SOURCES AND FATE OF PCDDs AND PCDFs IN RURAL AND URBAN ECOSYSTEMS AND FOOD CHAIN IN SOUTHERN VIETNAM

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ABSTRACT

Problems related to Dioxins contamination in Southern Vietnam are until now still a hot newsreel and controversy subject in international conferences as well as in many articles. The adverse effects of Dioxins residue from chemicals used by U.S. Army during the Vietnam War (Ranch Hand Operation 1961 to 1971) have caused many consequences not only to Vietnamese ecosystem and people but also to the U.S army veterans that participated in the war at that period as reported in many scientific reports.

In Vietnam, since 1980 many studies have been carried out in collaboration with overseas scientists and laboratories on Dioxin contamination levels as well as its influences on ecosystem and human health. However, regardless of many efforts, up to now the problem of Dioxin contamination and its consequences is not yet completely and appropriately solved. There is still relative high Dioxins residue in the areas named as “hot spot” in Southern Vietnam and its contamination is causing the adverse effects on local residents.

Nowadays, studies on this subject are relatively difficult to perform due to many factors: over 30 years passed; land use disturbance; degradation and transfer of dioxin into biological food web; population emigration; etc.. In addition, a lack of related documents and military secrets also contributed to this.

With the support of SDC in frame of a collaboration project between Vietnam and Switzerland, we have carried out the research named “Sources and Fate of PCDDs and PCDFs in rural and urban ecosystem and food chains of South-Vietnam”. Our research has examined integratelly the PCDD/Fs sources with special regard on the PCDD/Fs source from the war, but also consider the others possible sources such industrial and municipal combustions, agricultural used chemicals, etc.

The selected locations for our research have been set up based on collected document from Division 10-80 and Office 33 (two responsible organizations for Dioxins and related problems in Vietnam): CamLo District – QuangTri Province; DaNang City; MaDa Forest and BienHoa City (BienHoa Airforce Base and BienHung Lake) – DongNai Province; and industrial zone – Thu Duc – Hochiminh City. Seventeen 2,3,7,8-PCDD/Fs congeners have been chosen for our research due to their high toxicities. Soil, sediment, municipal waste incinerator (MWI) bottom ash, fish tissue, and human adipose are selected as the matrices to examine the PCDD/Fs residue.

The result showed that even after more than 30 years, the PCDD/Fs concentration based on i-TEQ value(especially for 2,3,7,8-TCDD) is still higher than guideline values in some countries: very high i-TEQ value in cultivate soil of CamLo district and DaNang City. For the area named “hot spot” such BienHoa Airforce Base and BienHung Lake, the i-TEQ value and 2,3,7,8-TCDD concentration in soil and sediment are superior than values proposed by Agency for Toxic Substance and Disease Registry (ATSDR), Canadian Council of Ministers of the Environment (CCME) and U.S Environmental Protection Agency (U.S. EPA. The PCDD/Fs concentration in fish tissue of BienHung Lake (catfish and snake-head) is superior in comparison with European Council (EC) standard. The dioxin contamination risk for local resident health is very high due to the lack of information and inconsiderable attention of responsible organizations about this problem.

As BienHoa was selected as a case-study for PCDD/Fs transfer in food chain, using statistic methods (cluster analysis and Principal Component Analysis - PCA), we have showed a high similarity of PCDD/Fs profiles pattern between BienHoa Airforce Base soils, BienHung Lake sediments and fish tissue.

The similarity in PCDD/Fs relative i-TEQ profiles between MWIs bottom ash and soil of industrial zone ThuDuc proves that industrial and municipal combustions are responsible sources for PCDD/Fs in the soil of such these zones. However in comparison with the sites contaminated by Agent Orange (A.O)/Dioxin, the PCDD/Fs contamination level in the industrial soil is lower and mainly dominated by PCDFs than PCDDs.

The result also showed comparables i-TEQ values in BienHoa residents with inhabitants of industrial countries, however Vietnam is only developing and agricultural country. Beside that, 2,3,7,8-TCDD has been detected in many tested cases. 2,3,7,8-TCDD is the most toxic compound and related to the A.O/Dioxins from the war, unfortunately we have not enough data to assess the relationship between A.O/Dioxin residue and these samples. To find out this relationship it is necessary an integrated large-scale investigation.

Result of our research serves as a base for set-up a reference laboratory for PCDD/Fs and dioxin-liked compounds research in the South of Vietnam. At present time, a small laboratory for PCDD/Fs analysis has been installed in IER and serves as a member of VietNam Dioxin Research Network.

RÉSUMÉ

Les problèmes liés à la contamination du Sud du Vietnam par les Dioxines sont jusq'aujourd'hui un sujet d'actualité et de polémique dans des conférences internationales et dans des scientifiques d'articles. Les effets des résidus de Dioxine présents dans les produits chimiques employés par l'armée des États-Unis pendant l'opération Ranch Hand (de 1961 à 1971) dans de la Guerre du Vietnam ont çu des conséquences nuisibles non seulement à l'écosystème vietnamien et aux habitants mais également aux vétérans de l'armée américaine que ont participé à la guerre.

Au Vietnam, depuis 1980 beaucoup d'études ont été effectués en collaboration avec les scientifiques et les laboratoires d'étranger à fin de mieux connaitre les niveaux de contamination de Dioxine et ses influences sur l'écosystème et la santé humaine. Cependant indépendamment de beaucoup d'efforts, jusqu'ici il n'est pas encore le complètement et convenablement résolution pour le problème de contamination par les Dioxiones et ses conséquences. Il reste encore des résidus des Dioxines dans les lieux appelés "le point chaud" au Sud du Vietnam et cette contamination provoque toujours des effets nuisibles sur les habitants des environs.

Aujourd'hui, les études à ce sujet sont relativement difficiles d'exécuter en raison de beaucoup de facteurs: plus 30 ans sont passés; perturbation dues à d'utilisation des sols; dégradation et transfert de dioxine dans la chaîne alimentaire; émigration de population; etc... En plus, un manque de documents et de secrets militaires également ont contribués à ceci.

Avec l'appui de la DDC et dans la cadre du projet de collaboration entre le Vietnam et la Suisse, nous avons effectué la recherche appelée "sources et destin des PCDDs et des PCDFs dans l'écosystème rural et urbain et les chaînes alimentaires du Sud-Vietnam". Notre travail a examiné les sources des PCDD/Fs, ayant donné plus d'importance aux sources de l'époque de la guerre, mais considéré également les autres sources possibles tels que combustions industrielles et municipales, produits chimiques utilisés dans l'agriculture, etc...

Les lieux choisis pour notre recherche proviennent du document rassemblé par la Division 10-80 et le Bureau 33 (deux organismes responsables pour la question Dioxines au Vietnam): CamLo district – QuangTri Province; DaNang Ville; MaDa forêt et BienHoa Ville (BienHoa Airforce Base et BienHung Lac) – DongNai Province; et zone industrielle - Thu Duc – Hochiminh Ville. 17 congeners 2,3,7,8-PCDD/Fs ont été choisis pour notre recherche due à leurs toxicités élevées en comparaison de d'autres. Des échantillons sol, sédiment, cendre inférieure des Incinérateurs de déchets municipaux, tissu de poissons, et graisse humaine sont choisis comme matrices pour examiner le résidu des PCDD/Fs.

Le résultat a prouvé que même après plus de 30 ans, la concentration de PCDD/Fs basée sur l'i-TEQ value (particulièrement pour 2,3,7,8-TCDD) est encore élévée et de passe les valeurs guides comme par exemple dans le sol cultivé des CamLo district et DaNang

Ville. Pour le secteur appelé "le point chaud" comme BienHoa Airforce Base et BienHung Lac, la valeur d'i-TEQ et la concentration de 2,3,7,8-TCDD dans sol et sédiment sont supérieures aux valeurs proposées par CCME (Canadian Council of Ministers of the Environment), ATSDR (Agency for Toxic Substance and Disease Registry) et U.S EPA (U.S Environmental Protection Agency). La concentration de PCDD/Fs dans le tissu de poissons du lac BienHung (poisson-chat et serpent-tête) est supérieure en comparaison avec la norme de la EC (European Council). Le risque de contamination en dioxin pour la santé des habitants locales est très haut dû au manque d'information et d'attention de la part des organismes responsables de ce problème.

BienHoa a été choisi comme cas-détudé pour le transfert de PCDD/Fs dans la chaîne alimentaire. En employant des méthodes statistique (analyse de faisceau et PCA), nous avons montré une similarité élevée basée sur les profils des PCDD/Fs entre les sols de BienHoa Airforce Base, les sédiments et le tissu de poissons de BienHung Lac. La similarité basée sur les profils relatifs d'i-TEQ des PCDD/Fs entre la cendre de MWIs et le sol de la zone industrielle ThuDuc prouve que des combustions industrielles et municipales sont les sources responsables pour PCDD/Fs contamination dans le sol de telles ces zones. Toutefois en comparaison avec les sites souillés par A.O/Dioxin, le niveau de contamination de PCDD/Fs dans le sol industriel plus ci-dessous et principalement est dominé par PCDFs que PCDDs.

Les résultats ont également montré une contamination comparable d'i-TEQ dans les résidants de BienHoa et aux des pays industriels. 2,3,7,8-TCDD a été détectée dans beaucoup de cas examinés. La 2,3,7,8-TCDD est le composé le plus toxique et lié à l'utisation de Agent Orange dans la guerre. Malheureusement nous avons pas assez de données pour évaluer le rapport entre le résidu d'A.O/Dioxin et ces échantillons. Pour découvrir ce rapport il est nécessaire une intégrée recherche à grande échelle.

Le résultat de notre recherche sert comme une base pour l'installation un laboratoire de référence pour PCDD/Fs dans le Sud du Vietnam. Actuellement, un petit laboratoire pour l'analyse de PCDD/Fs a été installé à IER et sert comme un membre de réseau de recherches de Dioxine du Vietnam.

ABBREVIATIONS

A.B	Agent Blue
A.O	Agent Orange
A.W	Agent White
ATSDR	Agency for Toxic Substance and Disease Registry
Carso	Laboratoire Sante Environnement Hygiene de Lyon
CCME	Canadian Council of Ministers of the Environment (CCME)
Cecotox	Laboratoire de chimie environnementanle et e'cotoxicologie – ISTE – ENAC – EPFL
CS	O-Chlorobenzalmalonitrile
d.w	dry weight
DCM	Dichlolromethane
Division10-80	
DL	Detection limit
EC	European Council
EI	Electron Impact
GPC	Gel-permeation chromatography
HPLC	
HRGC/HRMS	High resolution gas chromatography/high resolution mass spectrometry
HRGC/LRMS	High resolution gas chromatography/low resolution mass spectrometry
IARC	International Agency for Research on Cancer
IER	Institute for Environment and Resources, Vietnam National University of Hochiminh City (VNU of HCMC)
ISQG	Interim Sediment Quality Guideline (Canada)
MAYs	Mada Afforestation Yards
MWIs	Municipal Waste Incinerators
NCI	Negative Chemical Ionization
nd	non-detectable
OC	Organochlorine
Office33	
PCA	Principal Component Analysis
PCDDs	Polychlorinated dibenzo-p-dioxins
PCDFs	Polychlorinated dibenzofurans
PEL	Probable Effects Level
QA/QC	Quality Assurance/Quality Control
SIM	Selected ion monitoring
w.w	wet weight
WHO	World Health Organization

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INTRODUCTION

A. Context

Dioxin is the common name for polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), and is abbreviated as PCDD/Fs. Nowadays, it is more and more scientific evidence that dioxins can cause cancer and disrupt hormonal, reproductive and immune systems in people. The developing fetus and breast-feeding infants are particularly sensitive to the harmful effects of dioxins. Recent studies suggest that dioxins are also an "endocrine disrupter" – one of a number of toxic chemicals that interfere with our hormone systems by mimicking natural hormones and blocking or disrupting their normal action. Since it is has been referred to as “the most toxic man-made compound” and, therefore, has generated much concern over their potential health risks.

In the case of Southern Vietnam, during Vietnam War (Ranch Hand Operation from 1961 to 1971), the U.S Army has experimentally utilized an estimated amount of 11 million gallons (72 million litters) of defoliants, mainly Agent Orange (61%) and other herbicides such agents Blue, White and Purple. These chemicals were sprayed at high concentration, about 28 liters averaging 21 kg per hectare in term of active ingredients (*Cau, 2003*). The herbicidal attacks were inflicted upon some ten percent of South Vietnam’s total land area (some reports have suggested substantially higher values of 24-27 percent) with estimated quantity of 2,3,7,8-TCDD about 170 kilograms (*Westing, 2002*). Over 30 years has elapsed since the war’s formal conclusion in 1975, but it’s consequences on the environment and human health is still present and not yet completely solved.

Beside this formal source of PCDD/Fs as described above, dioxins and furans 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, drawing a big unexpected amount of toxic wastes, including the possible dioxin-contain wastes. 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. The agricultural boom has been accompanied by a huge increase in the use of pesticides. Although the use of chemicals does not affect the exportability of agricultural products, but the contaminants remain in the soil, affecting future crop yields. All of them are the additional source of PCDD/Fs and should be considered when doing the research on this subject.

B. Problem setting

These all possible sources of PCDD/Fs pose us a relatively complex problem. Vietnamese Government and related administrative organisations such Ministry of Science and Technology (MOST), Ministry of Resources and Environment (MORE), Ministry of Health (MOH) have supported for many research on dioxin domain. Two major areas of research should be addressed at: direct research on human health outcomes from exposure to dioxin and research on the environmental and ecological effects of dioxin and Agent Orange.

B.1 Areas of research to be developed

Priorities for Health Research

The primary concerns in Vietnam from prolonged exposure to dioxin are for reproductive and developmental disorders that may be occurring in the general population. The key areas for research in Vietnam include spontaneous abortions, miscarriage, premature birth, congenital malformations, endocrine disorders, neurological disorders, immunodeficiency, cancer, genetic damage and diabetes mellitus.

Priorities for Environmental Research

Dioxin contaminants from Agent Orange have persisted in the environment in Vietnam for over thirty years. In addition to a better understanding of outcomes of exposure, an improved understanding of residue levels and rates of migration of dioxin and other chemicals in the environment is needed. “Hot spots” containing high levels of dioxin in soil have been identified and others are presumed to exist but have yet to be located. Dioxin has migrated through soil and has been transported through natural processes such as wind-blown dust and erosion into the aquatic environment. Contamination of soil and sediments provides a reservoir source of dioxin for direct and indirect exposure pathways for humans and wildlife. Movement of dioxin through the food web results in bio-concentration and bio-magnification with potential ecological impacts and continuing human exposure.

Research is needed to develop approaches for more rapid and less expensive screening of dioxin residue levels in soil, sediment, and biological samples which can be applied in Vietnam. *Improvements in this first step of analysis should be complemented by efforts to upgrade capabilities of laboratory facilities and equipment to international standards required by the research needs*. These improved analytical capabilities can then be used to more readily determine locations of highly contaminated areas, monitor remediation and understand migration of dioxin in the natural environment. *Monitoring efforts need to be linked to modeling efforts to understand fate and transport of dioxin in the environment*. Innovative and cost-effective approaches to environmental remediation for application in Vietnam need to be developed, tested and applied. The is still the need for high quality research, development, and capacity building in these areas as a means to identify, characterize and mitigate dioxin ecological impacts and bridge knowledge gaps regarding human exposure both in the past and into the future. Coordination between health and environmental efforts will be necessary to achieve success in the efforts described above.

Two areas of research should be further developed: ecological and restoration research on a degraded upland forest; and research on the identification, characterization and remediation of hot spots.

*** The contribution aspects of the thesis to National Dioxin Research Plan (NDRP)**

The thesis will positively contribute to the NDRP: provide a general regard to many possible sources of Dioxin in Southern Vietnam. In other hand, the thesis examined and sets up a suitable and effective analytical method, according to the local conditions (to save the time, money, labors, etc.)

Through the obtained results, we have shown some clearer relationships between the PCDD/Fs sources as well as their transfer into environment and food chain. They are useful basic to orient the monitoring programs for PCDD/Fs in the future.

In the end, the result of the thesis is very useful for IER (Institute for Environment and Resources – Vietnam National University of Hochiminh City) to develop its own skill in scientific studies to participate on National Dioxin Research Plan and help to find a best solution for Dioxin contamination problem and its consequences in the Southern Vietnam.

B.2 Aim and structure of the thesis

This research was set up with the main objectives as presented below:

1. To identify clearly the different sources of PCDDs and PCDFs (PCDD/Fs) that still pollutes the environment in South Vietnam. The prior sources could be, for example: remaining traces of Agent Orange from the VN-US war, by-products residue of the organochloride pesticide use (chlordane, toxaphene), emissions from industries and incineration processes, etc.

2. To balance the importance of these different sources according to the socioeconomic activities (for instance: agriculture in/out areas affected by the war, urban areas with and without industries, etc) – To define the principal contaminated PCDDs/Fs sources.

3. To follow the transfer of PCDD/Fs from emission sources or polluted compartments into the food chain to man – to assess the ecosystem and human risks (acute and chronic effects)

Generally, these objectives correspond to the Viet Nam National Dioxin Research Plan and they satisfy the purposes of our collaboration project between CECOTOX and IER: to help IER to develop themselves their capacity in scientific research (familiarize with the new modern analytical techniques – GC/MS; and integrate and solve a completed problem such dioxin contamination). By the research we installed a laboratory for dioxin analysis in IER and we desire to develop our laboratory to be a reference laboratory for Dioxin/POP analysis in the Southern Vietnam.

The thesis is divided into 9 main chapters, first chapter – introduction presents the problems related to the PCDD/Fs contamination in Vietnam, aims of work and our methodology. Chapter I focused in general properties of PCDD/Fs that could be interested for our analytical procedure as well as for interpreting of obtained result. Chapter II showed a summary of previous studies on PCDD/Fs contamination in Vietnam from 1980 to 2000. 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 PCDD/Fs analysis for future reference laboratory. Chapter IV presents the analytical procedures for all types of samples, including soil, sediment, fly ash and biological sample. The result of methods validation by SRM is also showed in this chapter. Chapter V, VI, and VII present the analyzed result (PCDD/Fs concentration) in all tested samples and their comparison with guideline values. Chapter VIII shows the pattern of PCDD/Fs in our samples by using multivariate analysis techniques (cluster analysis and PCA). Based on PCDD/Fs relative and i-TEQ profiles we defined the sources of PCDD/Fs contamination in selected areas. Finally, some conclusions and suggestions have been shown in the end section. The experimental protocols and other supportive information for this work could find in Appendix.

C. Methodological frame work

Because our research is an experimental work, here we present the methodology for the research by fig. C.1.

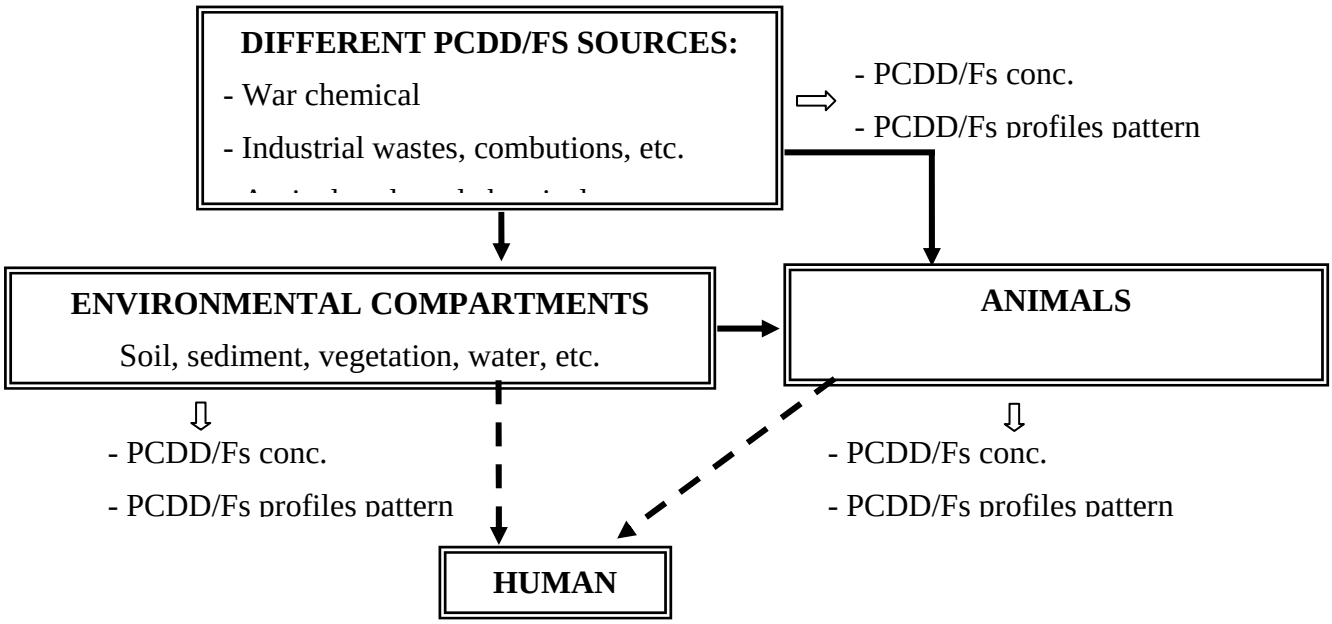

Fig. C.1 The PCDD/Fs cycle in nature

⟶Direct interaction

---->Indirect interaction

By comparison of PCDD/Fs concentration in sources, environmental compositions, animals, human body, etc. we can determinate the PCDD/Fs contamination level and assess their transfer from source to intermediate matrices and in the end to human. The PCDD/Fs profiles pattern together with additional data serve as raw data for identify the origin of PCDD/Fs principal sources by applying the statistic models.

The research steps and influence factor present it the table below: with the works (vertical direction from up to down) and influence factor (horizontal direction from left to right).

		WORKS
INFLUENCE FACTORS ⇨	- Lack of document and completed formalities	Define the selected zones and sample types for our research
	- Limit of time and budget - Limit of analytical equipment capacities	Set up the analytical methods – number of samples
	- Permit and formalities for sampling	Sampling
	- Lack of row data (concerning the diseases related to A.O/Dioxin) - Software for data treatment	Data treatment - interpreting – define PCDD/Fs principal sources

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CHAPTER I: PRINCIPAL PROPERTIES OF PCDD/Fs

1.1 Nomenclature

The polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are two series of almost planar trycyclic aromatic compounds with very similar chemical properties (*Figure 1.1*). Chlorine atoms can be attached to 8 different places on the molecule, numbered from 1 to 8. The term isomers refer to comparisons between compounds with the same empirical formula. Since we have 75 dioxins and 135 furans as presented in the Table 1.1.

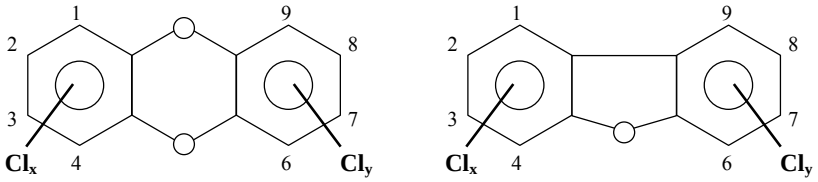

Fig. 1.1 Structural formula of PCDDs and PCDFs

These compounds differ from one another primarily by the location and number of chlorine atoms on the molecule, and their degree of toxicity varies greatly. The more chlorine atoms that are on the molecule, the lower the toxicity. The term "dioxin" is also used to refer specifically to 2,3,7,8-tetrachlorodibenzo-p-dioxin (commonly abbreviated as 2,3,7,8-TCDD, or just TCDD), the most toxic and thoroughly researched and widely publicized form of dioxins and furans. Dioxin-like compounds like polychlorinated biphenyls (PCBs) will be more and more considered together with dioxins in the future.

Table 1.1 Number of PCDD and PCDF isomers

Number of chlorine atoms	*Number of PCDD isomers*	*Number of PCDF isomers*
1	2	4
2	10	16
3	14	28
4	22	28
5	14	38
6	10	16
7	2	4
8	1	1
Total	***75***	***135***

(*Source: IPCS, 1989*)

1.2 Physical and chemical properties

The physical and chemical properties of dioxin compounds influent their availability, toxicity, fate and also the analytical methods. Knowledge of physical and chemical properties is essential to understanding and modeling the environmental transport and transformation of organic compounds such as the dioxin-like compounds. 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 PCDD/Fs, including water solubility (WS), vapor pressure (VP), octanol/water partition coefficient (K_{ow}), organic carbon partition coefficient (K_{oc}), and photolysis.

1.2.1 Solubility of PCDD/Fs

PCDD/Fs, particularly the higher chlorinated, are poorly soluble in water. They are capable to adsorb strongly to particles and deposit into sediment layer in aquatic – sink for PCDD/Fs. Thus, PCDD/Fs can hardly be identified in water. Not only for water, PCDD/Fs are also slightly soluble in most solvents. Tables 1.2 and 1.3 present the solubility of some PCDDs in organic solvents and water

Table 1.2 Solubility of 2,3,7,8-TCDD in various solvents

Solvent	*Solubility at 25 ^oC*	
	g/L	g/kg
O-Dichlorobenzene	1.8	1.4
Chlorobenzene	0.8	0.72
Pechloroethylene	0.68	0.48
Chloroform	0.55	0.37
Benzene	0.47	0.57
Acetone	0.09	0.11
Dimethylsulfoxide	< 0.1	< 0.1
Methanol	0.01	0.01
Water	2×10^{-7}	2×10^{-7}

(*Source: IPCS, 1989*)

Table 1.3 Water solubility of PCDDs

Compound	*Water solubility (g/L)*	
	20.0 ^oC	40.0 ^oC
1,3,6,8-TCDD	$(3.2\pm0.2) \times 10^{-7}$	$(3.9\pm0.4) \times 10^{-7}$
1,2,3,7-TCDD	$(4.3\pm0.1) \times 10^{-7}$	$(12.7\pm0.8) \times 10^{-7}$
1,2,3,4,7-PeCDD	$(1.2\pm0.1) \times 10^{-7}$	$(4.6\pm0.1) \times 10^{-7}$
1,2,3,4,7,8-HxCDD	$(4.4\pm0.1) \times 10^{-7}$	$(19.0\pm0.1) \times 10^{-7}$
1,2,3,4,6,7,8-HpCDD	$(2.4\pm0.3) \times 10^{-7}$	$(6.3\pm0.2) \times 10^{-7}$
OCDD	$(0.4\pm0.1) \times 10^{-7}$	$(2.0\pm0.2) \times 10^{-7}$

(*Source: IPCS, 1989*)

1.2.2 Vapor pressure

Vapor pressures are important parameters for the modeling of the environmental fate and incineration behavior of PCDD/Fs. Very few measured vapor pressure values are available in the literature for the PCDD/Fs, but the majority of the measured vapor pressures are for the 2,3,7,8-substituted congeners.

Vapor pressure can be used to assess the distribution of a chemical among air, air particles, water, soil and plants. It can be classified as solid vapor pressure (P_S) and (subcooled) liquid vapor pressure (P_L). P_L is of particular importance since it can well characterize the behavior of pollutants in the real environment.

Table 1.4 presents the P_S values of some PCDD/Fs at 25°C as well as some physico-chemical parameters such melting points T_m, ethalpie of vaporization (ΔH_{vap} at T_m), and change in molar heat capacities for the vaporization reaction (Δc_p).

Table 1.4 PCDD/Fs vapor pressure at 25°C

Compound	*Log p_S^o (Torr)*	*T_m (K)*	*ΔH_{vap} (kJ/mol^{-1})*	*Δc_p (J/mol^{-1}K^{-1})*
2,8-DiCDD	-4.95(±0.05)	424	84.1	-104.5
1,2,4-TriCDD	-5.42(±0.08)	402	83.9	-100.0
1,2,3,4-TCDD	-6.77(±0.11)	463	85.6	-95.6
2,3,7,8-TCDD	-8.38(±0.16)	579	79.9	-95.6
1,2,4,7,8-PeCDD	-7.71(±0.08)	479	91.8	-91.2
1,2,3,7,8-PeCDD	-8.00(±0.38)	514	88.7	-91.3
2,8-DiCDF	-5.10(±0.08)	458	74.2	-78.7
2,4,6-TriCDF	-5.16(±0.05)	390	84.2	-74.3
2,3,8-TriCDF	-5.73(±0.07)	464	78.8	-74.3
1,3,7,8-TCDF	-6.58(±0.09)	NA	NA	-69.8
2,3,7,8-TCDD	-6.90(±0.13)	501	80.3	-69.8
1,2,3,7,8-PeCDF	-8.09(±0.10)	500	83.8	-65.4
2,3,4,7,8-PeCDF	-7.31(±0.07)	470	85.8	-65.4

Note: NA=not available due to lack of melting point data (*Source: Mader and Pankow, 2003*)

The P_L is calculated based on the well-known Kirchoff equation:

$$\log p_L^0(T) = A - B/T + C\log T$$

with

$$A = \log p_L^0(298.15) + \frac{\Delta H_{vap}(298.15) - \Delta c_p.298.15}{2.303R.298.15} - \frac{\Delta c_p}{R}\log 298.15$$

$$B = \frac{\Delta H_{vap}(298.15) - \Delta c_p.298.15}{2.303R}$$

The values of A, B, and C for 17 2,3,7,8-PCDD/Fs are listed in the table 1.5 below:

Table 1.5 Parameter for the calculation of p_L^0 (Torr) for PCDD/F congener as a function of temperature by the Kirchoff equation

Congener	*A*	*B*	*C*
2378-TCDD	46.89	7061	-11.499
12378-PeCDD	44.87	7079	-10.971
123478-HxCDD	42.99	7132	-10.437
123678-HxCDD	42.96	7132	-10.437
123789-HxCDD	42.90	7130	-10.437
1234678-HpCDD	40.88	7142	-9.904
OCDD	38.26	6985	-9.370
2378-TCDF	35.91	6019	-8.399
12378-PeCDF	34.15	6081	-7.865
23478-PeCDF	33.99	6084	-7.865
123478-HxCDF	32.14	6106	-7.332
123678-HxCDF	32.12	6108	-7.332
123789-HxCDF	31.92	6114	-7.332
234678-HxCDF	31.99	6108	-7.332
1234678-HpCDF	30.03	6090	-6.798
1234789-HpCDF	29.69	6089	-6.798
OCDF	27.96	6148	-6.267

(*Source: Mader & Pankow, 2003*)

1.2.3 Henry's Law Constant

Henry's Law constants are used to estimate the volatilization of the PCDD/Fs from soil. They are also utilized in estimating the vapor-phase bio-concentration factor from air to plant leaves. Henry's law constants and sub-cooled liquid saturated vapor pressures at 298K were calculated for all PCDD/Fs (*Govers and Krop, 1998*). There is a strong relationship between Henry's law constants and sub-cooled liquid saturated vapor pressures (*Simcik, 2004*) for PCDDs as described in the equation below:

$\log H(298K) = 0.291 \log p_L^0 + 1.31 \quad r^2 = 0.844$:

The calculated Henry law constants for 17 2,3,7,8-PCDD/Fs are listed in the table 1.6

Table 1.6 Calculated Henry law constants of PCDD/Fs (Pa.m^3.mol^{-1})

No	*Compound*	*Log H*	*No*	*Compound*	*Log H*
1	2,3,7,8-TCDD	2.79	8	2,3,7,8-TCDF	2.57
2	1,2,3,7,8-PeCDD	2.83	9	1,2,3,7,8-PeCDF	2.72
3	1,2,3,4,7,8-HxCDD	2.84	10	2,3,4,7,8-PeCDF	2.59
4	1,2,3,6,7,8-HxCDD	2.84	11	1,2,3,4,7,8-HxCDF	2.72
5	1,2,3,7,8,9-HxCDD	3.08	12	1,2,3,6,7,8-HxCDF	2.72
6	1,2,3,4,6,7,8-HpCDD	3.08	13	1,2,3,7,8,9-HxCDF	3.02
7	OCDD	3.29	14	2,3,4,6,7,8-HxCDF	2.75
			15	1,2,3,4,6,7,8-HpCDF	2.85
			16	1,2,3,4,7,8,9-HpCDF	3.00
			17	OCDF	3.11

(*Sorce: Covers and Krop, 1998*)

1.2.4 Octanol/Water Partition Coefficient

The octanol/water partition coefficient (K_{ow}) 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.

Table 1.7 Calculated K_{ow} constants of PCDD/Fs

No	*Compound*	*Log K_{ow}*	*No*	*Compound*	*Log K_{ow}*
1	2,3,7,8-TCDD	7.02	8	2,3,7,8-TCDF	6.50
2	1,2,3,7,8-PeCDD	7.50	9	1,2,3,7,8-PeCDF	7.00
3	1,2,3,4,7,8-HxCDD	7.80	10	2,3,4,7,8-PeCDF	7.00
4	1,2,3,6,7,8-HxCDD	7.80	11	1,2,3,4,7,8-HxCDF	7.50
5	1,2,3,7,8,9-HxCDD	7.80	12	1,2,3,6,7,8-HxCDF	7.50
6	1,2,3,4,6,7,8-HpCDD	8.20	13	1,2,3,7,8,9-HxCDF	7.50
7	OCDD	8.60	14	2,3,4,6,7,8-HxCDF	7.50
			15	1,2,3,4,6,7,8-HpCDF	8.00
			16	1,2,3,4,7,8,9-HpCDF	8.00
			17	OCDF	8.80

(*Sorce: U.S. EPA, 1995*)

The log K_{ow} values increase with an increase in the number of chlorine substituents. Partition coefficients in the ranges of these reported values indicate that the substances tend to adsorb strongly to organic components in the soil and may bio-concentrate in those organisms exposed to the compounds.

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. Bioavailability and general behavior of PCDD/Fs are depending on their distribution between solid and liquid phases. Thus phase distribution is a major question with respect to assessment of environmental fate. For substances with low water solubility and high adsorption coefficient such PCDD/Fs is has been suggested that their natural occurrence in aquatic systems would be predominantly in a state of adsorption to (suspended) particulate matter (*Gotz et al., 1994*).

Log K_{oc} values for 2,3,7,8-TCDD have been measured in several studies. Lodge and Cook (1989) used contaminated sediments from Lake Ontario and distilled water in glass cylinders to measure the log K_{oc} of 2,3,7,8-TCDD. Log K_{oc} values ranged from 7.25 to 7.59. Jackson et al. (1986) used 10 contaminated soil samples in a batch extraction procedure to measure log K_{oc}. The average log K_{oc} of the 10 soils was reported as 7.39. Marple et al. (1987) used two uncontaminated soils spiked by two different methods with 2,3,7,8-TCDD to obtain the log

K_{oc} value. The soil was stirred with water in 2-liter flasks. The log K_{oc} values ranged from 5.96 to 6.54 for both soils, with an average value of 6.40 for the red clay soil and 6.02 for the alluvial soil. Puri et al., (1989) studied log K_{oc} of 2,3,7,8-TCDD with several other co-contaminants such as crankcase oils and surfactants. An average log K_{oc} value of 5.68 was reported for 2,3,7,8-TCDD in the presence of 0.01 percent surfactant. Walters and Guiseppi-Elie (1988) used several soils and water/methanol mixtures in a batch shake testing procedure to determine the log K_{oc} of 2,3,7,8-TCDD. The study resulted in a log K_{oc} value of 6.6.

Five studies for log K_{oc} of 2,3,7,8-TCDD were ranked number 1. The studies by Jackson et al. (1986) and Lodge and Cook (1989) had confirming values of 7.39 and 7.42, respectively. The studies by Walters and Guiseppi-Elie (1988), Walters et al. (1989), and Marple et al. (1987) had confirming values of 6.6, 6.66, and 6.4, respectively. The 6.6 value reported by Walters and Guiseppi-Elie (1988) was chosen by Syracuse Research Corporation (SRC) in the CHEMFATE Database (*SRC, 1991*) as the most definitive. This value was determined in a mixed solvent system, water and methanol; therefore, it is not considered as appropriate as a pure water equilibration system determined value. The values reported by Marple et al. (1987) and Walters et al. (1989) were determined in uncontaminated soil and with pure water; therefore, these values are selected as the most definitive for this document. Definitive values were not selected for other congeners because of few measured data points and, oftentimes, considerable differences in the reported values for those congeners with reported values.

Table 1.8 presents the log K_{oc} values measured in the Elber River and Baltic Sea by Golz et al (1994):

Table 1.8 Log K_{oc} of 2,3,7,8-PCDD/Fs

Compound	*Log K_{oc} measured*	*Compound*	*Log K_{oc} measured*
2,3,7,8-TCDD	6.4-7.2	2,3,7,8-TCDF	7.0-7.5
1,2,3,7,8-PeCDD	6.6-7.5	1,2,3,7,8-PeCDF	7.4-8.0
1,2,3,4,7,8-HxCDD	7.1-7.6	2,3,4,7,8-PeCDF	7.2-7.8
1,2,3,6,7,8-HxCDD	7.4-7.7	1,2,3,4,7,8-HxCDF	7.4-8.0
1,2,3,7,8,9-HxCDD	6.6-8.2	1,2,3,6,7,8-HxCDF	7.4-8.1
1,2,3,4,6,7,8-HpCDD	7.5-7.9	1,2,3,7,8,9-HxCDF	-
OCDD	6.8-7.9	2,3,4,6,7,8-HxCDF	6.8-7.7
		1,2,3,4,6,7,8-HpCDF	7.9-8.3
		1,2,3,4,7,8,9-HpCDF	6.7-7.9
		OCDF	7.4-8.3

(*Source: Golz et al., 1994*)

1.2.6 Photolysis of PCDD/Fs

Photo-degradation is an important abiotic transformation of the PCDD/Fs in the environment. Photolysis half-life ($t_{1/2}$) is one of the most important parameters characterizing photochemical reactions of PCDD/Fs and this parameter is indispensable for environmental risk assessment of these chemicals. The table 1.9 presents the photolysis half-life of 2,3,7,8-PCDD/Fs on spruce needle surfaces

Table 1.9 Photolysis half-lives of the 2,3,7,8-PCDD/Fs on spruce needle surfaces ([t] = h)

No	*Compound*	*Log $t_{1/2}$*	*No*	*Compound*	*Log $t_{1/2}$*
1	2,3,7,8-TCDD	1.74	8	2,3,7,8-TCDF	1.66
2	1,2,3,7,8-PeCDD	1.76	9	2,3,4,7,8-PeCDF	1.74
3	1,2,3,4,7,8-HxCDD	1.96	10	1,2,7,8-TCDF	-
4	1,2,3,6,7,8-HxCDD	1.88	11	1,2,3,4,7,8-HxCDF	-
5	1,2,3,7,8,9-HxCDD	1.97	12	1,2,3,6,7,8-HxCDF	1.87
6	1,2,3,4,6,7,8-HpCDD	1.92	13	1,2,3,7,8,9-HxCDF	1.90
7	ÔCDD	2.02	14	2,3,4,6,7,8-HxCDF	1.85
			15	1,2,3,4,6,7,8-HpCDF	1.93
			16	1,2,3,4,7,8,9-HpCDF	1.93
			17	OCDF	2.00

(*Sorce:Niu et al., 2005*)

Several works have noted that dechlorination is a major route for PCDD/Fs decomposed by direct photolysis, photo-catalytic process, and biologic treatment (*Chung-Hsin Wu et al, 2005*). Fig. 3 shown the proposed dechlorination pathways of 1,2,3,6,7,8-HxCDD :

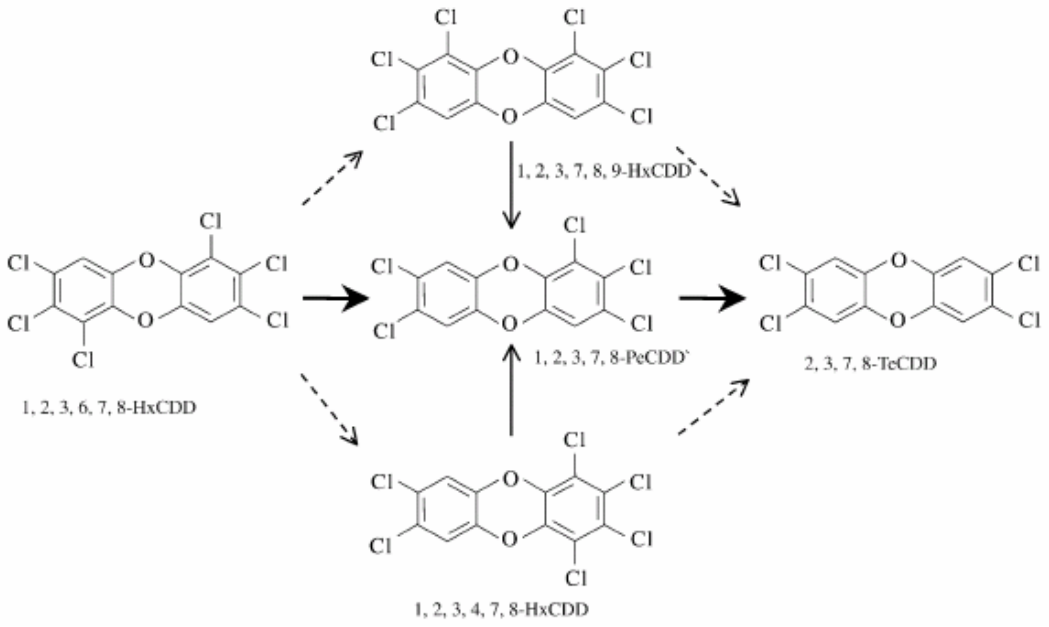

Fig. 1.2 Proposed dechlorination pathways of direct photolysis for 1,2,3,6,7,8-HxCDD
(Source: Wu et al, 2005)

Some authors had proposed that sunlight or ultraviolet light irradiation of PCDD/Fs in the presence of vegetable oil offers a potential method for the clean-up of contaminated soil (*Isosaari et al., 2001, 2004 & 2005; Namikaya et al., 2003; Bragato and El Seoud, 2003*). But follow them it is not yet clear whether the role of vegetable oils in photolytic treatment is simply to dissolve the PCDD/Fs molecules and facilitate their transport to the soil surface where they can be split by the light, or whether the oil itself and its quality plays any role in the photochemical reactions. Dechlorination of PCDD/Fs may take place when the molecule itself absorbs UV light or when it receives the required energy or electrons from other UV absorbing compounds as presented in the equations below:

$$\underset{}{ArCl} \xrightarrow{[h\nu]} {}^{1}ArCl \xrightarrow{[ISC]} {}^{3}ArCl \rightarrow Ar^{\cdot} + Cl^{\cdot}$$

$$ArCl^{*} + D \rightarrow ArCl^{\cdot -} + D^{\cdot +} \rightarrow Ar^{\cdot} + Cl^{-}$$

Note: $^{1}ArCl$, $^{3}ArCl$, $ArCl^{*}$ = single, triple and excited state aryl chloride, respectively; ISC=intersystem crossing, D=electron donor (Source: Isosaari et al., 2005)

The photodegradation products of PCDD/Fs are chlorinated dihydroxybiphenyls and hydroxydiphenyl ethers (chlorinated phenoxyphenols). In both reaction pathways, intermediate redical species are formed. An important mechanism of action of vegetable oils is probably to provide hydrogenatoms that stabilize the radicals. Hydrogen atoms are released via direct photooxydation of fatty acids.

$$RH \xrightarrow{[h\nu]} R^{\cdot} + H^{\cdot}$$

Note: RH = fatty acid (Source: Isosaari et al., 2005)

1.3 Source and fate of PCDD/Fs

Firstly we were interested in dioxin formation from herbicide synthesis processes such 2,4,5-T or 2,4-D. Due to this reason 2,4,5-T has not been produced in Europe since 1983 (*Gilber, 1994*). This is a great source of PCDD/Fs contamination for Southern Vietnam and we will discuss about this topic in a separated chapter of this thesis.

In present time, a big concern is the formation of PCDD/Fs through chemical and municipal waste incineration and other combustion processes. This can lead to atmospheric pollution, deposition onto soils and herbage uptake by animals and thus contamination of meat and dairy products. Chlorine bleaching of wood-pulp for paper making (now being phased out in some countries) has also been shown to be a source of contamination, particularly of PCDF-isomers, which can lead through discharge to the marine environment to contamination of fish and fish products (*Gilber, 1994*).

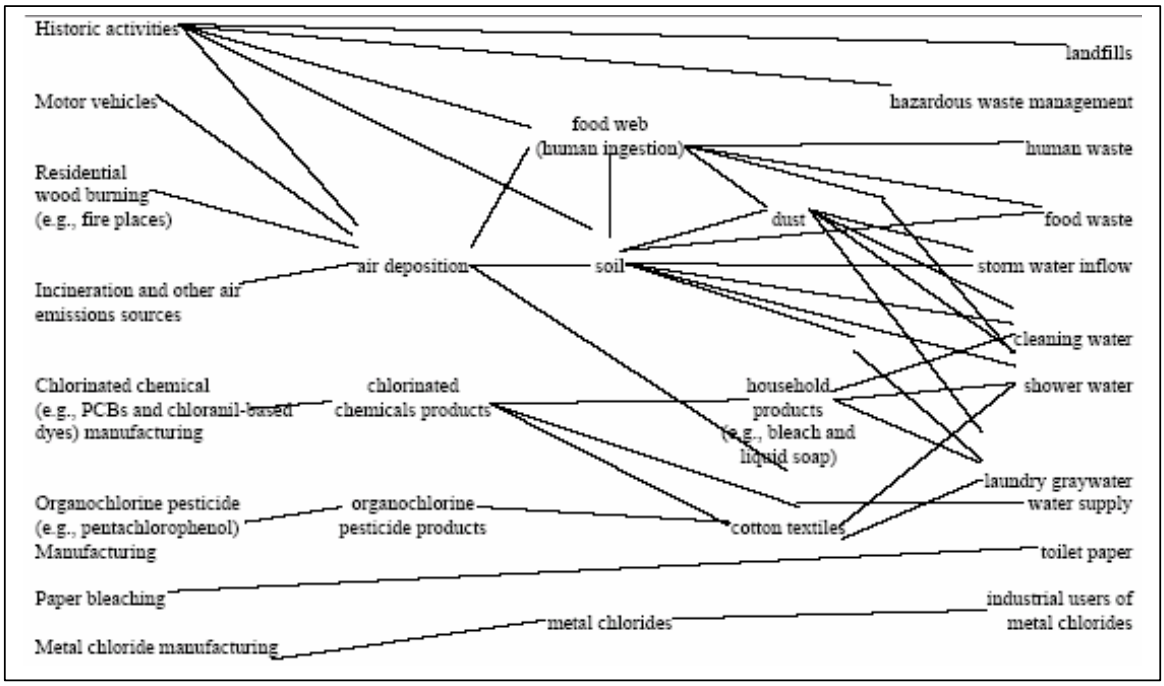

Fig. 1.3 Major sources of PCDD/Fs and their primary routes to the ecosystems (EIP, 1997)

Due to their very low water solubility and high lipophilicity, most of the CDD/CDFs occurring in water are expected to be associated with sediments or suspended material. Sediments may be an important, and ultimate, environmental sink for all global releases of

CDD/CDFs. Various studies have demonstrated that dioxins and furans are generally resistant to biodegradation. In addition, they are extremely stable compounds under most environmental conditions (*U.S. EPA, 2000a*).

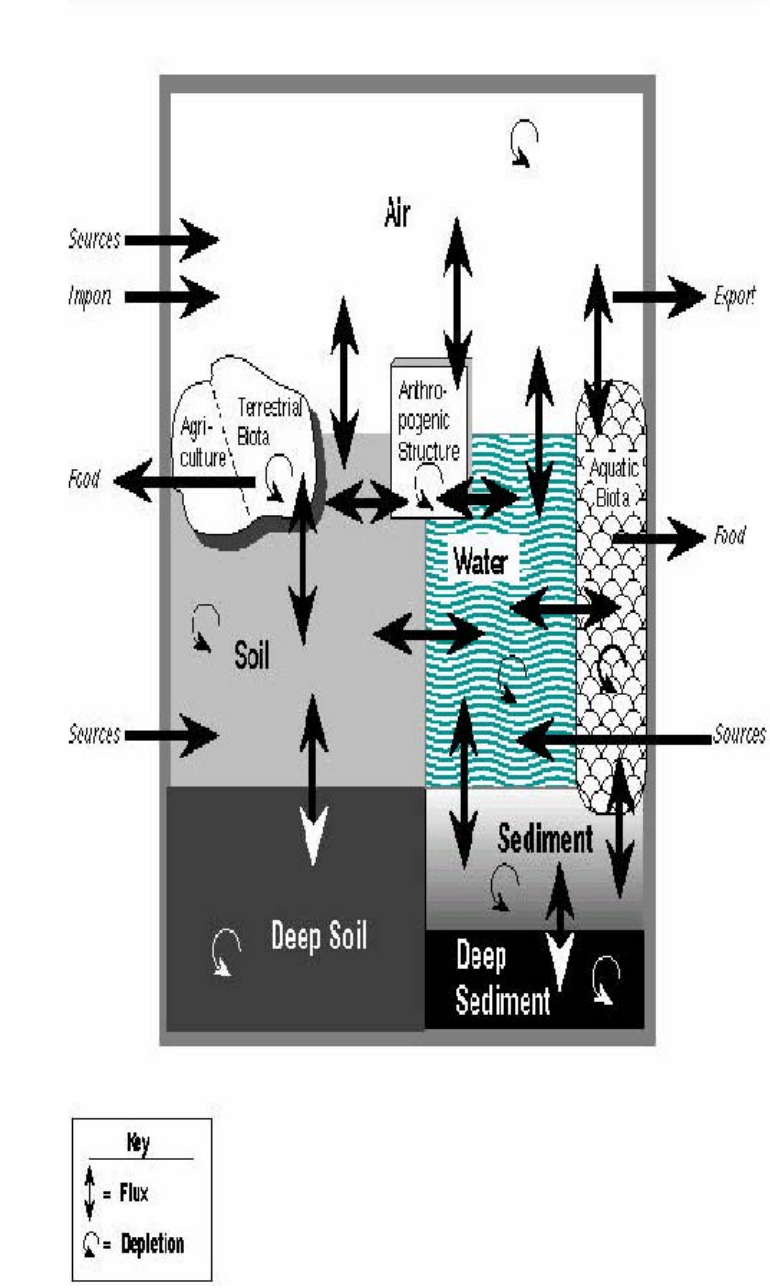

Fig. 1.4 Transport of PCDD/Fs among Media (Rifai, 2000)

If released to soil, PCDD/Fs are not expected to leach. For instance, the amount of TCDD detected more than 8 cm below the surface has been approximately 1/10 or less than that detected down to 8 cm (*U.S. EPA, 2000*). Being only slightly soluble in water, its migration in soil may have occurred along with soil colloids and particles to which it may have been bound. Volatilization from soil surfaces during warm conditions may be a major removal mechanism.

If released to the atmosphere, vapor-phase PCDD/Fs may be degraded by reaction with hydroxyl radicals and direct photolysis. Particulate-phase dioxins may be physically removed from air by wet and dry deposition (*U.S. EPA, 2000*)

Until now the half-live of PCDD/Fs compounds is still not yet defined clearly. Firstly the half-life of dioxin was estimated about 3 years, then changed on 7 to 8.2 years and for the organisms living in the sediment it could be longer, let say from 10 to 17 years (*Gros, 2005*). More then 30 years after utilization of Agent Orange in Vietnam we still find the considerable quantity of PCDD/Fs in the "hot spots" as shown in the results of this research and other related documents.

The studies by Geyer et al (2002) had shown the average half-life of TCDD in adult humans is approximately 2840 days, while in Sprague–Dawley rats the average $t_{1/2}$ of TCDD is 19 days. The $t_{1/2}$ of TCDD in humans is about 150 times that of rats. This factor was used to calculate the $t_{1/2}$ values of the other polychlorinated dibenzo-p-dioxins (PCDDs) in humans from the rat data. Another studies done by Masuda (2001) had reported the median half-life of 2,3,4,7,8-PeCDF in the Yusho patients is 2.9 years in the first 15 years after onset and 7.7 years in the next stage of 15 years. They also showed that the enzyme and/or hormone-mediated signs of high serum triglyceride, high serum thyroxin, immunoglobulin disorder and others are persistently maintained for 30 years. Studies done by Neuberger et al. (1999) on 159 cases of chloracne in TCDD-contaminated production of the herbicide 2,4,5-T had showed that chronic diseases related to TCDD exposure such chloracne are frequent in exposed even 23-25 years after the main exposure ended.

1.4 Toxicity and health aspects

1.4.1 Toxicity Equivalence (TEQ) and Toxicity Equivalency Factor

There are 210 different PCDDs and PCDFs with one to eight chlorines (75 PCDDs and 135 CPCDFs), but only 17 of these congeners are considered to be toxic. Toxicity and persistence are determined by structure, with lateral substitution (positions 2, 3, 7 and 8) imparting the highest degree of toxicity (*Wu, 2002; Huwe, 2002; C. Rappe, 1991*). The 17 2,3,7,8-PCDD/Fs are often found in complex mixtures. For risk assessment purposes, a toxicity equivalency procedure was developed to describe the cumulative toxicity of these mixtures (*U. S.EPA, 2000b*). This procedure involves assigning individual toxicity equivalency factors (TEFs) to the CDD, and CDF congeners. 2,3,7,8-TCDD is considered the most toxic of the dioxin-like congeners and is assigned a TEF of 1.0. All other congeners have lower TEF values ranging from 0.00001 to 0.5 (Table 1.10). To calculate the toxic equivalence (TEQ) of a mixture, the concentration of individual congeners is multiplied by their respective TEF and the sum of the individual TEQs is the TEQ concentration for the mixture. This is described mathematically as follows (*U.S. EPA, 2000b*):

$$TEQ = \sum_{i=1}^{n} (Congener_i \cdot TEF_i)$$

Table 1.10 Toxicity Equivalency Factor (TEF) for 17 2,3,7,8-PCDD/Fs

PCDD	*1988 NATO TEF*	*1998 WHO TEF*	*PCDF*	*1988 NATO TEF*	*1998 WHO TEF*
2378-TCDD	1.0	1.0	2378-TCDF	0.1	0.1
12378-PeCDD	*0.5*	*1.0*	12378-PeCDF	0.05	0.05
123478-HxCDD	0.1	0.1	23478-PeCDF	0.5	0.5
123678-HxCDD	0.1	0.1	123478-HxCDF	0.1	0.1
123789-HxCDD	0.1	0.1	123678-HxCDF	0.1	0.1
1234678-HpCDD	0.01	0.01	123789-HxCDF	0.1	0.1
OCDD	*0.001*	*0.0001*	234678-HxCDF	0.1	0.1
			1234678-HpCDF	0.01	0.01
			1234789-HpCDF	0.01	0.01
			OCDF	*0.001*	*0.0001*

(*Source: Huwe, 2002*)

The difference between WHO and NATO are in proposed TEF values for OCDD/Fs, normally we evaluate and calculate the i-TEQ based on TEFs of WHO.

1.4.2 Health effects of PCDD/Fs

Dioxin exhibits serious health effects when it reaches as concentration a few ppt in human body fat. Dioxin is a powerful hormone disrupting chemical. By binding to a cell's hormone receptor, it literally modifies the functioning and genetic mechanism of the cell, causing a wide range of effects, from cancer to reduced immunity to nervous system disorders to miscarriages and birth deformity. Because it literally changes the functioning of human cells, the effects can be very obvious or very subtle. Because it changes gene functions, it can cause so-called genetic diseases to appear, and can interfere with child development. In fact, the effects of PCDD/Fs on human health cold are divided on two aspects: short-term effects (acute toxicity) and long-term effects (chronic toxicity). Short-term exposure of human to high levels of PCDD/Fs may cause the diseases such chloracne, patchy darkening of the skin, altered liver function, etc. Long-term exposure is linked to impairment of the immune system, the developing nervous system, the endocrine system and reproductive functions. Chronic exposure of animals to dioxins has resulted in several types of cancer.

**Lethal Effect*

Studies of J.Yonemoto (2000) showed that toxicity of TCDD in experimental animals has been found to be greater in embryos than in dams. He also reported that the timing of TCDD administration is important in the occurrence of lethality (the day of gestation on which dosing occurred is an important factor). For example, when 24 μg/kg of TCDD was administered once to pregnant C57BL/6 mice on day 6 of gestation (GD6), the number of stillbirths increased. However, when administration took place on GD8, GD10, GD12 or GD14, there were no effects. The lethal dose of TCDD for hamsters and guinea pigs is 1157–5051 mg/kg and 1 mg/kg, respectively. Lethal toxicity in embryos is in the order of Rhesus monkeys~guinea pig>rat~rabbit>hamster~mouse.

The oral lethal dose LD_{50} of 2,3,7,8-TCDD for different animals are presented in the table 1.11 below:

Table 1.11 LD_{50} of 2,3,7,8-TCDD

Animal type	*LD_{50} (μg/kg)*	*Lethal time (day)*
Guinea-pig	0.6	5 – 200
Rat	22 – <100	9 – 43
Monkey	1 – 200	12 – 78
Dog	30 – 300	9 – 15
Chicken	25 – 30	17 – 21

(*Source:Division 10-80, 2000*)

*<u>*Half-life*</u>*

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

The average half-life of TCDD in adult humans is approximately 2840 days, while in Sprague-Dawley rats the average $t_{1/2}$ of TCDD is 19 days. The $t_{1/2}$ of TCDD in humans is about 150 times that of rats. This factor was used to calculate the $t_{1/2}$ values of the other polychlorinated dibenzo-p-dioxins (PCDDs) in humans from the rat data (*Geyer, 2002*).

The calculated half-lives ($t_{1/2}$) of some PCDDs in rats and adult humans are presented in the table 1.12:

Table 1.12 Half-lives ($t_{1/2}$) of some PCDDs in rats and adult humans

	Rats		Humans	
Congener	$t_{1/2}$ (days)	Mean	$t_{1/2}$ (years)	Mean
2,3,7,8-TCDD	15.0 – 23.7	18.7	5.8 – 9.7	7.78
1,2,3,7,8-PeCDD	27.2 – 33.1	30.9	12.6 – 45	
1,2,3,4,7,8-HxCDD	83 – 156	110	8.4 – 45	
1,2,3,4,6,7,8-HpCDD	200 – 314	251	3.7 – 102	
OCDD	173 – 413	322	6.7 - 132	

(*Source: Geyer, 2002*).

*<u>*Chloracne and skin diseases*</u>*

Chloracne is an acne-like eruption of blackheads, cysts, and pustules associated with over-exposure to certain halogenic aromatic hydrocarbons, such as chlorinated dioxins and dibenzofurans. Poland et al. (1971) had done a survey of 73 male workers in a 2,4,5-T factory. Chloracne, characterized by inclusion cysts, comedones and pustules, was found in 13 workers, and was correlated in severity with the presence of scarring, hyperpigmentation, hirsutism and complaints of eye irritation. Later, Schecter and Ryan (1988) had reported their studies on the adipose tissue of six workers who all developed chloracne and other illnesses following exposure to 2,3,7,8-TCDD in an industrial accident in Germany. The result showed an average PCDD/Fs concentration of 49 ppt, this value is about 15 times higher than current

population levels from this country. The studies of Beck et al. (1989) on adipose tissue samples of 45 occupationally exposed employees of a chemical plant and - as controls - from 21 persons resident in Hamburg, Germany had shown a correlations between chloracne and dioxin levels and confirmed the statement that chloracne is the most sensitive marker indicating exposure to dioxins. 193 cases of chloracne were reported among local residents after a chemical plant explosion in Seveso (*Eskenazzi et al., 2001*). To date, the only sustained toxic effect in humans associated with PCDD/PCDF exposure has been chloracne and related dermatological lesions (*Feeley et al, 1993*)

* *Carcinogenicity*

In 1985, the U.S. EPA declared 2,3,7,8-TCDD the most potent synthetic carcinogen yet tested. More recently, IARC has classified 2,3,7,8-TCDD as known human carcinogen (*IARC, 1997*).

Cancer Classification	*Toxic Effects*		
	Developmental	Reproductive	Immune System
2,3,7,8-TCDD: Group 1:carcinogenic to humans PCDDs (other than 2,3,7,8-TCDD): Group 3: unclassifiable as to carcinogenicity to humans. PCDFs: Group 3: unclassifiable as to carcinogenicity to humans.	Humans: - Foetuses exposed via placenta and breast milk showed muscles reflexes and thyroids dysfunction	Humans: Work exposure: - Reduced level of sex male hormone testosterone Monkey: (2,3,7,8-TCDD) chronic non toxic exposure: - Reduction of reproduction rate - Increase abortion	Animals: - Suppression of cell-mediated and humoral responses, suggesting that both innate and acquired immunities can be targeted

(*Source: IARC, 1997*)

For animal tests: 2,3,7,8-TCDD has been shown to be carcinogenic in several chronic studies at multiple sites in multiple species in both sexes. Short-term studies observed a lack of direct DNA-damaging effects including covalent binding to DNA by 2,3,7,8-TCDD, which underscores that 2,3,7,8-TCDD is not acting as an initiator of carcinogenesis. However, secondary mechanisms may be important in the observed carcinogenicity of 2,3,7,8-TCDD and related dioxin-like compounds. The lowest observed adverse effect of 2,3,7,8-TCDD in the Kociba study was the development of hepatic adenomas in rats at an intake of 10 ng/kg bw/day and the no observed effect level was 1 ng/kg/day. At the no observed effect level, body burdens were 60 ng 2,3,7,8-TCDD/kg bw. 2,3,7,8-TCDD also causes thyroid tumours in male rats. This has been indicated to proceed through a mechanism which involves altered thyroid hormone metabolism, and consequent increases in feedback mechanisms (TSH) which results in a chronic proliferative stimulation of thyroid follicular cells. Studies in the mouse skin support a lack of initiating activity and an ability to promote the growth of previously initiated lesions indicative of a promoting agent. Mouse skin tumor promotion indicates that the Ah receptor is involved in tumor promotion by 2,3,7,8-TCDD. Extensive examination of liver tumour promotion in the female rat liver also supports a non-genotoxic

mechanism for the induction of liver neoplasms by 2,3,7,8-TCDD. The ability of 2,3,7,8-TCDD to enhance proliferation and inhibit apoptotic processes in focal hepatic lesions further supports an indirect mechanism of carcinogenicity. Several PCDD/Fshave also been shown to be tumor promoters (*Who, 1998*).

Most information on the carcinogenicity of the dioxin 2,3,7,8-TCDD in humans comes from epidemiological studies of the accidentally exposed workers in herbicide plants and people living near it. However, most studies concern mixtures of several kinds of dioxins. As such, the evaluation of risks for individual dioxins is difficult. Herbicide plant workers heavily exposed to dioxins had more cancers of all types combined than the general population. The number of cancers increased with exposure (dose-response relationship). In Seveso, the number of deaths due to cancer has not increased since the accident, but it is still too early to reach definite conclusions. However, several studies showed excess risks for some specific cancers. A 22 year study of people in Japan who ate rice oil highly contaminated with PCBs and other dioxin-like compounds, showed an increase in liver cancer. No cancer increase was found after 12 years for another group in Taiwan who ate rice oil that was less contaminated. In summary, there is strong evidence that people accidentally exposed to the highest dioxin levels had an increased overall cancer risk (about 40% increase); there is less strong evidence of increased risks for specific cancers. In comparison, the average exposure of the general population is a hundred to a thousand times lower for TCDD and ten to hundred times lower for all dioxins combined (*Poland et al, 1971; Schecter and Ryand, 1988; Beck et al, 1989; Feeley, 1993; Yonemoto, 2000; Eskenazzi et al, 2001; etc.*).

**Teratogenicity and reproduction*

Up to now, the teratogenic and reproductive effects of PCDD/Fs and mainly 2,3,7,8-TCDD in man is still highly controversial, however there are already so many clear evidences to confirm it. The main teratogenic effects caused by TCDD are hydronephrosis and cleft palate. It is a widely held view that the teratogenic effects are mediated by the aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor. However, it has been suggested that a disturbance of epidermal growth factor (EGF) and its receptor (EGFR) function is also involved in the formation of cleft palate and hydronephrosis (*Miettinen et al., 2004*). Hydronephrosis was one of the most sensitive indicators of exposure in fetal hamsters, with an incidence of ll% and 42% when exposed to TCDD (I.5 pg/kg) on gd 9 and 7, respectively (*Olson and McGarrigle, 1992*). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is teratogenic in mice, inducing cleft palate and hydronephrosis at doses which are not overtly maternally toxic or embryotoxic (*Abbott and Birnbaum., 1990*). Hydronephrosis was observed at all dose levels, regardless of exposure day, and the incidence was close to 100% at 3 μg TCDD/kg and higher doses on GD 12 and earlier (*Couture et al., 1990*).

Several factors determine the deleterious effects of environmental toxicants on the functioning of the female reproductive system. Timing and intensity of exposure of a toxicant to the endocrine-immune systems contribute to the potential alteration in the normal functioning of the reproductive system. Susceptibility of target tissues are determined by the stage of development of the embryo, fetus, or adolescent, the duration, and often the cumulative amount of exposure as well as the host's immune resistance. 2,3,7,8-TCDD perturbs organogenesis and reduce fertility in wildlife and experimental animals (*Vasseur and Cossu-Leguille, 2005*). The reproductive capacity of rats ingesting TCDD was clearly affected at dose levels of 0.01 and 0.1 μg TCDD/kg/day, but not at 0.001 μg TCDD/kg/day, through three successive generations (*Murray et al., 1979*). Studies on zebrafish (*Wannemacher et al., 1992*) showed that single doses of >5 ng TCDD per fish (corresponding to 1.7 - 2.0 ~g/kg body weight) affected

spawning and suppressed it completely after 1 - 2 cycles. This effect preceded body weight reduction, suggesting a specific effect of TCDD on zebrafish reproduction. The offspring from spawnings after _> 5 ng TCDD showed a mortality of 100%, which was preceded by a severe thoracal edema and malformations of the Notocord. In rat, adverse effects on reproduction have been reported in animals treated with 2,3,7,8-TCDD (10 or 100 μg/kg/day). The NOAEL (non adverse effect level) for these effects was 1 ng/kg/day (*Ayotte et al., 1995*). Brower et al. (1995) studies reported that in utero and lactational exposure to PCDD/Fs can produce functional developmental effects in experimental animal offspring; these functional developmental effects observed in experimental animals can persist into adulthood and include: neurobehavioral effects (impairment of cognitive functioning, altered neuromotor behavior, altered sexual behavior), developmental reproductive effects (reduced sperm production and ejaculation, impairment of sex organ and urogenital tract development, reduced reproduction capacity of F1 generation), neurochemical effects (alterations in brain biogenic amine concentrations, reduced brain and circulating thyroid hormone levels, alterations in markers for glial and neuronal cell development).

**Immunotoxicity*

The immune system has been suggested to be one of the most sensitive organs to response to TCDD. 2,3,7,8-TCDD produces effects on the immune system as profound as thymic atrophy and impairment of the humoral-and cell-mediated system (*Nagarkatti et al., 1984*). The studies on male mice shown that the reduction in thymic weight, spleen B-cell functional response (per spleen), and bone marrow B-cell functional response to 14%, 35-54%, and 20-32% of control, respectively, at a dosage of 120μg/kg (*Chastain et al., 1985*). The immunotoxicity by 2,3, 7,8,-TCDD in animal models showed that 2,3,7,8-TCDD either indirectly (i.e., in the case ofT-cells) or directly (i.e., in the case of B-cells) affects the maturational or differentiative processes of immunocompetent cells and that such cells are sensitive to the effects of this xenobiotic during periods of activity associated with these events; the chronic, low level human exposures associated with PCDD/Fs contamination may adversely affect immune function (*Holsapple et al., 1991*). Studies of Lundberg et al. (1992) on adult male C57BL/6 mice exposed to TCDD (50μg/kg) shown that the antigen-specific T-cell proliferation and interleukin-2 (IL-2) production in response to ovalbumin (OVA) were significantly suppressed by TCDD, while the polyclonal response to anti-CD3 antibodies plus PMA was not affected. A differential effect on the immune system of rats and mice was observed using two models of host resistance, trichinella and influenza: rats and mice were challenged with Trichinella spiralis 7 days after TCDD treatment, then the proliferation of T cells in the splenic and mesenteric lymph nodes as well as effects on parasitemia were measured 7, 14, and 28 clays post parasite challenge. The result shown that there was a significant suppression of the lymphoproliferative response was observed both in the splenic and mesenteric lymph nodes in mice at > 1000ng/kg. Usingviral-induced mortality as the endpoint in mice, doses as low as 10 ng/kg TCDD significantly enhanced mortality. While the rat virus does not cause a lethal infection, an increase in the numbers of virus in the rat lung was observed at 10,000ng/kg, and a suppression of the viral-augmented NK activity was detected at 3000ng/kg (*Birnbaum, 1993*). The studies done by Grinwis et al. (2000) to elucidate the impact of aquatic pollution on fish health in the marine and estuarine environment had showed the effects on several organs (liver, gills, gastro-intestinal tract, thyroid gland, gonads, spleen and mesonephros): oral exposure of flounder to 0.0125 or 0.3125 μg TCDD/kg bw, or to 0.3125 mg TEQ:kg bw of a harbor sludge extract, weekly for 8 weeks, induced a significant increase in CYP1A immunoreactivity in hepatocytes; single

administration of higher doses (20, 100 and 500 μg/kg bw) of TCDD also induced a significant increase CYP1A immunoreactivity in the endothelium in all organs examined, and in the epithelium of the digestive tract, liver, and mesonephros; strong immunoreactivity was noted in a distinct cell population of the hematopoietic tissue in the mesonephros and spleen; and oral exposure to 20 μgTCDD/kg bw resulted in an increased mitotic activity, and an increased hepatosomatic index was found after exposure to 500 μgTCDD/kg bw. The developmental effects of perinatal exposure to low doses of TCDD on the major immune organs of offspring, thymus and spleen, were investigated focusing on weaning time (postnatal day (PND) 21), puberty (PND 49) and adulthood (PND 120) in male rats (*Nohara et al., 2000*), the result showed that dose-dependent CYP1A1 mRNA induction was clearly observed by maternal exposure to 50–800 ng TCDD/kg on PND 5. Recently, the studies of Kikuchi et al (2001) showed that low acute toxicity dioxin compounds such 2,3,7,8-TCDF, 1,2,3,4,6,7,9-HpCDD may actually exhibit strong immunotoxicity in humans. Walker et al. (2004) reported that the perinatal exposure to 1μg TCDD/kg or 3μg TCDD/kg dam weight resulted in long-term impairment of contact hypersensitivity responses in adult Fischer 344 rats. This suppression was associated with an increased proportion of CD4+ T cells expressing a naive phenotype in lymph nodes of female offspring of dams given 3μg TCDD/kg, suggesting an alteration in the generation, or maintenance of activated CD4+ T cells (and/or RTE) in peripheral lymphoid tissues of these animals.

**Metabolism and tissue distribution*

Metabolism or biotransformation through the phase I (cytochrome P-450 monooxygenase enzymes) and phase II (conjugating enzymes) pathway is a requisite for detoxification and excretion of lipophilic chemicals. TCDD resist metabolism by mammalian system, but hydroxylation by the cytochrome P-450 system and conjugation to form glucuronides, with subsequent elimination in the bile and urine, has been suggested from work in hamsters, rats and guinea-pig. There is no evidence that metabolic conversion is required for toxicity or that metabolism enhances the toxicity of any isomer (*IARC, 1991*).

Besides adipose tissue, the liver is the primary organ in small rodents, in which 2,3,7,8-substituted PCDDs and PCDFs accumulate (*van den Berg et al., 1989*). Studies of Curtis et al. (1990) provided in vivo evidence that an inducible, saturable system plays a predominant role in disposition of [14C]TCDD in female mice at doses between 5 and 20 μg/kg.

Greater detail about the metabolism of 2378-TCDD is desirable, but very difficult to obtain because the high toxicity (LD50 = 60 μg/kg for rats) and low metabolic turnover make conventional metabolism studies impractical (*Hakk et al., 2001*). So they used a relatively non-toxic dioxin congener, i.e., 1,2,7,8-tetrachlorodibenzo-p-dioxin (1278-TCDD), to gain a better understanding of mammalian metabolism of dioxins.

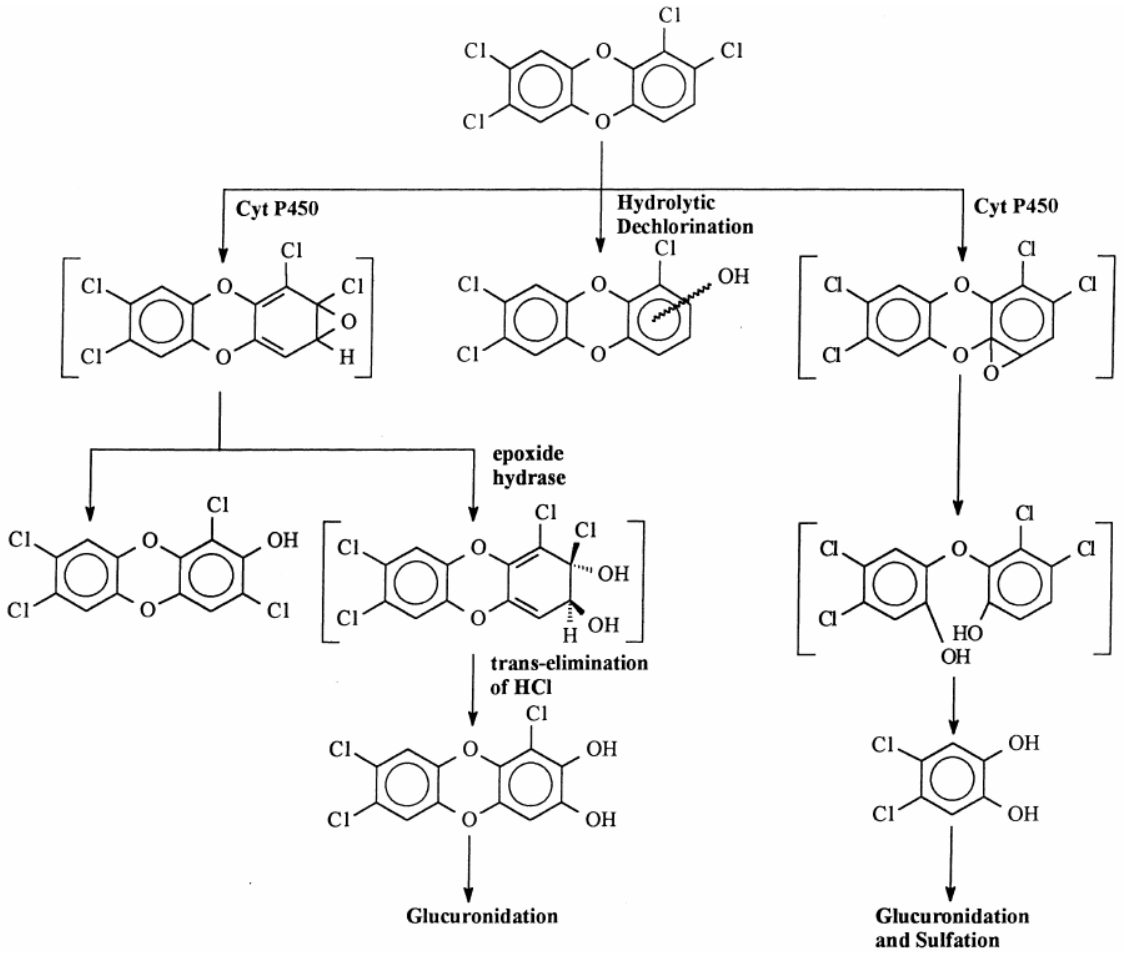

Fig. 1.5 The proposed pathway for the metabolism of 1,2,7,8-TCDD in male rats (Source: Hakk et al., 2001)

* *Mechanism of action of PCDD/Fs*

Environmental chemicals may alter female reproduction through direct (hormone disruption) or indirect (immune toxicant) mechanisms. Direct effects usually occur if an environmental chemical is structurally similar to an endogenous molecule and capable of entering reproductive organs. In this scenario, the chemical may alter normal cellular processes such as differentiation, mitosis, meiosis, programmed cell death, migration, intracellular communication, DNA repair, or mitochondrial function. Indirect effects may occur if a chemical requires metabolic conversion within the body before it is capable of exerting a toxic effect. Indirect effects also may occur if a chemical interferes with endogenous hormones. Some environmental chemicals may mimic or block natural hormone action, thereby adversely altering reproductive processes (*Sharara et al., 1998*).

The major toxicological impact of PCDD/Fs is related to the underlying assumption that many of the toxicological symptom result from compounds that can bind to the Ah receptor protein and translocate to the nucleus of the target cell (*IARC, 1991, Blankenship et al., 2003*).

Studies of Enan et al. (1996) indicated that TCDD may interrupt the endocrine function of human luteinized granulosa cells (hLGCs) through the blockage of the mitotic signal directly or indirectly through the interaction of protein tyrosine kinase/MAP2K (PTK/MAP2K) and protein kinase (PKA) signaling. TCDD induces production of Prostaglandin endoperoxide H synthase (PHS-2) but not PHS-1 mRNA in HUVEC (primary human epithelial cells) in a time- and concentration-dependent manner (*Liu et al., 1997*). TCDD causes rapid changes in the plasma-microsomal membrane levels and activity of $p60^{Src}$ in Hepa 1c1c7, Hepa c4 cell, and

SR3Y1 cell, a $p60^{v\text{-}Src}$ overexpressing cell line (*Blankenship and Matsumura, 1997*). TCDD clearly shortens LHR mRNA stability and may relate to the production of certain proteins that destabilize the LHR mRNA in granulosa cells (*Hirakawa et al., 2000*). Studies of Petroff et al. (2001) on rodents indicated that PCDDs interrupt ovulation through direct effects on the ovary in combination with dysfunction of the hypothalamo–hypophyseal axis.

The toxicity of TCDD and related compounds in birds has been studied by Blankenship et al. (2003), result showed that there are a number of potential mechanisms by which exposure to TCDD could result in developmental abnormalities, including: induction of specific cytochrome P450 enzymes ---> result in oxidative stress through production of H_2O_2 and/or other reactive oxygen species, result in activation of procarcinogen compounds into reactive metabolites; interact with critical cellular and molecular targets resulting in developmental toxicity; etc. They had provided the evidence of TCDD-induced oxidative DNA damage, increased production of reactive oxygen species, and a depletion of glutathione in chicken embryos.

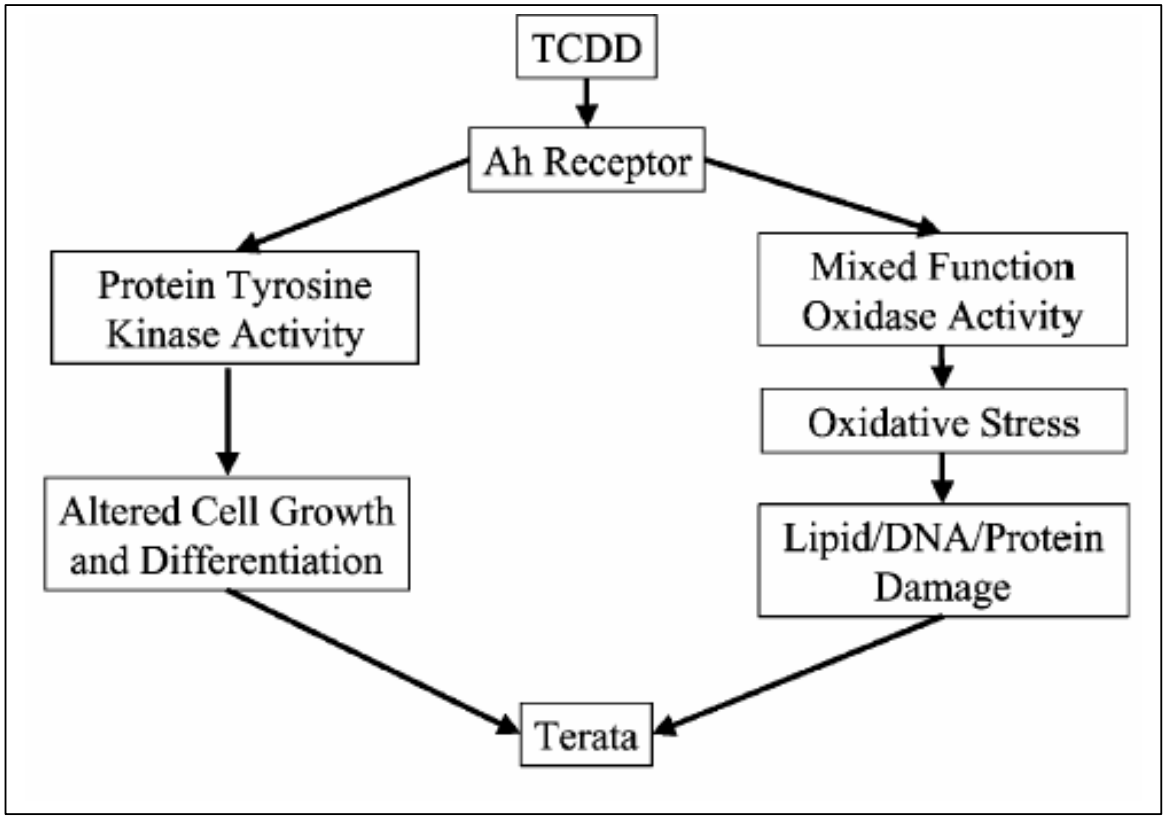

Fig. 1.6 Possible mechanisms for TCDD-induced abnormalities and embryolethality in birds (Blankenship et al. 2003)

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CHAPTER II: STUDIES ON DIOXIN CONTAMINATION IN VIETNAM

2.1 Introduction

Agent Orange is the name used to describe a particular type of herbicide blend used for military purposes in Vietnam from 1961 – 1971 during the Ranch Hand Operation done by U.S Army. In fact we could recognize this operation as an essential Chemical War. Up to now (after more than 30 years after the war), its adverse consequences still present and influence not only on the ecosystem over large areas in Vietnam (from 17th parallel to Camau peninsula), but also on human health. With the purposes to kill unwanted plants and remove leaves from trees which otherwise provided cover for the enemy, the US. Army had used three main agents sprayed by planes and motorized means (tanks, high-pressure pumps chemical units, etc), including Agent Orange (A.O), Agent White (A.W.) and Agent Blue (A.B). The sprayed surface is about 2,600 million hectares, reaching a percentage of about 10% of Southern Vietnam.

Table 2.1 Five chemicals used in Ranch Hand Operation

Chemical	*Date of spray*	*Quantity (million kg)*	*Area (million ha)*	*% Area*
A.O	1962 – 1970	57.0 (44.338L)	1.6	12
A.W	1966 – 1971	22.8 (19.835L)	0.7	5
A.B	1962 – 1970	10.7 (8.182L)	0.3	2
CS	1964 – 1970	9.0	5.0	37
Malathion	1967 – 1972	3.0	6.0	44
Total		102.5	13.6	100

(*Source: Cau, 2003*)

The used chemicals had devastated dreadfully the normally abundant floristic composition of Vietnam tropical forests, terrestrial forests and mangroves forests. As reported by many authors, the total area affected by used chemical included 70% coconut gardens, 60% rubber forests, 44.500ha pine forests, 66.000ha mangroves forests, 43% rice fields, and 2.5ha primitive forests (*Cau, 2003; Quy, 2002; Hieu et al., 2003; Dux and Young, 1986*).

From 1970, the problems caused by dioxin contamination have been studied and reported in many scientific magazines as well as in international conferences. The Division for mitigation of the consequences of the chemicals used during the war on human health has been established in 15/Oct./1980 (10-80 Division). This division belongs to the Ministry of Health and takes a mission to continue do the researches based on the results from previous studies done in decades 60' and 70'. In 1992, the National Steering Committee for the remediation of the consequences of toxic chemicals used by the U.S. Force during the war in Vietnam (Committee 33) has been established. The Committee 33 is more integrated and led by a committee with the participation of almost all important ministries of Vietnam, including Ministry of Science and Technology (MOST), Ministry of Natural Resources and Environment (MONRE), Ministry of Foreign Affairs (MOFA), Ministry of National Defense (MOND), Ministry of Labor, Invalids and Social Affairs (MOLISA), Ministry of Health (MOH), Ministry of Agriculture and Rural Development (MOARD), Ministry of Ministry of Finance (MOF), Ministry of Justice (MOJ), Ministry of Culture and Information (MOCI),

Committee 10-80, The Agent Orange Victim's Fund (Red Cross of Vietnam), National Center for Natural Science and Technology, and Vietnam – Russia Tropical Center.

Four big international conferences on dioxin and related subjects had been held in Vietnam:

- 1st International Conference on Herbicides in war – the Long-term effects on Man and Nature, January 13-20, Hochiminh City, Vietnam;
- 2th International Conference on Herbicides in war – the Long Term Effects on Man and Nature, November 15-18, Ha Noi, Vietnam;
- United States – VietNam Scientific Conference on human health and environmental effects of Agent Orange/Dioxin, March 3-6, HaNoi, VietNam;
- United States – VietNam Scientific Workshop on methodologies of dioxin screening, remediation and site characterization, November 3-5, HaNoi, VietNam;

The Division 10-80 has edited all studies carried out in period 1980 – 2000 in a big report in Vietnamese (*Division 10-80, 2000*). This means a lot of data as well as reports done by many scientists (Vietnamese and overseas). Because all data related to Agent Orange/Dioxin are legally considered as secret documents according to on Vietnamese regulation, we only summarized here the results extracted from documents of Division 10-80 without the names of authors (*see the reference for the title and author names of related documents).*

2.2 Studies on environment and ecology

The studies of Vietnamese scientists in collaboration with oversea laboratories showed that herbicides and defoliants had devastated dreadfully the normally abundant floristic composition of Vietnam tropical forests, terrestrial forests and mangroves forests. Especially, century-old higher foliage wood trees were seriously damaged, ranging from *Diptero-carpaceae, Pterocarpus Pedatus, Sindora Siamensis, Hopea Odorata* to *Afzelia Xylocarpa* species, etc...

The ravaged timber amount by war chemicals could rise up to 120 million cubic meters, not accounting other damaged resources such as resinous, essential oil trees, rattan, medicinal plants and trees...

Wherever there was not any human interference, the destroyed forests could regenerate. Almost all regenerating trees are of miscellaneous species with different economic values.

Over thirty years of the post war period, in many localities where virgin forests were ravaged, the actual spectacle still presents wildness with bare hills, waste land and even lateritzed soil; the frequent sights are bushes and herbs of the *Imperata cylindrica, Miscanthus japonnicus species* and wild herbs of the type *Pennisetum polystachyon*. These clearing and grass plots obstruct the growing of trees; there forest fires threaten quite often and no sign of indigenous vegetation is redeveloping

At present the destructed natural flora and fauna have been reviving but very slowly, with a tendency of deviation to a much poorer system. The former ecological system of humid tropical forests seems hardly to restore naturally. Some rate species could still be encountered, but scarcely; such as those of *Elephas maximus, Bosgaurus, Boar suscrofa, Pygathrix naemacus* species, and some birds of the *Phoenix* family, a few economic valuable survival wild animals such as *deers, monkeys, bears* whereas many rodents species have been developed (rats).

Vietnam mangroves forests consist of an abundant and diversified ecological system, with a valuable vegetation including *Nipa frauticans, Exoecaria agallocha, Rhizophora mucronata, Rhizophora apiculata* species...yielding an annual amount of timber about 20 to 30 cubic meters, and offer the dwelling for several wild animals such tiger, wild boars, dears, varans and iguansas and several big water fowls (*pelecanus anocrotalus, pharacrocorae carbo anastomus oscitans, egretta alba...)*. It was also the ideal site for rich aquatic resources development (fish, shrimps, cuttelefish, coral and brackish water species). The Ranch Hand Operation had devastated about 40% of the total mangroves forests area (i.e. at least 260.000 acres –according to the U.S. Federal Academic statistic), resulting in an annual lost about 20 to 40 cubic meters of timber, and 60/100 kg of shrimp harvest per hectares. The destruction of mangroves forests led to the means of livelihood deficiency for local residents.

Furthermore, the loss of mangroves forests has caused a lot of fluctuations upon the two parts of the seashores.

- The erosion on the eastern shores, or estuaries: being washed away an average width about 20 meters to 25 meters of the littoral each year, reaching a total area of about 388 hectares per year;
- - The illuviation of the western seashore of Mekong Delta;

The process of mangrove regeneration was very slowly, and its economic value remains at 8 to 20 times lower than that of virgin forests.

Just in the decade 60^{th}, the immediate consequences on the fauna and human were severe; some of victims were dead at once; the others had suffered from varied diseases; heavy reproductive disorders were happened.

2.3 Studies on environmental pollution

Researches have been conducted during over two decades (from 1980 to 2000) pursuing the aim of finding dioxin remaining in various samples such as:

- Soil and sediment of ponds, lakes, streams and rivers;
- Different kinds of foodstuff;
- Living specimens (muscles, fat, liver, blood, milk, entrails) from domestic and wild animal and from human;

Samples were taken from several localities in both southern and northern parts of the 17^{th} parallel.

The samples had been analyzed in Vietnamese and overseas laboratories, the results showed that:

- In natural environment, in the human and animal body of some parts of Vietnam is still present dioxin with relative high concentration;
- Dioxin concentration is extreme high in the former airforce bases (e.g Bien Hoa Airforce Base with 2,3,7,8-TCDD higher than 1×10^{6}ppm in soil next to chemical tanks) and they vicinities (*Division 10-80, 2000*);
- Dioxin concentration in natural environment, human and animal body of southern Vietnam (from the 17^{th} parallel) is higher in comparison with the northern part. The results also showed that there is a gradual decreasing of dioxin concentration from decade 70^{th} up to now. At present time, the dioxin concentration in natural

environment is not higher than the contaminated sites of developed industrial countries (except some special sites such former military bases, airport, etc) (*Cau, 2003*);

- The surveys on foodstuff of different places showed that there is still a residue of dioxin some where, but with very low concentration (under the WHO standard value). However in some sites named "hot spots", the dioxin residue is considerable and could threaten the human health of local residents (*Division 10-80, 2000; Cau, 2003*);
- There are some clear evidences that the dioxin residue at some "hot spots" is originated from chemical used by U.S Army during the war (Ranch Hand Operation) (*Division 10-80, 2000*);
- The dioxin residue in nature could cause the adverse effects on ecosystem and human health through food chain, even many years have pass since the end of Ranch Hand Operation (*Division 10-80, 2000; Cau, 2003; AAFV, 2005*).

2.4 Studies on biological changes

The studies showed the long-term effects as biological changes which can be found through ulterior changes of biological constants (*Cau et al (1) & (2), 1993*).

2.4.1 Hematological indices

In almost examined veterans who exposed to A.O/Dioxin the notable sign is the cellular immunological deficiency, associated with the following constants fluctuations.

- Decrease of monocyte;
- Decrease of lympho cyte Ta, Tt cells;
- Slight decrease of TCD_4; without or with slight increase of TCD_8 within a normal limit; the rate of TCD_4 compared with TCD_8 is balanced at over 1. In certain cases, if the rate is lower than 1, it would soon adjust after a short-term treatment;
- CD_{19} stands at its normal status or slightly increases;
- Slight decrease of cell function, manifesting through the rate of those who have the positive Mantoux reaction; and reduce the TNFβ secretion;
- Changes of cellular immunity also appear similarly in F1 generation that have apparent inborn deformities or not;
- No considerable changes on serous immunity: in term of IgA, IgB, IgM immune serum.

In several circumstances (about 10% of the analyzed instances) permeating dioxin in blood could be found, and so the dioxin origin of the disease be affirmed. However in the cases where dioxin doesn't exist in blood, the pathological dioxin originated manifestation on the F_1 and F_2 generations can not be excluded. By X-ray, in combination with hematological analysis for F and F_1 members of the family, a good deal of *spina bifida occulta* had been found (around 40% of the cases).

Table 2.1 Blood immunity pattern (mains constants) resulting from chronically exposed to war chemicals (Agent Orange)

F generation	*F_1 male generation*	*F_1 female generation*
Neutro white cell normal or decreased		Neutro white cell normal or increased
Lymphocytes increased	Lymphocytes normal	Lymphocytes decreased
Monocytes ↓		
Ta ↓		
Tt ↓		
$TCD_4/TCD_8 \geq 1$		
CD_{19} normal (may slightly increase)		

(*Source: Division 10-80, 2000*)

2.4.2 Biochemistry

Ordinary indices: including whole protein, GOT, GTP, GT, Cholesterol, HBsAg, TSH, T_3, T_4, free...are usually being used, and allow to set fouth the following observations (*Division 10-80, 2000*):

- The T_3, T_4, TSH hormone changes caused by dioxin depict the hypophyso thyroid imbalance, part of the hypothalamo hyhpophyso thyroid axis in particular and of the endocrine system in general;
- Dioxin has also caused the liver functional deficiencies; the *Enzym* indices change, entire *Lactate dehydrogenaza*; entire *Cretinkinaza*; entire *alcalophosphatase* and their *izozyms*, showing the harmful effects in destroying the diversified cell in various organs: the nervous system, the tissue with strong aerophil metabolism; mcardiac and muscular system; bones, liver and gall bladder system...

2.4.3 Genetic materials

Researches on bio-genetic showed that there existed a chromosomal aberrations such as the defects, the gaps, breaks, dicentric chromosomes, the translocation, the ring, the miniature chromosomes, and the chromatid sisters exchanges (*Tuyen et al, 1983; Trung et al, 1995*).

The chromosomal aberrations were one of the causes of cancer, and of the reproductive accidents during the embryonic phase.

Chromosome aberrations in the germ cells (in female, male) might transmit to subsequent generations.

2.4.4 Pathology - Histopathology

A. Macroscopically, the veterans spending a period of several years in-*service* in the south-bound part of the 17th parallel, have had various dermatitis, such as:

- Erythroderma maculo-desquamativum;
- Erythroderma bullo-prurigino-squamous;
- Diffuse scleredema associated with dysesthesia;
- Albinisme (achromatosis cutanea);
- Hyperpigmentation bullo-pilo-pruriginous;
- Eruption maculo-pririginous;
- Etc...

Skin injuries are usually polymorphic, associated on the same patient, and have never been solely one. All patients had acute dermatitis, and at the same time, other associated injuries (*see Fig. 2.1*).

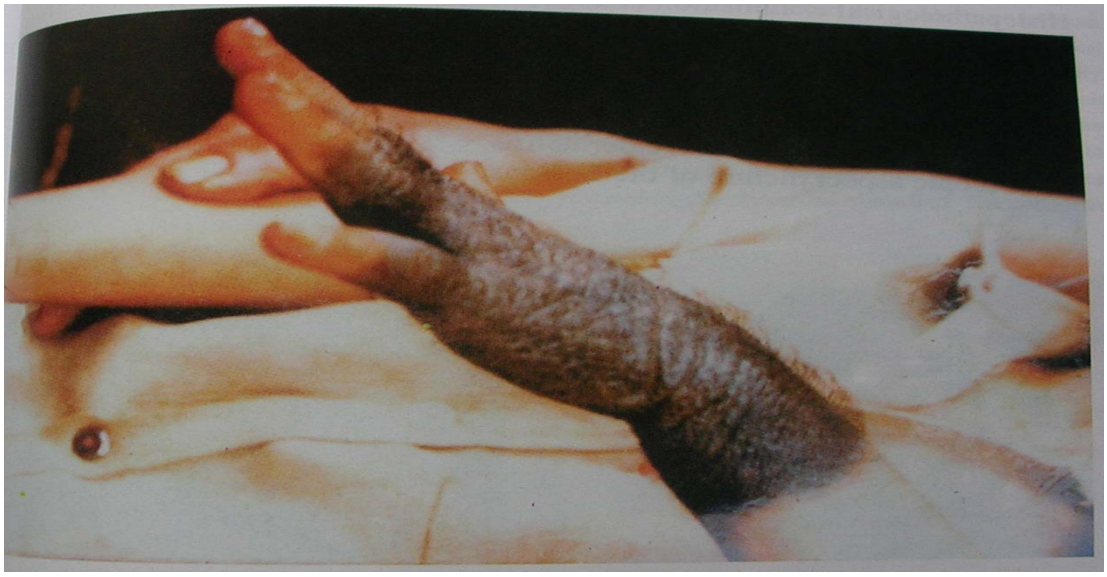

Fig. 2.1 Scleredeme pilo keratosique (source: Cau, 2003)

The interferences with several medicines (including Corticoid) and other ordinary physiotherapeutic means showed little or temporary efficiency. These injuries developed in periodicities, and are susceptible to the weather, and to strange food (like alcohol, seafood, etc...), durable, aggravating with the time, and having the characters of autoimmune diseases.

B. The microscopic examination (histo-pathology) on 30 male patients have gathered the injuries changes as diversified as those acquired on macroscopic observation. The images of acute dermatitis are intermingled with chronic dermatitis with cellular degeneration, and the invasion of melanin etc... Some of the specimens showed the evidence of the hyperplasia and the disorder of the cellular structure in consistent with intraepithelial *squamous neoplasia* type...

The observed injuries are diversified, which do not resemble to any typical pathological injuries as described in medicinal literature before. Perhaps this may be a characteristic on the dermatosis on the patients exposed to A.O/Dioxin in association with a diseases-pattern similar to an auto immune disease (linked to dioxin).

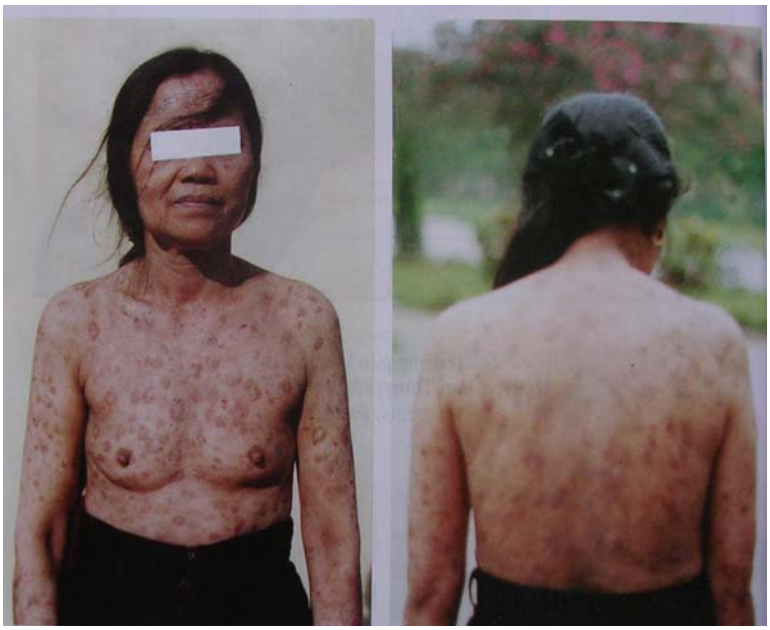

Fig. 2.1 Typical example of pathology (macro and microscopy) caused by Dioxin (source: Cau, 2003)

2.5 Studies on diseases presumably linked to Agent Orange/Dioxin exposure

Once penetrating into human body, dioxin affects various organs of the body due to the dioxin characteristics and the peculiarities of individual human body. Dioxin impacts mainly several organs and systems such as the nervous systems; the immunity system; endocrine glands, organs of sense, germ cell, etc...

Pathological researches on the people to A.O/Dioxin have been conducted in various residential communities throughout the country for more than 20 years. Some of their results are as follows:

1. In the military hospital 108, a research on 477 veterans non-exposed to A.O/Dioxin and on 523 veterans endangered with A.O/Dioxin exposure, has given the result below (*see table 2.2*).

Table 2.2 Morbidity in the two Vietnamese veterans groups

Kind of diseases	*Veterans exposed to A.O/Dioxin N=523 (%)*	*Veterans non exposed N=477 (%)*	*P*
Digestive System	78.3	69.0	< 0.01
Cirrhosis	1.9	1.2	
Colitis	23.5	16.7	
Nervous System:	48.9	20.3	< 0.05
Neurasthenia	29.6	13.2	
Vestibular disorders	2.48	1.04	

Kind of diseases	*Veterans exposed to A.O/Dioxin N=523 (%)*	*Veterans non exposed N=477 (%)*	*P*
Dermatosis:	30.4	17.6	< 0.05
Cancer:	17.4	11.7	< 0.05
Cancer of liver	2.67	2.09	
Cancer of pharynx	2.58	0.62	
Lung cancer	0.95	0.41	
Cancer of bladder	0.19	11.7	
Other cancers	14.5	14.2	
Leukemia; leucosis	0.95	0.2	
Hodgkin's disease:	0.38	0.0	
Pharyngitis	17.79	1.42	
Chronic sinusitis	3.05	1.4	
Atherosclerosis	5.73	2.3	
Hypertension	8.79	5.8	

(*Source: Division 10-80, 2000*)

2. Another research conducted on 293 non-exposed to A.O/Dioxin veterans, and 1647 veterans endangered of A.O/Dioxin exposure (*see table 2.3*) in VietYen district – BacGiang Province showed an existing difference with statistical significance on the respiratory, urological and cardio-vascular disease groups.

Table 2.3 Morbidity in the two veterans groups in VietYen district – BacGiang Province

Groups / *Disease*	*Veterans exposed to A.O/Dioxin N=1647 (%)*	*Veterans non exposed N=293 (%)*	*P*
Respiratory	42	23	< 0.05
Chronic bronchitis	29	17.6	
Digestive diseases	40.7	33.7	
Urological diseases	33	18.7	< 0.05
Dermatitis	22	14.6	
Nervous system diseases	20.9	17.3	
Neurasthenia			
Cardio Vascular diseases	7	0.3	< 0.05

(*Source: Division 10-80, 2000*)

3. The following epidemiological survey depicting a retrospective study on 19 communities including:

a. Five districts in the inner part of HaNoi City: BaDinh, HoanKiem, HaiBaTrung, DongDa, TayHo: over 4201 veterans A, 4953 veterans B.

b. Twelve communities in the delta (surrounding HaNoi City and other parts):

- TuLiem district (HaNoi): over 782 veterans A and 1300 veterans B;
- GiaLam district (HaNoi): over 1663 veterans A and 4504 veterans B;
- ThanhTri district (HaNoi): over 3800 veterans A, 8769 veterans B, and 75112 citizens;
- VinhTrung quater and HoaTho commune (DaNang City): 27956 citizens;
- NhuanDuc and PhuocVinhAn communes (CuTri district, Hochiminh City): 17139 citizens;
- ChanhPhuHoa commune (BinhDuong Province), TanPhong commune (Tayninh Province), DocBinhKieu commune (DongThap Province): 22011 citizens;
- TanPhuocKhanh commune (BinhDuong Province): 10997 citizens;

- LaiThieu commune (BinhDuong Province): 25159 citizens.

c. Two communes in the mountainous region:

- Aso and HuongLam communes (Aluoi district – Hue Province): 2252 citizens.

On analysis the morbidity of 19 residential communities, we could generalize Vietnam people disease into three main groups:

(1) The digestive, transmissible and respiratory diseases groups are pathological manifestation in an agricultural tropical country on its course of development.
(2) The circulatory and cardiological, muscle-bone-joint, and urological disease group are those of the initial phase of urbanization and industrialization.
(3) The nervous, digestive, dermatitis, cancer...can be considered as the imprints of the use of war chemicals, especially for military, paramilitary, and people linked to Agent Orange/Dioxin exposure.

The U.S government dragged its heels on investigating the health effects from Agent Orange exposure, so Congress shifted the responsibility away from federal agencies and to the National Academy of Sciences' Institute of Medicine (IOM). IOM formed an expert, independent panel in 1992, and it has produced three reports since then. The reports review all the available scientific literature and judge which diseases may be associated with Agent Orange exposure. The Department of Veterans Affairs can then pay disability compensation to any Vietnam veteran who develops that disease. The list of diseases follows.

- Chloracne
- Non-Hodgkin's lymphoma
- Multiple myeloma
- Respiratory cancers
- Lung
- Trachea
- Larynx
- Bronchus
- Prostate Cancer
- Soft Tissue Sarcoma
- Hodkins disease
- Porphyria cutanea tarda
- Peripheral neuropathy
- Spina Bifida in the children of Vietnam veterans
- Type 2 Diabetes
- Chronic Lymphocytic Leukemia (CLL)

2.6 Epidemiology in the study of Agent Orange

The study has been conducted with an epidemiological retrospective cohort method, in combination with a statistical case-control approach, clinical and laboratory examination (chemical, bio-chemistry, hematological and pathological, etc...).

1. The control group consist of people living on the north-bound part of the 17th parallel, the veterans operating in the said area, who are not endangered of exposure to war chemicals, among which , Agent Orange/Dioxin is the main substance.

2. The studied cases are the so-called veterans B, who had been in the south-bound part of 17th parallel, where the localities were seriously sprayed with war chemicals. These subjects can be divided into two sub-groups:
- (1): the sub-group one composes of those who prior to their service in the Southern part of the 17th parallel, had got married and had children that might or might not have congenital deformities. This period was named Bo phase. After certain years in service in the southern localities with much risks of exposure to A.O/Dioxin – this phase was called B1 – they were mobilized back to their homeland in the Northern part of the 17th parallel. The following reproductive period in their families was related to the B1 phase;
- (2): the sub-group two includes single youngsters who went into the Southern part of the 17th parallel. After demobilizing, they come back to their homeland, got married with whom having not in touch with A.O. Each individual in this sub-group had only one period in-service in the Southern part, as called the B1 phase. Reproductive accidents, if that happened could be considered as consequences from A.O/Dioxin.

During the survey, an establishing of the full-detail genealogy for each individual was done. Genealogy is a necessary document for the study (diagnostic, and assessment, etc...) the consequences on health caused by A.O/Dioxin (e.g. the obstetrical accidents, diseases, etc...).

In the Southern part of the 17th parallel, the classification of the residential population in two sub-groups for those who was exposed and those who was not exposed to A.O/Dioxin was more difficult, because of the environmental pollution, the mix-up of the population settlement, etc...In fact, it could be divided in two sub-groups:
- (1): the aborigine people living in the less sprayed area and they themselves were less endangered of being seriously infected;
- (2): the military and Para military participants (belong to both sides: the former SaiGon Government, and the Provisory revolutionary Government of the South VietNam) who usually in-service in the areas sprayed chemicals during the war.

2.7 Characteristics of the congenital anomalies presumably caused by Agent Orange/Dioxin

The strong toxicity of dioxin, after penetrating into the human body, generates several notorious features on congenital anomalies for F1 generation of those who were exposed to A.O/Dioxin.

2.7.1 High frequency

1. S. Harada and M. Kida during their survey in DocBinhKieu commune (ThapMuoi district, DongThap Province) and in Song Be Province, recorded that the frequencies of anencephalia, cleft-lip and cleft palate far-surpassed those of others countries in the wordl
2. The epidemiological survey in PhucLoi and QuyMong communes (YenBai Province); VinhTrung commune (DaNang City); NhuanDuc and PhuocVinhAn communes (CuChi district, Hochiminh City) showed that (*see table 2.4*) :

- The indices on the veterans' F1 children are so high compared with those on congenital anomalies of the sub-group who was not exposed to A.O/Dioxin recorded from the two communes in YenBai Province and the above mentioned in DaNang City and Hochiminh City;
- The frequencies congenital anomalies in the F1 generation recorded in the two communes (YenBai Province) are lower than in those communes in DaNang and HCMC.

Table 2.4 Dioxin and reproductive accidents in Phucloi, QuyMong communes (YenBai Province)

No	***Objects*** / ***Indices***	*Veterans A*	*Veterans B*	*Statistic indices of the two groups*
1	Veterans number	261	206	
2	Families number	261	206	
3	Pregnancy times: N	1170	1191	
	Children number/family	4.4	5.8	
4	Number of families having congenital anomalies children	7	67	$X^2 = 76.72$ RR = 12.13
	% N	2.7	32.5	5.69<RR<25.85 P<0.01
5	Obstetrical accidents (mainly miscarriage)	8	85	$X^2 = 64.93$ RR = 10.44
	% N	0.7	7	5.08<RR<21.45 P<0.01
6	Number of F1 children suffering from congenital anomalies	7	105	$X^2 = 88.17$ RR = 14.74 6.89<RR<31.54
	%N	0.6	8.8	P<0.01
7	Mortality rate of F1 generation suffering from congenital anomalies	0	28	
	% of congenital anomalies F1	0	27	
8	Reproductive accidents	15	190	$X^2 = 160.15$
	%N	1.3	16.0	RR = 12.44 740<RR<20.92 P<0.01

(*Source: Division 10-80, 2000*)

3. The congenital anomalies recorded from TuDu hospital: increased gradually during during the 70s and 80s decades, then reduced gradually in the 90s decade

2.7.2 Congenital anomalities are usually serious with its following manifestations:

1. *High rate of disabled; the most affected organ are:*

- the nervous system: anencephalia; hydrocephalia; cerebral palsy; epilepsy; mental redadation.
- The senses: anophtalmia (one or both sides): blindness (one or both eyes) cataract; deaf-mute, etc...
- Limbs deformities: phocomelia; dysmelia (two or four limbs), etc...

2. *Congenital anomalies gave on an individual with serious disabledness are usually relating to the following organs (as cited above):*

Several deformities on several organs can appear on an individual, making it a form of multi-deformities composing of a main physical defect and one or some associating ones. The most frequent complex-deformity was the form of mental retardation, in combination with deaf muteness, paralysis, talipes (Clubfoot), strabismus, etc...

The complex-deformities could reach 49% out of the tgotal number of congenital anomalies.

2.7.3 In a family whose parent (father or mother) was endangered of exposure:

- One child suffering from congenital anomalies and other obstetrical accidents (the most frequent was spontaneous abortion);
- Several children suffering from congenital anomalies with the same pattern (Squint-strabismus; mental retardation, blindness; harelip; cleft-lip and cleft palate, etc...) or each child bearing a different deformity (Squint-strabismus; deaf-muteness; mental retardation; neoplasm on a limb; etc...);

The families having their children suffering from the above disorders rose up to 25% of the total families exposed to A.O/Dioxin.

2.7.4 High possibility of generating monster:

Particulary only in QuyMong commune (YenBai Province) ten out of 206 veterns B families had given birth to monsters.

2.7.5 In a family if both parents were exposed to A.O/Dioxin

There would be a high possibility of obstetrical, especially serious congenital deformities in their F1 generation.

2.7.6 Epidemiological surveys in the communities

During the surveys, the physicians and medical officers could find out only the congenital anomalies apparent to the eyes after birth; obviously the discreet and hidden congenital anomalies had not been found, and had not caught their attention, such as:

- Congenital cardiological diseases;
- Defects relating to nervous system, senses, internal organs and entrails;
- Illnesses appearing a long time (many years) after birth: newborn children developed normally until a certain age, then occured the disorder;
- The spina bifida defect was a typical example. Epidemiological surveys statistic are usually 40% lower than real figures.

2.7.7 Follow – up of congenital anomalies

On keeping close follow-up congenital anomalies presumably linked to A.O/Dioxin exposure, the following things can be seen:

- The diagram showing congenital deformities of the veteran's F1 generation rose gradually in the ealy year of the 70s, reaching its highest point in the last years of the 70s and the early of 80s;
- It lowered down ahrply since the year 1985;
- The diagram showing that of the localities in the Southern part of the 17th parallel extended for 5 to 10 years, especially at the "hot spots";

One noticeable point was that during the 60s, 70s, 80s, and even 90s the type of similar serious congenital anomalies were still seen on individuals.

2.7.8 A roll of congenital anomalies presumably caused by Agent Orange/Dioxin

This roll collected the names of congenital anomalies recorded through clinical practice and through epidemiological surveys in several population communities, and on the individual endangered of being exposed to dioxin.

Attached to the roll was a scoring table that helped to assess the congenital anomalies cases

Table 2.5 Congenital anomalies suspected linked to A.O/Dioxin exposure among the F1 generation throughout the country (1995)

No	*Name of localities*	*Population*	*Total of injuries*	*Acquired injuries*	*Congenital anomalies*	*Congenital anomalies presumed by Dioxin*	*‰ of population*
Rural area							
1	30 communes throughout the country	263,007	1,041	165	876	477	1.8
2	3 districts in the Southern part from the 17th parallel	502,180	1,105	164	941	485	0.97
3	34 communes in GiaLam district (HaNoi)	296,318				505	1.70
4	22 communes in ThaiBinh Province	136,856			904	211	1.54
5	TanPhuocKhanh commune – LaiThieu district (BinhDuong Province)	36,156			202	79	2.2
6	Hue City						

No	*Name of localities*	*Population*	*Total of injuries*	*Acquired injuries*	*Congenital anomalies*	*Congenital anomalies presumed by Dioxin*	*‰ of population*
	ANgo commune – ALuoi district	2,185				11	5.0
	HuongLam communes – Aluoi district	1,339				21	15.7
	ASo commune – Aluoi district	922				20	21.69
	Sub-total	*1,238,963*			*2,923*	*1,809*	*1.46*
Urban area							
7	Hue City	220,176	698		546	265	1.2
8	Three towns in the Southern part from 17th parallel: ThuDauMot, BienHoa, Vungtau	624,356	915	323	593	311	0.53
9	Five districts in central of HaNoi City	871,080			853	181	0.21
10	Two communes in Hochiminh City	31,507			136	74	2.30
	Sub-total	*1,747,119*			*2,218*	*851*	*0.50*
	TOTAL	**2,986,082**				**2,660**	**0.9** ≈ **1‰**

(Source: Division 10-80, 2000)

2.8 Studies on congenital anomalies in grand children (F2) generation

In the early years of the 90s, here and there the congenital anomalies encountered at veterans'F2 generation exposed to the Ranch Hand Operation, Agent Orange/Dioxin. These were the F1 generation's children. They might get discreet and hidden defects of inner organs, or a slight injury which was cured (for instance harelip, polydactylism etc.); most of the victims had a normal appearance.

Among the others, Dioxin became a new causative agent, ever since. On identifying the cause of any congenital anomalies in the F2 generation, it is necessary to establish for each F2 object an individual genealogy including at least two higher generations (F1 and F) with full details for these generations and apply the assessment approach same to that for the F1 generation.

In the northern part of the 17th parallel, the identification of the cause for congenital anomalies from Dioxin was not too difficult.

But in the southern part of the 17th Parallel, the assessment is much more difficult, however, the similar approach could still be applied as having been implemented in the northern part.

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CHAPTER III: REVIEW OF METHODS FOR PCDD/Fs RESIDUE ANALYSES

3.1 Introduction

Recently the analytical methods for PCDD/Fs residues in different matrices have been developed very rapidly and the detection limit is lower and lower as well as the sample size required for analysis is less and less by the time.

Normally the PCDD/Fs residues are binded inside the structure of relative complicated matrices such soil, sediment, animal tissue, vegetable, etc. The analytical procedure for PCDD/Fs is generally similar for halogen micro-contaminants and should be divided in some basic steps, including:

1. Sample preparation
2. Extraction
3. Purification
4. Concentration – Isolation for final analysis
5. Identification and quantification

Due to the extremely high toxicity and biological activity of PCDD/fs as shown in chapter I, we should to consider choosing very sensitive, selective and specific analytical techniques to limit the risks of exposure to PCDD/Fs (*IARC, 1991*). Early searchers for PCDD/Fs in the environment were largely unsuccessful, mainly because of the lack of sufficiently methods. Nowadays with the development of analytical methods and equipments we could find very low concentration such nanogram per kilogram (ppt) levels and lower. These levels are sufficient to account for most of our body burden based on the existing standards.

In the beginning GC-ECD had been used for PCDD/Fs analysis (in 60’ and 70’ decades), so the results reported in this period have not very high precision (normally PCDD/Fs detected concentration was very high because the methods used were insufficient to select and separate the PCDD/Fs compounds from other halogen contaminants). Since years 80’ , the GC/MS has been used for PCDD/Fs detection as well as more advanced techniques for sample preparation and purification such multi-column chromatographic system, HPLC system, etc., so the trust of the results were higher. 2,3,7,8-TCDD or TCDD was the most concerned congener in the beginning, but now all 17 2,3,7,8-PCDD/Fs congeners are interesting with the TEF (*see chapter I*).

In this chapter we present all basic steps for PCDD/Fs analysis, the required equipment and possibility to apply it. We consider each advantage and also disadvantage to find a suitable analytical procedure according to our laboratory condition. There are some aspects that proposed analytical procedure should be satisfied: accorded to available instrument (LRMS); low consummation of chemicals/solvents (low price of analysis); and still satisfied QA/QC for PCDD/Fs analysis. As shown in the aims of this thesis, a supplementary and also important task is to establish and develop a laboratory for dioxin analysis/research (a reference laboratory), so a suitable analytical method is very important and necessary to fulfill it.

3.2 Sample handling and laboratory practice

Sample handling procedure for PCDD/Fs analyses are similar to those employed in the determination of other pesticides and residues (*IARC, 1991*). In fact we can use the same laboratory for PCDD/Fs and other organic micro-contaminants such PCBs, DDT, etc. Off-course it depend on the real conditions we could separate the laboratory for PCDD/Fs from the others to prevent cross-contamination due to ultra-trace-level of PCDD/Fs in our samples (especially for glassware and so all). The sampling procedure we not discus in detail here and we will present it in next chapters of this thesis.

The sample handling is very important if we should analyze a wide range of sample media (in our case). In such case, rigorous separation of equipment and glassware has to be made, and initial sample treatment may have to be carried out in separate laboratories.

In fact, the PCDD/Fs analyses are very complex, time-consuming and expensive (one individual analysis takes normally about 3-5 days, we always economize the time by the series analyses – usually 6 samples), so we should consider to limit the number of analyses but still to reach our purposes. Samples after retrieving should be stored properly: soil and sediment samples could be stored in room temperature or better in low temperature (5^oC) and in sun-light absence; biological samples such fish, adipose tissue, etc. should be stored in fridge (-15 to -20^oC).

Sample size depends on the availability of the medium and the detection threshold required. Two things should be considered: the possibility to do the sampling, especially for the biological samples such breast milk, blood, adipose; and the second that the representation of the sample. For example: to analysis the PCDD/Fs concentration in blood serum or breast milk, normally the analytical procedure requires a volume about 50-100mL of blood/brest milk, it is not always possible. Since for blood serum we often do sampling on a group of people, and then make the composite sample.

Usually, a large sample is sub-sampled (i.e. soil or sediment samples), after blending, mixing, homogenizating or comminuting. The sample size for different media is presented in the table below:

Table 3.1 Test portion size for various matrices

	Soil, sediment, paper pulp	Fly ash	Water	Sludges, fuel oil	Fish tissue	Human adipose tissue
Test portion weight (g)	10 – 50	10 – 20	1000	2	20	2 – 10
Final extract volume (μL)	10 – 50	50	10 – 50	50	10 – 50	10 – 50

(*US.EPA 1991, 1994, 1998; IARC, 1991; Sauvain, 1993; Paz et al., 1991*)

Sample size may also be affected by capacity limitations inherent in the sample clean-up scheme, i.e. surface and drinking water always need a large volume of sample for analyses due to their very low level of PCDD/Fs (lower than 0.01 pg/kg) (*IARC, 1991*); in contrary the contaminated samples such fly ash or industrial chemicals need only few gram for analyses. Sufficient sample size prevents contamination of glassware and equipment with excessive amounts of PCDD/Fs. Before we begin our test, it should to have as much as possible information about the samples – contamination potential.

To avoid the contamination/cross-contamination from solvents, reagents, glasswares, equipments we should follow strictly some criteria: i.e solvents used for analyses are ulta-trace-level (super purity solvents or pesticide grade); glassware used for analyses should be washed with special detergents and rinsed with proper solvents before use; the contacts with plastic materials should be limited/avoided; adsorbents should be solvent-washed and stored in clean glass bottles; gases used for analyses should be purified (99.9995%) (*US.EPA 1991, 1994, 1998; IARC, 1991*).

3.3 Extraction

Extraction is the first step to isolate PCDD/Fs from initial materials. Normally, solvents or solvent mixture are used in extraction techniques. The PCDD/Fs and other compounds which are adsorbed on matrix structure will dissolve into solvent and by this way are separated from initial matrix.

Two strategies can be considered (*IARC, 1991*): in the first approach, we try to recover all PCDD/Fs in a single fraction by a containment-enrichment procedure, with minimal or no isomer discrimination. Homologue and isomer separation are then carried out in the final HRGC analysis. In this way, complete isomer and homologue distribution patterns are obtained which aid in identifying the sources and origins of the compounds. This approach is very useful for global researches like our research. In the second approach, specific isomers are recovered, and then analyzed in different fractions, mainly after reversed-phase and normal-phase HPLC. This approach provides very clean and easily analyzable fractions, but the results are not integrated and in some case not enough data provided to assess the origin or distribution of PCDD/Fs. Normally, this approach is suitable for specific congeners such 2,3,7,8-TCDD.

* *Soil and sediment extraction*

For extractions of solid samples, Soxhlet is widely accepted as a robust liquid–solid extraction technique. Typical solvents in Soxhlet extraction of PCDD/Fs from soil and sediments have been toluene, hexane and hexane/acetone mixtures. The extraction time has varied in the range 12–24 h. The main drawback of this technique is the fact that refluxing with cold solvent is time consuming. Furthermore, solvent consumption is considerable, as a large amount of solvent has to be evaporated before the subsequent clean-up (*US.EPA 1991, 1994, 1998; IARC, 1991; Sauvain, 1993; Paz et al., 1991*). Soxhlet extraction has been considered as a reference technique in many works (usually used to compare the effectiveness with other methods). It has been largely used in the extraction of PCDD/Fs from soil, sediments, etc. as reported in many articles. 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 cheep.

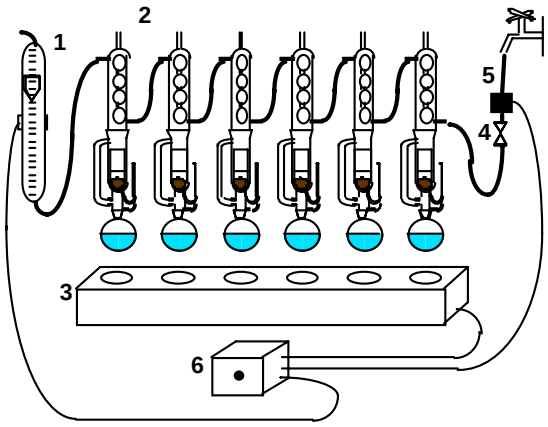

Fig 3.1 Soxhlet extraction system scheme (Source: Ferrary, 2003)

In the last few years, there have been efforts to develop extraction techniques that allow efficient extraction and reduced solvent volumes in shorter times, incorporating high levels of automation: accelerated solvent extraction (ASE) and supercritical fluid extraction (SFE). In these techniques a solvent is delivered into an extraction cell containing the sample, which is then elevated in temperature and pressure. Typically, fully automated ASE/SFE can be achieved in less than 30 min and with less than 30 mL of solvent per sample. Temperatures and pressures were in the ranges 100–150°C and 1000–1500 psi, respectively. Using these techniques, recoveries are similar to those using conventional Soxhlet extraction. However, lower standard deviations were found with it, probably because the system was automated (*Santosand Galceran, 2002; Eijarrat, and Barcelo', 2004; Ferrary, 2003; Friedrich and Kleiböhmer, 1997*). These techniques also have some problems such experimental costs, difficulty of handling, and so on (*Mannila et al., 2002; Chia et al., 2004*).

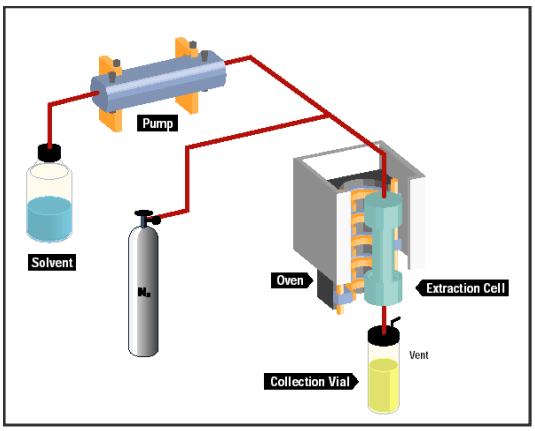

Figure 3.2 ASE Dionex™ system scheme (Source: Ferrary, 2003)

Recently, a new technique called solid-phase micro-extraction (SPME) has been applied. It is a fast, convenient, solvent-free extraction technique, originally developed by Pawliszyn and co-workers (*Chia et al., 2004*). This technique is dramatically increased and it allows efficient extraction, reduced solvent consumption and analysis time, and it is easily automated. SPME is applied to the determination of some PCDD/Fs congeners in complex solid samples, such as sludge. Unlike conventional methods, which involve solvent extraction and clean-up steps

before instrumental analysis, the proposed method uses headspace extraction, and particulate contamination of the chromatographic system is thus prevented. Due to the low volatility of dioxins and their strong binding to soil, it is difficult to extract these analytes from soil samples by SPME, so it should to improve the evaporation of the analytes from the soil to the headspace by heating and ultrasonic activating screener. The sensitivity of some dioxins (hepta and octachloro-substituted) in this technique is not so good in comparison with Soxhlet or ASE (*Chia et al., 2004*).

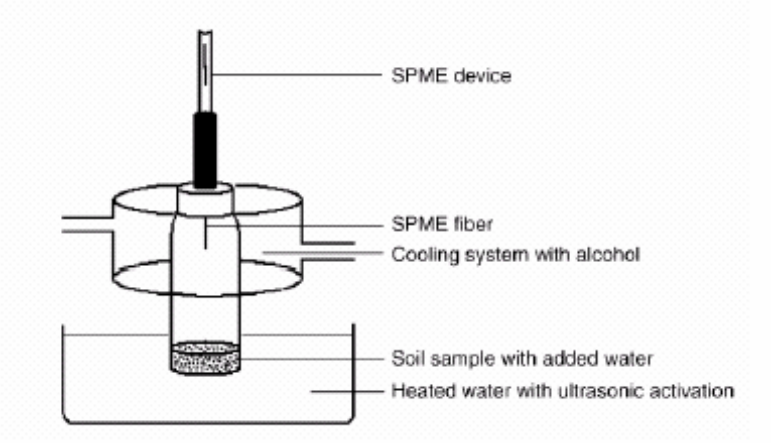

Fig. 3.3 Schematic diagram of extraction apparatus (Source: Chia et al., 2004)

* *Biological sample extraction*

Biological samples such fish 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 and the solvents used are different. The common solvents used for extraction are dichloromethane (DCM), dichloromethane/n-hexane (1:1), pentane/DCM (1:1), ethanol/n-hexane (1:3) (*Focant et al., 2004; Focant and De Pauw, 2002; Ryand and Mills, 1997*).

* *Fly ash extraction*

The acid treatment is commonly used to destroy the inorganic matrix of the samples and to make PCDD/Fs more accessible for extraction (*US.EPA 1991, 1994, 1998; IARC, 1991; Sauvain, 1993; Paz et al., 1991*). Normally we use the hydrochloric acid or acid sulfuric. The extraction procedure for fly ash samples is more complex than for soil and biological samples: the acid used for sample treatment is extracted by liquid-liquid procedure (usually with n-hexane and DCM) while the solid part (fly ash after acid treatment) is extracted by normal techniques as described above (Soxhlet, ASE, SFE, etc.) (*US.EPA 1991, 1994, 1998; IARC, 1991; Sauvain, 1993; Kemmochi, Y., and Tsutsumi, K., 2001; Richter et al., 1997*).

3.4 Sample purification

Purification of the extracts is carried out to remove co-extracted compounds which would prevent futher concentration and interfere in the final analysis. For this purpose, chromatographic procedures are most often used, sometimes preceded by acid treatment to destroy interfering compounds (triglycerides) (*IARC, 1991*)

Generally, in dioxin analysis procedure we use a series of the adsorbent chromatography columns to separate the PCDD/Fs from interfering compounds. Inevitably we could not separate completely because we never know all the compounds presented in our samples and even we know we have not enough time to do it. However we should consider and do as much as possible to limit the error of our analyses. The table 3.2 below presents the compounds that could interfere with the PCDD/Fs analyses.

Depend on the interfering compound types presented in our sample matrixes we will choose the suitable combination of the adsorbents. The common adsorbents used in purification for PCDD/Fs analyses are silica, florisil, alumina, celite or chemically modified adsorbents (celite and silica with H_2SO_4, KOH, CsOH, or $AgNO_3$) (*IARC, 1991*). Silica is used to retain fatty and oily components, whereas alumina is used to remove PCBs and other, less polar, chlorinated contaminants. Florisil (magnesium silicate) is used to separate the compounds such PCBs, PCNs, PCDEs, DDE and PAHs from interested PCDD/Fs.

Silica gel ($SiO_2 \times H_2O$) is the most widely used general purpose adsorbent for sample cleanup although it may irreversibly bind some strongly basic substances. Silica column used in PCDD/Fs analysis procedure is served like treatment column. Normally we use the combination of three silica types: activated silica (neutral silica activated by heating), basic silica (silica mixes with KOH, CsOH, $AgNO_3$) and acidic silica (silica mixed with $H_2SO_{2conc.}$). The extract is passed through silica combined column and the fatty compounds are retained in the silica structure and the interested compound are eluted by a suitable solvent (usually n-hexane) (*US.EPA, 1994; IARC, 1991; Fabrellas et al., 2004, etc.*). The silica treatment could be done manually or automatically. Manual silica treatment is carried in chromatography column with different diameter (depend on methods applied). The silica layers are filled

Table 3.2 Polychlorinated aromatic compounds potentially interfering in analyses of PCDD/Fs

Compound	Interference		Interfering ion	Molecular ion (M^+, ^{35}Cl only) m/z values									
	PCDDs	PCDFs		mono-chloro	di	tri	tetra	penta	hexa	hepta	octa	nona	deca
PCDDs	-	-	-	218	252	286	320	354	388	422	456		
PCDFs	+	-	-	202	236	270	304	338	372	406	440		
PCBs	-	-	M^+-Cl_2/Cl_4	188	222	256	290	324	358	392	426	460	494
PCTs	-	+	M^+ +6	264	298	232	366	400	434	468	502	536	570
PCNs	-	+	M^+ +6	162	196	230	264	298	332	366	400		
PCPAHs	+	+	M^+	236	270	304	338	372	406	440	474	508	542
PCBPs	-	-	M^+	186	220	254	288	322	356	390	424		
PCDPEs	+	+	M^+-Cl_2	204	238	272	306	340	374	408	442	476	510
Methoxy-PCBs	+	+	M^+/M^+-50	218	252	286	320	354	388	422	456	490	
Methylthio-PCBs	+	-	M^+ -50	234	268	302	336	370	404	438	472	506	
PCBPEs	+	-	M^+	218	252	286	320	354	388	422	456	490	524
PCXes	+	-	M^+ +2	216	250	284	318	352	386	420	454	488	522
PCXos	-	+	M^+ -CO	230	264	298	332	366	400	434	468		

(*Source: IARC, 1991*)

Note: PCTs: Polychlorinated terphenyls; PCNs: Polychlorinated naphthalenes; PCPAHs: Polychlorinated polynuclear aromatic hydrocarbons; PCBPs: Polychlorinated biphenylenes; PCDPEs: Polychlorinated diphenyl ethers; PCBPEs: Polychlorinated benzylphenyl ethers; PCXes: Polychlorinated xanthenes; PCXos: Polychlorinated xathones.

(dry or wet filled with n-hexane) and all process is carried out manually. The automatic silica treatment is usually done with Fluid Management System (FMS) and the silica column is provided by producers, available for use.

Fig. 3.4 The FMS system (in the left) and manual column (in the right)

Florisil is a registered name of U.S. Silica Co., is a magnesium silicate with basic properties. Florisil is prepared by precipitation from a mixture of magnesium sulfate and sodium silicate solutions and calcinated at about 1200°C. It is a very porous adsorbent with a surface area of about 200-250 m^2/g. Florisil has been used for the cleanup of pesticide residue and other chlorinated hydrocarbons; the separation of nitrogen compounds from hydrocarbons; the separation of aromatic compounds from aliphatic-aromatic mixtures; and similar application for use with fats, oils, and waxes. Additionally, florisil is considered good for separations with steroids, esters, ketones, glycerides, alkaloids, and some carbonhydrates. The florisil use for PCDD/Fs analyses has been reported in many documents (*IARC, 1991; US.EPA, 1994, 2000; etc*). In fact florisil is very effective to remove the undesired co-extracted compounds that multi-layer silica column could not retain. A disadvantage of florisil utilization is that we should use a large amount of solvent to elute the PCDD/Fs from it (about 30 mL/g florisil used) because florisil is very strongly holds the PCDD/Fs. Generally we use the relatively polar solvent such toluene or toluene/diethyl ether (9:1) to elute the PCDD/Fs from florisil compound and these solvents are contributed to the analysis cost. We can reduce the used solvent amount by de-activation by water (1-2%). In other hand by florisil treatment the extract is cleaner and our result is more precise, especially for the samples with relatively high fat content such human adipose and fish tissue (*Jasinski, 1989; Schramm et al., 1995; Ebert et al., 1999; Lundgren, 2003; etc*)

Alumina ($Al_2O_3 \times H_2O$) is prepared by the low temperature dehydration (< 700°C) of alumina trihydrate and is a mixture of γ alumina with smaller amount of α alumina (less active form) and sodium carbonate. By utilization of alumina column we can separate the PCDD/Fs from almost interfering compounds such chlorinated benzenes, PCBs and higher chlorinated diphenyls ethers. These compounds are eliminated in the first fractions (lower polar solvent mixture of n-hexane/DCM (2:98 or 5:95) (*US.EPA 1991, 1994, 1998; IARC, 1991; Sauvain, 1993; Paz et al., 1991; etc.*). PCDD/Fs are recovered by elution by polar solvent mixture (n-hexane/DCM 1:1 or 2:3). There are three available types of alumina in market: neutral, basic and acidic and we can use all for PCDD/Fs analyses. Many methods propose to use the basic alumina with very low/without water content (basic alumina Super I with 0% water). Normally, alumina column is served as the last step (or before the last) in purification procedure for PCDD/Fs analyses.

Depend on the methods used the alumina average quantity is about 4 -5g, alumina could be activated by heating from 130 – 180^{o}C for 3 – 8 hours to remove the water content or sometime it could be utilize directly without activating.

Carbon column is also utilized in PCDD/Fs analyses (*US.EPA 1991, 1994, 1998; IARC, 1991; Simon and walkeford, 2000; etc.*). The carbon quantity used is normally small (about 0.5g) and it is filled on Pasteur pipette. Planar aromatic compounds (including PCDD/Fs) are adsorbed on carbon surface and by this way they are separated from non-planar compounds. One big disadvantage of carbon column that its preparation is very complicated; the procedure demands in term of equipment and experience. The carbon used for this procedure is a specific activated carbon types such Amoco PX-21, carbopack B, C, etc. and it is relatively expensive. The carbon treatment is served as supplemental step and the multi-column cleanup procedure incorporating the carbon adsorption chromatography step is probably one of the best methods for isolation all PCDD/Fs congeners from complex sample matrices, with minimal isomer or homologue discrimination (*IARC, 1991*).

The High Performance Liquid Chromatography (HPLC) is also considered as a good alternative for purification mean of the PCDD/Fs extract (*IARC, 1991; US.EPA 1994, Lundgren, 2003; etc*). Though various substances which interfered with dioxin analysis can be removed efficiently with the multi-layer adsorbent columns as shown above, preparation of these columns is relative complicated and time-consuming, and there are problems with using and disposing many hazardous materials. Beside that, using many columns may has a lost of interested compounds due to solvent change (e.g. a loss could be happened during evaporating). Fractionation performance with the adsorbent columns is not always stable because of the presence of water and particle size distribution of used adsorbents (*Urano et al., 2001*). By HPLC system, the purification and the fractionation column could be done continuously and automatically, as well as the time consumed for analyses could be reduced considerably. One big disadvantage of this technique is that the primary investment cost for HPLC system is relatively high.

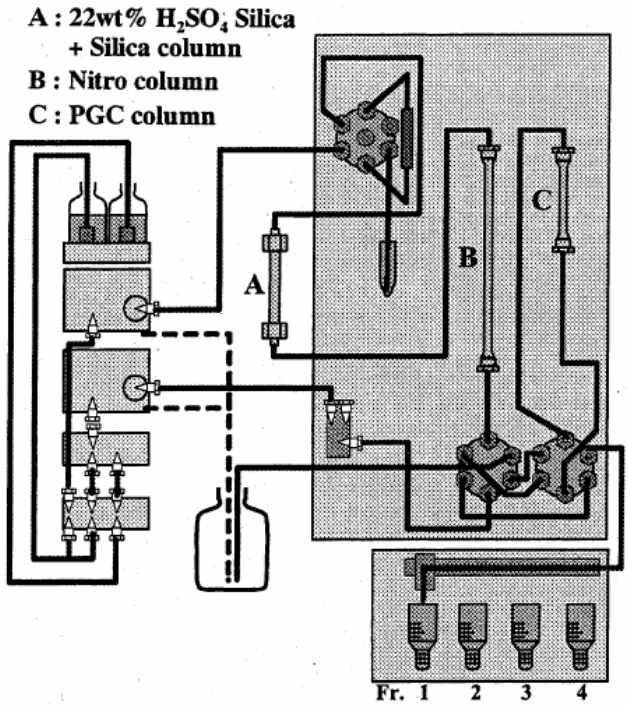

Fig 3.5 Diagram of HPLC system for purification in PCDD/Fs analyses (source: Urano et al., 2001)

3.5 Concentration – Isolation 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 10 – 50μL). By this way the concentration of PCDD/Fs in our extract is multiplied to 1.000.000 – 2.000.000 times and reached the detection limit of analytical equipment used for analyses (ppb, ppt or sub-ppt). Large volume of solvent is concentrated in a rotary evaporator at a temperature according to the solvent type used (e.g. 50 – 60^0C for toluene); the smaller volume of solvent is concentrated by a stream of purified nitrogen at room temperature to dryness and re-dissolved in recovery standard solution (*see next chapter for detail*). To minimize the loss during concentration by rotavapor (samples must not go to complete dryness), a small quantity of high-boiling point solvent is added to the extract (normally we use the pure organic solvents such dodecane, tetradecane, etc.).

Even if we take very complicated and carefully clean-up process, the final fraction may still contain other chlorinated contaminants such PCBs, PCNs, etc. These compounds may interfere in final analyses. So analyst should be well trained and have some experiences in result interpreting.

3.6 Identification and quantification of PCDD/Fs by GC/MS

The final purified extract is analyzed by GC/MS equipment: GC – gas chromatography is being used for homologue and isomer separation; and MS – mass spectrometry for the selective detection, identification and quantification of PCDD/Fs. Gas chromatography (GC) and mass spectrometry (MS) make an effective combination for chemical analysis. To effectively use GC/MS evidence one must understand the process.

3.6.1 GC/MS system

* *GC*

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 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.

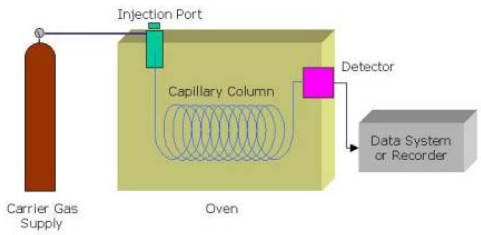

Fig. 3.6 Diagram of GC system

* *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):

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

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 t_R 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

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

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.

* *MS*

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*

1. Some common ionization types for GC/MS

A. ELECTRON IMPACT (EI)

Electron impact ionization is the classical ionization technique in mass spectrometry. In the ion source (10^{-7} - 10^{-5}mbar, 200°C – 250°C), the gaseous sample is bombarded with 70 eV electrons usually generated from a tungsten filament. 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 (almost) every mass spectrometer, too. Therefore, 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 a 8000 EI mass spectra library available on-line. (Nist Chemistry WebBook)

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.*

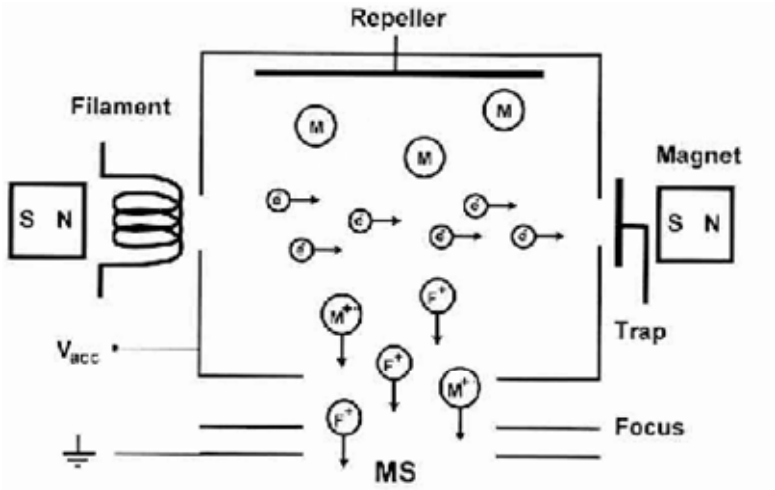

Fig. 3.7 Diagram of EI system

B. CHEMICAL IONIZATION –CI

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!).
PCI uses a reagent ion to react with the analyte molecules to form ions by either a proton or hydride transfer:
$MH + C_2H_5^+ \rightarrow MH_2^+ + C_2H_4$
$MH + C_2H_5^+ \rightarrow M^+ + C_2H_6$
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 CH4+ and CH3+ which further react with methane to form CH_5^+ and $C_2H_5^+$:
$CH_4^+ + CH_4 \rightarrow CH_5^+ + CH_3^+$
$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$

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 or 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.
$e^- + CH_4 \rightarrow e^-_{th}$
$M + e^-_{th} \rightarrow M^{-\cdot}$

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 $[M+NH_4]^+$ adducts*
- *basic compounds produce $[M+H]^+$ adducts*
- *non-polar and non-basic compounds are not ionized*

C *LASER IONIZATION (LIMS)*

A laser pulse ionizes some of the sample constituents. There is a number of LIMS techniques e.g. RIMS or MALDI.

Resonance ionization (RIMS)

One or more laser beams are tuned in resonance to transitions of a gas-phase atom or molecule to promote it in a stepwise fashion above its ionization potential to create an ion. Solid samples must be vaporized by heating, sputtering, or laser ablation.

Matrix-assisted laser desorption ionization (MALDI)

MALDI is a LIMS method for vaporizing and ionizing large biological molecules such as proteins or DNA fragments. The biological molecules are dispersed in a solid matrix such as nicotinic acid. A UV laser pulse ablates the matrix which carries some of the large molecules into the gas phase in an ionized form so they can be extracted into a mass spectrometer.

MALDI allows to determine the molecular weight of molecules up to 500 kDa, routinely 5 to 100 kDa (polymers, biomolecules, complexes, enzymes), depending on the analyzer. The MALDI techniques can be coupled with a time-of-flight analyzer (resolution and accuracy of the spectra are low but easy to handle and hence, most commonly used) or a Fourier-transform mass spectrometer (expensive, difficult to handle, low dynamical range, but very accurate).

MALDI characteristics in summary

- *Soft ionization method provides molecular weight information.*
- *Suitable for analyzing very large bio- or synthetic polymers.*
- *Sensitivity depends strongly upon the analyte.*
- *Suitable for analyzing polar and even ionic compounds (e.g. metal complexes).*
- *Less fragmentation.*
- *Pulsed ionization technique, in contrast to EI, CI, FAB, ESI, and APCI*

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

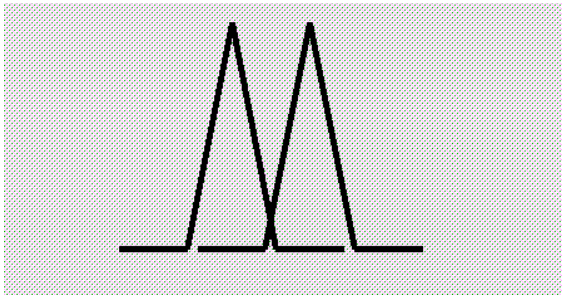

Fig. 3.8 Two peaks resolved to 10% valey

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. Some common MS types

All commonly used mass analyzers use electric and magnetic fields to apply a force on charged particles (ions). The relationship between force, mass, and the applied fields can be summarized in Newton's second law and the Lorentz force law:

F = ma (Newton's second law)

F= e(E+ v × **B)** (Lorentz force law)

where

- F is the force applied to the ion,
- m is the mass of the ion,
- a is the acceleration,
- e is the ionic charge,
- E is the electric field
- v x B is the vector cross product of the ion velocity and the applied magnetic field

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)

Principal of Operation

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.

A crude schematic of a quadrupole mass filter is shown below:

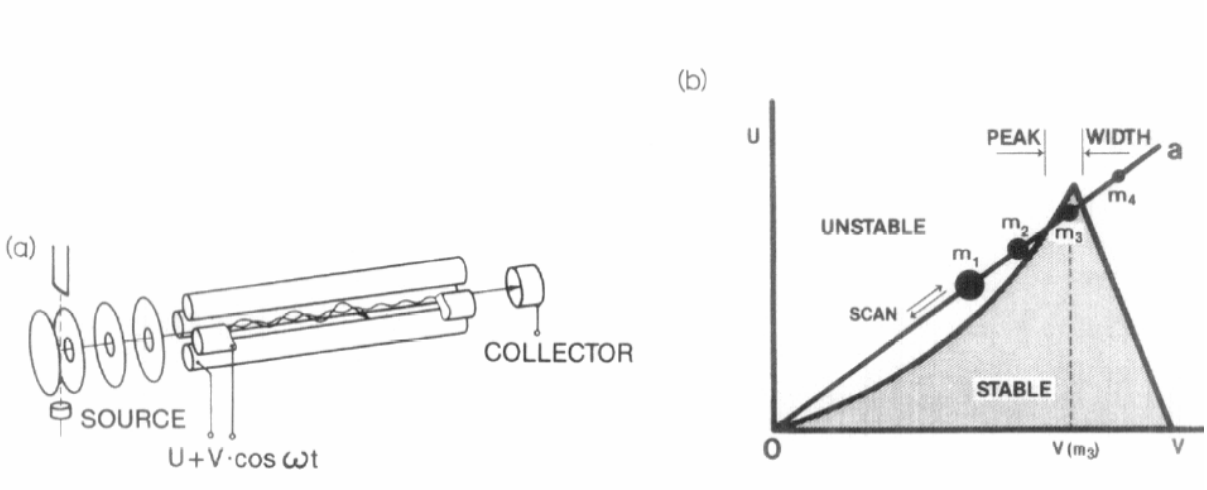

Fig.3.9 (a) Schematic of quadrupole analyzer. An rf voltage, V, with a superimposed d.c.voltage, U, is applied to the rods. The injected ions oscillate in the resulting quadrupole field . (b) The oscillations are stable only when the equation m = 2.83 eV/wri is fulfilled (mass = m, charge = e, circular frequency = w, field radius = ro). Ions having unstable oscillations are filtered out. According to the above equation, scanning of the masses can be performed by sweeping the rf voltage, V. The superimposed d.c. voltage, U, is also swept.while the ratio U/V is kept constant (slope o-a). The ions threaded" on the line o-a are then moving through the stable part of the stability diagram (m1 > m2 > m3 > m4).) (Source:Brunnee, 1987)

The operation of a quadrupole mass analyzer is usually treated in terms of a stability diagram that relates the applied DC potential (U) and the applied RF potential (V(t)) and the RF frequency (omega) to a stable vs. unstable ion trajectory through the quadrupole rods. A qualitative representation of a stability diagram for a given mass m is shown above: U and V are parameters that are proportional to U/m and V/m respectively.

Changing the slope of the scan line will change the resolution. Increasing the resolution decreases the number of ions that reach the detector (the region at the apex of the stable region that is bounded by the scan line). 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)

Principal of operation

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.

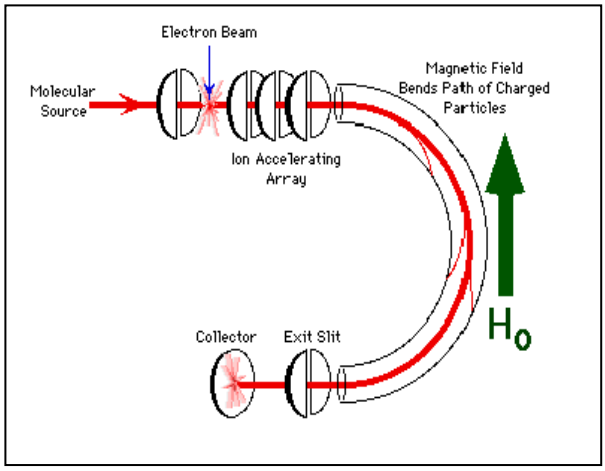

Fig. 3.10 Schematic of magnetic sector mass spectrometer (IVV, 2000)

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.

The dependence of mass-to-charge ratio on the electric and magnetic fields is easily derived. All ion formed in the ion source are accelerated to a kinetic energy, T of:

$$T = eV = \frac{mv^2}{2}$$

Solving for the velocity v we get:

$$v = \sqrt{\frac{2eV}{m}}$$

From the Lorentz force law, the magnetic field applies a force evB that must be equal to the centripetal force mv^2/r as the ions move in an arc through the magnetic sector:

$$evB = \frac{mv^2}{r}$$

Substituting for v, we arrive at the working equation for a magnetic sector mass spectrometer:

$$\frac{m}{e} = \frac{B^2 r^2}{2V}$$

As mentioned previously, the electric sector is usually held constant at a value which passes only ions having the specific kinetic energy, V. 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.

Linked scans, in which the magnetic and electric fields are scanned together, can be used to perform MS/MS experiments (product, precursor, and neutral loss) with a double focusing mass spectrometer.

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*

C. TIME-OF-FLIGHT MASS ANALYZERS(TOF-MS)

Principal of Operation

Linear

A time of flight mass spectrometer measures the mass-dependent time it takes ions of different masses to move from the ion source to the detector. This requires that the starting time (the time at which the ions leave the ion source) is well-defined. Therefore, ions are either formed by a pulsed ionization method (usually matrix-assisted laser desorption ionization, or MALDI), or various kinds of rapid electric field switching are used as a 'gate' to release the ions from the ion source in a very short time.

Recall that the kinetic energy of an ion leaving the ion source is:

$$T = eV = \frac{m v^2}{2}$$

The ion velocity, v, is the length of the flight path, L , divided by the flight time, t:

$$v = \frac{L}{t}$$

Substituting this expression for v into the kinetic energy relation, we can derive the working equation for the time-of-flight mass spectrometer:

$$\frac{m}{e} = \frac{2Vt^2}{L^2}$$

or, rearranging the equation to solve for the time-of-flight:

$$t = L\sqrt{\frac{m}{e}\frac{1}{2V}}$$

Reflectron

The ions leaving the ion source of a time-of-flight mass spectrometer have neither exactly the same starting times nor exactly the same kinetic energies (recall the "chromatic aberrations" discussed for magnetic sector mass spectrometers). Various time-of-flight mass spectrometer designs have been developed to compensate for these differences. A reflectron is an ion optic device in which ions in a time-of-flight mass spectrometer pass through a "mirror" or "reflectron" and their flight is reversed.

A linear-field reflectron allows ions with greater kinetic energies to penetrate deeper into the reflectron than ions with smaller kinetic energies. The ions that penetrate deeper will take longer to return to the detector. If a packet of ions of a given mass-to-charge ratio contains ions with varying kinetic energies, then the reflectron will decrease the spread in the ion flight times, and therefore improve the resolution of the time-of-flight mass spectrometer.

A curved-field reflectron ensures that the ideal detector position for the time-of-flight mass spectrometer does not vary with mass-to-charge ratio. This also results in improved resolution for time-of-flight mass spectrometers.

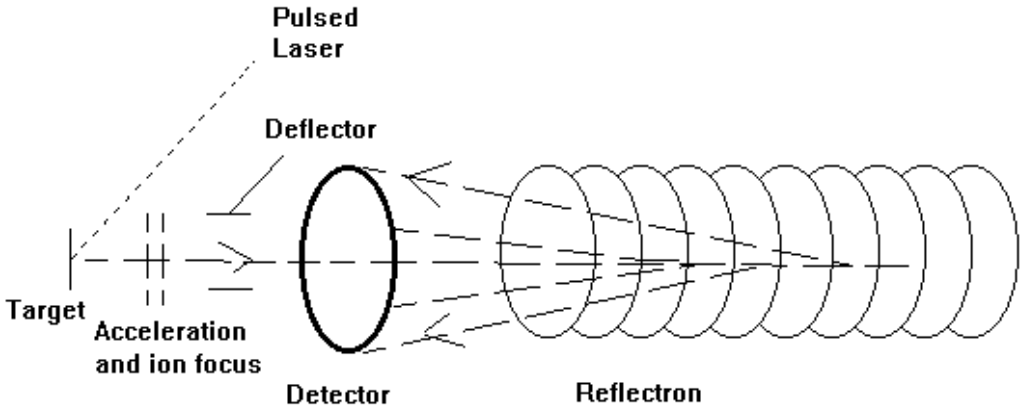

Fig. 3.11 Schematic of magnetic sector mass spectrometer (IVV, 2000)

Benefits

- *Fastest MS analyzer*
- *Well suited for pulsed ionization methods (method of choice for majority of MALDI mass spectrometer systems)*
- *High ion transmission*
- *MS/MS information from post-source decay*

- *Highest practical mass range of all MS analyzers*

Limitations

- *Requires pulsed ionization method or ion beam switching (duty cycle is a factor)*
- *Fast digitizers used in TOF can have limited dynamic range*
- *Limited precursor-ion selectivity for most MS/MS experiments*

Applications

- *Almost all MALDI systems*
- *Very fast GC/MS systems*

TOF-MS coupled with GC×GC technique is a new technique that developed dramatically in recent years and ***it seemed to be a good solution*** to reduce the cost and time for PCDD/Fs analyses (*we will discus about it later*).

3.6.2 Selection of GC column for PCDD/Fs analysis

Separation of PCDD/Fs homologue is easier than isomer separation and can be achieved by low-resolution technique, such as GC packed column. Isomer separation is more difficult, however so GC capillary column is ideal for PCDD/Fs isomer separation due to its very good efficiencies and sensitivities (*IARC, 1991*). The GC capillary column has higher resolution than GC packed column and so result is in less interference. However, the capillary column can handle only small (nanogram) amounts of sample due to its small diameter and thin film layer, so that the PCDD/Fs analysis much more stringent requirements in sample cleanup and purification procedure to prevent excessive contamination and overloading of the column by other components.

Glass or fused silica capillary column are the most utilized in PCDD/Fs analysis (*Gonnord et al, 1989; Tiernan et al., 1990; IARC, 1991; Sin and Kulshrestha, 1997; Abad et al 1&2, 1997; Chang-Chien et al, 2001; Fishman et al, 2004*). Glass capillary column are still used for PCDD/Fs analysis, however using fused silica is nowadays taking more predominance. There are commonly two types GC column used for PCDD/Fs analysis: non-polar/weakly-polar phases and polar phases. When selecting a capillary column for analysis, four basic parameters need to be considered. The order of relative importance is as follows:

- *Stationary Phase*
- *Column Internal Diameter*
- *Film Thickness*
- *Column Length*

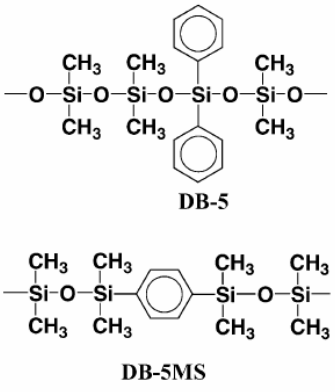

Fig. 3.12 Polymer structures of 5% phenyl methyl silicone (DB-5) and 5% phenyl silphenylene silicone-based (DB-5MS) GC columns (Source: Fishman et al., 2004)

The following GC capillary column types are commonly employed:

- Non-polar/weakly-polar phases: OV 1, SE 30, SE 52, SE 54, PS 255, DB-1, DB-5, OV 1701 and equivalents
- Polar phases: Silar 10C, SP 2330, SP 2331, SP 2340, CP SIL 88, DB-Dioxin and equivalents.

Column stationary phase

As we see in Fig. 3.12, for DB-5 column we have two types classified based on stationary phases: "conventional" type has the siloxane backbone with phenyl group attached to the side chains (DB-5) and "non-conventional" type has a phenyl ring in the polymer backbone intending to stiffen the polymer chain to reduce the amount of "back biting" and hence improve stability and lifetime (DB-5MS). Fishman et al. (2004) had done the studies on a series of 7 GC columns from variety of manufacturers (Varian CP-Sil 8 CB lowBleed/MS, Phenomenex ZB-5UMS, Agilent HP-5MS, Restek RTX-5MS, Supelco Equity-5, J&W Scientific DB-5 and DB-5MS). The result showed that none of the columns tested were able to separate all of seventeen 2,3,7,8-PCDD/Fs from other co-eluting isomers, which therefore leads to the overestimation of the TEQ value reported. However the using of DB-5MS and CP-Sil 8 columns done the lower TEQ values in comparison with DB-5, since DB-5MS is seemed to be more convenient for PCDD/Fs analysis.

Non-polar stationary phases allow the separation of PCDD/Fs homologues, but do not provide optimal isomer separation. The best solution for 2,3,7,8 PCDD/Fs substituted isomers is a DB-5 MS column with a length of 60m and the i.d is 0.25 mm, the film thickness is about 0.1 to 0.25 μm (*IARC, 1991; US.EPA, 1994; Fishman et al., 2004*). Normally, analysis by GC technique should use two columns with different polarity: one column for analysis and one served as complementary (confirmation). The US.EPA suggest the use of a DB-5 MS column, and DB-225 or SP 2330 (or equivalent) as a complementary column; Environment Canada suggest the use of a DB-5 MS column and DB-DIOXIN as a complementary column, and the VDI propose use of a DB-5 MS, and a Cp-Sil 88 confirmation column or equivalent (*Abad et al. 1&2, 1997*).

The study of Abad et al. (1997) has showed that all of 2,3,7,8 substituted PCDDs are well resolved on the DB-5 MS column, except 1,2,3,7,8,9-HxCDD and neighboring hexa-CDDs. For PCDFs, the most concern is the 2,3,4,7,8-PeCDF due to the its relatively high TEF (0.5). In this case, the Cp-Sil 88 column is better to resolve 2,3,4,7,8-PeCDF from non-toxic isomers (co-eluting isomers). These studies also reported that DB-5 MS column and DB-Dioxin are equally good in separation of 2,3,7,8-HxCDF , 1,2,3,4,7,8 and 1,2,3,6,7,8-HpCDF. However DB-5 MS is not good in separation of 2,3,4,6,7,8- and 1,2,3,7,8,9-HxCDF (for these compounds the Cp-Sil 88 is seemed to be better, even in comparison with DB-Dioxin column). Table 3.3 gives us a general overview for DB-5 MS in selection of 2,3,7,8-PCDD/Fs isomers.

The use of side-chain liquid crystalline polysiloxane polymer (PS3DBDE1) with high purity and high isotropic transition temperature served as a stationary phase in PCDD/Fs analysis had been reported by Chang-Chien et al. (2001). Result showed that the isomeric pair compounds 1,2,3,4-TeCDD vs. 2,3,7,8-TeCDD and 1,2,3,4,7,8-HxCDD vs. 1,2,3,6,7,8-HxCDD and the same substituted-chlorine number compounds 1,2,3,4,6,7,8-HpCDF vs. 1,2,3,4,6,7,8-HpCDD and1,2,3,4,6,7,8,9-OCDD vs. 1,2,3,4,6,7,8,9-OCDF had higher resolution than HP-5MS and RTX-5MS. Since this column could replace DB-225 column in confirmation of PCDD/Fs analysis.

Table 3.3 Isomer specific separation of 2,3,7,8-pCDD/Fs on DB-5 MS column

No	*Compound*	*DB-5 MS column*	*No*	*Compound*	*DB-5 MS column*
1	2,3,7,8-TCDD	++	8	2,3,7,8-TCDF	++
2	1,2,3,7,8-PeCDD	++	9	1,2,3,7,8-PeCDF	+
3	1,2,3,4,7,8-HxCDD	++	10	2,3,4,7,8-PeCDF	-
4	1,2,3,6,7,8-HxCDD	++	11	1,2,3,4,7,8-HxCDF	++
5	1,2,3,7,8,9-HxCDD	++	12	1,2,3,6,7,8-HxCDF	++
6	1,2,3,4,6,7,8-HpCDD	++	13	1,2,3,7,8,9-HxCDF	-
7	OCDD	++	14	2,3,4,6,7,8-HxCDF	++
			15	1,2,3,4,6,7,8-HpCDF	++
			16	1,2,3,4,7,8,9-HpCDF	++
			17	OCDF	++

(*Sorce: Abad et al 1, 1997*)
Note: ++: Baseline separated; +: Partially separated; -: Coelution with other congenenrs

Column internal diameter and film thickness

Column internal diameter (i.d) is closely related to its separation. Normally smaller i.d. column have higher separation efficiency, but its sample capacity is lower (that mean the sample quantity to be analyzed is smaller). The most common used column for PCDD/Fs analysis have i.d in the range of 0.22 to 0.35 mm. The film thickness is generally in the range of 0.15 to 0.25 μm (*IARC, 1991; Fishman et al, 2004*). Reduce the diameter by half could make the doubles of column efficiency. The thicker the stationary phase film, the greater the loading capacity. Overloading a column will always result in loss of resolution. If the column diameter is halved, while maintaining the same film thickness, then the loading capacity will also be halved. As a rule, a 0.32mm i.d column with a 0.25 micron film has a maximum loading capacity of approximately 100ng/component. As can be seen from the column selection area, most columns are offered in both 0.22mm and 0.25mm. A 0.22mm i.d × 25 m with 0.25μm film thickness column has 93000 theoretical plates whereas a 0.25mm i.d × 30m with 0.25μm film thickness column has 98000 theoretical plates. The shorter, smaller i.d column will give the same separations as the longer, larger i.d column in shorter time with only a difference in loading capacity of about 10%.

For samples with a large difference in solute concentrations, a thick film column is recommended. This will reduce the possibility of broad overloaded peaks co-eluting with other compounds of interest. If the separation of two solutes is sufficient and co-elution is still unlikely, even with large differences in concentration, then a thinner film can be used. A disadvantage of thin-film columns is that their capacity is very low for the least volatile compounds (OCDD/F) and therefore to lead to loss of the resolution for these compounds due to their overload. In other hand, the greater the film thickness has the greater the retention of a solute, therefore the higher the elution temperature. As a rule, doubling the film thickness could increase in elution temperature of approximately 15-200^{o}C, under isothermal conditions.

Column length

The capillary for PCDD/Fs analysis have a length of 15 to 60m, depend on the sample types, equipments used for analysis, etc. The longer column has more theoretical plates, so it is more effective for compound separation. Column dimensions (length, i.d and film thickness) determine the amount of stationary phase in a column and, together with the operating conditions (temperature, carrier gas flow), the retention time of the PCDD/Fs compounds.

There is a contradiction that we should consider: a long, narrow column shows the best separations, but also gives the longer retention times. And we know very well that we always try to shorter the time for the analyses, especially in the case if we have many samples that have to be analyzed. The best solution is that to select the shortest column length that will provide the required resolution for the analysis (12-30meters). If the maximum column length available is being used, and resolution of the sample mixture is still inadequate, then a possible change to the stationary phase being used or internal diameter may be needed. Resolution is proportional to the square root of the column efficiency. Therefore, doubling the column length will only increase the resolving power of the column by approximately 40%.

3.6.3 Retention time in PCDD/Fs analysis

Retention time is used as a parameter for PCDD/Fs identification (for identification, we should use both data: retention time and MS data). Retention time for various homologues and isomers show definite pattern related to molecular structure (*IARC, 1991*). Individual PCDD/Fs isomers can be identified by retention time matching with known standards and nowadays almost PCDD/Fs isomers pure standards could be found in the market.

Retention time depend on the GC column used for the analysis and also on the operating conditions of the analytical instruments. Since the retention time for each PCDD/Fs isomer is not a constant and varied between different columns. Elution temperatures of PCDD/Fs on GC columns are listed in the table below:

Table 3.4 Elution temperatures of PCDD/Fs on GC columns

PCDD/Fs group	*Elution temperature (^oC)*	
	25m SE 54 column	55m Silar 10C column
TCDD/Fs	203 – 212	203 – 225
PeCDD/Fs	215 – 224	214 – 234
HxCDD/Fs	229 – 236	226 – 246
HpCDD/fs	245 – 248	241 – 250
OCDD/F	260	259 – 260

(*Source: IARC, 1991*)

As we know that in PCDD/Fs we use the isotope-labelled (^{13}C, ^{37}Cl) as surrogates and internal standards. These standard compounds will be co-eluted with the native compounds, although they have higher molecular weight (up to 16 Da). However, due to operating conditions, the interactions between compounds in real matrix structure, etc., the retention time of native and labelled are not always the same and normally the labelled compounds will be eluted earlier than the native compound (e.g. relative retention time $^{12}C_{12}$-2,3,7,8-TCDD/$^{13}C_{12}$-TCDD = 1.002 – 1.006 for 55m Silar 10C column) (*IARC, 1991*).

Liang et al. (2000) has developed a model on relationships between chromatographic retentions and molecular structures of polychlorinated dibenzo-p-dioxins (PCDDs): log k'= A + B/T. They have found the quantitative relationships between the A, B values and the molecular structures. The A, B values of PCDDs with no standards available have been predicted according to these relationships. They are very useful in chromatographic identification and it is especially significant for the identification of these PCDDs of which standards are not available. Donnelly and Sovocool (1992) had showed a model which successfully predicts the order of elution and relative retentions of tetra-, penta- and hexa chlorodibenzo-p-dioxins (including 2,3,7,8-PCDDs) for gas chromatography (GC) columns

of different polarity, this model allows the correlation of GC retention time to dioxin substitution pattern and the prediction of dioxin elution order and relative retention time spacing for GC columns of different polarity. Normally, the 1,4 and 1,2,4 single rings and 1,9 ring/ring interactions tended to shift the relative retention times to higher normalized values with increasing GC column polarity while the l-, 2-, 1,2-, 2,3-, 1,2,3- and 1,2,3,4_substituted (chlorinated) single rings tended to shift the isomers to lower normalized relative retention times with increased GC column polarity. Generally, substitutions with vicinal chlorines (and to lesser degree vicinal hydrogen) show increased retention: isomers with no vicinal chlorines (1,3-substitutions) elute early, followed by those with only vicinal hydrogen (1,4-isomers), then by those with only vicinal chlorines (2,3-isomers). The last eluting isomers have both vicinal hydrogens and chlorines (1,2-substitution) (*IARC, 1991*). Based on this we have the window defining standard mixture for PCDD/Fs analysis that will be discussed later.

3.6.4 GC column temperature program and injection mode in PCDD/Fs 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 (*IARC, 1991*): e.g. for iso-octane 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 (160 – 200^{o}C, typically for tetra- groups of PCDD/Fs). The rest of PCDD/Fs 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 (*IARC, 1991*).

PCDD/Fs 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 PCDD/Fs 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 3.5 Recommended Linear Velocities and Flow Rates

Column i.d.	*Linear Velocity (cm/sec)*		*Flow rate (mL/min)*	
	Helium	Hydrogen	Helium	Hydrogen
0.18mm	30-45	45-60	0.5-0.7	0.7-0.9
0.25mm	30-45	45-60	0.9-1.3	1.3-1.8
0.32mm	30-45	45-60	1.4-2.2	2.2-2.9
0.53mm	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.

Both techniques are widely used in PCDD/Fs analysis, with split/splitless injection we can inject a larger volume of sample (generally 1.5 to 2μL) and we can use the solvent with relative high b.p such nonane, 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 (1μL) and uses the solvents with medium b.p such toluene or iso-octane. This technique is expected to be superior for accurate quantitative analyses; it results in larger sample transfer and less discrimination against less volative 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 then 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.

3.6.5 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) ionization is most commonly used for PCDD/Fs. Other ionization technique is NCI with very high sensitivity for higher chlorinated PCDD/Fs.

A. *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 PCDD/Fs in the presence of other chlorinated compounds. EI mass spectra of PCDD/Fs 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 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%. 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.

Based on the accurate mass values we could calculated exact mass for different PCDD/Fs: e.g the accurate mass of 2,3,7,8-TCDD is 319.8965 instead 320.

Fragmentation Patterns

The fragmentation of molecular ions into an assortment of fragment ions is a mixed blessing. The nature of the fragments often provides a clue to the molecular structure, since based on it we could identify the PCDD/Fs compounds by comparison the fragmentation pattern of unknown compound with the patterns given in libraries such Nist, Wiley, etc.

PCDD/Fs yield intense, characteristic, molecular ion clusters (M^+ and satellites). Major fragmentation of PCDDs occurs via loss of COCl to give M^+-COCl and M^+-2COCl, and of PCDFs via loss COCl and Cl_2, giving M^+-COCl and M^+-COCl-Cl_2. PCDD/Fs give a minor fragmentation via loss of Cl, Cl_2 and sometimes HCl from M^+ and major fragmentation ions, leading to M^+-Cl, M^+-Cl_2, M^+-$COCl_2$ and others. Table 3.6 gives the informations about the major fragmentation ions that is a basic for choosing of monitoring ions in SIM mode to identify and quantify the PCDD/Fs.

Generally, molecular ions and their satellites are the most important ions to identify PCDD/Fs and they are usually used in quantification methods for PCDD/Fs analysis. The m/z values and the relative abundances of the molecular ions are shown in table 3.7.

Table 3.6 Fragmentation ions for PCDD/Fs

Ion	***PCDDs***	***PCDFs***
M^+	Intense	Intense
M^+-COCl	m(-63)	m(-63)
M^+-COCl-Cl_2	-	m(-133)
M^+-2COCl	m(-126)	-
M^+-Cl	s(-35)	s(-35)
M^+-Cl_2	s(-70)	s(-70)
M^+-$COCl_2$	s(-98)	s(-98)
M^{2+}	m	m
M^{2+}-Cl_2	s	s
low mass ions		
$C_6H_{(4-n)}^+$	m	-
$C_8H_{(8-n)}^+$	-	-
$C_{11}H_{(8-n)}^+$	-	-

(Source: IARC, 1991)
Note: m-medium intensity; s-small intensity; number in parentheses-mass loss by M^+; n-number of halogen substituents in parent molecule.

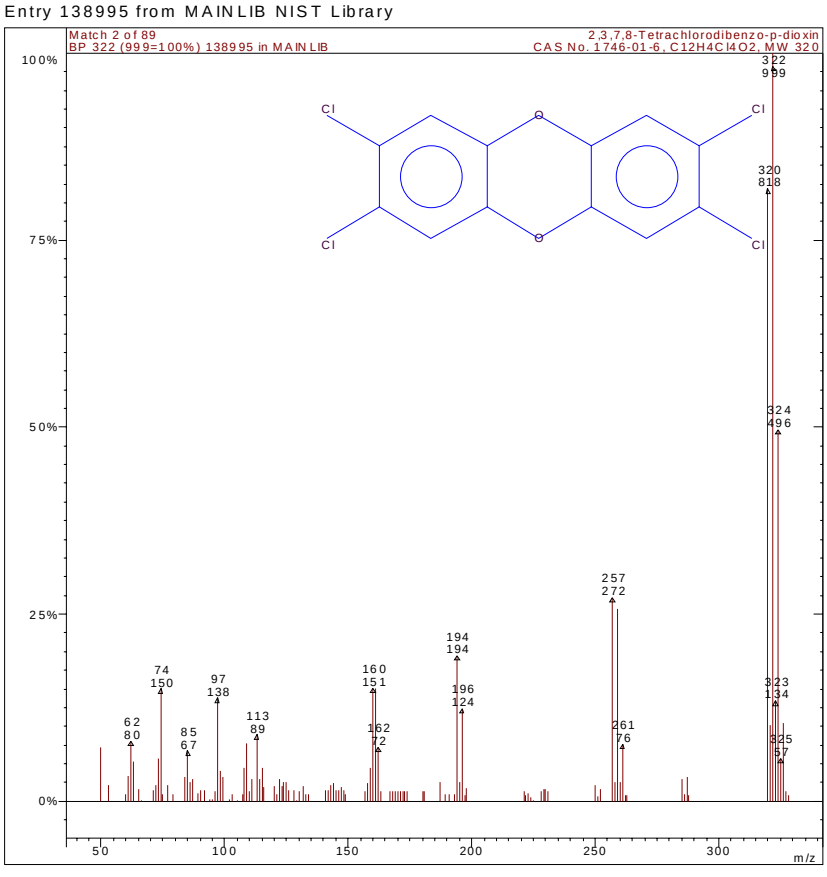

Fig. 3.13 Mass spectrum of 2,3,7,8-TCDD

Table 3.7 Molecular ion data (m/z relative abundance) for PCDD/Fs

Compound	*Number of isomers*		*Elemental composition*	*Molecular ion m/z values*[c]						*Relative abundance*				
	Total	*2,3,7,8-type*[a]		*Exact*[b]	M^+	*+2*	*+4*	*+6*	*+8*	M^+	*+2*	*+4*	*+6*	*+8*
mono-CDD	*2*	-	$C_{12}H_7O_2Cl$	*218.0134*	*218*	*220*				*100*	*33*			
di-CDD	*10*	-	$C_{12}H_6O_2Cl_2$	*251.9745*	*252*	*254*	*256*			*100*	*65*	*11*		
tri-CDD	*14*	-	$C_{12}H_5O_2Cl_3$	*285.9355*	*286*	*288*	*290*	*292*		*100*	*97*	*32*	*4*	
TCDD	*22*	*1*	$C_{12}H_4O_2Cl_4$	*319.8965*	*320*	*322*	*324*	*326*	*328*	*77*	*100*	*49*	*11*	*1*
PeCDD	*14*	*1*	$C_{12}H_3O_2Cl_5$	*353.8576*	*354*	*356*	*358*	*360*	*362*	*62*	*100*	*65*	*21*	*4*
HxCDD	*10*	*3*	$C_{12}H_2O_2Cl_6$	*387.8186*	*388*	*390*	*392*	*394*	*396*	*52*	*100*	*81*	*35*	*9*
HpCDD	*2*	*1*	$C_{12}HO_2Cl_7$	*421.7796*	*422*	*424*	*426*	*428*	*430*	*44*	*100*	*97*	*52*	*17*
OCDD	*1*	*1*	$C_{12}O_2Cl_8$	*455.7406*	*456*	*458*	*460*	*462*	*464*	*35*	*89*	*100*	*64*	*26*
mono-CDF	*4*	-	$C_{12}H_7OCl$	*202.0185*	*202*	*204*				*100*	*33*			
di-CDF	*16*	-	$C_{12}H_6OCl_2$	*235.9795*	*236*	*238*	*240*			*100*	*65*	*11*		
tri-CDF	*28*	-	$C_{12}H_5OCl_3$	*269.9406*	*270*	*272*	*274*	*276*		*100*	*97*	*32*	*4*	
TCDF	*38*	*1*	$C_{12}H_4OCl_4$	*303.9016*	*304*	*306*	*308*	*310*		*78*	*100*	*49*	*11*	*1*
PeCDF	*28*	*2*	$C_{12}H_{32}Cl_5$	*337.8626*	*338*	*340*	*342*	*344*	*346*	*62*	*100*	*65*	*21*	*3*
HxCDF	*16*	*4*	$C_{12}H_2OCl_6$	*371.8237*	*372*	*374*	*376*	*378*	*380*	*52*	*100*	*81*	*35*	*8*
HpCDF	*4*	*2*	$C_{12}HOCl_7$	*405.7847*	*406*	*408*	*410*	*412*	*414*	*45*	*100*	*97*	*52*	*17*
OCDF	*1*	*1*	$C_{12}OCl_8$	*439.7457*	*440*	*442*	*444*	*446*	*448*	*35*	*89*	*100*	*64*	*26*

(Source: IARC, 1991)

Note: a-number of 2,3,7,8-isomers; b-exact mass of M^+ ion (^{35}Cl isotope only); c-ions commonly used for SIM analyses are italicized

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 PCDD/Fs analysis a mass interval from 100 to 550 (or from 50 to 650) 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. Full scan mass spectrum of 2,3,7,8-TCDD in fig.3.13 is an example for this case.

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 PCDD/Fs analysis the SIM is predominated. With SIM, the mass spectrometers record only the mass spectrum of some fragmentation ions giving the biggest relative abundance (e.g for 2,3,7,8-TCDD, the monitoring ion mass is 320 and 322, *see table3.7 above*) 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 can not use the available library such Nist or Wiley to identify the compounds (not enough data to compare). Since for PCDD/Fs 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. The monitoring ions for EI-SIM are chosen based on the values presented in the table 3.7. For HRMS we will take the exact mass of M^+, M^++2 or M^++4 instead the values given by this table, e.g for 2,3,7,8-TCDD we will take the values of 319.8965 and 321.8936, respectively. Additionally, M^+-COCl ions are sometimes recorded for confirmation purposes.

B. NCI-MS

Even if EI-MS is the most common technique for PCDD/Fs 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 (*Simonsick et al., 1984; Oehme and Kirschmer, 1984; IARC, 1991; Crespin et al., 1999; Nakagawa et al., 2001; etc*). 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 PCDD/Fs 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 and we will discuss about it in next chapter.

As we showed above, CH_4 pure gas is commonly used as reagent gas. The sensitivity is very good for all PCDFs (from tetra- to octa-) and for higher chlorinated PCDDs (from penta- to octa-). Unfortunately, this techniques is not sensitive for TCDD, especially for 2,3,7,8-TCDD. For this compound we should use another techniques such EI-MS.

Table 3.8 Comparison of NCI (CH_4)- and EI-MS sensitivities for some PCDD/Fs

Compound	*Ratio of NCI/EI sensitivities*
2,3,7,8-TCDD	0.2
2,3,7,8-TCDF	22
1,2,3,6,7,8-HxCDD	75
OCDD	170
OCDF	440

(*Source: IARC, 1991*)

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, especially for PCDDs, with which lower ion source temperatures enhance the relative intensity of M^-. PCDFs generally show intense base peak molecular ions (M^-) and some fragmentation by loss of Cl and the unexpected addition of H, resulting in unusual M^-34 ions. Minor fragmentation ions are M^-68 and M^-102. All PCDFs isomers show very similar NCI mass spectra. PCDDs give conventionally the mass spectra with fragmentation ions M^-35 and M^-70. M^- ions were observed for almost higher chlorinated PCDDs (from pent- to octa-), but for some HxCDDs, HpCDDs and OCDD the M^- ions were less intense in comparison with M^--Cl ions. PCDD/Fs may yield M^+-19 ions (M^+-Cl+O) due to the presence of oxygen in the ion source (*IARC, 1991*).

* *GC×GC - TOFMS*

Recently, a new technique called GC×GC-TOFMS is developed and it is seemed to be a good effective and competitive tool in comparison with traditional MS techniques for PCDD/Fs analysis. This technique has been reported by many authors as an alternative method for PCDD/Fs analysis (*Lee et al, 2000; Korytar et al., 2002; Dimandja, 2003; Focant et al., 2005; etc*).

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. 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.

High-resolution magnetic sector and quadrupole mass spectrometers have recently been coupled to GC×GC, however the scan rates of these instruments (3–10 Hz) have resulted in protracted run times because these mass spectrometers are not fast enough to characterize chromatographic peaks whose widths (in normal GC×GC operation) range between 100 and 500 msec and thus require mass spectral acquisition rates of at least 20–50 Hz. Time-of-flight (TOF) mass spectrometers have always possessed the necessary speed to keep up with fast GC runs, and in recent years have experienced a resurgence due to improved data acquisition technology. In addition, the pulsed nature of TOF-MS ionization avoids the problem of

spectral skewing that is common in continuous ionization mode instruments (magnetic sector and quadrupole MS) due to the rapidly changing concentration flows into the source. The absence of a concentration-dependent MS signal allows for the mass spectral deconvolution of mixed spectra (corresponding to coeluting GC peaks), which is a significant advantage because the MS is capable of "separating" compounds in the mass spectral domain, and thus contributes to the overall resolution power of the analytical system. Even though a fast TOF is a low resolution MS, the separating power of GC×GC gives the potential to provide selectivity for dioxin and furan analysis. The focusing effect of GC×GC enhances sensitivity, so that sub- to low pg levels can be determined. The use of GC × GC–TOFMS for the isotope dilution measurement of dioxins and related compounds in environmental matrices such as soils and ashes showed to be correlated to GC–IDHRMS data (*Focant et al., 2005*).

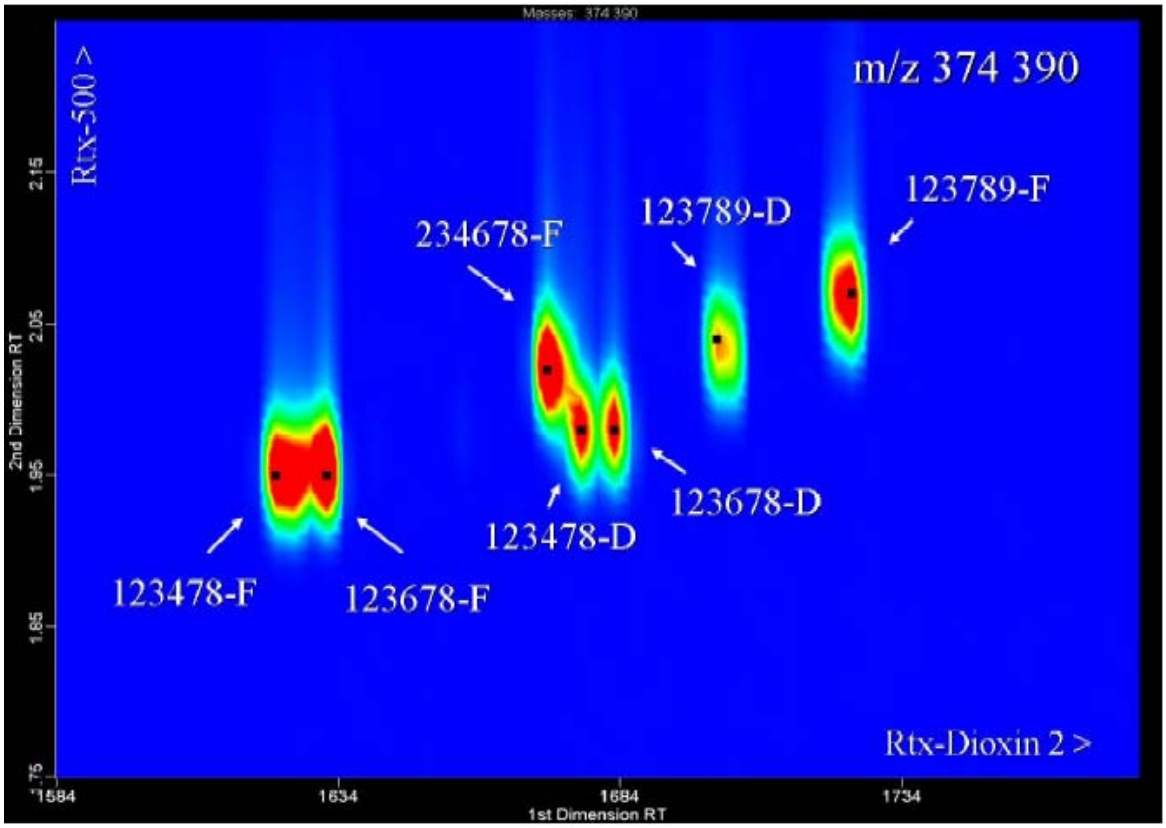

Fig. 3.14 Two dimensional chromatography graph of GC×GC-TOFMS for some PCDD/Fs (source: Cochran et al., 2004)

C. ***CONFIRMATION OF PCDD/Fs ANALYSIS BY MS TECHNIQUES***

Generally, both false-positive and false-negative results of PCDD/Fs analysis are the matters of potential concern. However, positive reports of PCDD/Fs which are false cause needless alarm to the public, since ***the analytical methods used should provide suitable quality assurance to ensure that false positives are minimized***. 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 PCDD/Fs analysis should be free of contaminants. The use of surrogate and internal standards (isotope labelled ^{13}C and ^{37}Cl) are indispensable for PCDD/Fs analysis. A serie of native and labelled PCDD/Fs, including most 2,3,7,8-substituted congeners are commercially available. Surrogate standards are added before extraction and cleanup procedure to determine recoveries in individual samples 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 PCDD/Fs 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 PCDD/Fs presented in some samples. The HRMS with accurate mass measurement is very useful and serves as official and recognized technique for PCDD/Fs analysis as reported in many documents (*US.EPA 1991, 1994, 1998*). LRMS-SIM (EI and NCI) could be sufficient for PCDD/Fs 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 (*IARC, 1991*):

(1) Correct retention time with respect to reference PCDD/Fs isomer on HRGC columns, preferably on both non-polar DB-5 MS and polar DB-Dioxin, or otheir 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.

A supplementary criterion is the confirmation by the loss of COCl (simultaneous signals for M^+ and M^+-COCl ions).

D. ***SAFETY IN PCDD/FS ANALYSIS***

As shown in *Chapter I,* PCDD/Fs could cause many adverse effects on human health so we should take a great attention when we work with them. For PCDD/Fs analysis we use the standards and surrogates that could be a source of PCDD/Fs exposure for laboratorians. The safety procedures in PCDD/Fs analysis are available in many official documents (*IARC, 1991; US.EPA, 1994, 1998; etc*). In addition, each laboratory has own regulation that the laboratorians/researchers should follow when working in. Here we discuss only the safety levels when we do the PCDD/Fs analysis.

Normally, we could classify the laboratories related to PCDD/Fs on three types (*IARC, 1991*):

- The Standards/Synthesis Laboratories (SSL), where the largest amounts of PCDD/Fs are handled. This laboratory type has the most stringent safety precaution and provides for the security of the standards;

- The Sample Preparation Laboratories (SPL), in which substantially lower levels of PCDD/Fs are handled and in which safety precautions are therefore more relaxed;
- The Sample Analysis Laboratories (SAL), which house the GC/MS systems used to analyze the prepared samples (our laboratory belongs to this type). This laboratory type has the least constraining safety protocol due to the small amount of PCDD/Fs used and the well contained nature of the samples and the analyzing equipments. This laboratory type should not be isolated and GC/MS used for PCDD/Fs analysis could place in the same laboratory with other analytical equipments employed for other analysis.

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) The concentration of sample/exacts and used standards rarely exceeds 1ng/μL (depend on the sensitivity of analytical equipments used for PCDD/Fs analysis);

(3) Use the autosampler for sample injection to minimize the exposure to laboratorians;

(4) 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);

(5) 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 PCDD/Fs as well as solvents used for analysis) have permit to do the work alone.

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65. US.EPA (1998). Method 8290A - Polychlorinated Dibenzo-p-Dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS).

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CHAPTER IV: SET-UP THE ANALYTICAL METHODS

4.1 Introduction

The selection and development of a proper analytical method is an important subject for the research. It can happen that the same method may be good for one case, but not for the other. Always one must keep in mind that the selected method is 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.

Furthermore, the method selected for analysis must be tested by several laboratories to verify that it meets criteria for validation that were previously established. The utilization of Standard Reference Materials (SRM) is very important to control the accuracy of the selected method. In fact, there are many methods available for PCDD/Fs analysis, however each method has own advantages and also disadvantages that we should consider when we apply it for our research. In addition, there are some limitations of real conditions (available equipments, finance, etc.) that can be limiting factors for our selection.

Firstly, we should recognize that HRMS is a very useful and it became reference equipment in PCDD/Fs analysis to confirm the results of analysis or to compare the effectiveness between different techniques. However, the investment cost for a GC/HRMS is very high and it exceeded the budget for our cooperation project. We chose the GC/LRMS due to some facilities:

- This equipment was available in CECOTOX Lab and IER Lab, so we could do the analysis in both laboratories with the same MS type ---> easier to compare the result, either to do the training work for utilization;
- The investment cost is adapted to our budget for second phase of our collaboration project (CECOTOX – IER);
- The LRMS is good for multipurpose analysis (not only for PCDD/Fs); in addition, with the NCI-SIM we could detect the low enough concentration, adapting to our purposes;
- All the analytical procedures applied for LRMS could be also applied for HRMS, so for the future if we want to develop a reference lab for PCDD/Fs analysis, it is just necessary to invest in the equipment (such HRMS).

4.2 Extraction and clean-up for soil and sediment

Soil and sediment are the subjects of our research as they act as a sink for PCDD/Fs. The analytical procedure is set up based on the certified methods (*IARC, 1991; US EPA, 1991,1994, 1998, and 2000*) , as well as modified from tested methods proposed by many authors (*Feola Paz et al., 1991; Sauvain, 1993;Maier et al., 1999; Oehme, 2001; Wu et al., 2002; etc*): The PCDD/Fs analysis was performed using an isotope dilution technique. This method is very similar to the method used in Carso laboratory (Carso – Centre d'analyse de traces – Laboratoire sante environnnement hygiene de Lyon) - a laboratory that is accredited by COFRAC (French Accreditation Committee) NF EN ISO 17025; approved by the Ministry of the Environment to control natural waters, industrial or urban waste waters (organic contaminants and metals in waters and sediments); and approved

by the Ministry of Social Affairs and Solidarity for lead and benzene (sampling and analyses).

* *Sample treatment before analysis*

After sampling, samples ware dried at room temperature for about 5-7 days. Dried soil and sediment were crushed by mean of a ceramic mortar than sifted through a 1×1mm stainless steel sieve to remove the stones, roots, etc. Finally, samples were labeled and stored in a brown glass flask at 4^{o}C until analysis.

* ***Extraction***

In the previous chapter we have presented many extraction methods for PCDD/Fs analysis, among them Soxhlet extraction which is always chosen as a basic, traditional technique to compare with others. Even if the time for Soxhlet extraction is relatively long (12 to 48 h depending on the matrix type), but this technique has two evidently advantages:

- ***Very effective comparison with other techniques;***
- ***Very easy to use and not requires any special equipments (minimize the investment cost).***

The ASE is also an effective method, but it requires costly system, so we consider it as *an alternative selection for the future investment* when we will set-up a reference laboratory for PCDD/Fs analysis.

Toluene is chosen as solvent for Soxhlet extraction as proposed by many certified methods. In fact we can use the mixture of ethanol/toluene (70:30) instead of pure toluene as proposed by Carso and some other authors. The use of this solvent mixture is better for swelling of fiber materials in the samples. The time for extraction is 48h because we want to be sure that all interested compounds are recovered in extract solution for our research. In real, we can reduce the time for extraction to one night (8-12 h), reducing the time of analysis, especially for the batch-analyses.

The quantity of soil analyzed is about 25g, this quantity is calculated based on the detection limit of the MS. With our LRMS system, the detection limit is more less 1ng/mL, that means if the final volume for PCDD/Fs analysis before injection is about 25 to 50µL then the 2,3,7,8-PCDD/Fs quantity in the sample should be bigger than 1×50/1000 = 0.05ng. As the minimum concentration of PCDD/Fs in soil of any types (*Mai et al., 2005*) in Vietnam is around 1-2pg/g, to have the quantity of 0.05ng we should take a soil quantity: (0.05×1000)/1 to 0.05×1000/2 of 25-50g to reach the detection limit. We will take the 50g of soil sample in the case if we want to re-analyze/control: after extraction, one half of the extract solution is analyzed and the other half is stored to re-analyze/control if we have the problems during the analysis process (this method is often applied in the laboratory doing the PCDD/Fs for clients –QA/QC procedure).

Sulfate anhydrous sodium (20g) is added to sample to remove the water content during the extraction process. The Cu powder is used for removing sulphur compounds. The extraction schema is shown in fig. 4.1. The filter paper is used instead extraction glass thimble. The use of filter paper is an advantage of the method because we can avoid the cross-contamination caused by thimble.

The water content in sample is controlled by the difference weight before and after drying by oven at 105^{o}C for 2h.

The used surrogate 2,3,7,8-PCDD/Fs standard is EDF-4053 solution (Cambridge Isotope Laboratories -CIL) diluted by factor 10 to have the concentration of ^{13}C-2,3,7,8-PCDD/Fs about 100ng/mL (200ng/mL for OCDD). This surrogate solution (50µL) is spiked directly on the sample in Soxhlet system just before extraction by a micro-syringe of 50µL. This syringe is *used only for spiking*, not for other purposes to avoid the cross-contamination.

The extraction flask is protected from sunlight by aluminum paper during the extraction process. As we know, the PCDD/Fs is relatively resistant in sunlight at normal temperature but it could be degraded at high temperature, especially in presence of sunlight and in very low concentration (conditions reached in extract flask).

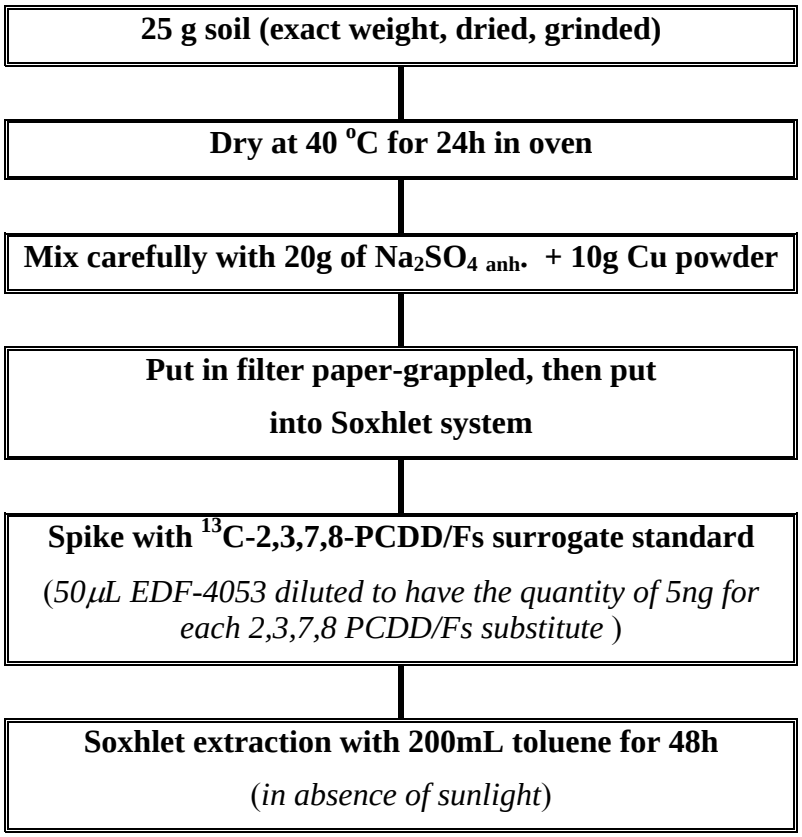

Fig. 4.1 Extraction schema for soil sample

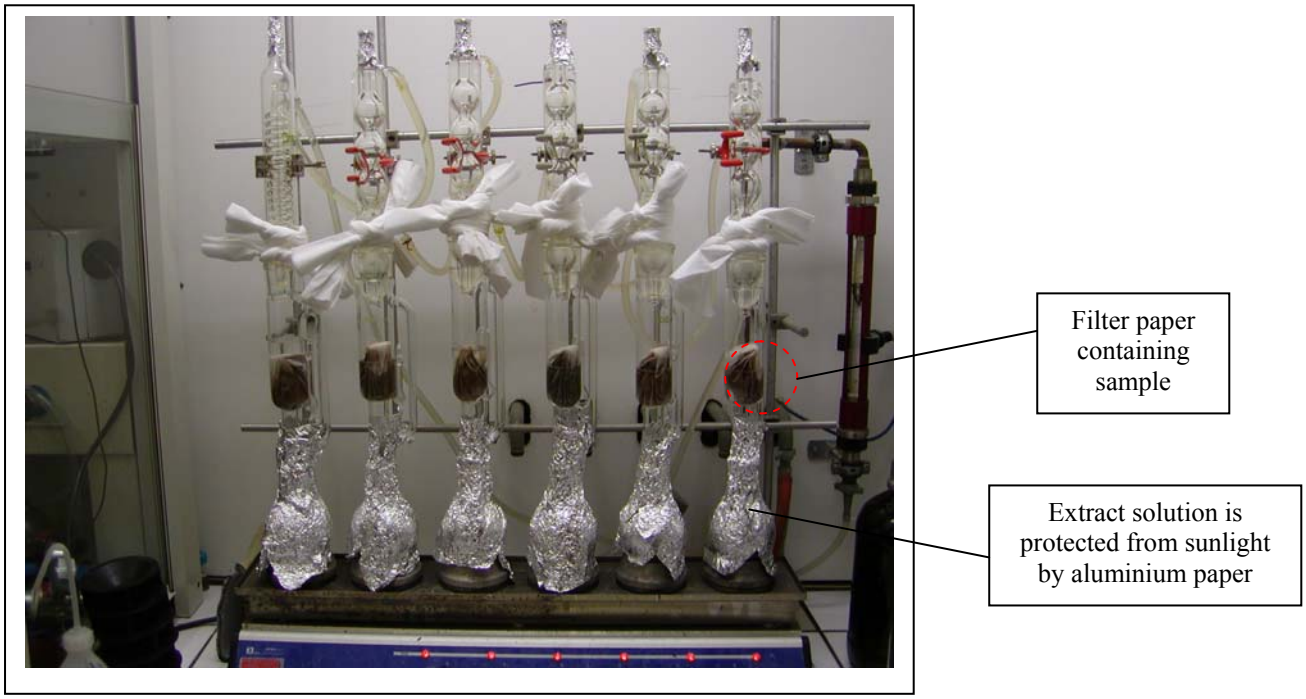

Fig. 4.2 Soxhlet extraction system used for PCDD/Fs analysis

* ***Purification***

Normally, the extract solution is colorless or slight yellow/green depends on the compositions of matrix. Sometimes the extract solution has a color of dark yellow/green, in this case we should to do the acid washing before clean-up by chromatography columns because the adsorbents used not sufficient to remove all the color.

The toluene extract is concentrated by rotavapor before clean-up. The vacuum pressure for toluene is about 77 mbar and the temperature of water bath is 50 – 55^{o}C. To minimize the loss during evaporation process (protection from dryness), a little quantity of high-boiling solvent is added to the extract just before evaporation (we use 100-200µL dodecane for this purpose). In the case of acid washing, after evaporation, the extract (less then 1 ml) is dissolved in 20-50mL n-hexane. This solution is washed with 3×20mL $H_2SO_{4,\ conc.}$ to have the colorless solution, then it is concentrated again to a volume of 1mL.

The concentrated extract containing interesting compounds is again cleaned-up by several steps described as follow:

*(1) **Silica mix column** (from bottom to top): 1g of activated silica, 2g of basic silica ($CsOH \times H_2O$), 1g of activated silica, 4g of acidic silica, 1g of activated silica. The colums was covered with 2g of Na_2SO_4 (anhydrous), and pre-washed with 50mL n-hexane. The sample was eluted with 90mL n-hexane;*

*(2) **Florisil column (6g)**, pre-washed with 50mL n-hexane. The sample was eluted with 180mL toluene/diethyl ether (9:1);*

*(3) **Basic alumina column** (3g of basic alumina Super I), pre-washed with 50mL n-hexane. The sample was eluted with:*

i) 10mL n-hexane

ii) 10mL n-hexane/dichloromethane (92:8)
iii) 15mL n-hexane/dichloromethane (2:3)
iv) 20mL dichloromethane

The adsorbent preparation and column filling is described in detail in appendices, here we want to discuss about some important points during the clean-up process:

- As we see, the multilayer silica column serves as treatment column (*see chapter I*), that means all colored compounds (undesired compounds) are held in the layers of this column while PCDD/Fs compounds are eluted in n-hexane solution together with another untreated interfering compound that will be treated by florisil column. Two columns are connected together that all elutes from the first column (multilayer silica colums) are passed directly into the second column (florisil column). By this way we economize the use of solvent (n-hexane) and also minimize the loss of interesting compounds (PCDD/Fs) during the column change (normally after first column we should concentrate the extract before to putting it to second column). After all n-hexane solution is passed through two column, the silica column is taken out and the PCDD/Fs kept in florisil columns are eluted by a solvent mix (toluene/diethyl ether 9:1);
- Because the solvent mix toluene/diethyl ether is relative polar we could not connect the second column with the third column. Before treatment by alumina column, a solvent exchange should be done: a small quantity of high-boiling solvent (nonane, dodecane, or tetradecane) is added to toluene/diethyl ether mix (normally 50-100µL is sufficient), then this mixture is concentrated by rotavapor to very small volume (less then 1mL).
- The anhydrous sodium sulfate is very important and indispensable for alumina column. This adsorbent is used to take out the water content or sometime residues of acid from acid treatment that may make adverse effects on GC-column and analytical instruments. Some time to be sure we use two layers of $Na_2SO_{4,\ anh.}$ (above and below the alumina layer), by this way we save our column and GC/MS from water, acid and other contaminated matters.

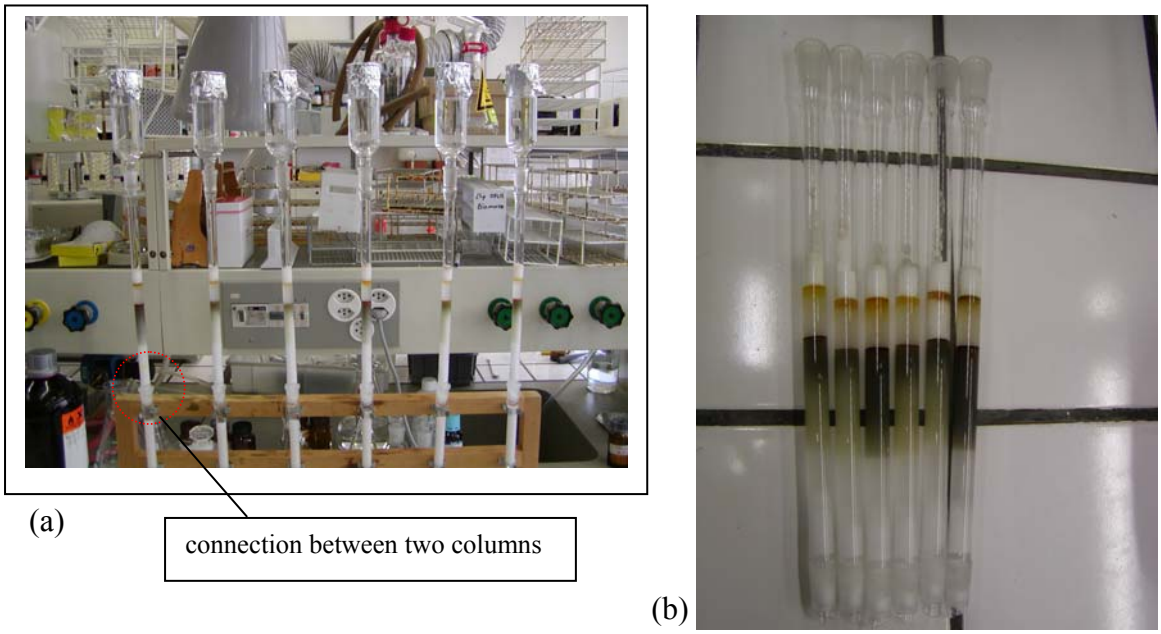

Fig. 4.3 (a) Multilayer silica column is conected with florisil column
(b)Colored compounds are held in silica column that is taken out after treatment

Normally, the extraction and clean-up steps take about 3-4 days. A grand part of time is extraction time, so if we reduce the extraction time from 48h to 8-12h, we could economize 1-1.5 days. In addition as we see in the method a big quantity of solvents (toluene, n-hexane, etc.) is used for the analysis. These solvents in one hand need a long time for concentration (especially for relative high-boiling solvent such toluene), in other hand they raise the analysis cost as well as the liquid waste. These disadvantages are not a big problem for our research because the number of analyses is limited, but they could be the subjects for consideration when we set-up a laboratory for PCDD/Fs research/analysis in the future. The investment for automatic instruments may be a good solution for this case.

4.3 Extraction and clean-up for biological sample

Biological matrices in our research are fish tissue and human adipose. Both types are very similar due to their high fat content, since the analytical method is the same for them.

* ***Extraction***

Normally biological samples are in the heterogeneous form that not facilitated for extraction (the solvent penetration into the structure of the matrix is very difficult). So, before extraction, the biological sample should be treated to change in free-flowing form. Generally, the samples are grounded with anhydrous sodium sulfate by a grinder. We have used the A11 basic IKA analytical mill with a cutting blade for pulverizing soft, fibrous grinding materials and the spare grinding container with effective volume of 80mL. For fish tissue the test portion weight is about 20g (*see chapter III*). Unfortunately, we can not choose the test portion weight for human adipose because it is depend on many real factors that we will discuss in more detail in next chapter. We should satisfy ourselves with the test portion weight from 1 - 5g.

The $Na_2SO_{4,\ anh}$ quantity mixed is normally equal 3-4 times of test portion weight, that means for 20g of fish tissue we should add 60 – 80g of $Na_2SO_{4,\ anh}$. Because our Soxhlet system has a volume of 250mL, so for the test portion for fish tissue is limited about 15g (if we want to take more test portion, then we should have the bigger Soxhlet system).

To have the equivalent condition for all tested samples, we have chosen the same $Na_2SO_{4,\ anh}$ quantity for each matrix type: 45g for fish tissue and 20g for human adipose, respectively.

As solvent for extraction we chose the mixture dichloromethane/n-hexane 1:1 (DCM/n-Hex 1:1). We have compared two solvents: pure DCM and mix DCM/N-Hex 1:1. A test was carried out with a samples of pork meat grinded with $Na_2SO_{4,\ anh}$ and spiked with surrogate standard EDF-4053. The result showed that extraction by pure DCM has very low recovery in comparison with mix DCM/n-Hex 1:1 for almost tested ^{13}C-2,3,7,8-PCDD/Fs. Since then we chosen mix DCM/n-Hex (1:1) for our research.

The extraction time is one night (8h) till 16 h as proposed by certified methods (*IARC, 1991; US.EPA, 1994*). Because the number of biological samples analyzed in our research was quiet big, we extracted our samples for one night (8-12h) only. The extraction procedure is presented in fig. 4.4 below.

Test portion – exact weight

(*15g fish tissue or 1-5g human adipose*)

↓

Grinded with Na_2SO_{4anh}

(*45g for fish tissue and 20g for human adipose*)

↓

Put in filter paper-grappled, then put into Soxhlet system

↓

Spike with ^{13}C-2,3,7,8-PCDD/Fs surrogate standard

(*50μL EDF-4053 diluted to have the quantity of 5ng for each 2,3,7,8 PCDD/Fs substitute*)

↓

Soxhlet extraction with 200 mL DCM/n-Hex 1:1 for 8-12h (one night)

(*in absence of sunlight*)

Fig. 4.4 Extraction schema for biological sample

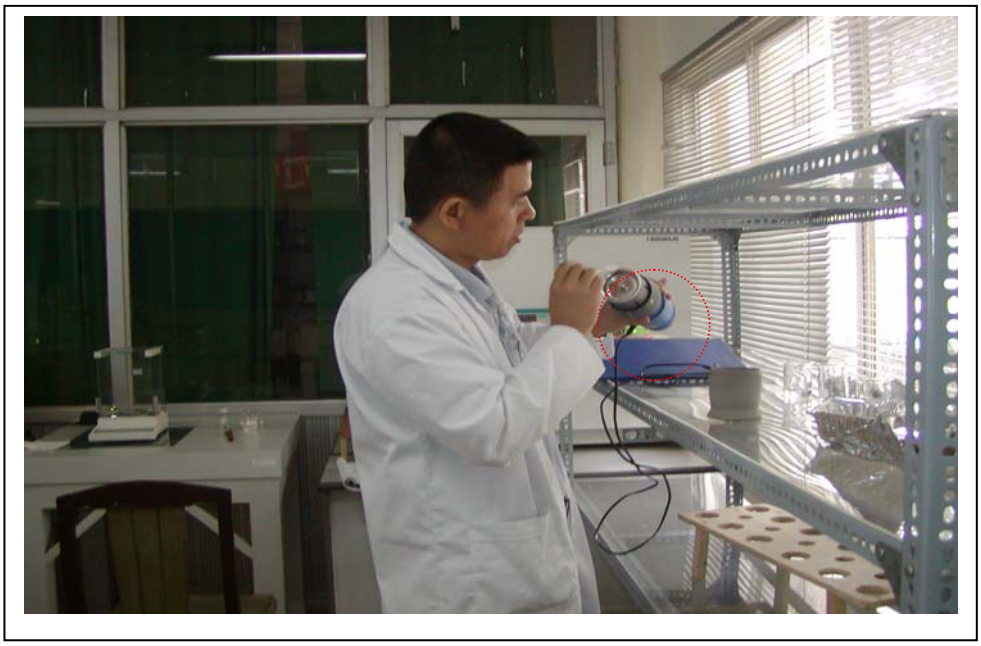

Fig. 4.5 Clean the grinder after use

* ***Purification***

The fish extract is divided on two portions: 2/3 (exact weight – M_1) is for PCDD/Fs analysis; 1/3 (exact weight – M_2) is for fat content determination by evaporating all solvent. The fat content is calculated as follow:

$F_{sample} = [(M_2 - M_3) \times (M_1 + M_2)]/M_2$

M_1-weight of extract portion for PCDD/Fs analysis; M_2-weight of extract portion for fat content determination; M_3-weight of extract portion for fat content determination after solvent evaporation.

The extract solution of fish tissue and human adipose contains a big quantity of fat that could make a overloading for chromatography columns used for purification. Hence we should do the acid treatment before adding the extract onto chromatography columns. Generally we use the $H_2SO_{4\ conc}$ for this purpose as told above. There are two techniques that could be applied:

a) Centrifuging treatment: add 30mL of $H_2SO_{4\ conc,}$ to the extract, the mixture is shaked for 3min, and then centrifuged for 20min (2000rpm) to separate the organic solvent and acid layer. The organic solvent is collected and the acid solution is extracted two times with 2x20mL n-hexane following the same procedure.

b) Washing treatment: the concentrated extract (about 20mL in n-hexane) is treated three times with $H_2SO_{4\ conc.}$ (3×20mL $H_2SO_{4\ conc}$). The separation is carried out in separator funnel by natural gravity.

The second technique consumes more acid, but it is very easy to do/control and no needs a specific instrument (centrifuger). In addition, this technique is very effective and the time consumed is shorter in comparison with centrifuging technique. Hence we had chosen this technique for our analytical procedure.

Fig. 4.6 Acid washing treatment for biological samples

After acid washing treatment, the extract is concentrated to a small volume of 1-2mL by rotavapor, the vacuum pressure for n-hexane is about 320mbar at 40°C. For biological samples we use only two columns: silica mix column and alumina basic column. The clean-up procedure is the same as described above: two columns are connected and all elute from

silica column is passed onto second column; then the silica mix column is taken out and the purification steps are continued with alumina basic column.

4.4 Extraction and clean-up for fly ash sample

The extraction and clean-up procedure for fly ash sample is very similar to the procedure for the soil/sediment sample. There is only a difference on the acid treatment of fly ash before extraction. As we told in the previous chapter, the acid treatment is common used to destroy the inorganic matrix of the samples and to make PCDD/Fs more accessible for extraction. The acid treatment and extraction is set up based on the methods proposed by many authors (*Paz et al., 1991; US.EPA 1991, 1994, 1998; IARC, 1991; Sauvain, 1993;*)

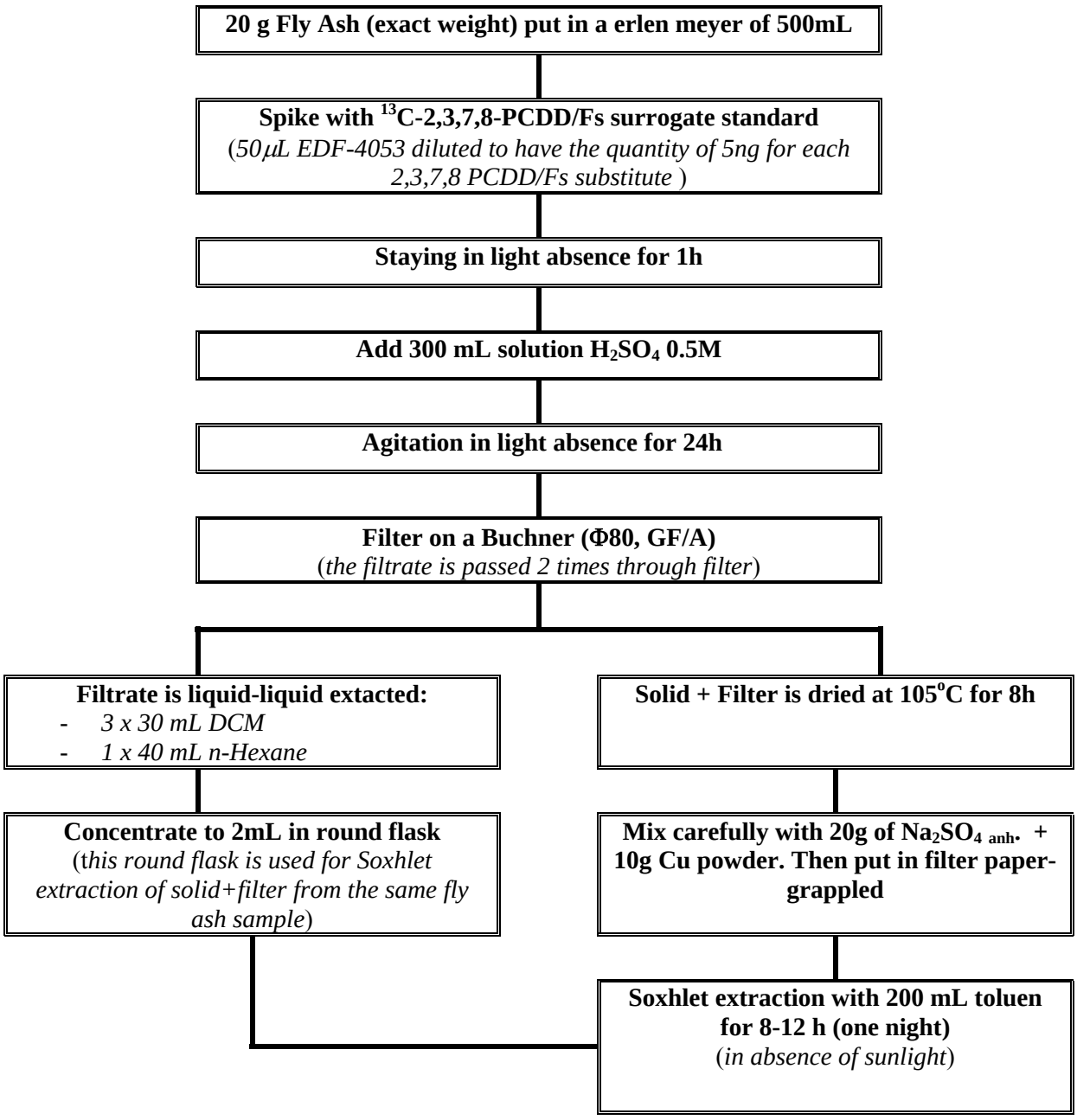

Fig. 4.7 Extraction schema for fly ash sample

There are some improvements that could do to shorten the time for analysis:

- *Use the HCl 3M instead the $H_2SO_{4,\ conc.}$ and shorten the acid treatment to 2-3h only;*
- *Use the air drying instead oven for solid+filter.*

With these improvements, the sample preparation is could be shortened.

The extract solution is purified by the same procedure for the soil/sediment samples as described above. Similar to the soil/sediment samples, if the extract is too colored (dark green or black), then the acid washing should be done before the treatment by chromatography columns.

4.5 Concentration and identification-quantification

4.5.1 Concentration

The fraction (iii) and (iv) after alumina column purification contain PCDD/Fs compounds of interest having a volume of 25mL mix DCM/n-Hex. This extract could be evaporated/concentrated directly by a pure nitrogen stream. But to economize the time for evaporation and also the pure nitrogen used, we always use the rotavapor firstly to reduce the extract volume to a half, then use the nitrogen stream to continue evaporate.

After evaporation by rotavapor, the extract is concentrated with nitrogen flow to about 200μL, transferred into glass insert and evaporated just to dryness under a stream of nitrogen at ambient temperature. The residue was immediately re-dissolved in 40μL iso-octane containing internal recovery standard (ED-2521, CIL). This internal recovery standard helps us to control the effectiveness (sensibilities) of the analytical instrument for PCDD/Fs compounds.

4.5.2 GC separation

The GC (Varian CP-3800) connected with MS (Varian 1200L quadrupole MS). The Varian 1200L GC/MS system 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)
- Column:

+ Guard column 5m×0.32mm (Restek Siltek Guard column 0.32mm ID)
+ For identification/quantification: J&W DB-5MS 60m×0.25mm×0.25μm (temp. max= 325^oC)
+ For confirmation: J&W DB-Dioxin 60m×0.25mm×0.25μm (temp. max=250^oC)

The guard column is connected to the GC column by glass connector. Generally, after a series of analysis (15 -20 samples) we have change the liner and cut a part of guard column (about 20cm in the end connected with liner).

- The GC condition
  - *Injection mode : on-column*
  - *Injection volume : 1 μL*
  - *Injector temperature : 250°C (85°C/(0.2min) --> 250°C/(100°C/min))*
  - *Constant Column flow: 1.0 mL/min*
  - *Temperature program: see table below*

GC column temperature program for 2,3,7,8-TCDD analysis

Temp. (°C)	*Rate (°C/min)*	*Hold (min)*	*Total (min)*
80	0.0	1.00	1.00
140	25.0	0.00	3.40
250	15.0	13.27	24.00
290	12.0	22.66	50.00

GC column temperature program for 2,3,7,8-PCDD/Fs analysis (except 2,3,7,8-TCDD)

Temp. (°C)	*Rate (°C/min)*	*Hold (min)*	*Total (min)*
80	0.0	1.00	1.00
140	25.0	0.00	3.40
250	15.0	40.00	50.73
270	10.0	32.27	85.00

The correct range of retention time for each group of ions (tetra- to octa-) has been checked with window standards ED-1732-B and EF-1731-B (CIL). These standards will show us the real retention times for each groups. Based on these retention times we can set-up the time for SIM.

Table 4.1 Mass selection for EI-SIM mode

Group	*Congener*	*m/z*	*Group*	*Congener*	*m/z*
I	TCDF	303.9	**IV**	HpCDF	407.8
		305.9			409.8
	TCDD	319.9		HpCDD	423.8
		321.9			425.8
	$^{13}C_{12}$-TCDF[a]	315.9		$^{13}C_{12}$-HpCDF[a]	419.8
		317.9			421.8
	$^{13}C_{12}$-TCDD[a]	331.9		$^{13}C_{12}$-HpCDD[a]	435.8
		333.9			437.8
	$^{37}Cl_4$-TCDD[b]	317.9		OCDF	441.7
		-			443.7
II	PeCDF	339.9	**V**	OCDD	457.7
		341.9			459.7
	PeCDD	355.9		$^{13}C_{12}$OCDF[a]	453.7
		357.9			455.7
	$^{13}C_{12}$-PeCDF[a]	351.9		$^{13}C_{12}$-OCDD[a]	469.8
		353.9			471.8
	$^{13}C_{12}$-PeCDD[a]	367.9			
		369.9			
III	HxCDF	373.8			
		375.8			
	HxCDD	389.8			
		391.8			
	$^{13}C_{12}$-HxCDF[a]	385.9			
		387.9			
	$^{13}C_{12}$-HxCDD[a]	401.9			
		403.9			

Note: the mass selection is taken from reported certified methods (IARC, 1991; US.EPA, 1994; Oehme, 2001; etc)
a-mass selection for surrogate internal standard; b-mass selection for clean-up standard (ED-2522, CIL)

Table 4.2 Mass selection for NCI-SIM mode

Group	*Congener*	*m/z*	*Group*	*Congener*	*m/z*
I	TCDF	303.9	**IV**	HpCDF	407.8
		305.9			409.8
	TCDD	-		HpCDD	388.8
					390.8
	$^{13}C_{12}$-TCDF	315.9		$^{13}C_{12}$-HpCDF	419.8
		317.9			421.8
	$^{13}C_{12}$-TCDD	-		$^{13}C_{12}$-HpCDD	400.8
	$^{37}Cl_4$-TCDD	-		OCDF	441.7
					443.7
II	PeCDF	339.9	**V**	OCDD	422.7
		341.9			424.7
	PeCDD	320.9		$^{13}C_{12}$OCDF	-
		322.9			
	$^{13}C_{12}$-PeCDF	351.9		$^{13}C_{12}$-OCDD	434.8
		353.9			
	$^{13}C_{12}$-PeCDD	332.9			
		334.9			
III	HxCDF	373.8			
		375.8			
	HxCDD	354.8			
		356.8			
	$^{13}C_{12}$-HxCDF	385.9			
		387.9			
	$^{13}C_{12}$-HxCDD	401.9			
		403.9			

Note: the mass selection is taken from reported certified methods (IARC, 1991; US.EPA, 1994; Oehme, 2001; etc)

The retention times for each 2,3,7,8-PCDD/Fs group were determinate by window defining mixture standards ED-1732-B and EF-1731-B and shown in the table 4.2

Table 4.3 Retention time for first and last isomers of PCDD/Fs

Isomer group	*Compound elutes*			
	First	Retention time (min)	Last	Retention time (min)
TCDD	1,3,6,8-	19.401	1,2,8,9-	22.769
TCDF	1,3,6,8-	18.544	1,2,7,9-	23.073
PeCDD	1,2,4,7,9-	24.683	1,2,3,8,9-	29.230
PeCDF	1,2,4,6,8-	26.295	1,2,3,8,9-	29.907
HxCDD	1,2,4,6,7,9-	32.937	1,2,3,4,6,7-	38.749
HxCDF	1,2,3,4,6,8-	31.439	2,3,4,6,7,8-	40.430
HpCDD	1,2,3,4,6,7,9-	48.692	1,2,3,4,6,7,8-	52.701
HpCDF	1,2,3,4,6,7,8-	47.070	1,2,3,4,7,8,9-	54.438

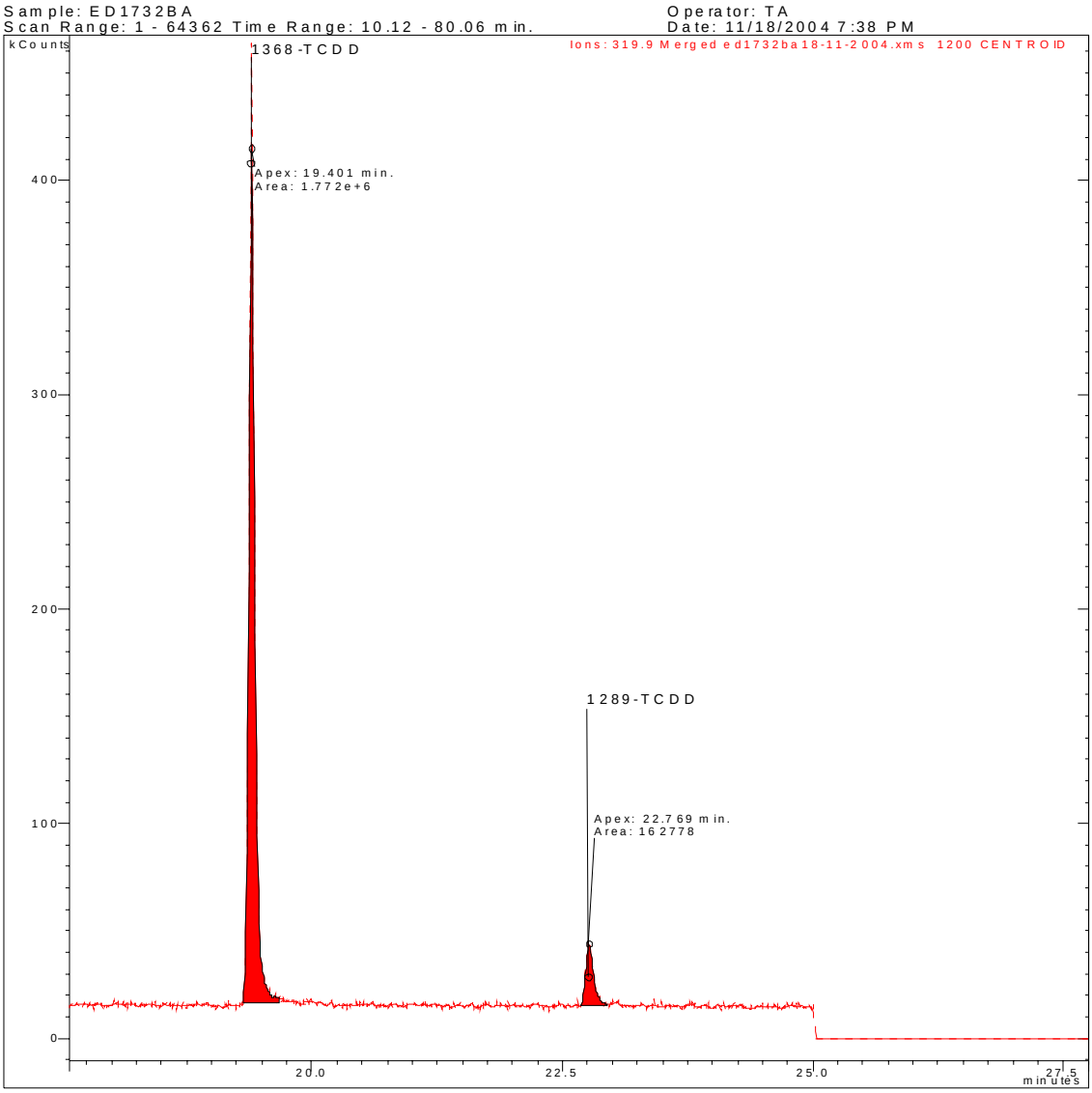

Fig. 4.8 Chromatogram of window defining standard ED-1732-BA

The data showed in the table 4.3 is not fixed, it depend on the type of GC column, type of GC/MS and real GC conditions. Generally, this data serves as a screening data. Based on it we can set-up and test the SIM mode. Fig. 4.8 shows the chromatogram of a window standard of a set of 8 window defining standards (*see annex for all chromatographs of this set*).

4.5.3 MS identification/quantification

Low resolution quadrupole MS (Varian 1200L) has been used for our research. 2,3,7,8-TCDD is identified and quantified by EI-SIM mode while NCI-SIM mode is used for the rest of 2,3,7,8-PCDD/Fs.

The MS condition for each mode is described as follow:

a) EI-SIM mode (for 2,3,7,8-TCDD determination)

Ionization mode:	*EI-SIM*
Electron energie:	*-70eV*
Detector:	*1200V (we can raie the detector voltage to have the bigger peak signal, but can not except the limit*
Filament current:	*150uA (typical)*
Manifold temperature:	*40^oC (typical)*
Ion source temperature:	*200^oC*
Transfer Line Temperature:	*250^oC*
Solvent cut-time:	*10min (typical)*
SIM with:	*0.500s*

Selected m/z	*Start at retention time*
317.9	10.00min (after solvent cut time)
319.9	
331.9	
333.9	
443.7	24min (based on data from window defining standards test)
459.7	
471.8	

Because this EI-SIM mode is used only for 2,3,7,8-TCDD, so for the first m/z group we chosen only the m/z values characterized for 2,3,7,8-TCDD and $^{13}C_{12}$-2,3,7,8-TCDD (*see table 4.1*). From table 4.2 we see that after 24min all TCDDs are eluted, so the m/z values for second group are the characteristic masses for high chlorinated compounds (OCDD/F and $^{13}C_{12}$-OCDD), serving as the monitoring indicators to control the GC column temperature program is enough for elution of high chlorinated compounds. If the peaks of these masses are not detected, then we should to improve the GC column temperature program (increase the end temperature or the time of this program until these peaks appeared).

Fig 4.9 showed the selected m/z = 319.9 chromatogram (for TCDD) and total chromatogram (TOC) of a calibration standard CC3 (EDF-2519-A, CIL) using EI-SIM mode. In this figure we see in plot 1 the selection between 1,2,3,4-TCDD and 2,3,7,8-TCDD while in plot 2 we see a relative big peak representative for OCDD/F (not very good separated).

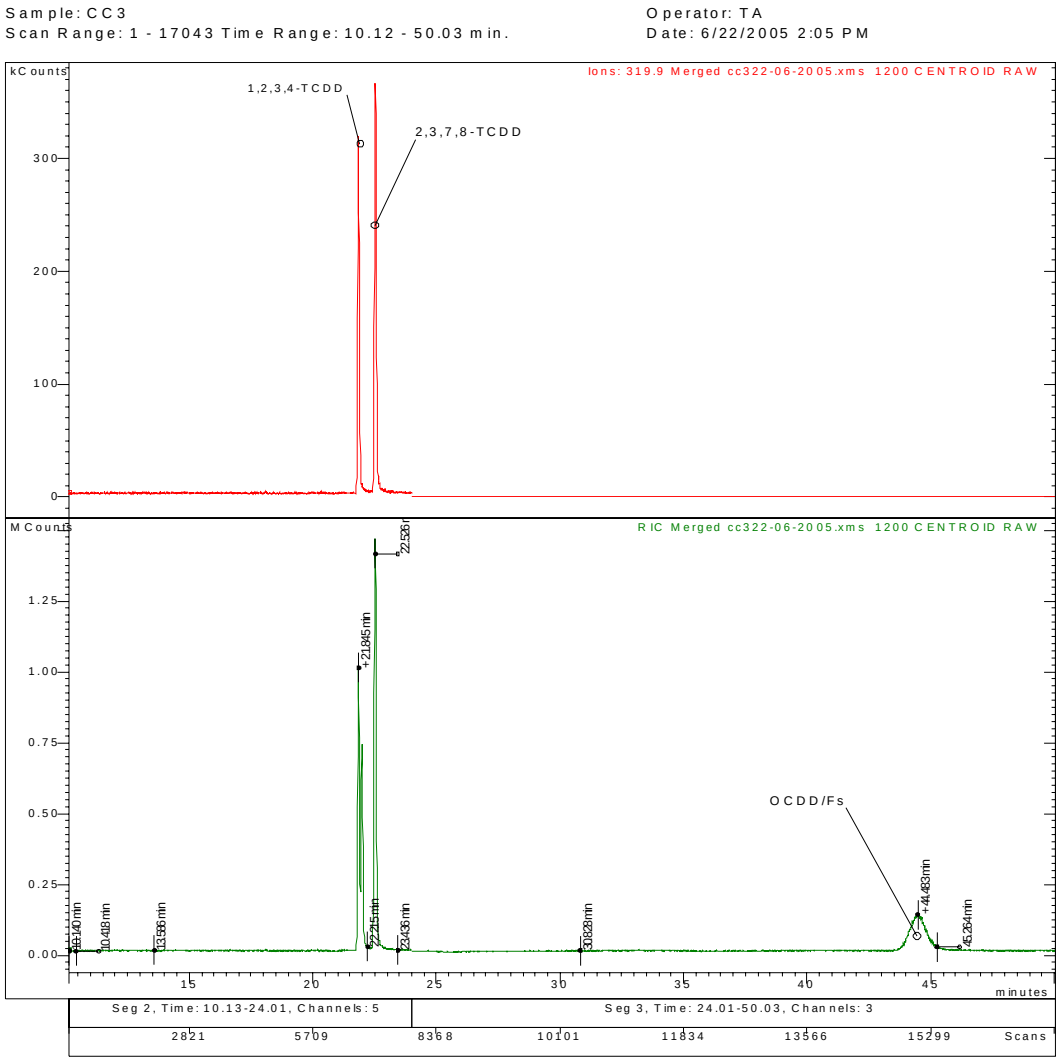

Fig. 4.9 EI-SIM chromatograph of CC3 solution (EDF-2519-A, CIL)

b) NCI-SIM mode (for 2,3,7,8-PCDD/Fs , except 2,3,7,8-TCDD)

Ionization mode:	*NCI-SIM*
Electron energie :	*-70eV*
Detector:	*1200V (we can raise the detector voltage to have the bigger peak signal, but can not except the limit*
Filament current:	*150uA (typical)*
Manifold temperature:	*40^{o}C (typical)*
Ion source temperature:	*200^{o}C*
Transfer Line Temperature:	*250^{o}C*
Solvent cut-time:	*10min (typical)*
SIM with:	*0.500s*

Selected m/z	Start at retention time (min)	Isomers for detection
303.9 305.9 315.9 317.9	10	TCDFs $^{13}C_{12}$-TCDF
320.0 322.9 - 339.9 341.9 353.9	24.50	PeCDD $^{13}C_{12}$-PeCDD PeCDF $^{13}C_{12}$-PeCDF[a]
354.8 356.8 401.9 403.9 373.8 375.8 385.9 387.9	34.50	HxCDD $^{13}C_{12}$-HxCDD HxCDF $^{13}C_{12}$-HxCDF[a]
388.8 390.8 - 407.8 409.8 419.8 421.8 373.8 375.8	47.00	HpCDD $^{13}C_{12}$-HpCDD HpCDF $^{13}C_{12}$-HpCDF HxCDF [b]
422.7 424.7 434.8 436.8 441.7 443.7	64.00	OCDD $^{13}C_{12}$-OCDD OCDF

Note: a- these m/z masses to control the retention times between TCDD and $^{13}C_{12}$-TCDF (having the same molecular/fragmentation ions mass); b- to be sure that no HxCDF eluted in this retention time section.

In fact the time for starting acquisition (start at retention time) is changed periodically because each time we change the liner and cut the pre-column, the retention time of the 2,3,7,8-PCDD/Fs isomers is also changed. So before the analysis we always inject a calibration standard containing all interested 2,3,7,8-PCDD/Fs isomers to control the SIM time program and correct it to obtain all peak of 2,3,7,8-PCDD/Fs presented in our standards.

Depending on the standard type used for analysis we will add or remove the selected m/z to make the higher sensitivity of the MS instrument. For example in our case we use the EDF-2519-A standard not containing $^{13}C_{12}$-PeCDD/F, but in surrogate internal standard EDF-4053 they have been presented, hence they will be theoretically presented in our final sample extract. Since here we not use the selected m/z for $^{13}C_{12}$-PeCDD, but we add only one m/z value for $^{13}C_{12}$-PeCDF (m/z 353.9) to control the retention time between ions mass from PeCDD and $^{13}C_{12}$-PeCDF because they have the same molecular mass.

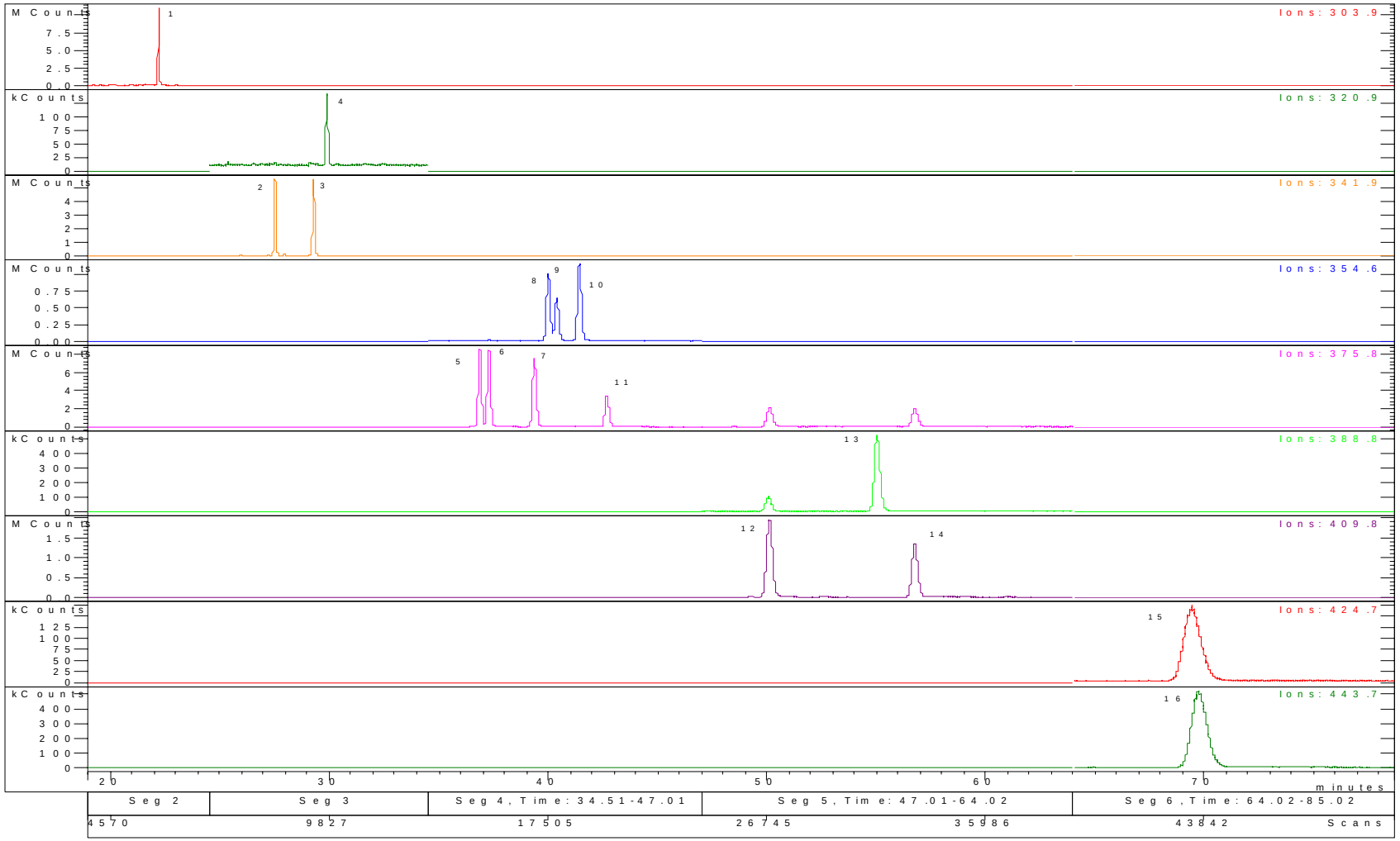

Fig. 4.10 NCI-SIM chromatograph of CC3 solution (EDF-2519-A, CIL) with all 2,3,7,8'PCDD-Fs (except 2,3,7,8-TCDD) 1-2,3,7,8-TCDF; 2-1,2,3,7,8-PeCDF; 3-2,3,4,7,8-PeCDF; 4-1,2,3,7,8-PeCDD; 5-1,2,3,4,7,8-HxCDF; 6-1,2,3,6,7,8-HxCDF; 7-1,2,3,7,8,9-HXCDF; 8-1,2,3,4,7,8-HxCDD; 9-1,2,3,6,7,8-HxCDD; 10-1,2,3,7,8,9-HxCDD; 11-2,3,4,6,7,8-HxCDF; 12-1,2,3,4,6,7,8-HpCDF; 13-1,2,3,4,6,7,8-HpCDD; 14-1,2,3,4,7,8,9-HpCDF; 15-OCDD; 16-OCDF

c)Identification and quantification procedure

The 2,3,7,8-PCDD/Fs are identified based on some criteria as follow:

1. *The retention time is correct in comparison with the retention time of native and labelled compound in external and internal standards;*
2. *Correct theoretical ion abundance ratios and their control limits as presented in the table 4.4 below:*

Table 4.4 Theoretical ion abundance ratios and control limits

Number of chlorine atoms	*Ion ratio*	*Theoretical value*	*Control limits*	
			Lower	*Upper*
4	M/M+2	0.77	0.65	0.89
5	M+2/M+4	1.55	1.24	1.86
6	M+2/M+4	1.24	1.05	1.43
6[a]	M/M+2	0.51	0.43	0.59
7[b]	M/M+2	0.44	0.37	0.51
7	M+2/M+4	1.04	0.88	1.20
8	M+2/M+4	0.89	0.76	1.02

(source: IARC, 1991; US.EPA, 1994). [a]: Used for ^{13}C-HxCDF; [b]: Used for ^{13}C-HpCDF

3. *Good separation between isomers, especially for 2.3.7.8-HxCDD/Fs*
4. *Response of the selected masses are greater than 3 time the noise level*

As we have showed on the previous chapter, the PCDD/Fs analysis is based on the method called isotope dilution method which uses the stable isotope ^{13}C-isomer as internal standard and the concentration of all interested PCDD/Fs isomers is calculated based on the response factor of these native isomers to correlative ^{13}C-isomer: e.g. for quantification of 2,3,7,8-TCDD we use its response of it to ^{13}C-2,3,7,8-TCDD.

Generally, the instrument manufacturers supply the quantification program and in our case we use the MS Work Station software to treat the raw data and quantify our result. The calculation is carried out according to the following principle:

- The response factor f_i of all single ^{12}C-2,3,7,8-PCDD/Fs are calculated relative to the ^{13}C-labelled congeners. The integrated single signal areas and concentration of the quantification standard are used as follow:

$$f_i = \frac{Conc.^{12}C_{isomer} \times area^{13}C_{isomer}}{Conc.^{13}C_{isomer} \times area^{12}C_{isomer}}$$

f_i-Response factor relative to $^{13}C_{isomer}i$; Conc.-Concentration in quantification standard

- The total amount of ^{12}C-2,3,7,8-PCDD/Fs in the sample is:

$$M_i = \frac{Amount^{13}C_i \times area^{12}C_i \times f_i}{Area^{13}C_i}$$

M_i-Total amount of ^{12}C-isomer i in the sample
Amount $^{13}C_i$-Total amount added to the sample

- The calculation of the recovery rate R_i (in %) of the ^{13}C-labelled 2,3,7,8-chlorine substituted congener i (added before extract clean-up as an internal standard) is carried out relative to the recovery standard (Rec.STD). The latter is added to the sample prior to quantification:

$$f_{rc} = \frac{Conc.^{13}C_i \times area\,\mathrm{Re}\,c.STD}{Conc.\,\mathrm{Re}\,cSTD \times area^{13}C - isomer}$$

f_{rc}-Response factor of ^{13}C-isomer i relative to recovery standard Rec.STD
Conc.-Concentration in internal standard

$$R(\%)_i = \frac{Amount\,\mathrm{Re}\,c.STD \times Area^{13}C_i \times f_{rc} \times 100}{Addedtot.amount^{13}C_i \times Area\,\mathrm{Re}\,c.STD}$$

R(%)-Recovery in % of the added ^{13}C-isomer i
Amount Rec.STD-Total amount of recovery standard added to the sample
Added tot.amount $^{13}C_i$-Total amount of the ^{13}C-isomer i added to the sample

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

d)Detection limit

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

$$DL = 3 \times conc._{std} \times \frac{Area_{noise}}{Area_{isomer}}$$

$Conc._{std}$-Concentration of standard
$Area_{noise}$-Area of the noise next to the isomer peak
$Area_{isome}$-Area of the isomer peak
The MSWS program allows us to calculate automatically the area of the peak and also the surrounding noise area. DL of all 2,3,7,8-PCDD/Fs analyzed by our LRMS (Varian 1200L) are presented in the table 4.5

Table 4.5 Calculated detection limit of 2,3,7,8-PCDD/Fs congeners

No	Congener	DL (ng/ml)	Note
1	2378-TCDD	0.05-0.1	EI-SIM
2	2378-TCDF	0.03-0.04	NCI-SIM
3	12378-PeCDF	0.02-0.04	--//--
4	23478-PeCDF	0.01-0.03	--//--
5	12378-PeCDD	0.1-0.3	--//--
6	123478-HxCDF	0.04-0.06	--//--
7	123678-HxCDF	0.05-0.07	--//--
8	123789-HxCDF	0.02-0.04	--//--
9	123478-HxCDD	0.08-0.1	--//--
10	123678-HxCDD	0.1-0.2	--//--
11	123789-HxCDD	0.05-0.07	--//--
12	234678-HxCDF	0.03-0.06	--//--
13	1234678-HpCDF	0.02-0.06	--//--
14	1234678-HpCDD	0.05-0.07	--//--
15	1234789-HpCDF	0.02-0.04	--//--
16	OCDD	0.04-0.06	--//--
17	OCDF	0.03-0.05	--//--

Note: the DL is calculated based on the chromatograph of calibration standard EDF2519-A (see annex for an example of calculation)

Based on the data in the table 4.5, we could calculate the detection limit for each real matrix type:

$DL_{matrix} = (DL_{isomer} \times V_{final})/(1000 \times M_{matrix})$ *[ng/g]*
DL_{matrix}-detection limit for real matrix typ; V_{final}-final volume of extract injected; M_{matrix}-weight of matrix analyzed
For example:

For 2,3,7,8-TCDD in soil sample, we have:

- DL_{isomer} = 0.1ng/ml

- V_{final} = 50μL

- M_{matrix} = 25g

$DL_{matrix} = (0.1 \times 50)/(1000 \times 25) = 2 \times 10^{-4}$ng/g = 0.2pg/g (0.2ppt)

The calculated detections limit for real matrix types are presented in the table 4.6 below:

Table 4.6 Calculated detection limit of 2,3,7,8-PCDD/Fs congeners

No	*Congener*	*DL_{matrix} (pg/g)*			
		Soil/sediment	Fish	Adipose tissue	Fly ash
1	2378-TCDD	0.2	0.5	1.0	0.25
2	2378-TCDF	0.08	0.2	0.4	0.1
3	12378-PeCDF	0.08	0.2	0.4	0.1
4	23478-PeCDF	0.06	0.15	0.3	0.08
5	12378-PeCDD	0.6	1.5	3.0	0.75
6	123478-HxCDF	0.12	0.3	0.6	0.15
7	123678-HxCDF	0.14	0.35	0.7	0.18
8	123789-HxCDF	0.08	0.2	0.4	0.1
9	123478-HxCDD	0.2	0.5	1.0	0.25
10	123678-HxCDD	0.4	1.0	2.0	0.5
11	123789-HxCDD	0.14	0.35	0.7	0.18
12	234678-HxCDF	0.12	0.3	0.6	0.15
13	1234678-HpCDF	0.12	0.3	0.6	0.15
14	1234678-HpCDD	0.14	0.35	0.7	0.18
15	1234789-HpCDF	0.08	0.2	0.4	0.1
16	OCDD	0.12	0.3	0.6	0.15
17	OCDF	0.1	0.25	0.5	0.13

As we see data from table 4.5, the NCI-SIM is not very sensitive for 1,2,3,6,6,8-HxCDD and 1,2,3,7,8-PeCDD. Unfortunately, ^{13}C-1,2,3,6,7,8-HxCDD is usually proposed as surrogate internal standard for PCDD/Fs analysis and it makes a difficulty for quantification of hexa-group of 2,3,7,8-PCDD/Fs in our research. This inconvenience could be solved by use of surrogate containing all 2,3,7,8-PCDD/Fs. In our case we not changed the standard solution due to limitation of time and budget for our research.

e)Linearity

For PCDD/Fs quantification we use the set of calibration standard solutions CC1 to CC5 (*see annex for the standards solutions used for analysis*) with the concentration varied from 20 to 2000 ng/ml. Generally, only CC3 solution is used for quantification, hence we should control the linearity of 2,3,7,8-PCDD/Fs to be sure that their abundances are linear with their concentrations in this concentration interval.

The linearity is controlled and calculated automatically by WSMS software for each series analysis (all calibration standard solutions CC1 to CC5 are injected). In the case of no linearity observed, the calibration standard having concentrations near concentrations in samples are used for quantification. Fortunately, in our case all series analyses had relatively good linearity. Fig. 4.11 showed an example for linearity of 2,3,7,8-TCDF using CC1 to CC5 calibration standards, as we see the linearity is very high with $R^2 = 0.997055$.

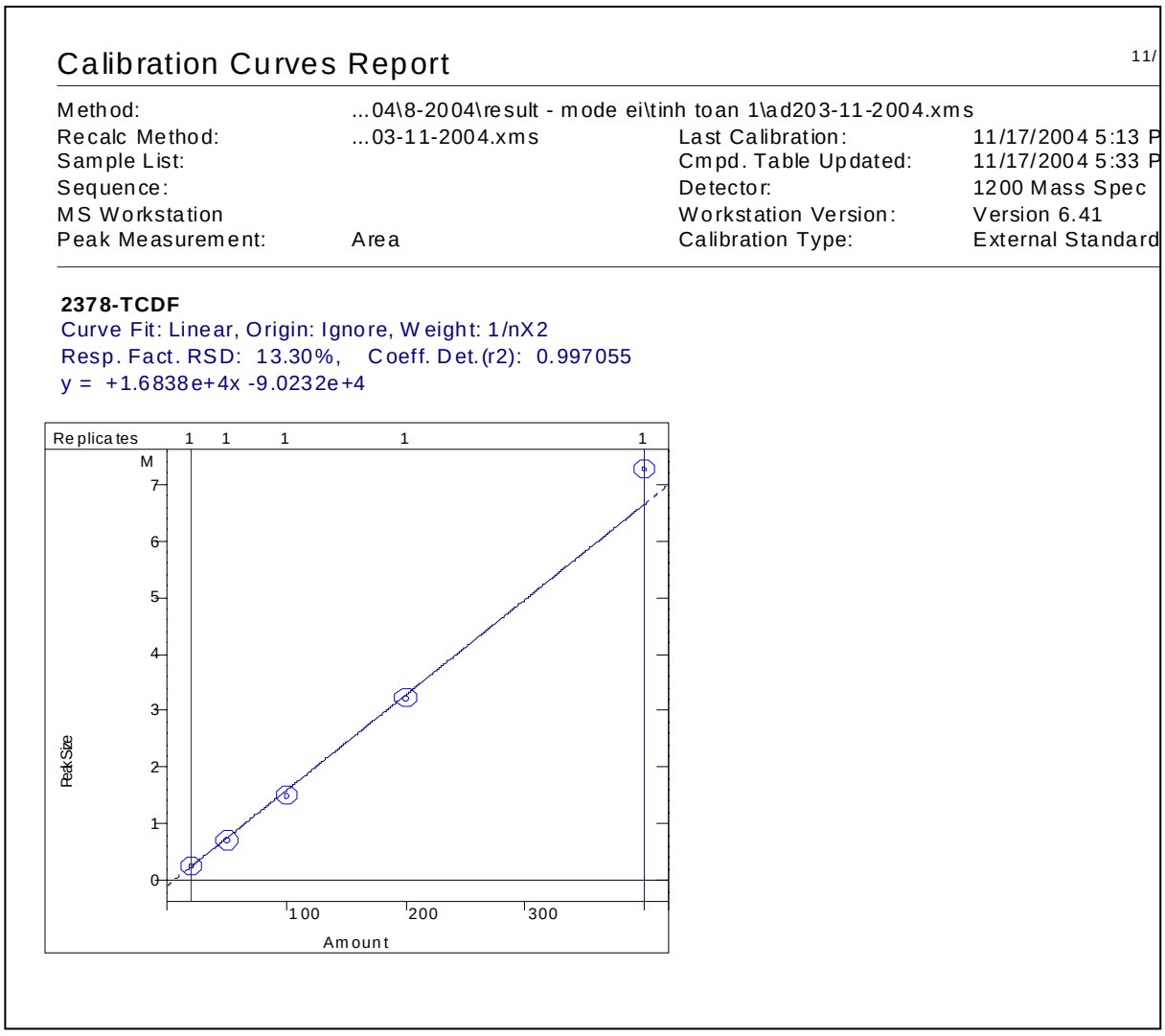

Fig. 4.11 Linearity of 2,3,7,8-TCDF with calibaration standards CC1 to CC5

4.6 Validation of selected analytical methods

The validation of selected analytical methods is carried out firstly with spiked samples (e.g: for testing the analytical method for soil/sediment analysis we used Merck quartz (clean sand) spiked with surrogate internal standards EDF-4053). After we obtained a good recovery, we continued to test the analytical method with SRMs. Here we present the result for SRM tests:

4.6.1 Testing analytical method for soil/sediment samples

EDF-2513: A consensus certified Fortified Natural Matrix Reference Material has been prepared for use as a Performance Evaluation for PCDD/Fs analysis of soils. The reference material is designed to be useful for both high and low resolution GC/MS analysis for all seventeen 2,3,7,8-PCDD/Fs.

CRM 529 (sandy soil): a certified reference material from industrial contaminated soil – use in the method validation and quality control of the complete analytical procedure for congener-specific determination of 2,3,7,8-substituted dioxins and furans and a number of chlorobenzenes and chlorophenols.

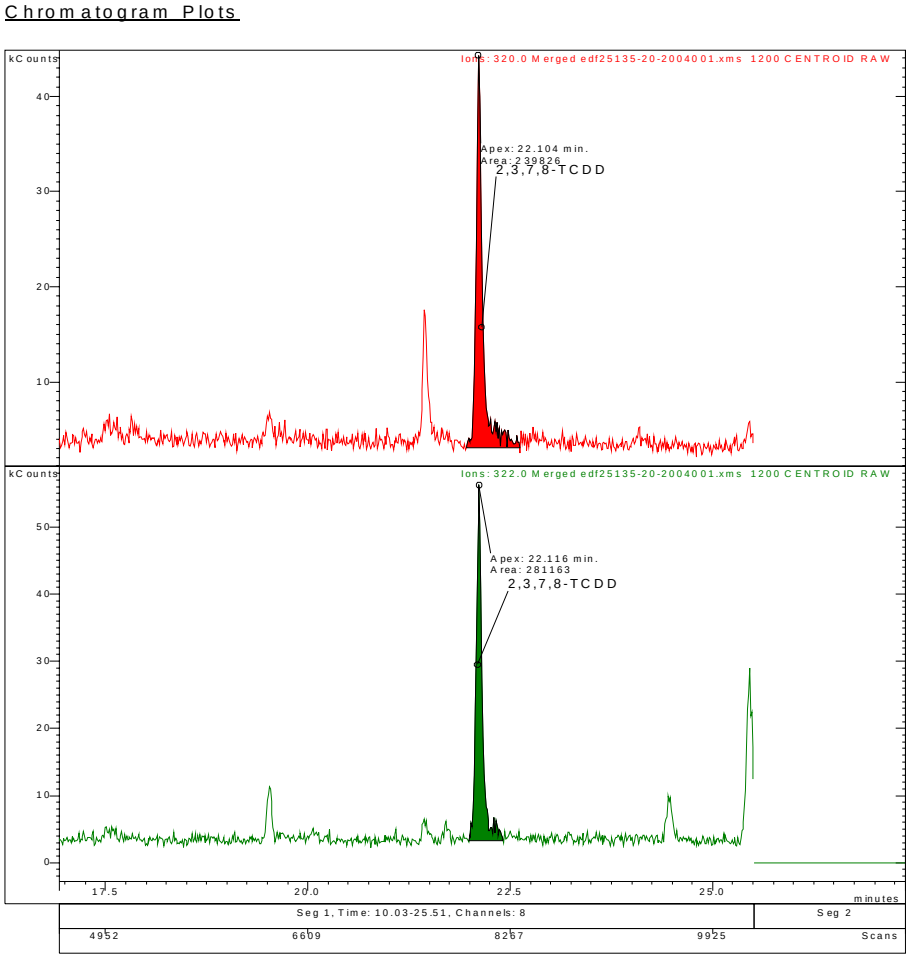

Fig. 4.12 EI-SIM chromatograph of EDF-2513 extract solution for 2,3,7,8-TCDD determination

The testing result of two SRM types is presented in the table 4.7 and 4.8. In fact the analyzed 2,3,7,8-TCDD and 2,3,7,8-TCDF concentrations for EDF-2513 samples ware relatively high in comparison with certified values (*see table 4.7*). The reason is that we had tested this material two times in March/2004, but the analytical method for soil/sediment was still in improving process (the clean-up procedure was not yet completed). However the analyzed concentrations for the rest 2,3,7,8-substituted PCDD/Fs were acceptable in comparison with the certified values done by producer. We could not re-analyze this SRM type because there was no stock (it is relatively expensive, about 500USD/10g). For CRM 529, the obtained recoveries (three times) were very good for all 2,3,7,8-PCDD/Fs, that mean the selected method is effective for such contaminated soil type. The 2,3,7,8-PCDD/Fs concentrations corrected with obtained recovery are also correlative with the values done by producer as presented in the table 4.8.

Table 4.7 Results of EDF-2513 test *(n=2)*

Congener	*Analyzed Conc. (ng/g)*	*Certified Conc. (ng/g)*	*Lower Value (ng/g)*	*Upper Value (ng/g)*
2378-TCDD	1.1 ± 0.22	0.5	0.26	0.67
2378-TCDF	0.7 ± 0.12	0.5	0.26	0.64
12378-PeCDF	1.6 ± 0.32	1.0	0.59	1.15
23478-PeCDF	1.1 ± 0.25	1.0	0.41	1.31
12378-PeCDD	2.0 ± 0.61	1.0	0.56	1.37
123478-HxCDF	1.2 ± 0.30	1.0	0.53	1.23
123678-HxCDF	1.9 ± 0.42	1.0	0.34	1.56
123789-HxCDF	1.7 ± 0.44	1.0	0.39	1.26
123478-HxCDD	0.8 ± 0.19	1.0	0.50	1.29
123678-HxCDD	1.9 ± 0.17	1.0	0.52	1.21
123789-HxCDD	2.0 ± 0.51	1.0	0.46	1.33
234678-HxCDF	1.5 ± 0.25	1.0	0.48	1.35
1234678-HpCDF	2.4 ± 0.36	1.5	0.52	2.01
1234678-HpCDD	1.9 ± 0.48	1.5	0.71	2.07
1234789-HpCDF	2.1 ± 0.31	1.5	0.25	1.98
OCDD	5.0 ± 1.07	2.5	1.98	5.03
OCDF	3.5 ± 0.75	2.5	1.17	3.33

Table 4.8 Results of CRM-529 test *(n=3)*

Congener	*Analyzed Conc. (ng/g)*	*Certified Conc. (ng/g)*	*Lower Value (ng/g)*	*Upper Value (ng/g)*
2378-TCDD	3.6 ± 0.9	4.5	3.05	5.90
2378-TCDF	1.65 ± 0.41	0.78	0.60	1.02
12378-PeCDF	0.27 ± 0.08	0.14	0.11	0.22
23478-PeCDF	0.35 ± 0.12	0.36	0.25	0.48
12378-PeCDD	0.3 ± 0.11	0.44	0.38	0.51
123478-HxCDF	4.5 ± 1.11	3.4	2.59	4.43
123678-HxCDF	1.79 ± 0.45	1.09	0.76	1.42
123789-HxCDF	0.07 ± 0.03	0.022	0.013	0.033
123478-HxCDD	1.06 ± 0.31	1.2	0.73	1.68
123678-HxCDD	5.5 ± 1.38	5.4	3.92	7.12
123789-HxCDD	4.1 ± 1.04	3.0	2.41	4.10
234678-HxCDF	0.71 ± 0.20	0.37	0.25	0.46

As we told above, the most difficult for quantification are the HxCDD/Fs, especially for 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD: the peaks of these compounds are not completely separated, beside that their abundance are relatively small in comparison with others. In fact for 2,3,7,8-PCDD/Fs identification/quantification by LRMS, beside the using of automatical software to identify/quantify such as WSMS we should also to regard to the chromatogram because the abundances of the interested compounds are usually very small. ***The PCDD/Fs analysis by LRMS is time-consuming and requiring an experience person***. HRMS is easier with very clear peak signal due to its high separation by exact mass.

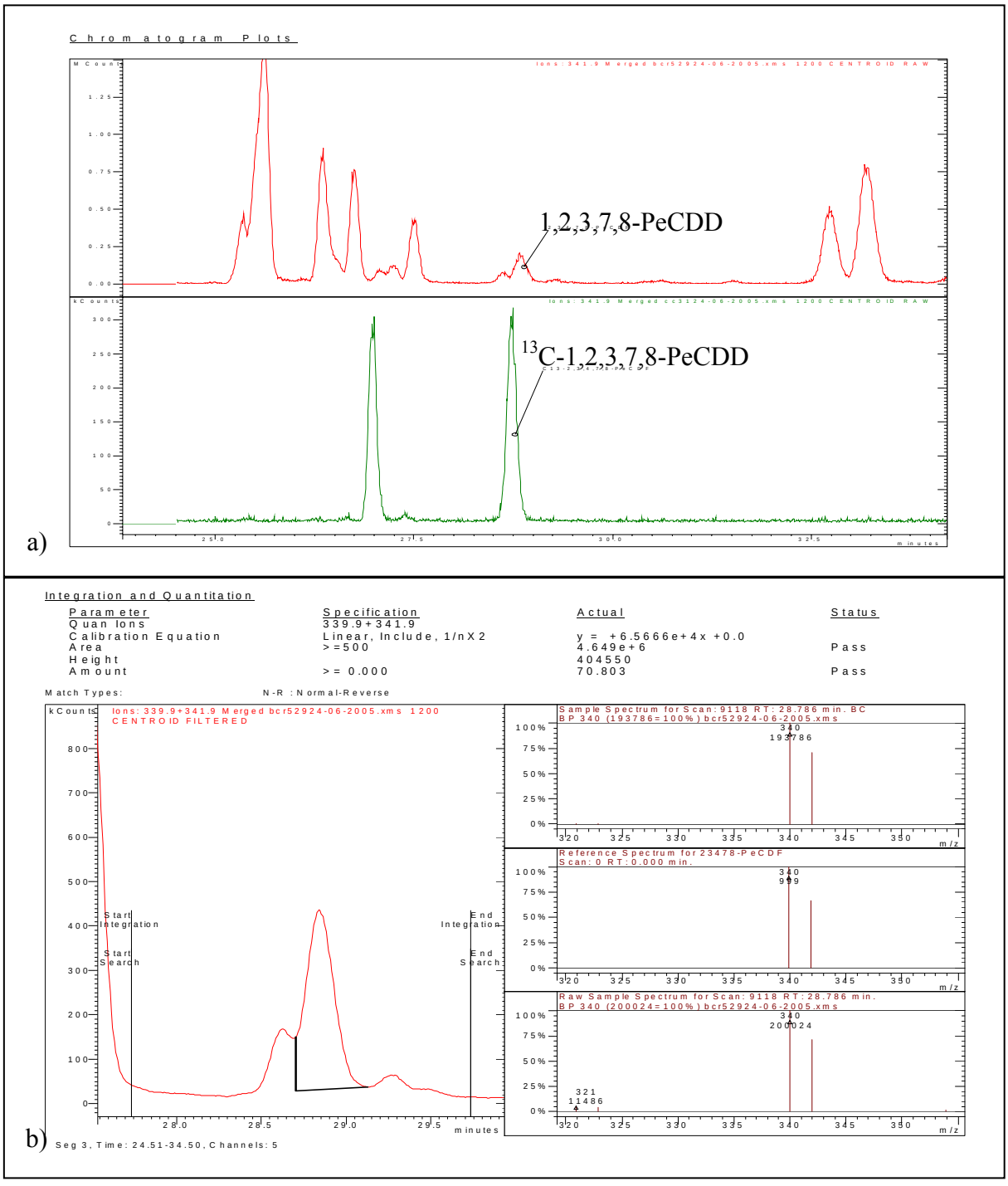

Fig. 4.13 An example for identification/quantification of 1,2,3,7,8-PeCDD in CRM-529: a) identify 1,2,3,7,8-PeCDD peak with regard to ^{13}C-1,2,3,7,8-PeCDD; b) quantify 1,2,3,7,8-PeCDD in CRM-529 by WSMS software

Based on the obtained results, we can see that our selected analytical method is acceptable for soil/sediment matrix. The standard deviation of controlled reference materials are about ±25%. In fact it could be better if we repeat the test for more times, but we not did it due to time and budget limits.

4.6.2 Testing analytical method for biological samples

Generally, biological SRM for LRMS are not available in the market because as we told in the previous chapter, LRMS is not proposed for PCDD/Fs analysis in this matrices. Almost all biological SRM's for PCDD/Fs test have very low 2,3,7,8-PCDD/Fs concentrations that we could use from our methods. In addition, the price of SRM is very expensive with very small quantity (10g to 50g). Hence we should try to find the maximum suitable SRM as possible to test our method as follow:

NIS-SRM 1588a: a cod liver oil is intended for use in developing and validating of analytical methods for determination of chlorinated biphenyls and chlorinated pesticides in cod liver oil or in other similar complex lipophilic matrices.

EDF-2526 (CIL): the fish tissue homogenates for use as QA/QC Reference Standards for on-going analytical programs; tools for new method development; and standards for training/certifying new analysts. We received this SRM ampoule in April/1994, but it was not yet analyzed due to some reasons. It was conserved in refrigerator (-15^{o}C). Even more then 10 years passed, but we though that it is still useful for us as a SRM to validate our analytical methods (to economize the budged for our research).

Table 4.9 Results of NIS-SRM 1588a test (*m=1.98g, n=1),*

Congener	*Analyzed Conc. (ng/g)*	*Non-Certified Conc.[a] (ng/g)*
2378-TCDD	0.25	0.21
123678-HxCDD	nd	0.39
123789-HxCDD	nd	0.22
OCDD	0.57	1.01
OCDF	1.13	1.00

Note: a- These concentrations are provided only as information values, not yet certified

For NIS-SRM 1588a, we have only the non-certified values of 2,3,7,8-PCDD/Fs concentrations because this SRM is used mainly for PCBs and pesticides test . 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD were non-detected because their concentration is lower than DL of our LRMS (*see table 4.5 & 4.6*). Concentrations of 2,3,7,8-TCDD, OCDD and OCDF are very comparative with the non-certified values as shown in table 4.9.

For fish tissue samples (EDF-2526), the obtained concentrations are relatively good. This is a fortified SRM with relative high concentrations of 2,3,7,8-PCDD/Fs, and furthermore after very long time storage the status of this sample ampoule was quiet good: when we opened the ampoule of EDF-2526, there was no gas inside neither bad smell.

Table 4.10 Results of EDF-2526 test *(n=1)*

Congener	*Analyzed Conc. (ng/kg)*	*Certified Conc. (ng/kg)*
2378-TCDD	32	19 + 1.4
2378-TCDF	30	17 + 1.5
12378-PeCDF	47	40 + 3.7
23478-PeCDF	39	38 + 3.5
12378-PeCDD	49	40 + 3.0
123478-HxCDF	111	80 + 8.4
123678-HxCDF	79	63 + 5.5
123789-HxCDF	71	58 + 7.0
123478-HxCDD	37	60 + 4.8
123678-HxCDD	31	56 + 4.8
123789-HxCDD	61	60 + 4.4
234678-HxCDF	72	60 + 5.5
1234678-HpCDF	87	83 + 9.2
1234678-HpCDD	85	76 + 5.9
1234789-HpCDF	90	73 + 7.7
OCDD	437	192 + 14
OCDF	280	190 + 22

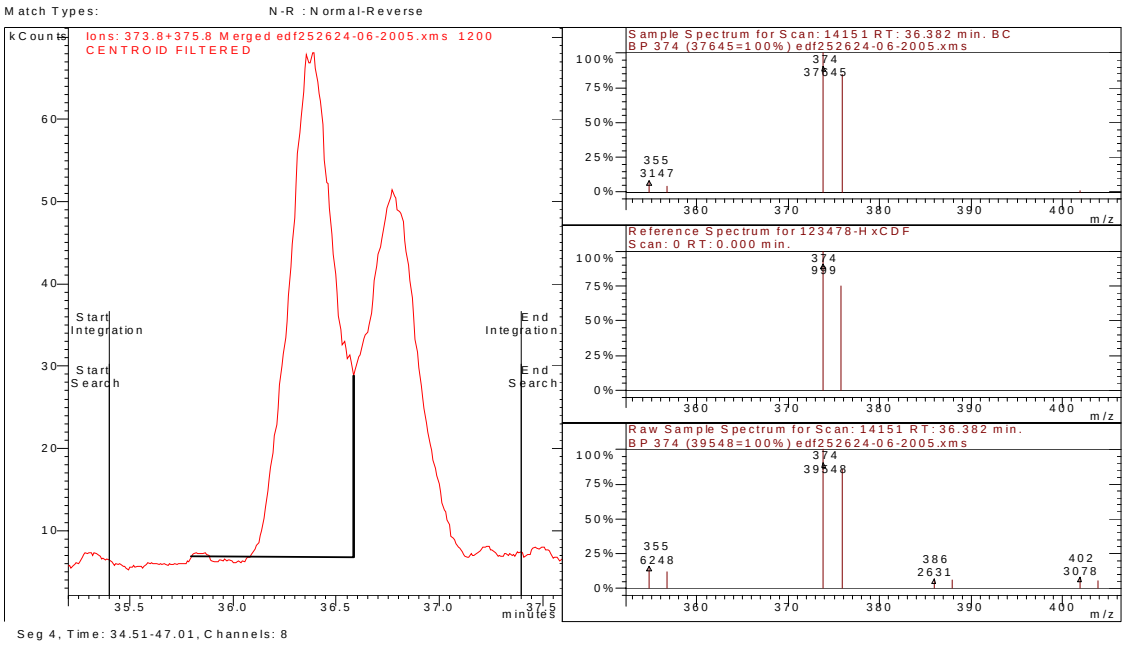

Fig. 4.13 quantify 1,2,3,4,7,8-HxCDF in EDF-2526

As we see in the fig. 4.13, the peak of 1,2,3,4,7,8-HxCDF is very net and clear after almost 10 years of conservation.

4.6.3 Testing analytical method for fly ash samples

BCR-615: a certified reference material of Community Bureau of Reference (BCR) – European Commission, has been prepared for use as a Performance Evaluation for PCDD/Fs analysis of fly ash (low level).

Table 4.11 Results of BCR-615 test, m=20.09g *(n=1)*

Congener	*Analyzed Conc. (pg/g)*	*Certified Conc. (pg/g)*	*Uncertainty (pg/g)*
2378-TCDD	30	27	5
2378-TCDF	120	86	28
12378-PeCDF	190	176	26
23478-PeCDF	160	125	20
12378-PeCDD	50	92	12
123478-HxCDF	230	203	21
123678-HxCDF	240	204	23
123789-HxCDF	17	13.3	2.0
123478-HxCDD	20	74	12
123678-HxCDD	20	103	13
123789-HxCDD	60	108	16
234678-HxCDF	70	130	15
1234678-HpCDF	800	0.75×10^3	0.09×10^3
1234678-HpCDD	910	0.87×10^3	0.13×10^3
1234789-HpCDF	60	61	6
OCDD	1520	1.75×10^3	0.20×10^3
OCDF	230	0.29×10^3	0.04×10^3

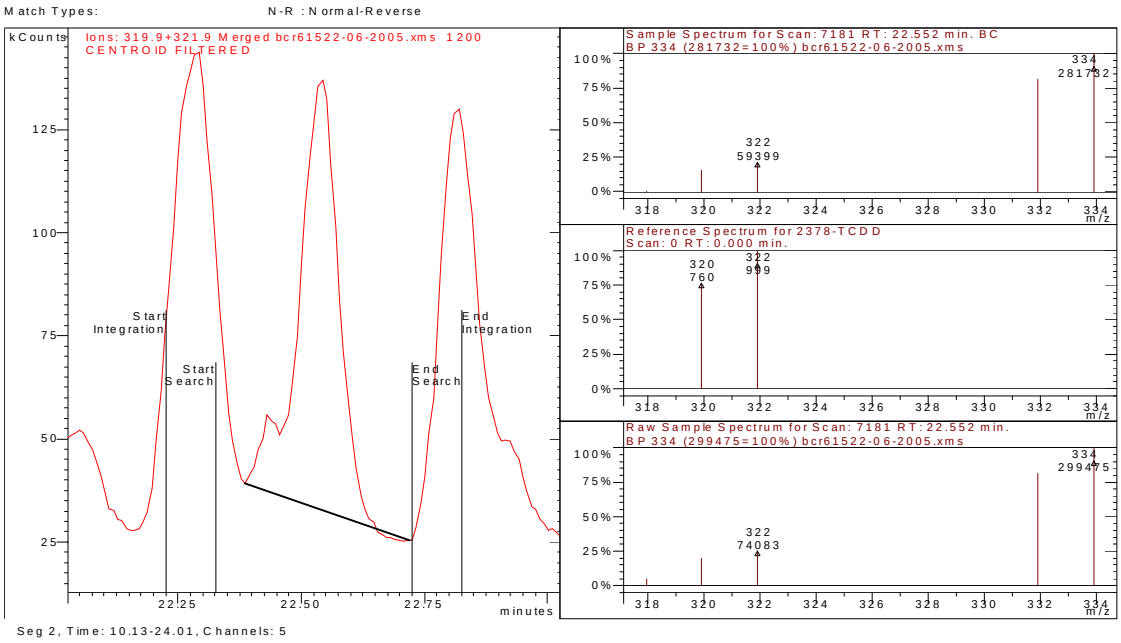

Fig. 4.14 Quantifying 2,3,4,7,8-TCDD in BCR-615

The analyzed concentrations of 2,3,7,8-PCDD/Fs presented in table 4.11 indicated that our analytical method is good for fly ash, except for hexa- group. There are two reasons: the LRMS is not enough sensitive for HxCDDs; and the use of ^{13}C-1,2,3,6,7,8-HxCDD as surrogate standard for all isomers of HxCDD/Fs is not very appropriate.

4.7 Conclusions

There are some important conclusions drawn from SRM tests:

1) ***The selected analytical methods are effective and good for soil/sediment and fly ash matrices; they are acceptable for biological matrices with relative high concentration of 2,3,7,8-PCDD/Fs (much higher than detection limits of such compounds as presented in table 4.6). With such analytical procedures, the estimated cost for one 2,3,7,8-PCDD/Fs analysis is reduced at minimum as possible;***
2) ***The identification/quantification of 2,3,7,8-PCDD/Fs by LRMS for relative complex matrices such animal tissue or liver oil, etc should be done very carefully. We should use the abundances (peak signal, retention time) in chromatographs of standard solution and sample extract, as well as the automatic software for identification/quantification such WSMS. Since the PCDD/Fs analysis procedure is time-consuming and requiring an experience person (long-time working with the GC chromatograph);***
3) ***Use the calibration standard solutions containing all ^{13}C-2,3,7,8-PCDD/Fs will be more helpful for quantification. Nowadays, these types standards are available, but unfortunately they are only for HRMS (with very small quantity 200-500μL and small concentration 0.1 -100ppb). Another way we should find and improve the detection limit for HxCDDs group: e.g. to find the other characteristic mass values.***

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CHAPTER V: DIOXIN CONTAMINATION IN THE SOILS AND SEDIMENTS

5.1 Introduction

As we have told above, soil and sediment were the first matrices selected for our research as they act as a sink for PCDD/Fs. In fact, studies on this subject are relatively difficult to perform due to many factors: over 30 years passed; land use disturbance; degradation and transfer of dioxin into biological food web. In addition, a lack of related documents and military secrets also contributed to this.

As dioxin contamination is a national problem and a whole investigation requires a big budget in the frame of our PhD thesis we should consider to select the appropriate areas according to our limited budget and time. Based on the defoliants sprayed map on the southern Vietnam, we have selected the areas for our research as follow:

1. MaDa Forest:

MaDa before 1975 was called Base D – it was a Vietnamese military base during the US-Vietnam war. This area is seriously affected by the chemical toxics and lethal weapons. In period 1964-1969, the defoliants ware sprayed on the Base D to destroy the local forest (*Trung,T.V. 1982)*. The forest along the road 322, from DongNai river bank to MaDa Stream (35 km) was mostly destroyed by the repeated sprays. The affected area is about 3,000 ha. In 1975 (after VN independence), the MaDa Afforestation Yards (MAYs) has been established to exploit the woods for economic purposes. Once again the forest is seriously injured. In 1982, after a long time of exploitation, the forest source was almost exhausted. The purpose of MAYs had been changed from the exploitation into the forest recovery. The afforesting activities focused on the economy-valuable vegetation types such *Dipterocarpaceae, Hopea Odorata*,etc to recover the forest source. The afforesting techniques were applicated to increase the wood production of the target vegetation. In 1985, the TriAn Hydroelectric Project had been carried out and in 1997 the TriAn Hydroelectric Reservoir formed and begun operates.

The sampling has been done in the area belonging to a former military airport – Rang Rang Airport (for helicopter only). This area is afforested, but the vegetation development is still poor. The samples were taken from a surface 15x30 m, in both left side (code RRT) and right side (code RRP), along road 322. The area has been afforested since 1984 with the selected tree type such *Acacia auriculaeformis, Acaciamagnum, etc* to remediate the soil. Under the main trees layer, there is a weed layer with the species such *Imperata conferta, Pennisetum polystachyon Pennisetum alopecuroides, Grewia paniculate, Pterospermum angustifolium, etc.*

2. **CamLo – Quang Tri Province**:

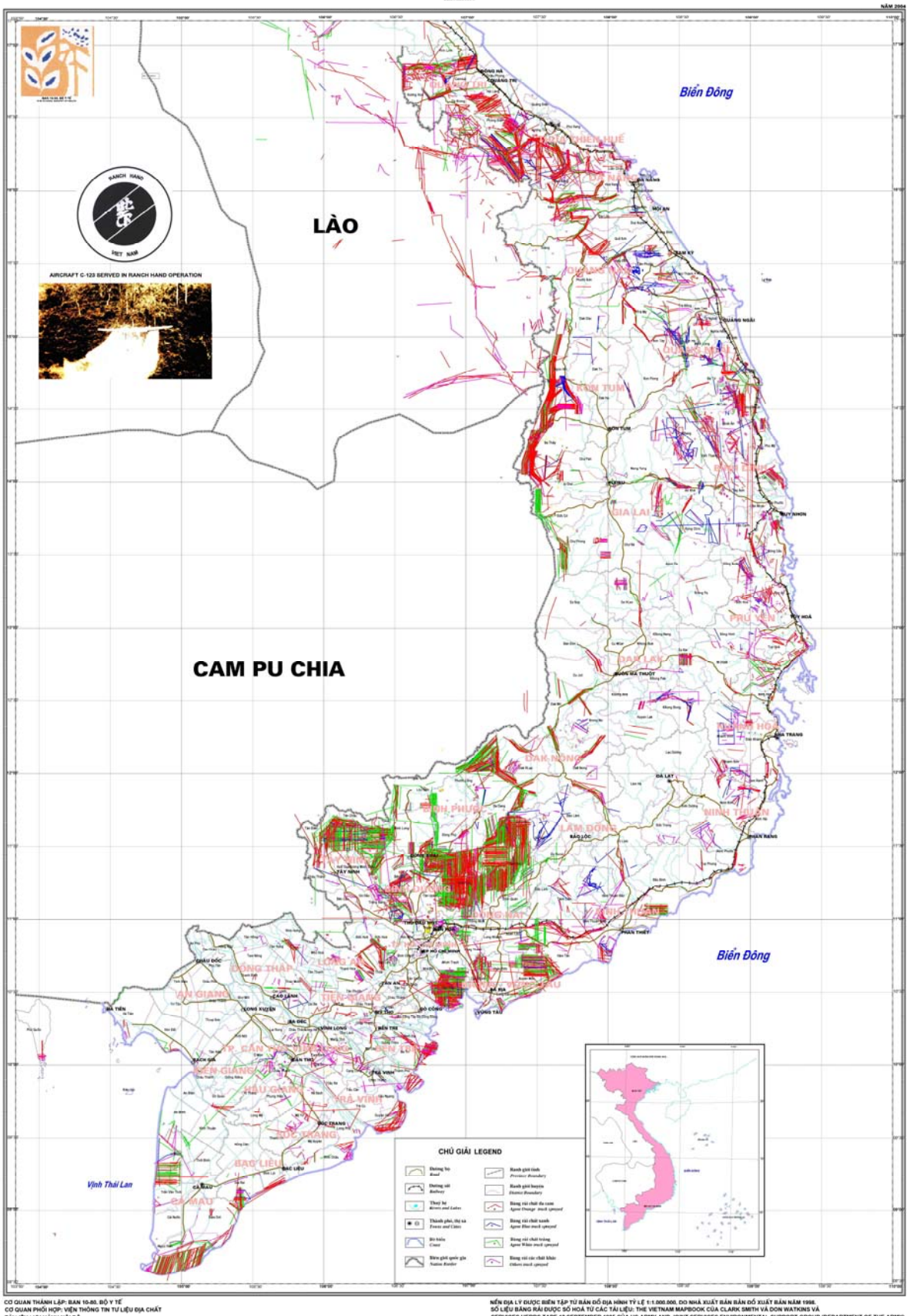
QuangTri lies just 30 kilometers north of the McNamara Line and 60 kilometers to the west of the HoChiMinh Trail, the most heavily bombed area in the war. The McNamara Line, named after the former US defense minister, refers to a borderline that the American armed forces set up at the 17th parallel separating North and South Vietnam. CamLo is a district in central QuangTri province – the most herbicide sprayed area in the Middle of Vietnam. It was hit hard during the war and today remains one of the country's poorest areas. A survey showed that 2,000 people from QuangTri have died from causes related to the chemical

BẢN ĐỒ BĂNG RẢI CHẤT DIỆT CỎ MỸ ĐÃ SỬ DỤNG TRONG CHIẾN TRANH VIỆT NAM

HERBICIDE SPRAYED MAP ON THE SOUTH VIETNAM (RANCH HAND OPERATION 1962-1971)

0 25 50
Kilometers

NĂM 2004



CƠ QUAN THÀNH LẬP: BAN 10-80, BỘ Y TẾ
CƠ QUAN PHỐI HỢP: VIỆN THÔNG TIN TƯ LIỆU ĐỊA CHẤT
TÀI LIỆU LƯU HÀNH NỘI BỘ

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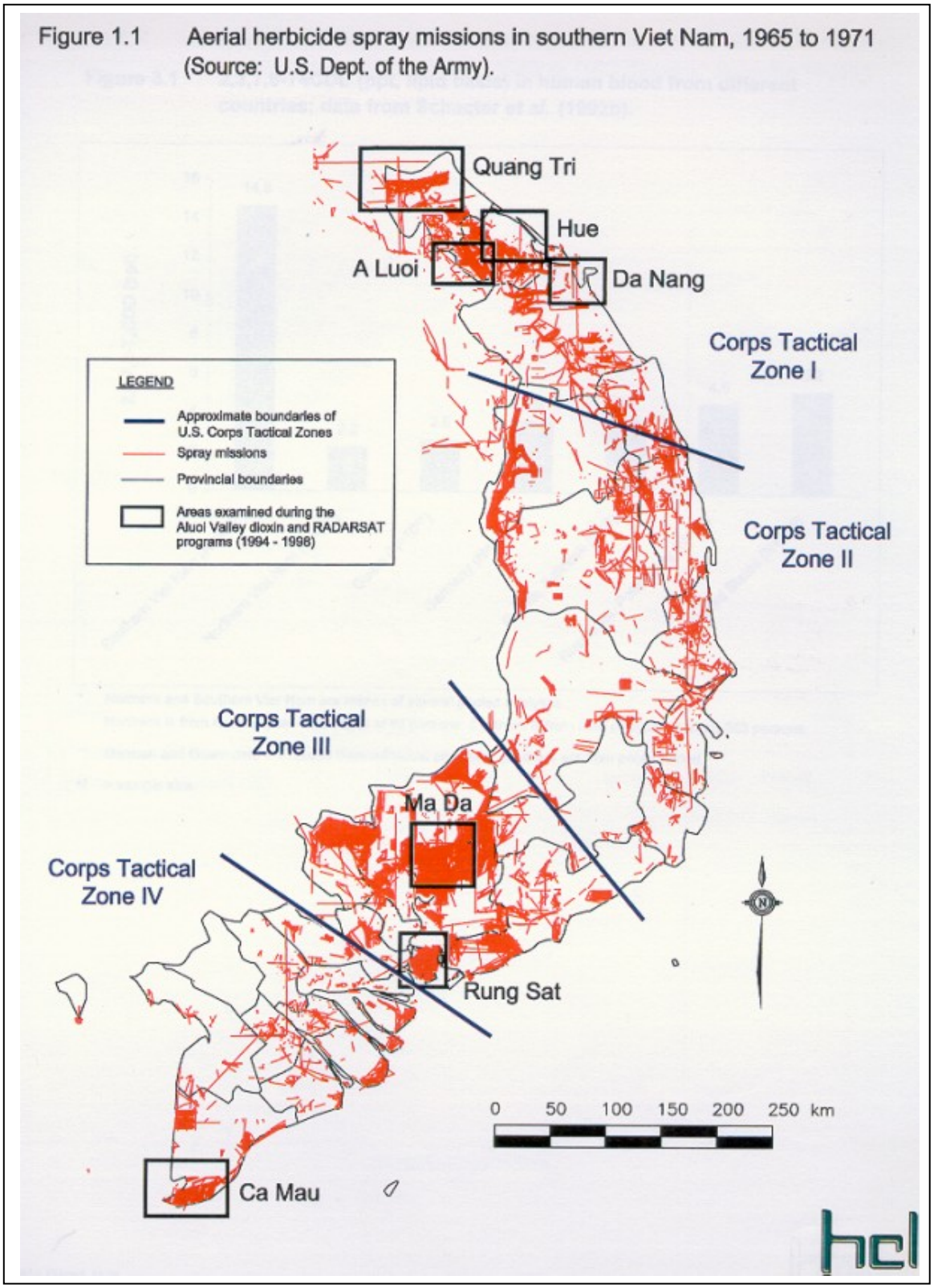
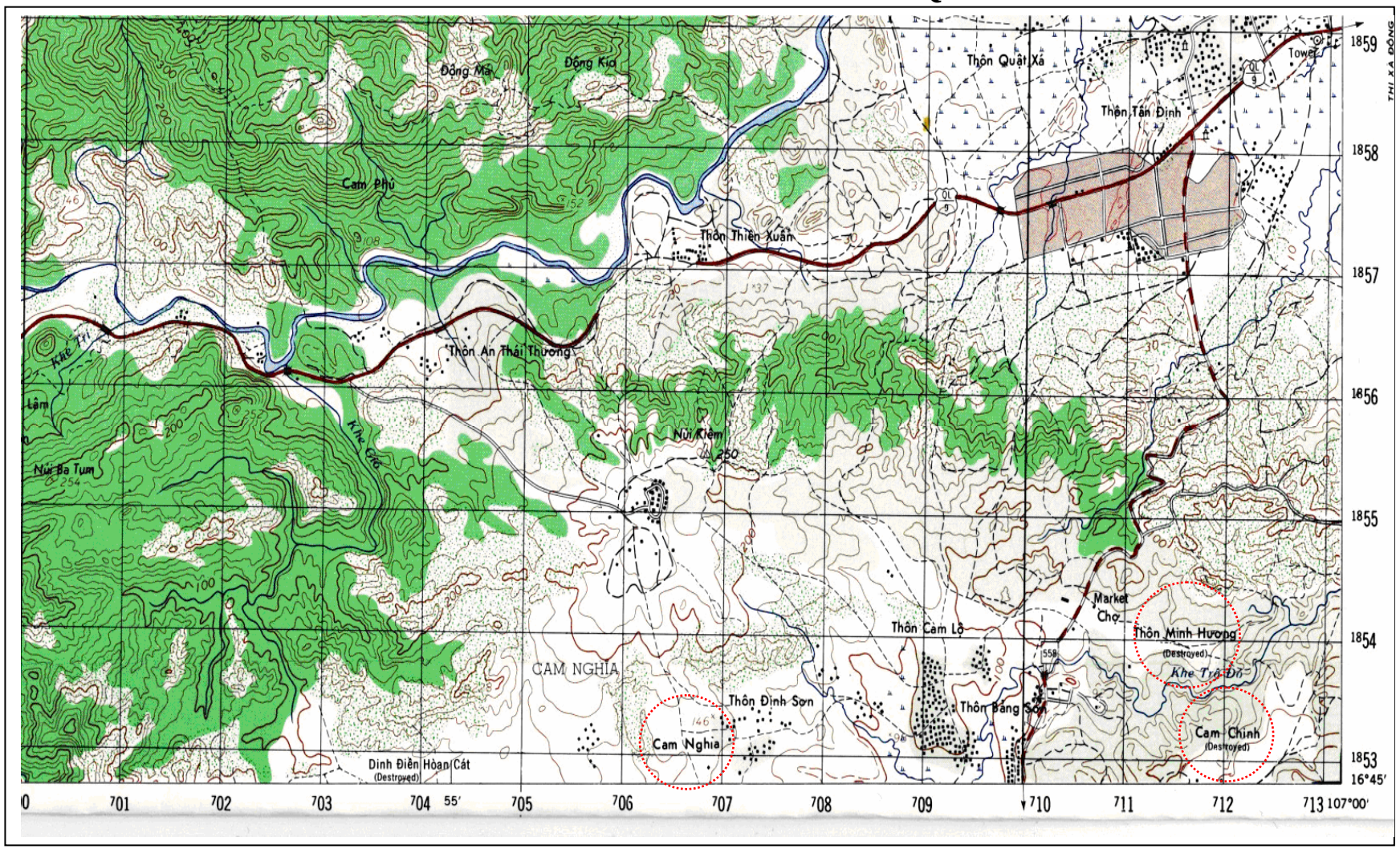
SỐ LIỆU BĂNG RẢI ĐƯỢC SỐ HOÁ TỪ CÁC TÀI LIỆU: THE VIETNAM MAPBOOK CỦA CLARK SMITH VÀ DON WATKINS VÀ SERVICES HERBS TAPE 12 SEPTEMBER 1985 CỦA US ARMY AND JOINT SERVICES ENVIRONMENTAL SUPPORT GROUP (DEPARTMENT OF THE ARMY)

MAP OF SELECTED SITES FOR OUR RESERACH



SAMPLING MAP OF CAMLO DISTRIC – QUANGTRI PROVINCE



N

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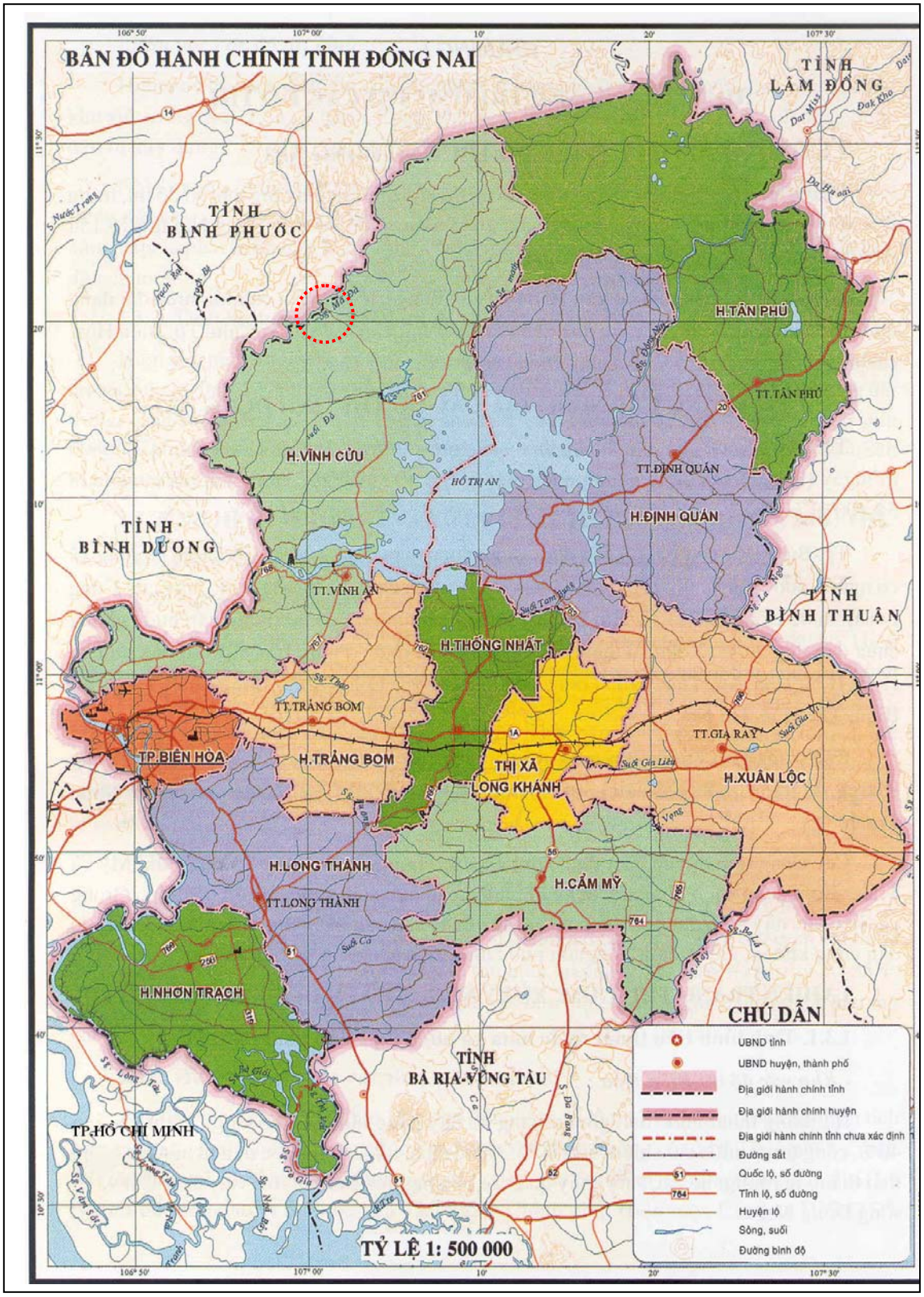
Sampling areas

SAMPLING MAP OF MADA FOREST – DONGNAI PROVINCE



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Sampling areas

SAMPLING MAP OF BIENHOA AIRFORCE BASE AND BIENHUNG LAKE – DONGNAI PROVINCE

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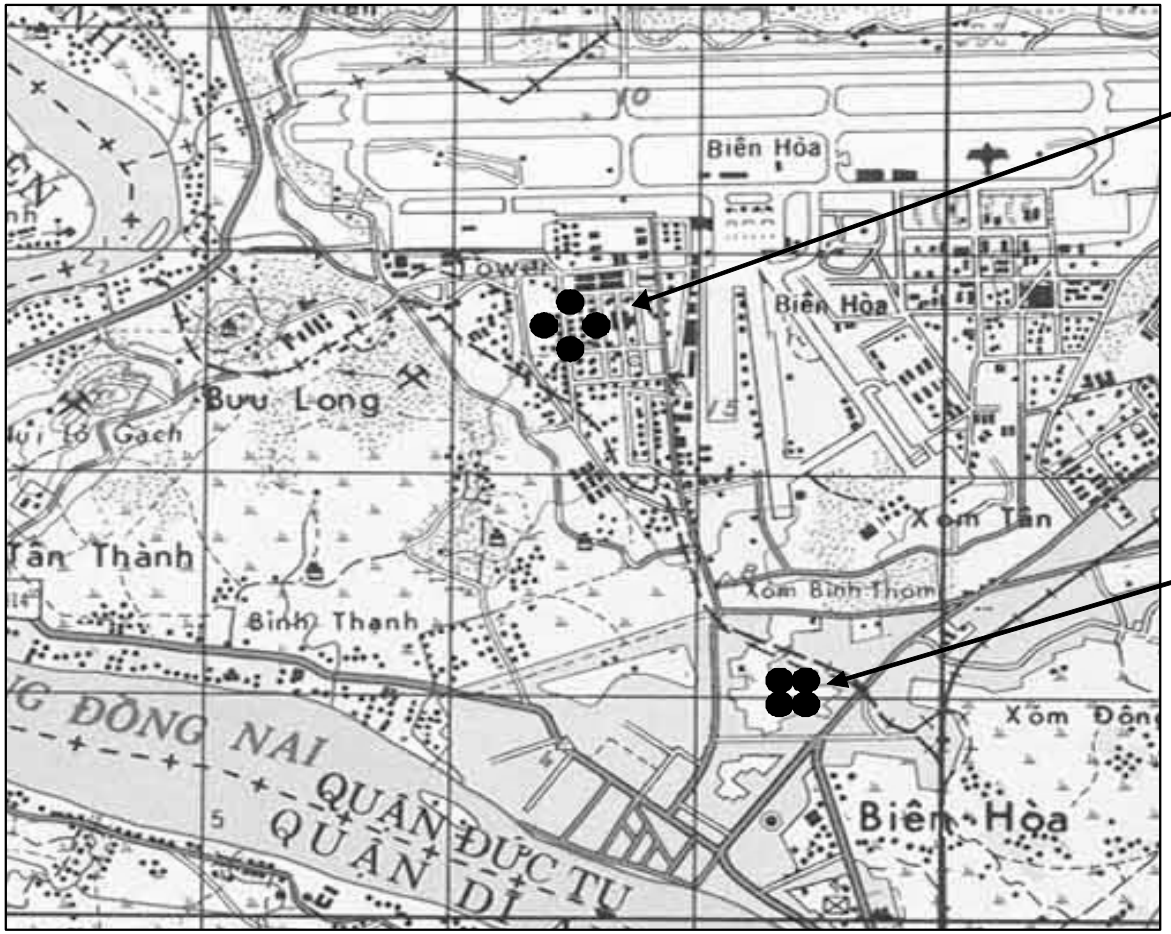


Soil sampling points (Bien Hoa Airbase)

Sediment sampling points (Bien Hung Lake)

defoliant, and 5,240 children whose parents were exposed in some ways to the chemical have been born with deformities; even a very small village as DongSon (CamChinh) has more than 272 birth-deformity children over 1078 residents (*Quy, 2002*).

The sampling was done in three sub-districts: CamChinh, CamNghia and MinhHuong. Follow the Local People Committee, these sub-districts with common name Cua are the most contaminated places by chemicals during the war. Samples were taken from the sites under a supervising of a local guide (an ancient veteran): in the gardens of the families having birth-deformity children, in the former helicopter military air port (MaiLoc – CamChinh) and near former military base (TroDo Bridge).

3. **DaNang Airport**:

DaNang Airport was used as a military base during the war and it is proposed as a testing model to assess and remediate the contaminated area. Due to the difficult formalities, we could not done our sampling inside the airport, so we have selected the sampling sites outside the airport - near the plane runway, where the waste from the planes were throw-out after the flights during war time. Nowadays, these sites are used for vegetable cultivation.

4. **BienHoa Air Force Base and BienHung Lake:**

BienHoa Air Force Base was a former storage depot for Agent Orange and the site of a large spill in 1970 (*Dwernychuk et al, 2002; Schecter et al., 2003*). The airport belongs to the sub district Tan Phong and is still military zone (entry forbidden). The soil sample was taken outside the airport near a radar station. Bien Hung Lake is located in the sub district TrungDung- BienHoa City, about 1 km from the airport and separated from the airport by the national highway No 15. This lake with a surface of about three hectares and an average depth of 2.5m is receiving runoffs from the Bien Hoa Airbase and its surrounding areas. Sediment samples were taken from 10 positions within the lake. Sampling period was during the end of the dry season

In addition, we have taken and analyzed some soil samples of ThuDuc – a industrial zone (power company, steel company, etc.) and MyThanhBac – TienGiang province (Mekong Delta). The data of these samples in one hand serves as a reference to assess the PCDD/Fs level; and in another by comparing the profile of PCDD/Fs in the samples we may find the origin of PCDD/Fs. With the same purposes, we have analyzed 6 bottom ash samples from different incinerators in HoChiMinh City and VungTau City.

5.2 Sampling technique

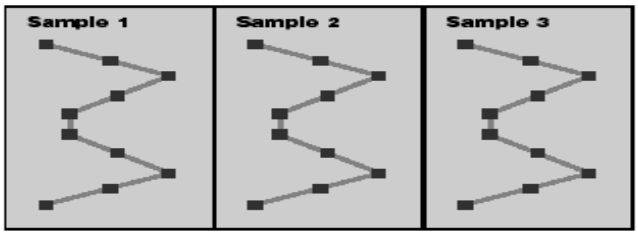
Considering that an acre (0.4 ha) of soil with a 15cm depth weighs about 1,000 ton, and that only few grams of soil will be used for the test in the laboratory, it is very important that the soil samples are characteristic of the entire site. There are two important points that we should consider when doing the soil sampling:

- ***The sampling site is open and not excessively covered by vegetation.***
- ***The area of interest is undisturbed during the time period of interest.***

We tried to follow these points, but as we have told about the difficulties in the beginning of this chapter, sometimes we have done the sampling even the conditions were not completely adapted to our criteria.

We have used a grid sampling - a systematic approach that divides the sampling site into squares of 30x30 m (usually referred to as "grid cells"). Soil is collected from within each of these "cells." The location of each "grid cell" was geo-referenced using global positioning

system technology (GPS). The soil samples ware taken by using soil hand-borer. The composite samples for analysis consisted of at least 10 cores collected from the same cell as described in the Fig.5.1. The composite soil was mixed thoroughly in a tray with a spoon (both stainless steel made), then put in to aluminum box for transport to laboratory.



a)

b)

Fig. 5.1:
a) W-shaped sampling Pattern (30×30m cell)
b) Cell measuring (RangRang Airport -MaDa forest)

As we known, topsoil is an important sampling medium for the investigation of the dioxin contamination due to its high organic content in comparison with other layer. PCDD/Fs mobility into soil is controlled by the equilibrium sorption/desorption process among several environmental compartments (air, water, mineral and organic matter). PCDD/Fs adsorbed strongly to soil particles with typical high organic concentrations - normally the first layer (topsoil) in the first few centimeters. In our research we had chosen the depth soil layer of 0 – 20cm.

Due to the laterization process in MaDa forest the soil is very compact; hence we taken only the topsoil in the depth of 0 – 10cm. To study the mobility of PCDD/Fs by the depth, we tried to taken the deeper soil layer in BienHoa Air Base.

Bed sediment was sampled with an Eckman Grab while deeper layer sediment was taken with a metal tube covered by a lid in the end. For each sampling position we took 3 samples: one from sediment bed (0-20 cm) and two from deeper layers (20-30 cm and 30-50 cm, respectively).

Fig. 5.2 Sampling by hand-borer (CamLo – QuangTri Province)

Fig. 5.3 Preparing the composite soil sample (MaDa Forest)

Fig. 5.4 Sediment sampling (BienHung Lake)

After sampling, samples ware dried at room temperature for about 5-7 days on the alum foil in laboratory. 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 4^{o}C until analysis.

Fig.5.5 Soil sampling (ThuDuc Industrial Zone)

5.3 PCDD/Fs level in soil of CamLo – QuangTri province

In May/2003, we have done an investigation and sampling in CamLo – QuangTri Province. With the help of the Local People Committee, we have taken 10 composite soil samples as described in the table 5.1 below:

Table 5.1 Description of CamLo soil samples

No	*Sample code*	*GPS positions*	*Sample Type*	*Note*
1	CL1	N: 16°44'56.5" E: 106°57'49.0"	Garden soil	Address: DocKinh village-sub-dist CamChinh Host-Tran Van Lan, having one birthdeform. son (12 years old)
2	CL2	N: 16°44'4.8" E: 106°57'12.8"	Soil	Address: sub-dist. CamChinh MaiLoc Former Military Airport (for helicopter only)
3	CL3	N: 16°44'6.6" E: 106°57'10.8"	Soil	--//--
4	CL4	N: 16°44'5.3" E: 106°57'13.9"	Soil	--//--
5	CL5	N: 16°44'26.9" E: 106°57'59.8"	Garden soil	Address: PhuongAn village, sub-dist CamNghia Host-Ng. th. Huyen, her husband died by a disease related to A.O, having three deform. children: - Daughter 27 year old (died) - Daughter 23 year old - Son 22 year old
6	CL6	N: 16°44'42.4" E: 106°56'55.1"	Garden soil	Address: PhuongAn village-sub-dist. CamNghia Host-Ng. v. Loc, having three birth-deform. children: - Son 26 year old (died) - Daughter 21 year old - Son 15 year old
7	CL7	N: 16°45'40.1" E: 106°58'40.7"	Garden soil	Address: MinhHuong village-sub-dist CamChinh (the place was recognized as a contaminated site by Agent Orange)
8	CL8	N: 16°45'32.4" E: 106°57'58.1"	Garden Soil	Address: TroDo Bridge, sub-dist. CamChinh (near the former military base)
9	CL9	N: 16°45'32.4" E: 106°57'48.5"	Garden Soil	Address: Tro Do Bridge, sub-dist. CamChinh (near the former military base)
10	CL10	N: 16°47'29.6" E: 106°58'47.2"	Rice field soil	about 3km from Cua – on the way to go to DongHa

The samples are analyzed two times by the analytical method described in chapter IV: first time in the end of 2003 and second time in 2005 (samples were re-analyzed because we have improved the clean-up procedure and used the new calibration standards). The extract of sample CL1 had been re-identified/re-quantified in Carso Lab to validate the obtained result.

The PCDD/Fs concentration presented in the table 5.2 below is the result of second analysis (2005):

Table 5.2 PCDD/Fs concentration in CamLo soil samples (ng/kg dry weight)

Isomer	*CL1**	*CL2*	*CL3*	*CL4*	*CL5*	*CL6*	*CL7*	*CL8*	*CL9*	*CL10*
2378-TCDD	nd	37.3	nd	64.1	1.0	5.2	1.6	nd	0.5	nd
12378-PeCDD	2.1	nd	nd	nd	2.9	nd	nd	nd	nd	nd
123478-HxCDD	1.7	8.3	nd	0.9	5.1	2.6	nd	0.3	nd	2.3
123678-HxCDD	5.9	31.5	nd	5.7	14.5	nd	nd	nd	nd	nd
123789-HxCDD	4.6	19.5	nd	nd	8.1	8.3	106.3	nd	nd	5.4
1234678-HpCDD	81.3	1034.3	14.4	167.7	372.1	9.2	3.9	65.1	14.6	132.3
OCDD	579.7	10253.2	162.2	1643.4	3514.2	67.9	34.9	719.2	246.2	2469.6
2378-TCDF	nd	1.1	0.1	0.5	1.2	0.7	nd	nd	nd	nd
12378-PeCDF	4.2	1.9	0.3	0.2	0.6	0.4	nd	0.1	nd	nd
23478-PeCDF	6.7	2.5	0.2	1.0	1.5	0.1	nd	0.2	nd	0.3
123478-HxCDF	9.3	9.5	0.7	1.0	8.3	0.2	1.0	0.7	0.6	1.3
123678-HxCDF	7.7	nd	nd	nd	nd	3.9	nd	nd	nd	nd
123789-HxCDF	8.8	6.7	0.3	2.2	4.4	0.4	0.4	0.5	0.3	1.5
234678-HxCDF	nd	2.8	0.9	1.2	2.8	nd	1.0	0.5	0.5	1.4
1234678-HpCDF	34.8	148.4	2.4	29.2	63.4	3.7	1.7	7.5	3.4	8.5
1234789-HpCDF	2.3	8.0	0.5	1.8	4.5	nd	nd	0.5	0.4	nd
OCDF	9.9	513.7	4.8	79.9	121.9	18.3	5.6	16.2	6.0	23.3
i-TEQ	**9.1**	**59.5**	**0.5**	**68.0**	**13.9**	**7.1**	**12.5**	**1.1**	**0.9**	**3.0**

Note:(*)- the result was validated by Carso Lab;
nd – non-detectable

We have detected all 17 2,3,7,8-PCDD/Fs with different concentrations as presented in the table 5.2.

- 2,3,7,8-TCDD is the most concerned compound, from table 5.2 we can see that this compound was detected in 6 of 10 samples with the concentration varied from 0.5 to 64.1ng/kg. There is one place of the interest – former MaiLoc aitport with relatively high TCDD concentration. The maximum concentration here (64.1ng/kg) is lower than max conc. of ASo former base (360pg.g), but higher than max conc. at TaBat former base (35ng/kg) (two places ASo and TaBat are recognized as contaminated places by Agent Orange) (*Dwernychuk, 2002*).

- 1,2,3,7,8-PeCDD is also considered as toxic compound (TEF=1); this compound has been presented in only two samples (Cl1 and CL5) at relatively low concentration 2.1 and 2.9ng/kg, respectively.

- 2,3,7,8-PeCDF is relative toxic with TEF = 0.5. This compound was detected in almost samples (8 of 10 samples), but at low concentration, varied from 0.1 to 6.7ng/kg.

- Group of average toxic isomers including 2,3,7,8-TCDF and 2,3,7,8-HxCDD/Fs presented in all samples: 2,3,7,8-TCDF in 5/10 samples, 2,3,7,8-HxCDD in 7/10 samples, and 2,3,7,8-HxCDF in 10/10 samples. The obtained concentration of these compound was also relatively low, except sample CL7 with high concentration of 1,2,3,7,8,9-HxCDD (106.3ng/kg)

- Group of low toxic isomer including 2,3,7,8-HpCDD/Fs and OCDD/F has been presented in all samples with high concentration (up to 10253.2ng/kg of OCDD – CL2). The presence of these compounds is maybe a reflecting of burning practice in agriculture as usual in Vietnam. In fact, these substances contributed very little to the total i-TEQ due to their very low TEFs.

In Vietnam there are not yet Guidelines for PCDD/Fs, so we should use the available values of other countries to assess our result

- Canada: In 2001, as part of their Environmental Quality Guidelines, the Canadian Council of Ministers of the Environment (CCME) set a soil quality guideline for residential/parkland of 4ng TEQ/kg (*CCME, 2001*). The same value also applies to agricultural, commercial and industrial land.
- US.EPA: Regions 6 (Louisiana, Arkansas, Oklahoma, New Mexico, Texas and 66 Tribes) and 9 (Arizona, California, Hawaii, Nevada, the Pacific Islands, and over 140 Tribal Nations) have set their own risk-based criteria for 2,3,7,8-TCDD for residential land use. The Region 6 criterion, referred to as a Human Health Medium-Specific Screening Level, is 3.9ng/kg for residential soil (*US.EPA R6, 2001*). Similarly, the Region 9 criterion, referred to as a Preliminary Remediation Goal (PRG), is also 3.9ng/kg for residential soil (*US.EPA R9, 2000*). These criteria are based on a one in 1,000,000 cancer risk, and take into consideration exposure via soil ingestion, inhalation of particles and dermal absorption. The method of their derivation allows for the criteria to be adjusted for different cancer risks. For a one in 100,000 cancer risk, the adjusted criteria for 2,3,7,8-TCDD become 39ng/kg.
- Agency for Toxic Substances and Disease Registry (ATSDR): The guideline specifies a screening level of ≤50ng TEQ/kg, an evaluation level of >50 but <1,000ng TEQ/kg and an action level of ≥1,000ng TEQ/kg. The screening level is based on a minimal risk level (MRL) of 1 picogram/kilogram body weight/day (1 pg/kg bw/day) for 2,3,7,8-TCDD. When concentrations exceed 50ng TEQ/kg, site specific evaluations are needed. Evaluation levels consider site specific factors such as bioavailability, ingestion rates,

pathway analysis, soil cover, community concerns, background exposures. When exposures to dioxin concentrations in residential soils exceed 1,000ng TEQ/kg, public health actions such as surveillance, research, health studies and exposure investigations are considered (*ADSTR, 1998*).

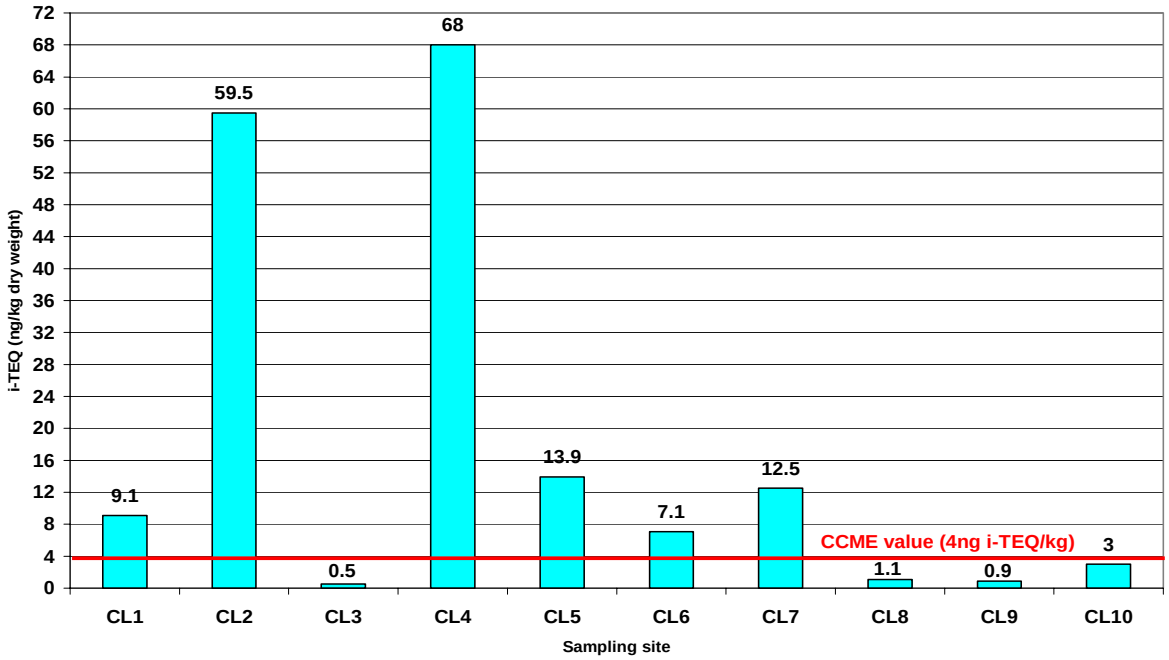


Fig. 5.6 PCDD/Fs level in soil of CamLo in comparison with Canadian Standard

Fig. 5.6 showed that there are 6 sites having superior i-TEQ values then Canadian Standard. Because these sites are using for agricultural purposes (cultivation of cassava, vegetable, pepper, etc.), so the risk for human health is considerable (especially for former MaiLoc Airport with very high i-TEQ, 17 times higher than CCME value).

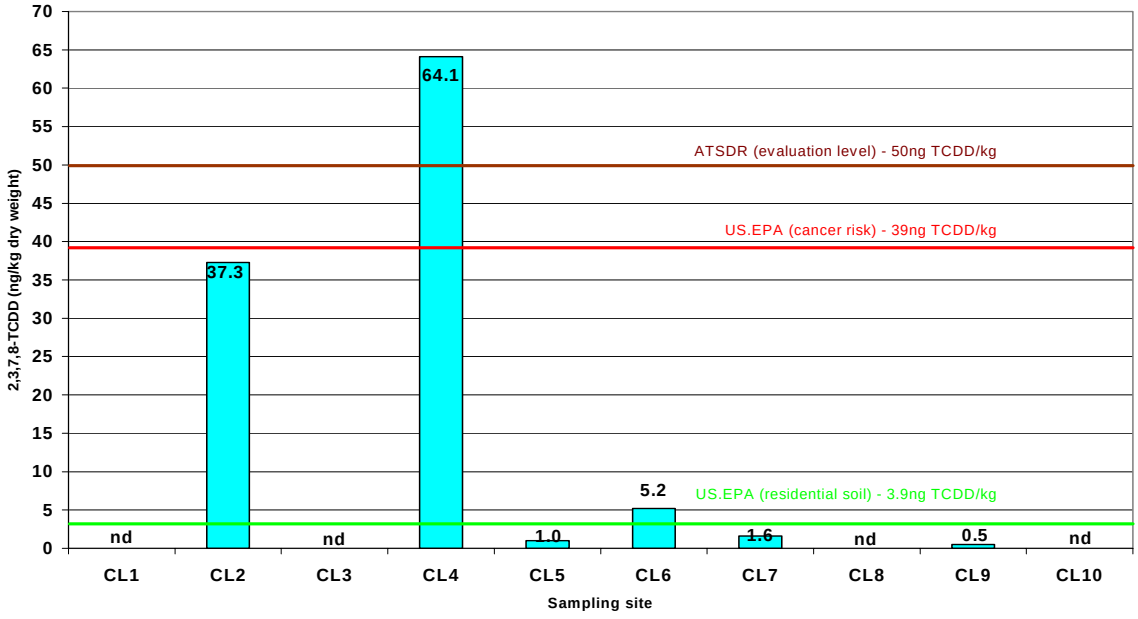


Fig. 5.7 PCDD/Fs level in soil of CamLo in comparison with US.EPA and ATSDR Standard

From Fig.5.7 we see that there are only 3 sites not reaching the standard for residential soil, among them again former MaiLoc airport is the most contaminated place with very high 2,3,7,8-TCDD concentration, more superior than cancer threshold and belong to the evaluation level.

5.4 PCDD/Fs level in soil of DaNang Airport

In DaNang, due to the security formalities we could not done the sampling inside the airport. With a supervising of a person from Environmental Department of DaNang City, we have taken only two composite soil samples outside the airport as described below:

Table 5.3 Description of DaNang Airport soil samples

No	*Sample code*	*GPS Position*	*Sample Type*	*Note*
1	DN1	N: 16°03'38.0" E: 108°11'26.3"	Vegetable field	Da Nang Airport : near the air port wall – in the top of the plane runway
2	DN2	N: 16°04'1.1" E: 108°11'2.9"	Vegetable field	Da Nang Airport : about 2,000 m from the air port wall (place collected runoff water from airport)

The samples were extracted and identified/quantified in Cecotox Lab, then the extracts were validated by Carso Lab. PCDD/Fs concentration in two samples are presented in the table 5.4

Table 5.4 PCDD/Fs concentration in DaNang Airport soil samples (ng/kg dry weight)

Isomer	*DN1*	*DN2*
2378-TCDD	4.2	0.2
12378-PeCDD	nd	nd
123478-HxCDD	nd	1.8
123678-HxCDD	nd	2.1
123789-HxCDD	nd	4.8
1234678-HpCDD	18.5	327.0
OCDD	1116.4	13906
2378-TCDF	nd	0.1
12378-PeCDF	nd	nd
23478-PeCDF	nd	nd
123478-HxCDF	nd	nd
123678-HxCDF	nd	nd
123789-HxCDF	nd	nd
234678-HxCDF	nd	nd
1234678-HpCDF	nd	1.2
1234789-HpCDF	nd	0.3
OCDF	2.1	30.8
i-TEQ	**4.5**	**4.1**

nd: non-detectable

From table 5.4 we can see that 2,3,7,8-TCDD presented in both samples, but the site DN1 is more contaminated than site DN2. It is reasonable because as we told above, site DN1 is next to the airport and was the place for the rubbish from the airport in the pass time. The 2,3,7,8-TCDD concentration is a little bit higher than US.EPA standard value for residential soil, but still below the criteria for cancer risk.

In comparison with Canadian standard, both sites have i-TEQ values near value proposed by CCME. However with regard to the data in table 5.4, the i-TEQ of site DN2 is contributed mainly by OCDD and 1,2,3,4,6,7,8-HpCDD – the compounds considered as low toxic (with TEFs of 0.01 and 0.0001, respectively). These compounds come rather from sources such burning ash and so all the used defoliants.

5.5 PCDD/Fs level in soil of MaDa Forest

The investigation in MaDa forest was done in April/2003 with the supervising of a forestry engineer – eng. Nguyen Huu Tuan (The Research Center for Natural Ecology). Eng. N.H. Tuan has participated in many investigations carried out in this area. The soil samples were taken along road 322 in both left and right sides (code RRT-left and RRP-right). We have also taken the soil in the bed of a local stream - MaDa stream (because the sampling was done in the dry season, so there was no water in the stream - *see Fig. 5.8).* The sampling site belongs to the former helicopter military airport – RangRang Airport. The area is afforested from 1982, but the forest not yet recovered as origin. Soil samples from surrounding natural forest also taken to compare the contamination level.

The sampling sites are described in the table 5.5 below:

Table 5.5 Description of MaDa Forest soil samples

No	*Sample code*	*GPS Position*	*Sample Type*	*Note*
1	RRT	N: 11°20'28.8" E: 107°00'57.4"	Topsoil (0-15cm)	Left side of 322 road – RangRang Airport, afforested area
2	RRP	--//--	--//--	Right side of 322 road – RangRang Airport, afforested area
3	S92-98	N: 11°20'10.8" E: 107°01'17.8"	--//--	MaDa stream, next to RangRang Airport. The soil is the sediment of surrounding soil, washed-out during raining season.
4	RTN	N: 11°19'58.0" E: 107°01'31.5"	--//--	Natural forest, about 1 km from RangRang Airport

Fig.5.8 MaDa stream in dry season (MaDa Forest)

In this sampling, we have taken 16 samples (10 from RangRang Airport, 3 in the MaDa stream, and 3 in natural surrounding forest). Due to the time limit, we have analyzed only 6 samples (three of them were validated by Carso Lab and one of them was analysed in Carso Lab). The PCDD/Fs concentration in these samples are presented in the table 5.6

Table 5.6 PCDD/Fs concentration in MaDa Forest soil samples (ng/kg dry weight)

Isomer	*RRT1**	*RRT2*	*RRP1*	*RRP2***	*S92-98**	*RTN**
2378-TCDD	6.3	1.9	4.4	5.3	1.9	1.2
12378-PeCDD	nd	nd	nd	0.1	0.3	nd
123478-HxCDD	nd	nd	nd	0.05	0.3	nd
123678-HxCDD	nd	nd	nd	0.3	1.3	nd
123789-HxCDD	1.9	nd	nd	0.5	1.3	nd
1234678-HpCDD	7.7	nd	nd	1.8	7.4	4.0
OCDD	46.1	14.0	93.1	53.8	38.6	74.9
2378-TCDF	0.6	nd	nd	0.4	0.5	nd
12378-PeCDF	nd	nd	nd	0.08	0.3	nd
23478-PeCDF	nd	nd	nd	0.1	0.4	nd
123478-HxCDF	nd	nd	nd	0.09	0.7	nd
123678-HxCDF	nd	nd	nd	0.07	0.7	nd
123789-HxCDF	nd	nd	nd	0.07	0.8	nd
234678-HxCDF	nd	nd	nd	nd	0.1	nd
1234678-HpCDF	0.7	nd	nd	0.3	7.3	0.9
1234789-HpCDF	0.2	nd	nd	0.04	0.3	nd
OCDF	1.2	nd	nd	4.8	1.5	0.6
i-TEQ	**6.7**	**1.9**	**4.4**	**5.7**	**2.7**	**1.3**

Note: (*)-Result was validated by Carso Lab;
(**) Sample was analysed in Carso Lab;
nd – non-detectable

Because Carso Lab use HRMS for PCDD/Fs analysis (the extraction and clean-up are similar to our method), so they can detect very small values as shown in table 5.6 (sample RRP2).

The 2,3,7,8-TCDD concentration varied from 1.9 to 6.3pg/kg in the RangRang airport soil samples. The lower concentration of 2,3,7,8-TCDD in MaDa stream bed soil (sediment) means that there is not an accumulation of this compound. This fact is logical because as we see, this stream is normally dried in dry season, but in contrary in raining season the flow is very fast due to local sloping terrain. Since there are not a sedimentation soil organic particles with the PCDD/Fs compounds fixed on. The presence of 2,3,7,8-TCDD even in small concentration is an evidence to confirm that this place is effected by defoliants.

The penta- to hexa- groups of 2,3,7,8-PCDD/Fs were presented in very small quantity (*nd* to 1.3ng/kg). There is only the clear presence of low toxic groups (hepta- to octa-), but also in low concentration in comparison with another investigated sites means that the burning activities here is not so big.

PCDD/Fs level in MaDa soil samples in comparison with standard values are showed in Fig. 5.9 and 5.10 below.

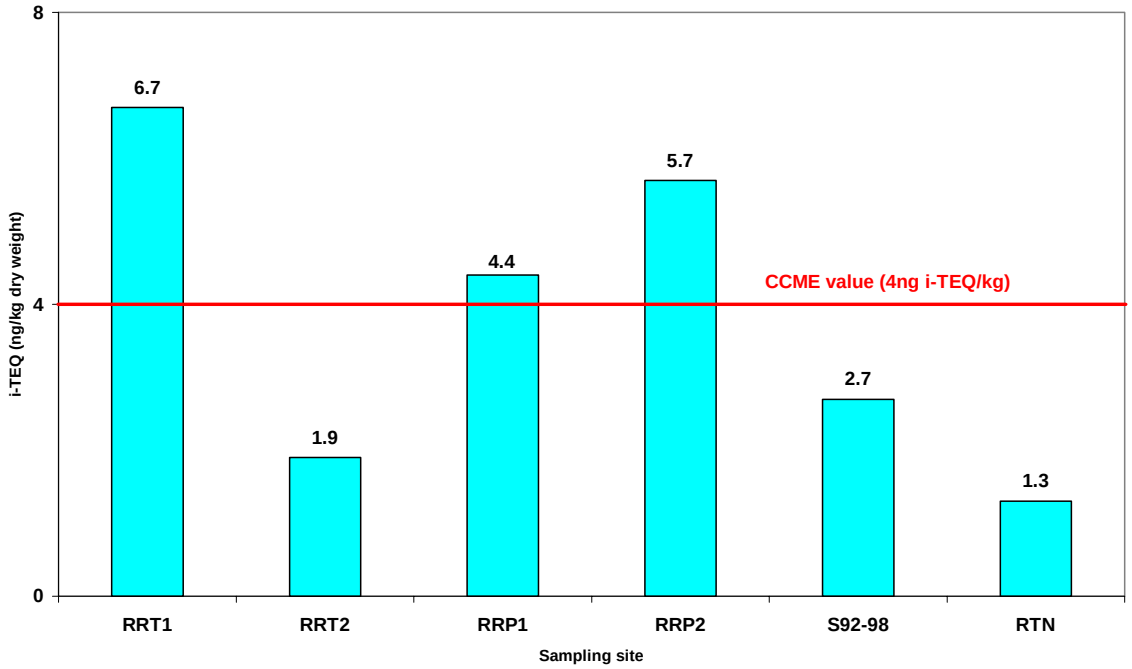


Fig. 5.9 PCDD/Fs level in soil of MaDa in comparison with Canadian Standard

Even after more than 30 years the soil of former RangRang airport still has the i-TEQ values higher than value proposed by CCME (both left and right sides). The obtained i-TEQ values were not very high due to soil disturbance of afforesting activities and washing-out by the time. However these i-TEQ values could be used as an explanation for the poor development of local forest (*Division 10-80, 2000*). The Fig. 5.10 again confirmed this evidence with the 2,3,7,8-TCDD concentration of this place superior than value proposed by US.EPA.

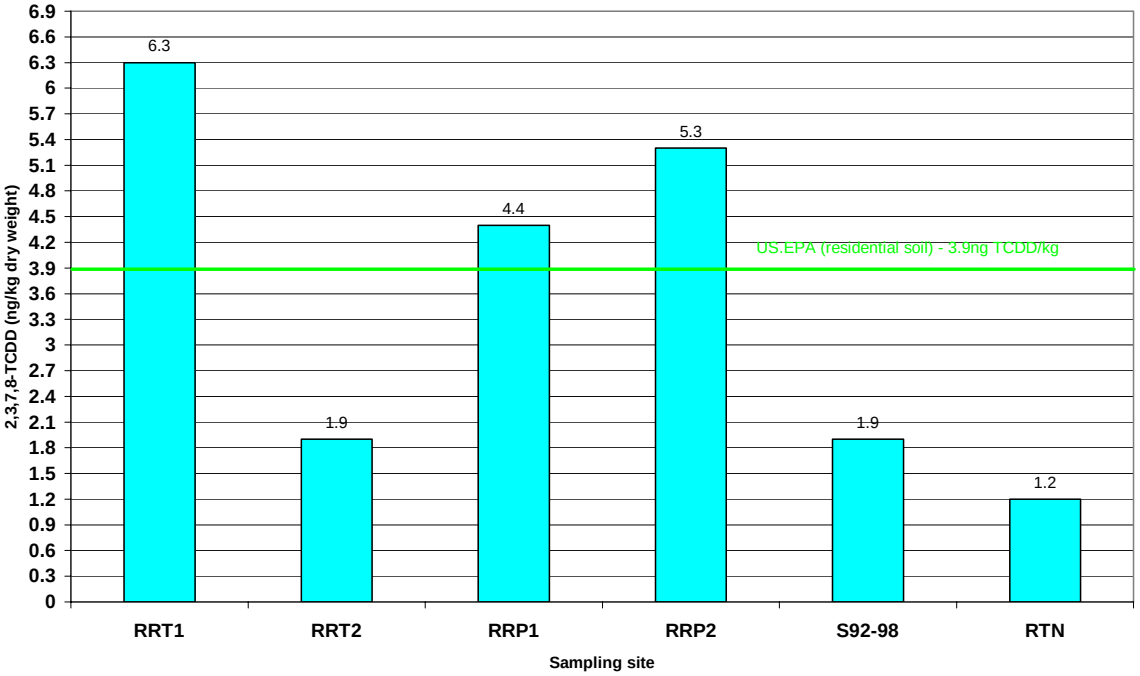


Fig. 5.10 PCDD/Fs level in soil of MaDa in comparison with US.EPA and ATSDR Standard

5.6 PCDD/Fs level in soil of BienHoa Air Force Base and sediment of BienHung Lake

4.6.1 PCDD/Fs contamination level in soil of BienHoa Airforce Base

Until now the BienHoa Air Force Base is still belongs to the Vietnamese Army, so sampling there is very difficult (in fact the official sampling is fobiden). With a help of one person of Environmental Department of DongNai Province, we have entered and sampled (June/2004) in the area near the radar station of the airport. At present time, the construction activities inside this area are very intensive with an effort to change it to a residential zone (for the soldier families). In very near future there will be houses, buildings, gardens and parks with many inhabitants living within.

The sampling was done in two layers: 0-20cm and 50-80cm. In this sampling event we have taken 10 composite soil samples as described in the table 5.7.

Table 5.7 Description of BienHoa Airforce Base soil samples

No	*Sample code*	*GPS Position*	*Sample Type*	*Note*
1	SDi1 (i =1- 4)	N: 10°57'34.5" E: 106°48'40.4"	Topsoil (0-30cm)	Near the radar station of airport
2	SDi2 (i = 1 – 4)	--//--	Deeper layer (50 – 80cm)	--//--
3	SRi (i = 1 – 2)	N: 11°57'25.9" E: 106°48'40.2"	Field topsoil (0-30cm)	Soil of a sunken field (in direction to BienHung Lake)

The samples were analyzed two times to confirmed the obtained result, the PCDD/Fs of the samples are presented in the table 5.8. In general, we can see that the PCDD/Fs concentration decreased by the depth with a significant defference between two layers.

- 2,3,7,8-TCDD presented in almost all samples (except SD21) with relative high concentration 3.8 to 146.3ng/kg. This concentration is comparable with the concentration of ASo Base – one of the most contaminated site by Agent Orange as reported in many documents (*H.C.L, 1998; Division10-80, 2000; Cau, 2003; etc*). The 1,2,3,7,8-PCDD was also detected in 7/10 samples, but with lower concentration (0.1 – 13.1ng/kg).

- 2,3,4,7,8-PeCDF had presented in all samples with concentration varied from 0.1 to 3.2ng/kg. Similarly, 2,3,7,8-TCDF and 2,3,7,8-HxCDD/Fs ware detected in all samples in low concentration (0.1 -11.2pg.g and *nd* -13.3ng/kg).

- 2,3,7,8-HpCDD/Fs and OCDD/Fs were detected with very high concentration. However these compounds are considered as low toxic, but in the case of sample SD11 the OCDD contributed a significant amount onto i-TEQ value. For this site, we could tell that beside the contamination by ancient chemicals such A.O, it is also contaminated by another undetermined sources (*we will discuss about the origin of PCDD/Fs in late chapters*).

Fig. 5.11 showed that all sampling sites not reach the Canadian standard for residential soil. With the regard to the construction activities here without any investigation for the possible reverse effects. Since polluted soil could affect people by many direct or indirect exposure routes due to the relative high i-TEQ level found, it is really a serious problem of public health and a suitable solution needs to be found as soon as possible.

Table 5.8 PCDD/Fs concentration in BienHoa Airforce Base soil samples (ng/kg dry weight)

Isomer	*$SD1_1$*	*$SD2_1$*	*$SD3_1$*	*$SD4_1$*	*$SD1_2$*	*$SD2_2$*	*$SD3_2$*	*$SD4_2$*	*SR1*	*SR2*
2378-TCDD	25.4	nd	83.2	113.3	18.5	3.8	9.7	66.3	43.1	146.3
12378-PeCDD	13.1	nd	6.0	2.7	0.4	nd	0.1	0.3	nd	0.7
123478-HxCDD	6.7	1.7	11.0	2.8	0.3	nd	nd	1.0	0.2	0.1
123678-HxCDD	7.3	nd	nd	13.3	2.9	0.9	0.9	2.9	1.3	1.1
123789-HxCDD	7.3	1.7	nd	6.7	1.1	0.3	0.4	1.7	0.2	0.3
1234678-HpCDD	4824.3	289.1	323.8	988.5	142.0	48.9	100.4	290.3	6.0	8.9
OCDD	44972.8	2214.3	3604.8	8410.9	1463.0	384.3	811.5	2644.2	35.1	47.8
2378-TCDF	1.3	0.1	2.5	4.8	0.6	0.1	0.2	1.1	2.8	11.2
12378-PeCDF	1.6	nd	1.1	nd	nd	nd	nd	nd	nd	nd
23478-PeCDF	2.3	0.6	1.7	3.2	0.6	0.1	0.3	1.0	0.2	0.6
123478-HxCDF	1.2	0.9	2.1	7.6	1.8	0.4	0.5	2.3	0.1	0.3
123678-HxCDF	4.5	1.5	4.1	5.4	nd	nd	nd	1.5	nd	nd
123789-HxCDF	2.8	1.4	2.7	6.3	1.2	0.2	0.5	1.9	0.1	0.2
234678-HxCDF	1.1	0.3	0.6	1.9	nd	nd	nd	0.6	nd	nd
1234678-HpCDF	278.9	30.6	54.8	137.0	17.3	4.4	10.1	42.9	2.5	1.6
1234789-HpCDF	11.6	2.4	2.4	9.2	1.4	0.2	0.7	2.7	nd	nd
OCDF	1134.2	76.2	154.9	471.9	38.4	11.1	30.9	140.1	7.4	5.3
i-TEQ	**98.7**	**4.5**	**96.6**	**134.7**	**21.8**	**4.6**	**11.3**	**72.0**	**43.7**	**148.8**

Note: nd – non-detectable

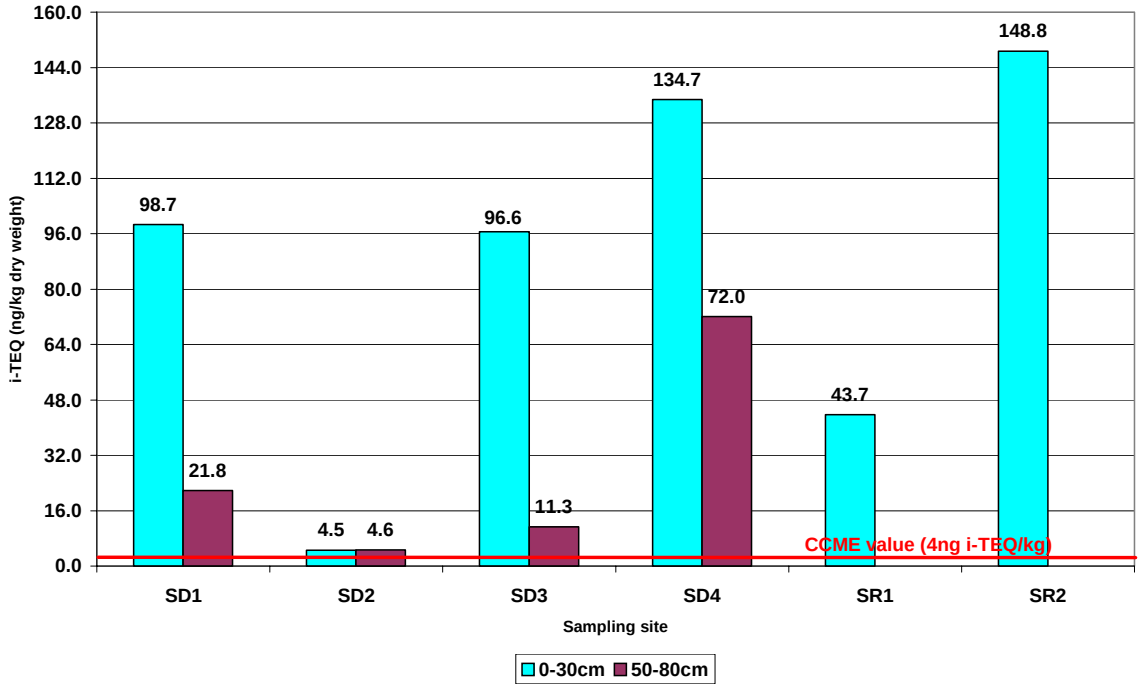


Fig. 5.11 PCDD/Fs level in soil of BienHoa Airforce Base in comparison with Canadian Standard

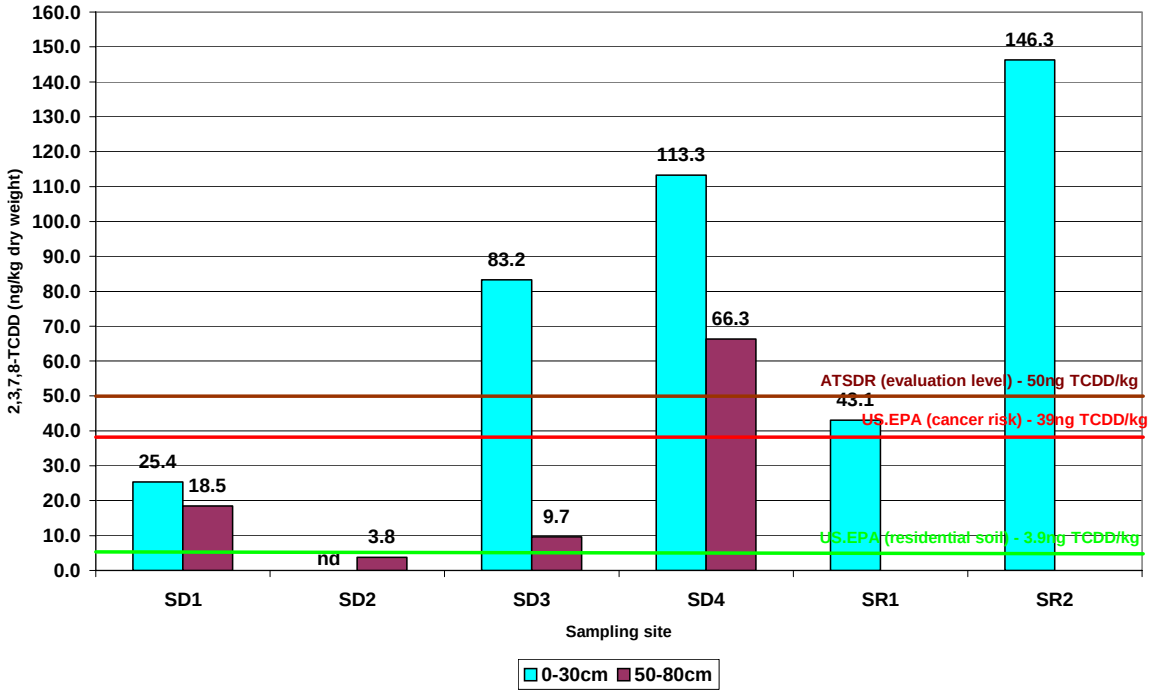


Fig. 5.12 PCDD/Fs level in soil of BienHoa Airforce Base in comparison with US.EPA and ATSDR Standard

Fig. 5.12 again confirmed the possible risks for human health of the investigated sites as we have told above: 4/6 sites have the 2,3,7,8-TCDD concentration superior than US.EPA threshold for cancer risk and three of them have the contamination level for evaluation activities.

4.6.2 PCDD/Fs contamination level in sediment of BienHung Lake

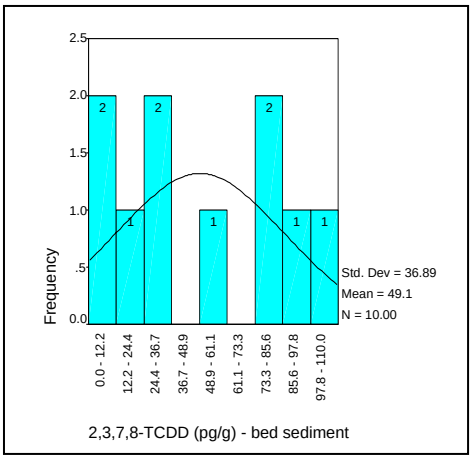
The sediment sampling was done the same time that the soil sampling. We have collaborated with the group of Faculty of Geology and Petroleum – Hochiminh City University of Technology to carry out the sampling by the technique as described above. In this sampling event we have taken 29 samples in 3 layers: bed sediment (surface), 20-30cm and 30-50cm. The description of sediment samples are presented in table 5.9.

Table 5.9 Description of BienHung sediment samples

No	*Sample code*	*GPS Position*	*Sample Type*	*Note*
1	SSMi (i =1- 10)	N: 10°57'15.5" E: 106°48'40.3"	Bed sediment	Sediments were taken around BienHung lake, a receiving source of runoff from BienHoa Airforce Base and surrouding
2	SSi_1 (i = 1 – 10)	--//--	20-30cm layer	--//--
3	SSi_2 (i = 2 – 10)	--//--	30-50cm layer	--//--

Samples were dried and treated and stored in IER Lab (in low temperature fridge, -20°C). In 2005, the samples were transported to Cecotox lab and analyzed here. The PCDD/Fs concentrations of the samples are presented in table 5.10, 5.11 and 5.12.

By using a statistical software (SPSS for window), we can find the difference of PCDD/Fs concentrations in distribution as well as their levels between three sediment layers.



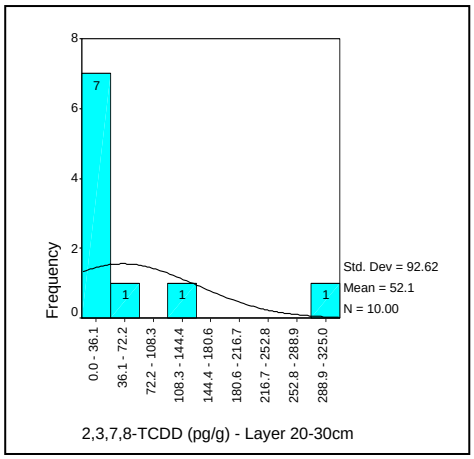


Fig. 5.13 Distribution of 2,3,7,8-TCDD in three sediment layers of BienHung Lake

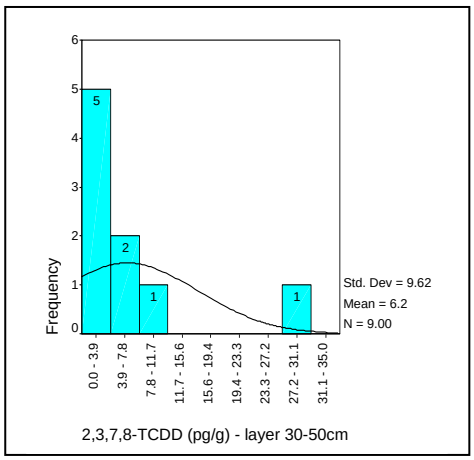


Table 5.10 PCDD/Fs concentration in BienHung bed sediment samples (ng/kg dry weight)

Isomer	*SSM1*	*SSM2*	*SSM3*	*SSM4*	*SSM5*	*SSM6*	*SSM7*	*SSM8*	*SSM9*	*SSM10*
2378-TCDD	99.2	81.9	29.3	86.7	56.7	83.9	18.9	27.9	nd	6.5
12378-PeCDD	nd	18.6	2.6	5.7	3.1	4.7	0.6	508.5	109.7	nd
123478-HxCDD	nd	2.2	3.2	3.8	3.6	4.1	6.3	9.4	11.5	6.5
123678-HxCDD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
123789-HxCDD	nd	4.2	4.8	8.6	5.5	6.7	2.0	21.0	nd	nd
1234678-HpCDD	52.0	209.2	110.4	132.5	62.2	162.7	37.7	147.4	404.4	654.1
OCDD	nd	222.5	688.9	1996.8	959.1	2825.2	415.3	1513.4	4782.5	nd
2378-TCDF	21.7	23.8	nd	22.3	20.7	26.1	5.0	1.1	6.2	5.9
12378-PeCDF	0.4	1.4	nd	nd	nd	nd	nd	8.1	4.8	8.0
23478-PeCDF	0.6	3.2	0.8	2.8	0.6	2.4	1.2	nd	4.3	17.4
123478-HxCDF	0.8	4.1	2.7	0.7	3.1	3.6	1.2	nd	nd	nd
123678-HxCDF	nd	nd	nd	3.4	nd	nd	nd	4.6	4.0	6.1
123789-HxCDF	0.5	5.2	1.4	2.3	0.7	3.1	0.8	18.2	4.2	10.9
234678-HxCDF	1.3	2.4	6.7	3.8	1.1	2.3	0.7	28.3	2.4	8.8
1234678-HpCDF	nd	nd	nd	nd	nd	nd	7.4	38.3	79.4	107.5
1234789-HpCDF	nd	2.2	1.9	22.6	0.6	1.5	nd	137.3	121.8	502.9
OCDF	nd	21.8	82.3	101.3	32.0	105.3	7.6	60.9	203.1	nd
i-TEQ	**102.5**	**108.5**	**35.4**	**100.0**	**64.3**	**96.4**	**22.2**	**548.5**	**121.5**	**32.0**

Note: nd – non-detectable

Table 5.11 PCDD/Fs concentration in BienHung sediment layer 20 to 30cm (ng/kg dry weight)

Isomer	***$SS1_1$***	***$SS2_1$***	***$SS3_1$***	***$SS4_1$***	***$SS5_1$***	***$SS6_1$***	***$SS7_1$***	***$SS8_1$***	***$SS9_1$***	***$SSM10_1$***
2378-TCDD	12.8	122.2	290.3	67.4	2.0	1.4	nd	5.6	nd	18.8
12378-PeCDD	5.4	12.2	15.5	7.4	117.1	12.2	nd	nd	nd	nd
123478-HxCDD	nd	nd	nd	nd	nd	nd	1.4	nd	4.4	5.8
123678-HxCDD	nd	7.2	10.3	14.1	8.5	5.7	1.9	nd	nd	nd
123789-HxCDD	nd	0.7	0.5	nd	9.8	0.8	nd	3.4	0.9	nd
1234678-HpCDD	14.5	80.2	57.4	37.6	12.8	24.3	8.0	35.7	7.5	38.2
OCDD	253.2	1307.5	857.7	805.0	741.7	272.6	39.0	187.2	92.9	450.7
2378-TCDF	8.6	32.3	98.5	25.8	1.8	0.1	0.8	2.8	2.0	5.4
12378-PeCDF	0.3	0.2	4.3	0.5	0.2	0.4	nd	nd	0.4	0.3
23478-PeCDF	0.2	1.2	1.1	1.1	0.1	0.5	0.1	1.3	0.4	0.2
123478-HxCDF	nd	nd	nd	nd	nd	0.3	nd	0.4	nd	0.5
123678-HxCDF	nd	4.9	5.2	1.2	nd	nd	nd	nd	nd	nd
123789-HxCDF	nd	2.1	1.7	6.0	nd	0.2	nd	nd	0.1	0.6
234678-HxCDF	3.5	8.1	2.3	12.8	2.2	nd	0.5	8.1	0.3	1.2
1234678-HpCDF	nd	12.9	7.9	7.3	nd	nd	nd	nd	nd	8.4
1234789-HpCDF	nd	2.2	nd	0.7	nd	nd	nd	nd	nd	1.7
OCDF	nd	46.8	26.2	nd	nd	5.0	nd	nd	5.0	14.0
i-TEQ	**19.7**	**141.7**	**319.1**	**81.9**	**121.6**	**14.9**	**0.6**	**8.1**	**1.1**	**20.8**

Note: nd – non-detectable

Table 5.12 PCDD/Fs concentration in BienHung sediment layer 30 to 50cm (ng/kg dry weight)

Isomer	***$SS2_2$***	***$SS3_2$***	***$SS4_2$***	***$SS5_2$***	***$SS6_2$***	***$SS7_2$***	***$SS8_2$***	***$SS9_2$***	***$SSM10_2$***
2378-TCDD	nd	29.8	7.3	nd	9.1	nd	7.3	1.9	nd
12378-PeCDD	4.5	2.2	nd	5.6	0.1	5.0	nd	0.4	0.1
123478-HxCDD	nd	nd	nd	nd	nd	nd	0.1	nd	nd
123678-HxCDD	1.5	3.4	nd	nd	nd	nd	1.9	2.5	nd
123789-HxCDD	2.4	2.2	nd	nd	0.9	2.2	3.9	1.0	nd
1234678-HpCDD	59.6	27.5	54.7	15.3	33.3	50.2	57.0	2.3	12.3
OCDD	658.8	432.2	468.2	371.5	381.8	862.1	653.8	29.8	119.4
2378-TCDF	4.0	6.1	2.5	1.2	nd	1.5	nd	nd	nd
12378-PeCDF	0.8	nd	nd	0.3	nd	nd	nd	0.1	nd
23478-PeCDF	0.1	0.3	0.2	0.2	0.1	nd	0.1	nd	0.1
123478-HxCDF	0.5	0.6	0.9	1.4	0.2	0.8	0.2	nd	0.2
123678-HxCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd
123789-HxCDF	0.4	0.5	nd	0.3	nd	0.2	0.2	0.1	0.3
234678-HxCDF	5.4	2.7	nd	1.0	1.5	1.4	16.6	nd	0.6
1234678-HpCDF	5.8	3.1	1.4	1.8	nd	7.6	nd	nd	1.7
1234789-HpCDF	1.5	1.0	nd	nd	nd	2.4	0.3	nd	0.5
OCDF	31.1	12.0	5.3	4.2	1.3	82.6	1.8	0.6	4.1
i-TEQ	**6.7**	**34.0**	**8.4**	**6.4**	**9.9**	**6.3**	**10.3**	**2.7**	**0.4**

Note: nd – non-detectable

From Fig.5.13 we can see that the distribution of 2,3,7,8-TCDD in bed sediment is regular and divided in three parts with different concentrations: nd – 36.7ng/kg, 48.9-61.1ng/kg, and 73.3 – 110ng/kg. This variation could be related to the organic content in this sediment layer because as we told above the PCDD/Fs are the hydrophobic compounds, so they will be accumulated in the site having higher organic content. The 2,3,7,8-TCDD distribution of deeper sediment layers (20-30cm and 30-50cm) is irregular and having the tendency to the left (lower concentration) with the ranges of nd-72.2ng/kg and nd-11.7ng/kg, respectively.

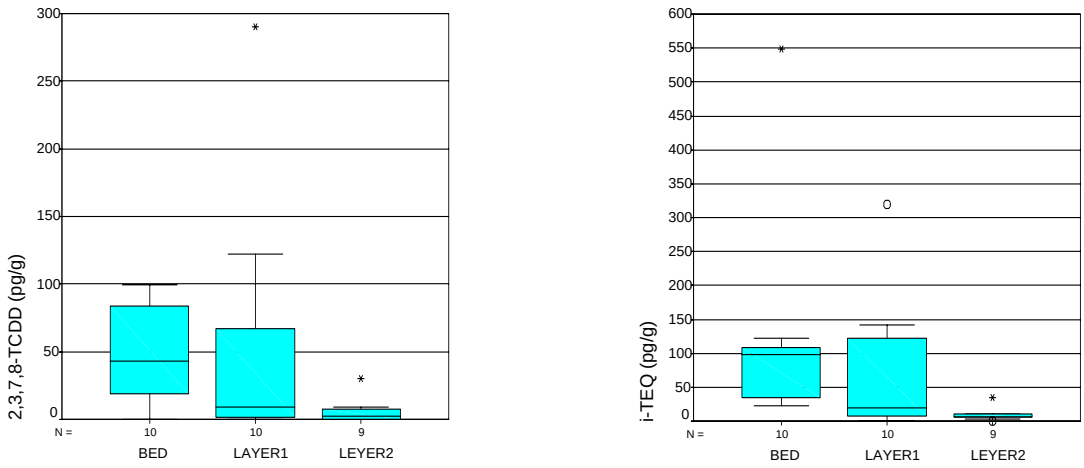


Fig. 5.14 PCDD/Fs level in three layer of BienHung sediments (layer1=20-30cm, layer2=30-50cm)

Median is the middle value in a dataset, i.e. half the variables have values greater than the median and the other half values which are less. The median is less sensitive to outliers (extreme scores) than the mean and thus a better measure than the mean for highly skewed distributions: the median and the interquartile range (IQR) may be better than other measures to indicate where the observed data are concentrated. For this reason we use median values of TCDD and i-TEQ to assess the PCDD/Fs contamination level change between different sediment layers. The boxplot given by SPSS with 95% of confidence is used for this assessment as showed in fig. 5.14.

From fig.5.14 we can see that the PCDD/Fs contamination level is reduced by the depth with the regard to both 2,3,7,8-TCDD concentration and i-TEQ value: the median values of 2,3,7,8-TCDD in turn are 43.0ng/kg, 9.2ng/kg and 1.9ng/kg; the median values of i-TEQ are 98.2ng/kg, 20.3ng/kg and 6.7ng/kg. It is logical due to fact that the upper layers always contain a big quantity of organic matter settled that facilitates dioxin adsorption. The dioxin level in the third layer is lowest because it is mainly composed of inorganic matters such as clay and sand.

Beside that, we can see that the 2,3,7,8-TCDD and i-TEQ variance of sediment layer 1 (20-30cm) is larger than bed sediment (some sites of this layer have the 2,3,7,8-TCDD concentration and i-TEQ values equal or superior than the obtained values in bed sediment - case of site SS2). That means the compositions of this layer is more heterogeneous than bed sediment.

By statistic method, we can also see the unusual data: e.g the 2,3,7,8-TCDD concentration in sample $SS3_1$ or the i-TEQ value of sample SSM8 (caused mainly by 1,2,3,7,8-PeCDD, not by 2,3,7,8-TCDD).

To assess the PCDD/Fs contamination level in sediment, we use the Canadian and US.EPA standards:

- Canada: For freshwater sediments two values have been developed (based on the scientific literature and toxicity tests): the Interim Sediment Quality Guideline (ISQG) is that value below which adverse effects on sediment fauna are unlikely. This value is 0.85ng i-TEQ/kg dw. The Probable Effects Level (PEL) is that concentration above which adverse ecological effects are likely to occur and is 21.5ng i-TEQ/kg dw (*CCME, 2001*).
- US.EPA Region 5 (Illinois, Indiana, Michigan, Minnesota, Ohio, and Wisconsin): the PCDD sediment level is 11ng i-TEQ/kg dw. This value is intended to provide guideline level against which site-specific media data can be screened for ecological risk. By implication, site concentrations that exceed this value could incur risks to ecological receptors.

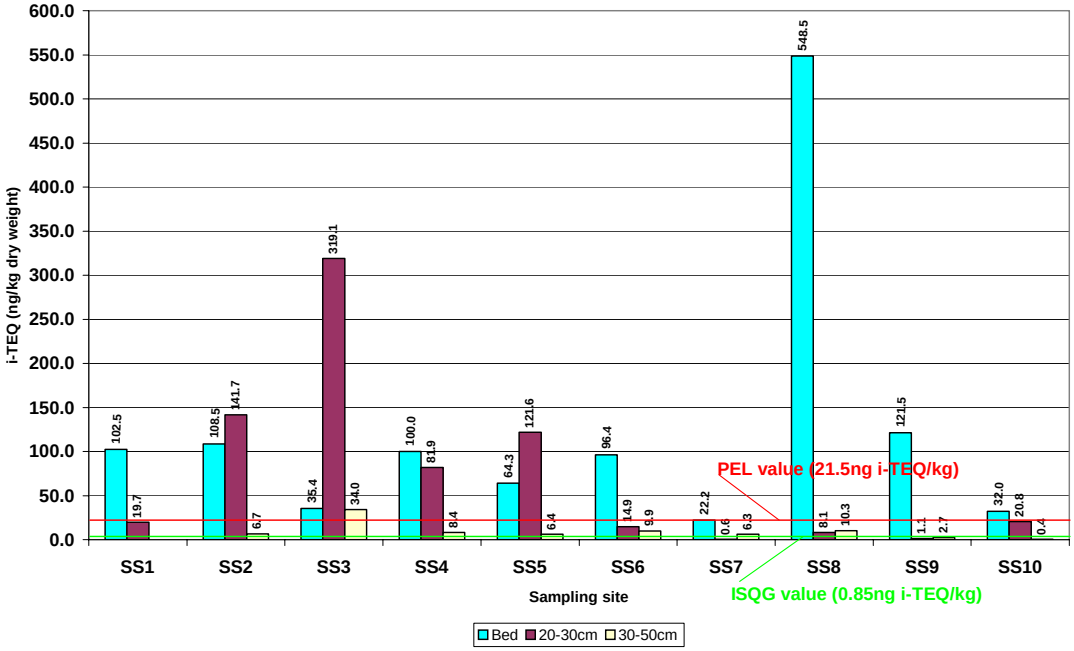


Fig. 5.15 PCDD/Fs level in sediment of BienHung Lake in comparison with Canadian Standard

Fig. 5.15 showed that almost all sampling sites have the i-TEQ value higher than ISQG value; all bed sediment samples have i-TEQ higher than PEL value while only 5/10 samples for first sediment layer (20-30cm). The median i-TEQ values of bed sediment is higher 115.5 times than ISQG and 4.6 times than PEL.

In comparison with the i-TEQ value proposed by US.EPA Region 5 (Fig. 5.16), all bed sediment samples and almost all first layer sediment samples (7/10 samples) have the higher i-TEQ (up to 49.3 times higher – bed sediment of SS8 or 28 times higher – first sediment layer of SS3). The obtained result again confirm that the relative high toxic level of the sediment of BienHung Lake (from surface to 30cm depth).

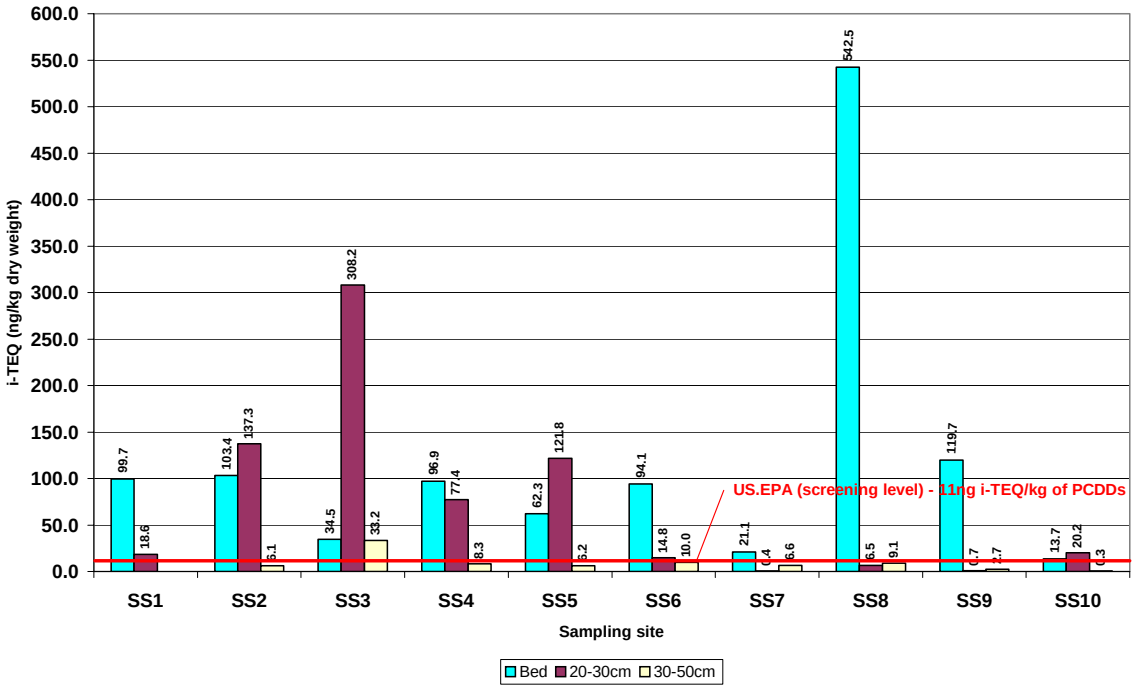


Fig. 5.16 PCDDs level in sediment of BienHung Lake in comparison with US.EPA Standard

Since the bed and first sediment layers are the exchangeable layers, so the contaminants could be associated with a wide range of impacts on the plants and animals that live within and upon bed sediments. Sediment-associated contaminants also have the potential to accumulate in the tissue of aquatic organisms. Elevated tissue concentration in benthic or other aquatic organisms can result in the bioaccumulation of chemicals in higher levels of the aquatic food web. The bioaccumulation of toxic compounds such as dioxin in aquatic organisms presents a potential hazard to sensitive wildlife species, bird, and humans that rely on these organisms for food as reported by many authors (*Fattore et al., 2002; Eljarrat et al., 2001; Schecter at al., 2001 and 2003*). Furthermore, the presence of PCDD/Fs in the surface layer sediment of Bien Hung Lake shows that the dioxin contamination by washout/runoff from the potential source (probably Bien Hoa Airforce Base) still exists and threatens the local ecosystem and human health.

5.7 PCDD/Fs level in soil and sediment of some comparative sites

To compare the PCDD/Fs contamination level in soil and sediment of selected sites with other sites serving as a background level, as well as to find the origin of these compounds we have analyzed some soil samples of ThuDuc industrial zone, MyThanhBac rice field (TienGiang province), and ThamLuong Canal sediment.

ThuDuc industrial zone with the factories such power, steel processing, cement, etc that could be the possible of PCDD/Fs emission sources (*see chapter I*). The rice field at MyThanhBac – Tien Giang Province is a place that several pesticides are using, including also the organochlorinated pesticides (in the past time). ThamLuong Canal is one of most contaminated canal in Hochiminh City by the untreated wastewater (domestic and industrial)

and its sediment contains many contaminants such heavy metals, PCBs, PAHs, etc (*Phuong et al., 1998; Anh et al., 1999*). All samples were taken in June/2004.

The description of the samples is shown in table 5.13 below

Table 5.13 Description of comparative samples

No	*Sample code*	*Sample Type*	*Note*
1	TDi (i =1- 5)	Topsoil (0-30cm)	Soil of ThuDuc industrial zone – Hochiminh City
2	MTBi (i = 1 – 2)	Rice field topsoil (0-30cm)	Soil of rice field at MyThanhBac – TienGiang Province, about 70km from HCMC
3	BTL	Sediment (0-20cm)	Sediment of ThamLuong Canal – Hochiminh City

The PCDD/Fs concentrations in these samples are presented in the table 5.14

Table 5.14 PCDD/Fs concentration in comperative samples (ng/kg dry weight)

Isomer	*TD1**	*TD2***	*TD3*	*TD4**	*TD5*	*MTB1*	*MTB2**	*BTL*
2378-TCDD	1.0	0.2	nd	0.3	1.2	nd	0.1	nd
12378-PeCDD	6.9	0.7	nd	2.4	nd	nd	0.3	13.8
123478-HxCDD	15.1	1.3	nd	6.0	5.6	nd	0.2	4.1
123678-HxCDD	43.4	5.4	nd	15.94	18.3	nd	0.8	5.9
123789-HxCDD	30.1	3.2	nd	11.2	7.8	nd	1.6	4.0
1234678-HpCDD	712.6	85.2	2.8	320.4	345.3	14.4	22.5	89.2
OCDD	6444.9	738.9	72.5	2204.3	2985.5	306.6	187.3	921.2
2378-TCDF	4.0	0.9	0.2	1.6	4.9	nd	0.8	2.3
12378-PeCDF	5.1	1.0	nd	1.8	nd	2.5	1.8	1.3
23478-PeCDF	9.1	1.5	nd	3.9	3.4	nd	0.4	4.4
123478-HxCDF	21.5	2.2	0.4	6.8	15.8	nd	0.3	1nd
123678-HxCDF	12.6	1.9	nd	6.3	9.9	nd	0.3	nd
123789-HxCDF	21.8	1.7	0.3	6.2	16.3	nd	0.2	6.4
234678-HxCDF	0.7	0.7	nd	nd	4.9	17.5	nd	6.5
1234678-HpCDF	109.6	17.3	0.7	79.31	77.4	14.5	1.1	22.0
1234789-HpCDF	12.4	1.2	0.7	6.3	5.5	nd	0.1	1.5
OCDF	103.4	23.6	2.7	87.91	128.0	2.7	0.9	78.3
i-TEQ	**33.3**	**4.5**	**0.1**	**13.2**	**15.8**	**2.2**	**1.4**	**21.2**

*Note: *)-the sample was extracted in Cecotox and identified/quantified in Carso;*
(**) The sample was extracted and identified/quantified in Carso;
nd – non-detectable

From table 5.14 we can see that all 2,3,7,8-PCDD/Fs compounds are detected in industrial soil samples (except site TD3 with the high sand content and low organic matter). The i-TEQ value of these sites is higher than CCME value (4ng i-TEQ/kg), but we easily see that this i-TEQ values are not contributed by 2,3,7,8-TCDD concentration, but from other compounds such 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF. Since these sites reach the US.EPA Standards because this standard focusing only on 2,3,7,8-TCDD.

However we detected many 2,3,7,8-PCDD/Fs in rice field soil sample (MTB2) due to the high sensitivity of HRMS, but their concentrations were very low (except OCDD) leading to the low value of i-TEQ. Because these samples were taken from the rice field using many pesticide types, so we can say that the pesticides used for agriculture is not a big concern for 2,3,7,8-PCDD/Fs contamination. These PCDD/Fs concentrations could be served as reference values for contamination assessment.

The canal sediment has i-TEQ superior than values proposed CCME and US.EPA, but this value is not contributed by 2,3,7,8-TCDD. It is a big difference of BienHung sediments and we will return to this subject later.

5.8 PCDD/Fs level in soil and sediment of selected sampling sites in comparison with the obtained results by other studies

To have an overview about the PCDD/Fs contamination levels of our selected sampling with the other sites of Vietnam, we have use the results summarized by Division 10-80 from many documents (*Division 10-80, 2000*). Because 2,3,7,8-TCDD it the most toxic compound of PCDD/Fs and furthermore it is evidentially presented in defoliants used by US. Army during the war (*Young and Reggiani, 1988*), hence we take its concentration for our comparison.

From table 5.15 we see that the contamination level in soil of CamLo is comparable with CanGio Forest and an unnamed place of Hochiminh City; the contamination in soil of DaNang Airport and MaDa Forest is comparable with the Chujor-Pleiku/Giarai; and the contamination in soil of BienHoa Airforce Base is comparable with TaBat-Aluoi/Hue. We also see very high concentration of TCDD in BienHoa Airforce Base from Division10-80 document (up to 1.16×10^6pg/g). Follow this document, these soil samples were taken from the areas next to the former chemical storage tanks, so a leakage could be a reasonable explanation for this concentration. Unfortunately, these concentrations are not yet announced as official documents, as well as this area is a prohibited zone and we could not do the sampling here without permit from responsible governmental organizations.

The contamination in sediment of BienHung Lake is very high and it could cause the risks for human health through the food chain as we discussed above. We continue this subject in next chapter – the PCDD/Fs contamination level in the fish of BienHung Lake.

Table 5.15 PCDD/Fs contamination level in comparison with the results obtained by others

No	*Sampling time*	*Place/Province*	*2,3,7,8-TCDD (pg/g dw.)*	*Note*
		SOIL		
1	1990	ThanhTri/HaNoi	nd	topsol (0-10cm)
2	1991	Hochiminh City (unnamed place)	3.0 – 59	topsoil (0-10cm)
3	1987	CanGio Forest/Hochiminh City	1.5 – 22.7	topsoil (0-5cm)
4	1987	Chujor-Pleiku/Giarai	nd – 9.2	topsoil (0-20cm)
5	1989	Mekong Delta (TienGiang, VinhLong, HauGiang, CaMau)	nd	topsoi (0-10cm) and 40-60cm
6	1990	TaBat-Aluoi/Hue	nd – 115.2	topsoil (0-10cm)
7	1990	BachMa/Hue	62.7	topsoil (0-10cm)
8	1992	BienHoa Airforce Base	nd – 1.16×10^{6}	topsoil (0-20cm)
9	**Our result**			
10	May/2003	CamLo/QuangTri Province	nd – 64.1	topsoil (0-20cm)
11	May/2003	DaNang Airport	0.2 – 4.2	topsoil (0-20cm)
12	April/2003	MaDa Forest/DongNai Province	1.2 – 6.3	topsoil (0-10cm)
13	June/2004	BienHoa Airforce Base/DongNai Province	nd – 146.3	topsoi (0-20cm) and 50-80cm
14	June/2004	ThuDuc Industrial Zone/Hochiminh City	nd – 1.2	topsoil (0-20cm)
15	June/2004	MyThanhBac rice field/TienGiang Province	nd – 0.1	topsoil (0.20cm)
		SEDIMENT		
1	1985	DongNai River/DongNai Province	nd	bed sediment
2	1985	Red River/HaNoi	nd	bed sediment
3	1995	Aluoi/Hue	nd-1.9	sediment of fish pond
4	1999	GioLing/QuangTri Province	nd	sediment of fish pond
	Our result			
5	June/2004	ThamLuong Canal/Hochiminh City	nd	bed sediment
6	June/2004	BienHung Lake-BienHoa City/DongNai Province	nd - 99.2 nd – 290.3 nd – 29.9	bed sediment 20 – 30 cm 30 – 50cm

(*Source: Division 10-80, 2000*)

5.9 PCDD/Fs level in bottom ash samples of some incinerator in Hohiminh City and VungTau City

As we told in chapter I the waste from combustion processes such exhausts, fly ash and bottom ash from municipal waste incinerators (MWIs) is a big concern for PCDD/Fs sources. Hence we have taken and analyzed 5 bottom ash samples to have an idea about the PCDD/Fs contamination levels. This data also serves as a base for the assessment of PCDD/Fs source (origin) in chapter VIII.

The description of bottom ash samples is presented in the table 5.16 below

Table 5.16 Description of bottom ash samples

No	*Sample code*	*Sample Type*	*Note*
1	BHH	Ash	Bottom ash of BinhHungHoa MWI/Hochiminh City
2	RYT1	Ash	Bottom ash of an incinerator for medical waste/Hochiminh City
3	RYT2	Ash	--//--
4	PH1	Ash	Bottom ash of PhuHoa MWS/Vungtau City
5	TCN	Ash	Bottom ash of an incinerator for industrial waste/Hochiminh City (applying catalytic method called photoresist)

Fig. 5.17 Bottom ash collection and packing for transport in BinhHungHoa incinerator

The PCDD/Fs concentrations in bottom ash samples are presented in the table 5.16. From the first view we can see that the PCDD/Fs concentration in bottom ash is 1000 times higher than values detected in soil samples with the presence of all 2,3,7,8-PCDD/Fs compounds (except sample TCN having very low content of 2,3,7,8-PCDD/Fs due to its low organic content and a catalytic method - photoresist).

Similar to soil and sediment, there is not an official standard on PCDD/Fs value for the waste from incinerator in Vietnam. So for comparison, we use some results reported in the documents (*Jiang et al, 1997; Wunderli et al, 2000; Osako etal, 2002; Abad et al, 2003; Kim et al, 2005*). The comparison is presented in table 5.18.

Table 5.17 PCDD/Fs concentration in bottom ash samples (ng/g)

Isomer	*BHH*	*RYT1*	*RYT2*	*PH1*	*TCN*
2378-TCDD	0.037	0.006	0.034	0.053	nd
12378-PeCDD	2.083	0.121	4.392	7.318	nd
123478-HxCDD	1.093	0.070	2.037	3.582	nd
123678-HxCDD	1.469	0.087	2.815	7.298	nd
123789-HxCDD	0.183	0.014	0.472	0.284	nd
1234678-HpCDD	1.395	0.237	5.314	15.083	0.006
OCDD	1.172	0.285	4.912	14.587	nd
2378-TCDF	1.342	0.249	5.072	18.644	nd
12378-PeCDF	0.326	0.056	1.046	3.847	nd
23478-PeCDF	0.065	0.040	0.321	1.905	nd
123478-HxCDF	0.196	0.053	1.251	7.783	nd
123678-HxCDF	0.282	0.103	1.035	7.902	nd
123789-HxCDF	2.212	0.223	7.274	29.009	0.007
234678-HxCDF	0.226	0.029	0.615	2.415	nd
1234678-HpCDF	0.465	0.015	1.463	14.056	0.038
1234789-HpCDF	1.643	0.308	3.510	4.921	0.040
OCDF	0.959	0.405	3.048	8.604	0.040
i-TEQ	**1.724**	**0.184**	**4.443**	**12.328**	**0.001**

Note: nd – non-detectable

Table 5.18 Comparison the PCDD/Fs in bottom ash with other studies

Country	Type of samples	i-TEQ (ng/g)	Source of document
Chine	Bottom ash of WSI	0.087	*Jiang et al, 1997*
Korea	Bottom ash of industrial incinerator	0.091	*Kim et al, 2005*
Spain	Bottom ash of WSI	0.02-0.03	*Abad et al, 2003*
Japan	Bottom ash of WSI	0.059 – 4.5	*Osako etal, 2002*
Switzerland	Bottom ash of incinerator for waste wood	0.004 – 0.011	*Wunderli et al, 2000*
Our result			
	Bottom ash of industrial incinerator	0.001	
	Bottom ash of WSI (for municipal and medical waste)	0.184 – 12.328	

Table 5.18 showed the relative high PCDD/Fs concentration of bottom ash in our samples in comparison with the results of other publications. That means this type of waste is a considerable source of PCDD/Fs and needed a strict regulation to manage it. In fact up to know in Hochiminh City, the bottom ash from governmental administrative incinerators such BinhHungHoa is considered as toxic solid waste. It is collected in packed in special containers (*see Fig. 5.17*) and transported to the special place for toxic solid waste treatment. However we should recognize that the management of this type of the waste is very difficult, especially for the small scale incinerators: e.g incinerators for hospitals. In addition, almost current incinerators in Vietnam now have not the separated system for fly ash treatment like electrostatic precipitator - ESP (that why we can not take the fly ash for our tests). The exhaust from incinerator is treated by a wet filter system mainly for the gas such CO_2, SO_2, NO_x, etc., not yet considering to the toxic parameters such PCDD/Fs and so al. As reported by Abad et al (2003), the solid waste from the gas treatment system containing a lot of PCDD/Fs (*see Fig. 5.18*). Hence this waste is should be counted to minimize the possible risks caused this PCDD/Fs source.

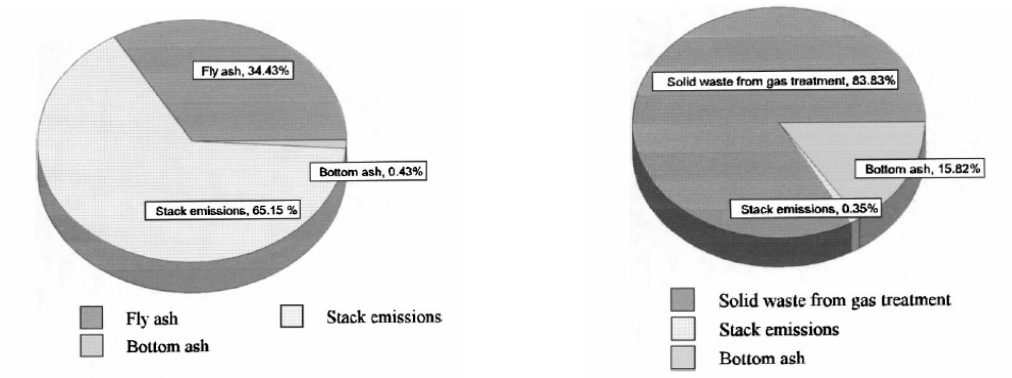


Fig. 5.18 Percentage distribution of dioxins and furans in combustion residues before and after implementation of the new Air Pollution Control System – APCS (Source: Abad et al, 2003)

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CHAPTER VI: DIOXIN CONTAMINATION IN THE FISH TISSUE

6.1 Introduction

One of our aims was to follow the PCDD/Fs in food chain at contaminated sites and from the obtained results we can see that BienHung Lake could serve as a case-study for this purpose. Fish is known to accumulate lipophilic chloro-organic environmental pollutants (*Karl et al, 2002*). Furthermore, as reported in some articles (*Schecter et al, 2001; Schecter et al, 2003(2); Dwernychuk, 2005*) the dioxin concentration in fish tissue here is relative high and BienHoa Airforce Base is one of dioxin "hot spots" in southern Vietnam.

As already described BienHung Lake serves as a receiving reservoir for runoff from BienHoa Airforce Base and surrounding area (*see Fig. 6.1*). This lake with a surface of about three hectares and an average depth of 2.5m belong to the TrungDung subdistrict in the center of BienHoa City. Underground and runofff are the main water source for the lake. A half of BienHung Lake border line is located in BienHung Park and the rest is residential zone. In fact, the access to BienHung Lake is open.

We have selected two kinds of fish for our research: catfish and snake-head – both are the fish predator: catfish lives in the bottom while snake-head lives in middle water layer. In addition, these fish kinds are used as food for local residents.

Fig. 6.1 The sewer mouth for run-off from BienHoa Airforce Base in BienHung Lake

6.2 Sampling technique and sample treatment

The fish samples were fished by the help of a local person who living in the border next to BienHung Lake. He uses a fishing-net to fish every night for his family and to sell. We have taken 8 catfishes (coded FT) and 5 snake-heads (coded FL). The sampling time was June/2004. Parallely, we have analyzed 5 catfish samples bought at HaNoi market (coded FTH) to compare the PCDD/Fs contamination level. All fishes were conserved in alum box at low temperature (-20^{o}C).

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

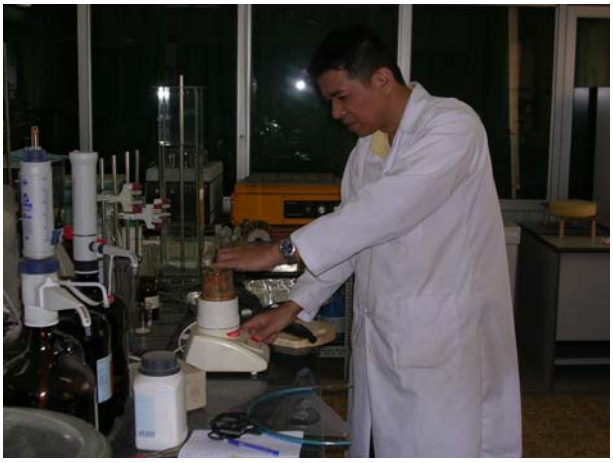
Table 6.1 Description of fish samples

No	*Code*	*Length (cm)*	*Weight (g)*	*No*	*Code*	*Length (cm)*	*Weight (g)*
1	FT1	29	151	10	FL2	42	416
2	FT2	32	260	11	FL3	32	295
3	FT3	28	146	12	FL4	35	282
4	FT4	28	147	13	FL5	39	432
5	FT5	37	350	14	FTH1	15	47
6	FT6	30	245	15	FTH2	21	53
7	FT7	29	160	16	FTH3	17	34
8	FT8	26	117	17	FTH4	22	59
9	FL1	35	273	18	FTH5	18	48

Note: all fish samples were still live at sampling time

a)

b)



Sample treatment: whole fish were cut and grinded by a meat-grinder with glass grinding chamber and inox cutting blade. After grinding, the sample was mixed again carefully with a spoon, and then a part of sample (about 100g) was taken and put into a glass brown flask with screw lid. The flask containing sample was labelled and stored in fridge at low temperature (-20^{o}) until analysis.

The fish samples were extracted and analyzed in Cecotox lab by the analytical procedure described in chapter IV.

Fig. 6.2 a) A catfish of BienHung Lake
b) Grinding the fish sample with a meat-grinder

6.3 PCDD/Fs level in fish tissue of BienHung Lake

The content of lipid (fat) is determined by method described in chapter IV, then PCDD/Fs concentration is calculated based on lipid content. The lipid content and PCDD/Fs concentration in fish tissue of BienHung Lake are presented in the table 6.2 and 6.3.

Table 6.2 PCDD/Fs concentration in snake-head tissue of BienHung Lake (ng/g lipid)

Sample	***FL1***	***FL2***	***FL3***	***FL4***	***FL5***
Lipid content (%)	2.7	4.7	4.7	3.4	3.6
	PCDD/Fs concentration (ng/g lipid)				
Isomer					
2378-TCDD	0.611	0.072	nd	0.033	0.267
12378-PeCDD	nd	0.084	nd	nd	nd
123478-HxCDD	nd	nd	nd	nd	nd
123678-HxCDD	nd	nd	nd	nd	nd
123789-HxCDD	nd	nd	0.018	nd	nd
1234678-HpCDD	0.136	nd	0.003	nd	nd
OCDD	1.043	0.232	0.099	nd	0.351
2378-TCDF	0.030	0.027	0.015	0.051	0.053
12378-PeCDF	nd	0.006	nd	nd	0.028
23478-PeCDF	0.041	0.008	0.007	0.008	0.007
123478-HxCDF	0.071	0.024	0.019	0.039	nd
123678-HxCDF	nd	nd	nd	nd	0.028
123789-HxCDF	0.070	nd	0.021	0.049	nd
234678-HxCDF	nd	nd	0.013	nd	nd
1234678-HpCDF	nd	nd	0.003	nd	nd
1234789-HpCDF	nd	nd	0.006	nd	nd
OCDF	0.187	0.075	0.058	nd	0.088
i-TEQ	**0.650**	**0.166**	**0.012**	**0.051**	**0.280**

Note: nd – non-detectable

5 catfish samples were purchared from HaNoi market (in the North of Viet Nam) – the place considered as a zone not affected by A.O to compare the obtained PCDD/Fs contamination level. These catfishes were originated from rice field of surrounding areas of HaNoi. As we can see in table 6.1, these catfishes have a small size in comparison with catfishes of BienHung Lake due to different weather between South and North of VietNam.

The PCDD/Fs concentration of HaNoi catfishes is presented in the table 6.4 and from BienHung Lake in the table 6.2 and 6.3. Based on data from these tables we see that almost all samples have the lipid content varied from 2 to 5%. The difference of lipid content between samples is caused by inhomogeneity during sample preparation (because we taken only a part of whole fish for our analysis and we have not equipment for homogeneity). Hence we could not detect the relationship between lipid content and PCDD/Fs concentration, neither the relationship between their size and PCDD/Fs concentration.

Table 6.3 PCDD/Fs concentration in catfish tissue of BienHung Lake (ng/g lipid)

Sample	FT1	FT2	FT3	FT4	FT5	FT6	FT7	FT8
Lipid content (%)	3.9	10.6	3.3	3.3	4.1	8.5	2.6	2.6
	PCDD/Fs concentration (ng/g lipid)							
Isomer								
2378-TCDD	1.015	0.065	nd	0.285	0.238	0.136	0.029	0.152
12378-PeCDD	nd	0.047	nd	nd	nd	nd	nd	nd
123478-HxCDD	0.136	nd	nd	nd	nd	0.071	nd	0.049
123678-HxCDD	nd	0.019	nd	nd	nd	nd	nd	0.017
123789-HxCDD	nd	nd	nd	nd	nd	nd	nd	nd
1234678-HpCDD	0.294	0.049	nd	0.149	0.073	0.016	0.007	0.008
OCDD	2.237	0.292	0.528	nd	0.356	nd	0.219	nd
2378-TCDF	0.025	0.031	0.033	0.035	0.036	0.036	nd	0.016
12378-PeCDF	0.031	nd	0.019	nd	0.014	nd	0.013	0.011
23478-PeCDF	0.024	0.006	0.008	0.016	0.016	0.003	nd	0.008
123478-HxCDF	0.164	0.016	0.017	nd	0.032	0.014	0.013	0.019
123678-HxCDF	nd	nd	nd	nd	nd	nd	0.019	nd
123789-HxCDF	nd	nd	nd	nd	nd	nd	0.007	0.009
234678-HxCDF	nd	nd	nd	nd	nd	nd	nd	nd
1234678-HpCDF	0.201	0.017	nd	nd	nd	nd	nd	nd
1234789-HpCDF	nd	nd	nd	nd	nd	nd	nd	nd
OCDF	1.685	0.212	0.421	0.160	0.323	0.053	0.040	nd
i-TEQ	**1.067**	**0.122**	**0.010**	**0.298**	**0.254**	**0.150**	**0.033**	**0.168**

Note: nd – non-detectable

Table 6.4 PCDD/Fs concentration in catfish tissue of HaNoi market (ng/g lipid)

Sample	***FTH1***	***FTH2***	***FTH3***	***FTH4***	***FTH5***
Lipid content (%)	2.6	2.6	2.4	3.1	5.4
	PCDD/Fs concentration (ng/g lipid)				
Isomer					
2378-TCDD	nd	nd	nd	nd	nd
12378-PeCDD	nd	nd	nd	nd	nd
123478-HxCDD	nd	nd	nd	nd	nd
123678-HxCDD	nd	nd	nd	nd	nd
123789-HxCDD	nd	nd	nd	nd	nd
1234678-HpCDD	nd	nd	nd	nd	nd
OCDD	0.110	nd	0.159	nd	nd
2378-TCDF	nd	nd	nd	nd	nd
12378-PeCDF	nd	nd	nd	nd	nd
23478-PeCDF	0.016	nd	0.015	0.010	nd
123478-HxCDF	0.041	nd	nd	nd	nd
123678-HxCDF	nd	nd	nd	nd	nd
123789-HxCDF	0.052	nd	nd	nd	nd
234678-HxCDF	nd	nd	nd	nd	nd
1234678-HpCDF	nd	nd	nd	nd	nd
1234789-HpCDF	nd	nd	nd	nd	nd
OCDF	0.100	nd	0.057	nd	nd
i-TEQ	**0.017**	**nd**	**0.008**	**0.005**	**nd**

Note: nd – non-detectable

We have detected 2,3,7,8-TCDD/F in both fish types of BienHung Lake with relative high concentration: 2,3,7,8-TCDD concentration varied from *nd* – 0.611ng/g lipid for snake-heads and *nd* – 1.015ng/g lipid for catfishes while these compounds were not presented in the catfish samples of HaNoi market. The other 2,3,7,8-PCDD/Fs compounds were also detected in analyzed samples, but in lower concentration and not contributed so much in final i-TEQ values.

The i-TEQ and 2,3,7,8-TCDD concentration in fish tissue were calculated and compared by statistic method, result is showed in table 6.5 and fig. 6.3 and 6.4 below.

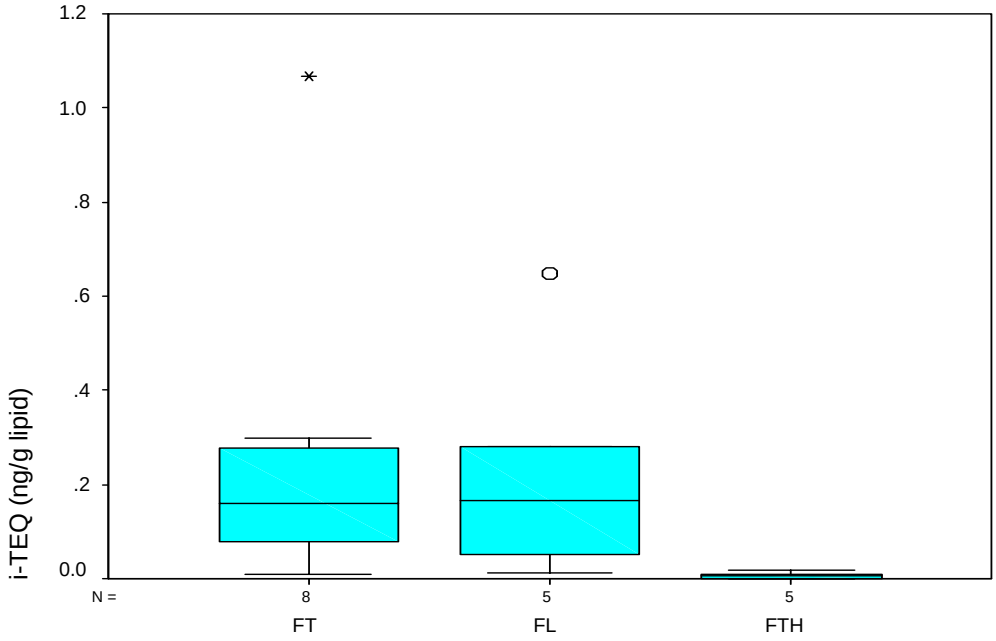


Fig. 6.3 Comparing PCDD/Fs levels based on i-TEQ

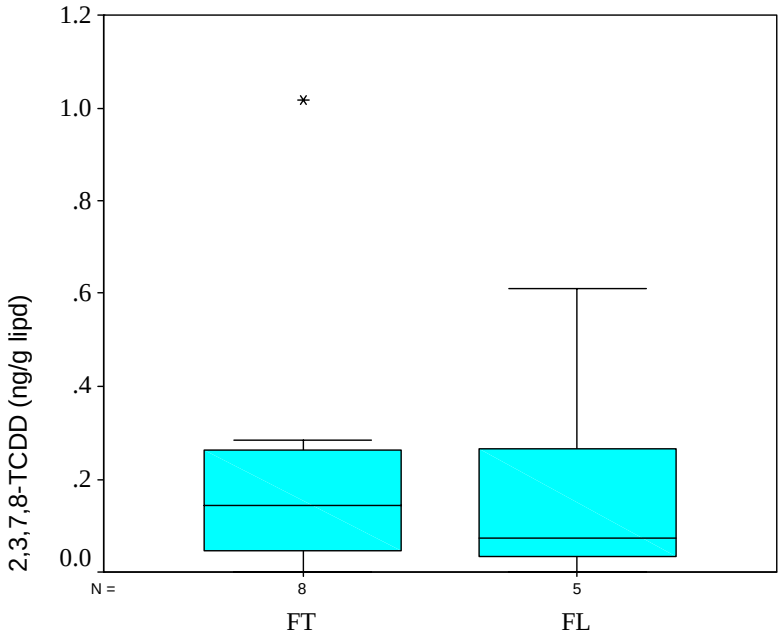


Fig. 6.4 Comparing PCDD/Fs levels based on 2,3,7,8-TCDD conc.

Table 6.5 Statistic calculation for i-TEQ and TCDD conc. in fish tissue

Sample type		*N*	*Mean*	*Median*	*Std. Deviation*	*Variance*	*Range*	*Min*	*Max*
FT	i-TEQ (ng/g lipid)	8	0.263	0.159	0.339	0.115	1.057	0.010	1.067
	TCDD (ng/g lipid)		0.240	0.144	0.328	0.108	1.015	nd	1.015
FL	i-TEQ (ng/g lipid)	5	0.232	0.166	0.256	0.066	0.637	0.012	0.650
	TCDD (ng/g lipid)		0.197	0.072	0.254	0.064	0.611	nd	0.611
FTH	i-TEQ (ng/g lipid)	5	0.006	0.005	0.007	nd	0.017	nd	0.017

Note: nd – non-detectable

Fig. 6.3 showed that there is not a significant difference in i-TEQ between two fish species (catfish and snake-head) of BienHung Lake. Both have relative high i-TEQ values in comparison with the catfish of HaNoi market. In contrary, fig. 6.4 showed that the 2,3,7,8-TCDD contamination level in catfish tissue is higher than in snake-head tissue (median TCDD concentration of 0.144ng/g lipid for catfish and 0.072ng/g lipid for snake-head). It is seemed to be logical because catfishes live in the bottom, since they are easy affected by the contaminants presented in the sediment layers while snake-heads lives in the middle water layer and they are influenced mainly through food chain (preys).

6.4 PCDD/Fs level in BienHung fish tissue in comparison with obtained results by others and standard values

The PCDD/Fs concentration in fish samples of different areas in VietNam has been surveyed and reported by some authors (*Division 10-80, 2000; Dwernychuk et al, 2002; Schecter et al, 2003; etc*). The results are compared with our obtained values are presented in the table 6.6 below:

Table 6.6 PCDD/Fs contamination level in fish tissue of BienHung Lake in comparison with obtained values by others

Place	*Fish kind*	*i-TEQ (pg/g w.w)*	*2,3,7,8-TCDD (pg/g w.w)*	*Source*
HaNoi	Catfish	0.2	0.13	*Division 10-80, 2000*
Aluoi, Hue	Grass carp	17 - 29	17 - 29	*H.C.L, 1998*
Aluoi, Hue	Grass carp	nd – 9.1	nd – 8.7	*Division 10-80, 2000*
Aso, Hue	Carp	-	21 - 51	*Dwernychuk et al, 2002*
Hochiminh City	Catfish	-	0.13 – 0.31	*Son, 2003*
BienHung lake, BienHoa City, DongNai Province	Snake-head	66	65	*Scheter et al (2), 2003*
		Our Results		
BienHung lake	Catfish	0.7 – 87.7 (16.18)	nd – 83.4 (14.6)	
	Snake-head	1.5 – 76.0 (16.3)	nd – 71.5 (7.1)	

Note: nd – non-detectable
Values in parenthesis: median values

From table 6.6 we can see that our results are very comparable with the result reported by Schecter at al (2003). The PCDD/Fs contamination levels in fish tissue from BienHung Lake

are very high in comparison with other places of Vietnam (even higher than Aso/Aluoi – Hue Province, a place is most surveyed and recognized as an example for the A.O contamination).

With regard to the standard values Vietnam has not yet the limit standard for dioxin, so we have used the EC Standard for fish as food 4ng PCDD/F-TEQ/kg wet weight (w.w). Fig. 6.5 showed the PCDD/Fs concentration based on i-TEQ of our fish samples in comparison with EC standard (*EC, 2001*)

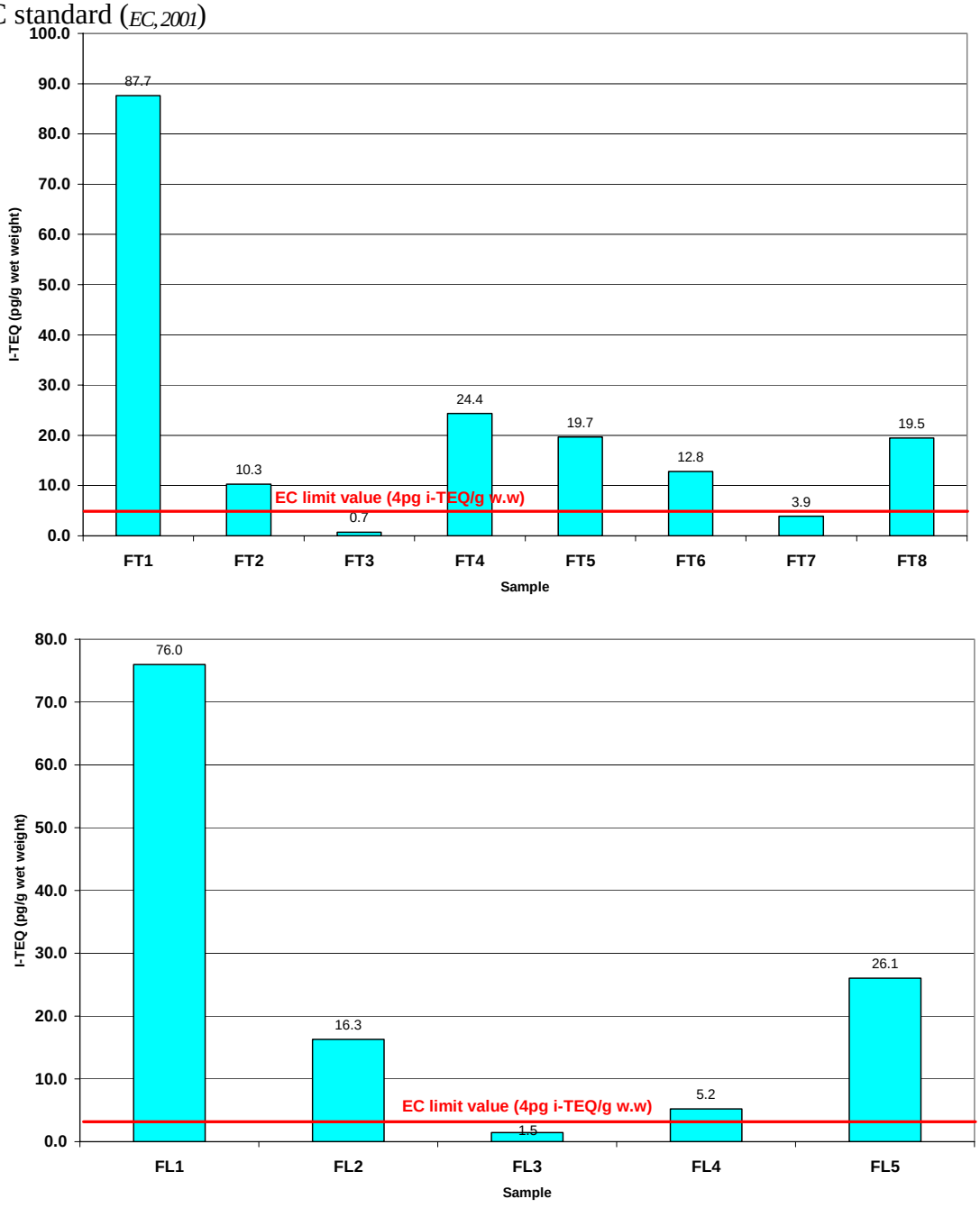


Fig. 6.5 PCDDs level in fish tissue of BienHung Lake in comparison with EC Standard

From fig. 6.5 we can see that almost all fish samples of BienHung Lake have i-TEQ higher than EC limit values (6/8 samples for catfish and 4/5 samples for snake-head). Since these kinds of fish are still used as food, so it causes a big risk for the health of local residents.

The origin of PCDD/Fs in fish tissue will be examined and discussed in chapter VIII.

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CHAPTER VII: DIOXIN CONTAMINATION IN HUMAN ADIPOSE TISSUE

7.1 Introduction

Human adipose tissue is an important matrix because as we presented in chapter I, the PCDD/Fs are lipophilic compounds, hence when they penetrated into human body via direct/indirect contact they will be accumulated mainly in the human body part with high lipid content. Estimating dioxin concentrations in human tissues is the first step to know the critical level of toxic responses for human, and then for risk assessment.

In fact to assess the dioxin accumulation and transfer through 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 sample and analyze the PCDD/Fs at different levels of the same food chain (e.g.: sediment --> fish-->eating-fish bird --> ....--->human body). That will be an ideal strategy for sampling, but it is very difficult to realize and depends on many factor as listed below:

- The limits of time and budget;
- The difficulties to obtain the completed formalities for sampling permit: as we have mentioned above, all data of related to dioxin is consider as extreme secret according Vietnamese law. Since our research is only a scientific study in the frame of collaboration project between Swiss (Cecotox) and VietNam (IER) and is not an official governmental research, we could not done our sampling as we wanted to do.

Fortunately, with the help of the vice director of DongNai Polyclinic we got a permit from Department of Health of DongNai Province to carry-out our sampling in this hospital. The human adipose samples were taken from patients passed a surgery in period from 3/2004 to 12/2004. There are some important points for sampling:

- Adipose sample is taken under belly skin;

- The sample weight was 0.5- 8 g (or more, depends on reality);

- After sampling, sample was put in glass flask with lid (10mL) and storage in a fridge (- 10^{o}C) inside the hospital;

- Every week, the samples were transported to IER Lab. In IER Lab, samples were stored in fridge (- 25^{o}C) until analysis;

- All samples with the hospital given information related to the patient: name, age, sex, etc.

We have taken about 200 samples, but due to the limit of time we have analyzed only 90 samples. In fact, with the sampling model like this we could not choose the person of our interest (e.g. the person who has the diseases liked to A.O/Dioxin). Since the result will give us only an overview about the PCDD/Fs contamination level in the human body of BienHoa City and its surrounding area.

The samples were coded with the name ADi (i = 1 – 91) and analyzed following the method presented in chapter IV.

7.2 PCDD/Fs concentration in human adipose tissue of BienHoa residents

The PCDD/Fs concentration in adipose tissue of BienHoa residents are presented in in table 3 (*see annex*). We divided the samples onto two groups: group1 with the age varied from 19 to 30 and group2 with the age varied from 31 to 59. Using the statistic calculation for 2,3,7,8-TCDD concentration and i-TEQ value we obtained the result as presented in table 7.1 and fig 7.1 below:

Table 7.1 Statistic comparison between two groups in TCDD concentration and i-TEQ (pg/g w.w)

	Group1 (age: 19 – 30)		*Group2 (age: 31 – 59)*	
	2378-TCDD	*i-TEQ*	*2378-TCDD*	*i-TEQ*
N	40	40	41	41
Mean	26.5	54.9	35.5	110.7
Median	1.7	27.0	1.7	26.6
Std. Deviation	44.0	65.8	68.5	217.0
Range	177.6	255.5	300.0	1148.0
Minimum	nd	nd	nd	0.7
Maximum	177.6	255.5	300.0	1148.7

Note: nd-non-detectable

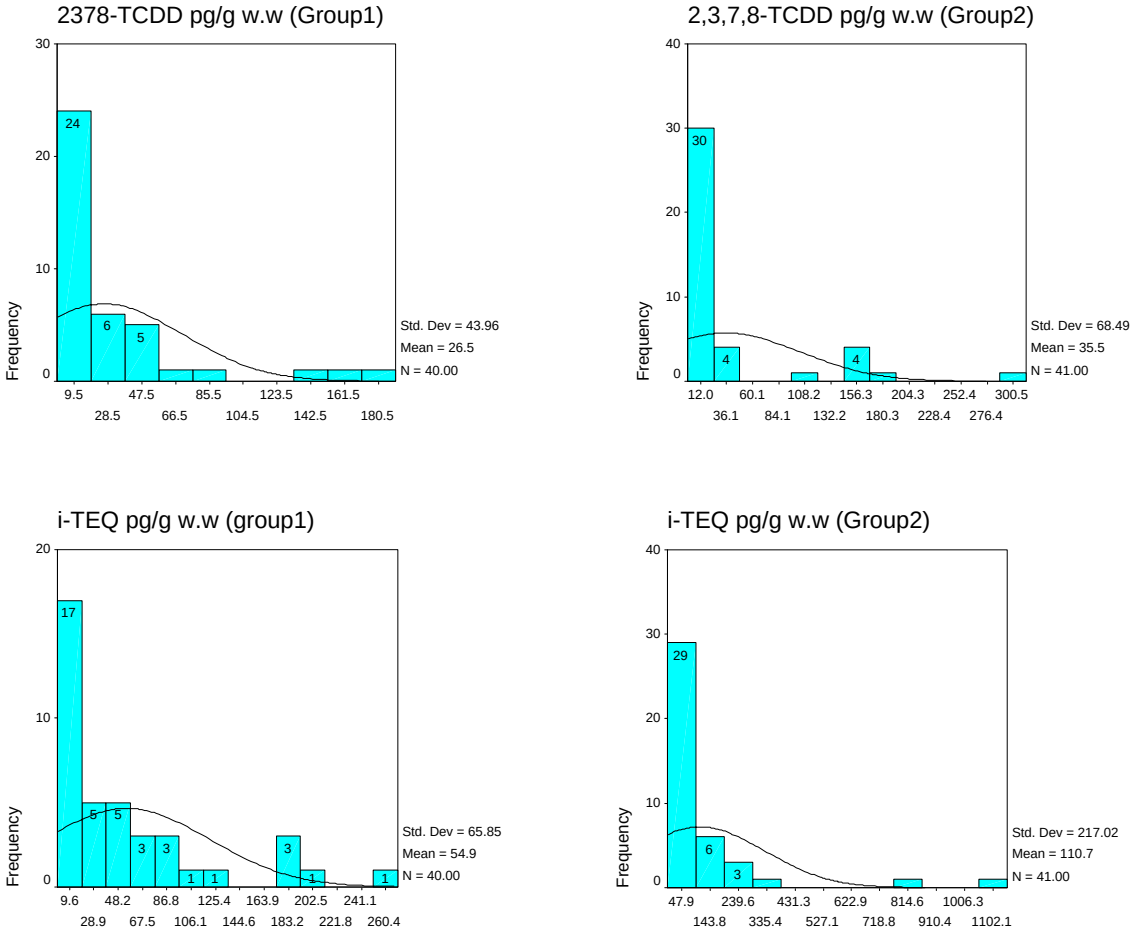

Fig. 7.1 Distribution of 2,3,7,8-TCDD conc. and i-TEQ in samples of two groups

Fig. 7.1 shows that the distributions of 2,3,7,8-TCDD concentration and i-TEQ of two groups: group1 has highest density in the left (more cases with low 2,3,7,8-TCDD and i-TEQ), then gradually decreased to the right; group2 has highest density in the left, but its density in the middle is increased a little bit (6/41 cases – 15%). The median values of 2,3,7,8-TCDD concentration and i-TEQ values of two groups are equal, but the mean values of group2 are higher for both 2,3,7,8-TCDD and i-TEQ (*see table 3 - annex*). That means group2 has more exceptional cases with higher values than upper bound as showed in the fig. 7.2 below.

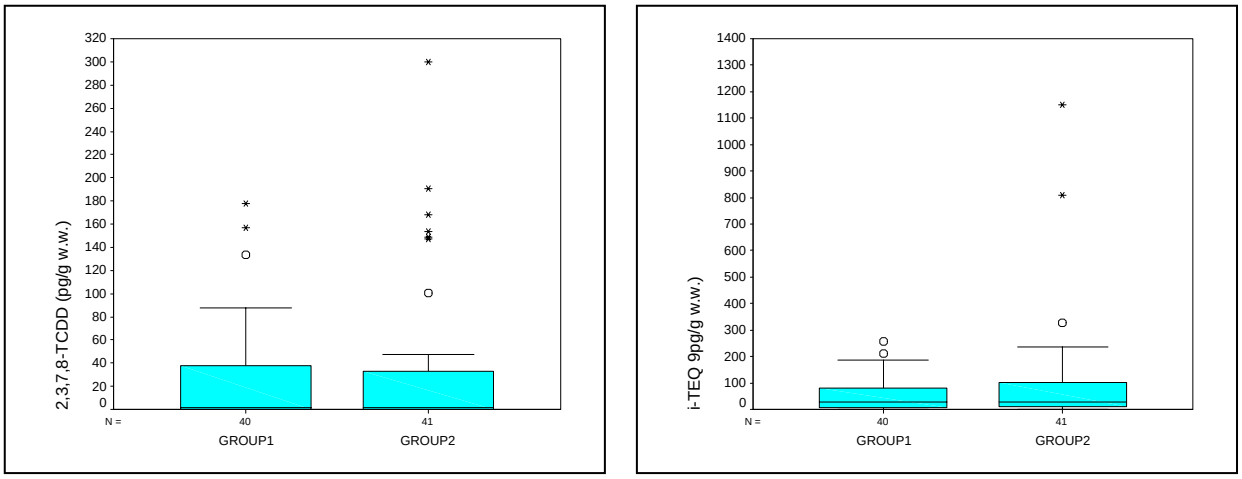

Fig. 7.2 PCDD/Fs levels in human adipose samples of BienHoa residents based on i-TEQ and 2,3,7,8-TCDD conc.

With regard to 2,3,7,8-TCDD – the most toxic compound of 2,3,7,8-PCDD/Fs, it is detected in 43/81 cases (53%) of analyzed samples, this is an evidence that 2,3,7,8-TCDD is a principal important component for PCDD/Fs contamination in adipose tissue of BienHoa residents. Its contribution onto i-TEQ value is used for evaluate the source of PCDD/Fs and it will be discuss later in next chapter.

We have also analyzed 9 adipose samples of non-BienHoa residents (*see table 7.2*). Because these persons come from different places of VietNam, so the meaning of analyzed result presented here is only for consulting and we could not apply the statistic calculation for these samples. 2,3,7,8-TCDD was detected in 6/9 samples. Two samples having highest 2,3,7,8,-TCDD concentration (AD16 and AD41) were from two women with relative high age (35 and 40). It is interesting that both were born and lived in the middle of VietNam (QuangNgai and QuangNam Provinces) – areas affected seriously by A.O and another chemicals during the war. Of course we could not say more with only two samples because these samples were not yet the representative samples for whole population overthere, however from the obtained results we can suggest to the responsible organizations such Committee 33, Division 10-80, etc. to consider and carry-out more studies on these areas.

Table 7.2 PCDD/Fs concentration in human adipose tissue of non-BienHoa residents (pg/g w. w)

Sample	*AD5*	*AD10*	*AD16*	*AD17*	*AD33*	*AD37*	*AD40*	*AD41*	*AD79*
Age	39	34	35	20	36	32	23	40	29
Isomer									
2378-TCDD	nd	nd	773.4	nd	10.2	55.7	51.3	65.8	1.7
12378-PeCDD	nd	nd	nd	47.0	19.1	nd	nd	374.7	nd
123478-HxCDD	nd	nd	63.3	9.3	nd	nd	nd	nd	nd
123678-HxCDD	nd	nd	468.8	nd	nd	nd	nd	nd	nd
123789-HxCDD	nd	nd	342.2	nd	nd	nd	nd	nd	nd
1234678-HpCDD	14.6	8.0	nd	176.3	29.5	109.2	nd	nd	nd
OCDD	10.1	nd	3125.1	10.8	26.6	917.9	561.1	nd	nd
2378-TCDF	0.6	0.3	12.0	nd	nd	nd	nd	nd	nd
12378-PeCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd
23478-PeCDF	1.7	2.4	8.0	9.4	8.1	nd	nd	37.8	nd
123478-HxCDF	nd	nd	375.0	nd	12.3	107.5	112.9	nd	40.9
123678-HxCDF	nd	nd	nd	nd	nd	116.0	158.1	nd	nd
123789-HxCDF	0.1	nd	nd	0.7	nd	72.6	nd	27.8	nd
234678-HxCDF	nd	nd	nd	2.8	nd	70.3	nd	nd	nd
1234678-HpCDF	nd	nd	nd	nd	8.1	80.3	nd	nd	nd
1234789-HpCDF	0.6	0.6	nd	nd	nd	100.6	nd	nd	nd
OCDF	nd	nd	857.5	6.6	30.9	460.9	423.3	255.5	nd
i-TEQ	**1.1**	**1.3**	**903.9**	**54.7**	**24.8**	**39.7**	**27.2**	**396.3**	**4.1**

Note: nd – non-detectable

7.3 PCDD/Fs level in human adipose of BienHoa residents in comparison with obtained results by others

For PCDD/Fs concentration in human tissue there is no suggested value, neither the limit value. In fact we have only few documents related to this subject in VietNam due to the reason as we have told above. The PCDD/Fs level in human adipose of BienHoa residents is compared with results obtained by others and presented in table 7.3

Table 7.3 Our result in comparison with results obtained by others

City/Country	*2,3,7,8-TCDD*	*i-TEQ (pg/g)*			*Source*
	(pg/g)	Min	Max	Mean	
Paris/France (8 cases)	-	-	-	32.1	*Petersen, 1999*
Germany (28 cases)		25.4	107.4	50.0	
Madrid/Spain (17 cases)		4.1	82.9	41.8	
Tarragona/Spain (15 cases)		13.4	69.4	31.0	
Sweden (19 cases)		-	-	24.0	
Hochiminh City/Vietnam (93 cases)					*Phiet et al., 1997*
- Age < 40				9.2	
- Age > 40				16.3	
Vietnam	-	-	-		*Division 10-80, 2000*
- South (44 cases)				22.41	
-North (11 cases)				4.8	
Hochiminh City/VietNam (27 cases)	11.4	-	-	-	*Division 10-80, 2000*
Our result: BienHoa City – DongNai Province					
Age: 19 – 30 (40 cases)	*26.5 (1.7)*	*nd*	*255.5*	*54.9 (27.0)*	
Age: 31 – 59 (41 cases)	*35.5 (1.7)*	*0.7*	*1148.7*	*110.7 (26.6)*	

Note: nd-non-detectable
Values in parenthesis: median values

From table 7.3 we can see that median i-TEQ in adipose tissue of BienHoa residents is comparable with the industrial countries while mean i-TEQ was higher for both groups, especially for group2 - older people. This group has some exceptional cases with i-TEQ value up to 1148.7pg/g.

Compared with the studies done by Phiet et al. (1997) and Divison 10-80 (2000), our result showed the higher i-TEQ value. For 2,3,7,8-TCDD, because study of Division 10-80 not showed the median value, so it is very difficult to compare two results. However, our result is correlated with the comments of previous studies showed in chapter II: at present time, the dioxin concentration in natural environment and human body is only a residue and not higher than in developed industrial countries

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CHAPTER VIII: PATTERNS AND SOURCES OF PCDD/FS IN OUR SAMPLES

8.1 Introduction

One of our aims is to find out the origin and principal sources of PCDD/Fs for the South of Vietnam. In fact it is quite difficult because our collected and analyzed data is inadequate to solve such big problem. In addition, the question of PCDD/Fs origin in Southern VietNam is still a subject of controversy between scientists even if many studies have been done and some real evidences have been presented as we showed in chapter II.

In our research, we tried to apply the statistic method called multivariate analysis to interpret the obtained result. Multivariate analysis is a very useful tool to assess the data with many variables such our 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 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 hierarchical schemes, we are predicting how one quantity affects another, while recognizing that they are different variables quantified by different measures. These strategies are used to predict a criterion variable (as in Multiple Regression); group membership (as in Discriminant Analysis); group comparison (as in Analysis of Variance); and structure (for example Factor Analysis). By contrast, single-level Cluster Analysis attempts to relate response variables. Cluster analysis is the grouping of similar objects into a subset of objects. In cluster analysis scores are grouped together with no predetermined group membership, and we attempt to optimize a group, rather than a route. This is the opposite of Multiple Discriminant Analysis where the variables have known group membership and we determine which of the variables are good descriptors of the group. Cluster analysis may also be exploratory in the sense that we are processing a number of variables in the hope of establishing some pattern from which we may develop a theory. Typically researchers use cluster analysis as a check on the validity of their findings. In this case, they might 'pretend' that they have no idea of the group membership of the individuals under consideration, and see what groupings are suggested by the clustering procedure. Then, a Multiple Discriminate Analysis may be performed on the clusters to verify the validity or otherwise of the categories found.

In the case of PCDD/Fs, to find the patterns and sources of these compounds two techniques often used are cluster analysis and principal component analysis (PCA) as reported by many authors (*Lindstrom et al, 1989; Fielder et al, 1996; Gotz et al (1)&(2), 1998; Oberg , 2004; etc*). At present time, there is much available software programmed to treat automatically the data and show us very fast the calculated result. However to apply these software we should to know the algorithm as well as the process to treatment the data before using their models to interpret the result after calculation. The algorithm of cluster analysis and PCA are presented below in next section.

8.2 Cluster analysis

In cluster analysis we search for patterns in a data set by grouping the observations into clusters. The goal is to find an optimal grouping for which the observations or objects within each cluster are similar, but the clusters are dissimilar to each other. We hope to find the natural groupings in the data, groupings that make sense to the researcher.

Generally speaking, clustering algorithms fall into two categories:

1. Partitioning Algorithms. A partitioning algorithm describes a method that divides the data set into k clusters, where the integer k needs to specify. Typically, you run 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 you to select the "best" value of k afterwards. Algorithms of this type described in this chapter are used by the functions *kmeans*, *pam, clara, and fanny*.

2. Hierarchical Algorithms. A hierarchical algorithm describes a method yielding an entire hierarchy of clusterings for the given data set. Agglomerative methods start with the situation where each object in the data set forms its own little cluster, and then successively merges sters until only one large cluster remains which is the whole data set. The functions *agnes, mclust*, and *hclust* use agglomerative methods. Divisive methods start by considering the whole data set as one cluster, and then split up clusters until each object is separate. Algorithms of this type are used in the functions *diana* and *mona*.

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.

Data sets for clustering can have either of the following structures:

1. $n \times p$ data matrix:

$$\begin{bmatrix} x_{11} & \cdots & x_{1p} \\ \vdots & \cdots & \vdots \\ x_{n1} & & x_{np} \end{bmatrix}$$

2. $n \times n$ dissimilarity matrix:

$$\begin{bmatrix} 0 & & & & \\ d(2,1) & 0 & & & \\ d(3,1) & d(3,2) & 0 & & \\ A & A & A & & \\ d(n,1) & d(n,2) & \cdots & \cdots & 0 \end{bmatrix}$$

Where d(i,j) = d(j,i) measures the "difference" or dissimilarity between the objects and. This kind of data occurs frequently in the social sciences and in marketing.

Many of the clustering algorithms considered here operate on a dissimilarity matrix. If the data consist of an $n \times n$ data matrix, the algorithm first constructs the corresponding

dissimilarity matrix. The functions *kmeans, clara, mona,* and *mclust* operate on a data matrix. The *hclust* function operates on a dissimilarity matrix. The functions *pam, fanny, diana*, and *agnes* will take either a data or dissimilarity matrix.

The dissimilarity between two objects measures how different they are. Sometimes we can use an actual metric (distance function) between objects, but a dissimilarity function is not necessarily a metric. Often only the following three axioms of a metric are satisfied:

1. d(i,i) = 0
2. d(i.j) ≥0
3. d(i,j) = d(j,i)

If all variables are interval-scaled, we can use an actual metric such as:

$$d(i,j) = \sqrt{\sum_{f=1}^{p} (x_{if} - x_{jf})^2} \qquad \text{(Euclidean distance)}$$

$$d(i,j) = \sum_{f=1}^{p} |x_{if} - x_{jf}| \qquad \text{(Manhattan distance)}$$

Note that the choice of measurement units strongly affects the resulting clustering. The variable with the largest dispersion will have the largest impact on the clustering. If all variables are considered equally important, the data need to be standardized first.

Put $m_f = \frac{1}{n}\sum_{i=1}^{n} x_{ij}$ and $s_f = \frac{1}{n}\sum_{i=1}^{n} |x_{if} - x_f|$; then the standardized measurements are defined as follows:

$$z_{if} = \frac{x_{if} - m_f}{s_f}$$

Here we have used s_f , the *mean absolute deviation* instead of the usual standard deviation, because the former is more robust: since the deviations are not squared, the effect of outliers is somewhat reduced. Of course, there are more robust measures of dispersion, such as the median absolute deviation (the function mad). The advantage of using a robust measure of dispersion is that the *z-scores* of outliers do not become too small, hence the outliers remain detectable and visible in the clustering.

The two most widespread clustering techniques are k-means and agglomerative hierarchical clustering

K-Means : One of the most well-known partitioning methods is k-means. In the k-means algorithm the observations are classified as belonging to one of k groups. Group membership is determined by calculating the centroid for each group (the multidimensional version of the mean) and assigning each observation to the group with the closest centroid. The k-means algorithm alternates between calculating the centroids based on the current group memberships, and reassigning observations to groups based on the new centroids. Centroids are calculated using least-squares, and observations are assigned to the closest centroid based on least-squares. This use of a least-squares criterion makes k-means less resistant to outliers than the medoidbased methods which will be discussed in later sections. The *kmeans* function performs k-means clustering. It is an older function that does not have special plot or summary methods. The main arguments to *kmeans* are dissimilarities as produced by *daisy* or dist and the number of clusters. Alternatively, a matrix of starting centroids may be specified

in place of the number of centroids. If starting values are not specified the initial centroids are obtained using the hierarchical clustering algorithm in *hclust*.

Agglomerative Nesting: As 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, consisting of all the objects.

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:

1. Merge the two clusters with smallest between-cluster dissimilarity;

2. 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.

1. *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.

2. *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.

8.3 Principal Component Analysis (PCA)

For investigations involving a large number of observed variables, it is often useful to simplify the analysis by considering a smaller number of linear combinations of the original variables. For example, scholastic achievement tests typically consist of a number of examinations in different subject areas. In attempting to rate students applying for admission, college administrators frequently attempt to reduce the scores from all subject areas to a single, overall score. If the reduction can be done with minimal information loss, all will be the better.

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 eparated 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.

One obvious choice for the overall score is the mean over all subject areas. For three subject areas s_1, s_2, and s_3 the mean corresponds to the linear combination $s_1/3 + s_2/3 + s_3/3$, , or equivalently l's, where l' is is the vector of coefficients (1/3, 1/3, 1/3). A linear combination with $\Sigma l_i^2 = 1$ is called a standardized linear combination, or SLC. By restricting attention to SLCs, you can make meaningful comparisons between various choices of linear combinations. For example, with the test scores, you can seek the combination with the greatest variance as a way of ranking the students and separating them.

Principal components analysis finds a set of SLCs, 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 (from Mardia, Kent, and Bibby (1979)):

If x is a random vector with mean μ and covariance matrix Σ, then the principal component transformation is the transformation

$$x \to y = \Gamma'(x - \mu)$$

where Γ is orthogonal, $\Gamma'\Sigma\Gamma = \Lambda$ is diagonal, and $\lambda_1 \geq \lambda_2 \geq \ldots \geq 0$. The *ith principal component* of x may be defined as the ith element of the vector y , namely, as

$$y_i = \gamma'_i(x-\mu)$$

Here γ_i is the ith column of Γ and may be called the ith vector of *principal component loadings*.

8.4 Applying Cluster Analysis and PCA for our data

To apply the cluster analysis and PCA our data, firstly we should normalize the variables (PCDD/Fs concentration) to comparable. There are some strategies to normalize the variables (*Ding et al, 1989; Fiedler et al, 1996; Sakurai et al, 2002; etc*): 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; so, the congener pattern is more stable than the homologue profile and should be better preserved in the sample.

a) Relative concentration of PCDD/Fs homologues

The concentration of each of the tetra- through octa- dioxin and furan homologue is divided by the total sum of dioxins/furans, i.e. 2,3,7,8-TCDD/Σ2,3,7,8-PCDD/Fs,.. OCDD/Σ2,3,7,8-PCDD/Fs, 2,3,7,8-TCDF/Σ2,3,7,8-PCDD/Fs, ...OCDF/Σ2,3,7,8-PCDD/Fs. Thus, a single sample is characterized by 10 ratios – PCDD/Fs relative profiles.

b) Relative amount of 2,3,7,8-substituted congeners to the total i-TEQ
The contribution of each 2,3,7,8-substituted congener to the total TEQ of the sample is calculated by weighing the congener's concentration according to its relative toxicity expressed as I-TEF :
(2,3,7,8-TCDD× $TEF_{2,3,7,8\text{-TCDD}}$)/i-TEQ, ...(OCDF×$TEF_{OCDF}$)/i-TEQ

Thus, 17 ratios (PCDD/Fs relative i-TEQ profiles) characterize the sample not only by simple concentration ratios but considers also the toxic potential of each 2,3,7,8-substituted congener present in the sample. TEF values are in the range from 0.0001 for OCDD to 1 for 2,3,7,8-TCDD, the most toxic congener.

For all normalizing methods, concentrations below the detection limit were treated as zero.

8.4.1 Comparison of soil samples

For cluster analysis we use S-PLUS® 6.2 for Windows (Insightful Corp.), applying the normalization process (a) for data of soil samples and Euclidean model we received the agglomerative hierarchical clustering tree as showed in the fig. 8.1 below:

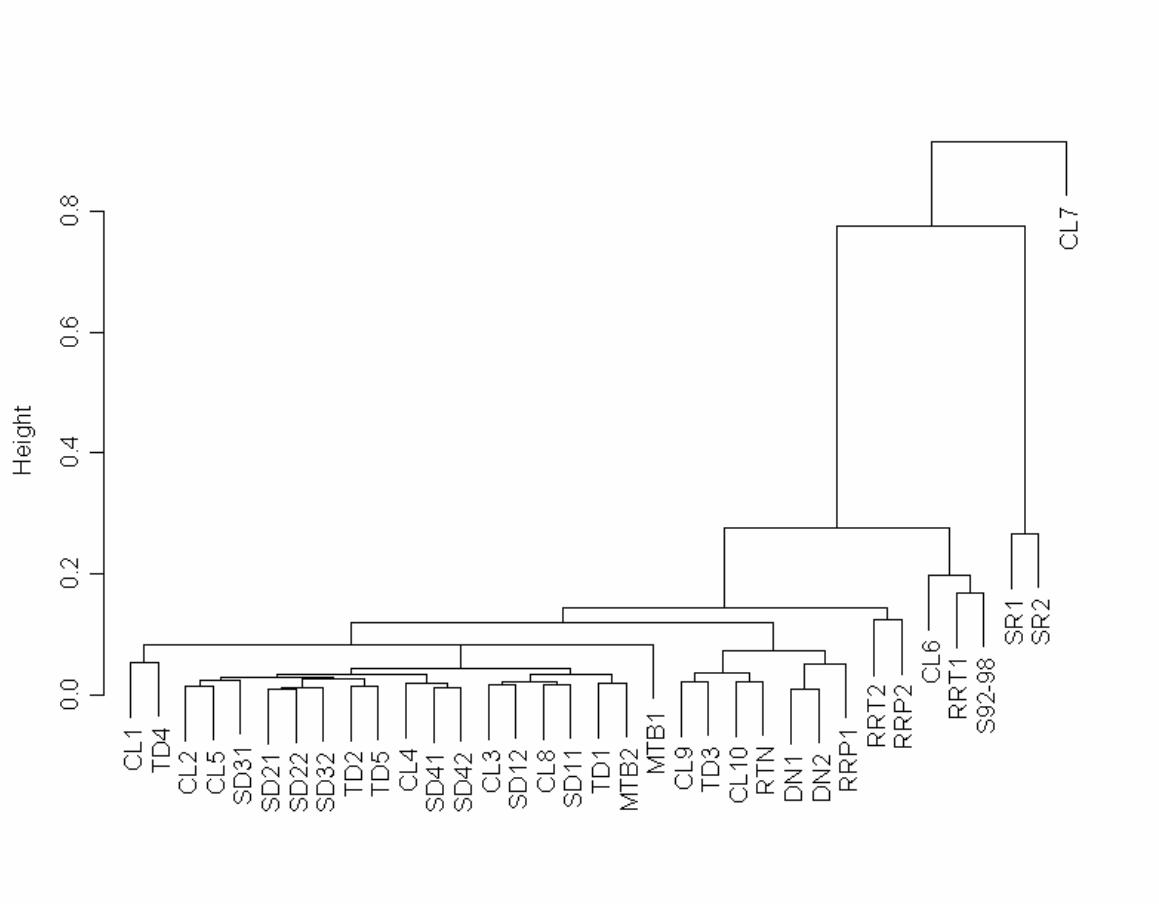


Fig. 8.1 Cluster analysis based on PCDD/Fs relative profiles; 35 soil samples analyzed

From Fig. 8.1 we can see that in general we can divided our soil samples on four groups (from up to down): group1 (CL7), group2 (SR1, SR2), group3 (CL6, RRT1, S92-98), and group4 (the rest of soil samples). In fact there is no visible separation between soil from contaminated sites such CamLo (CL_i, i = 1-10) or BienHoa (SD_{ij}, i =1-2 and j = 1-10) and soil from non-contaminated such MyThanhBac (MTB_i, i = 1-2).

For PCA analysis we use the SIMCA-P version 10.0 (Umetric AB) to understand better the classification of soil samples by relative concentration of PCDD/Fs (PCDD/Fs profiles). The PCDD/Fs profiles are treated by PCA and done in two new variables with 60.4% of the variation. Fig. 8.2 below showed the result of PCA, the ellipse represents the Hottelling T2 with 95% confidence.

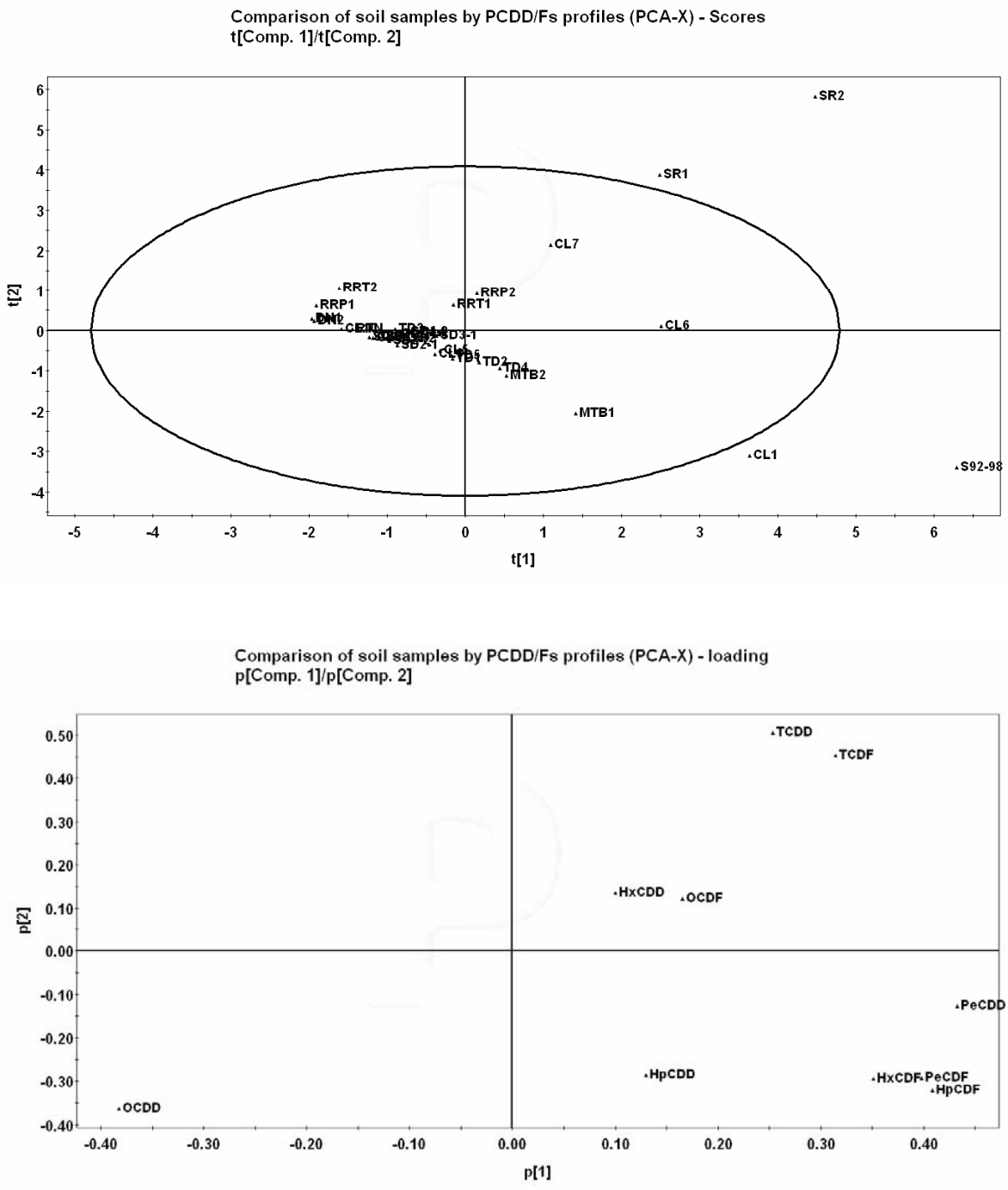


Fig. 8.2 PCA analysis based on relative PCDD/Fs profiles; 35 soil samples analyzed

By PCA analysis again we can see that by PCDD/Fs relative profiles it is difficult to separate the soil samples from contaminated sites by A.O/Dioxins and soil samples from other sites (industrial, agricultural, etc.). In general we can divide soil samples into three group: upper-right group (RRT1, RRP2, CL7, CL6, SR1, SR2) with the interaction of HxCDD, OCDF, TCDD, TCDF); lower-right group (TD2, TD4, MTB2, MTB1, CL1, S92-98) with the interaction of PeCDD, HpCDD, PeCDF, HxCDF, HpCDF; and the rest (center and left) with the interaction of OCDD.

Applying the normalization process (b) for the same samples we received the result showed in fig 8.3 and 8.4 bellow. With considering to the i-TEQ value, we can divide our soil samples to three groups (up to down):

- Group1 (including CL2, CL9,...SD22): this group contains almost samples from contaminated sited by A.O such CamLo, RangRang, DaNang, etc.;

- Group2 (CL7);

- Group3 (including CL1,..., MTB1): this group contains almost non-affected by A.O/Dioxins sites (industrial, rice field;

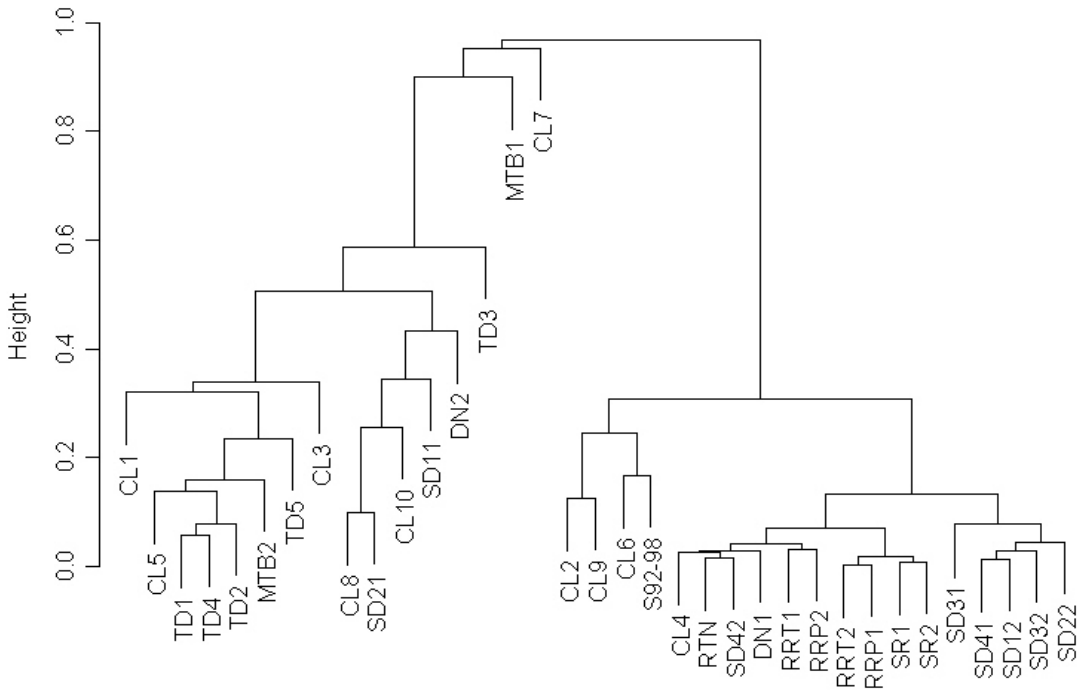
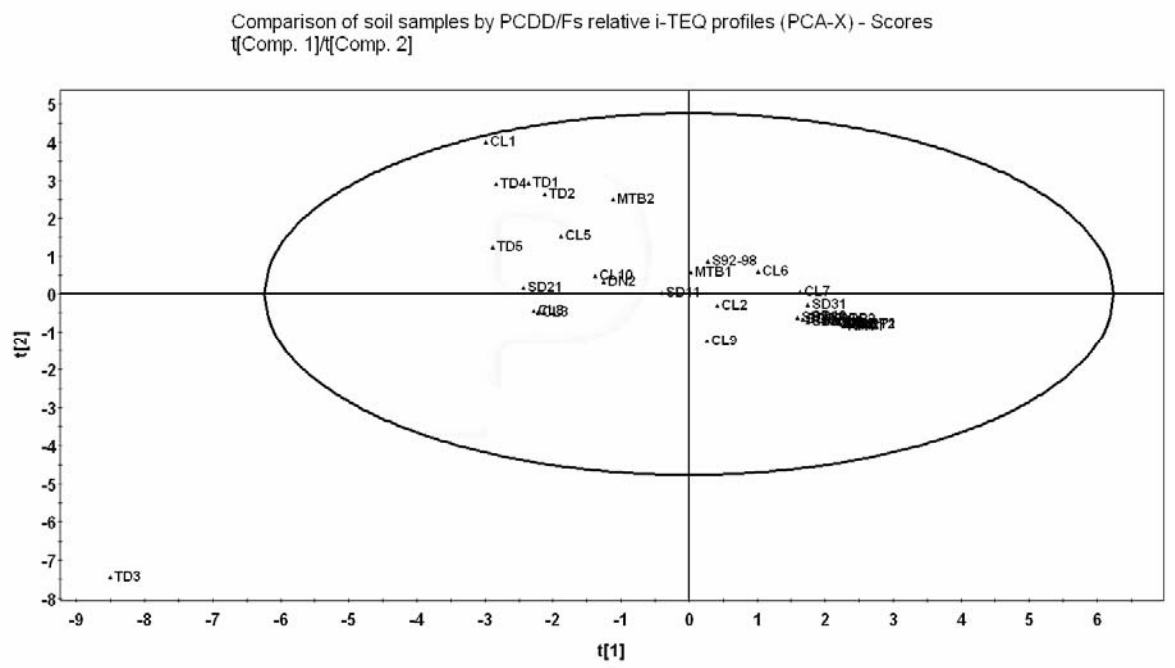


Fig. 8.3 Cluster analysis based on PCDD/Fs relative i-TEQ profiles; 35 soil samples analyzed

Use the PCA for PCDD/Fs relative i-TEQ profiles we have the result as show in fig. 8.4 (variables of 55.1%). The result confirms the grouping by clustering above. There are in principle three groups: lower-right group includes almost contaminated sites by A.O/Dioxin with the interaction of 2,3,7,8-TCDD; upper-right group with interaction of 1,2,3,7,8,9-HxCDD and 2,3,4,6,7,8-HxCDF; upper-left group with interaction of 1,2,3,7,8-PeCDF, 1,2,3,4,7,8-HxCDD,...as showed in fig. 8.4.

We see that normalization process (b) taken the advantage for clustering than process (a) because it taken in account the TEF values, so by this way we can eliminate the influence of the high chlorinated compounds such OCDF, OCDD, 1,2,3,4,7,8,9-HpCDF, etc, especially in the cases of their very high concentration presented.



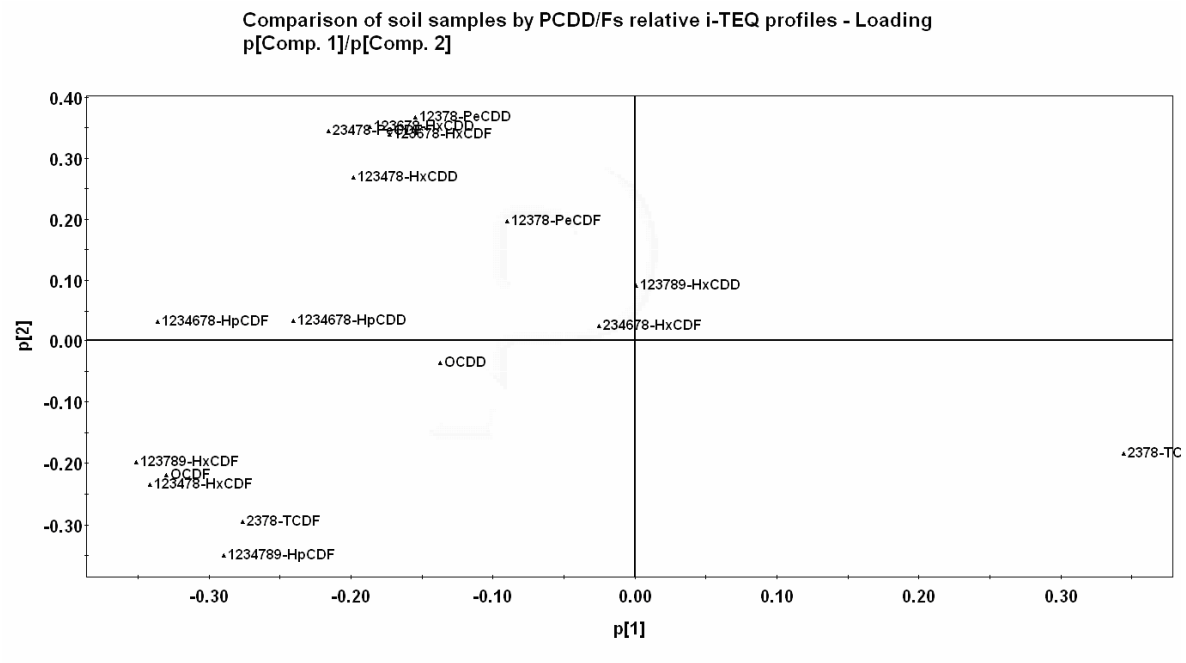


Fig. 8.4 PCA analysis based on PCDD/Fs relative i-TEQ profiles; 35 soil samples analyzed

8.4.2 Comparison of soil and sediment samples

With a hypothesis that the PCDD/Fs contamination in BienHung Lake sediment is related to the soil of BienHoa Airforce Base, we applied the multivariable analysis technique to compare the obtained data of these samples. The PCDD/Fs concentration of BTL sample (sediment of ThamLuong Canal) served as a reference in our comparison. The result of cluster analysis and PCA is showed in Fig. 8.5 and 8.6.

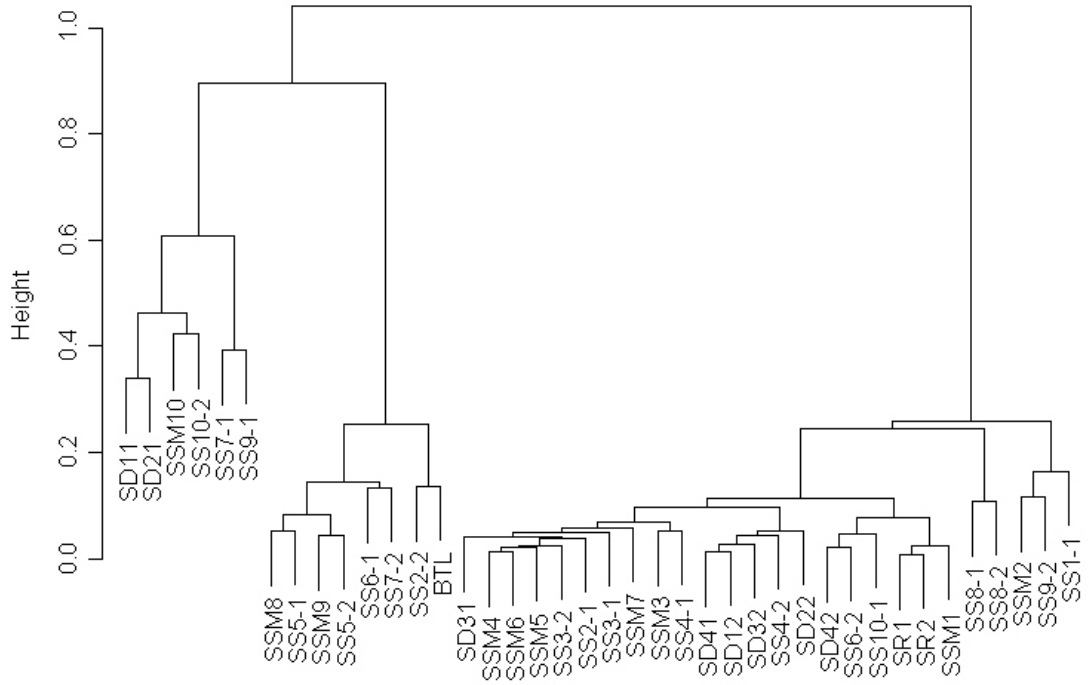


Fig. 8.5 Cluster analysis based on PCDD/Fs relative i-TEQ profiles; 40 soil and sediment samples analyzed

From cluster analysis (Fig. 8.5) our soil and sediment samples could divided onto three groups:

- Group1: SD11, SD21, SSM10, SS10-2, SS7-1, SS9-1;
- Group2: SSM8, SS5-1, SSM9, SS5-2, SS6-1, SS7-2, SS2-2, BTL;
- Group3 (a biggest groups): SD31, SSM4, SSM6, ..., SS1-1.

By PCA analysis (variables of 54.6%), we can see in general there is only two group: right group with very concentrated similarity values, mostly interacted by 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD concentrations and slightly interacted by 1,2,3,6,7,8-HxCDD concentration; and left group with dispersive similarity values, interacted by different 2,3,7,8-PCDD/Fs concentration as showed in fig 8.6

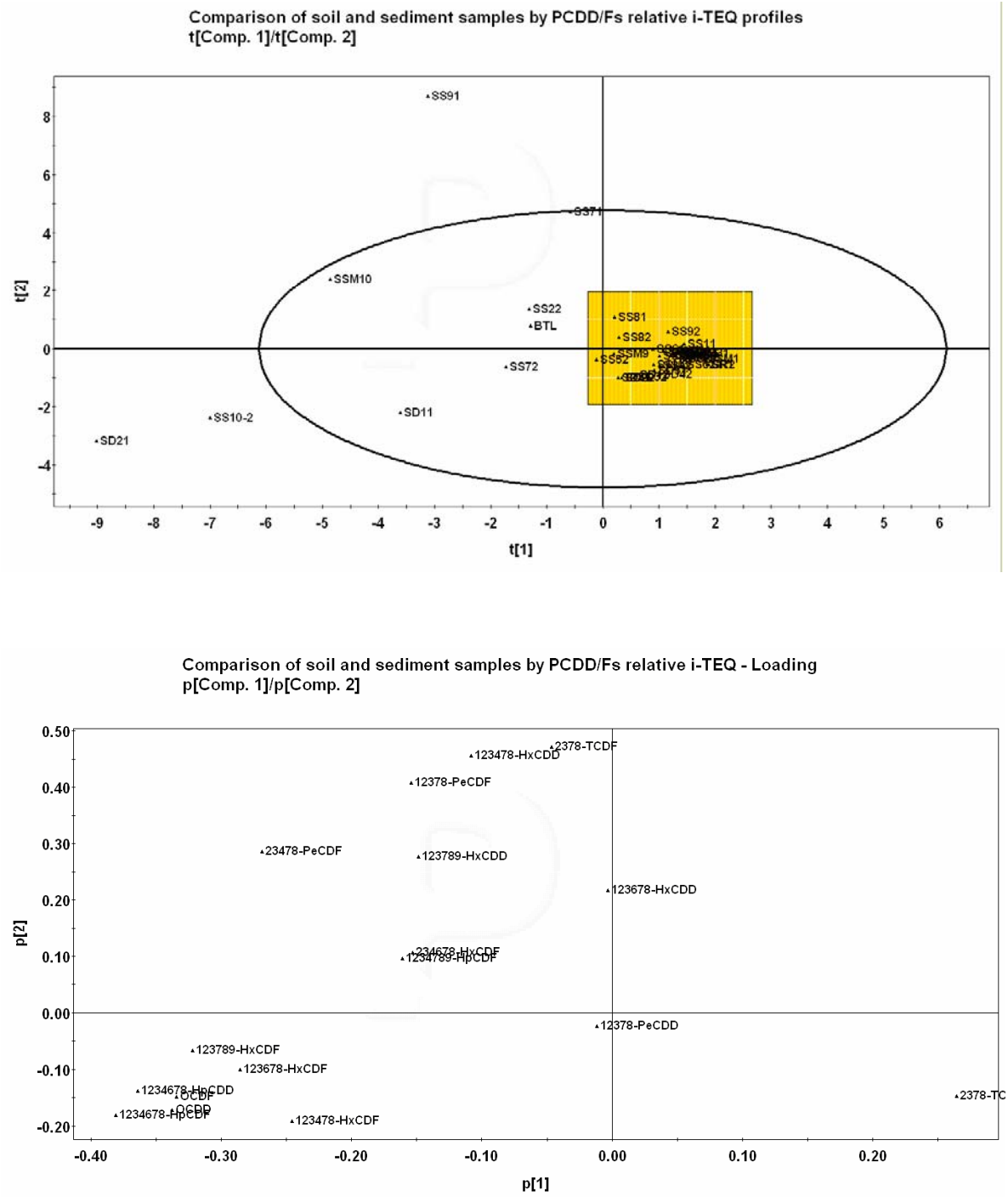


Fig. 8.6 PCA analysis besd on PCDD/Fs relative i-TEQ profiles; 40 soil and sediment samples analyzed

The result of cluster analysis and PCA showed that there is a high similarity between soil samples of BienHoa Airforce Base and sediment of BienHung Lake. Their similarity is mostly affected by 2,3,7,8-TCDD concentration and partially affected by 1,2,3,7,8-PeCDD and 1,2,3,6,7,8-HxCDD concentration.

Now we try to compare the soil and sediment samples by PCDD/Fs relative profiles, result is presented in fig. 8.7 below

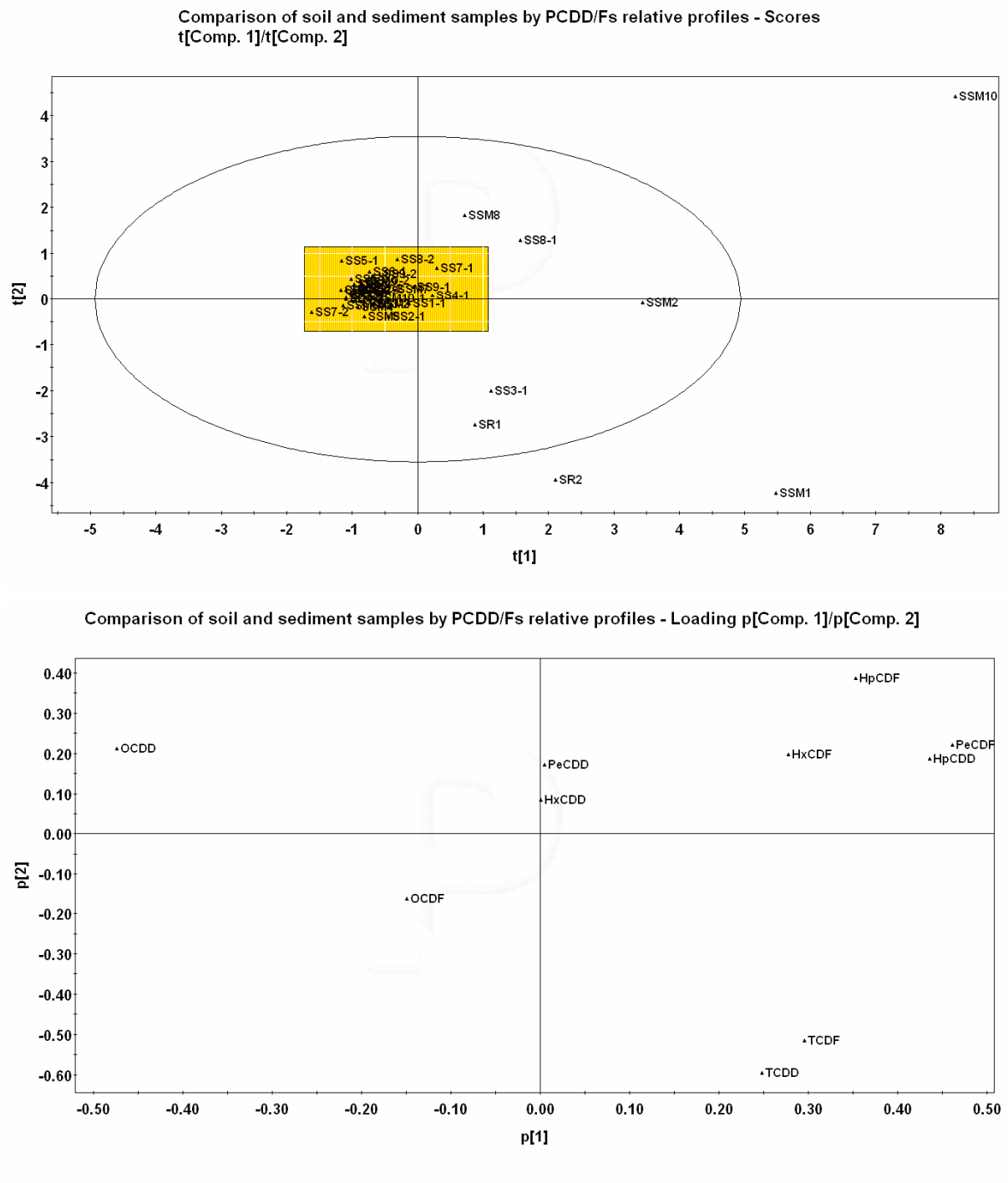


Fig. 8.7 PCA analysis besd on PCDD/Fs relative profiles;
40 soil and sediment samples analyzed

The obtained result again showed the high similarity between soil and sediment samples (yellow zone). Ofcourse there is a different between fig. 8.6 and 8.7 for the samples SR1, SR2, SM1, etc. because interaction of 2,3,7,8-TCDF now is near equivalent in comparison with 2,3,7,8-TCDD. Hence it separated these samples from others.

8.4.3 Comparison of sediment, soil and fish tissue samples

Since the PCDD/Fs concentration in fish tissue of BienHung Lake is relative high and could pose a risk for local residents as showed in chapter VI, so with the same hypothesis as presented above we used the cluster analysis and PCA to find the relationship between them. For comparison we used only the soil and sediment samples with near similarity (yellow group – *see Fig 8.6*). The result presented in the fig. 8.7 and 8.8.

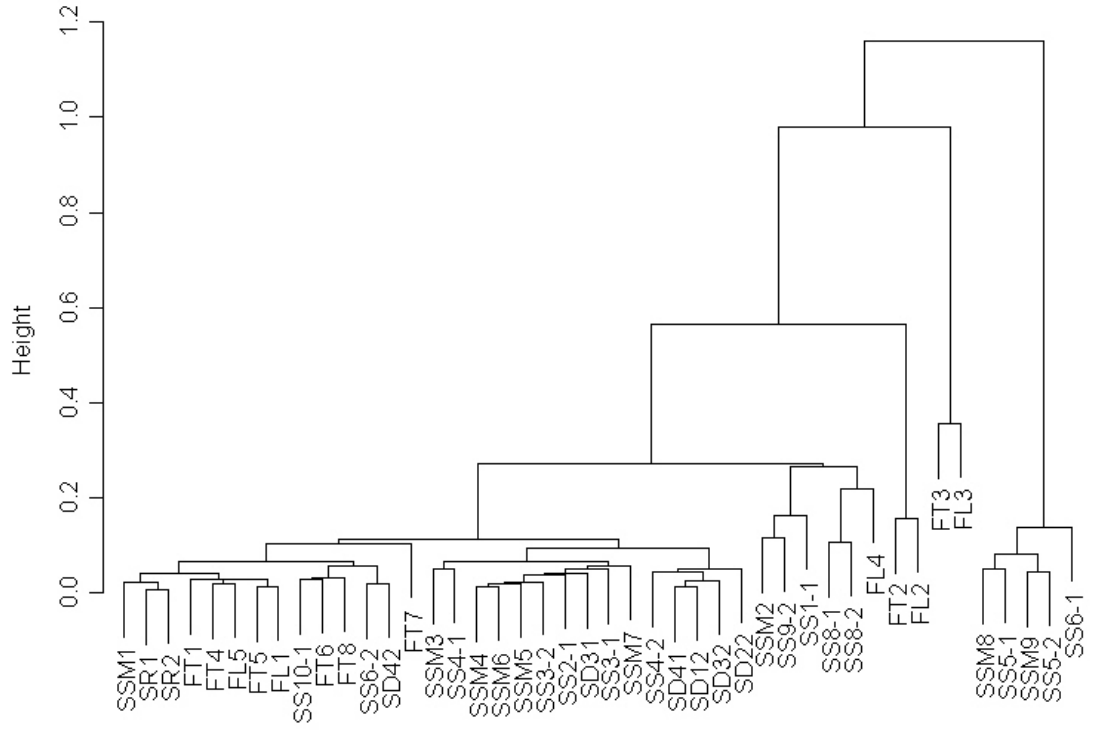
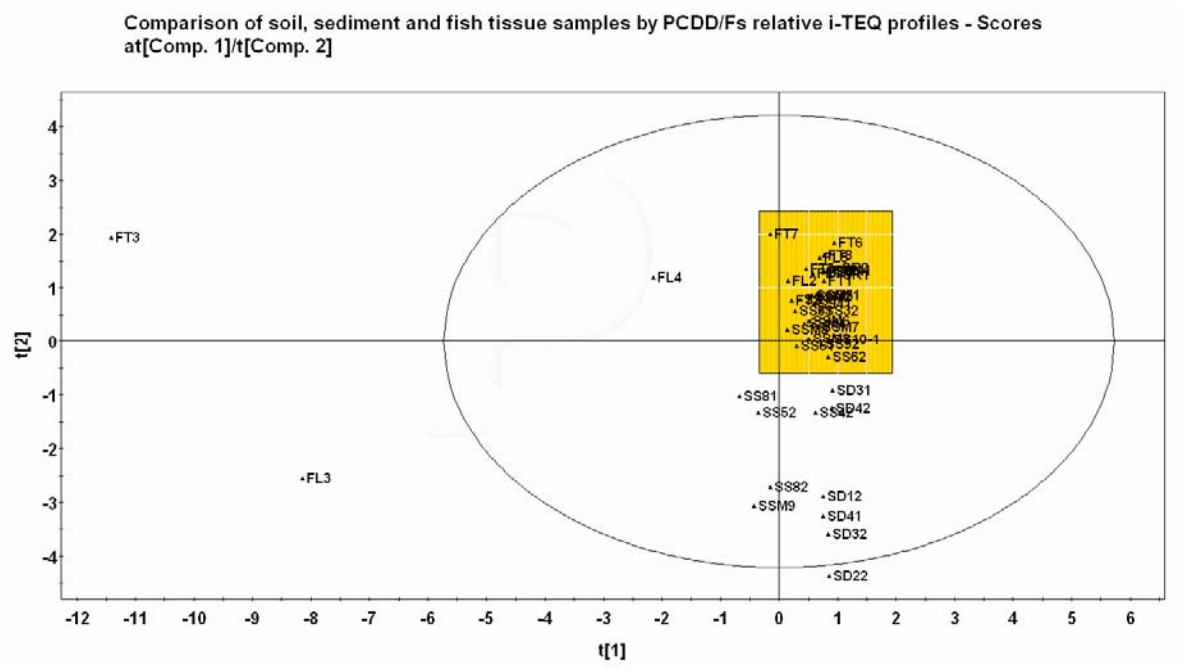


Fig. 8.8 Cluster analysis based on PCDD/Fs relative i-TEQ profiles ;
8 soil, 23 sediment and 13 fish tissue samples analyzed

By cluster we can divide samples into three groups:

- Group1: SSM8, SS5-1, SSM9, SS5-2, SS6-1;
- Group2: FL3, FT3 (two fish tissue samples);
- Group3: SSM1, SR1, ...FL4 (almost fish tissue samples belong to this group)

Result of PCA (variables of 46,2%) is presented in fig. 8.9, from this we can see that there are in principle three groups: group1 (yellow zone – *see fig. 8.9*) contains almost all sediment samples and fish tissue samples (10/13 samples), interacted mostly by 2,3,7,8-TCDD concentration and slightly 1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDF concentration; group2 (lower-right) contains 6 soil samples and 5 sediment samples, interacted by mostly by 1,2,3,4,6,7,8-HpCDD and 1,2,3,4,6,7,8-HpCDF concentration and slightly by 1,2,3,7,8-PeCDD and 1,2,3,6,7,8-HxCDD concentration; and group3 (left) with very dispersive similarity contains 3 fish tissue samples (FL4, FL3 and FT3).



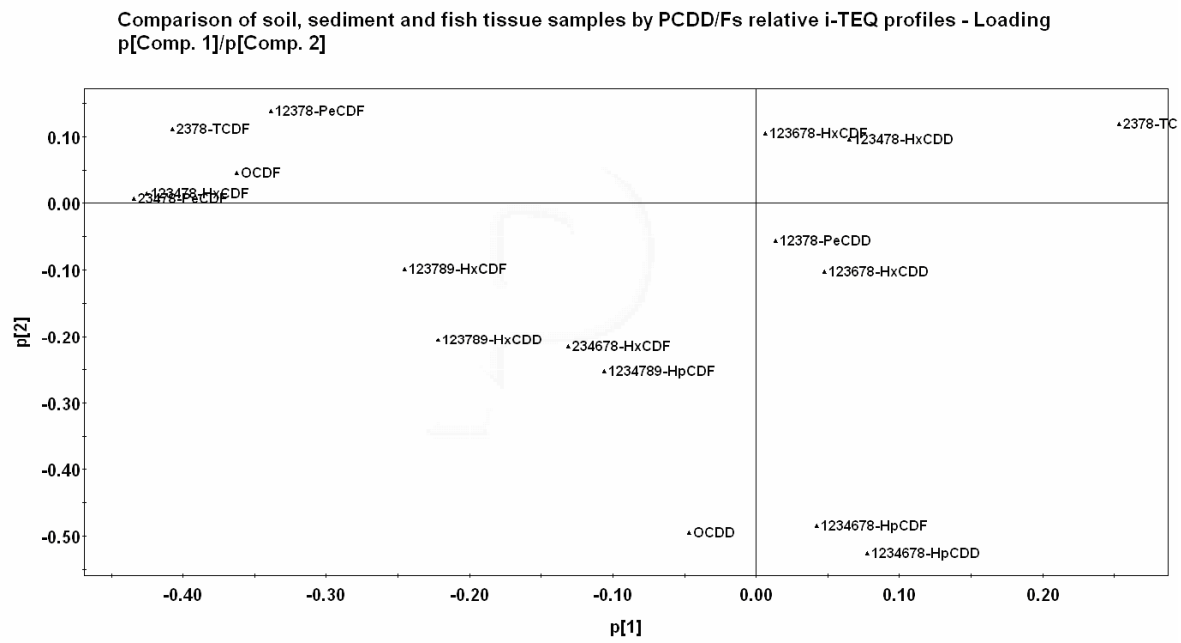


Fig. 8.9 PCA analysis based on PCDD/Fs relative i-TEQ profiles ;
8 soil, 23 sediment and 13 fish tissue samples analyzed

Apply the PCDD/Fs relative profiles for PCA, we received the result as showed in fig. 8-10. In contrary with fig. 8.9, the fish tissue samples are separated from soil and sediment samples: almost all soil and sediment samples belong to the group interacted by PeCDD and OCDD, while fish tissue samples are more dispersive by interaction of the rest of PCDD/Fs concentration as showed in fig 8.10.

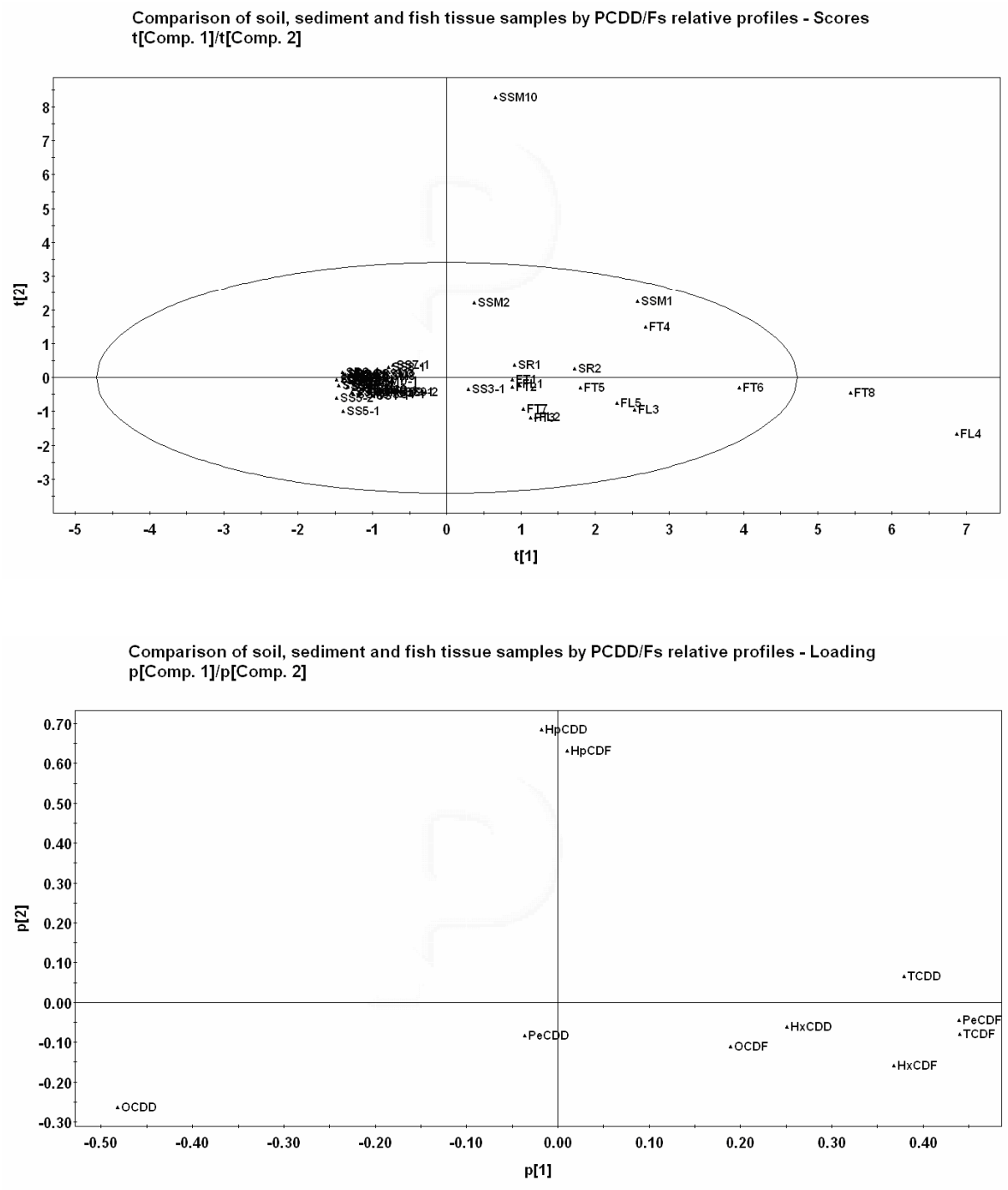


Fig. 8.10 PCA analysis based on PCDD/Fs relative i-TEQ profiles ;
10 soil, 29 sediment and 13 fish tissue samples analyzed

Some general conclusions can be drawn as follow:

- Applying PCA for PCDD/Fs relative i-TEQ profiles we can see that there are different pattern for different types of soil: soil of contaminated sites by A.O/Dioxins have similar pattern affected mostly by 2,3,7,8-TCDD concentration; the reference sites (including industrial and agricultural) have similar pattern affected by hexa- to octa- 2,3,7,8-substituted congeners.

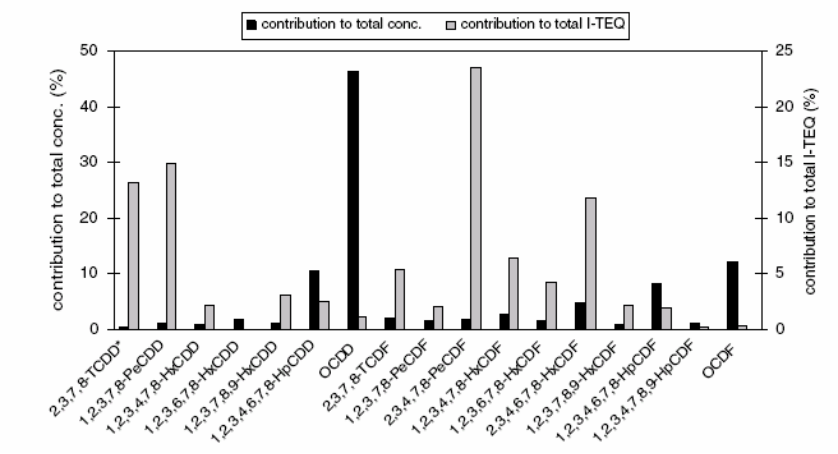
- There is a high similarity of BienHoa Airforce soil samples and BienHung sediment samples (based on both PCDD/Fs relative profiles and relative i-TEQ profiles, especially for the group affected by 2,3,7,8-TCDD concentration and partially affected by 1,2,3,7,8-PeCDD and 1,2,3,6,7,8-HxCDD concentration). Hence logically we can say that the PCDD/Fs contamination in BienHoa Airforce soil could be identified as a principal PCDD/Fs source for sediment of BienHung Lake
- There is a similar PCDD/Fs relative i-TEQ profile pattern between fish tissue and sediment of BienHung Lake (10/13 fish tissue samples). However for PCDD/Fs relative profiles, there is relative high dissimilarity between fish tissue and soil/sediment samples. This difference could be reasoned by the effects of many factors such metabolism and elimination processes in fish body, etc. Since we may say that the sediment of BienHung Lake (and farther BienHoa Airforce Base soil) is responsible for the toxic 2,3,7,8- TCDD presented in fish tissue here, especially for the fish types such catfish and snake-head (predator fish).

We take an example from study by Bakoglu et al (2005) to compare with our data (fig. 8.11): soil samples of BienHoa Airforce Base represent for site contaminated by A.O/Dioxin and soil samples of ThuDuc represent for site contaminated by industries. Data presented in this figure is the average PCDD/Fs relative and i-TEQ profiles calculated in %.

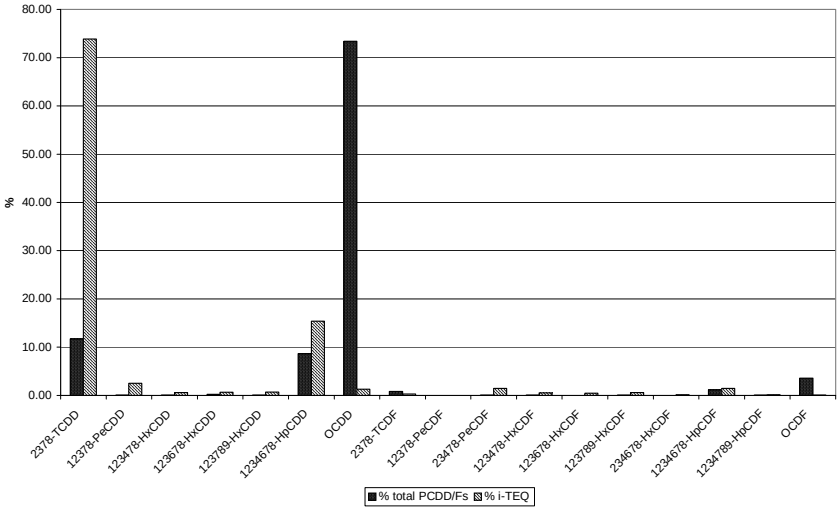
- Based on PCDD/Fs relative profiles we can see the similarity of three types of soil: all of them is mostly dominated by OCDD, then partially dominated by 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8-HpCDF and OCDF;

- Based on i-TEQ, there is a big difference between three soil types: soil of Kocaeli is mostly contaminated by 2,3,4,7,8-PeCDF, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,4,6,7,8-HxCDF; soil of BienHoa Airforce Base is mostly contaminated by 2,3,7,8-TCDD, then partially contaminated by 1,2,3,4,6,7,8-HpCDD; and soil of ThuDuc is contaminated mostly 1,2,3,4,6,7,8-HpCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,7,8,9-HxCDF. In general we can say that soil of ThuDuc is more similar to soil of Kocaeli than soil of BienHoa Airforce Base.

a)



b)



c)

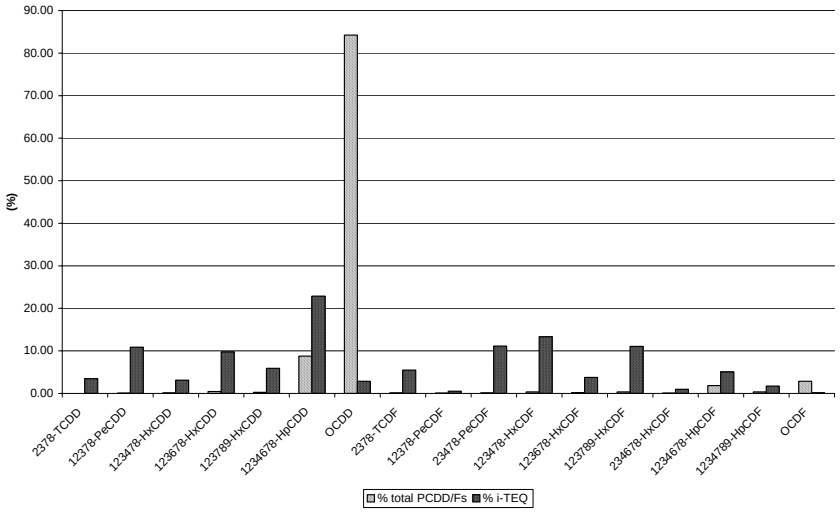


Fig. 8.11 PCDD/Fs relative and i-TEQ profiles (%) in soil : a) soil of Kocaeli-Turkey; b) soil of site contaminated by A.O/Dioxin (BienHoa); c) soil of industrial zone (ThuDuc)

8.4.4 Comparison of soil and incinerator bottom ash samples

Incinerator bottom ash is not considered as a direct source of PCDD/Fs contamination, but their PCDD/Fs profiles pattern could be used as a representative for the type of PCDD/Fs contamination originated from industries. By using PCA we compared 5 bottom ash samples with our soil samples and result showed in the fig. 8.11 and 8.12.

Fig. 8.12 showed us all bottom ash samples are separated very far from the soil samples. A grand part of soil samples are rested in the right and some of them are near the center by interaction of OCDD (mostly) and TCDD (partially). Bottom ash samples are divided themselves into two groups: group1 includes RRT1, RRT2, BHH and PH1 is far in the left by interaction of HxCDF, PeCDF, PeCDD, and TCDF; group2 has only one sample – TCN is in upper-left, interacted by OCDF and HpCDF. In general we can say that the bottom ash is contaminated by more PCDFs than PCDDs.

By using PCDD/Fs relative i-TEQ profiles (Fig. 8.13) again we can see the separation of bottom ash samples from soil samples, especially for the soil from contaminated sites by A.O/Dioxin. Three of them (RYT1, BHH, PH1) are separated into the groups containing the soil samples from industrial zone (TD1, TD2, TD4, TD5), Only sample RYT1 is rested in the group with the contaminated soil samples by 2,3,7,8-TCDD (right group). Sample TCN is still separated from others by interaction of high chlorinated PCDFs such 1,2,3,7,8,9-HxCDF, 1,2,3,4,7,8-HpCDF, 2,3,,4,6,7,8-HpCDF, etc.

Combination of fig. 8.12 and 8.13 we can draw some important conclusions:

- The PCDD/Fs contamination caused by industrial sources such incinerator combustion is different from PCDD/Fs contamination caused by A.O/Dioxin for soil samples. These sources contain more PCDFs than PCDDs;

- From the toxicity point of view based on PCDD/Fs relative i-TEQ profiles, industrial sources such incinerator combustion are responsible for the PCDD/Fs contamination in the soil of industrial zones (presented by the similarity of bottom ash samples and industrial soil samples as shown in fig. 8.13).

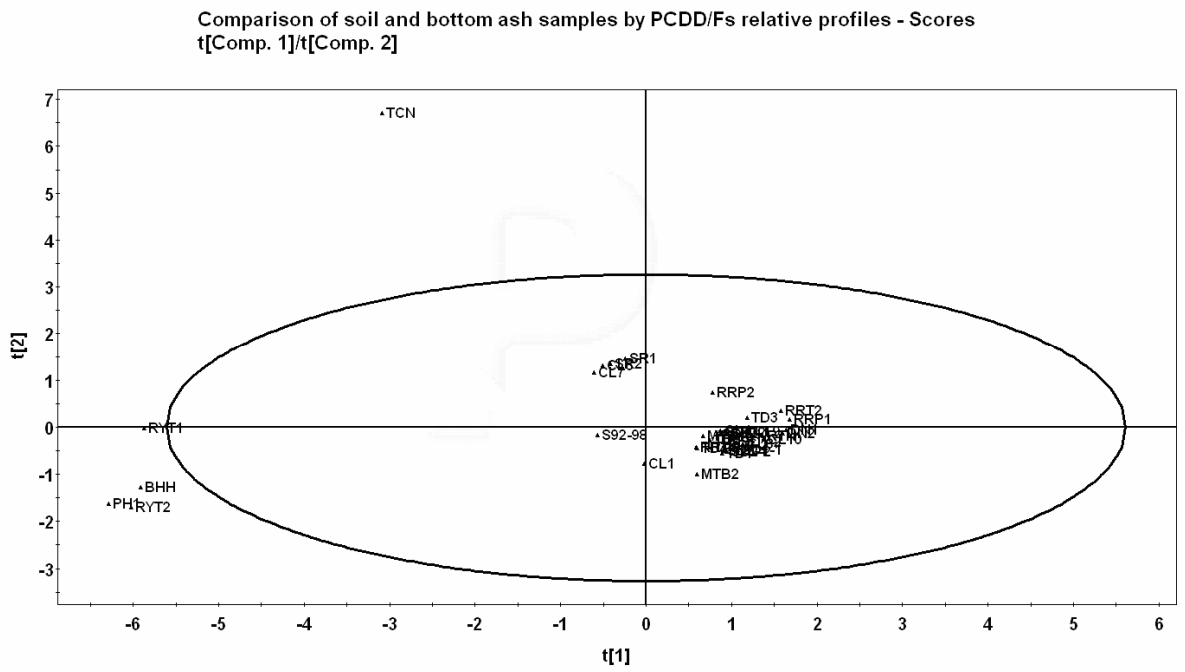


Fig. 8.11 PCA analysis based on PCDD/Fs relative profiles; 35 soil and 5 bottom ash samples analyzed

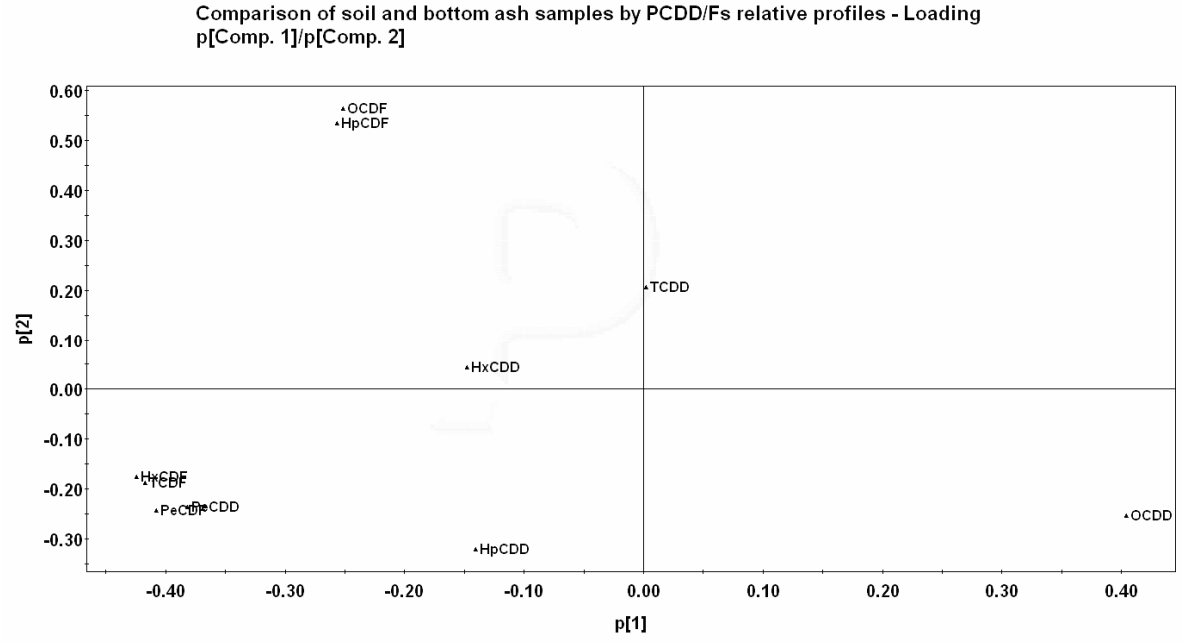


Fig. 8.12 PCA analysis based on PCDD/Fs relative profiles; 35 soil and 5 bottom ash samples analyzed

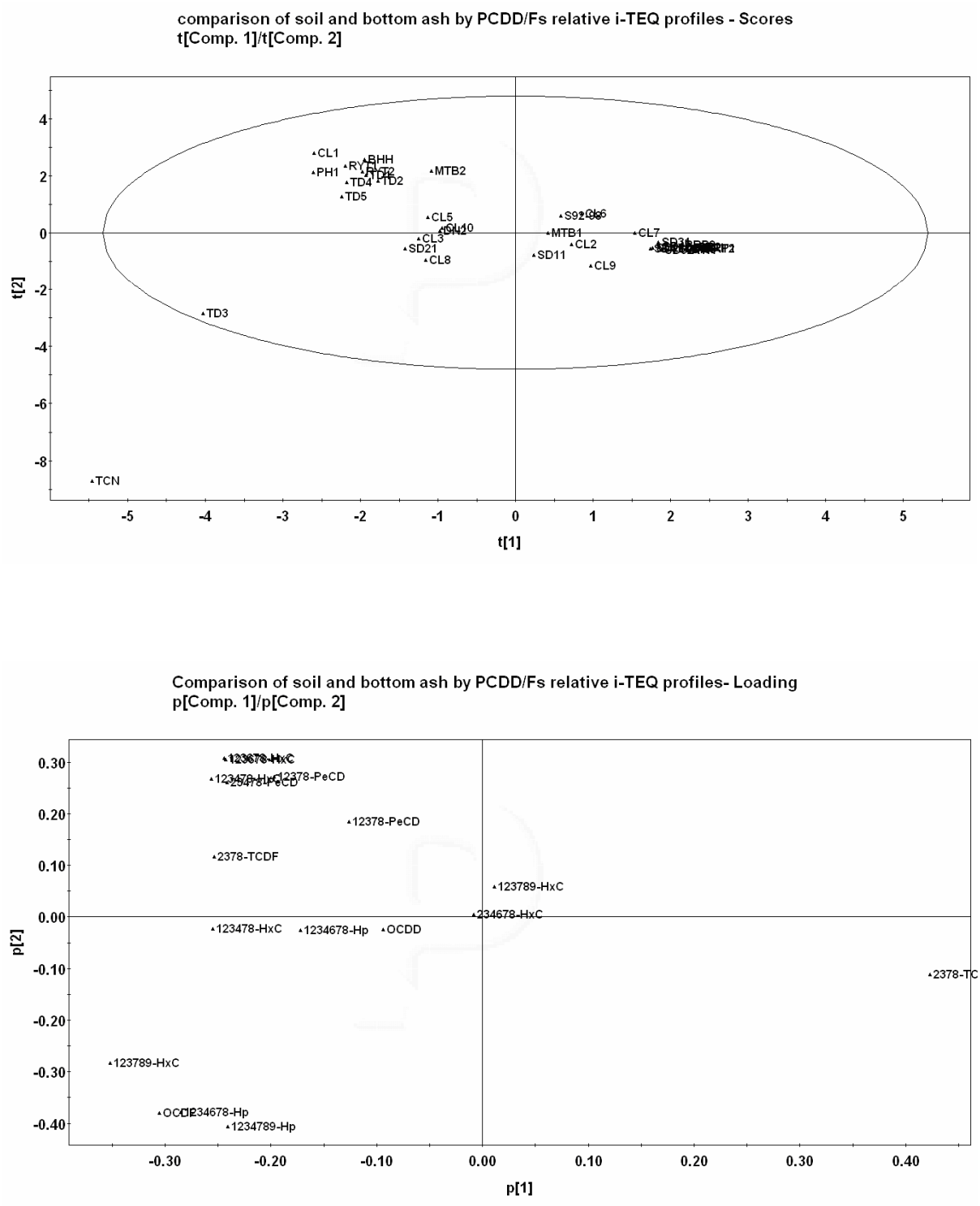


Fig. 8.13 PCA analysis based on PCDD/Fs relative i-TEQ profiles; 35 soil and 5 bottom ash samples analyzed

8.4.5 Comparison of human adipose samples

Using PCA for two groups of BienHoa residents we obtained the results showed in fig. 8.13 and 8.14. In general we can see that the samples are not clearly grouped as for soil or sediment samples.

For younger group (group1 - fig 8.14), the biggest group of samples (lower-left) is interacted mostly by OCDD, HxCDF and partially by OCDF; second group (lower-right) contains the samples interacted mostly by TCDF, HpCDF and partially by TCDD and PeCDF; third group (upper-right) contains the samples interacted by HpCDD and HpCDF; last group (upper-left) contains the samples interacted by HxCDD and PeCDD.

For older group (group2 - fig 8.15), there are two big groups: one in the lower-left contains the samples interacted mostly by OCDD, OCDF and partially by TCDF; another in upper-right contains the samples interacted by HpCDF, HxCDF and PeCDD. Smaller group of samples (upper-left) contains the samples interacted mostly by HpCDD and partially by HxCDD. The rest samples (lower- right) are very dispersive, interacted by TCDD and PeCDF.

By combination of two fig. 8.14 and 8.15 we can say that OCDD, OCDF and TCDF are important factors to separate the human adipose samples each other based on PCDD/Fs relative profiles.

Using PCA for PCDD/Fs relative i-TEQ profiles we obtained the result shown in fig. 8.16 and 8.17. The PCA was done with regard to 2,3,7,8-TCDD – the most toxic compound and also the compound is recognized originated from A.O/Dioxin.

Group1 showed more concentration of samples by interaction of 2,3,7,8-TCDD, 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF while group2 was dispersive with less interaction of 2,3,7,8-TCDD and more interaction of hexa- and penta- 2,3,7,8-substituted PCDD/Fs as showed in fig. 8.17.

By combination of fig. 8.16 and 8.17 we can say that 2,3,7,8-TCDD taking an important part of i-TEQ for younger people than for older people.

However this conclusion should be confirmed on larger scale study with more data and on a selected group of people.

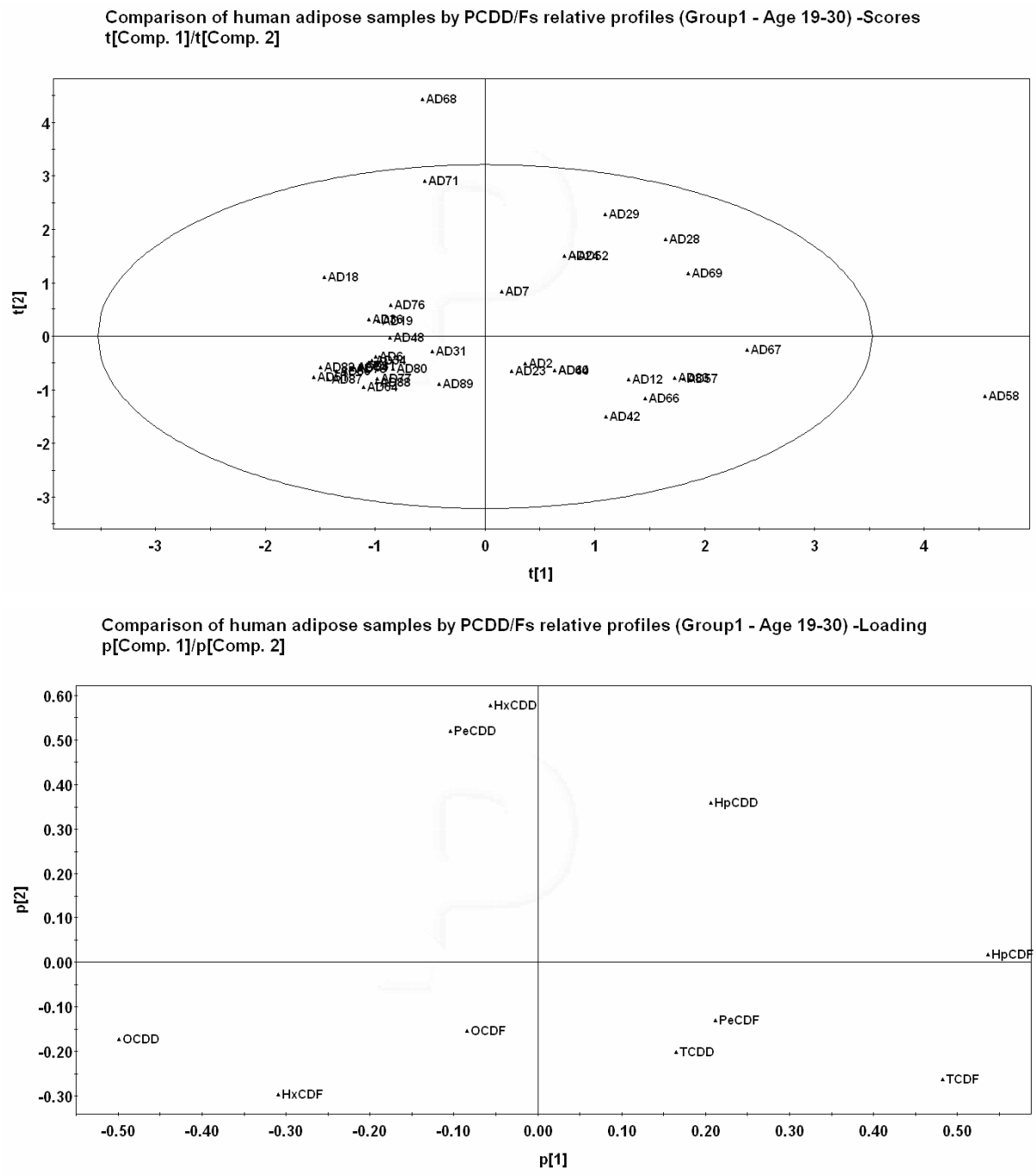


Fig. 8.14 PCA analysis based on PCDD/Fs relative profiles (group1 – age 19-30)

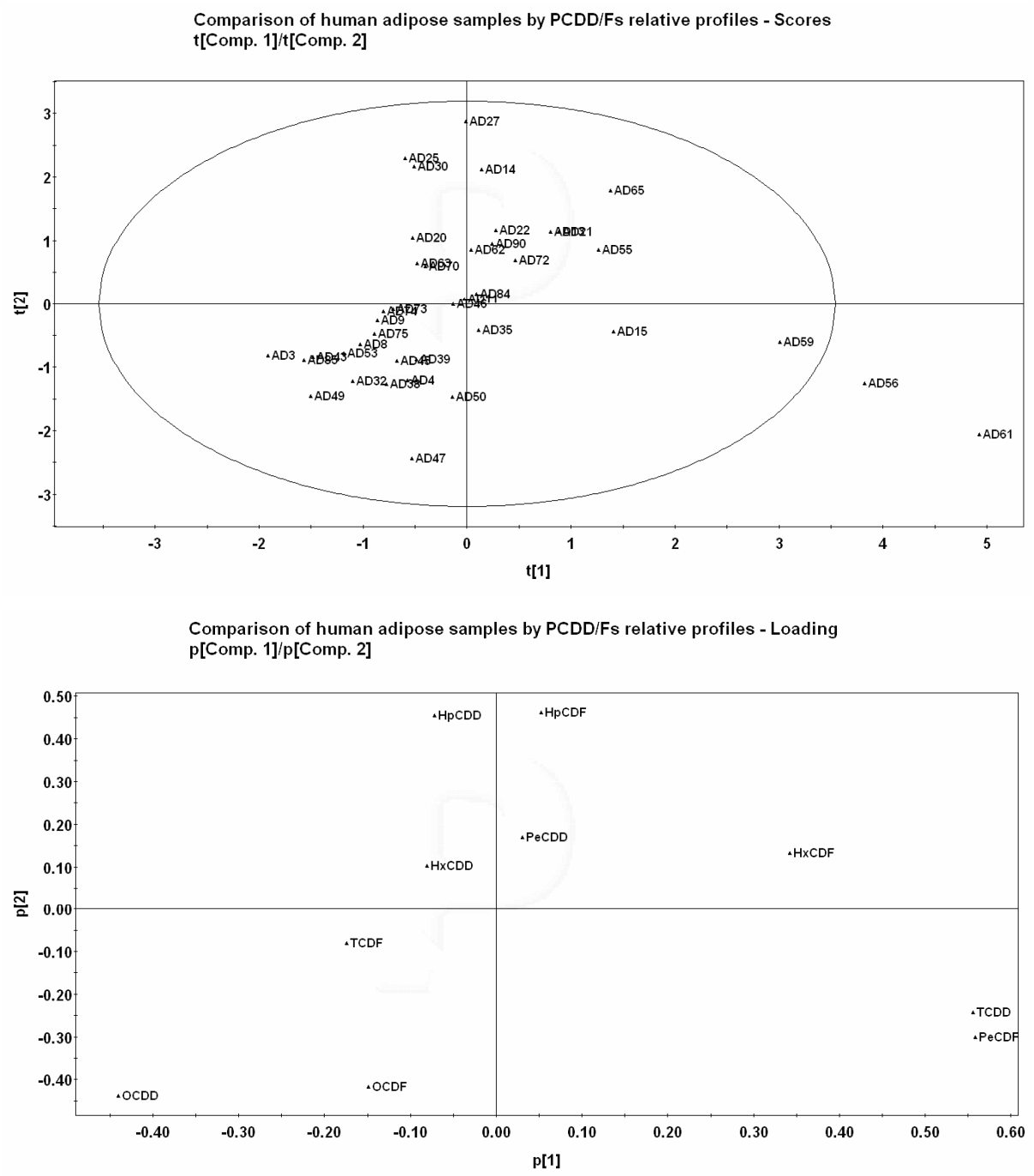


Fig. 8.15 PCA analysis based on PCDD/Fs relative profiles (group2 – age 31-59)

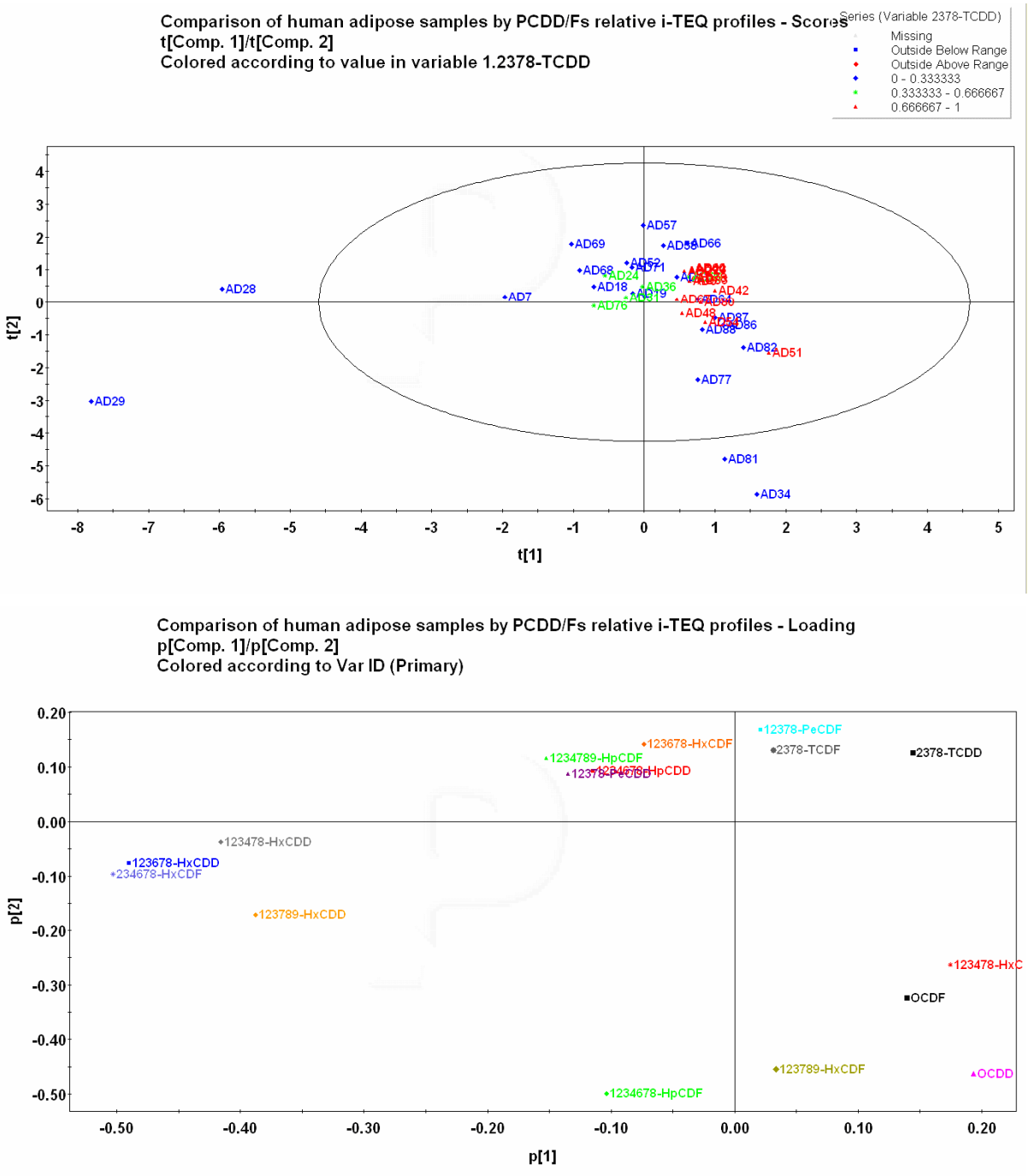


Fig. 8.16 PCA analysis based on PCDD/Fs relative i-TEQ profiles (group1 – age 19-30)

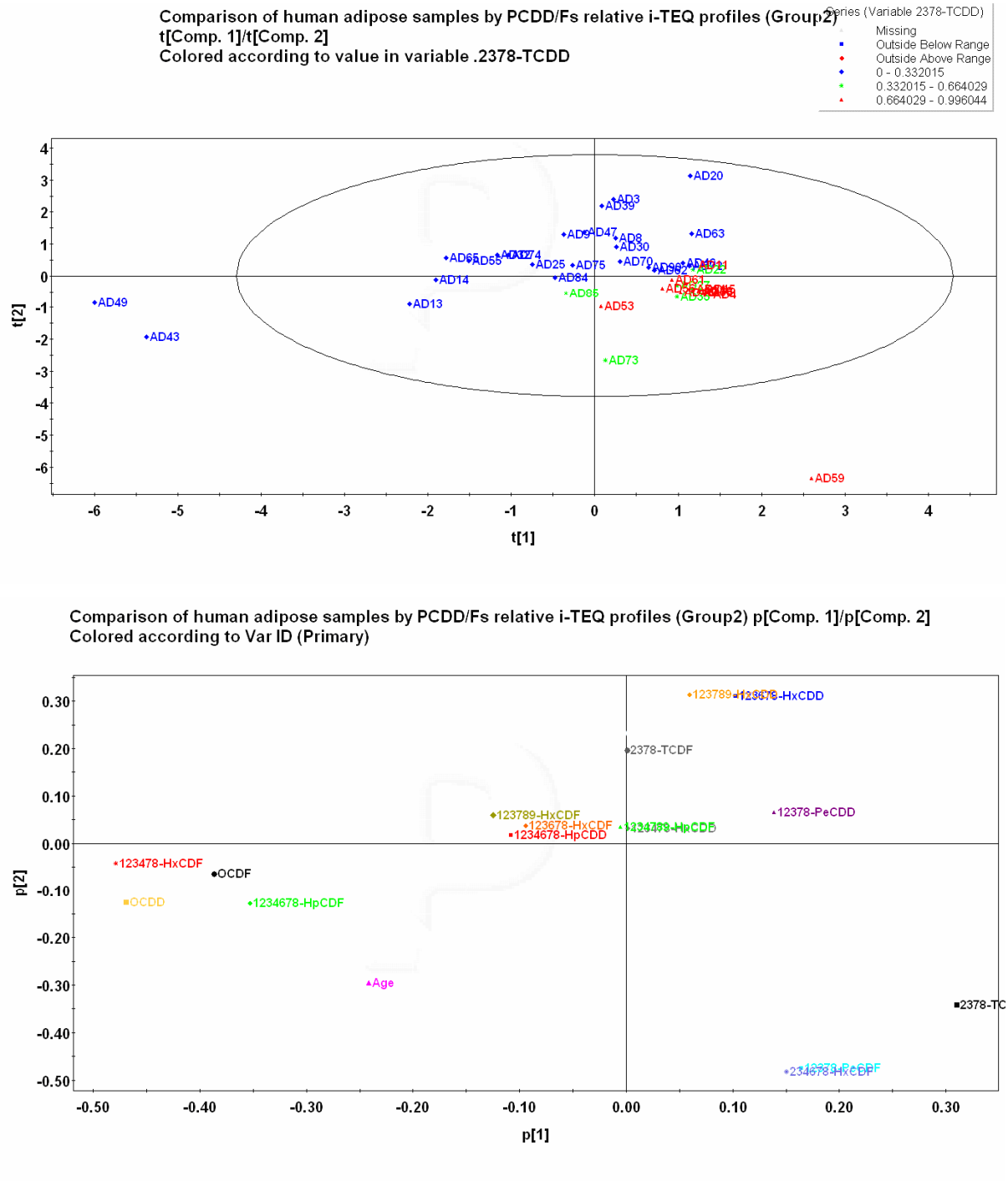


Fig. 8.17 PCA analysis based on PCDD/Fs relative i-TEQ profiles (group2 – age 31-59)

Reference

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9 CONCLUSIONS AND SUGGESTIONS

9.1 Conclusions

Our research has examined integratelly the PCDD/Fs sources with special regard on the PCDD/Fs source from the war, but also consider the others possible sources such industrial and municipal combustions, agricultural used chemicals, etc... The obtained result positively contributes to National Dioxin Research Plan to better understand the consequences of the chemicals used by U.S Army during Vietnam War (Ranch hand Operation from 1961 – 1971). Considering the initial aims stated in the beginning, we can make the following comments:

- The principal responsible source for PCDD/Fs contamination in the soil of Southern VietNam (for selected locations of our research) is the residue from chemicals (herbicides and defoliants) used by U.S. Army during Ranch Hand Operation (from 1961 to 1971). Even after more than 30 years, the PCDD/Fs concentration based on i-TEQ (especially for 2,3,7,8-TCDD) value is still higher than values done by Canadian guideline and U.S EPA standard as showed in chapter V.

- Our result shown very high i-TEQ value in the cultivate soil of CamLo district and DaNang City. For the area named “hot spot” areas such BienHoa Airforce Base and BienHung Lake, the detected i-TEQ value and 2,3,7,8-TCDD concentration in soil and sediment are more superior than values proposed by CCME, ATSDR and U.S EPA. The dioxin contamination risk for local resident health requires the responsible organizations to consider urgently a complete solution to solve the problem for this area.

- The PCDD/Fs concentration in fish tissue of BienHung Lake (catfish and snake-head) is superior in comparison with EC standard. Since these kinds of fish are still used as food for local residents, it causes a big risk for their health as presented in chapter VI.

- By using statistic methods (cluster analysis and PCA) for obtained data, we have showed a high similarity of PCDD/Fs profiles pattern between BienHoa Airforce Base soils and BienHung Lake sediments. Therefore BienHoa Airforce soil is could be identified as PCDD/Fs principal contamination source for this lake. Based on relative i-TEQ profiles, we also obtained the similarity between a grant part of BienHung sediment samples and fish tissue samples by interaction of 2,3,7,8-TCDD. Logicaly we can say that there is a transfer of this toxic compound from soil (BienHoa Airforce Base) to sediment (BienHung Lake) and to fish.

- The result of cluster analysis and PCA showed the different PCDD/Fs pattern for different types of soil: soil of sites contaminated by A.O/Dioxin is dominated mainly by 2,3,7,8-TCDD and partially by higher chlorinated 2,3,7,8-PCDD/Fs such OCDD, and HpCDD/Fs while soil of industrial sites are dominated strongly by PCDFs. The similarity in PCDD/Fs profiles of incinerator bottom ash (MWIs) and industrial soil samples showed that the incinerator combustion process is responsible for PCDD/Fs contamination for these zones. However PCDFs compounds are not very high toxic in comparison with 2,3,7,8-TCDD, but with the rapid industrialization at present time, these PCDD/Fs contamination sources should be controlled and minimized to avoid the possible risks in the future.

- The PCDD/Fs concentration based on i-TEQ value in human adipose of BienHoa residents is comparable with the industrial countries. However from toxicity point of view there is still the residue of 2,3,7,8-TCDD in human body (49/90 cases delaminated), especially at very

high concentration in some cases. The result of PCA showed that this compound affects more in PCDD/Fs profiles of younger people than older. Since 2,3,7,8-TCDD is the most toxic compound and related to the A.O/Dioxins from the war, so a large-scale investigation on selected group of people (people affected/having diseases related to A.O/Dioxins) should be done to confirm this conclusion.

- In addition, the research has set up the suitable analytical methods for PCDD/Fs residue in many matrices such soil, fly ash, fish tissue, etc with minimum and basic analytical equipment (HRGC/LRMS), still reaching the required conditions. A laboratory for PCDD/Fs analysis has been installed in IER during research time and serves as a member of VietNam Dioxin Research Network. Furthermore, in frame of the research we have organized a workshop with the name "Studies on Dioxins contamination and related problems" that held in IER –Hochiminh City (06/August/2004) with about 40 participants from different institutions and related governmental organizations of Hochiminh City and surrounding provinces. A part of research had been presented in Dioxin2004 (Berlin, September/2004).

9.2 Suggestions

From our obtained result, we would like to suggest:

- For the "hot spot" identified zone such BienHoa Airforce Base, the local government should take on their responsibility and find urgently and responsibly a complete solution for this zone and similar areas, e.g. to dredge and treat the contaminated sediment and soil with suitable methods; to inform the local residents about the toxic level of dioxin and its consequences; to isolate and emigrate the residents of the territories with high risk; etc.

- To continue a large-scale study on dioxins transfer in food chain with more matrix types, including also the people belonging to this food chain to get more precise assessment. Parallely, to apply the statistic model such cluster analysis or PCA, it should have more data of different organizations such Department of Health, Department of Natural Resources and Environment, etc.

- Vietnam should set up as soon as possible a guideline for dioxins and dioxin-liked compounds for soil, foodstuff, etc.

- To set up a reference laboratory for PCDD/Fs research and related problem in the South of VietNam, we should consider investing in reference analytical equipments such HRMS or MS/MS to reach the standard level and comparable result withy other laboratory in the world.

ANNEX 1
Absorbent preparation

1. 1 Sodium sulphate, anhydrous granular, (Na_2SO_4): Sodium sulfate (Na_2SO_4) granular, anhydrous. Merck 1.06639.0500

1.2 Florisil: 100 - 200 mesh. Fluka
- Heat to 180ºC for 3h in an open dish -->
- Cool to room temperaturein desiccator
- Add 1.2% (w/w) de-ionized H_2O (bidistilled)
- Shake for 30' ---> use within the same day.

1.3 Alumina Basic Super I: 50-200μm. SAI (maintained in desiccator)

1.4 Silicagel 60: 70 - 230 mesh (0.063 -0.200mm). Merck.
- Activate for 8h (one night)
- Maintain in desiccator

1.5 Silicagel acid
- Heat 102g Silicagel 60 to 80^{o}C for 30'
- Cool to room temperaturein desiccator
- Rinse with 230mL Methanol, then 170mL Dichlomethane
- Heat to 50^{o}C for 30', then rise gradually to 180^{o}C for 90'
- Cool to room temperaturein desiccator
- Add $H_2SO_{4\ conc.}$ to have the concentration 44% (w/w): 80g H_2SO_4 for 100g Silicagel
- Shake for 1h
- Dry by liquid N_2--> maintain in desiccator

1.6 Silicagel basic
- Dissolve 30g $CsOH \times H_2O$ in 100mL Methanol in round bottom flask 500mL
- Add 100mL Methanol and 50g Silicalgel 60
- Evaporate the solvent by rotavapor (without vacuum) at 55^{o}C (temperature of water bath) for 90'
- Rinse with 100mL Methanol, then 100mL Dichlomethane
- Dry by liquid N_2

ANNEX 2
Solvents, chemicals and standards solutions

2.1 Solvents and chemicals

1. Dichlomethane: Romil - SpS (Super Purity Solvent) H202
2. n-Hexane 95%: Romil -SpS H389
3. Methanol 205: Romil -SpS H410
4. Toluene: Romil -SpS H771
5. Diethyl Ether: Romil-SpS H220
6. Acetone: Romil-SpS H031
7. Casium monohydroxide: Fluka -PA
8. Cupper powder: Merck - PA
9. Filter paper: Schleicher&Schuell 595 1/2, Φ240

2.2 Standard solutions

A. Stock standard

1. Native Stock Response Factor Solution for EN-1948-2

Code: EDF-4175 - Cambridge Isotope Laboratories (CIL)

0.5 mL in nonane

		Conc.			Conc.
No	**Congener**	ug/mL	**No**	**Congener**	ug/mL
1	2378-TCDD	1.0 ± 0.082	8	2378-TCDF	1.0 ± 0.082
2	12378-PeCDD	1.0 ± 0.082	9	12378-PeCDF	1.0 ± 0.082
3	123478-HxCDD	1.0 ± 0.082	10	23478-PeCDF	1.0 ± 0.082
4	123678-HxCDD	1.0 ± 0.082	11	123478-HxCDF	1.0 ± 0.082
5	123789-HxCDD	4.0 ± 0.328	12	123678-HxCDF	1.0 ± 0.082
6	1234678-HpCDD	2.0 ± 0.164	13	123789-HxCDF	1.0 ± 0.082
7	OCDD	2.0 ± 0.164	14	234678-HxCDF	1.0 ± 0.082
			15	1234678-HpCDF	2.0 ± 0.164
			16	1234789-HpCDF	2.0 ± 0.164
			17	OCDF	2.0 ± 0.164

2. Method 23 Internal Standard Stock Solution

Code: EDF 4053 Cambridge Isotope Laboratories (CIL)

1.2mL in nonane

No	***Congener***	**Conc. (ng/mL)**
1	^{13}C-2378-TCDD	1000 ± 82
2	^{13}C-12378-PeCDD	1000 ± 82
3	^{13}C-123678-HxCDD	1000 ± 82
4	^{13}C-1234678-HpCDD	1000 ± 82
5	^{13}C-OCDD	2000 ± 164
6	^{13}C-2378-TCDF	1000 ± 82
7	^{13}C-12378-PeCDF	1000 ± 82
8	^{13}C-123678-HxCDF	1000 ± 82
9	^{13}C-1234678-HpCDF	1000 ± 82

3. Method 8280 Recovery Standard

Code: ED 2521 Cambridge Isotope Laboratories (CIL)
1.2mL in nonane

No	*Congener*	Conc. (ug/mL)
1	^{13}C-1234-TCDD	5.000 ± 0.41
2	^{13}C-123789-HxCDD	5.000 ± 0.41

4. Method 8280 Cleanup Standard

Code: ED 2522 Cambridge Isotope Laboratories (CIL)
1.2mL in nonane

No	*Congener*	Conc. (ug/mL)
1	^{37}Cl-2378-TCDD	4.902 ± 0.08

5. TetraCDF - HeptaCDF Window Defining Mixture

Code: DF-1731-B Cambridge Isotope Laboratories (CIL)

No	*Congener*	Quantity (ng)
1	1368-TCDF	400
2	1289-TCDF	400
3	13468-PeCDF	400
4	12389-PeCDF	400
5	123468-HxCDF	400
6	123489-HxCDF	400
7	1234678-HpCDF	400
8	1234789-HpCDF	400

6. TetraCDF - HeptaCDD Window Defining Mixture

Code: DF-1732-B Cambridge Isotope Laboratories (CIL)

No	*Congener*	Quantity (ng)
1	1368-TCDd	400
2	1289-TCDD	400
3	12468/12479-PeCDD	400
4	12389-PeCDD	400
5	1244679/124689-HxCDD	400
6	123467-HxCDD	400
7	1234678-HpCDD	400
8	1234679-HpCDD	400

8. Method 8280 Calibration Solution

Code: EDF 2519-A Cambridge Isotope Laboratories (CIL)

5×0.2mL in nonane

No	Congener	Concentration (ng/uL)				
		CC1	CC2	CC3	CC4	CC5
	Native					
1	2378-TCDF	0.10 ± 0.01	0.25 ± 0.02	0.50 ± 0.04	1.00 ± 0.08	2.00 ± 0.16
2	2378-TCDD	0.10 ± 0.01	0.25 ± 0.02	0.50 ± 0.04	1.00 ± 0.08	2.00 ± 0.16
3	12378-PeCDF	0.10 ± 0.01	0.25 ± 0.02	0.50 ± 0.04	1.00 ± 0.08	2.00 ± 0.16
4	23478-PeCDF	NA	NA	0.50 ± 0.04	NA	NA
5	12378-PeCDD	0.10 ± 0.01	0.25 ± 0.02	0.50 ± 0.04	1.00 ± 0.08	2.00 ± 0.16
6	123478-HxCDF	NA	NA	1.25 ± 0.10	NA	NA
7	123678-HxCDF	0.25 ± 0.02	0.63 ± 0.05	1.25 ± 0.10	2.50 ± 0.21	5.00 ± 0.41
8	123789-HxCDF	NA	NA	1.25 ± 0.10	NA	NA
9	123478-HxCDD	NA	NA	1.25 ± 0.10	NA	NA
10	123678-HxCDD	0.25 ± 0.02	0.63 ± 0.05	1.25 ± 0.10	2.50 ± 0.21	5.00 ± 0.41
11	123789-HxCDD	NA	NA	1.25 ± 0.10	NA	NA
12	234678-HxCDF	NA	NA	1.25 ± 0.10	NA	NA
13	1234678-HpCDF	0.25 ± 0.02	0.63 ± 0.05	1.25 ± 0.10	2.50 ± 0.21	5.00 ± 0.41
14	1234678-HpCDD	0.25 ± 0.02	0.63 ± 0.05	1.25 ± 0.10	2.50 ± 0.21	5.00 ± 0.41
15	1234789-HpCDF	NA	NA	1.25 ± 0.10	NA	NA
16	OCDD	0.50 ± 0.04	1.25 ± 0.10	500	2.50 ± 0.21	10.0 ± 0.82
17	OCDF	0.50 ± 0.04	1.25 ± 0.10	500	2.50 ± 0.21	10.0 ± 0.82
	Isotope					
1	^{13}C-1234-TCDD	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04
2	^{13}C-2378-TCDD	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04
3	^{37}Cl-2378-TCDD	NA	NA	0.25 ± 0.02	NA	NA
4	^{13}C-2378-TCDF	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04
5	^{13}C-123678-HxCDD	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04
6	^{13}C-123789-HxCDD	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04
7	^{13}C-1234678-HpCDF	1.00 ± 0.08	1.00 ± 0.08	1.00 ± 0.08	1.00 ± 0.08	1.00 ± 0.08
8	^{13}C-OCDD	1.00 ± 0.08	1.00 ± 0.08	1.00 ± 0.08	1.00 ± 0.08	1.00 ± 0.08

B. Working standard

1. Native Response Factor Solution

Code: EDF-4175 - dil. 8x

in iso-octane

		Conc.				Conc.
No	**Congener**	ug/mL	**No**	**Congener**	ug/mL	
1	2378-TCDD	150	8	2378-TCDF		150
2	12378-PeCDD	150	9	12378-PeCDF		150
3	123478-HxCDD	150	10	23478-PeCDF		150
4	123678-HxCDD	600	11	123478-HxCDF		150
5	123789-HxCDD	150	12	123678-HxCDF		150
6	1234678-HpCDD	300	13	123789-HxCDF		150
7	OCDD	300	14	234678-HxCDF		150
			15	1234678-HpCDF		300
			16	1234789-HpCDF		300
			17	OCDF		300

2. Method 23 Internal Standard Solution
Code: EDF 4053 - dil. 10x
in dodecane

No	*Congener*	Conc. (ng/mL)
1	^{13}C-2378-TCDD	100
2	^{13}C-12378-PeCDD	100
3	^{13}C-123678-HxCDD	100
4	^{13}C-1234678-HpCDD	100
5	^{13}C-OCDD	200
6	^{13}C-2378-TCDF	100
7	^{13}C-12378-PeCDF	100
8	^{13}C-123678-HxCDF	100
9	^{13}C-1234678-HpCDF	100

3. Method 8280 Recovery Standard
Code: ED 2521 - dil. 50x
in iso-octane

No	*Congener*	Conc. (ug/mL)
1	^{13}C-1234-TCDD	100
2	^{13}C-123789-HxCDD	100

4. Method 8280 Cleanup Standard
ED 2522 - dil. 50x
1.2mL in nonane

No	*Congener*	Conc. (ng/mL)
1	^{37}Cl-2378-TCDD	100

5. TetraCDF - HeptaCDF Window Defining Mixture
Code: DF-1731-B Cambridge Isotope Laboratories (CIL)

No	*Congener*	Conc. (ng/mL)
1	1368-TCDF	100
2	1289-TCDF	100
3	13468-PeCDF	100
4	12389-PeCDF	100
5	123468-HxCDF	100
6	123489-HxCDF	100
7	1234678-HpCDF	100
8	1234789-HpCDF	100

6. TetraCDF - HeptaCDD Window Defining Mixture
Code: DF-1732-B Cambridge Isotope Laboratories (CIL)

No	*Congener*	Conc. (ng)
1	1368-TCDd	100
2	1289-TCDD	100
3	12468/12479-PeCDD	100
4	12389-PeCDD	100
5	1244679/124689-HxCDD	100
6	123467-HxCDD	100
7	1234678-HpCDD	100
8	1234679-HpCDD	100

8. Method 8280 Calibration Solution

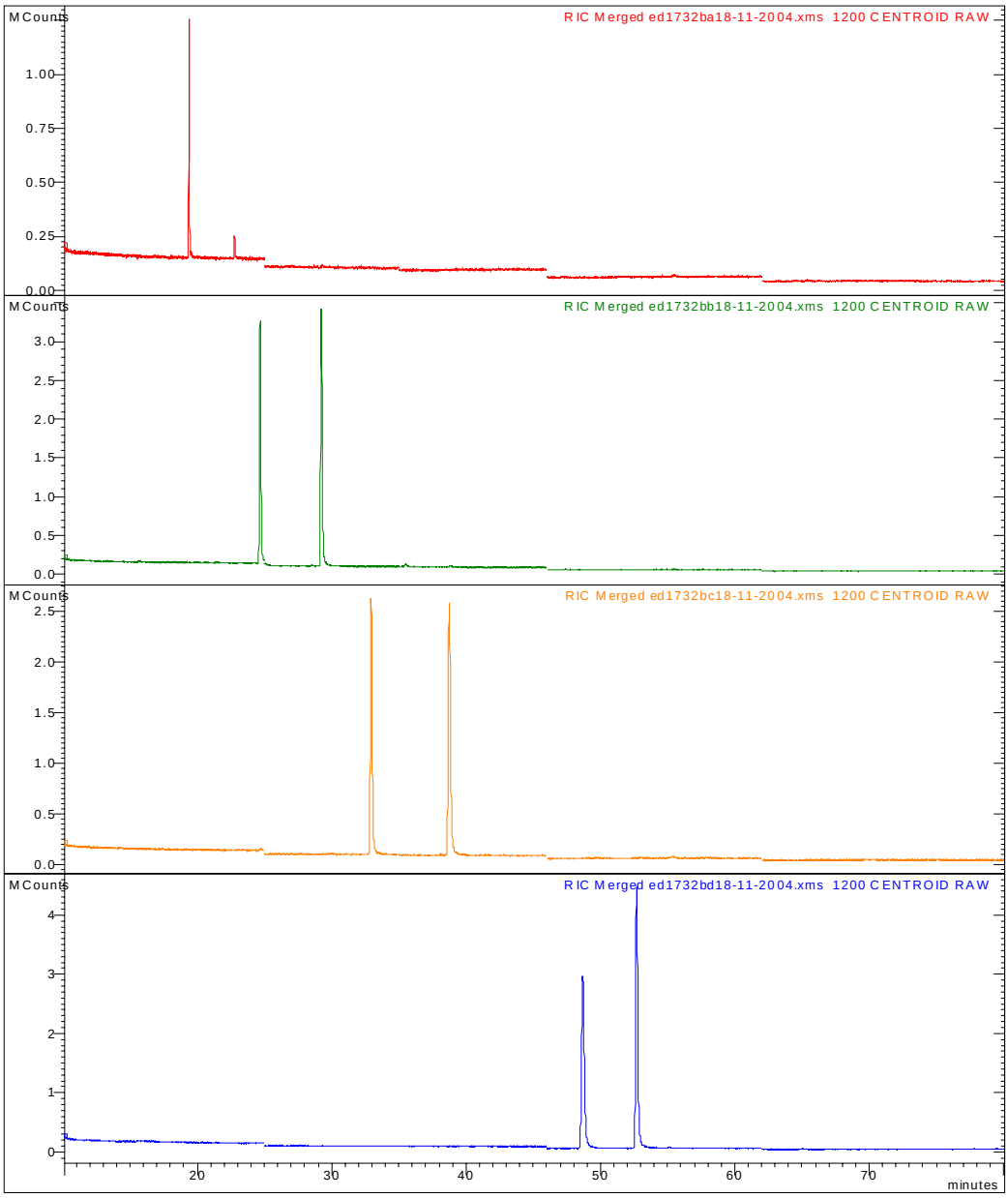
Code: EDF 2519-dil. 5x

5×0.2mL in nonane

No	Congener	Concentration (ng/uL)				
		CC1	CC2	CC3	CC4	CC5
	Native					
1	2378-TCDF	20	50	100	200	400
2	2378-TCDD	20	50	100	200	400
3	12378-PeCDF	20	50	100	200	400
4	23478-PeCDF			100		
5	12378-PeCDD	20	50	100	200	400
6	123478-HxCDF			250		
7	123678-HxCDF	50	125	250	500	1000
8	123789-HxCDF			250		
9	123478-HxCDD			250		
10	123678-HxCDD	50	125	250	500	1000
11	123789-HxCDD			250		
12	234678-HxCDF			250		
13	1234678-HpCDF	50	125	250	500	1000
14	1234678-HpCDD	50	125	250	500	1000
15	1234789-HpCDF			250		
16	OCDD	100	250	500	1000	2000
17	OCDF	100	250	500	1000	2000
	Isotope					
1	^{13}C-1234-TCDD	100	100	100	100	100
2	^{13}C-2378-TCDD	100	100	100	100	100
3	^{37}Cl-2378-TCDD			50		
4	^{13}C-2378-TCDF	100	100	100	100	100
5	^{13}C-123678-HxCDD	100	100	100	100	100
6	^{13}C-123789-HxCDD	100	100	100	100	100
7	^{13}C-1234678-HpCDF	200	200	200	200	200
8	^{13}C-OCDD	200	200	200	200	200

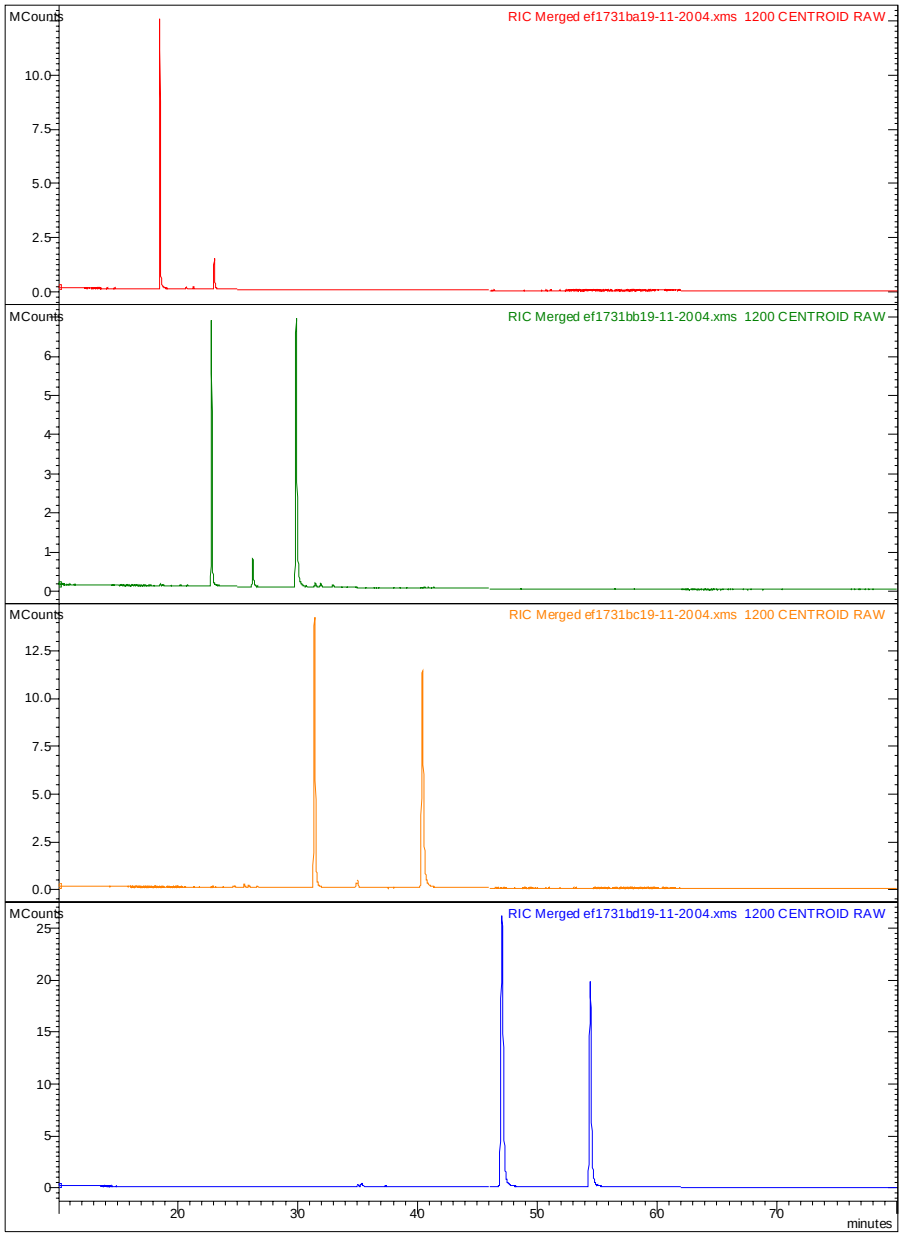
ANNEX 3
Chromatograms of window defining mixtures

Chromatogram Plots



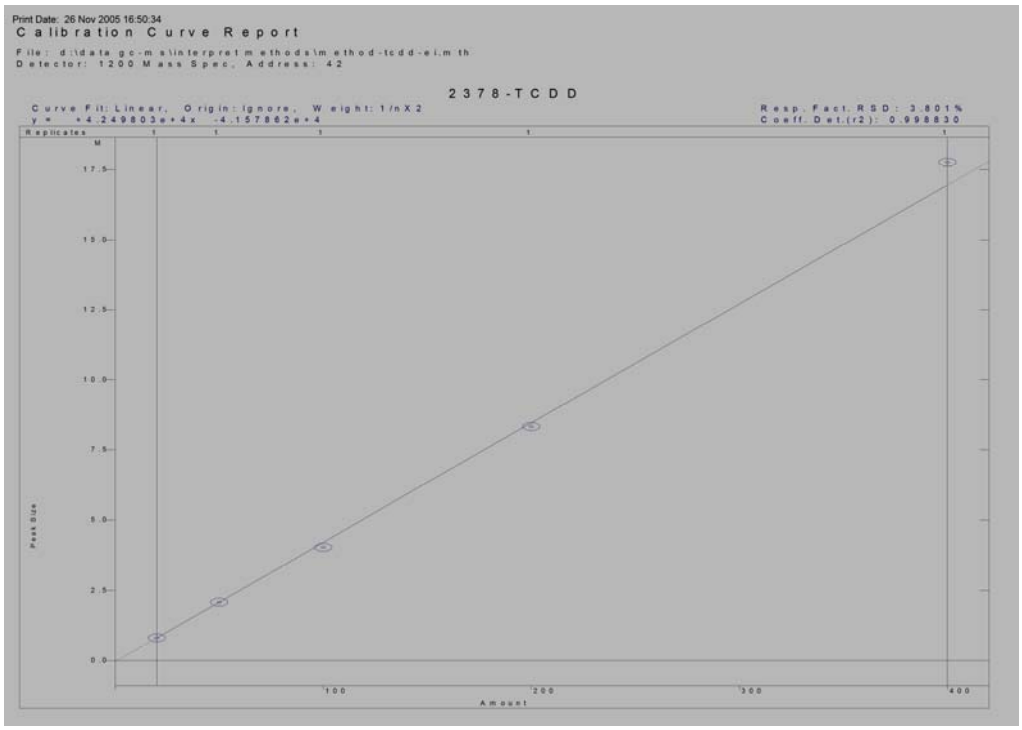
Chromatogram of PCDDs Window Defining Mixtures

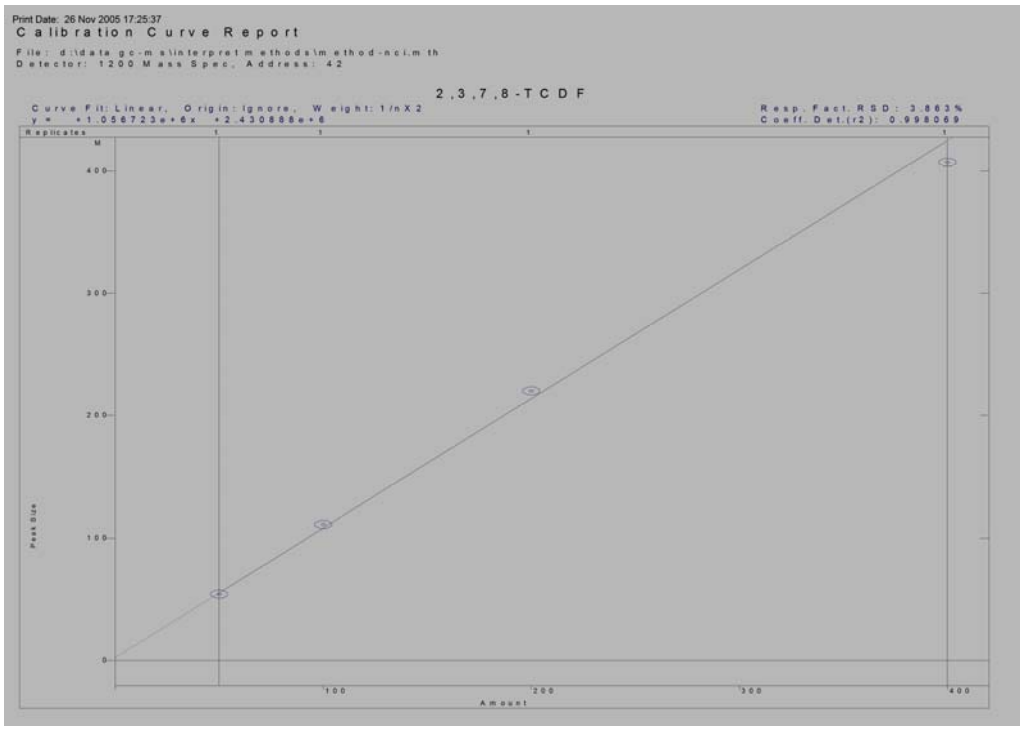
Chromatogram Plots

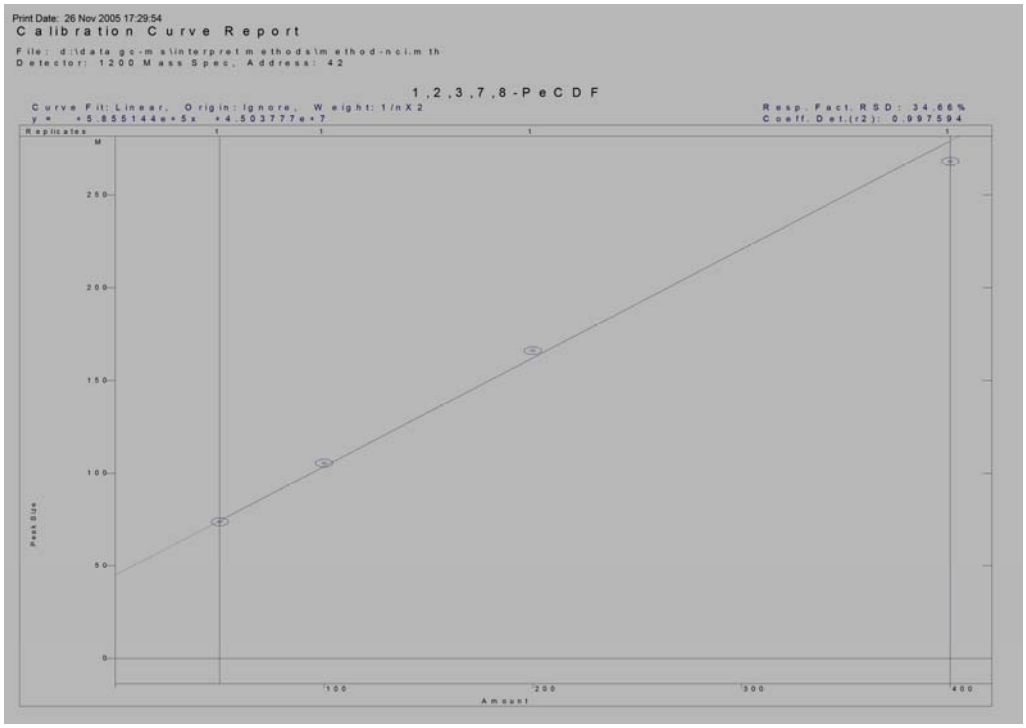


Chromatogram of PCDFs window defining mixtures

ANNEX 4
Calibration curves for linearity check



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Calibration Curve Report
File: d:\data gc-ms\interpret methods\method-nci.mth
Detector: 1200 Mass Spec, Address: 42
1,2,3,7,8-PeCDF
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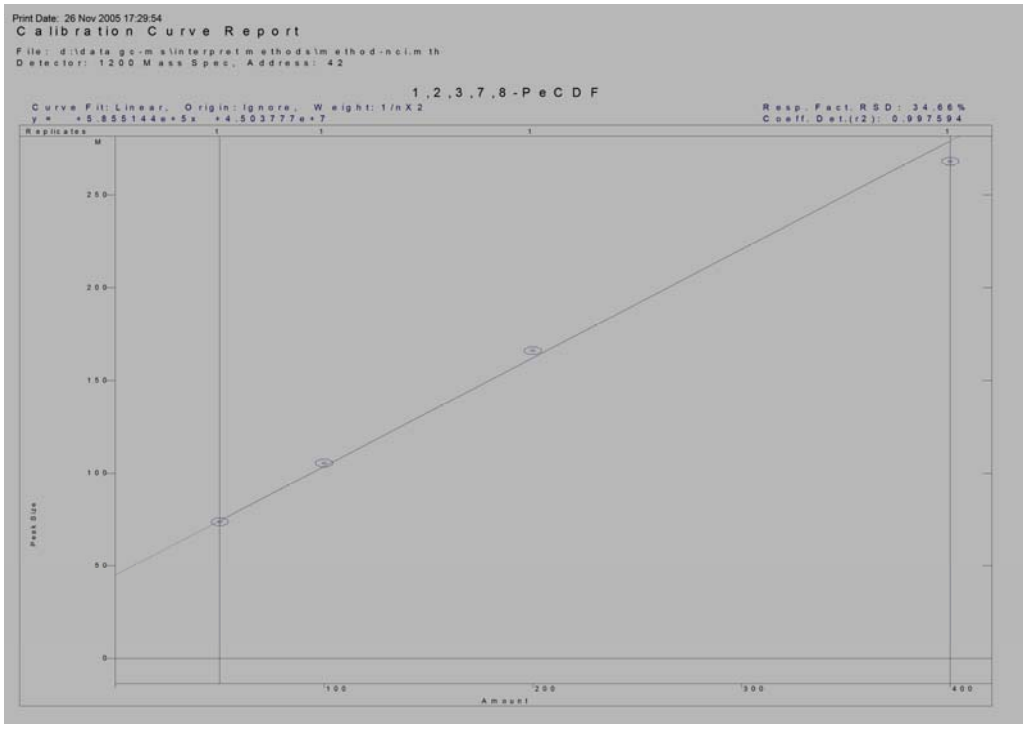
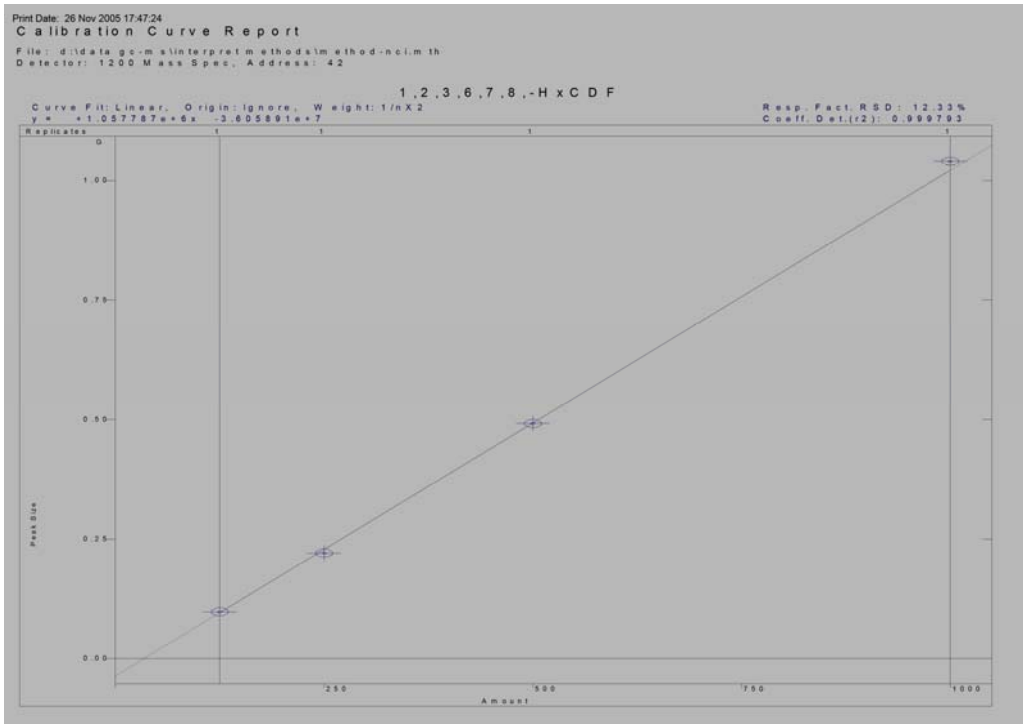
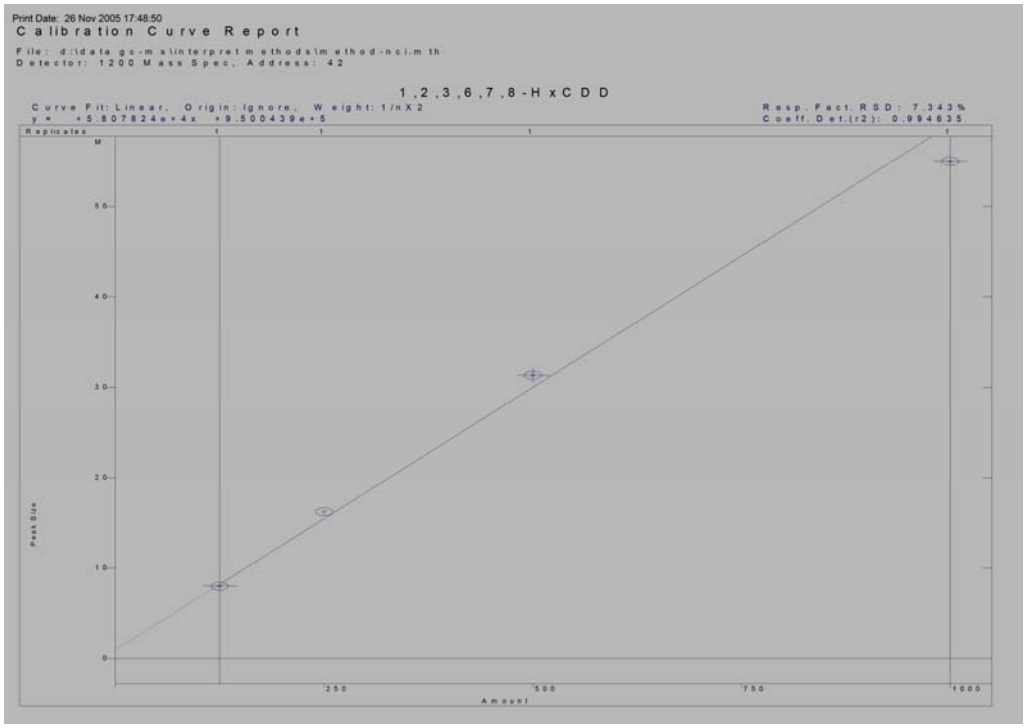
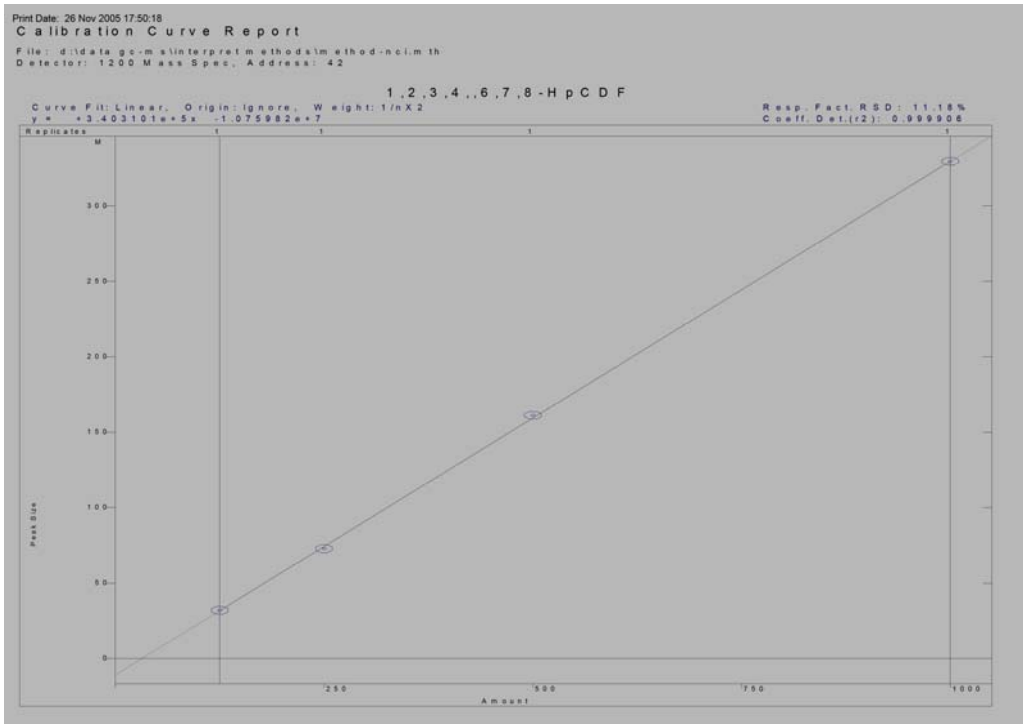
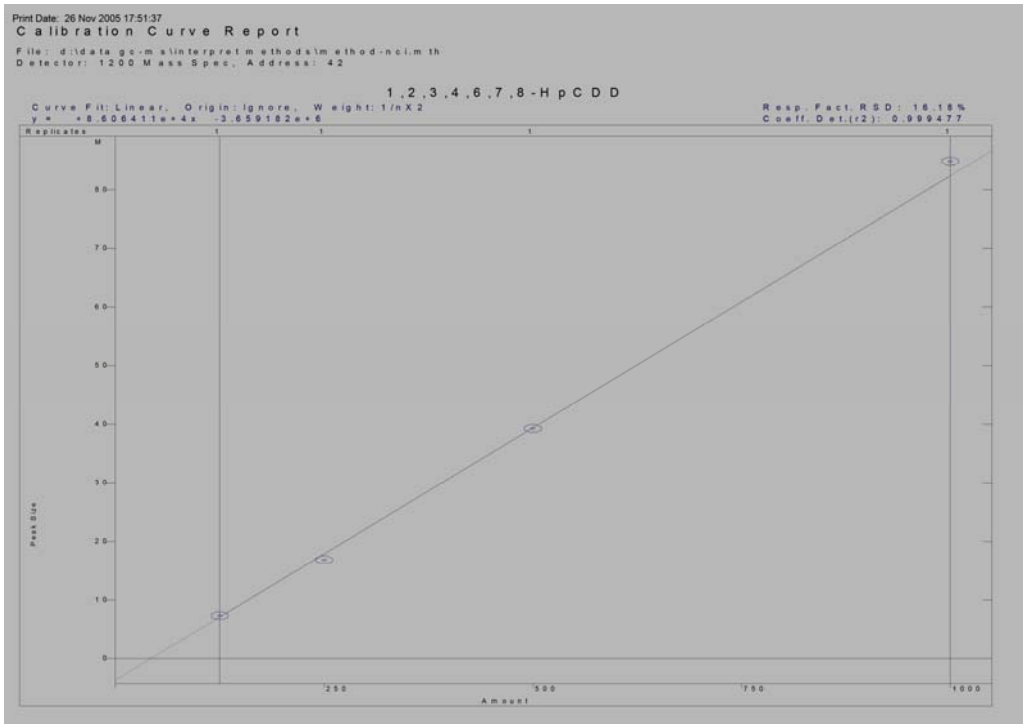
y = +5.855144e+5x +4.503777e+7
Resp. Fact. RSD: 34.66%
Coeff. Det.(r2): 0.997594
Replicates
Peak Size
Amount

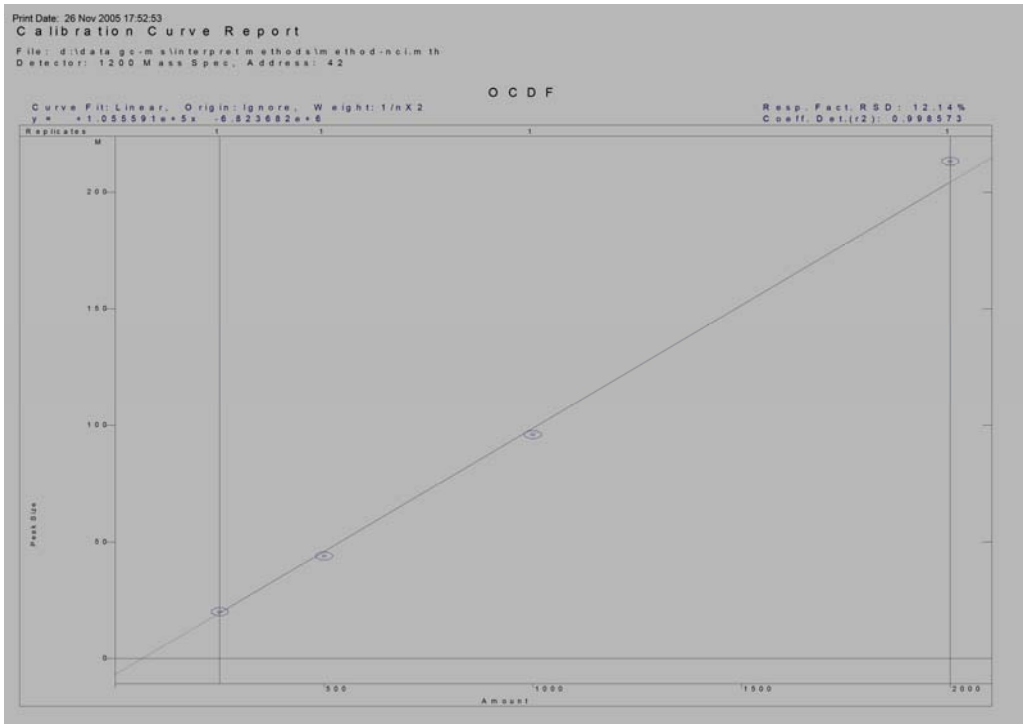

Print Date: 26 Nov 2005 17:29:54
Calibration Curve Report
File: d:\data gc-ms\interpret methods\method-nci.mth
Detector: 1200 Mass Spec, Address: 42
1,2,3,7,8-PeCDF
Curve Fit: Linear, Origin: Ignore, Weight: 1/nX2
y = +5.855144e+5x +4.503777e+7
Resp. Fact. RSD: 34.66%
Coeff. Det.(r2): 0.997594
Replicates
Peak Size
Amount

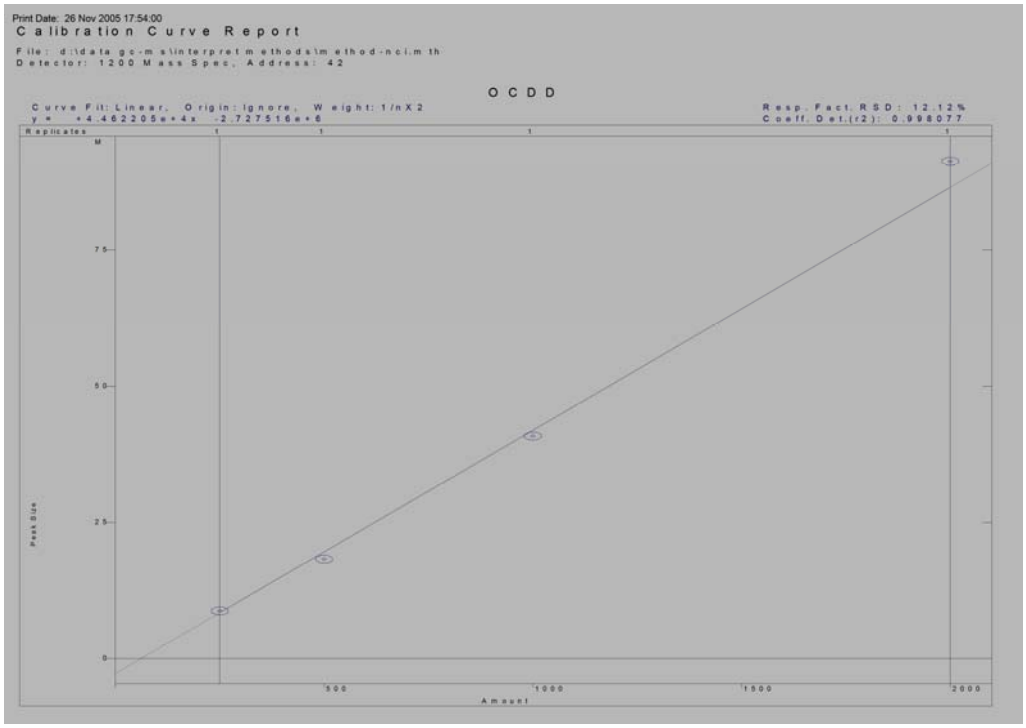

Print Date: 26 Nov 2005 17:47:24
Calibration Curve Report
File: d:\data gc-ms\interpret methods\method-nci.mth
Detector: 1200 Mass Spec, Address: 42
1,2,3,6,7,8,-HxCDF
Curve Fit: Linear, Origin: Ignore, Weight: 1/nX2
y = +1.057787e+6x -3.605891e+7
Resp. Fact. RSD: 12.33%
Coeff. Det.(r2): 0.999703
Replicates
Peak Size
Amount
250
500
750
1000


Print Date: 26 Nov 2005 17:48:50
Calibration Curve Report
File: d:\data gc-ms\interpret methods\method-nci.mth
Detector: 1200 Mass Spec, Address: 42
1,2,3,6,7,8-HxCDD
Curve Fit: Linear, Origin: Ignore, Weight: 1/nX2
y = +5.807824e+4x +9.500439e+5
Resp. Fact. RSD: 7.343%
Coeff. Det.(r2): 0.994635
Replicates
Peak Size
Amount
250
500
750
1000


Print Date: 26 Nov 2005 17:50:18
Calibration Curve Report
File: d:\data gc-ms\interpret methods\method-nci.mth
Detector: 1200 Mass Spec, Address: 42
1,2,3,4,,6,7,8-HpCDF
Curve Fit: Linear, Origin: Ignore, Weight: 1/nX2
y = +3.403101e+5x -1.075982e+7
Resp. Fact. RSD: 11.18%
Coeff. Det.(r2): 0.999906
Replicates
Peak Size
Amount


Print Date: 26 Nov 2005 17:51:37
Calibration Curve Report
File: d:\data gc-ms\interpret methods\method-nci.mth
Detector: 1200 Mass Spec, Address: 42
1,2,3,4,6,7,8-HpCDD
Curve Fit: Linear, Origin: Ignore, Weight: 1/nX2
y = +8.606411e+4x -3.659182e+6
Resp. Fact. RSD: 16.18%
Coeff. Det.(r2): 0.999477
Replicates
Peak Size
Amount


Print Date: 26 Nov 2005 17:52:53
Calibration Curve Report
File: d:\data gc-ms\interpret methods\method-nci.mth
Detector: 1200 Mass Spec, Address: 42
OCDF
Curve Fit: Linear, Origin: Ignore, Weight: 1/nX2
y = +1.055591e+5x -6.823682e+6
Resp. Fact. RSD: 12.14%
Coeff. Det.(r2): 0.998573
Replicates
Peak Size
Amount


Print Date: 26 Nov 2005 17:54:00
Calibration Curve Report
File: d:\data gc-ms\interpret methods\method-nci.mth
Detector: 1200 Mass Spec, Address: 42
OCDD
Curve Fit: Linear, Origin: Ignore, Weight: 1/nX2
y = +4.462205e+4x -2.727516e+6
Resp. Fact. RSD: 12.12%
Coeff. Det.(r2): 0.998077
Replicates
Peak Size
Amount

ANNEX 5

Table 1 List of Congenital Anomalies presumably linked to A.O/Dioxin exposure

I. Congenital anomalies relating to nervous system	
Q 00	Anencephaly
Q 01	Ancephalocele
Q 75.1	Lacuna of cranium (cranio-lacuna)
F 19.1	Oligophrenia – Imbecility
F 70	Mental retardation; mental incompletence
G 81	Hemiplegia, general paralysis
Q 02	Microcephaly
Q 03	Congenital hydro cephalus
Q 05	Spina bifida
Q 07	Meningo encephalomyelocele
F 21.1	Schizophrenia
F 29.1	Psychosis. Neurosis
G 43	Migrain – hemicrania
G 44	Cephalalgia (headache Syndrome)
G 51	Bell's palsy; facial palsy
G 82	Diplegia. Tetraplegia
G 82	Monoplegia
G 83	Paralysis by decerebration; decelebrate paralysis
G 40.1	Congenital epilepsy
G 90.1	Cerebral palsy
II. Congenital anomalies relating to audition organ	
Q 16.3	Multi – Deafness – deafmutism
Q 17.7	Anisoauricle, anisopinna
Q 16	Hypacusis, Hypo-acusis
Q 16.1	Congenital deafness
Q 16.2	Mutism
Q 17.2	Anotia
Q 17.3	Alobulus auriculae
Q 17.4	Bifid lobulus (auriculae)
Q 17.5	Supernumerary auricle, polyauricle
Q 17.6	Bat pinna, bat auricle
Q 17.8	Deformities of auricle
Q 17.9	Atresia of auditory canal
III. Congenital anomalies of eyes	
Q 11.1	Anophthalmos (unilateral)
Q 11.2	Anophthalmos (bilateral)
Q 15.2	Blindness Amourosis (unilateral)
Q 15.3	Blindness Amourosis (bilateral)
Q 13.2	Nystagmus
Q 13.3	Congenital glaucoma

Q 14	Degeneration of retina; atrophy of macula retinae
Q 15.1	Ambliopia
Q 10.1	Obliteration (Atresia) of lachrymal duct
Q 10.2	Not occulusio of eyelids
Q 10.3	Aniso pphthalmos, Aniso ophthalmia
Q 12	Congenital myopia, presbyopia, presbytia
Q 13.1	Strabismus
IV. Congenital anomalies of the circulatory system	
C 91	Leukemia
Q 27	Angionma
C 83	Lymphoma
Q 27	Splenic anemia
Q 20	Congenital cardiopathy
Q 21	Congenital malformations of cardiac septa: Vetricular, Atrial septal defect
Q 24	Dextro cardia
V. Congenital anomalies of respiratory and larynx system	
Q 32.1	Larynx fistula
Q 30.1	Deformities of nose; Snub-nosed; Flat nose
R 47.1	Dysphonia-Dysphemia
R 47.2	Dyslalia
R 49	Hoarseness
VI. Harelip; cleft palate	
Q 37	Harelip and cleft palate
Q 35	Cleft palate
Q 36	Harelip
VII. Congenital malformation of digestive system	
Q 38.1	Macro glossia
Q 38.2	Oligoglossia (shortening glossia)
Q 38.3	Enlargement of commissura labiorum Macrostomia
Q 42.5	Atressia of large intestine
Q 43.1	Ombilico-intestinal fistula
R 17.1	Congenital unspecified jaundice
R 18.1	Ascites
Q 42.1	Magacolon
K 45.1	Abdominal hernia
Q 42.3	Anal polyp (polypus)
Q 42.4	rectum fistula
Q 45.1	Anodotia-Hypodotia
Q 87.1	Visceral situs inversus
K 40	Inguinal hernia
K 42	Exomphalos. Exomphalocele
VIII. Congenital malformations of genital organs	
Q 52	Malformation of female genitalia
Q 52.1	Anelytria (absence of vagina)
Q 55.1	Microphllus (micro penis)

Q 55.2	Anisorchis
Q 55.3	Cryptorchidism
Q 55.6	Malformation of male genitalia
QV 56	Ambiguous external genitalia; Pseudo-hermaphroditism
Q 55.4	Cyst of spermatic cord
IX. Congenital anomalies of urinary system	
Q 54.1	Hypospadias
Q 54.2	Epispadias
Q 64.1	Extrophy of urinary bladder (ectopia vesicae)
R 32.1	Enuresis/bed wetting
X. Congenital anomalies and deformities of the muscle-skeleton-connective tissue	
Q 71.3	Amelia
M 62.2	Progressive muscular atrophy; progressive amyotrophia
Q 74.3	Luxation (Luxatio) of knee; elbow
Q 75.2	Acrania
Q 76.1	Kyphosis
Q 76.3	Kypho-Scoliosis
Q 76.4	fat-chested (unilateral)
Q 76.5	Amusculus pectoralis major (absence of musculus)
Q 76.6	Protrusion of thorax
Q 76.7	Deformities of bony thorax
Q 76.8	Sternocostal deformities
Q 73.3	Oligo-Syndactylia
M 62.1	Amyotrophy of lower limbs
M 83.1	Ostreomalacia
M 94.1	Dyplasia chondro – osseous
Q 65	Congenital deformities of hip
Q 66.1	Talipes; Clubfoot
Q 66.2	Talipes calcaneovalgus; Talipes equinovalgus
Q 66.3	Metatarsus varus; Talipes equinovarus
Q 67.1	Macrocephalia; Megacephalia
Q 68.3	Anisomelia
Q 71.1	Dysmelia (fore arm, leg)
Q 71.2	Phocomelia
Q 71.4	Deformities of upper limbs
Q 72.2	Deformities of lower limbs
Q 74	Constricting bands (of limbs)
Q 74.1	Dislocation of patella
Q 74.2	Apatella (absence of patella)
Q 74.4	Ankylosis of knee
Q 76.2	Scoliosis
Q 68.1	Genu varum
Q 68.2	Genu valgum
Q 73.1	Oligodactylia; Oligodactyly
Q 73.2	Oligophalangy (phalangia)

Q 69	Polydactylism; Polydactyly
Q 70	Syndactyly
Q 70.1	Polysyndactyly
XI. Other congenital anomalies	
E 88.1	Dysmature of stature; Statural dysmature
E 23.1	Pituitary dwarfism (nanism)
L 44.2	Congenital straphulus itching eruption
L 51	Pruritus; itching erythrodermia
L 63.1	Alopecia areata
L 81.1	Melanodermia phlyctenoid
Q 85	Phacomatosis
L 81	Itching leukoderma
E 66	Obesity
L 67.1	Congenital leucotrichia
D 36	Benign tumors on all sites
L 30	Congenital dermatitis
L 80	Congenital leukoderma
Q 80	Congenital ichtyosis
XII. Chromosomal abnormalities, not elsewhere classified	
Q 91	Apert's syndrome; Acro cephalo syndactylia
Q 92	Edward's syndrome
Q 93	Klinefelter's syndrome
Q 94	Patau's syndrome
Q 95	Poland's syndrome
Q 96	Turner's syndrome
Q 97.1	Sex chromosome anormalies male phenotyp
Q 97.2	Sex chromosome anormalies female phenotyp
Q 99	Poly-anomalia in one individual
Q 98	Poly abnormalities in sole corpse
Q 99.1	Sole monster
Q 99.2	Twin monster
Q 100	Adhersive twin fetus
Q 100.1	Craniopagus
Q 100.2	Cephalo didymus
Q 100.3	Thoracopagus
Q 100.4	Duplicitas posterior
Q 100.5	Ischiopagus
Q 100.6	Pyopagus
Q 90	Down syndrome

(*Source: Division 10-80, 2000*)

Table 2 Reproductive accidents making table

No	*Criteria*	*1965 – 1975*		*1976 – 1990*		**Since 1991**
		Serious Dioxin-affected area	Slight Dioxin-affected area	Serious Dioxin-affected area	Slight Dioxin-affected area	Serious Dioxin-affected area
1	Geographic region (Maximum = 5 marks)	5 marks	3	3	1	1
	South Laos, Cambodia		3		1	
2	Fist time of possible contact with and iffected by dioxin (Maximum 5 marks):					
	- Aborigines	5 marks	3	3	1	1
	- Ectogenes (alien)	5	3	3	1	1
3	Residence duration (max: 10 marks):					
	- Aborigenes (since the first contact until the first reproductive accident)	3 years: 1 mark	3 years: 1 mark	3 years: 1 mark	3 years: 1 mark	5 years: 1 mark
	- Immigrants (alien)	1 year: 1 mark	1 year: 1 mark	1 year: 1 mark	1 year: 1 mark	3 year: 1 mark
4	Obstetrical acidents (max: 10 marks):					
	- Spontaneous	3	3	3	2	2
	abortion	4	4	4	3	3
	- Preterm delivery	4	4	4	3	3
	- Intrauterine death of fetus					
	(fetus dead in utero)	4	4	4	3	3
	- Intrauferine death of fetus with congenital anomalies	6	6	6	4	4
	- Mole					
5	Congenital malformations, deformities, anomalies (max: 10 marks)	- In case of clear correlation with A.O/Dioxin: 7-8-9 marks - In case of unclear correlation with A.O/Dioxin: 5 marks				

(*Source: Division 10-80, 2000*)

ANNEX 6

PCDD/Fs concentration in human adipose of BienHoa residents (pg/g w. w.)

Sample	*AD2*	*AD3*	*AD4*	*AD6*	*AD7*	*AD8*	*AD9*	*AD11*	*AD12*	**AD13**
Age	30	38	32	28	25	40	34	32	26	55
Isomer										
2378-TCDD	156.7	nd	32.6	72.2	nd	nd	nd	17.8	nd	nd
12378-PeCDD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
123478-HxCDD	11.9	nd	nd	11.7	nd	nd	nd	nd	nd	nd
123678-HxCDD	nd	47.3	11.2	nd	4.1	44.0	nd	47.1	nd	nd
123789-HxCDD	nd	10.9	3.0	nd	nd	nd	nd	2.6	nd	nd
1234678-HpCDD	36.8	21.3	16.3	6.4	8nd	7.4	57.5	37.4	2.5	97.1
OCDD	121.3	156.5	168.6	151.5	nd	153.0	105.4	45.8	nd	31.2
2378-TCDF	58.4	12.9	nd	nd	nd	nd	2.4	nd	3.4	nd
12378-PeCDF	nd	nd	1.5	nd	nd	nd	nd	nd	nd	0.7
23478-PeCDF	27.2	5.0	7.5	0.7	6.1	5.3	8.1	6.8	6.9	2.8
123478-HxCDF	nd	28.9	nd	nd	nd	12.4	24.0	nd	4.1	766.5
123678-HxCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
123789-HxCDF	1.1	0.4	nd	nd	nd	0.3	1.1	nd	nd	8.0
234678-HxCDF	1.0	1.1	nd	nd	0.8	0.7	nd	0.2	nd	nd
1234678-HpCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	130.9
1234789-HpCDF	nd	nd	2.1	1.7	nd	nd	nd	1.3	0.8	nd
OCDF	16.4	3.6	58.9	51.0	5.2	nd	9.4	nd	1.9	37.5
i-TEQ	**177.9**	**12.9**	**38.0**	**73.8**	**4.4**	**8.5**	**7.4**	**26.6**	**4.3**	**81.2**

Sample	*AD14*	*AD15*	*AD18*	*AD19*	*AD20*	*AD21*	*AD22*	*AD23*	*AD24*	**AD25**
Age	42	33	26	22	32	33	32	22	22	31
Isomer										
2378-TCDD	nd	190.2	nd	nd	nd	300.0	37.9	177.6	47.6	nd
12378-PeCDD	nd	nd	41.3	32.1	nd	nd	47.1	nd	nd	nd
123478-HxCDD	46.5	154.0	nd	nd	nd	nd	nd	nd	nd	nd
123678-HxCDD	nd	nd	12.3	nd	153.3	nd	nd	nd	47.9	nd
123789-HxCDD	nd	nd	20.3	37.7	74.1	nd	3.5	nd	nd	nd
1234678-HpCDD	171.9	13.0	nd	nd	nd	510.3	121.4	nd	2281.9	13747.4
OCDD	40.1	29.8	129.5	42.2	10.1	18.6	3.0	23.1	40.1	22.9
2378-TCDF	1.8	nd	nd	5.2	nd	nd	nd	nd	nd	nd
12378-PeCDF	nd	nd	0.2	0.7	nd	nd	nd	0.2	0.8	nd
23478-PeCDF	2.7	0.7	0.4	3.4	0.3	0.9	1.2	0.4	1.2	4.9
123478-HxCDF	251.3	nd	nd	178.6	nd	192.3	nd	72.2	nd	5.1
123678-HxCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
123789-HxCDF	6.8	nd	2.2	4.8	5.0	nd	nd	nd	3.8	nd
234678-HxCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1234678-HpCDF	136.1	1.9	nd	nd	nd	nd	nd	nd	nd	420.5
1234789-HpCDF	236.3	4.2	nd	nd	58.4	141.7	nd	17.8	nd	nd
OCDF	9.1	3.9	10.5	24.2	14.6	9.1	2.3	nd	10.0	nd
i-TEQ	**37.5**	**206.2**	**45.0**	**56.5**	**24.0**	**326.2**	**87.2**	**185.2**	**76.3**	**144.7**

Sample	*AD27*	*AD28*	*AD29*	*AD30*	*AD31*	*AD32*	*AD34*	*AD35*	*AD36*	**AD38**
Age	32	20	26	32	25	43	30	34	24	32
Isomer										
2378-TCDD	146.9	44.4	nd	nd	53.2	nd	nd	37.6	43.4	100.7
12378-PeCDD	41.7	83.7	6.4	81.2	24.1	nd	nd	8.2	25.8	nd
123478-HxCDD	nd	432.3	54.7	50.4	nd	nd	nd	nd	41.4	nd
123678-HxCDD	nd	305.5	67.7	87.9	nd	nd	nd	nd	nd	nd
123789-HxCDD	58.2	nd	187.5	148.7	nd	nd	nd	nd	nd	nd
1234678-HpCDD	1314.5	2355.3	814.7	6574.6	12.1	nd	39.2	40.9	nd	nd
OCDD	15.5	11.9	1.2	5.0	161.1	220.6	184.1	139.9	147.2	432.6
2378-TCDF	nd	7.6	nd	0.6	nd	nd	nd	nd	nd	nd
12378-PeCDF	nd	2.2	0.3	0.3	nd	nd	nd	2.9	nd	nd
23478-PeCDF	0.8	3.7	0.1	0.2	7.0	7.5	8.9	12.1	9.7	nd
123478-HxCDF	nd	nd	nd	320.7	21.1	19.6	26.8	51.2	31.1	nd
123678-HxCDF	10.4	28.8	nd	nd	nd	nd	nd	nd	nd	nd
123789-HxCDF	11.9	9.6	27.3	11.6	12.9	6.7	9.9	7.8	12.8	nd
234678-HxCDF	38.0	98.0	19.5	nd	10.5	nd	nd	nd	nd	nd
1234678-HpCDF	230.9	nd	67.3	87.9	24.7	nd	13.5	13.2	nd	32.4
1234789-HpCDF	2060.6	1367.6	168.3	158.2	12.8	nd	nd	8.0	nd	nd
OCDF	2.3	18.2	0.7	5.3	21.0	42.6	50.8	39.6	52.9	186.5
i-TEQ	**237.0**	**255.5**	**52.7**	**211.5**	**85.8**	**6.4**	**8.7**	**58.6**	**82.6**	**101.1**

Sample	*AD39*	*AD42*	*AD43*	*AD44*	*AD45*	*AD46*	*AD47*	*AD48*	*AD49*	**AD50**
Age	35	30	59	30	34	33	34	29	34	32
Isomer										
2378-TCDD	nd	28.4	nd	133.8	148.9	153.8	nd	87.6	nd	168.1
12378-PeCDD	nd	nd	nd	nd	nd	632.9	nd	nd	nd	nd
123478-HxCDD	nd	nd	nd	nd	nd	172.5	nd	nd	nd	nd
123678-HxCDD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
123789-HxCDD	54.7	nd	nd	nd	nd	nd	nd	52.1	nd	nd
1234678-HpCDD	nd	nd	150.6	nd	nd	70.0	nd	100.9	nd	nd
OCDD	123.5	nd	1627.7	nd	814.0	233.1	nd	437.7	329.3	365.0
2378-TCDF	nd	72.0	nd	nd	nd	nd	nd	nd	nd	nd
12378-PeCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
23478-PeCDF	15.8	nd	nd	nd	nd	nd	9.9	11.1	nd	13.8
123478-HxCDF	26.4	nd	45.1	nd	100.8	nd	nd	nd	8.6	42.3
123678-HxCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	22.9
123789-HxCDF	nd	nd	nd	nd	nd	24.3	nd	8.6	nd	nd
234678-HxCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	12.9
1234678-HpCDF	11.3	nd	45.8	nd	56.1	nd	nd	27.9	nd	nd
1234789-HpCDF	nd	nd	nd	nd	nd	21.6	nd	nd	nd	nd
OCDF	63.3	120.3	178.3	nd	102.1	156.2	141.9	148.6	145.2	299.6
i-TEQ	**16.1**	**35.6**	**6.7**	**133.8**	**159.6**	**807.3**	**5.0**	**100.6**	**0.9**	**182.9**

Sample	*AD51*	*AD52*	*AD53*	*AD54*	*AD55*	*AD56*	*AD57*	*AD58*	*AD59*	**AD60**
Age	28	24	52	30	35	39	26	21	44	28
Isomer										
2378-TCDD	25.0	nd	47.3	55.6	nd	9.7	nd	nd	4.0	2.7
12378-PeCDD	nd	nd	nd	10.4	nd	nd	nd	nd	nd	nd
123478-HxCDD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
123678-HxCDD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
123789-HxCDD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1234678-HpCDD	nd	47.8	139.0	nd	nd	nd	nd	nd	nd	nd
OCDD	358.2	nd	1295.1	498.7	nd	nd	nd	nd	nd	nd
2378-TCDF	nd	nd	nd	nd	nd	nd	1.3	4.2	nd	nd
12378-PeCDF	nd	nd	nd	nd	nd	nd	2.2	nd	1.2	nd
23478-PeCDF	nd	nd	25.9	nd	nd	3.8	5.4	nd	nd	nd
123478-HxCDF	12.8	nd	21.5	nd	5.7	6.1	1.7	nd	1.5	nd
123678-HxCDF	nd	nd	nd	nd	nd	nd	1.5	nd	nd	nd
123789-HxCDF	nd	nd	nd	nd	1.2	1.5	nd	nd	nd	nd
234678-HxCDF	nd	nd	nd	nd	nd	nd	nd	nd	4.4	nd
1234678-HpCDF	nd	nd	36.0	30.4	nd	nd	nd	nd	nd	nd
1234789-HpCDF	nd	nd	nd	14.5	nd	nd	2.6	3.4	nd	nd
OCDF	135.0	nd	nd	98.5	nd	nd	nd	nd	nd	nd
i-TEQ	**26.4**	**0.5**	**64.3**	**66.5**	**0.7**	**12.4**	**3.3**	**0.5**	**4.7**	**2.7**

Sample	*AD61*	*AD62*	*AD63*	*AD64*	*AD65*	*AD66*	*AD67*	*AD68*	*AD69*	**AD70**
Age	36	42	33	22	36	28	24	30	28	38
Isomer										
2378-TCDD	5.9	8.6	1.7	nd	0.7	0.7	19.4	nd	nd	nd
12378-PeCDD	nd	155.5	3.5	nd	nd	nd	nd	195.0	nd	nd
123478-HxCDD	nd	nd	nd	nd	nd	nd	nd	147.3	nd	152.1
123678-HxCDD	nd	nd	52.1	nd	nd	nd	nd	nd	nd	nd
123789-HxCDD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1234678-HpCDD	nd	nd	nd	nd	nd	nd	nd	nd	29.2	nd
OCDD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2378-TCDF	nd	nd	nd	nd	nd	2.9	nd	nd	nd	nd
12378-PeCDF	nd	nd	nd	nd	nd	3.3	nd	nd	nd	nd
23478-PeCDF	4.0	nd	nd	nd	16.6	3.4	0.8	1.9	nd	2.8
123478-HxCDF	2.7	nd	nd	2.5	41.6	3.4	nd	nd	nd	5.7
123678-HxCDF	nd	nd	nd	nd	29.6	nd	nd	nd	nd	nd
123789-HxCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
234678-HxCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1234678-HpCDF	nd	nd	nd	nd	80.6	nd	14.4	nd	nd	nd
1234789-HpCDF	nd	nd	nd	nd	14.0	nd	nd	nd	10.3	nd
OCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
i-TEQ	**8.1**	**164.1**	**10.4**	**0.3**	**17.1**	**3.2**	**20.0**	**210.7**	**0.4**	**17.2**

Sample	*AD71*	*AD72*	*AD73*	*AD74*	*AD75*	*AD76*	*AD77*	*AD78*	*AD80*	**AD81**
Age	29	32	50	31	35	24	19	24	23	20
Isomer										
2378-TCDD	nd	2.1	12.2	nd	nd	28.3	nd	20.7	18.7	nd
12378-PeCDD	174.3	92.9	2.0	nd	3.5	nd	nd	2.2	nd	nd
123478-HxCDD	nd	nd	7.1	24.4	nd	54.2	nd	nd	nd	nd
123678-HxCDD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
123789-HxCDD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1234678-HpCDD	nd	nd	34.1	85.8	nd	nd	nd	nd	15.8	26.6
OCDD	nd	nd	163.0	354.6	90.4	120.9	53.9	50.5	60.7	142.0
2378-TCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
12378-PeCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
23478-PeCDF	2.8	4.7	0.5	5.4	0.7	2.9	nd	nd	nd	nd
123478-HxCDF	3.1	1.8	22.8	98.2	27.6	82.9	139.7	24.4	86.3	86.4
123678-HxCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
123789-HxCDF	nd	nd	8.5	15.5	nd	nd	19.4	nd	nd	37.0
234678-HxCDF	nd	12.2	12.7	nd	nd	nd	nd	nd	nd	nd
1234678-HpCDF	nd	nd	37.3	14.1	5.4	17.9	13.3	nd	7.4	11.9
1234789-HpCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
OCDF	nd	nd	35.6	nd	nd	nd	nd	nd	17.4	12.2
i-TEQ	**176.0**	**98.8**	**20.3**	**17.6**	**6.7**	**43.7**	**16.1**	**25.3**	**27.6**	**12.7**

Sample	*AD82*	*AD83*	*AD84*	*AD85*	*AD86*	*AD87*	*AD88*	*AD89*	*AD90*	**AD91**
Age	25	25	34	36	26	29	20	24	41	20
Isomer										
2378-TCDD	nd	32.9	23.7	6.5	nd	nd	4.9	6.3	nd	nd
12378-PeCDD	nd	nd	nd	nd	nd	0.5	nd	nd	64.7	nd
123478-HxCDD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
123678-HxCDD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
123789-HxCDD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1234678-HpCDD	19.1	nd	42.9	25.2	15.0	nd	15.9	nd	nd	nd
OCDD	157.8	nd	375.3	635.4	91.6	54.3	74.8	nd	nd	nd
2378-TCDF	nd	7.1	nd	nd	nd	nd	nd	nd	nd	nd
12378-PeCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
23478-PeCDF	nd	nd	nd	nd	nd	nd	nd	6.4	nd	nd
123478-HxCDF	106.5	7nd	530.1	4nd	126.1	101.0	811.8	28.2	26.0	nd
123678-HxCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
123789-HxCDF	nd	nd	nd	nd	nd	nd	59.2	nd	nd	nd
234678-HxCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1234678-HpCDF	nd	5.7	19.3	19.8	nd	nd	19.0	nd	nd	nd
1234789-HpCDF	nd	nd	nd	nd	nd	nd	6.2	nd	nd	nd
OCDF	nd	nd	nd	nd	nd	nd	10.0	nd	nd	nd
i-TEQ	**10.9**	**40.7**	**77.4**	**11.0**	**12.8**	**10.6**	**92.4**	**12.3**	**67.3**	**nd**

Note: nd – non-detectable

CURRICULUM VITAE

1. PERSONAL DATA		
Family Name: MAI	First Name: TUAN ANH	
Year of Birth: 19/08/1963		
Civil status: married		
Number of children (below 18 years of age): 02		
Nationality: Vietnamese		
Country of Permanent Residence: Vietnam		
Permanent address: 22/16 Cu Xa Lu Gia – Dist. 11 – HCMC – Viet Nam Tel : +84 8 8654076 MP: +84 9 03803101		
2. EMPLOYMENT RECORD (Most recent employment first)		
Employer's Company Name, Telephone, Fax, Address. Contact Person (Manager/ Personnel Officer)	Period of service and length:	Position with the Company:
Center for Environmental Technology (CEFINEA) – Institute for Environment and Resources (IER) – National University of HCMC	From 1991 up to now	Lab. Supervisor
Tel: +84 8 8651132		
Fax: +84 8 8655670		
Email: tuananh@hcmier.edu.vn tuananh.mai@epfl.ch mtanh@hotmail.com		
Address: IER - 142 To Hien Thanh St., Dist. 10, HCMC-VietNam		
Director : Prof.Dr. Huynh thi Minh Hang		
3. EDUCATION		
Institution (University, etc.), City and Country	Length of education (Date: from (month/year) to (month/year)	Degree/Diploma obtained
HCMC University of Technology - National University of HCMC	1986-1991	Engineer of Chemical Engineering and Foods
HCMC University of Technology - National University of HCMC	1993-1995	Master of Environmental Engineering
Ecole Polytechnique Federale de Lausane	1997-1999	Master of Environmental Science
Ecole Polytechnique Federale de Lausane	2002 up to now	Ph.d student

4. LANGUAGE SKILLS OF RELEVANCE TO THE ASSIGNMENT

(State language, formal language education, and level of speaking, reading and writing skills (mother tongue, perfect, average, poor)

Language	Formal Education	Speaking skills	Reading skills	Writing Skills
English		Good	Good	good
French		Good	Good	Good
Czech		Good	Good	Good
Rusia			Good	

5. PROFESSIONAL EXPERIENCE

(Key Experience and Qualifications of relevance to the assignment/project)

- Environmental Analytical chemistry (air, water, sediment quality, etc)
- Water and wastewater treatment
- EIA report making
- Environmental Quality Assessment

6. SPECIAL EXPERIENCE FROM DEVELOPING COUNTRIES

(List projects of relevance to the assignment/project)

Year	Project Name	Country	Area of responsibility	Name of Client
1991-1995	Project KT02.04 – application of some typical technologies for water and air pollution control in shouthern provinces	Vietnam	Industries	Ministry of Science, Technology and Envoronment (MOSTE)
1999-2000	Project KH07.17 -Study the scientific base for investigated management of Resources of Dongnai river Basin	Vietnam		MOSTE
1995 up to now	Environmental Monitoring Program for HCMC and Mekong Delta	Viet Nam		MOSTE
1994-1998	Study and Apply some models of Clean Water Supply for rural areas of HCMC and Mekong Delta	Vietnam		Department of Science, Technology and Envoronment (DOSTE) of HCMC
1993-1994	Investigate the Industrial Pollution of HCMC and Suggest the Treatment Measures (Black Book for Industrial	Vietnam		DOSTE of HCMC
1998-1999	Study on Domestic Wasterwater composition and Properties Serviced for Wastewater Discharge and Treatment of HCMC	Vietnam		DOSTE of HCMC
1998-1999	Study on Measures for Environmental Ensure of HCMC Chanel Sediment dredging, Transport and Disposal – Sediment Treatment and Reuse	Vietnam		DOSTE of HCMC
Etc.				

7. OTHER INFORMATION OF RELEVANCE TO THE ASSIGNMENT

Published research:

- PAHs Contamination Levels in Air Particles and Sediments of Hochiminh City, Vietnam - "Bulletin of Environmental Contamination and Toxicology (1999) 63: 728-735;
- PAHs Contamination in Hochiminh City, Vietnam –Inter. Conference on PAHs– Bordeaux October 25-29, 1999;
- PAHs Contamination in transport points and industrial parks of Hochiminh City – Inter. Conference on Environ. Anal. Chemistry – Hensinki June 11-16, 2000;
- Determination of Chlorophenol contamination in wastewater of pulp and paper industries – Ministry of Training and Education (MOTE) Project
- Report on Monitoring water, air and solid waste quality of HCMC and Mekong delta (yearly) – MOSTE Project
-Pesticide Contamination Level in HCMC Canals – Nation. Conference – Hanoi 2000;
- Heavy metal Contamination in HCMC Canals – Nation. Conference – Hochiminh City 2000;
- Report on Pesticide and Heavy Metal Contamination of Saigon-Dongnai River System – Pilot Project of Vietnam – Swiss Collaboration Project (1996-2000);
- Micropollutants in the Sediment of Saigon-Dongnai River: Situation and Ecological Risk – Chimia 2003, 57: 537- 541;
- Dioxin Contamination in Southern Vietnam – 2nd Asian Pacific Inter. Conference on Pollutans Analysis and Control – Hochiminh City December 1-3, 2003;
- Anal. Methods for Dioxin Analysis and Results – Workshop on Dioxin Study and Related Problem – Hochiminh City August 6, 2004;
- Dioxin Contamination in the soil of Southern Vietnam - 24th International Symposium on Halogenated Environmental Organic Pollutants and POPs – Berlin, September 6-10, 2004 (http://www.dioxin2004.org/frameset.htm).

- Dioxin Contamination in soils of Southern Vietnam-Chemosphre (Submitted)

Other Activities:

- Teaching on Environmetal Chemistry for Graduated and Postgraduated students;
- Carrying out the water and wastewater treatment plants
- Supervising for Posgraduated thesis
- Doing the IEA Report for the new project investments in Southern Vietnam

etc.