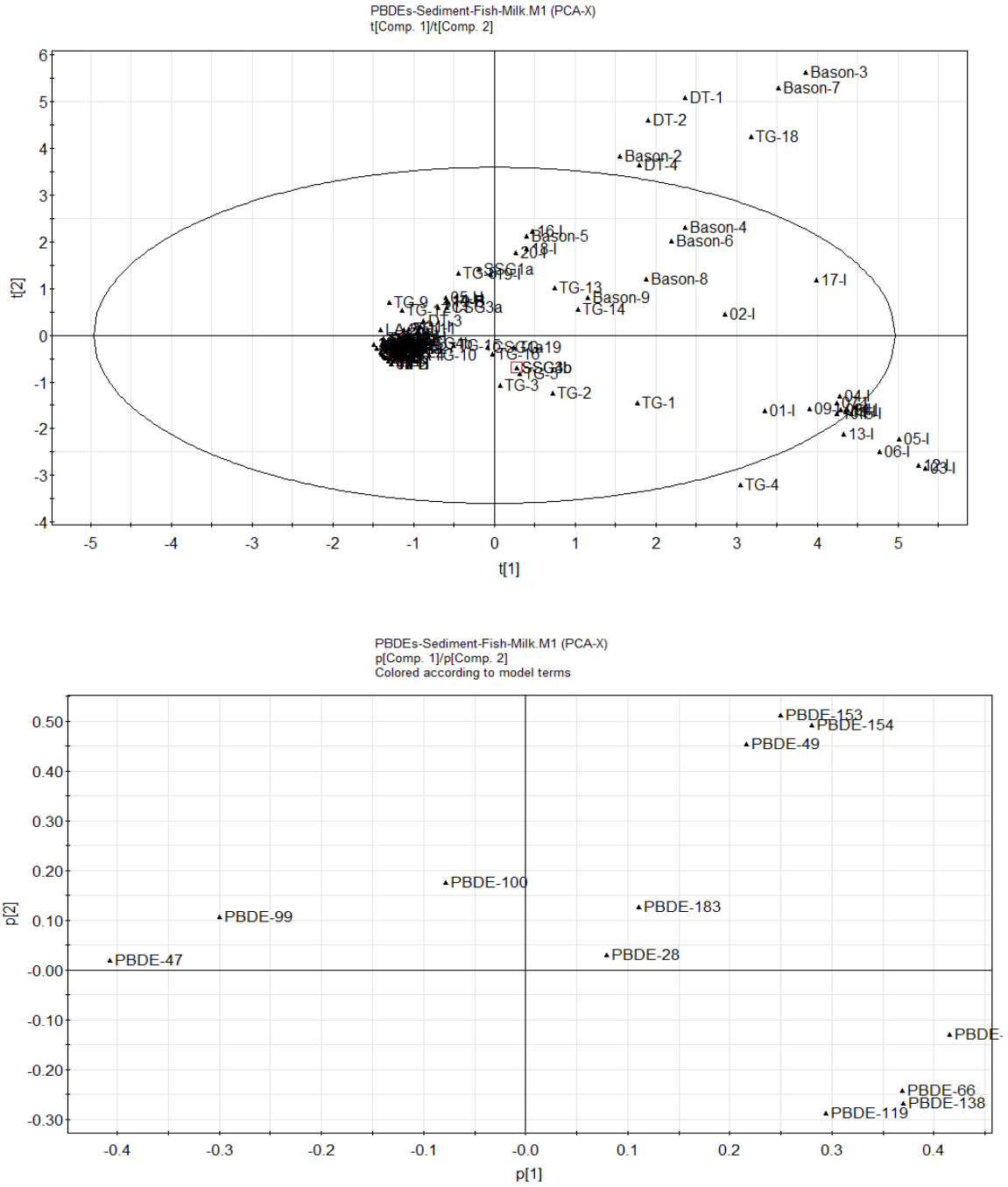


Figure 8.24 PCA analysis based on PBDEs relative concentrations, 8 sediment, 9 soil, 8 fish and 87 human milk sample analyzed

In the PBDEs case, it is similar to OCls pesticides case. From the Fig 8.24 (variables of 30.3%) we can divide into two groups: group1 (left) is interacted mainly by PBDE47 and 99; group 2 (right) is interacted by PBDE49, 100, and 153. From dendogram of cluster analysis (Fig8.23) we can see all soil, sediment from landfill and Saigon River grouped together with half of the urban milk samples.

With the hypothesis that PTSs contamination in landfill and HCMC canals can related to fish and may be to human breast milk, we applied the multivariate analysis with cluster and PCA analysis technique presented above and compared all soil, sediment, fish and human breast milk in HCMC each other. We can draw some conclusions as follow:

- There is a high similarity in PTSs pattern of landfill soil (Dongthanh landfill) and Thinghe canal-Saigon river sediment.
- In other word, the pattern of PTSs in soil samples from landfill, sediment from canals, and fishes from Saigon River could be correlated each other.
- In chapter 6, we also found a similarity of PCBs congener's patterns in comparison PCBs profile in fish from Saigon River with those in landfill soil and TN-SG River sediment. These samples were interacted by CB 118, 101, 149, 153 and 138 (Fig. 6.8).
- Hence logically, we can admit that Dongthanh landfill can be a primary potential source of PTSs for HCMC's canals and fish in Saigon River.

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Chapter 9: CONCLUSIONS AND RECOMMENDATIONS

9.1 Conclusions

Our study endeavors to draw the current state of the PTSs contamination levels in southern Vietnam. The obtained results will positively contribute to the understanding of PTSs pollutants pattern and their impacts to environment and food chain as well as contribute to the improvement of PTSs research capability of Vietnam. This is highly necessary when Vietnamese government approved the National Implementation Plan for participating, performing and validating Stockholm Convention. Considering the thesis' objectives and results, we come up with the conclusion as follows:

- The research has established suitable analytical methods, which is able to analyze simultaneously PCBs, PBDEs, OCs pesticides and especially, mirex and toxaphene, which are analyzed for the first time in Vietnam, in various matrices such as soil/sediment, fish and human breast milk samples by HRGC/LRMS. All analysis works were carried out in IER lab (Vietnam) with GC/MS and in CEAL lab - EPFL with GC/MS and GC/ECD instruments. The analytical method is in accordance with real conditions of IER lab and Vietnam.

- Our result shown that PTSs levels of soil and sediment in southern Vietnam are not so high. For instance, PCBs levels are 11.8 ng/g dry wt. in TN-SG River sediment, 17.2 ng/g dry wt. in landfill soil and 6.1 ng/g dry wt. in Tien River sediment; DDTs levels are 17.6 ng/g dry wt. in TN-SG River sediment, 13.9 ng/g dry wt. in landfill soil, 20.5 ng/g dry wt. in Longan agrichemical warehouse soil, and 6.1 ng/g dry wt. in paddy fields soil from Tien River basin. Toxaphene was only found in Longan agrichemical warehouse soil samples with relatively high level (40.3 ng/g dry wt.) In addition, the widespread endosulfans contamination was suggested due to their large usage in agriculture in southern Vietnam until end of 2006. Other PTSs such as HCHs, CHLs, HCB and dieldrin were detected at low levels (< 5 ng/g dry wt.) and heptachlor, heptachlor epoxide, aldrin, endrin, endrin aldehyde levels were lower than LOD of the analytical method. Meanwhile, PTSs levels in Thivai River sediment are much lower than those in other areas in our study as well as lower than those in previous studies in Vietnam. This indicates that PTSs contamination levels of Thivai River is low and industrial zones and ports along riversides could be insignificant PTSs contamination sources of Thivai River basin.

- PTSs concentrations in wild fish samples from Saigon and Tien Rivers are lower than those in previous studies in Vietnam and in the world. However, unlike DDTs, PCBs showed slow decreasing in fish samples and this suggests an continuous contamination. Besides, due to large usage in agricultural activities (Chapter 5), endosulfans were also found in wild fish samples from Saigon and Tien River at the levels of 23.9 and 8.3 ng/g lipid wt., respectively.

- In general, PTSs levels in human breast milk of HCMC residents are lower than those in previous study (except PCBs and CHLs). The higher PCB residues observed in human breast milk from HCMC suggests continuous exposure to PCBs through the food chain to man. In this study, we observed higher concentration of PTSs in human breast milk of primiparous mother as compared to multiparous mothers. This trend well agrees with previous study in Hanoi and HCMC.

- Based on the obtained results by using statistic methods (cluster analysis and PCA), we have shown the similar PTSs profiles pattern of landfill soil, TN-SG River sediments and Saigon River fish samples by interacting PCBs with CB 118, 101, 149, 153 and 138; PBDEs with BDE 47, 99 and 100; OCl pesticides with pp'-DDE, pp'-DDT and HCB. Logically, we might suggest that there is a transfer of PTSs compounds from the landfill and TN-SG River to fish collected from Saigon river. With the results from chapter 5 and 6, our study clearly demonstrates that municipal and industrial waste from landfill and urban activities of HCMC could be considered as PTSs pollution sources.

- In general, PCBs and DDTs are still two major PTSs contaminants in the environment. Comparison PTSs levels in our study in various matrices with those in previous studies in Vietnam, we suggest a decreasing trend of PTSs levels in the environment in southern Vietnam.

9.2 Recommendations

From our obtained results, we would like to suggest:

- Despite the possible decrease in PTSs contamination in the future, the rapidly growth industrial zones and urban areas may continue to be a potential sources for PTSs contaminants. Comprehensive and long-term monitoring programs especially for the new PTSs such as PeCB, HeBB, and PFOSs in industrial activities are still urgent requirement. In this context, well-designed monitoring of temporal trends of PTSs residues in Vietnam over an extended period is crucial for tracing the unrevealed sources and predicting future prospects of their pollution.

- To continue a large-scale study on PTSs transfer into food chain with more matrix types, including adipose tissue and blood of human to get more precise assessment.

- Performing analytical research on the transport, exposures, and inter-media partitioning if each PTS compound helps researchers to understand more clearly about the partitioning tendencies between soil, sediment, water, and biota of PTSs as well as the unequal importance of bioaccumulation and biomagnification mechanism in inter-media. In the future, this kind of analysis may help researchers to put their data in the context of the chemical processes that transport and degrade PTSs in environment and human.

- Based on the results of our research as well as previous studies in Vietnam, more research the analysis of transport, exposures, and inter-media partitioning of each PTS compound can be carried out to have new and more intensive knowledge about the partitioning tendencies between soil, sediment, water, and biota. In the future, this kind of analysis may help researchers to put their data in the context of the chemical processes that transport and degrade PTSs in environment and human.

- To set up a reference laboratory for PTSs research and related problem in the South of VietNam, we should consider investing in reference analytical equipments such GC×GC coupled with ECD or TOFMS and HRMS to reach the standard level and comparable result with other laboratories in the POP monitoring network in the frame of Stockholm Convention.

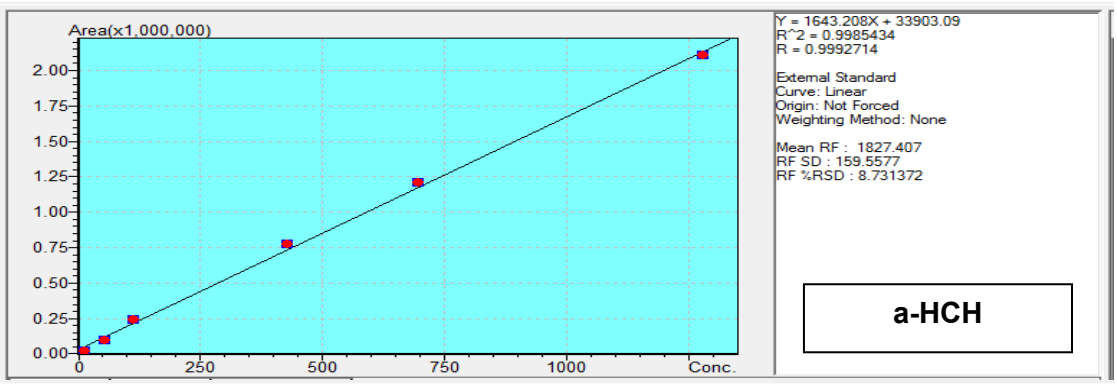
- To perform further research on breast milk for monitoring the temporal trend of DDT as well as other PTSs in breast milk and for more comprehensive confirming this research conclusion on breast milk analysis results.

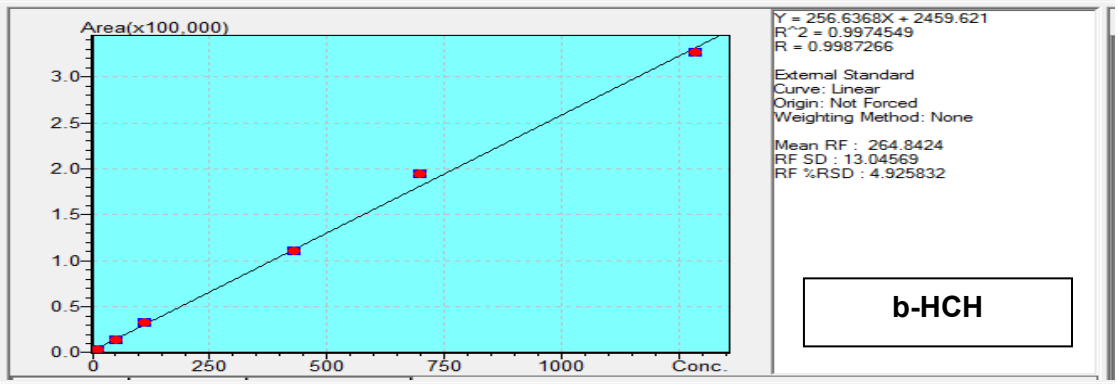
- Vietnam authorities must soon establish a PTSs guideline for soil, foodstuff, and aquatic environment, etc.

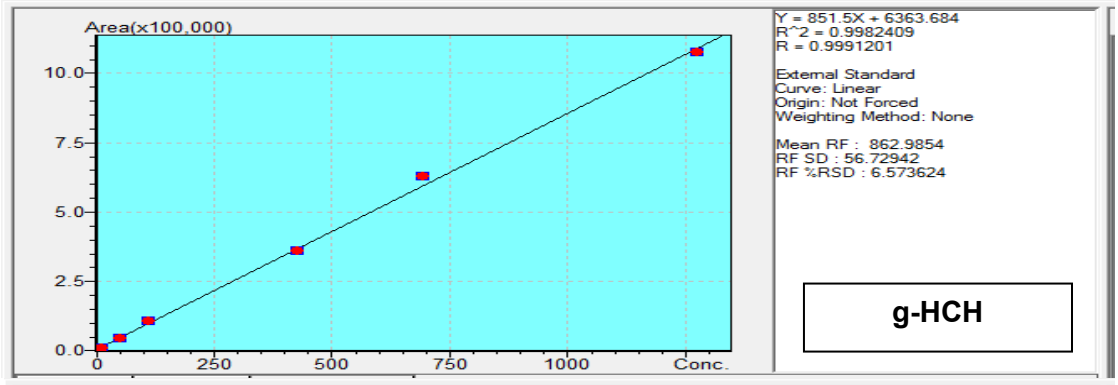
- CEAL Lab is one of laboratory is equipped with state-of-the-art instrument and experienced in the field of PTSs analysis. In the frame of the SDC cooperation project between the Switzerland and Vietnam governments, CEAL and IER have established a long-time cooperation through the support of CEAL in improving research capability of IER. It has been CEAL who helps setting the PTSs analyzing lab, facilitating the equipment as well as training for IER staffs. This research is also a part of these cooperative activities. We highly recommend strengthening the cooperation through scientific research activities, especially in analyzing PTS with new POPs like PBDEs, OSn, PFOSs, the compounds almost have not been researched in Vietnam.

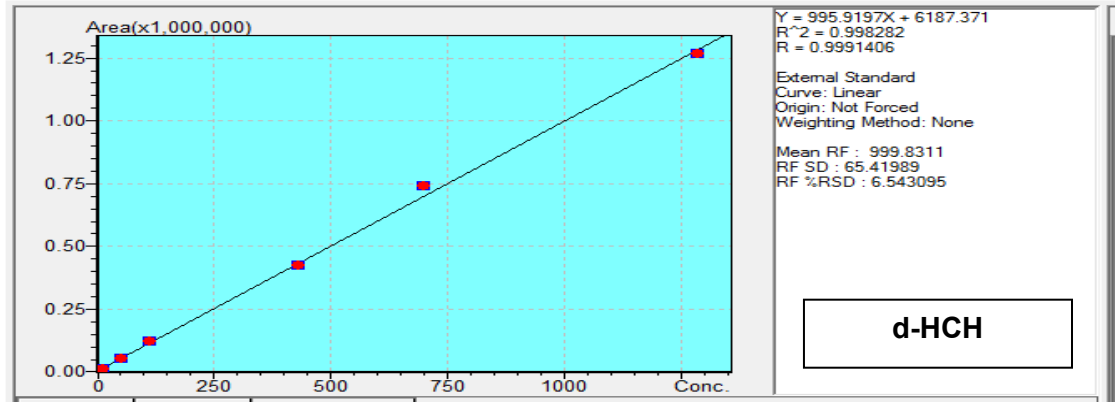
- Furthermore, under the National Implementation Plan – Stockholm Convention, IER lab was approved to be a partner in southern Vietnam POPs monitoring network of Vietnam Environment Administration (VEA). This affirms the improved capability of IER lab, the effectiveness of the support from CEAL and again asks for further cooperation in new POPs analysis as well as other scientific research activities.

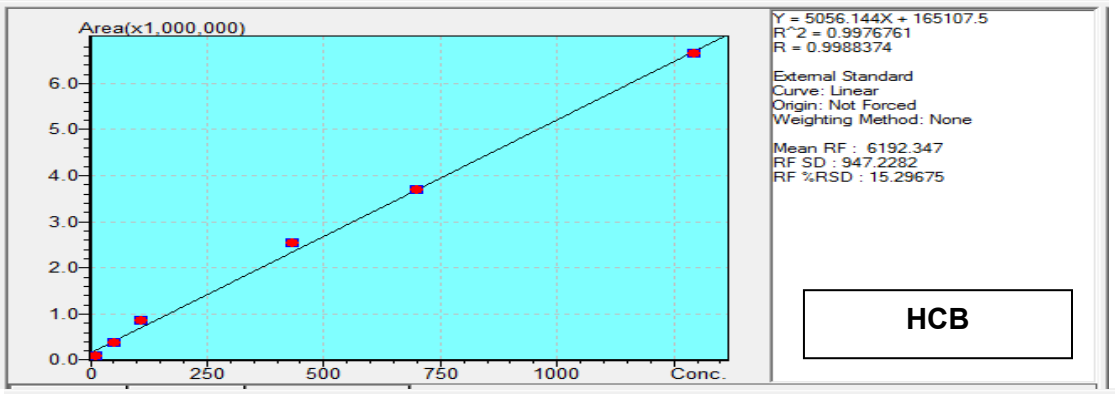
Annex-1
Calibration curve for linearity check

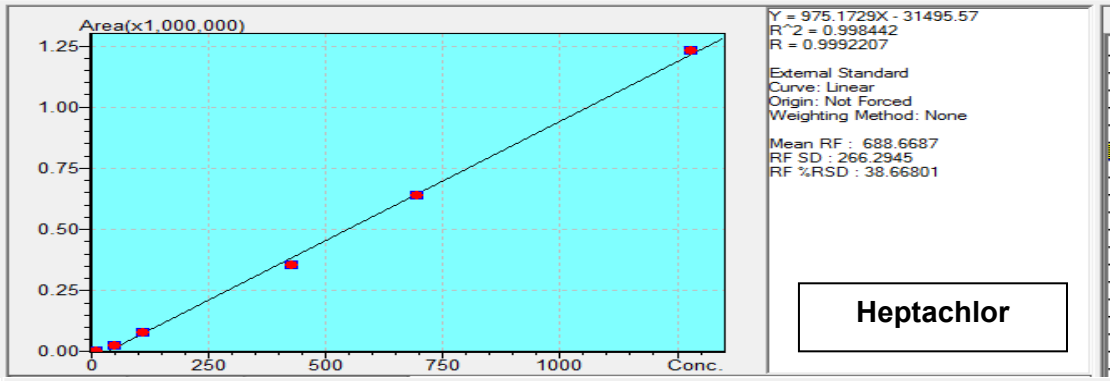


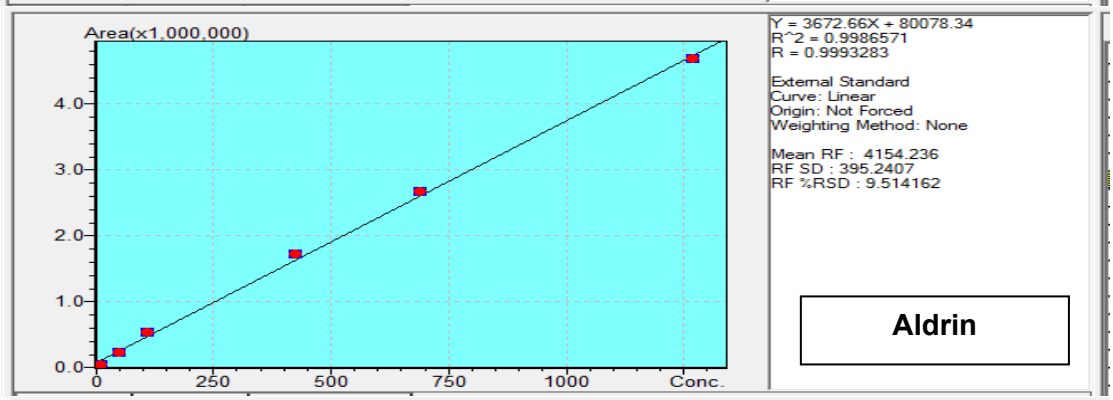


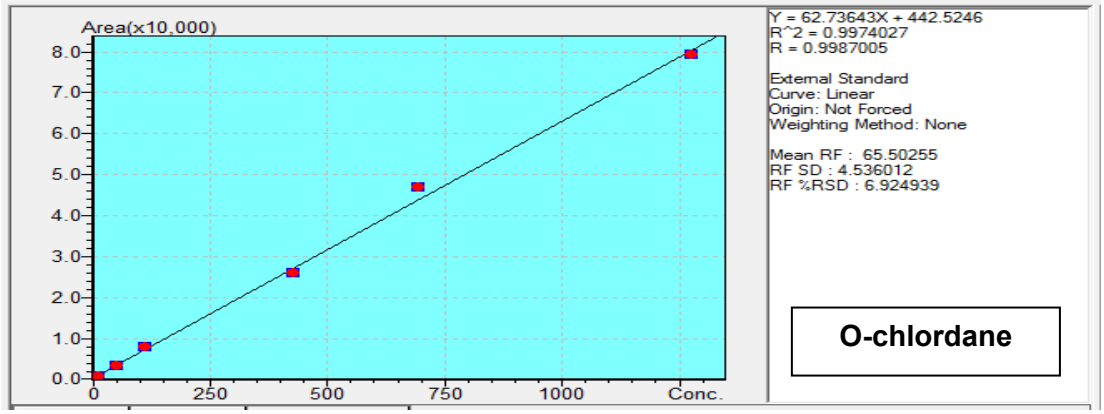


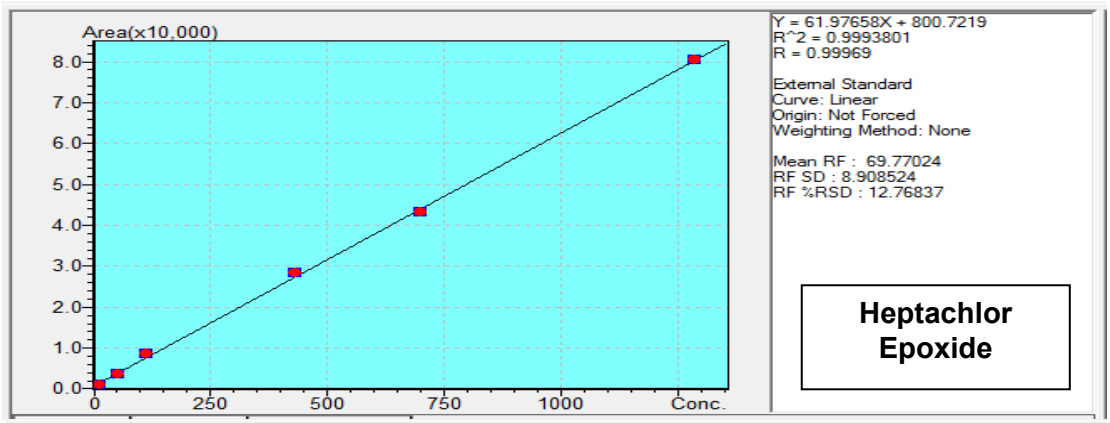


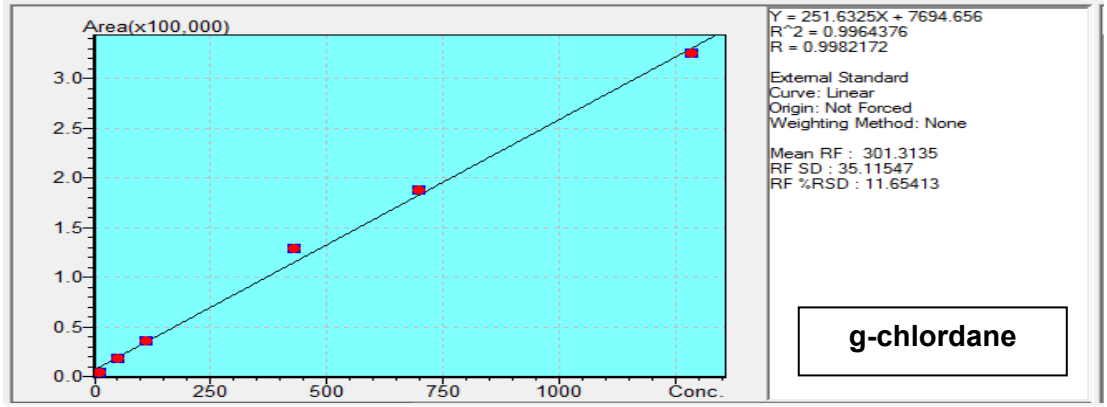

Area(x1,000,000)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
250
500
750
1000
Conc.
Y = 5056.144X + 165107.5
R^2 = 0.9976761
R = 0.9988374
External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None
Mean RF : 6192.347
RF SD : 947.2282
RF %RSD : 15.29675
HCB

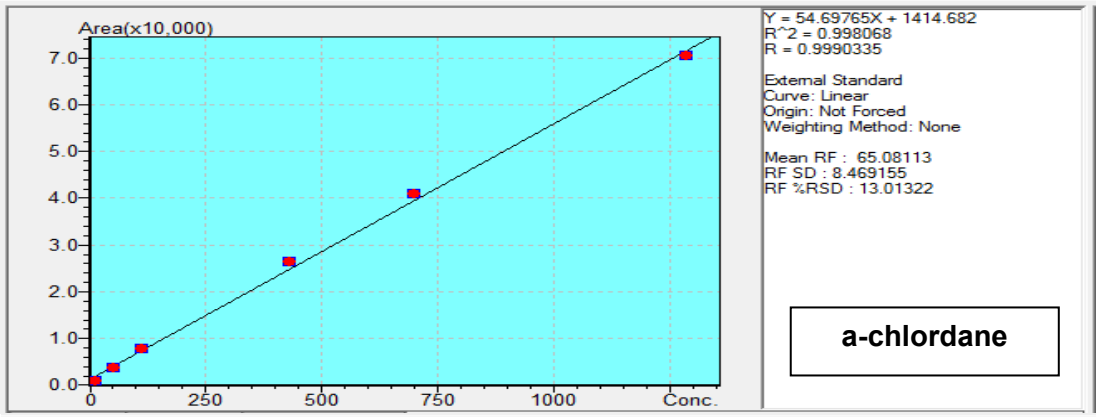

Area(x1,000,000)
1.25
1.00
0.75
0.50
0.25
0.00
0
250
500
750
1000
Conc.
Y = 975.1729X - 31495.57
R^2 = 0.998442
R = 0.9992207
External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None
Mean RF : 688.6687
RF SD : 266.2945
RF %RSD : 38.66801
Heptachlor

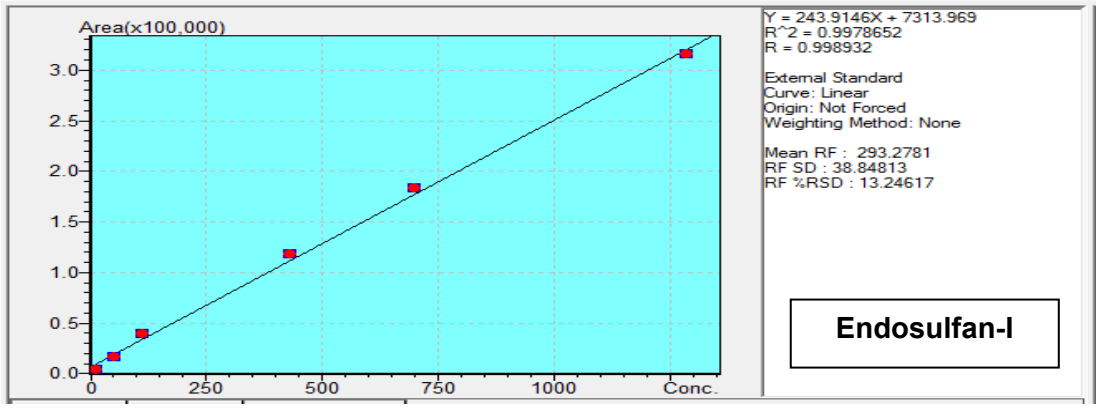

Area(x1,000,000)
4.0
3.0
2.0
1.0
0.0
0
250
500
750
1000
Conc.
Y = 3672.66X + 80078.34
R^2 = 0.9986571
R = 0.9993283
External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None
Mean RF : 4154.236
RF SD : 395.2407
RF %RSD : 9.514162
Aldrin

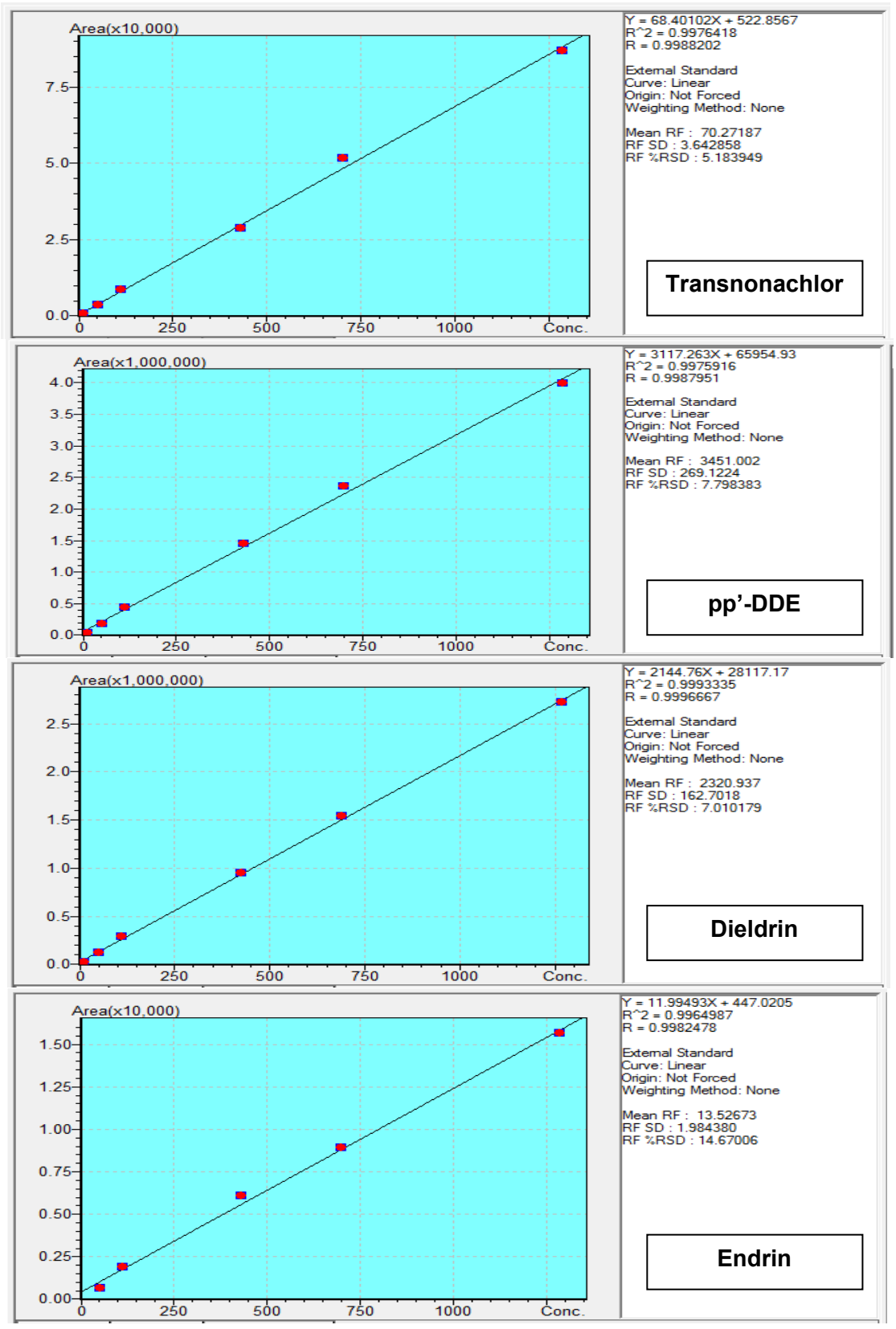

Area(x10,000)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
250
500
750
1000
Conc.
Y = 62.73643X + 442.5246
R^2 = 0.9974027
R = 0.9987005
External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None
Mean RF : 65.50255
RF SD : 4.536012
RF %RSD : 6.924939
O-chlordane

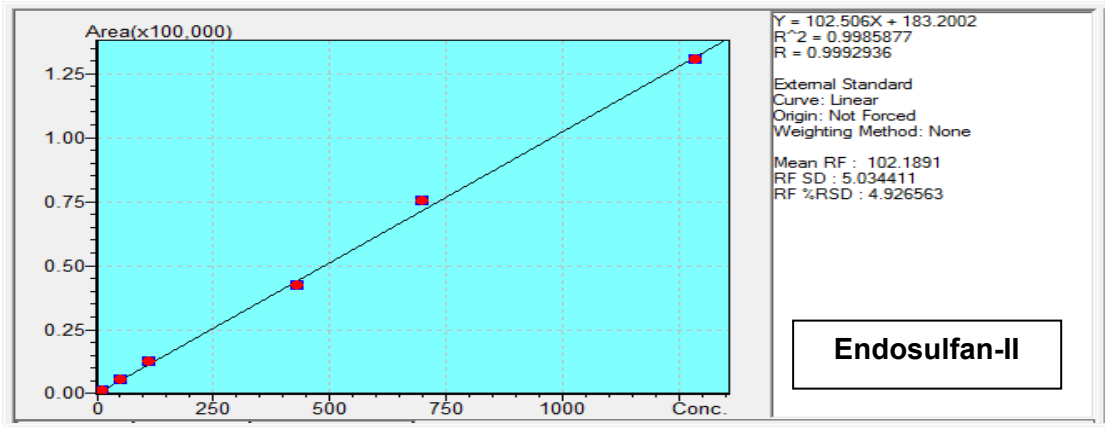

Area(x10,000)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
250
500
750
1000
Conc.
Y = 61.97658X + 800.7219
R^2 = 0.9993801
R = 0.99969
External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None
Mean RF : 69.77024
RF SD : 8.908524
RF %RSD : 12.76837
Heptachlor Epoxide

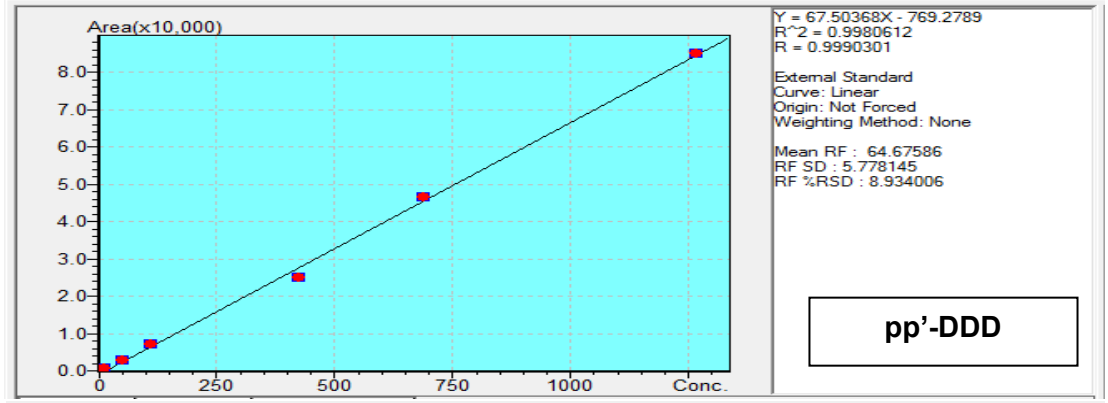

Area(x100,000)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
250
500
750
1000
Conc.
Y = 251.6325X + 7694.656
R^2 = 0.9964376
R = 0.9982172
External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None
Mean RF : 301.3135
RF SD : 35.11547
RF %RSD : 11.65413
g-chlordane

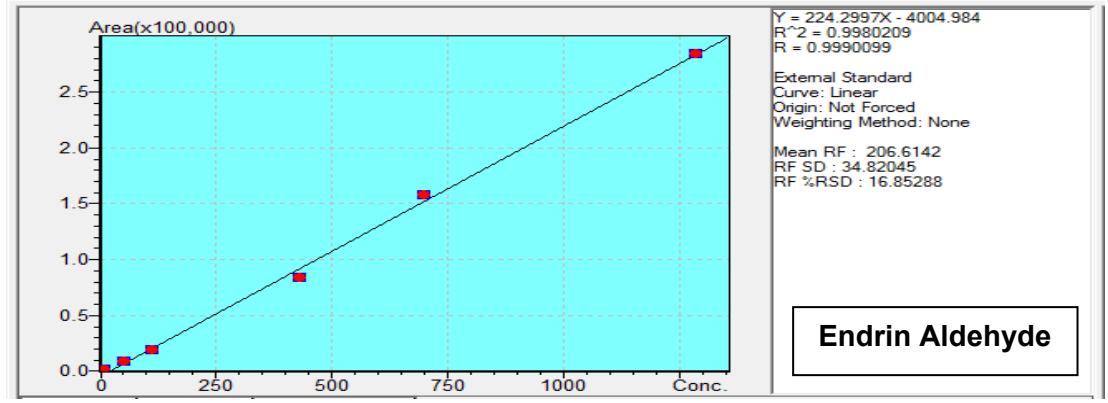

Area(x10,000)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
250
500
750
1000
Conc.
Y = 54.69765X + 1414.682
R^2 = 0.998068
R = 0.9990335
External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None
Mean RF : 65.08113
RF SD : 8.469155
RF %RSD : 13.01322
a-chlordane

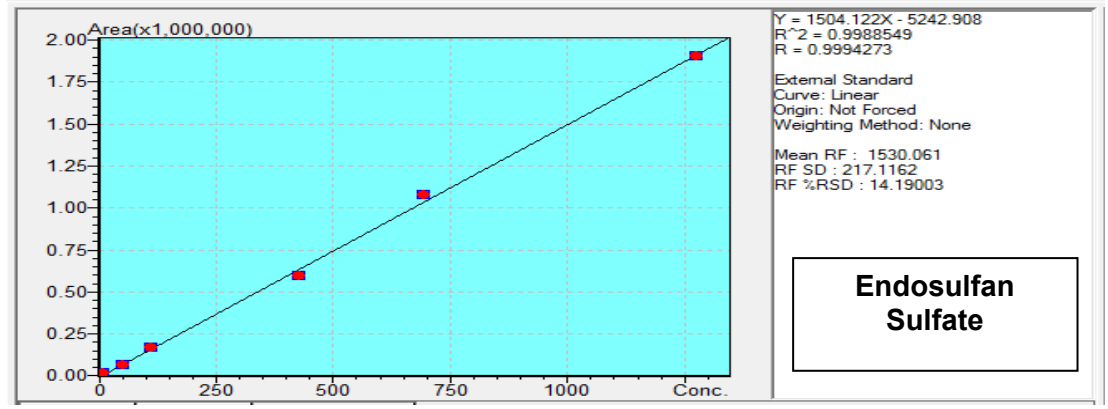

Area(x100,000)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
250
500
750
1000
Conc.
Y = 243.9146X + 7313.969
R^2 = 0.9978652
R = 0.998932
External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None
Mean RF : 293.2781
RF SD : 38.84813
RF %RSD : 13.24617
Endosulfan-I

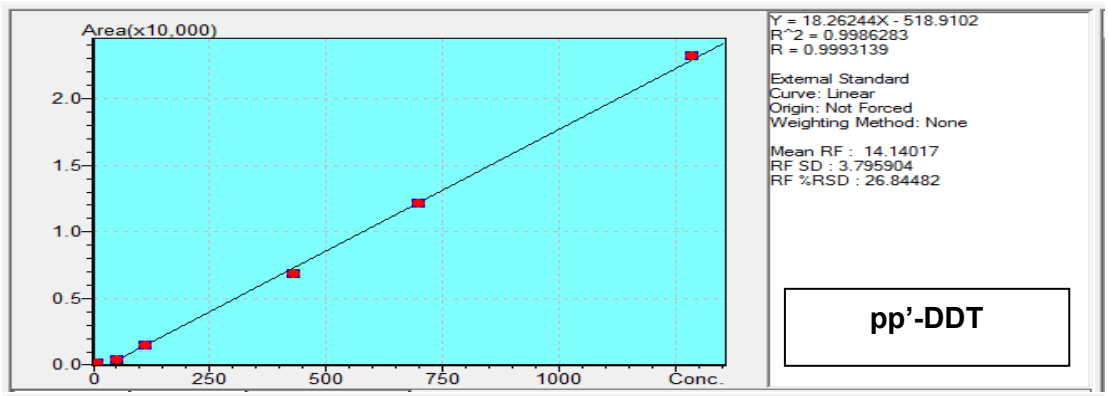

Area(x10,000)
7.5
5.0
2.5
0.0
0
250
500
750
1000
Conc.
Y = 68.40102X + 522.8567
R^2 = 0.9976418
R = 0.9988202
External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None
Mean RF : 70.27187
RF SD : 3.642858
RF %RSD : 5.183949
Transnonachlor
Area(x1,000,000)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
250
500
750
1000
Conc.
Y = 3117.263X + 65954.93
R^2 = 0.9975916
R = 0.9987951
External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None
Mean RF : 3451.002
RF SD : 269.1224
RF %RSD : 7.798383
pp'-DDE
Area(x1,000,000)
2.5
2.0
1.5
1.0
0.5
0.0
0
250
500
750
1000
Conc.
Y = 2144.76X + 28117.17
R^2 = 0.9993335
R = 0.9996667
External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None
Mean RF : 2320.937
RF SD : 162.7018
RF %RSD : 7.010179
Dieldrin
Area(x10,000)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
250
500
750
1000
Conc.
Y = 11.99493X + 447.0205
R^2 = 0.9964987
R = 0.9982478
External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None
Mean RF : 13.52673
RF SD : 1.984380
RF %RSD : 14.67006
Endrin

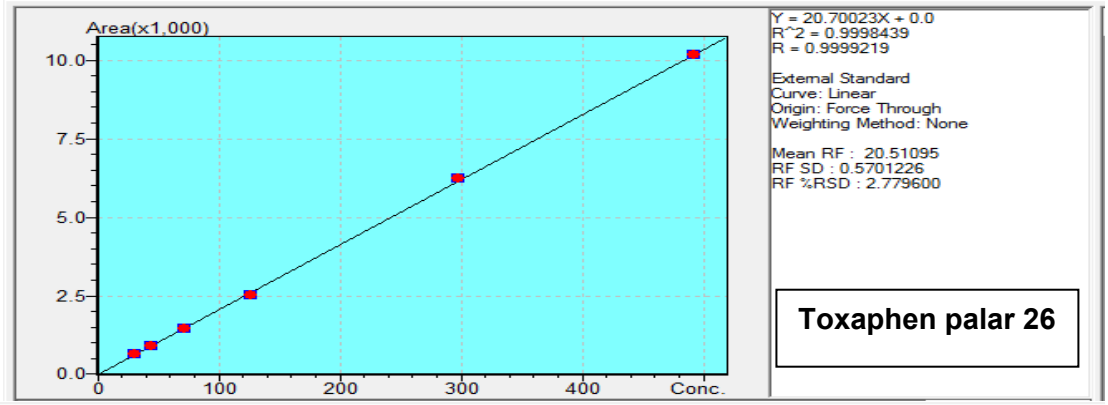

Area(x100,000)
1.25
1.00
0.75
0.50
0.25
0.00
0
250
500
750
1000
Conc.
Y = 102.506X + 183.2002
R^2 = 0.9985877
R = 0.9992936
External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None
Mean RF : 102.1891
RF SD : 5.034411
RF %RSD : 4.926563
Endosulfan-II

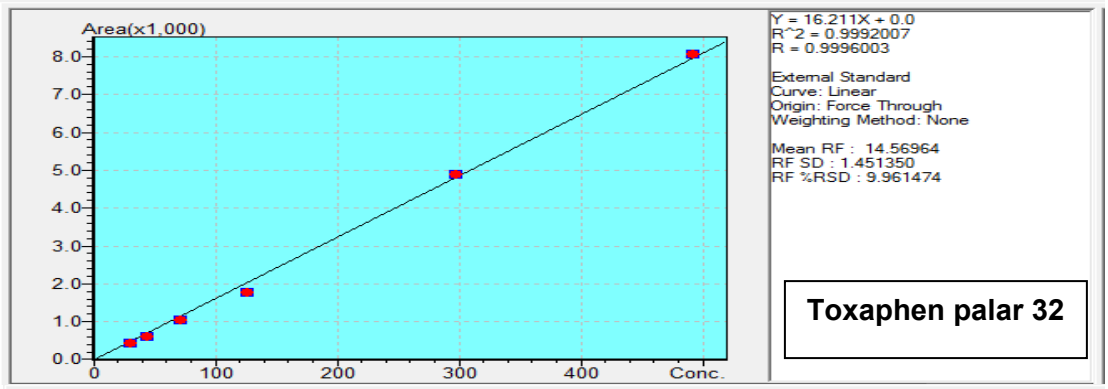

Area(x10,000)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
250
500
750
1000
Conc.
Y = 67.50368X - 769.2789
R^2 = 0.9980612
R = 0.9990301
External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None
Mean RF : 64.67586
RF SD : 5.778145
RF %RSD : 8.934006
pp'-DDD

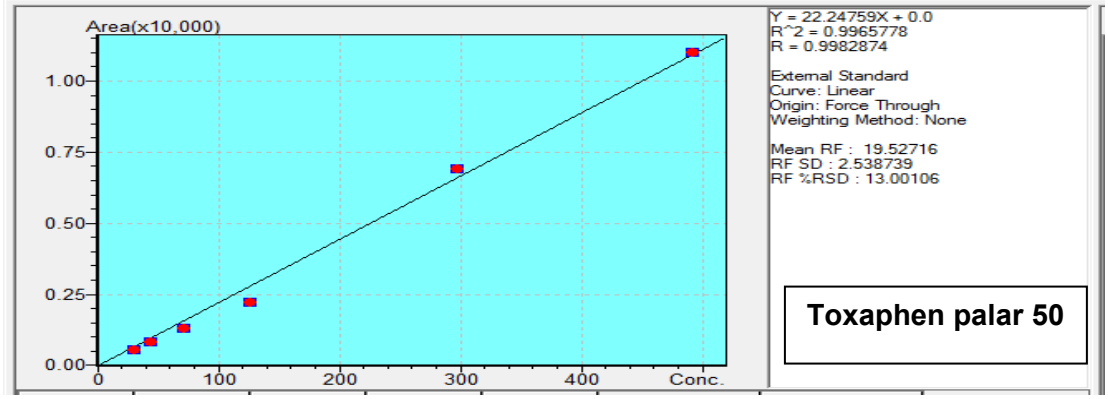

Area(x100,000)
2.5
2.0
1.5
1.0
0.5
0.0
0
250
500
750
1000
Conc.
Y = 224.2997X - 4004.984
R^2 = 0.9980209
R = 0.9990099
External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None
Mean RF : 206.6142
RF SD : 34.82045
RF %RSD : 16.85288
Endrin Aldehyde

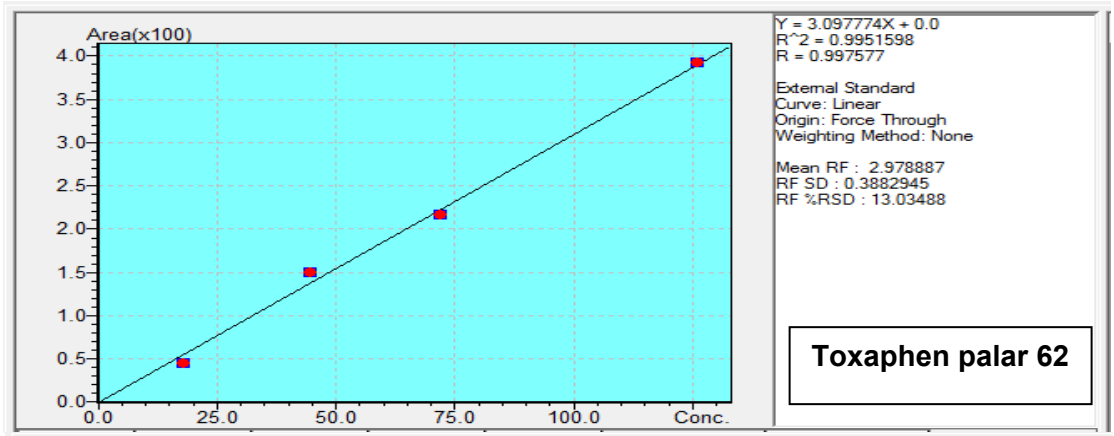

Area(x1,000,000)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
250
500
750
1000
Conc.
Y = 1504.122X - 5242.908
R^2 = 0.9988549
R = 0.9994273
External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None
Mean RF : 1530.061
RF SD : 217.1162
RF %RSD : 14.19003
Endosulfan Sulfate

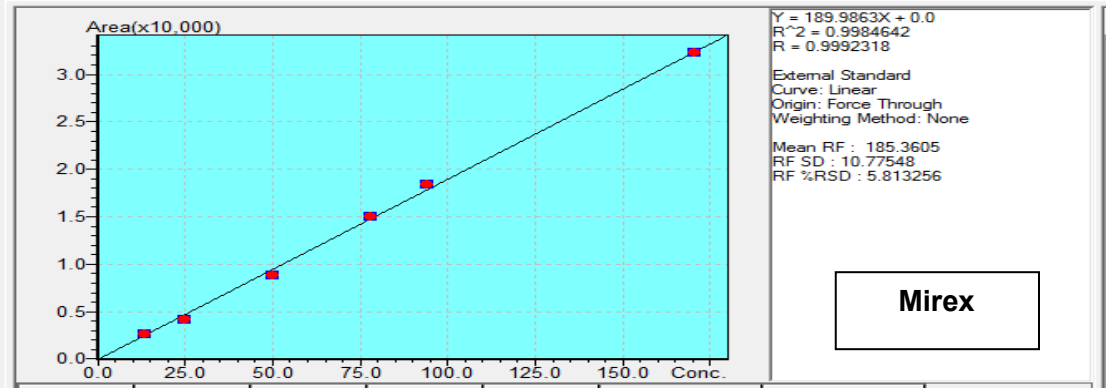

Area(x10,000)
2.0
1.5
1.0
0.5
0.0
0
250
500
750
1000
Conc.
Y = 18.26244X - 518.9102
R^2 = 0.9986283
R = 0.9993139
External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None
Mean RF : 14.14017
RF SD : 3.795904
RF %RSD : 26.84482
pp'-DDT


Area(x1,000)
10.0
7.5
5.0
2.5
0.0
0
100
200
300
400
Conc.
Y = 20.70023X + 0.0
R^2 = 0.9998439
R = 0.9999219
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 20.51095
RF SD : 0.5701226
RF %RSD : 2.779600
Toxaphen palar 26

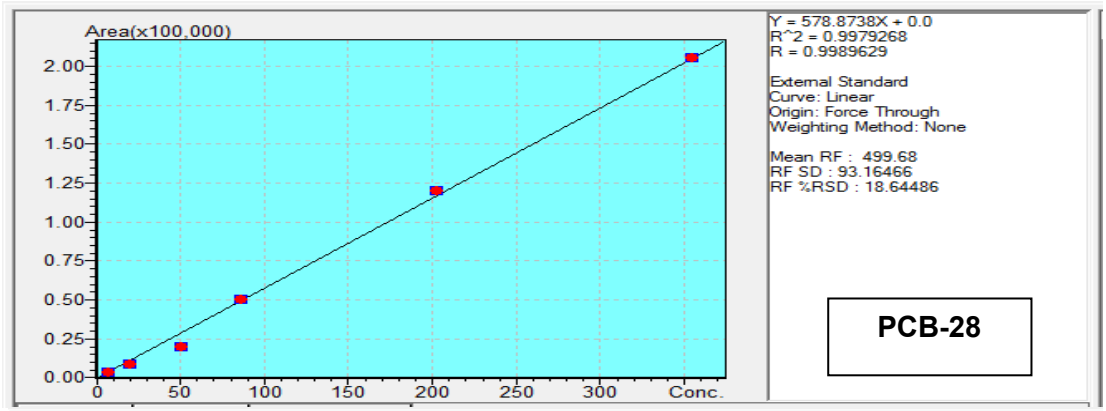

Area(x1,000)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
100
200
300
400
Conc.
Y = 16.211X + 0.0
R^2 = 0.9992007
R = 0.9996003
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 14.56964
RF SD : 1.451350
RF %RSD : 9.961474
Toxaphen palar 32

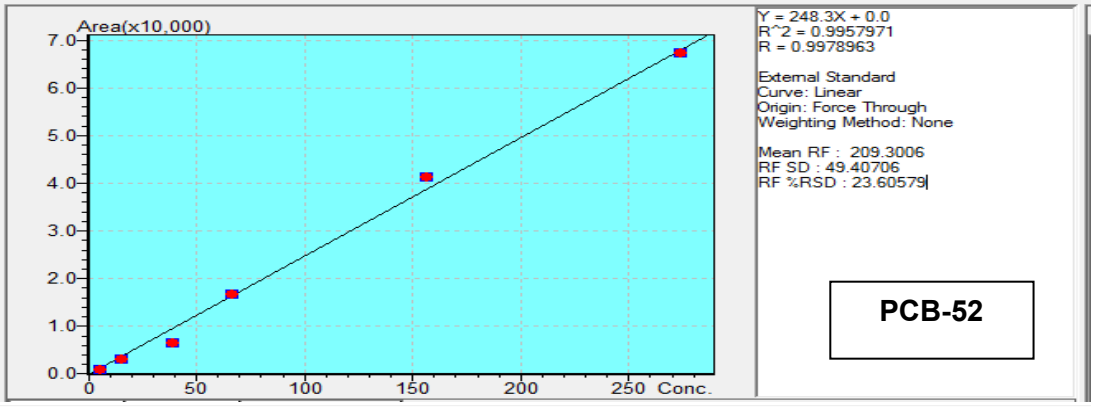

Area(x10,000)
1.00
0.75
0.50
0.25
0.00
0
100
200
300
400
Conc.
Y = 22.24759X + 0.0
R^2 = 0.9965778
R = 0.9982874
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 19.52716
RF SD : 2.538739
RF %RSD : 13.00106
Toxaphen palar 50

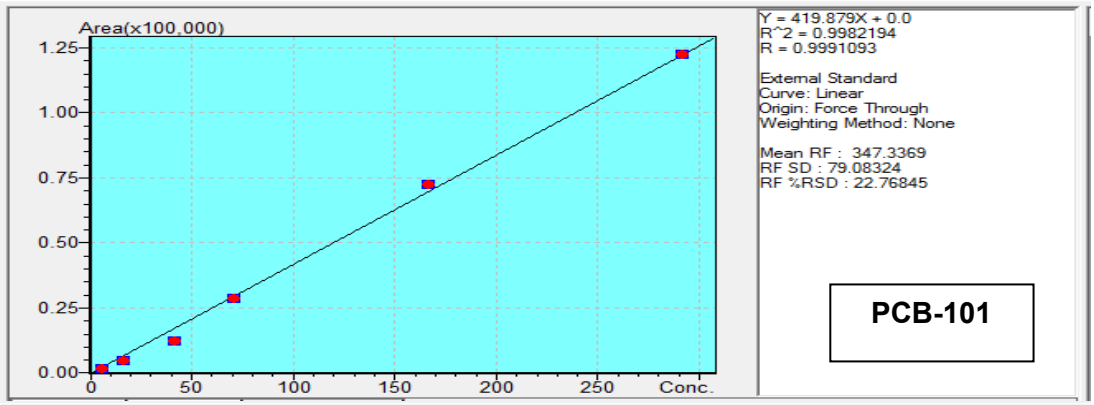

Area(x100)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
25.0
50.0
75.0
100.0
Conc.
Y = 3.097774X + 0.0
R^2 = 0.9951598
R = 0.997577
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 2.978887
RF SD : 0.3882945
RF %RSD : 13.03488
Toxaphen palar 62

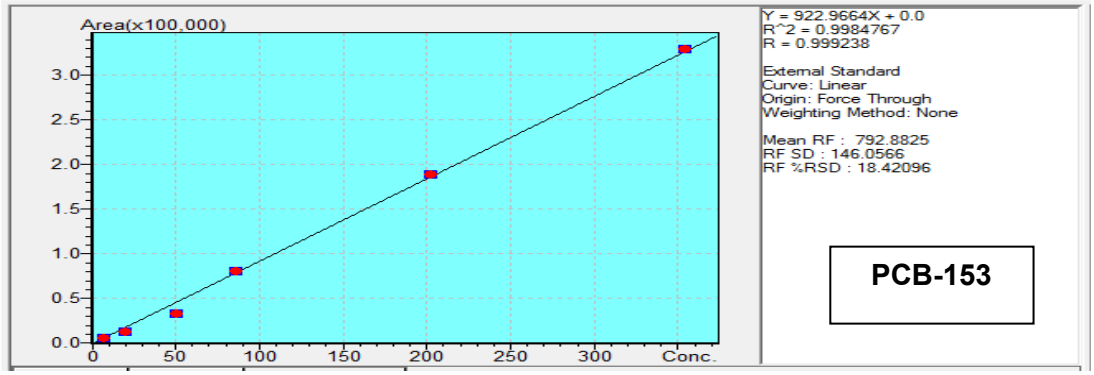

Area(x10,000)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
25.0
50.0
75.0
100.0
125.0
150.0
Conc.
Y = 189.9863X + 0.0
R^2 = 0.9984642
R = 0.9992318
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 185.3605
RF SD : 10.77548
RF %RSD : 5.813256
Mirex

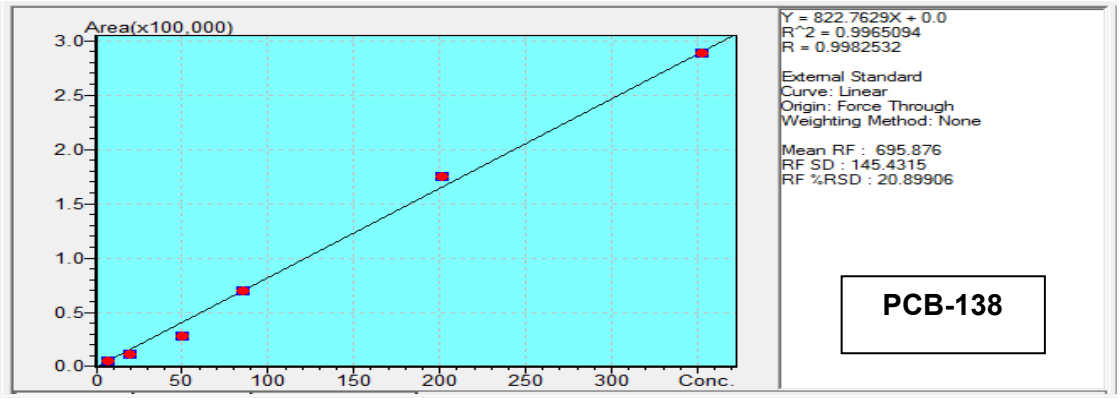
PCBs

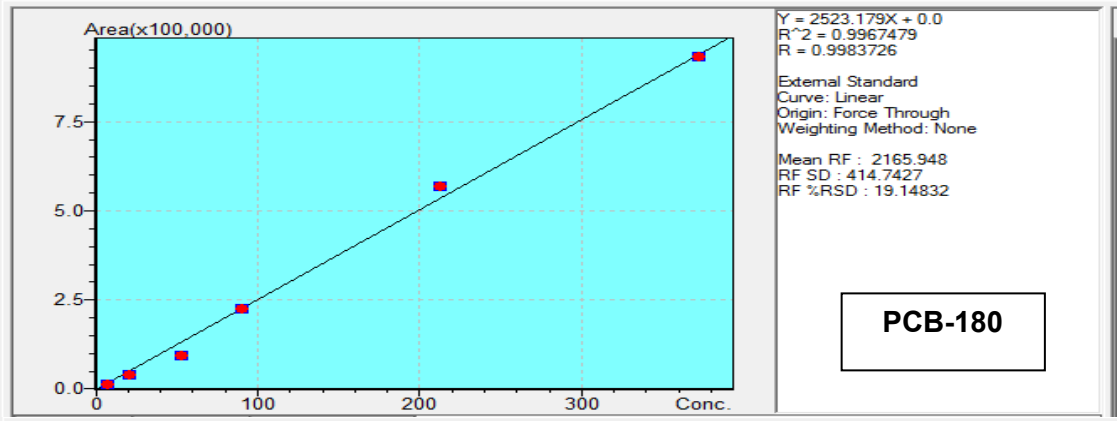

Area(x100,000)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
50
100
150
200
250
300
Conc.
Y = 578.8738X + 0.0
R^2 = 0.9979268
R = 0.9989629
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 499.68
RF SD : 93.16466
RF %RSD : 18.64486
PCB-28

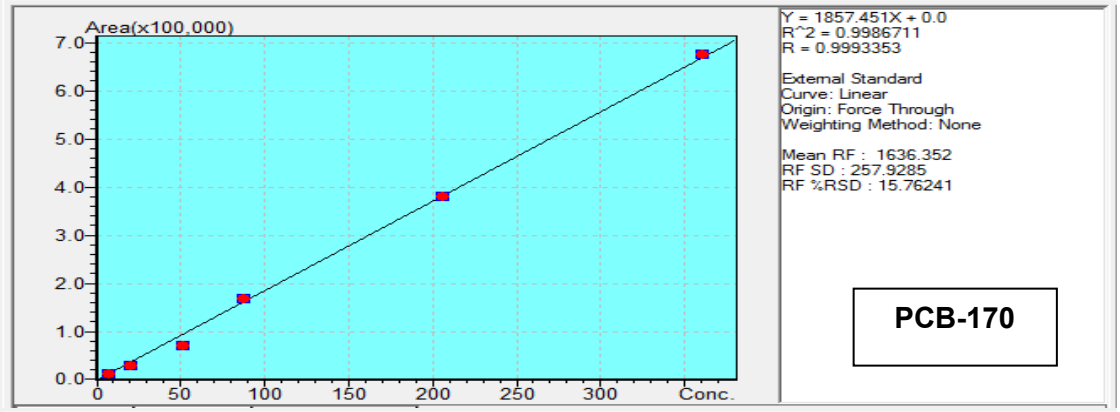

Area(x10,000)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
50
100
150
200
250
Conc.
Y = 248.3X + 0.0
R^2 = 0.9957971
R = 0.9978963
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 209.3006
RF SD : 49.40706
RF %RSD : 23.60579
PCB-52

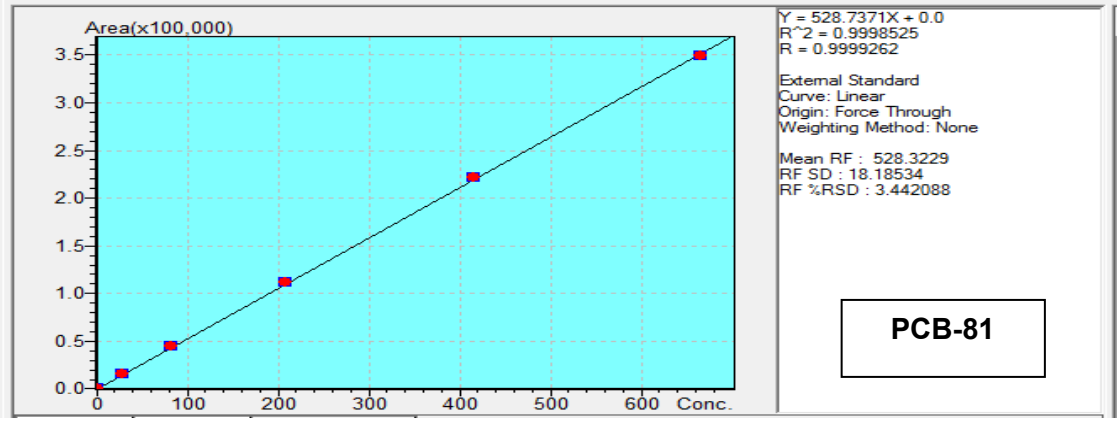

Area(x100,000)
1.25
1.00
0.75
0.50
0.25
0.00
0
50
100
150
200
250
Conc.
Y = 419.879X + 0.0
R^2 = 0.9982194
R = 0.9991093
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 347.3369
RF SD : 79.08324
RF %RSD : 22.76845
PCB-101

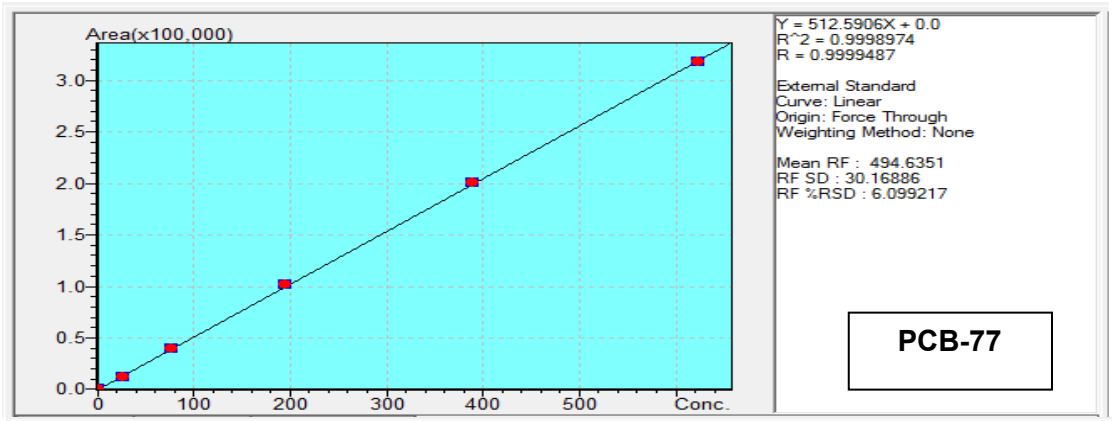

Area(x100,000)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
250
300
Conc.
Y = 922.9664X + 0.0
R^2 = 0.9984767
R = 0.999238
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 792.8825
RF SD : 146.0566
RF %RSD : 18.42096
PCB-153

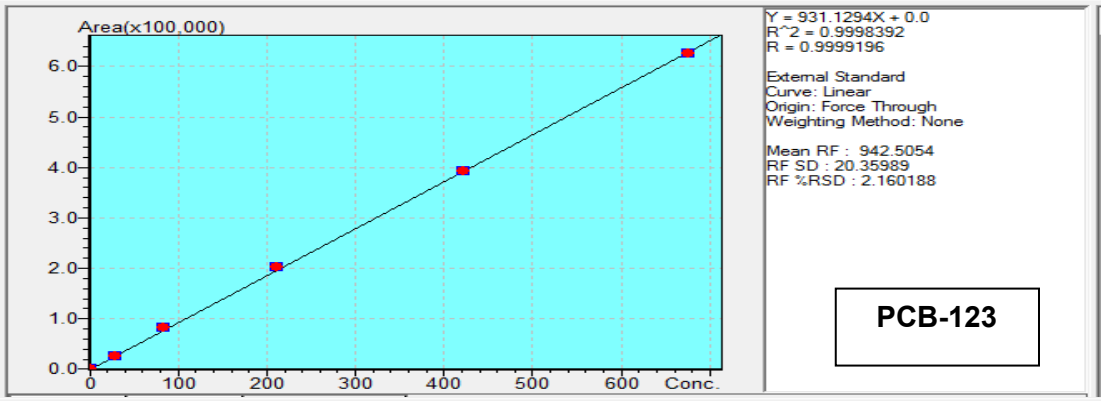

Area(x100,000)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
250
300
Conc.
Y = 822.7629X + 0.0
R^2 = 0.9965094
R = 0.9982532
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 695.876
RF SD : 145.4315
RF %RSD : 20.89906
PCB-138

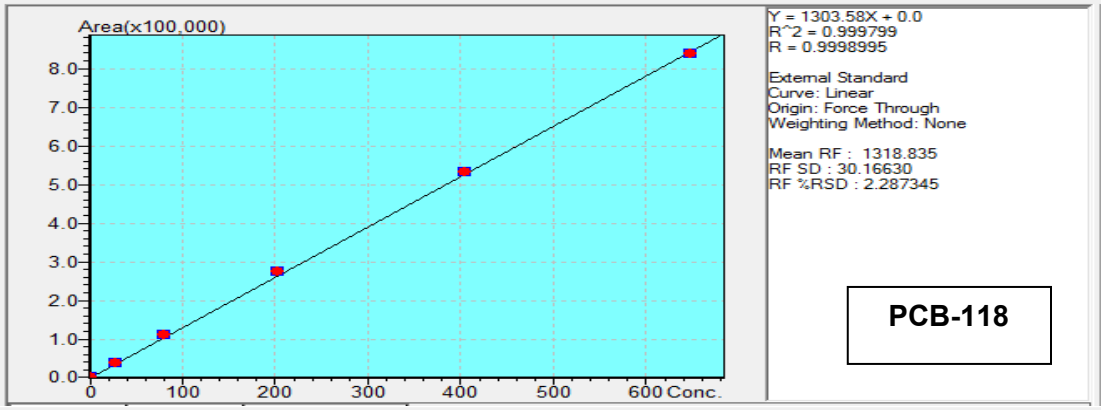

Area(x100,000)
7.5
5.0
2.5
0.0
0
100
200
300
Conc.
Y = 2523.179X + 0.0
R^2 = 0.9967479
R = 0.9983726
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 2165.948
RF SD : 414.7427
RF %RSD : 19.14832
PCB-180

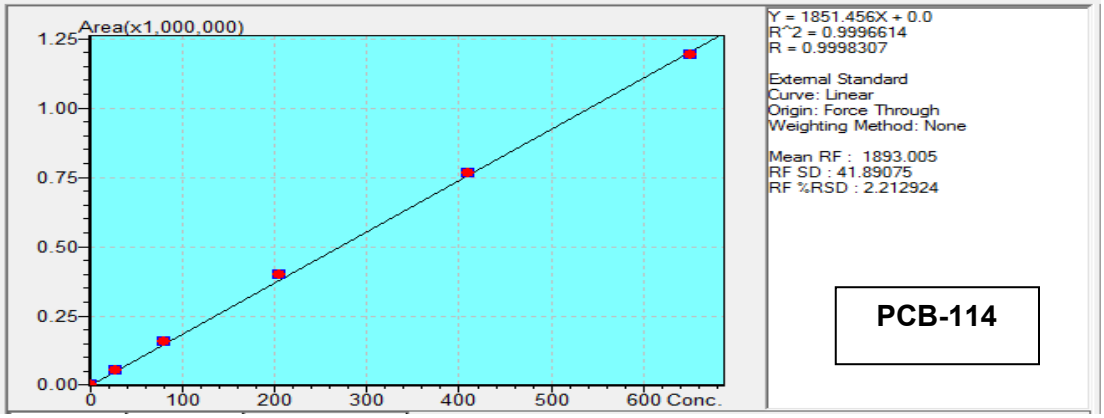

Area(x100,000)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
50
100
150
200
250
300
Conc.
Y = 1857.451X + 0.0
R^2 = 0.9986711
R = 0.9993353
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 1636.352
RF SD : 257.9285
RF %RSD : 15.76241
PCB-170

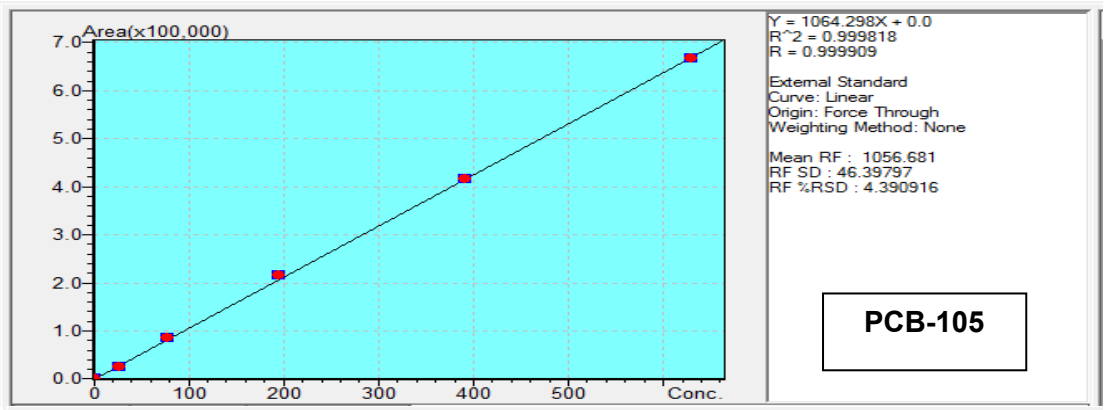

Area(x100,000)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
500
600
Conc.
Y = 528.7371X + 0.0
R^2 = 0.9998525
R = 0.9999262
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 528.3229
RF SD : 18.18534
RF %RSD : 3.442088
PCB-81

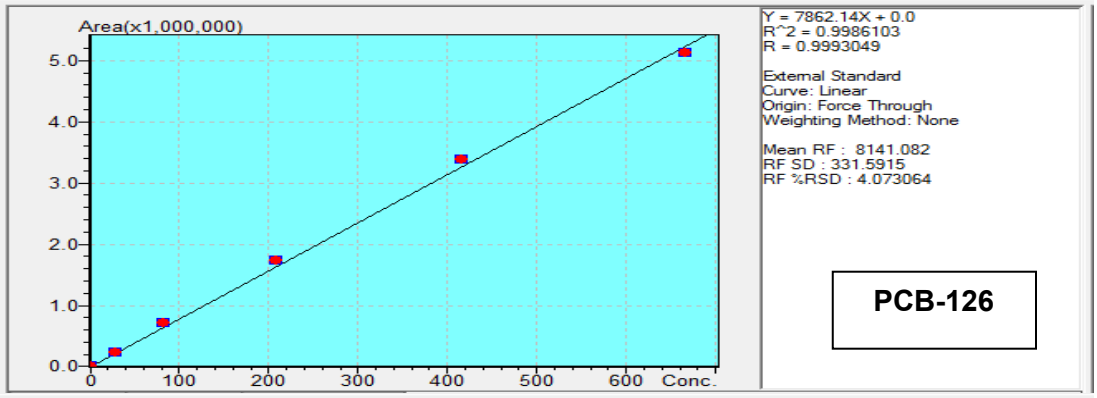

Area(x100,000)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
500
Conc.
Y = 512.5906X + 0.0
R^2 = 0.9998974
R = 0.9999487
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 494.6351
RF SD : 30.16886
RF %RSD : 6.099217
PCB-77

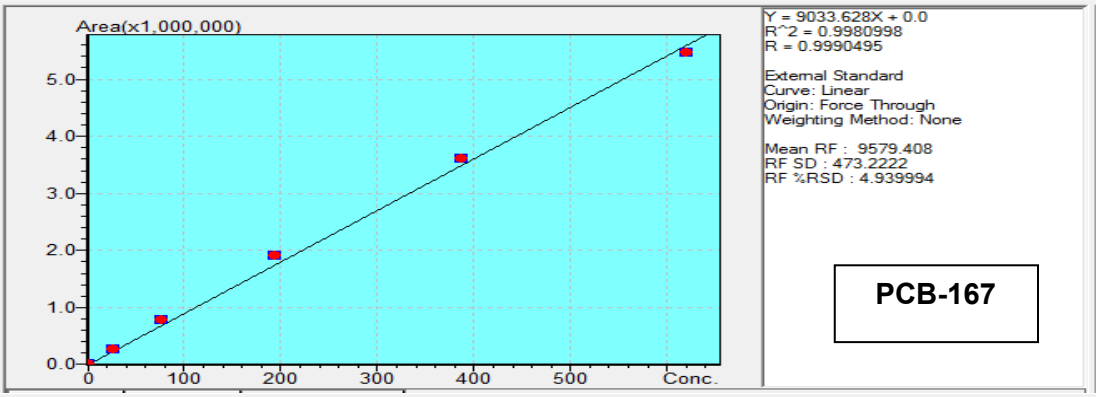

Area(x100,000)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
100
200
300
400
500
600
Conc.
Y = 931.1294X + 0.0
R^2 = 0.9998392
R = 0.9999196
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 942.5054
RF SD : 20.35989
RF %RSD : 2.160188
PCB-123

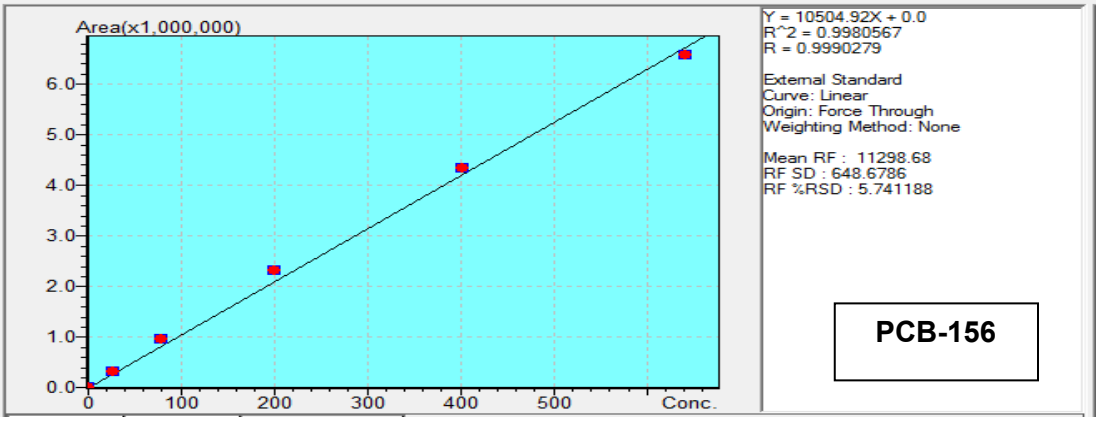

Area(x100,000)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
100
200
300
400
500
600
Conc.
Y = 1303.58X + 0.0
R^2 = 0.999799
R = 0.9998995
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 1318.835
RF SD : 30.16630
RF %RSD : 2.287345
PCB-118

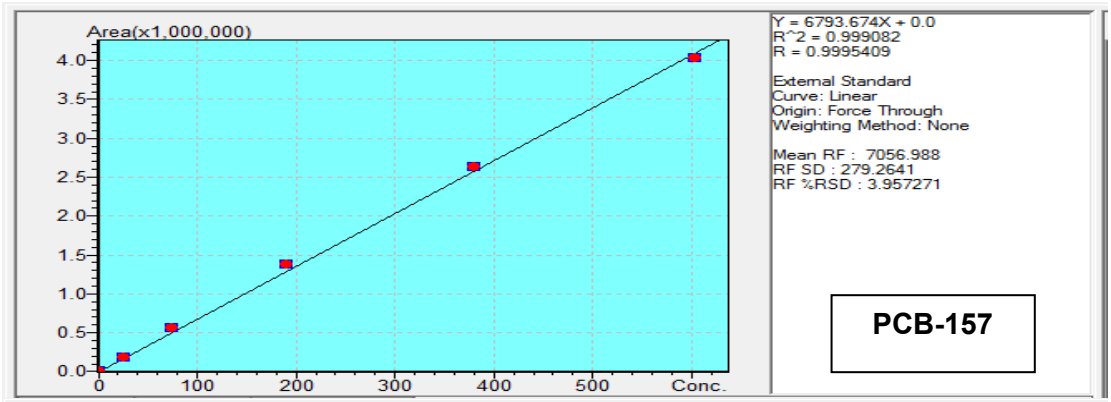

Area(x1,000,000)
1.25
1.00
0.75
0.50
0.25
0.00
0
100
200
300
400
500
600
Conc.
Y = 1851.456X + 0.0
R^2 = 0.9996614
R = 0.9998307
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 1893.005
RF SD : 41.89075
RF %RSD : 2.212924
PCB-114

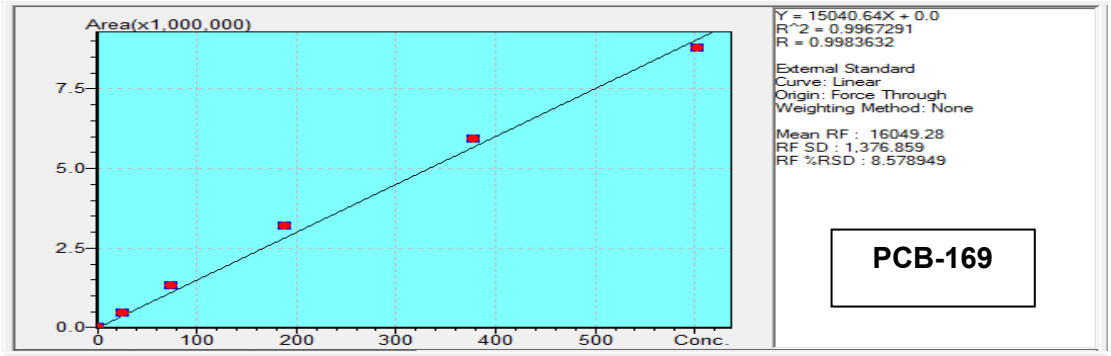

Area(x100,000)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
100
200
300
400
500
Conc.
Y = 1064.298X + 0.0
R^2 = 0.999818
R = 0.999909
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 1056.681
RF SD : 46.39797
RF %RSD : 4.390916
PCB-105

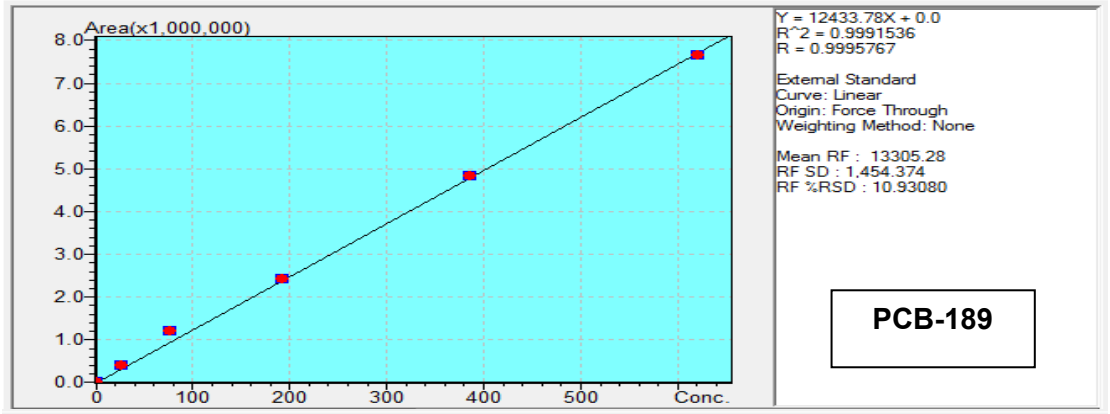

Area(x1,000,000)
5.0
4.0
3.0
2.0
1.0
0.0
0
100
200
300
400
500
600
Conc.
Y = 7862.14X + 0.0
R^2 = 0.9986103
R = 0.9993049
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 8141.082
RF SD : 331.5915
RF %RSD : 4.073064
PCB-126

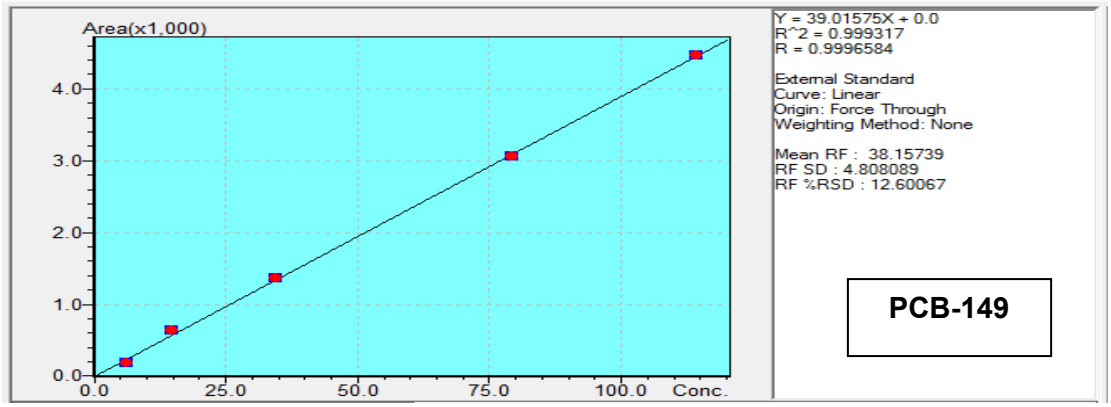

Area(x1,000,000)
5.0
4.0
3.0
2.0
1.0
0.0
0
100
200
300
400
500
Conc.
Y = 9033.628X + 0.0
R^2 = 0.9980998
R = 0.9990495
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 9579.408
RF SD : 473.2222
RF %RSD : 4.939994
PCB-167


Area(x1,000,000)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
100
200
300
400
500
Conc.
Y = 10504.92X + 0.0
R^2 = 0.9980567
R = 0.9990279
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 11298.68
RF SD : 648.6786
RF %RSD : 5.741188
PCB-156

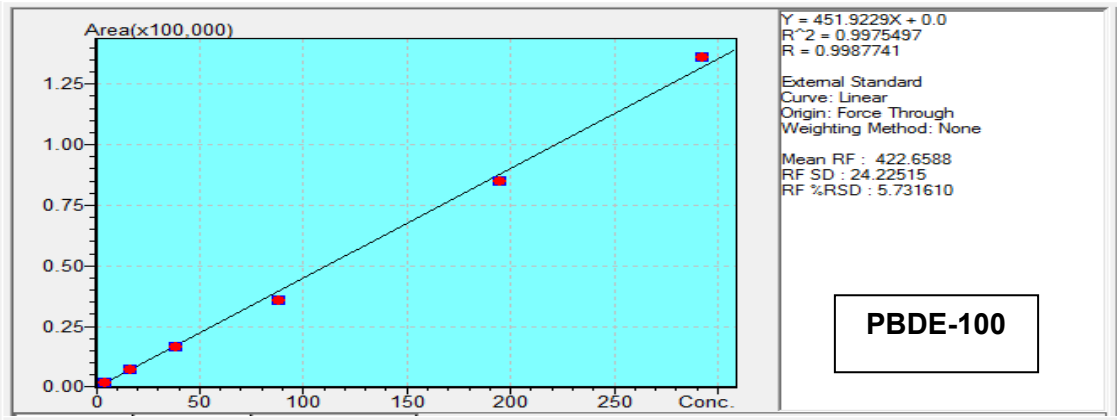

Area(x1,000,000)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
500
Conc.
Y = 6793.674X + 0.0
R^2 = 0.999082
R = 0.9995409
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 7056.988
RF SD : 279.2641
RF %RSD : 3.957271
PCB-157

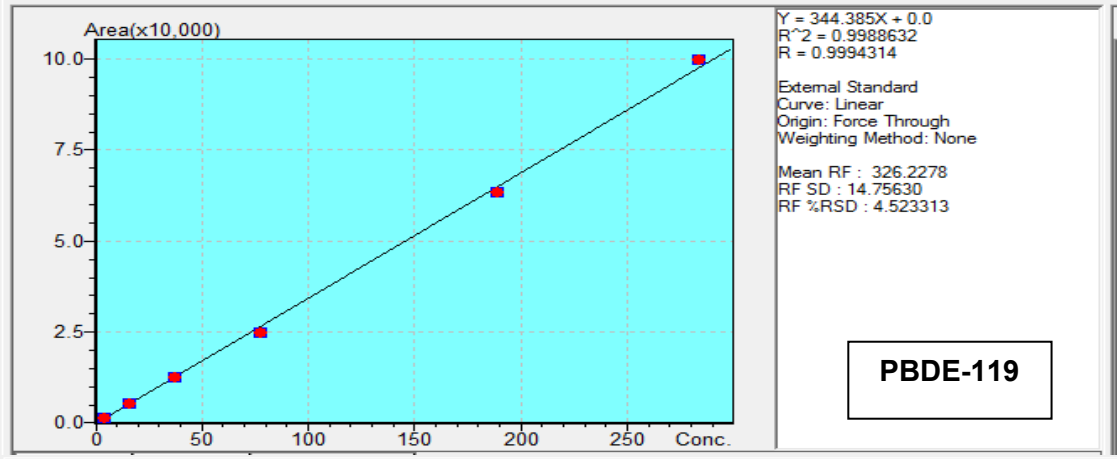

Area(x1,000,000)
7.5
5.0
2.5
0.0
0
100
200
300
400
500
Conc.
Y = 15040.64X + 0.0
R^2 = 0.9967291
R = 0.9983632
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 16049.28
RF SD : 1,376.859
RF %RSD : 8.578949
PCB-169

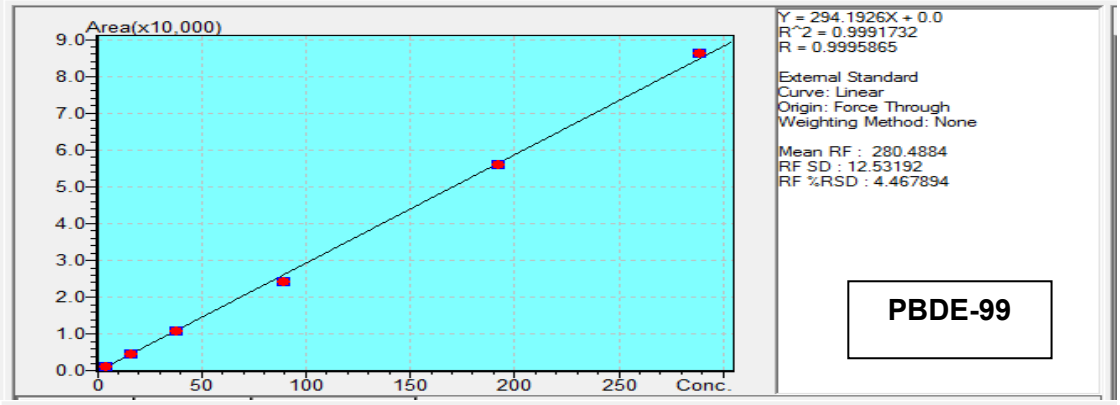

Area(x1,000,000)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
100
200
300
400
500
Conc.
Y = 12433.78X + 0.0
R^2 = 0.9991536
R = 0.9995767
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 13305.28
RF SD : 1,454.374
RF %RSD : 10.93080
PCB-189

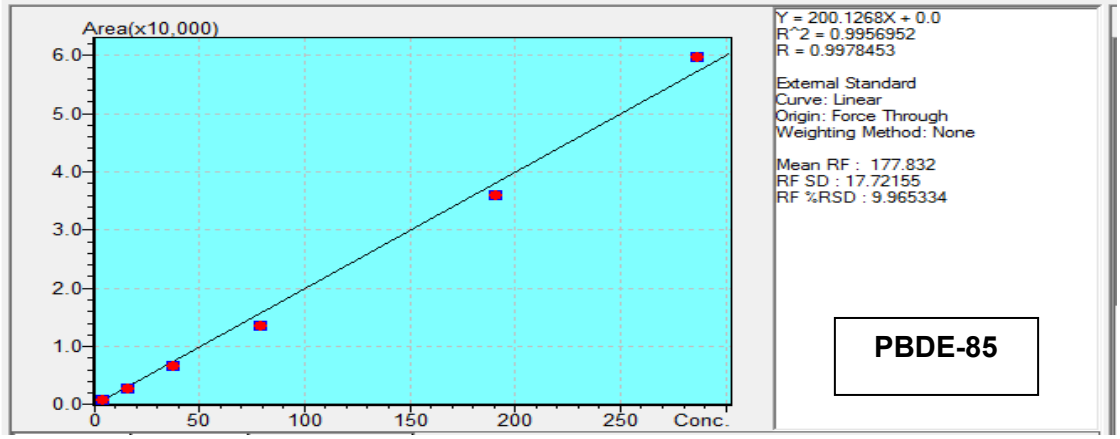

Area(x1,000)
4.0
3.0
2.0
1.0
0.0
0.0
25.0
50.0
75.0
100.0
Conc.
Y = 39.01575X + 0.0
R^2 = 0.999317
R = 0.9996584
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 38.15739
RF SD : 4.808089
RF %RSD : 12.60067
PCB-149

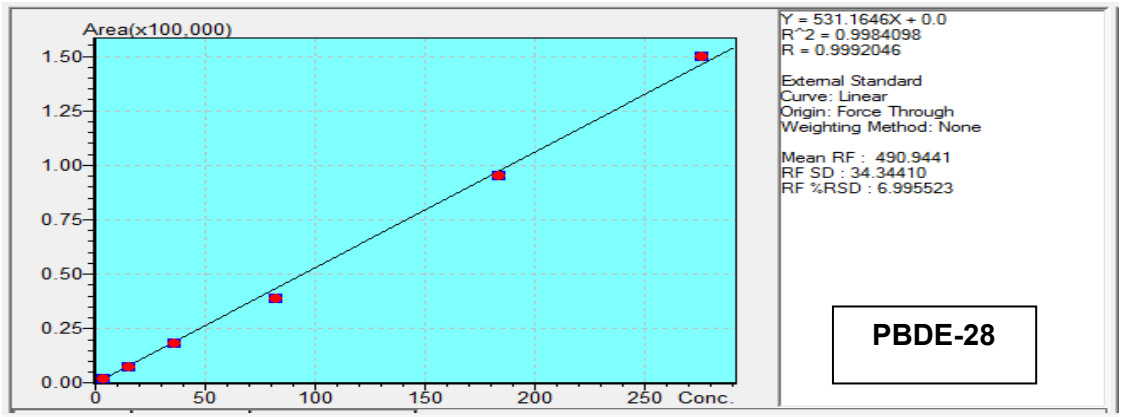
PBDEs

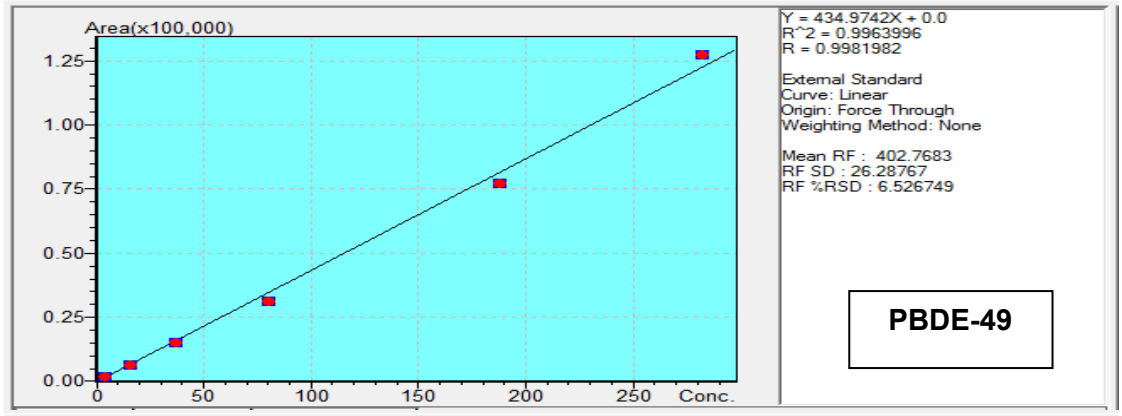

Area(x100,000)
1.25
1.00
0.75
0.50
0.25
0.00
0
50
100
150
200
250
Conc.
Y = 451.9229X + 0.0
R^2 = 0.9975497
R = 0.9987741
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 422.6588
RF SD : 24.22515
RF %RSD : 5.731610
PBDE-100

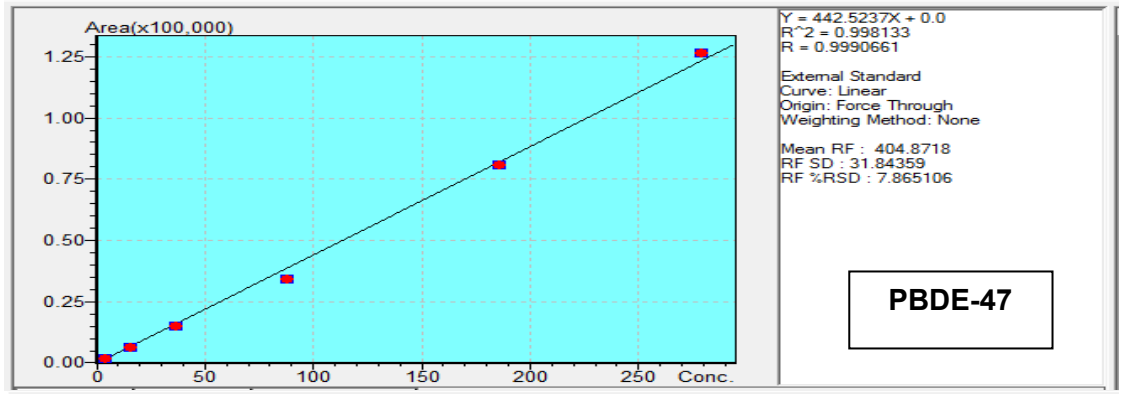

Area(x10,000)
10.0
7.5
5.0
2.5
0.0
0
50
100
150
200
250
Conc.
Y = 344.385X + 0.0
R^2 = 0.9988632
R = 0.9994314
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 326.2278
RF SD : 14.75630
RF %RSD : 4.523313
PBDE-119

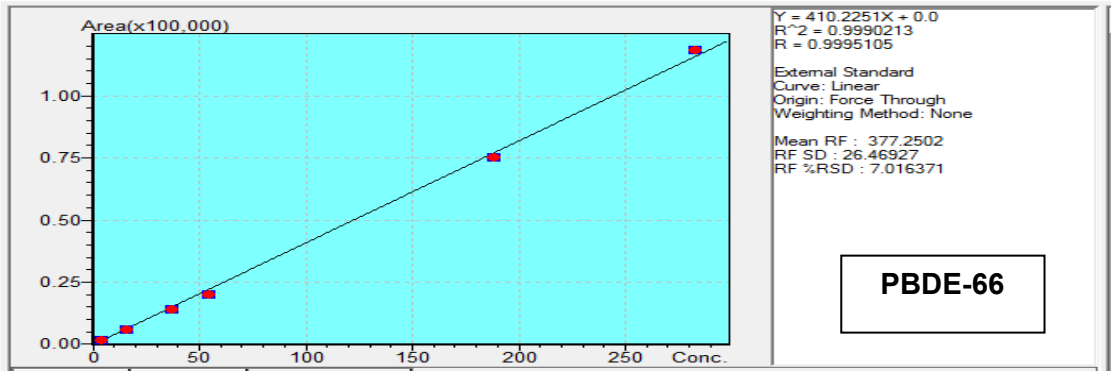

Area(x10,000)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
50
100
150
200
250
Conc.
Y = 294.1926X + 0.0
R^2 = 0.9991732
R = 0.9995865
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 280.4884
RF SD : 12.53192
RF %RSD : 4.467894
PBDE-99

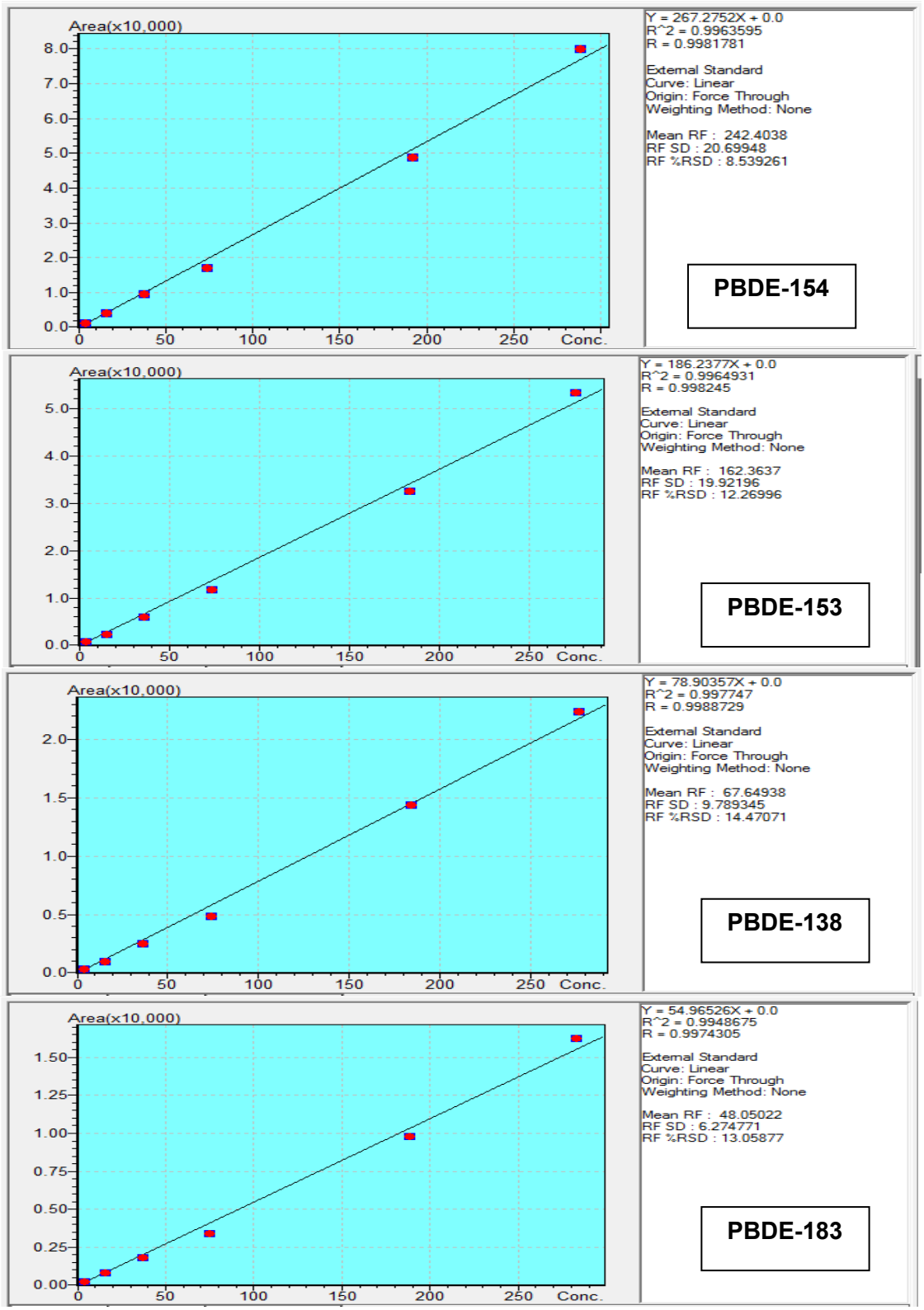

Area(x10,000)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
50
100
150
200
250
Conc.
Y = 200.1268X + 0.0
R^2 = 0.9956952
R = 0.9978453
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 177.832
RF SD : 17.72155
RF %RSD : 9.965334
PBDE-85


Area(x100,000)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
50
100
150
200
250
Conc.
Y = 531.1646X + 0.0
R^2 = 0.9984098
R = 0.9992046
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 490.9441
RF SD : 34.34410
RF %RSD : 6.995523
PBDE-28


Area(x100,000)
1.25
1.00
0.75
0.50
0.25
0.00
0
50
100
150
200
250
Conc.
Y = 434.9742X + 0.0
R^2 = 0.9963996
R = 0.9981982
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 402.7683
RF SD : 26.28767
RF %RSD : 6.526749
PBDE-49


Area(x100,000)
1.25
1.00
0.75
0.50
0.25
0.00
0
50
100
150
200
250
Conc.
Y = 442.5237X + 0.0
R^2 = 0.998133
R = 0.9990661
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 404.8718
RF SD : 31.84359
RF %RSD : 7.865106
PBDE-47


Area(x100,000)
1.00
0.75
0.50
0.25
0.00
0
50
100
150
200
250
Conc.
Y = 410.2251X + 0.0
R^2 = 0.9990213
R = 0.9995105
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 377.2502
RF SD : 26.46927
RF %RSD : 7.016371
PBDE-66


Area(x10,000)
Conc.
Y = 267.2752X + 0.0
R^2 = 0.9963595
R = 0.9981781
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 242.4038
RF SD : 20.69948
RF %RSD : 8.539261
PBDE-154
Y = 186.2377X + 0.0
R^2 = 0.9964931
R = 0.998245
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 162.3637
RF SD : 19.92196
RF %RSD : 12.26996
PBDE-153
Y = 78.90357X + 0.0
R^2 = 0.997747
R = 0.9988729
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 67.64938
RF SD : 9.789345
RF %RSD : 14.47071
PBDE-138
Y = 54.96526X + 0.0
R^2 = 0.9948675
R = 0.9974305
External Standard
Curve: Linear
Origin: Force Through
Weighting Method: None
Mean RF : 48.05022
RF SD : 6.274771
RF %RSD : 13.05877
PBDE-183

Annex-2

PTSs concentration in human breast milk of Hochiminh City residents (ng/g lipid wt.)

PTSs	01-I	02-I	03-I	04-I	05-I	06-I	07-I	08-I	09-I	10-I	11-I	12-I	13-I	14-I	15-I
Lipid (%)	1.3	3.7	1.8	1.5	3.8	2.1	2.4	2.5	2.3	0.7	1.8	4.1	1.2	1.3	1.9
PCBs	128.18	85.54	155.36	226.53	58.31	81.20	121.49	98.43	143.19	257.76	293.32	72.52	210.14	198.99	108.47
PBDEs	1.32	1.16	0.16	0.20	0.14	0.15	0.86	0.81	0.82	1.17	0.70	0.28	0.42	0.84	0.24
HCB	2.69	2.01	2.93	0.92	1.08	0.99	2.23	2.09	3.06	2.88	1.39	2.61	2.31	3.50	2.00
HCHs	19.09	8.53	20.62	6.35	2.71	9.87	14.49	6.13	7.46	14.76	13.76	29.06	5.62	21.05	6.28
Heptachlor	1.69	0.00	0.16	0.00	0.00	0.00	0.58	0.63	0.74	1.15	1.40	0.23	0.34	0.90	0.34
Hept Epox	2.27	0.37	0.44	0.85	0.21	0.45	1.78	1.10	1.73	1.51	0.85	0.59	0.53	1.78	0.87
Aldrin	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.70	0.00	0.00	0.00	0.23	0.00	0.00	0.00
Dieldrin	39.04	1.91	1.22	6.58	1.52	2.10	4.40	4.90	5.63	8.27	9.27	2.54	3.56	7.40	2.25
Endrin	2.81	0.00	0.00	0.00	0.00	0.00	0.97	1.31	1.30	1.26	3.29	0.33	0.00	1.67	0.00
EndAld	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chlordanes	17.41	2.07	5.09	5.96	1.84	2.53	9.45	3.54	9.82	7.12	3.85	2.52	2.83	7.65	5.27
Endosulfans	34.76	0.92	0.68	4.20	0.51	0.63	2.49	3.19	3.77	4.31	6.28	1.02	1.51	5.45	1.21
pp'-DDE	82.55	97.25	220.85	138.71	67.87	115.72	185.37	68.50	116.59	307.20	80.52	99.04	110.94	328.51	169.56
pp'-DDD	5.12	5.61	9.50	4.58	3.82	4.11	19.65	4.99	9.36	12.76	5.06	8.78	3.29	17.59	6.85
pp'-DDT	19.85	72.33	93.88	19.12	22.55	50.83	186.48	27.23	40.47	106.99	27.54	84.30	22.36	152.41	62.47
DDTs	107.52	175.19	324.22	162.41	94.24	170.66	391.50	100.72	166.43	426.95	113.13	192.12	136.59	498.51	238.87
Mirex	0.00	0.61	0.57	0.19	0.18	0.25	0.42	0.18	0.50	0.46	0.00	0.34	0.50	0.00	0.26
Tcxaphenes	11.22	1.96	4.02	2.44	1.89	1.11	2.06	2.38	3.28	5.33	2.03	2.92	2.24	5.43	3.79

16-I	17-I	18-I	19-I	20-I	21-I	22-I	23-I	24-I	25-I	26-I	27-I	28-I	29-I	30-I
4.5	4.6	1.7	3.7	3.4	1.7	2.2	2.1	4.3	4.5	1.6	5.3	4.3	2.4	2.5
337.83	44.04	214.84	142.71	213.14	202.33	56.85	75.35	45.03	45.97	64.69	11.45	36.83	225.59	80.64
1.70	20.02	3.77	4.27	2.68	5.59	1.52	0.37	0.27	0.28	3.93	0.02	0.04	0.19	0.15
2.55	0.21	1.31	1.01	1.19	0.76	0.24	2.32	0.60	0.11	1.14	0.12	0.18	1.13	0.47
19.09	6.87	30.90	47.23	34.63	18.24	34.19	43.20	10.81	20.42	24.72	5.70	1.76	10.01	4.09
0.00	0.00	0.19	0.22	0.13	0.28	10.20	17.42	6.30	8.33	11.79	2.74	2.29	10.59	6.12
0.23	1.34	1.46	1.52	0.51	1.42	4.20	3.35	7.40	1.77	10.72	0.26	4.80	30.21	10.43
0.00	0.00	0.00	0.00	0.96	0.00	5.59	8.84	2.85	4.81	6.08	1.17	0.86	12.82	2.50
4.35	7.88	14.57	19.71	13.45	33.63	7.07	14.80	4.44	8.61	7.79	1.94	1.63	12.13	3.51
0.00	0.42	1.36	1.00	0.00	1.06	5.71	19.74	2.30	10.76	5.91	2.78	0.00	0.16	0.71
0.00	0.00	0.00	0.00	0.00	0.00	15.03	23.68	7.10	14.82	13.99	1.53	2.38	8.40	2.53
5.04	4.49	7.27	10.84	6.03	13.63	11.21	21.49	15.64	15.39	10.44	1.87	6.56	35.00	13.50
0.47	7.11	17.77	24.31	6.91	47.26	25.54	41.57	11.55	63.62	27.19	3.87	10.63	23.16	8.31
291.60	113.16	238.38	239.54	353.18	276.83	39.37	29.43	25.48	39.22	40.25	13.91	15.88	26.34	41.78
2.38	4.56	8.21	13.44	4.84	17.26	7.03	14.18	8.96	5.52	17.49	1.77	3.07	2.35	5.11
104.73	33.56	59.67	106.20	86.51	230.42	6.59	18.37	4.02	3.12	35.76	3.58	2.36	6.66	2.84
398.71	151.28	306.26	359.17	444.53	524.51	53.00	61.98	38.46	47.86	93.50	19.25	21.31	35.35	49.73
0.00	0.00	0.38	0.83	1.37	10.26	8.16	18.35	2.86	6.30	2.21	0.98	1.06	6.37	1.44
1.47	0.65	0.87	1.21	1.03	0.85	3.29	6.10	9.53	2.84	2.78	1.39	0.41	4.37	3.62

31-I	32-I	33-I	34-I	35-I	36-I	37-I	38-I	39-I	40-I	41-I	42-I	43-I	44-I	45-I
2.4	1.3	2.0	2.0	1.7	2.5	3.1	1.5	1.9	1.7	2.4	2.4	4.2	2.1	1.4
99.52	237.84	85.31	146.78	199.34	182.93	10.00	334.57	155.99	45.42	13.70	62.31	35.01	42.75	69.83
0.75	0.42	0.19	0.21	0.14	0.15	0.10	0.65	0.13	0.04	0.13	0.08	0.13	0.04	0.03
0.86	2.88	0.44	0.85	1.27	0.47	0.92	1.99	1.70	0.42	0.54	0.80	0.23	0.32	0.34
5.89	9.61	2.73	9.33	14.50	1.98	17.56	19.46	14.99	7.48	2.94	5.39	2.52	6.16	11.46
10.67	13.77	6.61	10.05	14.99	8.71	13.27	18.37	50.95	38.25	9.65	10.92	4.86	16.13	15.12
13.74	33.89	19.82	19.91	31.34	31.88	1.75	45.53	17.44	2.99	1.90	9.47	4.75	3.50	8.37
2.72	16.78	0.58	15.91	24.18	0.90	25.49	55.00	56.93	1.81	3.07	4.82	1.52	4.93	11.59
4.81	12.64	6.37	7.86	10.14	10.29	0.94	15.20	6.26	2.00	0.80	3.68	2.29	2.43	4.45
0.30	0.28	0.00	0.09	11.41	0.00	0.00	0.18	1.64	2.25	0.96	3.06	2.05	2.49	4.54
4.04	8.18	2.14	18.70	10.07	30.53	8.62	18.98	54.68	1.97	11.12	21.02	13.10	10.23	30.78
15.02	37.19	22.89	22.74	33.59	33.64	4.91	49.96	17.76	10.23	7.50	15.28	7.78	13.80	19.93
14.05	18.38	0.00	19.36	28.45	52.22	11.66	4.40	45.65	16.35	4.55	10.65	7.62	7.98	24.04
34.31	40.08	26.84	39.29	52.60	33.45	30.59	59.74	14.32	76.69	69.07	58.44	42.30	62.92	74.90
6.11	1.85	4.79	9.27	13.16	13.14	2.74	11.94	9.32	3.04	1.31	7.29	4.30	3.77	5.98
3.65	6.74	2.22	7.57	6.89	4.87	4.36	4.62	4.78	6.33	6.44	2.27	1.64	2.76	2.46
44.07	48.67	33.85	56.13	72.65	51.46	37.69	76.31	28.43	86.05	76.82	68.00	48.23	69.45	83.33
3.21	7.40	3.24	5.76	14.42	5.06	0.92	11.53	6.87	1.73	0.37	0.22	0.88	0.33	0.96
2.01	5.60	3.31	3.58	12.08	3.04	5.57	7.03	1.63	1.17	0.21	1.44	0.97	2.17	3.88

PTSs	01-H	02-H	03-H	04-H	05-H	07-H	08-H	09-H	10-H	11-H	12-H
Lipid (%)	2.4	2	1.2	2.7	1.8	4.2	1.1	0.5	1.3	7.3	2.3
PCBs	52.91	116.72	194.06	101.97	34.30	36.79	58.22	82.12	65.52	43.70	72.80
PBDEs	0.41	0.10	5.17	0.00	0.89	0.14	0.45	1.81	0.33	0.07	0.53
HCB	0.36	1.67	1.57	1.44	0.78	0.33	0.70	1.59	0.67	0.03	0.15
HCHs	6.13	14.36	21.56	9.69	4.21	3.69	5.26	34.42	7.81	2.26	1.59
Heptachlor	13.91	20.86	35.58	17.67	16.66	4.94	26.73	63.89	20.57	3.14	5.76
Hept Epox	23.09	54.19	87.19	47.54	1.21	5.32	2.44	6.57	9.07	0.39	18.80
Aldrin	4.29	11.35	14.54	6.91	4.21	3.13	8.05	6.01	4.61	0.20	1.62
Dieldrin	7.35	14.97	24.22	12.55	1.17	2.17	3.37	9.18	7.61	0.41	5.43
Endrin	7.21	14.56	24.59	13.13	0.54	1.94	0.77	10.42	3.47	0.41	5.11
EndAld	48.76	115.01	177.10	89.09	3.47	13.12	8.54	17.42	35.24	3.52	1.61
Chlordanes	37.00	76.23	118.14	63.87	6.97	8.56	8.78	23.33	29.90	2.00	30.18
Endosulfans	34.93	5.04	9.67	20.18	9.15	4.59	20.99	31.32	12.42	1.89	0.00
pp'-DDE	28.87	37.41	55.08	29.96	49.44	23.90	84.78	188.89	154.77	13.95	19.93
pp'-DDD	4.52	18.30	30.14	13.96	1.56	1.95	1.30	7.82	7.97	2.47	6.61
pp'-DDT	14.20	8.23	6.63	5.12	6.39	4.24	3.20	17.85	3.77	3.61	2.27
DDTs	47.59	63.94	91.85	49.04	57.39	30.09	89.28	214.57	166.51	20.03	28.82
Mirex	3.01	7.07	13.58	7.27	0.89	0.41	1.45	2.60	1.42	0.15	0.42
Tcxaphenes	7.63	6.49	11.21	5.16	0.98	2.24	0.85	4.41	3.70	0.35	7.31

13-H	14-H	15-H	16-H	17-H	18-H	19-H	20-H	21-H	22-H	23-H	24-H
4.7	3.0	1.8	2.4	1.5	4.0	0.6	3.2	3.7	6.9	2.0	2.6
30.51	27.33	132.19	84.50	27.32	63.40	39.18	84.30	78.17	51.08	30.16	68.53
0.03	0.84	0.34	0.34	0.66	0.03	0.72	0.50	0.19	0.21	0.31	0.34
0.05	0.09	0.16	0.21	0.30	0.13	0.73	0.14	0.12	0.03	0.05	0.10
2.96	2.54	4.58	4.70	7.00	1.62	8.90	1.77	1.10	0.37	1.81	1.63
0.32	0.55	0.37	0.21	3.31	0.18	1.02	0.18	0.15	0.22	0.07	0.08
0.38	3.78	1.11	1.30	0.25	0.44	3.69	0.65	0.16	0.35	0.93	0.18
2.68	2.20	6.84	1.69	0.94	30.85	5.68	1.20	0.90	0.54	1.75	7.37
2.74	3.08	1.56	0.58	6.48	1.73	5.85	1.44	0.48	0.76	2.19	0.09
3.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.06	1.86	0.88	23.86	26.33	11.93	83.75	15.76	13.01	1.10	2.09	0.43
1.05	21.00	48.22	29.32	147.77	55.69	52.64	14.21	28.31	9.23	43.18	27.32
31.61	12.65	15.43	20.97	26.08	10.74	39.61	7.44	9.96	2.31	8.61	6.63
30.94	37.76	33.07	72.11	92.88	40.29	181.23	34.04	25.21	10.31	40.98	35.82
1.69	9.52	4.16	3.47	8.15	2.08	9.83	2.80	5.30	2.83	5.96	5.34
7.51	11.14	9.31	11.85	16.79	2.99	36.38	7.15	6.42	2.79	10.80	6.49
40.15	58.42	46.54	87.42	117.83	45.36	227.43	43.99	36.94	15.93	57.75	47.65
0.00	3.78	1.09	0.33	1.54	0.86	2.02	0.42	0.47	0.09	0.31	0.19
3.35	2.29	0.68	0.65	1.68	0.38	2.06	0.42	0.08	0.25	1.78	0.37

PTSs	02-B	03-B	04-B	05-B	06-B	07-B	08-B	09-B	10-B
Lipid (%)	1.0	1.9	3.3	3.2	1.9	2.6	1.6	1.8	1.9
PCBs	19.73	14.39	46.27	12.13	132.19	84.50	27.32	63.40	39.18
PBDEs	0.31	0.07	0.03	0.05	0.47	0.73	0.45	0.23	0.14
HCB	0.19	0.17	0.03	0.06	0.04	0.04	0.00	0.02	0.08
HCHs	3.36	2.01	0.72	0.96	2.29	2.12	1.22	3.67	1.38
Heptachlor	0.00	0.00	0.05	0.00	0.19	1.06	0.00	0.00	0.00
Hept Epox	2.02	1.54	0.04	0.53	5.88	1.15	2.80	0.84	1.84
Aldrin	1.91	2.39	0.70	1.67	3.19	8.67	3.19	2.80	4.59
Dieldrin	4.31	2.35	0.62	2.76	8.12	7.25	5.46	7.19	7.90
Endrin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EndAld	20.34	7.69	0.09	10.27	10.17	9.48	8.29	6.66	6.27
Chlordanes	25.81	29.26	6.62	37.87	39.38	6.95	44.12	52.85	45.05
Endosulfans	26.92	9.84	5.37	11.26	20.95	16.33	15.76	16.99	14.35
pp'-DDE	78.71	30.19	12.23	43.98	53.03	34.71	51.34	50.83	40.02
pp'-DDD	9.04	2.33	2.62	1.87	23.23	4.21	13.18	12.33	18.66
pp'-DDT	21.01	13.58	5.19	8.41	8.67	12.50	11.90	21.40	12.66
DDTs	108.77	46.10	20.04	54.26	84.92	51.42	76.41	84.56	71.33
Mirex	0.63	0.20	0.35	0.63	9.04	5.87	6.01	4.11	5.97
Tcxaphenes	1.20	0.67	0.15	1.01	1.55	1.51	0.98	0.80	1.13

11-B	12-B	13-B	14-B	15-B	16-B	17-B	18-B	19-B	20-B
2.0	1.2	1.0	2.4	1.6	2.0	1.8	2.0	1.2	2.0
84.30	45.08	24.10	13.81	32.43	108.19	41.06	46.69	420.88	303.25
0.04	0.00	0.06	0.07	0.31	0.18	0.04	0.03	1.98	0.11
0.08	0.00	0.00	0.05	0.00	0.02	0.17	0.02	0.56	0.22
0.35	1.33	0.99	1.54	4.40	6.73	6.40	5.00	18.24	18.97
0.89	0.00	0.00	0.25	1.42	0.00	1.66	3.33	16.72	14.08
0.78	0.30	0.29	0.06	2.17	2.73	2.92	5.45	52.49	39.26
0.36	0.94	1.28	0.24	1.43	20.92	2.02	1.05	2.78	16.26
0.56	4.23	0.00	2.73	2.83	9.55	4.88	2.50	32.61	24.64
0.00	0.00	0.00	0.00	0.00	0.00	8.52	4.87	33.88	0.00
1.47	6.95	1.12	7.88	7.27	4.23	11.19	11.84	37.14	26.29
7.60	4.49	12.13	5.46	3.04	2.83	24.14	5.94	74.62	57.70
7.83	31.15	8.83	4.24	19.23	16.60	13.04	17.82	34.02	28.75
33.24	103.53	31.79	13.79	42.52	30.72	30.41	24.48	93.35	67.96
1.38	8.93	4.64	2.81	5.83	29.85	4.68	3.22	41.95	25.71
9.26	15.22	22.19	9.68	13.76	14.43	5.57	5.68	32.02	18.05
43.88	127.69	58.62	26.28	62.10	75.00	40.66	33.38	167.32	111.72
0.53	4.95	0.28	1.05	4.26	11.88	2.10	2.09	13.23	7.74
0.80	0.77	3.74	0.82	0.96	2.37	1.16	0.26	6.83	4.81

CURRICULUM VITAE

Mr Ngoc Vinh NGUYEN

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Born: 30.08.1968; Vietnamese citizen

Married; 2 children.

Personal interests: sports (tennis, swimming & soccer), music, and travel.

PROFFESSIONAL EXPERIENCE

1997 up to date

Environmental Analytical Chemistry Researcher

Institute for Environment and Resources (IER); Vietnam National University – Ho Chi Minh City (VNU-HCMC), Vietnam

General duties:

- Sampling and analysing air, water, sediments and soil micro pollutants using gas chromatography, gas chromatography/mass spectrometry and high performance liquid chromatography
- Improvement of analytical methods for analysing organic micro pollutants such as Pesticides, PCB's, Chlorophenols etc.
- Environmental Quality Assessment
- Organisation and implementation of training courses on analytical techniques for external participants from research organisations in Vietnam

Specific assignments:

2002 -2007:

Monitoring of air pollution in Industrial and Export – Processing zones in Hochiminh city by Mobile Air Pollution Monitoring Station within the framework of the IER's cooperation project with Vietnam Environment Protection Agency - VEPA

1999 -2000:

Water and rice field soil sampling; analysis of pesticides in water and sediment; monitoring and creation of a sampling map; and reporting within the framework of the IER's cooperation project "Study of pesticides and heavy metals in water and sediment of the Saigon - Dongnai River" with the Laboratory of Environmental Chemistry and Ecotoxicology (CECOTOX), Swiss Federal Institute of Technology (EPFL)

1999-2000:

Analysis of pesticides in surface water and reporting (one part) for the "Natural resources management of the Dongnai river Basin" project of the Vietnam Ministry of Science, Technology and Environment (MOSTE)

1998-1999:

Water sampling; monitoring and creating of a sampling map for the project "Study of Ho Chi Minh City's domestic wastewater of the Service for Wastewater Discharge and

Treatment" of Ho Chi Minh City's Department of Science, Technology and Environment (DOSTE)

1998-1999:

Water and sediment sampling; analysis of pesticides in sediment; monitoring and creating of a sampling map; and reporting for HCMC-DOSTE's project entitled "Channel sediment dredging, transport and disposal; sediment treatment and reuse in Ho Chi Minh City".

1997-2000:

Sampling and analysing pesticides in surface water, data processing and reporting for MOSTE's Environmental Monitoring Program for Ho Chi Minh City and the Mekong Delta Area

1997 – 1998:

Identification of sampling sites; sampling of water and sediment; analysing and reporting within the framework of the IER's cooperation project "Assessment of organochloride levels in water and sediment in the basin of the Saigon - Dongnai River" with the CECOTOX, EPFL.

1992 – 1996:

LABORATORY RESEARCHER

Centre for Applied Chemistry, University of Natural Sciences - Ho Chi Minh city, VNU-HCMC

General duties:

- Management of the Organic Chemistry Laboratory
- Research of synthesis organic chemicals for specific applications in industrial and agricultural production
- Supervision of bachelor students in training in the lab

Specific assignments:

1995: Synthesized Cycocel, a plant growth regulator

1994: Participated in trial production project of chocolate from Vietnamese cocoa bean

1993: Extracted lecithin from soybean residue of vegetarian oil production.

EDUCATION

Dec. 2005 – until now: PhD Student, CEAL, ISTE, EPFL, Lausanne, Switzerland

- Majored in Environmental Analytical Chemistry
- Thesis title:" **Persistent Toxic Substances in Southern Vietnam, Development of Analytical Method, Bioaccumulation and Modelling**"

1994 – 1996: Master of Science in Chemistry, University of Natural Sciences, Vietnam National University - Ho Chi Minh City (VNU-HCMC)

- Majored in Organic Synthesis Chemistry
- Graduation Thesis "Synthesizing Cycocel, a Plant Growth Regulator, from soybean residue"

1987 – 1991: **Bachelor of Science in Chemistry, University of Natural Sciences, Vietnam National University - Ho Chi Minh City (VNU-HCMC)**

- Majored in Organic Synthesis Chemistry
- Seminar work: "Extraction of rotenone from the tree *Derris Eliptica*"

OTHER SKILLS

Skill	Level of Expertise
English	Advanced
French	Beginner
Vietnamese	Expert (mother tongue)
Microsoft Office (Word, Excel, PowerPoint)	Expert
Statistical Package For Social Sciences (SPSS), SIMCA-P	*Advanced*

PUBLICATIONS

- Mai T. A., Do H. L. C., Nguyen N. V., Tu T. C. L., Lam M. T., K. Becker-Van Slooten and J. Tarradellas, (**2003**). Micro pollutants in the Sediment of SaiGon – DongNai River: Situation and Ecological Risks. *Chimia,* 57, 537 – 541.
- Mai T. A., Nguyen N. V., Nguyen T. H., Lam M. T., J. J. Sauvain, J. Tarradellas, Preliminary evaluation of the contamination of organo chloride and phosphorous pesticides at SaiGon – DongNai valley. Workshop on *Management, Use and Assessment of Environmental Pollution of Pesticides, Hanoi Sept. 28 – 29, 2000*
- Current situation of Organo chloride pesticide level in water and sediment in the basin of Saigon – Dongnai river (Pilot Project, 1997 - 1998)
- Nguyen N. V., Lam M. T., 'Annual report: Environmental monitoring of HCMC and Mekong delta', HCMC, 1997 – 1999.
- Tran Kim Qui, Nguyen Ngoc Vinh, (**1999**). Synthesizing Plant Growth Regulator. *Journal of Chemistry,* 3, 1-2. National Centre for Natural Science and Technology of Vietnam.