

**HEALTH RISK ASSESSMENT OF PERSISTENT ORGANIC POLLUTANTS
THROUGH CONSUMPTION OF CHICKEN MEAT**

by

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LIST OF ABBREVIATIONS

ADI	Acceptable daily intake
ASE	Accelerated solvent extraction
ATSDR	Agency for Toxic Substances and Disease Registry
BBP	Butylbenzyl phthalate
DBP	Dibutyl benzyl phthalate
DEP	Diethyl phthalate
DEHP	Diethylhexyl phthalate
DMP	Dimethyl phthalate
DOP	Diisooctyl phthalate
EPA	Environmental Protection Agency
EDI	Estimated daily intake
FAO	Food and Agricultural Organization
GC-ECD	Gas chromatograph-electron capture detector
GC-FID	Gas chromatograph –flame ionization detection
LD ₅₀	Acute oral toxicity in rats-lethal concentration 50
LOD	Limit of detection
MAE	Microwave-assisted extraction
MDL	Method Detection Limit
MRLs	Minimum risk levels
OCPs	Organochlorine pesticides

OPPs	Organophosphorus pesticides
PAE	Phthalate esters
PCBs	Polychlorinated biphenyls
PCDF	Polychlorinated dibenzo-p-furans
PLE	Pressurized-liquid extraction
POPs	Persistent organic pollutants
SFE	Supercritical fluid extraction
TDS	Total diet study
TEF	Toxic equivalent factor
TEQ	Toxic equivalent quotient
UNEP	United Nations Environment Programme
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

PENILAIAN RISIKO KESIHATAN BAHAN-BAHAN PENCEMAR ORGANIK PERSISTEN MELALUI PENGAMBILAN DAGING AYAM

Abstrak

Bahan-bahan pencemar organik persisten (POPs) di dapati tersebar dengan meluasnya di alam sekitar termasuk makanan. Bahan pencemar organik persisten mempunyai ciri-ciri terkumpul dan kesan sampingan kepada kesihatan terutamanya sistem endokrin manusia melalui pemakanan. Makanan seperti ikan dan ayam di dapati mengandungi bahan pencemar organik persisten.

Empat kumpulan bahan pencemar organik persisten termasuk polychlorinated biphenyls (PCB), organochlorine pesticides (OCP), organophosphorus pesticides (OPP) dan phthalate esters telah dianalisis dalam daging ayam. Kepekatan bahan pencemar persisten dalam ayam yang diperolehi dari kajian kemudiannya digunakan untuk mengangkar tahap penilaian risiko kesihatan melalui pengambilan daging ayam yang diperolehi dari sepuluh tempat pensampelan di Perlis. Anggaran pemakanan harian (EDI) yang diperolehi dalam kajian ini adalah rendah berbanding pemakanan harian yang dibenarkan (ADI) mengikut ketetapan Organisasi Kesihatan Sedunia (World Health Organization) dan organisasi-organisasi kesihatan lain. Oleh itu, berdasarkan kajian ini, daging ayam adalah selamat untuk dimakan oleh penduduk Malaysia.

Kaedah analisis yang digunakan termasuk pengekstrakan menggunakan soxhlet, diikuti dengan pembersihan sampel menggunakan florisil kromatografi turus yang kemudiannya dianalisis menggunakan GC dengan pengesan penangkapan elektron (ECD) bagi sebatian PCB dan OCP serta pengesan pengionan nyalaan (FID) bagi sebatian OPP dan phthalate esters. Pelbagai parameter telah dioptimumkan dan prosedur analisis disahkan menggunakan pakuan perolehan semula analit. Nilai TEQ tertinggi bagi PCB 126 (3.86) dan PCB 169 (1.37) pg/g berat basah. PCB 77, PCB 126 dan PCB

169 didapati menjadi penyumbang utama bagi nilai keseluruhan TEQ di dalam sampel ayam. Kepekatan OCP dalam ayam didapati rendah, 0.5-2.3 $\mu\text{g}/\text{kg}$ berat basah . Di antara sebatian OCP yang penting, DDT ditemui pada kadar (0.49-3.14) $\mu\text{g}/\text{kg}$; chlordane (0.22-2.12) $\mu\text{g}/\text{kg}$; HCH termasuk lindane (0.99-4.19) $\mu\text{g}/\text{kg}$ dan endosulfan (0.92-3.12) $\mu\text{g}/\text{kg}$. Sebatian OPP yang dominan dalam semua sampel ayam ialah dichlorvos manakala dibutyl phthalate, butylbenzyl phthalate, diisooctyl phthalate dan diethyhexyl phthalate adalah sebatian phthalate esters yang dominan dalam sampel ayam yang dikaji.

Penentuan sebatian PCB dalam ayam menunjukkan hasil yang setanding dengan kajian PCB dalam ikan. Kiraan pengambilan makanan harian (EDI) bagi PCB adalah rendah berbanding dengan pemakanan harian yang dibenarkan (ADI) iaitu 4 pg TEQ/kg bw/day. Anggaran pengambilan makanan harian (EDI) untuk OCP dalam kajian ini juga adalah lebih rendah berbanding pemakanan harian yang dibenarkan (ADI). Bagi OPP, pengambilan makanan harian untuk dichlorvos adalah lebih tinggi berbanding pemakanan harian yang dihadkan di tiga lokasi. DEHP, BBP, DOP dan DEP untuk pengambilan makanan harian dalam semua sampel ayam adalah lebih rendah dari MRL (minimum risk level).

HEALTH RISK ASSESSMENT OF PERSISTENT ORGANIC POLLUTANTS THROUGH CONSUMPTION OF CHICKEN

Abstract

Persistent organic pollutants (POPs) are widely dispersed throughout the environment including food. Due to their accumulative properties and well documented endocrine disruptive toxicities, there are concerns on health effects on human through dietary intake. Foods such as fish and chicken have been found to contain significant levels of POP chemicals.

Four groups of POPs consisting of polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), organophosphorus pesticides (OPPs) and phthalate esters were analyzed in chicken meat. The concentration levels were determined and then used to assess the health risk which would arise through daily consumption of chicken collected from ten sampling locations in Perlis. The estimated daily dietary intakes in this study were generally lower than the acceptable daily intake (ADI) recommended by World Health Organization (WHO) and other health governing organizations, suggesting that chickens are safe to Malaysians through daily intake.

The analytical methods for analyses of these contaminants in chicken involved soxhlet extraction, sample clean-up using florisil column chromatography and quantitation using GC-ECD for PCB and OCP compounds while GC-FID for OPP and phthalate esters. Various parameters were optimized and the analytical procedure was validated using spiked standard solutions of target analytes. The highest TEQ value was PCB 126 (3.86) and PCB 169 (1.37) pg/g ww. PCB 77, PCB 126 and PCB 169 were found to be the major contributor for the high TEQ value of PCB contamination in the chicken samples. Concentration of OCPs in chicken were found to be lower in the range

of 0.5-2.3 $\mu\text{g}/\text{kg}$ ww compared to reported concentration of OCPs in fish from several studies in Malaysia. Among the important OCPs, DDT were found in the range of 0.49-3.14 $\mu\text{g}/\text{kg}$; chlordane in the range 0.22-2.12 $\mu\text{g}/\text{kg}$; HCH including lindane in the range 0.99-4.19 $\mu\text{g}/\text{kg}$ and endosulfan in the range 0.92-3.12 $\mu\text{g}/\text{kg}$. For the OPP compounds, dichlorvos was dominant in all the chicken samples. Dibutyl phthalate, butylbenzyl phthalate, diisooctyl phthalate and diethylhexyl phthalate were the dominant phthalate esters in the chicken samples.

Concentrations of PCB compounds in chicken were comparable to results on fish. The calculated daily intakes of PCB through consumptions of chicken meat were generally lower than the upper limit of the ADI (4 pg TEQ/kg bw/day). The estimated daily intakes for each of the OCPs in chicken were much lower than the acceptable daily intake (ADI). For OPPs, the estimated daily intake for dichlorvos was slightly higher than the acceptable daily intake at three locations. The estimated daily intakes for DEHP, BBP, DOP and DEP in all the chicken samples were much lower than the minimum risk level (MRL).

CHAPTER 1

INTRODUCTION

1.0 Overview

Persistent organic pollutants (POPs) such as organochlorines pesticides (OCPs), organophosphate pesticides (OPPs) and polychlorinated biphenyls (PCBs) have been extensively used worldwide (Cairns & Siegmund, 1981). Applications of OCPs and OPPs as pesticides and PCBs as industrial fluids have however been limited since 1970s when these chemicals were discovered to be highly toxic to the environment and human health as well as being highly persistent in nature. Other negative properties such as bioaccumulation and the ability of long-range migration have placed these chemicals in a class of toxic substances called persistent organic pollutants or POPs.

When human are exposed to these POPs, either through inhalation or ingestion, their endocrine systems, paracrine systems, nervous systems, immune systems and brain functions may be affected (Johnson et al., 1998, Dewailly et al., 2000; Longnecker et al., 2001). Studies have revealed that measurable quantities of persistent organochlorines were found in human adipose tissue, blood and human milk (Chikuni et al., 1991; Lederman, 1996; UNEP, 2002). A study in China reported relatively high concentrations of dichlorodiphenyltrichloroethane (DDT), chlordanes and PCBs in human milk (Kunisue et al., 2004). High concentrations of DDTs were also found in human tissues from China (Nakata et al., 2002). Concentrations of hexachlorocyclohexanes (HCHs) as high as 1400 µg/kg were also reported in food such as fish and eggs (Chen & Gao, 1993).

Another common POP chemicals are the phthalate esters which are mainly used as fillers or plasticizers in polyvinyl chloride (PVC) and other polymers. Their uses in medical devices, toys, cars, vinyl products and plastic bags explains their ubiquity in the environment. In recent years, studies on phthalates contamination revealed that even at low concentration levels, they affected the endocrine system (Petrovic et al., 2001).

In Malaysia, organochlorine and organophosphate pesticides have been largely used in the agricultural sector by farmers. Although Malaysia is heading towards becoming an industrial nation, agriculture still remains a major economic activity. About 41% of the land area is cultivated with various crops such as oil palm, rubber, rice, cocoa, coconut, fruits and other cash crops (FAO, 2004). Some POP chemicals such as DDT, dieldrin and endrin had been in use following the Second World War until they were banned or de-registered. For example, the rice fields were profusely sprinkled with organochlorines such as dieldrin, lindane, and endosulfan to control pests and DDT was used to control malaria (Tan & Vijayaletchumy, 1994; Tan et al., 1973).

Other POP chemicals such as PCB compounds was used predominantly in electrical transformers and capacitors until prohibited in 1995 (Hashim, 2001). Even though most countries including Malaysia have restricted or banned the use of some of these POP chemicals for more than a decade, relatively significant amount were still found in river water, sediments and even in food such as fish and marine organisms (Iwata et al., 1994; Abdullah, 1995; Tanabe, 2002; Muhammad, 2006).

There is limited information concerning the sources, fates and effects of POPs in Malaysia. A number of studies have been carried out on the contamination of POP pesticides in the northern area with pesticide residues remaining highly abundant in soils

and water (Abdullah, 1995; Hossain et al., 1999). DDT (0.8 µg/kg) and endosulfan (5.4 µg/kg) were also detected in rice fields and marine fish (Abdullah, 1995). Hossain et al. (1999) reported concentration of OCPs in sediments were found to be 1.7 µg/kg for endosulfan and 0.9 µg/kg for HCH. Levels of OCPs in several rivers in the northern area conducted in our laboratory were found to be in the range of 0.09 µg/L (Tan, 2001). Fishes are suitable indicators for environmental pollutions because they concentrate pollutants in their tissues directly from the aquatic environment, and since these aquatic animals live in contaminated water, studies had also been carried out in our laboratory on levels of POPs in fish and marine organisms (Muhammad, 2006).

Fishes are one of the main sources of protein in Malaysia and Southeast Asia Region. However, chicken meat and its processed chicken products have become a popular option for source of protein in Malaysia. Chicken consumption on a per person basis rose from 20.1 kg in 1990 to 31.8 kg in 2005 (FAO, 2007). Chicken meat is the main meat type consumed in Malaysia, accounting for more than 60 % of total meat consumption. Thus it is important that the level of persistent organic pollutants in chickens to be known in order to assess the risk of POPs exposure through consumption of chicken.

1.1 Objective of research

The objective of this study is to investigate the levels of OCPs, OPPs, PCBs and phthalate esters in chicken meat, and to determine the health risk arises from daily consumption of chicken. The data on OCPs and PCBs levels in chicken will be compared to other collected data on food such as fish and marine organisms. This study includes validation of method for the analysis of OCPs, PCBs, OPPs and phthalate esters in chicken meat. Health risk assessment of these POP chemicals on Malaysian population will be calculated and compared to the available Acceptable Daily Intake (ADI), Standards and Minimum Residual Level (MRL) when applicable. Chicken samples from various secondary sources available in northern states of West Malaysia will be analysed.

CHAPTER 2

LITERATURE SURVEY

2.0 Introduction to persistent organic pollutants (POPs)

Persistent organic pollutants (POPs) are defined as organic compounds that are extremely difficult to break down, accumulate in the fatty tissue of living organisms, toxic and have become widely dispersed throughout the environment (UNEP, 2002). POP chemicals include pesticides such as OCPs and OPPs, and non-pesticides such as PCBs and phthalate esters. Pesticides are chemical substance used for preventing, destroying, repelling or mitigating pests such as insects, weeds, and rodents. The use of pesticides throughout the world became popular in agriculture to obtain higher yields. Currently about 759 chemical and biological pesticides are used worldwide in the agriculture and health sectors. Among the compounds, 33 pesticides have been classified by World Health Organization (WHO, 1998) as extremely hazardous to human health, 48 pesticides as highly hazardous, 118 pesticides as moderately hazardous, 239 pesticides as slightly hazardous and 149 pesticides have been considered as unlikely to cause hazard in normal use (Xavier et al., 2004). According to World Resource Institute, in 1995, world pesticide consumption was 2.6 million metric tons, and 85 % of this consumption was used in agriculture (WHO, 1998). These pesticides were mainly used in developed countries like North America, Western Europe and Japan. Although the use of pesticides is small in developing countries relative to developed countries, it is rapidly growing and substantial to cause long term effect to the environment and human health.

PCBs and phthalate esters have wide applications in the industries, and they are commonly used in capacitors, transformers, lubricants and plasticizers. Both of these groups of persistent chemicals can enter the environment through improper disposal of industrial and domestic wastes, such as transformer fluids; through leaks or fugitive emissions from electrical transformers containing PCBs; and by disposal of PCB-containing consumer products in municipal landfills. The chemical stability of these synthetic compounds accounts for their persistence in the environment. Low levels of PCBs can be found throughout the world often far away from known sources of PCB. PCBs in water or on soil surfaces evaporate and return to earth by rainfall or settling of dust particles. Indirect contamination can occur such as leaching by PCBs which eventually led to their presence in the food chain (Biros et al., 1970). PCBs are lipophilic; they preferentially separate from water and adsorb to sediment at the bottoms of lakes and rivers. Bottom feeders and other aquatic organisms then ingest and accumulate the compound in their fat tissues. Food can be a major source of human PCB exposure, usually from fish and animal fat. Contamination of PCBs in fish and wildlife was first reported by Jensen in the 1966. When PCBs are burned, they can produce dioxins and furans, which increase the risk of exposure to these toxic compounds.

There are twelve POP chemicals that have been selected under the Stockholm Convention in 2001. The convention is an international treaty to phase out harmful chemicals that persist in the environment. Under this treaty, the signatory countries agree to reduce or eliminate the production, use or release of these 12 POPs. The twelve POPs are aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, toxaphene, polychlorinated biphenyl, mirex, polychlorinated dibenzo-*p*-dioxins (PCDD) and

polychlorinated dibenzo-*p*-furans (PCDF) (USEPA, 2002). Out of the twelve chemicals, nine are organochlorine pesticides. However, many insecticides and nematicides of the organophosphate group, in some cases with much higher toxicity, have not been included on the POP chemicals list (Garrido et al., 2006). Table 2.1 shows status of Stockholm convention POPs in Malaysia, all of which have been banned (UNEP, 2002).

Table 2.1: Status of Stockholm convention POPs in Malaysia

POPs	Status
Aldrin	Banned in 1994
Chlordane	Banned in 1998
DDT	Banned in 1999
Dieldrin	Banned in 1994
Endrin	Withdrawn by company
Heptachlor	Banned in 1990
HCB	Never registered
PCB	Banned 1995

Since there are no manufacturing facilities for these POP pesticides in Malaysia, most of them have been either banned or voluntarily withdrawn by the local importers (Ibrahim, 2007). However, the use of endosulfan and lindane was still being allowed but limited to certain sectors. Endosulfan was not allowed to be used in cocoa and pepper plantations while lindane was only allowed in the oil palm and coconut plantations before it was totally deregistered in 2003 (Sufian Yek, 2005).

Humans are exposed to these toxic chemicals through air, water, soil and food. Many of these POPs become incorporated into the food chain as one animal eats another and the residues accumulate up the animal food chain. Due to the high K_{ow} (partition coefficient in octanol/water system), these compounds accumulate in the fatty tissues of the species. Studies indicated that older and larger species have higher levels of POPs, resulting from bioaccumulation and bioamplification through the food chain (Goldberg et al., 1978; Richardson & Waid, 1980; Hossain et al. 1999; UNEP, 2002). Species higher in the food trophic were found to have much higher levels of POPs resulting in harmful effects to species such as birds and even human. Even though most POPs have low acute toxicities, the accumulative property increases the human health risk when such contaminated foods are consumed on a regular basis.

2.1 Health risk assessment

In order to evaluate the potential of chemical to cause health problems, a method called “risk assessment” was introduced (USEPA, 1993). Health risk assessment generally consists of four basic steps; hazard or toxicity identification, exposure assessment, dose-response assessment and risk characterization. Hazard identification relies on toxicity studies either on exposed human or laboratory animals. Exposure assessment requires concentration data of contamination sources while dose-response assessment estimate the amount of the chemical that is likely to result in a particular adverse health effect in human. Finally, risk characterization is determined based on the available information to describe the resulting health risks that are expected to occur in

the exposed population (California EPA, 2001). Risk is normally characterized as cancer and non-cancer health effect.

Health risk assessment through dietary intake requires information on the toxicity of the chemical contaminants, environmental levels of pollutants, population characteristic such as eating habits, type and amount of food consumed and physical features of the population. Estimated daily intake reported as microgram per kilogram ($\mu\text{g}/\text{kg}$) body weight per day may be calculated from the contamination levels in the food, the amount of food consumed daily and the average body weight of the general population.

In general, for each contaminant, the average daily exposure level through food consumption for a population is calculated using the following equation;

$$\text{Average daily exposure} = \text{Food consumption} \times \text{Contamination concentration}$$

$(\mu\text{g}/\text{kg body weight}) \quad (\text{g}/\text{kg body weight}) \quad (\mu\text{g}/\text{g})$

where food consumption is expressed as daily consumption divided by the body weight.

Estimation of dietary intake plays an important role in ensuring safe food for the human population. In calculating compound residue intake, long-term food consumption habits and not day-to-day variations should be considered to permit valid comparison with the acceptable daily intake (ADI), which is based on intake over a lifetime. Thus, average daily food consumption values are used in predicting residue intake for long-term hazards. Food consumption patterns vary considerably from country to country and even within a country; thus, to a large extent, individual countries must estimate their own consumption pattern. The foods are not necessarily in the forms that people consume, but

rather in raw or semi-processed forms. The best available food consumption data should be used for estimation of pesticide residue intake at the national level.

2.1.1 Acceptable Daily Intake

The Acceptable Daily Intake (ADI) is a measure of the quantity of a chemical that can be consumed everyday for a lifetime without any adverse effect. The Joint Food and Agricultural Organization (FAO) and World Health Organization (WHO) consultation in 1996 agreed on a list of ADI for a number of chemicals, most of which were pesticides (FAO/WHO, 1997). A more comprehensive and up to date list of ADI has been listed by the Office of Chemical Safety, Australia (2006). The starting point for the derivation of the ADI is usually the 'no observed adverse effect level' (NOAEL) that has been observed in animal studies for toxicity. The NOAEL is the highest level of continual exposure to a chemical which causes no significant adverse effect on morphology, biochemistry, functional capacity, growth, development or life span of individuals of the target species used in the toxicology study (PRC, 2006). This is then divided by an uncertainty factor (most often 100) to allow for the possibility that animals may be less sensitive than humans and also to account for possible variation in sensitivity between individuals. In other words, the ADI value has an uncertainty margin of 100 times less for the chemical to cause harm in human as compared to the animal in the toxicology study. The studies from which NOAEL, and hence ADI, are derived take into account any impurities in the pesticide active ingredient when it is manufactured, and also any toxic breakdown products of the pesticide.

2.1.2 Risk characterization

The minimal risk levels (MRLs) were formulated by the Agency for Toxic Substances and Disease Registry (ATSDR) to estimate the maximum daily human exposure to a hazardous substance that will not result in adverse non-cancer health effects. MRLs are more detail in estimating the effect of exposure by defining different exposure duration such as acute (1-14 days), intermediate (15-364 days) and chronic (365 days and longer) and either oral or inhalation routes of exposure. Inhalation MRLs are exposure concentrations expressed in units of parts per million (ppm) for gases and volatiles, or milligrams per cubic meter (mg/m^3) for particles. Oral MRLs are expressed as daily human doses in units of milligrams per kilogram per day ($\text{mg}/\text{kg}/\text{day}$). ATSDR had issued MRLs for 346 hazardous substances including some of the POP chemicals under study as shown in Table 2.2 (ATSDR, 2005).

The MRLs is useful as reference value in evaluating human health from exposure to substances found at hazardous waste sites. However, most MRLs contain some degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants and elderly) to the effects of hazardous substances. There are a number of other approaches to the exposure-response assessment such as Reference Dose (RfD) and Reference Concentration (RfC) set by the US-EPA, Maximum Residual Levels (also MRLs) by Codex Alimentarius commission (CODEX) and pesticide control organization of several countries. For Malaysia, regulation on the food contamination by chemicals is governed by the Food Acts, 1983 and Food Regulation 1985. Unfortunately, the MRL are not completely listed for chemicals mentioned in these Acts (Ibrahim et al., 2006).

Table 2.2: Minimal Risk Level (MRL) values for some OCP, OPP and PAE

OPP	Minimal Risk Levels (mg/kg/day)
Chlorpyrifos	0.003
Dichlorvos	0.004
Disulfoton	0.001
Parathion methyl	0.0007

PAE	Minimal Risk Levels (mg/kg/day)
Diethyl phthalate	7
Di-n-butyl phthalate	0.6
Di-n-octyl phthalate	3
Diethylhexyl phthalate	1

OCP	Minimal Risk Levels (mg/kg/day)
Aldrin	0.002
Chlordane	0.001
p,p'-DDT	0.0005
Dieldrin	0.0001
Endosulfan	0.005
Endrin	0.002
Heptachlor	0.0001
Alpha-HCH	0.008
Beta-HCH	0.05
Gamma-HCH	0.003
Methoxychlor	0.005

2.2 Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls are a class of non-polar compounds that are chemically stable, non-flammable with electrical insulating properties suitable as dielectric fluids in transformers and capacitors, hydraulic and heat exchange fluids, and as lubricating and cutting oils (Ahmed, 2003). PCBs were first synthesized by Grieffs in 1867 and manufactured in 1929 by Monsanto Chemical Company, USA. Since then, over 2 million tons of PCBs were produced as commercial mixtures of 60–90 congeners with trade names such as Aroclors in the United States and Great Britain, Kaneclors in Japan, Clophens in Germany, Fenclore in Italy, Phenoclor in France, and Soval in USSR. The principal manufacturer in the U.S. was the Monsanto Company, the producer of the commercial PCB mixtures known by the trade name Aroclor[®]. Aroclor PCB mixtures were manufactured by chlorinating the biphenyl to specific weight percentages of chlorine. The most common aroclor[®] preparations are Aroclors 1242, 1248, 1254, and 1260. The first two digits signify the biphenyl base while the last two digit represent percentage of chlorine by weight, for example Aroclor 1242 is 42 % chlorine by weight of polychlorinated biphenyl (Sawhney, 1986). One exception to this generality was Aroclor 1016, a distillation product of Aroclor 1242, containing 41 % chlorine by weight but with 1 % of the congeners contain five or more chlorine atoms. The numbering system for Clophen A60, Phenoclor DP6 and Kaneclor 600 indicate an average of six chlorine atoms per molecule, ie. 59 % chlorine by weight. The congener composition of commercial PCB mixtures varies from batch to batch since the extent of their chlorination ranges from 21 to 68 % (w/w). Consequently, commercial PCBs are not based upon composition per se but on the batch's physical properties. In addition, the

composition and amount of impurities also vary from batch to batch, and among manufacturers. For example, most commercial PCBs, except Arochlor 1016, contain polychlorinated dibenzofurans (PCDFs) (Nicholson & Landrigan, 1994).

PCBs are either oily liquids or solids that are colorless to light yellow. Some of the PCBs with lower number of chlorines can exist as vapour in air. PCBs have no known smell or taste. Table 2.3 lists the physical properties of PCBs.

Table 2.3: Physical properties of PCBs

PCB isomer group	Melting point °C	Boiling point °C	Vapor pressure (PA) at 25 °C	Water solubility (g/m ³)	Log octanol-water partition coefficient	Approximate evaporation rate (g/(m ² h))
Biphenyl	71	256	4.9	9.3	4.3	0.92
Mono	25-77.9	285	1.1	4.0	4.7	0.25
Di	24.4-149	312	0.24	1.6	5.1	0.065
Tri	28-87	337	0.054	0.65	5.5	0.017
Tetra	47-180	360	0.012	0.26	5.9	0.0042
Penta	76.5-124	381	0.00026	0.099	6.3	0.0010
Hexa	77-150	400	0.00058	0.038	6.7	0.00025
Hepta	122.4-149	417	0.00013	0.014	7.1	0.000062
Octa	159-162	432	0.000028	0.0055	7.5	0.000015
Nona	182.8-206	445	0.000006	0.0020	7.9	0.0000035
Deca	305.9	456	0.000001	0.00076	8.3	0.00000085

The mean molecular weight of PCBs varies from 188.7 for C₁₂H₉Cl to 498.7 for C₁₂Cl₁₀. Although the physical and chemical properties vary widely across the class, generally PCBs have low water solubility and low vapor pressure. They are highly soluble in non-polar solvents, oils and fats. Due to their high thermodynamic stability, environmental and metabolic degradation is generally very slow (Erickson, 1997).

Theoretically, polychlorinated biphenyls are mixtures of a total of 209 individual chlorinated compounds. Individual PCBs are called congeners, and differ by the positions and numbers of chlorine atoms. However, there are only about 130 of these congeners found in commercial products (UNEP, 2003). The biphenyl backbone of PCBs and the numbering of chlorine position is shown in Figure 2.1.

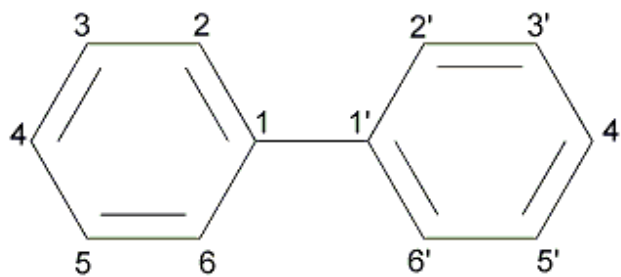


Figure 2.1: Numbering in the biphenyl system.

There are ten possible chlorine positions in the biphenyl rings; 3 monochloro-, 12 dichloro-, 24 trichloro-, 42 tetrachloro-, 46 pentachloro-, 42 hexachloro-, 24 heptachloro-, 12 octachloro-, 3 nonachloro-, and 1 decachloro- giving a total of 209 possible congeners of PCBs. They are normally classified based on the reactivity with respect to the chlorine atom being on the ortho or non-ortho positions. Non-ortho and mono-ortho- substituted PCB congeners can achieve coplanar conformations because the phenyl groups are free to rotate. For easy identification each congener is given a “PCB number”. A full list of the 209 congeners of PCBs are listed in Appendix A.

2.2.1 Sources of exposure to PCBs

PCBs was thought to be the perfect fluid for coolants and lubricants in transformers, capacitors, and other electrical equipments. However, the manufacture of PCBs was stopped in the U.S. in 1977 because it was found that they build up in the environment and caused harmful health effects. Products made before 1977 often contain PCBs such as old fluorescent lighting fixtures and electrical devices containing PCB capacitors, and various hydraulic oils. The estimated half-lives of PCBs range from three weeks to two years in air, more than six years in aerobic soils and sediments with the exception of mono- and di- chlorobiphenyls. PCBs were found to have extremely long half-lives in adult fish. For example, an eight-year study of eel showed that the half-life of PCB 153 was more than ten years (UNEP, 2002).

Humans can be exposed to PCBs either by inhalation, dermal and through consumption of food. PCBs have been known to volatilize and be transported for deposition at sites far from their origin for many years (Reibman et al., 1996). Small amounts of PCBs may leak into the air when old fluorescent lighting fixtures and electrical appliances such as television sets and refrigerators made 30 or more years ago get hot during operation causing skin exposure. PCBs contamination was also the result from intensive industrial use and inadequate disposal over few decades (Tanabe, 1998). PCBs were released to the environment from hazardous waste sites through illegal or improper disposal of industrial wastes and consumer products, and burning of these wastes in incinerators. PCBs could also be released during repair and maintenance of PCB transformers, fires or spills involving transformers, fluorescent lights, and disposal of PCB materials. Food contamination such as in the Yusho incident (1968) in Japan, Yu-

Cheng incident (1979) in Taiwan, and animal feed incident in Belgium caused wide concern on the ill-effects of PCBs to human health (Covaci et al., 2002).

The main dietary sources of PCBs are through consumption of fish especially from contaminated lakes or rivers. In water, a small amount of PCBs may remain dissolved, but most adhere to organic particles and bottom sediments. PCBs also bind strongly to soil. Breathing air near hazardous waste sites and drinking contaminated well water are other sources of PCB exposure (ATSDR, 2000).

2.2.2 Health risk assessment of PCBs

Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) constitute a group of polyhalogenated aromatic hydrocarbons which are toxic environmental contaminants frequently detected at the parts-per-trillion (ppt) level in lipid tissues of animals and humans (Zook & Rappe, 1994). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic congener within this group of compounds and has been associated with a wide variety of health effects including dermal, immunologic, carcinogenic, reproductive, developmental and endocrinologic effects in animals and humans (Birnbaum, 1994, 1995; IARC, 1997). It is believed that the mechanism of TCDD toxicity is via binding with the aryl hydrocarbon receptor (AhR) (Whitlock, 1993).

Several PCDDs, PCDFs, and PCBs have been shown to cause toxic effects similar to TCDD (IARC, 1997). Since TCDD and other dioxin-like compounds exist as complex mixtures of various congeners throughout the environment, calculating total TCDD toxic equivalent (TEQ) concentration is the most relevant exposure measure in studies of

health effects of dioxins and dioxin-like compounds (Van den Berg et al., 1998; Farland et al., 2000). TEQs are calculated values that allow us to compare the toxicity of different combinations of dioxins and dioxin-like compounds. In order to calculate a TEQ, a toxic equivalent factor (TEF) is assigned to each member of the dioxin and dioxin-like compounds category. The TEF is the ratio of the toxicity of one of the compounds in this category to the toxicity of the two most toxic compounds in the category, which are each assigned a TEF of 1 i.e 2,3,7,8-tetrachlorodibenzo-p-dioxin and 1,2,3,7,8-pentachlorodibenzo-p-dioxin. TEFs that have been established through international agreements currently range from 1 to 0.0001. To evaluate the potential risk of PCBs, the concept of toxic equivalence factors (TEFs) has been applied by the World Health Organization (WHO) to four non-ortho substituted PCBs and eight mono-ortho substituted PCBs in 1998 (WHO-ECEH, 1999). Three non-ortho coplanar PCBs, namely PCB-77, PCB-126, and PCB-169 are similar in structure to the most toxic dioxin (Miller, 1983).

TEFs can be used to establish the total toxic equivalence (TEQ) of PCB mixtures present in various matrices such as animal tissues, soil, sediments and water (Danielsson et al., 2004). To determine the TEQ in a sample due to PCB contamination, the measured concentration is multiplied with the TEF value of the corresponding congener (Alcock et al., 1998).

$$\text{TEQ} = \sum \text{PCB} \times \text{TEF}$$

The TEQ values will later be applied in the calculation of the EDI. Estimates of TEQ of PCB exposure can be used in risk equations with the same toxicity metrics (e.g., lethal concentration, cancer slope factor) as would be used for TCDD exposure. For example, if exposure is measured in pg/g chicken, or pg/kg human bodyweight then multiplication of the compound being measured by the TEF expresses the exposure in pg TEQ/g chicken, or pg TEQ/kg human body weight/day which is equivalent to the amount of TCDD to cause similar toxicological effects (Judd et al., 2004).

TEF values are based on 2,3,7,8-tetrachlorodibenzo-p-dioxin (2378-TCDD) toxicity which is set to equal to 1.000. Toxicity studies have shown that not all of these PCB congeners are toxic to living organism. The coplanar configuration or ‘dioxin-like PCBs’ comprised of 12 congeners that had shown some toxicological effects on studied animals and in the determination of human health risk due to PCB (Ahlborg et al., 1994; Van den Berg et al., 1998). These congeners and their toxicity equivalent factor (TEF) are listed in Table 2.4.

Table 2.4: Toxic Congeners of Polychlorinated Biphenyls

PCB Congeners	TEF
PCB 77	0.00010
PCB 81	0.00030
PCB 105	0.00003
PCB 114	0.00003
PCB 118	0.00003
PCB 123	0.00003
PCB 126	0.10000
PCB 156	0.00003
PCB 157	0.00003
PCB 167	0.00003
PCB 169	0.03000
PCB 189	0.00003

The UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) has recommended the use of TEF to assess the potential toxicity of selected PCB congeners present in food (COT, 1997). The daily intake of PCB in food can be calculated based on the TEQ values, the consumption rate of food and the average body weight of the population (Alcock et al., 2000).

$$\text{Daily intake} = \frac{\text{Consumption rate (g/day)} \times \text{toxicity equivalent (TEQ)}}{\text{Body weight (kg)}}$$

The daily intakes calculated were then directly compared with the Acceptable Daily Intake (ADI) issued by WHO for dioxin-like pollutants such as PCB to be between 1 to 4 pg TEQ per kg body weight per day (van Leeuwen et al., 2000).

Malaysia has one of the highest consumption rates in the world for chicken. Per capita consumption of chicken is reported at 32 kg (USDA, 2006) which is equivalent to daily consumption of about 88 g per person. The average weight for Malaysians regardless of gender and ethnic origin is 58 kg (UNU, 1992).

A study in Netherlands on the daily intake by the populations was 0.58 pg TEQ/kg bw/day (Freijer et al., 2001). The estimated dietary intake of PCB TEQ for adults (age 16 and above) in United Kingdom in 1992 was 0.9 pg TEQ/kg bw/day and about 28 % of the total PCB TEQ, was from fish (Alcock et al., 2000). In Japan, a study on the daily dietary intake level for the mean total intake for a total diet study in 1999 and 2000 was 2.25 pg TEQ/kg bw/day for an adult weighing 50 kg. In this estimates, the total intakes were highest from fish and shellfish food group with the value of 1.73 pg TEQ/kg bw/day (Tsutsumi et al., 2001).

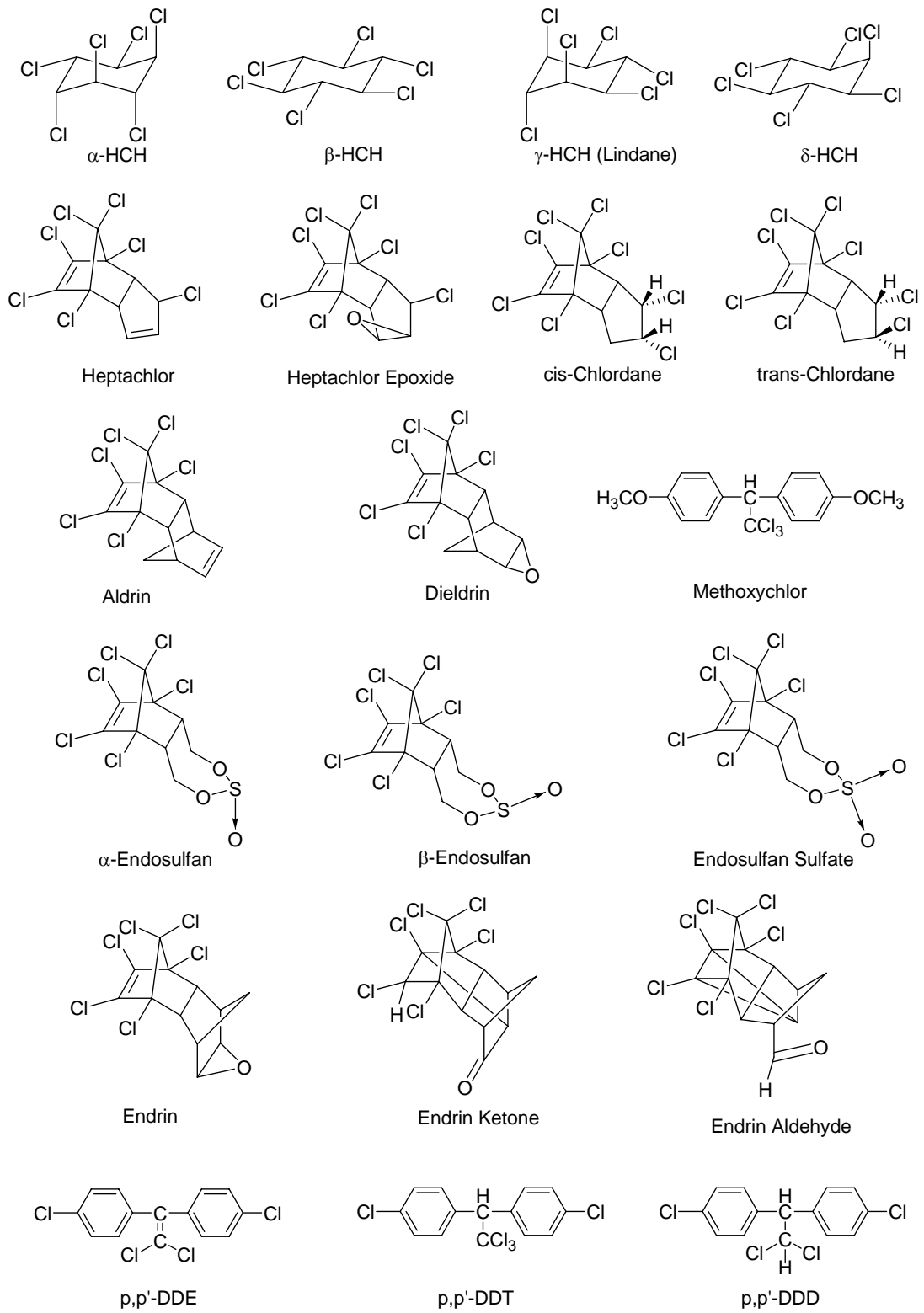
2.3 Organochlorine pesticides (OCPs)

Organochlorine pesticides (OCPs) are organic compounds composed of primarily carbon, hydrogen, and chlorine (Ware, 1991). Structures of some of the OCP compounds in this study are shown in Table 2.5. OCPs were introduced in the 1940s and were mostly used as insecticides in the form of pellet application in field crops and sprays for seed coating and grain storage. Some organochlorines were applied to surfaces to kill insects like the spraying of interior home walls with DDT to control mosquitoes and malaria. Other organochlorines such as chlordane and heptachlor were used to treat wood to prevent pest damage.

Organochlorine compounds are generally very slightly soluble in water but they are very soluble in lipids. For this reason, OCP compounds tend to be stored in animal tissues at levels that depend on the intake, the metabolic rate and the fat contents of the species concerned.

Some aquatic organisms may acquire levels of organochlorine compounds exceeding 10 000 times of the concentration in the water they live (IARC, 1974). The primary concern for the presence of pesticide residues in the environment arise from their toxicity to all living organisms. Hence, in addition to the intended target pests, non-target organisms including humans are exposed to the toxic effects of pesticides, which may include mutagenic and carcinogenic effects. The negative impact of these OCP compounds on the environment and human health have become evident since 1950s (Wong, 2005).

Table 2.5 : Structure of some OCP compounds



USEPA had identified between 18-20 OCPs which are either parent pesticides or more toxic metabolites as OCP priority pollutants. Some of these OCPs have been banned under the Stockholm Convention on Persistent Organic Pollutants (POPs). Nine of the twelve Stockholm Convention POPs are organochlorine pesticides. The well-known organochlorine is the insecticide DDT (Dichlorodiphenyltrichloroethane). In 1939, Muller discovered that DDT was effective as an anti-malaria insecticide in controlling the spread of anopheles mosquito. During World War II, DDT was used excessively to control insects that carry diseases like malaria, dengue fever and typhus. After the war, DDT was widely used on a variety of agricultural crops (NRDC, 2001; Whyllie et al., 2003). This insecticide has also been applied in the developing countries of Asia and Africa because of its low cost and versatility against various pests (Kannan et al., 1997; Konishi et al., 2001). DDT was found to be highly persistent in the environment with a half life of 2–15 years (USEPA, 1989). The technical grade DDT is a mixture of about 70–80 % *p,p'*-DDT and 20 % *o,p'*-DDT. This compound is metabolised mainly to *p,p'*-DDD and *p,p'*-DDE in the environment. Thus, total amount of DDT in the environment would include *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD and *p,p'*-DDD. Degradation product of DDT; *p,p'*-DDE, was found to be more toxic than DDT and highly persistent in fatty tissues (ATSDR, 2002).

The publication of Silent Spring by Rachel Carson in 1962 raised public awareness about the dangers of pesticides and increased interest on persistent organochlorines, especially DDT (Carson, 1962). DDT was banned in many countries since the 1970s in response to public concern and scientific evidence linking DDT with

damage to wildlife. Inclusion of DDT as one of the Stockholm Convention POPs resulted in total ban worldwide on the manufacture and use of DDT.

Other highly persistent organochlorines that have been banned include aldrin, dieldrin, toxaphene, chlordane and heptachlor. Other pesticides listed as priority pollutants such as lindane, endosulfan, and methoxychlor are still being used in some part of the world. 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 epoxide is a more stable breakdown product of heptachlor and would normally be analysed as total heptachlor contamination. Endrin was mostly used against a wide range of agricultural pests. Endrin may be metabolised to endrin aldehyde and endrin ketone which are normally monitored together with endrin. Technical mixture of HCHs (hexachlorocyclohexanes) is a mixture of various isomers, including α -HCH (70 %), β -HCH (7 %) and γ -HCH or commonly called lindane (13 %) and δ -HCH (5 %). Lindane by itself was widely used as insecticide to control a wide range of insects to protect crop seeds, pest control in forests, on livestock and household pets, for control of ticks and other pests, and in homes to control ants and other household pests. It is also the active ingredient in many medicated shampoos and soaps to control head lice and scabies (UNEP, 2003)

Endosulfan was first registered as an insecticide and miticide in the U.S. in 1954. The technical grade endosulfan contains at least 94 % of two isomers, α and β -endosulfan (UNEP, 2003). It was used to control pests in vegetables, fruits, cereal grains, and cotton, as well as ornamental shrubs, trees, and vines. In Africa, it was used in cotton farms while in India it was applied to control pests on cashew plantations. Endosulfans are still