ANALYSIS OF PERSISTENT ORGANIC POLLUTANTS IN FISH AND SEAFOOD: HEALTH RISK ASSESSMENT THROUGH DIETARY INTAKE

by

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LIST OF ABBREVIATIONS

ADI	Acceptable daily intake
ASE	Accelerated solvent extraction
Cl	Chlorine
FAO	Food and Agricultural Organization
GC-ECD	Gas chromatograph-electron capture detector
GC-MS	Gas chromatograph-mass spectrometer
GEF	Global environment facility
GPC	Gel permeation chromatography
$K_{o/w}$	Lipid/water partition coefficient
LD_{50}	Acute oral toxicity in rats-lethal concentration 50
LOD	Limit of detection
LOQ	Limit of quantification
MAE	Microwave-assisted extraction
MLD	Median lethal dose
MSPD	Matrix solid-phase dispersion
OCPs	Organochlorine pesticides
PCBs	Polychlorinated biphenyls
POPs	Persistent organochlorine pollutants
SFE	Supercritical fluid extraction
SIM	Selected ion monitoring
TEF	Toxic equivalent factor
TEQ	Toxic equivalent quotient
UNEP	United Nations Environment Programme
USE	Ultrasonic extraction
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

ANALISIS BAHAN-BAHAN PENCEMAR ORGANIK PERSISTEN DI DALAM IKAN DAN MAKANAN LAUT:
PENILAIAN RISIKO KESIHATAN MELALUI PEMAKANAN

Abstrak

Profil enam sebatian aroklor telah dibina menggunakan kromatografi gas-jisim spektrometer dan pemetaan puncak kongener bifenil politerklorin kepada masa penahanan masing-masing telah dilakukan dengan tahap keyakinan yang tinggi. Kromatografi gas dengan pengesan penangkapan elektron telah digunakan untuk tujuan kuantifikasi sebatian-sebatian pestisid organoklorin dan bifenil politerklorin di dalam kajian ini.

Eksperimen perolehan semula pakuan digunakan untuk mengoptimumkan prosedur pemisahan analit daripada lipid dan seterusnya untuk proses pengesahan kaedah bagi memastikan kemantapan kaedah analisis yang digunakan di dalam kajian ini. Masa pengaktifan florisil ditetapkan selama 3 jam dan florisil panas didapati adalah mod padatan paling sesuai bagi kaedah pembersihan ini. Pemisahan PCB, OCP dan lipid telah dicapai dengan menggunakan 25 g florisil dan heksana dan diklorometana sebagai pelarut pengelusi.

Penentuan sebatian pestisid OCP dan PCB di dalam tisu ikan, udang dan kerang dilaporkan sebagai berat lemak dan berat basah menunjukkan keputusan yang setanding dengan beberapa keputusan kajian yang telah dilaporkan. Endosulfan dan metabolitnya didapati merupakan sebatian OCP yang tertinggi kepekatan di dalam kebanyakan sampel diikuti oleh DDT dan metabolitnya. Bagi PCB, berdasarkan faktor kesetaraan toksik (TEF), keputusan kiraan per berat lemak dan berat basah adalah hampir sama dengan kebanyakan laporan. PCB 126 dan PCB 169, yang mempunyai ketoksikan lebih tinggi daripada sebatian PCB lain, didapati memberi sumbangan paling tinggi dari pertimbangan ketoksikan setara bagi sampel yang dikaji.

Menggunakan tahap kepekatan bagi pencemar di dalam makanan laut dan ikan sungai ini, penilaian risiko ke atas kesihatan manusia melalui pemakanan telah dianggarkan berdasarkan pendekatan pemakanan harian yang dihadkan (acceptable daily intake). Data pemakanan diet harian yang telah dikirakan di dalam kajian ini adalah secara keseluruhannya lebih rendah berbanding pemakanan harian yang dihadkan mengikut ketetapan Badan Kesihatan Sedunia (World Health Organization) dan badan-badan kesihatan lain yang diperakui. Dengan membandingkan jumlah pengambilan yang telah diterbitkan di dalam sumber literatur yang berkaitan, keputusan-keputusan di dalam kajian ini masih konsisten walaupun menunjukkan tahap yang lebih rendah untuk sebatian-sebatian pestisid organoklorin dan bifenil politerklorin.

Kesimpulannya, berdasarkan kajian ini, kehadiran sebatian OCP dan PCB di dalam ikan, udang dan kerang tidak memberikan ancaman kepada penduduk-penduduk di Malaysia melalui pengambilan diet seharian.

ANALYSIS OF PERSISTENT ORGANIC POLLUTANTS IN FISH AND SEAFOOD: HEALTH RISK ASSESSMENT THROUGH DIETARY INTAKE

Abstract

The profiles of six aroclors were mapped out using gas chromatograph-mass spectrometer and the assignments of chlorinated biphenyl congeners peaks to their respective retention times were carried out with very good degree of certainty. Gas chromatograph with electron capture detector was employed for the quantification of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs).

Spike recovery experiments were employed to optimise the procedure of separating the targeted analytes from lipid and subsequently to validate the analytical methods used in this study. The time for activation of florisil was set at 3 hours and hot florisil was found to be the most suitable mode of packing for this clean-up procedure. Separation of PCBs, OCPs and lipid was achieved using 25 g of florisil and hexane and dichloromethane as eluting solvents.

Determination of OCP and PCB compounds in fish tissue, shrimp and cockle reported as per lipid weight and wet weight gave comparable results with several reported studies. Endosulfan and its metabolites were found to be the highest concentration of OCPs detected in most samples followed by DDT and its metabolites. In the case of PCBs, based on toxicity equivalent factors (TEFs), calculated results in lipid and wet weight were comparable with literature reports. PCB 126 and PCB 169, which are higher in toxicity than the other dioxin-like PCBs, were found to contribute the most in term of toxicity equivalent of the samples studied.

Using the residual levels of these pollutants in the seafood and fresh water fish, risk assessment on human health through consumption was estimated based on the acceptable daily intake (ADI) approach. The estimated daily dietary intake exposure data calculated in this study were generally lower than the ADI imposed by World

Health Organization (WHO) and other health governing organizations. In comparison with the intake estimates published in related literature sources, the results in this study were still consistent despite lower results for both OCPs and PCBs.

In summary, based on this study, the presence of OCPs and PCBs in fish, shrimp and cockle did not pose a threat to the Malaysians through daily dietary intake.

CHAPTER 1

INTRODUCTION

1.0 Overview

Most chemicals find their way into the environment via various products and processes. Once in the environment, they can persist for long periods of time or break down into other chemicals with their own risk (UNEP, 2003). They may also produce potential adverse effects on the environment and human health when they act together with other natural or manufactured chemicals that are already in the environment. Effective risk management for chemicals depends on tracking the pathways, fate and exposure implications of chemicals, yet data on the pathways, emissions, environmental fate and exposure for risk assessment are only available for very few chemicals (UNEP, 2002).

Special attention has been given to the persistent toxic organic substances, which are widely found in the environment. These substances can travel through air, water and migrating species, be released into the environment in one part of the world, and, through a repeated process of release and deposit, emerge in regions far away from their original sources (UNEP, 2003). They can become increasingly concentrated in the tissues of animals at higher levels of the food chain which include human through bioaccumulation. Therefore, realizing the potential risks of long-term adverse effects of these chemicals, environmental toxicologists have extensively studied the exposure, fate and effects of their presence in our environment since the early sixties (van der Oost *et al*., 2003).

For practical reasons, human health and environmental risk assessment methodologies have developed independently. Classical risk assessment has evaluated health and ecological risks independently, typically assessed and reported by researches from different disciplines. However, with increased recognition of the need to protect both humans and the environment more effectively, an integrated

approach to risk assessment that addresses real-life situations of multi-chemicals, multi-media, multi-route and multi-species exposures is needed. In response to this need, the UNEP/ILO/WHO, International Programme on Chemical Safety (IPCS), in collaboration with the U.S. Environmental Protection Agency, European Commission and other international and national organizations, have developed a working partnership to foster the integration of assessment approaches to evaluate human health and ecological risks (Sekizawa and Tanabe, 2005). In 1997, the United Nations Environment Programme (UNEP) Governing Council decided that immediate international action should be initiated to protect human and the environment through measures which will reduce and/or eliminate the emissions and discharges of an initial set of twelve ''persistent organic pollutants'' (POPs) nine of which are organochlorine pesticides (UNEP, 2003).

In practice, threshold levels indicating predicted no effect concentration or other approach such as Acceptable Daily Intakes (ADIs) outlined by national and international organizations have been estimated for specific compounds in their environments. However, it should be realised that such approach to risk assessment cannot be applied across the regions of the world as several geographical and climatic factors affect the assessment significantly. Furthermore, certain chemicals, particularly those that were released as mixtures of compounds such as polychlorinated biphenyls (PCBs), would pose greater challenge in setting up critical levels for risk assessment evaluations.

Health risk assessment of persistent organic pollutants is relatively new in Malaysia. Data on human exposure to POP chemicals such as organochlorine pesticides (OCPs) and PCBs in Malaysia are limited. However, data on environmental levels of these POPs are sufficient to correlate with the exposure levels for health risk assessment. Realizing that the main exposure of POP chemicals is through food consumption, we embarked on this project on health risk assessment of OCPs and PCBs through dietary intake.

1.1 Objectives

The main objective of this study is to assess the health risk involved in consuming food such as fish and seafood contaminated with OCP and PCB. Current data on OCP and PCB levels in fish and other seafood need to be generated. Method for analyses of OCP has been developed in this laboratory but no method has been developed and validated for the analysis of PCB in tissue. Therefore, other objectives for this project are

1. To develop and validate a method to analyse PCB in fish tissues;
2. To modify and validate method to analyse OCP in fish tissues;
3. To determine the OCP and PCB levels in local fish and other marine organisms and lastly;
4. To assess the health risk involved in consuming these fish through dietary intake.

CHAPTER 2

LITERATURE REVIEW

2.0 Introduction

Extensive literature surveys have been made before carrying out this project. The preliminary part of these surveys mainly focused on the fundamental aspects of understanding the subjected persistent organic contaminants undertaken in this study; OCPs and PCBs. Properties, chemistry, usage, toxicity and environmental case histories are presented in the early section of this chapter followed by evaluation of the analytical methods for the analyses of OCP and PCB. Literature reports have provided several analytical techniques which can be modified and validated for the current project. Some of these analytical procedures are discussed in the following sections to lay the ground for our method of choice for this study.

2.1 Persistent organic pollutants

2.1.1 Organochlorine pesticides

Currently, there are over 500 compounds registered worldwide as pesticides, or metabolites of pesticides (Van der Hoff and Van Zoonen, 1999). Pesticides can be classified based on functional groups in their molecular structures (e.g. inorganic, organonitrogen, organohalogen, or organosulphur compounds), or their specific biological activities or target species such as insecticides, fungicides, herbicides, acaricides, etc. (Van der Hoff and Van Zoonen, 1999). Herbicides are by far the most commonly used chemical in the agricultural field followed by insecticides, fungicides and others. Pesticide used in agriculture has progressively increased after World War II leading to increased world food production. Nevertheless, this use and additional environmental pollutions due to industrial emissions during their productions have resulted in the release of these chemicals into food commodities, living organisms,

water and soil. Legislations were enacted in the USA, the European Union (EU) and other countries to regulate pesticides in food products (Ahmed, 2001).

Even though there are numerous chemicals being input into the environment, only a few of them are considered to be ecotoxic and have long term effect on the environment and human health. These chemicals exhibited characteristics of environmental persistence so that long term exposures might result and effects may be felt some distance from the point of production or release. Under the Stockholm Convention of 2001, there are 12 designated persistent organic pollutants or better known as “Stockholm POPs” which are given priorities in the assessment of pollutants in our environment and out of the 12 pollutants, 9 are pesticides. However, it is crucial to recognise that the exclusion of certain chemicals from worldwide assessment does not imply that other persistent toxic substances are not important (UNEP, 2003).

Aldrin, dieldrin, endrin, heptachlor and DDT are all included in the 12 “Stockholm POPs” while HCHs and endosulfan were omitted even though they are considered as priority pollutants by US EPA (UNEP, 2003). However, in most POP monitoring studies, these compounds were also assessed.

Aldrin has been manufactured commercially since 1950 and used to control soil pests and in the protection of wooden structures against termites. It is readily metabolised to dieldrin by both plants and animals. Heptachlor is another pesticide primarily used against soil insects and termites although it had also been used in controlling malaria mosquitoes. Heptachlor epoxide is a more stable breakdown product of heptachlor and would normally be assessed together with heptachlor. As for endrin, its usage was mostly against a wide range of agricultural pests and as rodenticide. Endrin may be metabolised to endrin aldehyde and endrin ketone. DDT appeared during World War II to control insects that spread diseases like malaria, dengue fever and typhus. The technical grade DDT is a mixture of about 85 % *p,p*’-DDT and 15 % *o,p*’-DDT. This compound is metabolised mainly to *p,p*’-DDD and *p,p*’-

DDE in the environment, which unfortunately are more toxic than their parent compound (UNEP, 2003).

HCHs or hexachlorocyclohexanes are one of the compounds which are frequently monitored by chemists in the investigation of POPs toxicity impact on human health. It exists in two principle formulation: “technical HCH”, which is a mixture of various isomers, including α-HCH (55 – 80 %), β-HCH (5 – 14 %) and γ-HCH (8 – 15 %), and “lindane”, which is essentially pure γ-HCH. Lindane was one of the most widely used insecticides in the world in controlling a wide range of sucking and chewing insects.

Another pesticide, endosulfan was first introduced in 1954 and used as a contact and stomach insecticide and acaricide in a great number of food and non-food crops. This compound has been formulated to be used in commercial agriculture, home gardening and for wood preservation. The technical grade endosulfan contains at least 94 % of two isomers, α and β-endosulfan. Table 2.1 shows the properties of some of the OCPs (UNEP, 2003).

Table 2.1: OCPs and its properties.

Name of compounds	*Half lives (years)*	*Toxicity in rats (oral) LD_{50} (mg/kg)*	*FAO/WHO tolerance limit (mg/kg/day)*
HCHs	>1 – 2	60 – 250	0.1 – 0.5
Heptachlor	0.75 – 2	40 – 119	0.2
Aldrin	0.05 – 1.6	67	0.2
Endosulfan	0.1 – 0.4	18 – 160	0.2
DDT	15	113 – 118	5.0
Dieldrin	3 – 4	40 – 70	0.2
Endrin	<12	3 – 43	0.05

Organochlorine pesticides had been extensively used in malaria control programs (vector borne diseases) and against livestock ectoparasites and agricultural pests (Fytianos *et al*., 1985). Public concern over organochlorine pesticides (OCPs) contamination of the environment had risen over recent years to the extent that it has now become a significant food safety issue. These compounds are known to disrupt the hormone endocrine system and induce cancer in a range of organisms, thereby posing a significant risk to natural ecosystems and human health (Patlak, 1996). The use of OCPs is tightly regulated in the developed world, but OCPs, including DDT and hexachlorocyclohexane are still widely used in many developing countries (Tkalin, 1996). Generally, OCPs have very low solubility in water, highly lipophilic, resist metabolic degradation and have a propensity to bioaccumulate in the food chain. High concentrations of OCPs have been detected in bird raptors, marine organisms and human breast milk (Harris *et al*., 1999). Owing to their high lipophilic properties, OCPs have contaminated the environment and food chain, particularly in those organisms having high fat content.

2.1.2 Polychlorinated biphenyls

PCBs are a class of synthetic chlorinated organic compounds with biphenyl as the basic structural unit. Chlorination of the biphenyl moiety can produce 209 possible polychlorobiphenyls (congeners) substituted with 1 to 10 chlorine atoms. Systematic numbering and structures of these chlorobiphenyls are given in Table 2.2 (Ballschmiter and Zell, 1980).

Table 2.2: Systematic numbering of PCB compounds.

PCB No.	Structure	PCB No.	Structure
	Monochlorobiphenyls		Tetrachlorobiphenyls
1	2	40	2,2',3,3'
2	3	41	2,2',3,4
3	4	42	2,2',3,4'
		43	2,2',3,5
	Dichlorobiphenyls	44	2,2',3,5'
4	2,2'	45	2,2',3,6
5	2,3	46	2,2',3,6'
6	2,3'	47	2,2',4,4'
7	2,4	48	2,2',4,5
8	2,4'	49	2,2',4,5'
9	2,5	50	2,2',4,6
10	2,6	51	2,2',4,6'
11	3,3'	52	2,2',5,5'
12	3,4	53	2,2',5,6'
13	3,4'	54	2,2',6,6'
14	3,5	55	2,3,3',4
15	4,4'	56	2,3,3',4'
		57	2,3,3',5
		58	2,3,3',5'
	Trichlorobiphenyls	59	2,3,3',6
16	2,2',3	60	2,3,4,4'
17	2,2',4	61	2,3,4,5
18	2,2',5	62	2,3,4,6
19	2,2',6	63	2,3,4',5
20	2,3,3'	64	2,3,4',6
21	2,3,4	65	2,3,5,6
22	2,3,4'	66	2,3',4,4'
23	2,3,5	67	2,3',4,5
24	2,3,6	68	2,3',4,5'
25	2,3',4	69	2,3',4,6
26	2,3',5	70	2,3',4',5
27	2,3',6	71	2,3',4',6
28	2,4,4'	72	2,3',5,5'
29	2,4,5	73	2,3',5',6
30	2,4,6	74	2,4,4',5
31	2,4',5	75	2,4,4',6
32	2,4',6	76	2',3,4,5
33	2',3,4	77	3,3',4,4'
34	2',3,5	78	3,3',4,5
35	3,3',4	79	3,3',4,5'
36	3,3',5	80	3,3',5,5'
37	3,4,4'	81	3,4,4',5
38	3,4,5		
39	3,4',5		

Cont. of Table 2.2

PCB No.	Structure	PCB No.	Structure
	Pentachlorobiphenyls		Hexachlorobiphenyls
82	2,2',3,3',4	128	2,2',3,3',4,4'
83	2,2',3,3',5	129	2,2',3,3',4,5
84	2,2',3,3',6	130	2,2',3,3',4,5'
85	2,2',3,4,4'	131	2,2',3,3',4,6
86	2,2',3,4,5	132	2,2',3,3',4,6'
87	2,2',3,4,5'	133	2,2',3,3',5,5'
88	2,2',3,4,6	134	2,2',3,3',5,6
89	2,2',3,4,6'	135	2,2',3,3',5,6'
90	2,2',3,4',5	136	2,2',3,3',6,6'
91	2,2',3,4',6	137	2,2',3,4,4',5
92	2,2',3,5,5'	138	2,2',3,4,4',5'
93	2,2',3,5,6	139	2,2',3,4,4',6
94	2,2',3,5,6'	140	2,2',3,4,4',6'
95	2,2',3,5',6	141	2,2',3,4,5,5'
96	2,2',3,6,6'	142	2,2',3,4,5,6
97	2,2',3',4,5	143	2,2',3,4,5,6'
98	2,2',3',4,6	144	2,2',3,4,5',6
99	2,2',4,4',5	145	2,2',3,4,6,6'
100	2,2',4,4',6	146	2,2',3,4',5,5'
101	2,2',4,5,5'	147	2,2',3,4',5,6
102	2,2',4,5,6'	148	2,2',3,4',5,6'
103	2,2',4,5',6	149	2,2',3,4',5',6
104	2,2',4,6,6'	150	2,2',3,4',6,6'
105	2,3,3',4,4'	151	2,2',3,5,5',6
106	2,3,3',4,5	152	2,2',3,5,6,6'
107	2,3,3',4',5	153	2,2',4,4',5,5'
108	2,3,3',4,5'	154	2,2',4,4',5,6
109	2,3,3',4,6	155	2,2',4,4',6,6'
110	2,3,3',4',6	156	2,3,3',4,4',5
111	2,3,3',5,5'	157	2,3,3',4,4',5'
112	2,3,3',5,6	158	2,3,3',4,4',6
113	2,3,3',5',6	159	2,3,3',4,5,5'
114	2,3,4,4',5	160	2,3,3',4,5,6
115	2,3,4,4',6	161	2,3,3',4,5',6
116	2,3,4,5,6	162	2,3,3',4',5,5'
117	2,3,4',5,6	163	2,3,3',4',5,6
118	2,3',4,4',5	164	2,3,3',4',5',6
119	2,3',4,4',6	165	2,3,3',5,5',6
120	2,3',4,5,5'	166	2,3,4,4',5,6
121	2,3',4,5',6	167	2,3',4,4',5,5'
122	2',3,3',4,5	168	2,3',4,4',5',6
123	2',3,4,4',5	169	3,3',4,4',5,5'
124	2',3,4,5,5'		
125	2',3,4,5,6'		
126	3,3',4,4',5		
127	3,3',4,5,5'		

Cont. of Table 2.2

PCB No.	Structure	PCB No.	Structure
	Heptachlorobiphenyls		Octachlorobiphenyls
170	2,2',3,3',4,4',5	194	2,2',3,3',4,4',5,5'
171	2,2',3,3',4,4',6	195	2,2',3,3',4,4',5,6
172	2,2',3,3',4,5,5'	196	2,2',3,3',4,4',5',6
173	2,2',3,3',4,5,6	197	2,2',3,3',4,4',6,6'
174	2,2',3,3',4,5,6'	198	2,2',3,3',4,5,5',6
175	2,2',3,3',4,5',6	199	2,2',3,3',4,5,6,6'
176	2,2',3,3',4,6,6'	200	2,2',3,3',4,5',6,6'
177	2,2',3,3',4',5,6	201	2,2',3,3',4',5,5',6
178	2,2',3,3',5,5',6	202	2,2',3,3',5,5',6,6'
179	2,2',3,3',5,6,6'	203	2,2',3,4,4',5,5',6
180	2,2',3,4,4',5,5'	204	2,2',3,4,4',5,6,6'
181	2,2',3,4,4',5,6	205	2,3,3',4,4',5,5',6
182	2,2',3,4,4',5,6'		
183	2,2',3,4,4',5',6		Nonachlorobiphenyls
184	2,2',3,4,4',6,6'	206	2,2',3,3',4,4',5,5',6
185	2,2',3,4,5,5',6	207	2,2',3,3',4,4',5,6,6'
186	2,2',3,4,5,6,6'	208	2,2',3,3',4,5,5',6,6'
187	2,2',3,4',5,5',6		
188	2,2',3,4',5,6,6'		Decachlorobiphenyls
189	2,3,3',4,4',5,5'	209	2,2',3,3',4,4',5,5',6,6'
190	2,3,3',4,4',5,6		
191	2,3,3',4,4',5',6		
192	2,3,3',4,5,5',6		
193	2,3,3',4',5,5',6		

According to the IUPAC definitive rules for nomenclature of organic chemistry, one ring system in the biphenyl ring assembly is assigned unprimed numbers and the other primed numbers as illustrated in Figure 2.1.

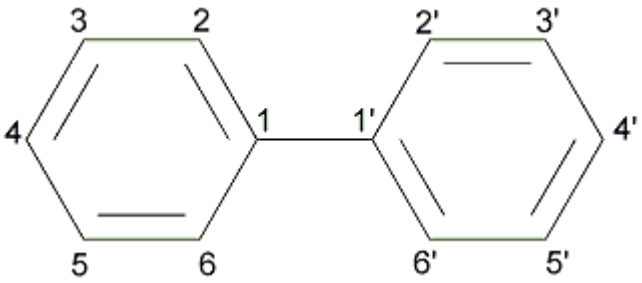

FIGURE 2.1. Numbering in the biphenyl system.

The order for assigning priorities to the substituents in the ring assembly, as Cl in PCBs is (i) unprimed number is assigned lower order than the corresponding primed number, as 2 vs. 2'; (ii) lower number is assigned to a point of attachment in equivalent position, as 2 vs. 6, for a substituent in one of the *ortho* positions; (iii) when the number of substituents in the two ring systems is the same, unprimed numbers are assigned to the ring system with smaller numbered substituents (Sawhney, 1986). In theory, the total number of possible compounds resulting from chlorination of the biphenyl nucleus is 209 as shown in Table 2.3 (Cairns *et al*., 1986).

Table 2.3: Number of possible congeners of PCBs.

Chlorine Substitution	Number of Possible Isomers
Mono-	3
Di-	12
Tri-	24
Tetra-	42
Penta-	46
Hexa-	42
Hepta-	24
Octa-	12
Nona-	3
Deca-	1
Total:	209

Syntheses of most of the 209 chlorobiphenyls individually have been accomplished and many of these are now available commercially. Methods used for the preparation of individual chlorobiphenyls include (1) phenylation or arylation of aromatic compounds, (2) condensation reactions, and (3) controlled chlorination of biphenyl. Chlorination of biphenyl in the presence of a catalyst, such as iron fillings or iron chloride is used for industrial preparations of PCB mixtures. The chlorination process produces mixtures of chlorobiphenyls which are influenced by the ratio of chlorine to biphenyl. The crude product resulting from chlorination of biphenyls is

purified to remove colour, catalyst, and traces of HCl by alkali treatment and distillation. However, PCBs can also be unintentionally produced as by-products in a wide variety of chemical processes that contain chlorine and hydrocarbon sources, during water chlorination and by thermal degradation of other chlorinated organics.

The purified PCB mixtures are generally viscous liquids. In the U.S., PCBs have been manufactured by Monsanto under the trade name aroclors®. The most common aroclor® preparations include 1242, 1248, 1254, and 1260. For example, for aroclor® 1242 blend, the first two digits: 12 represents the number of carbon atoms in the biphenyl group while the last two digits, 42 gives approximate estimate for the wt % of chlorine in the blend preparation (Sawhney, 1986).

Even though the synthesis of polychlorinated biphenyls (PCBs) was first described in the late nineteenth century by Schmidt and Schultz, the potential industrial applications were not fully realized until about 1930 (Cairns *et al*., 1986). The increased use of PCBs as important industrial chemicals for use as non-flammable oils in a host of products gave birth to a series of commercially available raw materials marketed under various trade name; e.g., Aroclor® (Monsanto, U.S.), Clophen® (Bayer, West Germany), Phenoclor® (Caffaro, Italy), Kanechlor® (Kanegafuchi, Japan), Pyralene® (Prodelec, France), and Sovol® (U.S.S.R.), just to name a few. These mixtures of PCBs quickly gained wide acceptance as industrial products where non-flammability and heat-resistant properties were highly desired. For reference purposes, the aroclor® series manufactured in U.S. can be taken as representative of the range of PCB mixtures available on the world marketplace. Foreign counterparts had different names but their PCB contents were very similar to the corresponding description of the aroclor® series (Cairns *et al*., 1986).

PCBs are among the most stable organic compounds known. They have low dielectric constants and high heat capacities which render them ideal for use in electrical capacitors and transformers. While most individual chlorobiphenyls are solids at room temperature, commercial preparations are generally resins or viscous liquids of

density greater than water. Table 2.4 gives amounts of chlorobiphenyls of different chlorine contents in the aroclors® (Sawhney, 1986; Cairns *et al*., 1986) and Table 2.5 shows some selected properties of 8 common aroclors®.

Table 2.4: Molecular weight, Cl content, and amounts of chlorobiphenyls in aroclors®.

			% in aroclors®				
Chlorobiphenyl	**Mol wt**	**% Cl**	**1232**	**1242**	**1248**	**1254**	**1260**
Monochlorobiphenyl	188.65	18.79	31	3	ND	ND	ND
Dichlorobiphenyl	223.10	31.77	24	13	2	ND	ND
Trichlorobiphenyl	257.54	41.30	28	28	18	ND	ND
Tetrachlorobiphenyl	291.99	48.56	12	30	40	11	ND
Pentachlorobiphenyl	326.43	54.30	4	22	36	49	12
Hexachlorobiphenyl	360.88	58.93	<0.1	4	4	34	38
Heptachlorobiphenyl	395.32	62.77	ND	ND	ND	6	41
Octachlorobiphenyl	429.77	65.98	ND	ND	ND	ND	8
Nonachlorobiphenyl	464.21	68.73	ND	ND	ND	ND	1

Note: *ND means none detected*

Table 2.5: General physical and toxicological properties of various aroclors®.

Aroclor® no.	**Form**	**Sp. gravity**	**Distillation Range (˚C)**	**Rats oral LD_{50} (mg/kg)**	**Rabbits skin MLD (mg/kg)**
1221	Clear, mobile oil	1.182-1.192	275-320	3,980	>2,000
1232	Clear, mobile oil	1.270-1.280	290-325	4,470	>1,260
1242	Clear, mobile oil	1.381-1.392	325-366	8,650	>794
1248	Clear, mobile oil	1.405-1.415	340-375	11,000	>794
1254	Light yellow viscous oil	1.495-1.505	365-390	11,900	>1,260
1260	Light yellow, soft sticky resin	1.555-1.566	385-420	10,000	>1,260
1262	Light yellow, soft sticky viscous resin	1.572-1.583	395-425	11,300	>1,260
1268	White to off-white powder	1.804-1.811	435-450	10,900	>2,510

PCBs undergo several processes of degradation either through biological or physicochemical means. Investigations on biodegradation of PCBs in soils, sediments, lakes, and rivers showed that both aerobic and anaerobic micro organisms decompose and metabolize PCBs. A number of reports showed that microbial degradation of the lower chlorinated biphenyls occur at a faster rate than the higher chlorinated biphenyls (Furukawa and Matsumura, 1976; Metcalf *et al*., 1975; Baxter *et al*., 1975; Clark *et al*., 1979; Liu, 1982; Hankin and Sawhney, 1984). Not only does the degree of chlorination influence biodegradation, but the environment also affects biodegradation (Hankin and Sawhney, 1984; Iwata *et al*., 1973). As PCBs are soluble in lipids, they can be accumulated by a number of organisms according to their lipid/water partition coefficients ($K_{o/w}$). Chlorobiphenyls show selective bioaccumulation and degradation, which are affected by both the chlorobiphenyls and the animal species. Hansen and co-workers had reviewed various factors, including animal species, size of adipose compartments, enzyme activity etc., that affect bioaccumulation and biodegradation by animals. Accumulation ratios of chlorobiphenyls vary among different animals as well as in lipids from different parts of an animal (Hansen *et al*., 1983).

Another possible route of environmental breakdown of PCBs is photochemical degradation. Photochemical degradation of PCBs is influenced by the degree of chlorination (Hannan *et al*., 1973), positions of chlorine substitutions in the ring (Bunce *et al*., 1978; Ruzo *et al*., 1974; Wagner, 1967), and the presence of organic compounds that sensitize the photo reaction (Zepp *et al*., 1981; Occhiucci and Patacchiola, 1982; Nordblom and Miller, 1974; Carey *et al*., 1976).

Because of the stability and potential toxicity of PCBs, numerous laboratory experiments have been conducted for the combustion and complete destruction of these compounds for safe disposal of industrial wastes and used products. While pyrolisis at below 700 ˚C produces various toxic materials, higher temperatures decompose PCBs completely (Buser and Rappe, 1979; Buser *et al*., 1978). Many

chemical procedures of complete dechlorination of PCBs using catalysts have also been developed (Berg *et al*., 1972; Dennis *et al*., 1979).

Grave concerns about the environmental fate of such vast quantities of PCBs and their resultant toxicological effects were alerted by the first reported findings of PCBs in fish and wildlife by Jensen in the 1966. Soon it became obvious that the widespread of PCBs usage had led to their incorporation as persistent and ubiquitous contaminants on a global scale (Richardson and Waid, 1982). In the ecosystem, PCBs had become the most abundant of the chlorinated aromatic pollutants (Risebrough *et al*., 1968), rivalling DDE. Though the US environmental Protection Agency (US EPA), under the provisions of the Toxic Substances Control Act, specifically banned most of the uses of PCBs in 1997, their presence are not decreasing since they have half-lives in the order of several years. PCBs circulate in the environment from soil to air and back to soil again (Ahmed, 2003). Unfortunately, indirect contamination such as leaching by PCBs had eventually led to their presence in the food chain (Biros *et al*., 1970).

Shortly after Jensen's report of PCBs, an unfortunate accident happened in Japan in 1968. PCBs had accidentally leaked from a heat exchanger used in the production of rice oil. Resultant levels of PCBs in the rice oil, when ingested, produced a spectrum of adverse symptoms: chloracne, discoloration of the gums and nail beds, swelling of the joints, waxy secretions of the glands in the eyelids, as well as more general manifestations such as lethargy joint pain (Cairns *et al*., 1986). This single historical event ultimately prompted regulatory monitoring and intervention by various bodies. The U.S. Food and Drug Administration (FDA) initiated a national survey to determine the exact extent and levels to which PCBs might have made their way into the food chain by indirect use of PCB contaminated animal feed, industrial and environmental sources, and the use of PCB-containing paper food packaging materials. Several accidents similar to the "Yusho" incident had, however directly contaminated animal feed and subsequently the poultry and eggs intended for human

consumption. Other parts of the survey indicated that the use of PCB-containing coatings on the inner walls of grain silos had been responsible for PCB residues in milk derived from dairy cows who fed on the grain stored in such silos. Results on food packaging were revealing; 67 % of the samples tested contained PCBs with the highest value found being 338 ppm, and of these samples only 19 % of the actual foods were contaminated at the maximum level of 0.1 ppm (Cairns *et al*., 1986).

In spite of scanty knowledge of toxicological effects of PCB in 1973, the FDA decided to reduce human low-level exposure to PCBs in food containing unavoidable residues from environmental or industrial sources by the establishment of temporary tolerances until additional toxicological data might cause reconsideration (Cairns *et al*., 1986). From then on, numerous tolerance and regulatory limits were set up e.g. national regulatory limits for PCBs in finfish and shellfish range from 500 µg/kg to 5000 µg/kg (with various countries setting their limits depending on fishing grounds and species) and regulatory limits for PCBs in dairy milk in various countries vary from 20 µg/kg to 60 µg/kg (Ahmed, 1999). Coincidentally, recent events, quite similar to the "Yusho" incident occurred in Belgium (dioxin crisis) which prompted the government to impose a norm for PCB (200 ng/g fat) level in foodstuffs containing more than 2 % animal fat (Pirard *et al*., 2002).

Up to the present day, there have been reports (Ahmed, 1999) of PCBs being involved in plethora of short-term and long-term toxicological effects, including skin rashes (chloracne), itching and burning, eye irritation, skin and fingernail pigmentation changes, disturbances in liver function; as endocrine disruptors and environmental estrogens; and, as inducers of cancer, neurobehavioral changes, cognitive dysfunction, reproductive and developmental defects, and immunological abnormalities.

The effect of PCB congeners is not the result of direct DNA reactivity, but involves epigenetic mechanisms based on the induction and their binding to an intercellular protein, the Ah receptor (Ahmed, 1999; Safe, 1995; Alcock *et al*., 1998). A relationship between PCB structure and its stability to stimulate oncogene expression,

reduce the gap-junction-protein level in rat livers and induce mini-satellite mutations in the germ-line of male mice have been reported (Gribaldo *et al*., 1998; Bager *et al*., 1997). There is evidence to suggest that there is a common mechanism of action of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and the coplanar group of PCBs in human biological systems as stated above (Safe, 1995). The so-called 'coplanar or dioxin-like PCBs' include 3 non-*ortho* (PCB 77, PCB 126 and PCB 169), 8 mono-ortho (PCB 105, PCB 114, PCB 118, PCB 123, PCB 156, PCB 157, PCB 167 and PCB 189) and 2 di-*ortho* (PCB 170 and PCB 180) substituted congeners which have been shown in experimental systems to exert a number of responses similar to those observed for 2,3,7,8-TCDD (Alcock *et al*., 1998).

The UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) has recently recommended the use of Toxicity Equivalent Factors (TEFs) to assess the potential toxicity of complex mixtures of dioxin and the coplanar PCBs present in food (COT, 1997). In this way, an Acceptable Daily Intake (ADI) of 10 pg TEQ/kg b.w./day can be used to assess the health risks of intake of mixtures of PCDDs/Fs and PCB congeners. The Toxicity of Equivalents (TEQs) concept uses available toxicological and biological data to generate a set of weighing factors (TEFs) each of which expresses the toxicity of 'dioxin-like' compounds in terms of the equivalent amount of 2,3,7,8-TCDD. Multiplication of the concentration of a congener by its TEF gives a TCDD Equivalent or TEQs (Alcock *et al*., 1998). Table 2.6 shows the coplanar PCBs which have been assigned the following TEFs by WHO (Ahlborg *et al*., 1994).

Table 2.6: Coplanar PCBs with its TEFs.

PCB No	TEF value
77	0.0001
105	0.0001
114	0.0005
118	0.0001
123	0.0001
126	0.1
156	0.0005
157	0.0005
167	0.00001
169	0.01
170	0.0001
180	0.00001
189	0.00001

Since part of the problem with PCBs was the vulnerability of food and feed commodities to direct contamination through accidental causes, the EPA issued rules governing the continued deployment of PCBs in certain industrial applications. These regulatory controls were made under the Toxic Substances Control Act of 1976 and proposed the discontinued use of PCBs in heat transfer systems in plants manufacturing or processing food, drugs, and cosmetics (Cairns *et al*., 1986). Also, a European Community Directive 96/59/EC on disposal of PCBs, with its requirements for the preparation of inventories, labelling of all significant PCB holdings and the tighter regulation of PCB treatment facilities, is committed to phasing out identifiable PCBs by 2010 (Ahmed, 2003).

2.2 Aspects of environmental sampling

Monitoring of aquatic pollution can be carried out by means of bioindicator organisms because hydrophobic compounds such as OCPs show high affinities for lipids. Assuming that bioconcentration is primarily a result of water-lipid partitioning, the pollutant levels in aquatic biota should reflect concentrations in their environment (Pastor *et al*., 1996). Bivalve have been used extensively for this purpose (Goldberg *et*

al., 1978), but fish have also been selected for monitoring because firstly they concentrate pollutants in their tissues directly from water and also through diet which enable the assessment of transfer of pollutants through the trophic food web (Bruggeman, 1982). Secondly they generally exhibit low metabolism for organochlorines and consequently should reflect the levels of parent pollutants in the aquatic environment (Muir *et al*., 1990) and, thirdly they occupy different habitats in the same ecosystem and have different feeding behaviour, thus offering the potential to study the influence of environmental and biological factors on the bioaccumulation of pollutants (Porte and Albaigés, 1993).

Samples of each species should be divided into different size group to investigate the influence of growth on bioaccumulation. Animal size is recognized to be of importance in determining the rate of physiological processes and the allometry of tissue growth, thus influencing uptake, distribution and elimination of hydrophobic pollutants (Swackhamer and Hites, 1988; Barron, 1990; Gutenmann *et al*., 1992).

2.3 Analytical methods for determination of POPs in tissue samples

Analysis of organic pollutants in animal tissue is quite a challenge. Most organic compounds have high log K_{ow} such that they are very soluble in the fatty tissue and tend to accumulate in the lipid. Various methods have been developed and variation of these methods revolved on three main procedure in each method namely extraction of pollutants in tissue, separation of contaminants from lipid and quantitative analysis of the target analytes.

2.4 Extraction techniques

Most extraction techniques have been developed on the basis of a specific requirement. For example, column extraction, where the solvent percolates through the sample in a chromatographic style column was developed to cope with large sample masses. Provided the correct conditions of extraction rate and solvent polarity are

observed, most techniques have been shown to be adequate for this purpose. All extraction techniques must be validated either by spiking (Wells, 1994) or by exhaustive extraction (Wells, 1993). Some techniques are specifically not recommended such as the vapour-phase (Bleidner) method for tissue samples (Hess *et al*., 1995).

Extractions generally rely on favourable partition of OCPs and PCBs from the sample into the extraction matrix. The more favourable the partition coefficient, the higher the extraction efficiency. Efficiency is also improved by repeated extractions (Ahmed, 2003). The character of the food matrix, especially the fat content of the commodity, dictates the method(s) to be employed for isolation, clean-up and analysis of sample (Motohashi *et al*., 1996). Since OCPs and PCBs are highly lipophilic, the extraction methods are based on the isolation of lipids from the sample matrix (Erickson, 1997). The extraction step is the rate-limiting factor when analyzing a large number of samples, taking into account recent advances in the detection capabilities of instruments (Ahmed, 2003). Extractions of lipophilic organic contaminants from the matrix are both kinetically and thermodynamically controlled. Thus, increasing the polarity or matching the solvent to determinant, e.g. dichloromethane or toluene, may not significantly reduce the required contact time. Organic contaminants do not just reside on the outside surfaces of the matrix to be “washed” off. Cells in tissues must be penetrated by the solvent which must subsequently be replaced to effect a complete extraction (Hess *et al*., 1995).

2.4.1 Soxhlet extraction

Soxhlet extraction is one of the most frequently used liquid-solid extraction method developed in the late nineteenth century and is still routinely used for extraction of PCBs from several food matrices. Soxhlet extraction has been used for the isolation of non-polar and semi-polar trace organics from a wide variety of sediments, soils, animal and plant tissues (Hess *et al*., 1995). The size of the soxhlet systems can vary,

but the more common configurations use between 100 and 200 mL solvent to extract between 20 and 200 g of sediment and 1 and 100 g of biological tissue. Larger systems can be used, but require proportionally more solvent which would be quite costly (Hess *et al*., 1995). Soxhlet extraction normally uses polar organic solvents or mixture (dichloromethane, hexane-acetone, hexane-dichloromethane) which would require effective clean-up procedures (Ahmed, 2003).

Non-polar solvents like *n*-hexane have also been used to extract non-polar contaminants like OCPs and PCBs. While these solvents are relatively efficient for removing organochlorines from fatty tissues which have a predominance of triglycerides, they are not completely extracted from low fat tissue. de Boer made a comparative study of the extraction efficiency of different solvents for PCBs in fatty and lean fish tissues. The comparison was made between pike perch, perch, bream, roach and eel and the solvents were *n*-pentane, *n*-pentane–diethyl ether (1:1), dichloromethane and acetone–*n*-hexane (1:9). He also compared these extractions with the saponification of the tissue with 40 % potassium hydroxide in ethanol (1:1) at 90 ºC for 4 hours prior to extraction. He found that treatment lead to loss of some PCBs, and dechlorination and hydrolysis of highly chlorinated PCBs though higher recoveries were obtained. The main conclusion of this work was that samples should be left for a minimum of 2 hours to dry completely after grinding with sodium sulphate and a longer time was unnecessary. Extractions with non-polar solvents like *n*-alkanes took considerably longer time (> 6 hours) and were not as effective as polar solvents such as dichloromethane in removing the PCBs and the lipid (de Boer, 1988). This was less evident for fatty (triglyceride) tissue, reflecting the relative distribution of PCBs bound onto the phospholipids and the partition into the neutral lipids (Hess *et al*., 1995).

2.4.2 Matrix solid-phase dispersion (MSPD)

Matrix solid-phase dispersion is a more recent technique for extraction and clean-up of PCBs and pesticides in fish tissue (Ling *et al*., 1994). This technique allows the extraction of these contaminants from homogeneously dispersed food samples in a solid support such as the synthetic magnesium silicate (florisil) or silica (C_8 or C_{18}). The homogenized matrix is placed in a glass-syringe-barrel column and PCBs are selectively eluted with organic solvents. Thus, sample extraction and clean-up are carried out in the same step with good recovery and reproducibility, reducing the analysis time and the amount of solvent employed.

Multi-residue methods based on MSPD using alumina, silica and florisil were known to be employed for analyses of pesticide residues in vegetables. It has been reported that although recoveries using all sorbents were similar, extracts from florisil were the cleanest (Viana *et al*., 1996).

An alternative application for MSPD has been employed. This extraction was applied to analysis of 24 organochlorine pesticide residues from milk dispersed on solid matrix diatomaceous material (e.g. hydromatrix) fitted into disposable cartridges by means of light petroleum saturated with MeCN and ethanol. The extraction procedure took about 30 min, without the need for sample clean-up and no emulsions occurred. These developments with new adsorption materials are suited for extraction of fatty samples (Muccio *et al*., 1996).

2.4.3 Supercritical fluid extraction (SFE)

SFE has attracted intense interest during the past 20 years, mainly for extraction of solid samples, because it offers short extraction times and minimum use of organic solvents (Ahmed, 2003). Apart from these advantages, SFE is also popular because of its high degree of selectivity (Lehotay, 1997). This technique uses supercritical fluid which is defined as an element or compound above its critical pressure and temperature (Ahmed, 2003). It has similar densities to liquids, but lower

viscosities and higher diffusion coefficients. This combination of properties results in a fluid that is more penetrative has a higher solvating power and extracts solutes faster than liquids (Hess *et al*. 1995). Using automation, SFE reduces labour and laboratory space. In principle, SFE allows for a higher degree of selectivity in extraction as compared with solvent-based procedures (Ahmed, 2001). This extraction when combined with solid sorbent traps will give a single-step extraction and clean-up (Hajslová, 1999). An advantage of SFE is that extracts are very clean and require only moderate additional clean-up or none at all in certain cases. However, the small volume of the extractor, which contains only a few grams of the material, is a disadvantage when a higher sample mass is required (Ahmed, 2001).

CO_2 is frequently used as a supercritical fluid because of its low cost, availability and safety, and its suitable critical temperature (31.2 ºC) and pressure (72.8 atm; 1 atm=101.325 Pa). CO_2 can easily be removed by reducing the pressure. A CO_2 density of 0.8 – 0.9 g/mL appears to be adequate for most pesticides (Ahmed, 2001). Extraction volumes vary from 1.3 to 7.2 vessel volumes depending on the instruments, pesticides and matrices (Lehotay, 1997). Other less commonly used fluids include nitrous oxide, ammonia, fluoroform, methane, pentane, ethanol, sulphur hexafluoride and dichlorofluoromethane. Most of these fluids are clearly less attractive as solvents in terms of toxicity or as environmental friendly chemicals.

Satisfactory extraction efficiencies were reported for non-polar to low polar pesticides such as organochlorine and organophosphorus pesticides. For pesticides of high polarity (e.g. metamidophos and amethoate) and especially for metabolites of pesticides, the addition of polar modifiers such as methanol or water to CO_2 enhances its dissolving power. Since SFE with CO_2 also extracts lipids from the matrix, further clean-up may be necessary to remove lipids before GC analysis. For meat and fatty material, separation of lipids from lipophilic insecticides is essential for accurate analysis. Water must be removed before performing SFE because a highly water soluble analyte will prefer to partition into the aqueous phase and its SFE recovery will

be low (Ahmed, 2001). Several drying agents (e.g. Celite 545, a palletized hydromatrix, alumina, florisil, $MgSO_4$ and $NaSO_4$) have been used to control water content, each with its advantages and disadvantages (Lehotay, 1997). For some analytes, a basic modifier (e.g. pyridine or triethyl amine) was found to be more suitable (Wan and Wong, 1996).

Fish and seafood have been the food matrices most commonly investigated with SFE to determine PCBs by employing supercritical CO_2 as the extraction solvent. PCBs dissolve easily in CO_2 and are co-extracted with lipids (Ahmed, 2003). Fat retainers are usually introduced into the extraction thimble to achieve a fat-free extract; the most commonly used fat retainers are basic alumina, neutral alumina, florisil and silica (Järemo *et al*., 2000). In a study carried out by Johansen and co-workers, fish tissue were ground with anhydrous sodium sulphate and basic alumina prior to extracting with CO_2 at a fluid density of 0.57 g cm^{-3} and recoveries ranged between 70 and 86 %. The extracts were cryofocused prior to reinjection by thermal desorption with little or no interference from any lipid. This method of extraction give promising evidence of improved selectivity, but interferences from other co-extracted materials still required further separation in most samples prior to the final determination (Johansen *et al*., 1992). An option for reducing fat co-extraction was the use of milder extraction conditions; however these conditions often result in incomplete extraction of PCBs (Björkland *et al*., 2002).

Solid-phase traps were used with florisil in the extraction thimble as a fat retainer. Clean-up step was achieved by rinsing with *n*-heptane following moderate SFE extraction (pure CO_2 at 60 ºC and 218 bars) to remove fat before on-column injection into gas chromatography. A sulphuric acid clean-up on acidified silica without any fat retainer present in the extraction thimble was employed when too much fat was present in the matrix. Relatively strong SFE conditions for raw, homogenized fish samples contaminated with PCB employed pure CO_2 (0.5 min static step followed by a 20 min dynamic step at 100 ºC and 345 bar) with solid-phase trapping on C_{18}-modified