METHOD DEVELOPMENT FOR THE DETECTION OF DICHLORODIPHENYLTRICHLOROETHANE (DDT) METABOLITES IN MECONIUM USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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2007

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by

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Theses submitted in fulfillment of the requirements for the degree of Master of Science

April 2007

ACKNOWLEDGEMENTS

First of all and after all, I acknowledge this work to Allah, The Most Gracious, and The Most Merciful for lightening my way in all my life.

Secondly, several persons have directly or indirectly contributed to my work. They have helped me to bring this work to a fruitful completion. I would like to thank them all, with special thanks and sincere gratitude to the following persons:

My academic supervisor, Professor Dr. Rahmat Awang , Director of National Poison Center, for his guidance, helpful discussions, directive comments and constant support and encouragement along the way.

My academic co-supervisor, Professor Dr. Aishah A. Latif Director of Doping Control Center for giving advice, kind support, directive comments, and fruitful discussions.

Che Nin Man for her help, advising and facilitating the laboratory work. All staff of National Poison Center and Doping Control Center. Dr. T. Arumainathan and Jeleha B. from Maternity hospital, Pulau Penang. My colleagues in PRN Yanie and Abdullah Al-dahbali, my friends Adel Alhaj and Mohammed Aldoh from School of Chemical Science.

Finally, my father who made me reach this level of education, my mother who never stop praying for me, my uncle Abdul-Rahman A.H, my aunt Sh. Alhaj my wife and my children Nada and Almutasem Bellah for their patience, prayers and support.

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CH_2O_2	Formic acid
Comp.	Compound
Con.	Concentration
CV	Coefficient of variation
DCC	Doping Control Centre
DDA	2,2-bis(4-chlorophenyl)ethanoic acid
DCM	Dichloromethane
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethene
DDT	Dichlorodiphenyltrichloroethane
DEP	Diethylphosphate
DETP	Diethylthiophosphate
DMP	Dimethylphosphate
DEDTP	Diethyldithiophosphate
EI	Electron Impact
EISC	Environmental Illness Society of Canada
EPA	Environmental Protection Agency
Extoxnet	Extension Toxicology Network
g	Gram
GC/MS	Gass chromatography-Mass spectrometry
H_3BO_3	Boric acid
HCL	Hydrochloric acid
H_2SO_4	Sulphuric acid
IgG	Immunoglobulin G
IFCS	Intergovernmental Forum on Chemical Safety
INC	Intergovernmental Negotiating Committee
IPCS	International Program of Chemical Safety
IPEN	International POPs Elimination Network
IS	Internal standard
kg	Kilogram
L	Liter

LD50	Lethal Dose, 50% kill
LOD	Limit of Detection
LOQ	Limit of Quantitation
M	Molar
mm	Millimeter
min	Minute
*µ*g	Microgram
mg	Milligram
ml	Milliliter
*µ*L	Micro liter
m/z	Mass to Charge
ng	Nanogram
NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
NTP	National Toxicology Program
OC	Organochlorine
POPs	Persistent Organic Pollutants
QC	Quality Control
RT	Retention Time
rpm	Round per Minute
SAICM	*Strategic Approach to* International Chemicals Management
SD	Standard deviation
SIM	Selective Ion Monitoring
STD	Standard
WFPHA	World Federation of Public Health Associations
WHO	World Health Organization

APPENDICES

PEMBANGUNAN KAEDAH PENGESANAN METABOLIT DIKLORODIFENILTRIKLOROETANA (DDT) DI DALAM MEKONIUM DENGAN MENGGUNAKAN KROMATOGRAFI GAS-SPEKTROMETRI JISIM

ABSTRAK

DDT ialah sejenis racun perosak organoklorin yang telah digunakan secara meluas di seluruh dunia untuk tujuan pertanian dan kawalan malaria sekitar tahun 1950an dan 1960an. DDT telah ditemui oleh Dr. Paul Muller, seorang ahli sains dari Switzerland, pada tahun 1942. Kebimbangan mengenai kesan DDT keatas hidupan liar terutamanya burung pemangsa telah menyebabkan penggunaannya diharamkan di kebanyakan negara dalam tahun 1970an. Namun demikian sesetengah negara masih menggunakan DDT untuk tujuan kawalan malaria.

Tujuan kajian ini dijalankan adalah untuk membangunkan satu kaedah analisis yang mudah, sensitif, dan cepat menggunakan kromatografi gas-spektrometri jisim untuk mengukur metabolit DDT, *o,p*'-DDE dan *p,p*'-DDE. Metabolit tersebut dan bahan-bahan piawai (*o,p*'-DDE C13 and *p,p*'-DDE C13) dalaman telah dimasukkan ke dalam sampel mekonium, diikuti dengan tambahan asid sulfurik. Kemudian proses pengekstrakan cecair-cecair dengan menggunakan heksana telah dijalankan. Natrium sulfat nyahhidrat telah dimasukkan untuk mengeringkan ekstrak. Ekstrak tersebut telah disuntik secara terus ke dalam kromatografi gas-spektrometri jisim dengan menggunakan mod Pemonitoran Ion Selektif. Graf-graf kalibrasi untuk *o,p*'-DDE and *p,p*'-DDE di dalam mekonium telah dilakarkan dengan pekali korelasi (r^2) melebihi 0.996. Pengesanan terendah untuk *o,p*'-DDE dan *p,p*'-DDE ialah 5 ng/g. Pengesanan

kuantitatif untuk *o,p*'-DDE dan *p,p*'-DDE ialah 10 ng/g. Hasilan untuk *o,p*'-DDE adalah dalam lingkungan 89–96%, manakala hasilan untuk *p,p*'-DDE adalah dalam lingkungan 92–97%. Ketepatan di antara satu esei dengan yang lain dan juga di antara setiap ukuran bagi setiap esei adalah kurang daripada 15% manakala kejituan diantara satu esei dengan yang lain dan juga di antara setiap ukuran bagi setiap esei adalah kurang daripada 10% untuk *o,p*'-DDE dan *p,p*'-DDE.

Penggunaan kaedah ini telah diaplikasikan dengan menganalisa 20 sampel mekonium. Kaedah ini boleh digunakan untuk menganalisa DDT di dalam mekonium. Ia menggambarkan pendedahan bayi secara kronik terhadap DDT semasa dalam kandungan. Kaedah ini boleh membantu dalam kajian mengenai ralat metabolisme semulajadi yang berkaitan dengan DDT.

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ABSTRACT

DDT is an organochlorine pesticide that was used heavily worldwide in the 1950s and 1960s both in agricultural production and for malaria control. It was discovered by a Swiss Scientist Dr. Paul Muller in 1942. Concerns about the impact on wildlife population particularly predatory birds led to the phasing out of DDT in many countries in the 1970s, but it is still used for malaria control in some countries.

The aim of this study was to develop a simple, sensitive, and rapid gas chromatography-mass spectrometry (GC-MS) assay for the quantitation of DDT metabolites, *o,p*'-DDE and *p,p*'-DDE. The analytes and internal standard (*o,p*'-DDE C13 and *p,p*'-DDE C13) were spiked into meconium samples; sulphuric acid was added, followed by liquid-liquid extraction using hexane and addition of anhydrous sodium sulfate for drying purposes. The extract was directly injected into the GC-MS where Selective Ion Monitoring (SIM) mode was utilized. Calibration curves of the analytes in meconium were established with linear correlation coefficients (r^2) greater than 0.996. The limit of detection and limit of quantitation for both metabolites were 5 ng/g and 10 ng/g respectively. The recovery of *o,p*'-DDE and *p,p*'-DDE were in the range of 89-96% and 92-97% respectively. Within and between assay accuracy was less

than 15% for the metabolites while within and between assay precision was less than 10% for these metabolites.

The applicability of the assay was demonstrated in 20 meconium samples. This assay can be used for the determination of DDT in meconium. The analysis is useful in determining the chronic exposure of newborns to DDT during the gestation period. This assay can contribute in studies investigating DDT- associated inborn errors of metabolism.

CHAPTER 1

INTRODUCTION

1.1. Pesticides

According to the definition of the Food and Agriculture Organization (FAO) a pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with production, processing, storage, transport or marketing of food, agricultural commodities, wood and woolen products or animal feedstuffs, regulators, or which maybe administered to animal for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as plant-growth regulator, defoliant, desiccant, or fruit tinning agent for preventing of premature fall of fruit and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport (Kaloyanova and El Batawi, 1991).

There are five basic classes of pesticides: Insecticides (e.g organochlorines**,** organophosphates**,** carbamates, pyrethroids, botanical insecticides), herbicides (e.g chlorophenoxy compounds**,** bipyridyl derivatives), fungicides (e.g hexachlorobenzene**,** organomercurials, pentachlorophenol), rodenticides (e.g zinc phosphide**,** anticoagulants**),** fumigants (e.g phosphine**,** ethylene dibromide), (Lavalle, 2003).

Most of pesticides are synthetic but few of them are naturally occurring either as inorganic compounds or plant-derived organic compounds. Historically, human used sulphur as a fumigant pesticide as early as 1000 B.C. Arsenic was used as an insecticide by the year 79 A.D., and by 900 A.D., the Chinese were using arsenic, among other inorganic chemicals as insect killers. Nicotine was the first naturally occurring insecticide used. It was extracted from tobacco leaves by the seventeenth century. Few decades later, mercuric chloride was used as a wood preservative, and a century later copper sulfate was used as insecticide (Waxman, 1998).

By the late 19th century rotenone, pyrethrum, formaldehyde, copper arsenate and lead arsenate were introduced as insecticides. The beginnings of the 20th century noted the discovery and use of organomercurials in the field of pest control. These beginnings and for the first time revealed another side of the story when people were concerned about the possible poisoning that could result from the extensive application of arsenates. Arsenate poisonous residuals were found in fruits and vegetables treated with arsenicals. Research were then carried out to find safer pesticides and consequently organic compounds such as tar, petroleum oils and nitro-o-cresol were discovered and put in to use. Since then, the era of synthetic organic pesticides started. Less dangerous compounds were introduced and these include alkyl thiocyanate, dithiocarbamate and dichlorodiphenyltrichloroethane (DDT). Among these DDT became the most widely used single synthetic pesticide after Muller in 1939 discovered its insecticidal effect (Waxman, 1998).

Advances in the field of synthetic pesticides led to the introduction of organochlorines such as aldrin, dieldrin, heptachlor, and endrin. Organophosphates are also synthetic insecticides. Older compounds in this group were schardan and parathion which were developed in World War II as warfare agents. These compounds were replaced by malathion which was more selective and less poisonous as an insecticide. In fact, malathion was the first organophosphate insecticide that possesses low mammalian toxicity (Waxman, 1998).

Nowadays there are more than 1500 individual pesticidal chemical compound distributed in 55 chemical classes. Pesticides have gained a widespread use in all countries due to their proven effect in vector control and their high effectiveness in agriculture (Kaloyanova and El Batawi, 1991).

However, today pesticide poses a very serious health and environmental problem. When it was introduced about more than fifty years ago there was no knowledge about the basic similarities that realized between humans and other forms of life at a subcellular level. There was also no knowledge of the ability of these chemicals to move from one environment to the next. Today we have clear knowledge and evidence of its properties bioaccumulation, biomagnification; hormone disruptive impact, immunotoxic, neurotoxic, carcinogenic and other cumulative multigenerational health damaging impact (Environmental Illness Society of Canada, 2000).

1.1.1 Health effects

The health effects of pesticides depend on the type of pesticide. Some, such as the organophosphates and carbamates, affect the nervous system. Others may irritate the skin or eyes. Some pesticides may be carcinogens. Others may affect the hormone or endocrine system in the body such as organochlorine (U.S. Environmental Protection Agency (EPA), 2006).

Health effects of pesticides can be the result of both acute and chronic exposures. Acute health effects appear shortly after exposure to these pesticides and include: skin and eye irritations, headaches, dizziness, shaking, stomach cramps, diarrhea, sweating and nausea, weakness, difficulty breathing, mental confusion and disorientation, seizures, coma, and death (Kamrin, 1997).

Chronic health effects may not be apparent until months or years after exposure. Such health effects include reproductive, teratogenic, mutagenic and carcinogenic. Upon these effects many studies in animals were conducted from early time worldwide. Some of these studies have linked pesticides to the effects mentioned above. For example: malathion caused a decrease in the number of pregnancies, litter size, and surviving offspring and also decreases the cholinesterase activity of the fetus, diazinin has some potential to cause mutagenic and teratogenic effects (Kamrin, 1997); dichlorvos has been classified as a possible human carcinogen (Extension toxicology network (Extoxnet), 1996). Fertility was reduced by about 50% at a dose of 22 mg/kg/day of chlordane, it has caused liver cancer in mice (Extoxnet, 1993),

the EPA has classified it as a probable human carcinogen. Hexachlorobenzene (HCB) and dieldrin, have been shown to cause birth effects. In a rat study with HCB, some offspring had an extra rib and cleft palates. Endosulfan was found to be mutagenic to bacterial and yeast cells (Extoxnet, 1993). In several chronic high-dose exposure rat studies with organochlorine compounds such as chlordane, heptachlor, and pentachlorphenol, there were increased incidences of liver tumors (Extoxnet, 1993, 1996; Agency for Toxic Substances and Diseases Registry (ATSDR), 2005). The effect of notorious organochlorine (DDT) will be detailed in the next chapter.

1.1.2 Ecological effects

1.1.2.1. Effect on birds

The avian toxicity of pesticides varies from slightly toxic to highly toxic. However, a majority of organophosphate compounds such as coumaphos, dichlorvos, fonofos, methidathion, and parathion are highly toxic to wild birds, mallard duck and pheasants (Kamrin, 1997). Organochlorine compounds are only slightly acutely toxic to bird. For example, the LC_{50} value for DDT is 1869 ppm in mallard duck The evidence of bioaccumulation is most notable at the top of the food chain in the terrestrial community. Predatory birds contain the highest body burdens and thus suffer the most effects, generally reproductive failure. DDT and other organochlorines can cause reproductive failure by disrupting the bird's ability to mobilize calcium, thus resulting in thin, brittle eggshells that may be crushed by the parents during incubation or attacked by bacteria (International Program of Chemical Safety (IPCS), 1989).

1.1.2.2 Effects on aquatic organism

Pesticides such as pyrethroids, organophosphates , carbamates and the organochlorines range its toxicity to aquatic organisms from moderate to highly toxic. Among these, organochlorine is the only class of pesticides that bioaccumulates. The evidence of bioaccumulation is most notable at the top of the food chain in the aquatic community. Predatory fish contain the highest body burdens and thus suffer the most from reproductive failure. DDT for example concentrates in the egg sac and affects the fish reproduction. In laboratory experiments DDT residue level of 2.4 mg/kg, causes the eggs of the winter flounder to contain abnormal embryos (IPCS, 1989).

1.1.2.3. Breakdown in vegetation

The effects of pesticides in plants depend on several factors such as the rate and frequency of application, the nature of the plant surface, and the weather conditions. Plants absorb pesticides mainly through the roots and translocate them to other parts of the plant. However, these chemicals do not bioaccumulate with the exception of the organochlorines. Organochlorines have been found to accumulate in fruits and vegetables, and as an example, chlorobenzilate residues an organochlorine compound have been found in the peels of citrus (Kamrin, 1997). DDT was found in carrots, radishes, and turnips. There was some accumulation of DDT reported for rice, grain and wheat but the material was found in the roots, not the grain (Extoxnet, 1996).

Years of research that look into the health effects of pesticide use have generated many evidence indicating its adverse effects to human. Despite this, pesticides continue to be used and depended upon for control of various types of pests in both the developed and the developing countries. While its use is considered to be the only way of controlling pests, there need to be strict regulation and enforcement especially in the developing countries whereby access is easy and use is poorly handled.

The concepts of strict regulation and training in the sound management of pesticides have been introduced in many countries, but these initiatives are by no means universal. Detrimental effects on the environment continue to occur particularly as a result of misuse. Ways of counteracting adverse environmental effects have been devised including sophisticated application techniques to reduce the quantities of pesticides applied as well as the introduction of integrated pest control (Awang *et al*., 2004)**.**

The presence of strict control of pesticides is not the absolute solution to the problem. It, however, minimizes the risk of use and decreases the adverse effects on human health. Irrespective of whether the use is with control or with out control, eventually all pesticides will reach into our food and become an important source of human exposure.

1.2 Children being a vulnerable group

Among the humans, children are at a greater risk of exposure to pesticides than adults. Their unique environment and their characteristic behavioural patterns and diets are two important reasons that explain why they differ greatly from adults. Childhood patterns of behaviour often lead to increased levels of exposure to pesticides compared to the adults. For example, infants and young children hands-to-mouth activities are very prominent in children. Infants and children also spend more time at home than adults, often crawling or playing at ground level where pesticide residues in household air, dust, carpets and even toys may be higher. The main difference in the exposure of adults and children to pesticides is in their respective diets and children tend to consume more food per kg of body weight than do adults. Though their diet is less diverse, they, however, have a relatively higher intake of some food items than adults do. In addition, average water consumption, both as drinking-water and as a food component, is relatively higher in children than in adults (Tirado, 2002).

Breast milk and infant formula can be also contaminated with pesticide residues. Women may accumulate fat soluble chemicals during their lifetime. Increased energy expenditure during and after pregnancy can release these fat soluble compounds, exposing the fetus and infant. To compound this problem further almost all pesticides and other pollutants cross the placenta, the natural protective shield for the fetus thus increasing the possibility of children exposure to toxicants (Nuriminen, 1995).

The major hazard of pesticides exposure in children is their chronic effects represented by neurodevelopmental and reproductive effects on human body. These effects are captured by the human body in its fetal life as well as in the neonatal period. It is in these two periods of human life during which all the organ systems are formed and developed.

In the embryo stage, cell growth is particularly rapid and primary differentiation occurs, providing more opportunity for toxicants to cause mutations and congenital anomalies.

In the neonatal stage, organs and tissues, including the nervous system, lung, blood, somatic cells and epithelium, continue to undergo rapid growth or have rapid turnover, thus increasing their vulnerability to toxicants**.** The neonatal stage is also characterized by a highly permeable gastrointestinal tract, which multiply the dangerous impact of toxicants. During the early years of life, most of the development of the nervous system takes place. The nervous system has a limited capacity to repair any structural damage, and therefore brain cells destroyed by chemicals. Failure to establish vital connections between nerve cells may result in dysfunction, which is permanent and irreversible. Children's metabolic pathways, especially in the first few months after birth, are immature and therefore, they may be less able to detoxify and excrete chemicals than adults (Tamburlini, 2002).

However, the major concerns are still the exposure of children to pesticides. It has been recognized that evaluation of children exposure is one of

the ways to control their exposure and consequently to protect them from the harmful effects of pesticides.

Many researches were conducted to measure children exposure to pesticides using several methodologies such as analysis of pesticides in some biological fluids including blood, urine, cord blood and amniotic fluid. Though analysis of pesticides in blood, urine, cord blood can be done to determine exposure, its application is rather limited because it does not reflect long-term exposure. Amniotic fluid is considered to be a better tool as biomarker to measure fetal exposure to pesticides, but its collection is associated with many difficulties and risk to the fetus. Amniotic fluid can be collected easily at delivery; the sample can be easily contaminated.

However, the detection of fetal exposure to environmental toxins still remains a major challenge. Researchers propose that meconium analysis is a promising tool to meet this challenge.

A measurement of pesticides and other chemicals in meconium is likely to be a useful biomarker of direct fetal exposure, because it starts to form by the 12^{th} gestational week and remains in the fetus bowel until after delivery (Moriya et *al*., 1994). A measurement of pesticides in this medium may represent cumulative exposures. In addition, the pesticide metabolites appear stable in meconium over 12 hours at room temperature, which should facilitate ease of incorporation of meconium measurements into research protocols (Whyatt and Barr, 2001).

1.3. International and governmental initiatives in chemical safety

Considering the concern over safety issues involving the use of pesticides, many initiatives at the international level were taken. The first initiative happened in Brazil year 2000, when the Intergovernmental Forum on Chemical Safety (IFCS) meeting convened. The meeting ended up with declaration called "The Bahia Declaration on Chemical Safety" in which IFCS reaffirms government's commitment to the promotion of sound chemical management.

In February 2002, the Governing Council of the United Nations Environment Programme (UNEP) realized the need to develop a Strategic Approach to International Chemicals Management (SAICM). This approach was founded on Bahia declaration and Priorities for Action Beyond 2000 of the Intergovernmental Forum on Chemical Safety.

The World Summit on Sustainable Development (the Johannesburg Summit) in September 2002 endorsed SAICM initiative and set a goal that, by 2020, chemicals are used and produced in ways that lead to the minimization of significant adverse effects on human health and the environment.

In it's forth forum in Bangkok in 2003, IFCS called for conducting surveillance and implementing monitoring systems on the use and impact of pesticides on health and environment. It also called for assessing chemical exposure during preconception, throughout gestation, infancy, childhood and adolescence as a means for children protection.

So far, Stockholm convention is the only regulatory action taken to eliminate the use and production of Persistent Organic Pollutants” (POPs). Stockholm Convention emerged in the 5th session of the Intergovernmental Negotiating Committee (INC) for an" International Legally Binding Instrument for Implementing International Action on certain Persistent Organic Pollutants” (POPs) in 2000 in Johannesburg. INC concluded the need for a convention which objective is to protect human health and the environment from POPs, this convention took place in Stockholm in 2001.

The Stockholm Convention addressed the issue of toxic chemicals by starting with the twelfth most dangerous POPs. Nine of these POPs are pesticides: aldrin, chlordane, DDT (famous for decimating bald eagles, ospreys, and other predatory birds and for contaminating the milk of nursing mothers), dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, and toxaphene. Stockholm Convention adapted an international treaty aimed at restricting and ultimately eliminating POPs’ production, use, release and storage. This convention became an international law by May, 2004. By April 2005, over 90 countries have joined this convention. The convention permits the use of some POPs in countries that requested for such exemption. But it encourages the use of safer alternatives and dictates monitoring the effects, implementing measures for reducing the risk of exposure and assessing the effectiveness of these measures. For example, DDT use was restricted for controlling disease vectors such as malarial mosquitoes, as an intermediate in the production of dicofol in some countries like: Algeria, Bangladesh, China, Costa Rica, Ecuador, Ethiopia, India, Iran, Kenya, Malawi, Morocco, Mozambique, Papua New Guinea,

Russian Federation, Saudi Arabia, South Africa, Sudan, Swaziland, Venezuela, Yemen, Zambia, Zimbabwe. The convention called the countries to report on used amounts of DDT (International POPs Elimination Network (IPEN), 2002).

1.4. Organochlorines as one example of pesticides

Organochlorine (OC) pesticides are based on the benzene ring with one or more chloride atoms attached. They include DDT, aldrin, dieldrin, toxaphene, chlordane, heptachlor. lindane, endosulfan, dicofol, methoxychlor, pentachlorophenol and others. OCs act on neuronal membrane, interfering with the permeability gradients involving the passage of sodium and potassium ions.

Acute poisoning in humans causes dizziness, nausea, twitching of arms and legs, tremors and convulsions, and finally cessation of breathing (Kamrin,1997).

Organochlorines are particularly harmful for all living systems, owing to their high affinity to fatty tissue, and to their persistence in the environment. Their half-lives have been found to be at least 20 years in both soil and water, with some soils retaining as much as 38% of the amount originally applied. Because of their solubility in fats, this group of pesticides can accumulate and transfer from one food chain to another, e.g. from insects to birds, to fish, and thence to larger mammals, including humans. This accumulative properties of OC pesticides, its potential to cause adverse effect in the environment, ecological damage, (decreasing fertility, egg-shell thinning on birds population), led to their environmental ban in 1970s in many countries (Lavalle, 2003).

Among this persistent group of pesticides, DDT is one the pesticide that is heavily used and misused. DDT was first used during World War II for the control of lice and mosquitoes that spread infection involving typhus and malaria. It has also been extensively used for insect control in forestry and agriculture. It accumulated in plants and soil, and transformed through food chain, due to its high persistence (Hardell *et al*., 2002).

The publication of Silent Spring by Rachel Carson in 1962 raised public awareness about the dangers of pesticides, with a specific focus on persistent organochlorines and DDT. Shortly after this, attention has been paid to DDT use as a pesticide. DDT was banned in many countries in the 1970s in response to public concern and mounting scientific evidence linking DDT with damage to wildlife (World Health Organization (WHO), 2004).

DDT was banned In Malaysia in 1999. However, DDT is still used in some developing countries due to the facts that it is cheap, effective and slow in the development of resistance by the targeted pests (www.pesticideinfo.org.; WHO, 2004).

The method developed in this study is a valuable tool to detect DDT and to quantify its presence during the fetal life. As such, it can be used to evaluate the long term exposure to DDT. Longitudinal studies can be designed to find a possible association between DDT exposure *in utero* and the congenital defects.

CHAPTER TWO

LITERATURE REVIEW

2.1. Dichlorodiphenyltrichloroethane (DDT)

DDT is a colourless crystalline organochloride insecticide, that was first created by the reaction of trichloromethanal with chlorobenzene (C_6H_5Cl) in 1874 by a German graduate student Zeidler but its properties as insecticide were discovered only in the late 1930's by the chemist Paul Muller. (http://en.wikipedia.org)

Technical grade DDT is actually a mixture of three isomers of DDT, principally the *p,p*'-DDT isomer (63-70%), with the *o,p*'-DDT(8-21%) and *o,o*'-DDT(0.1-1%) isomers typically present in much lesser amounts (ATSDR, 2003).

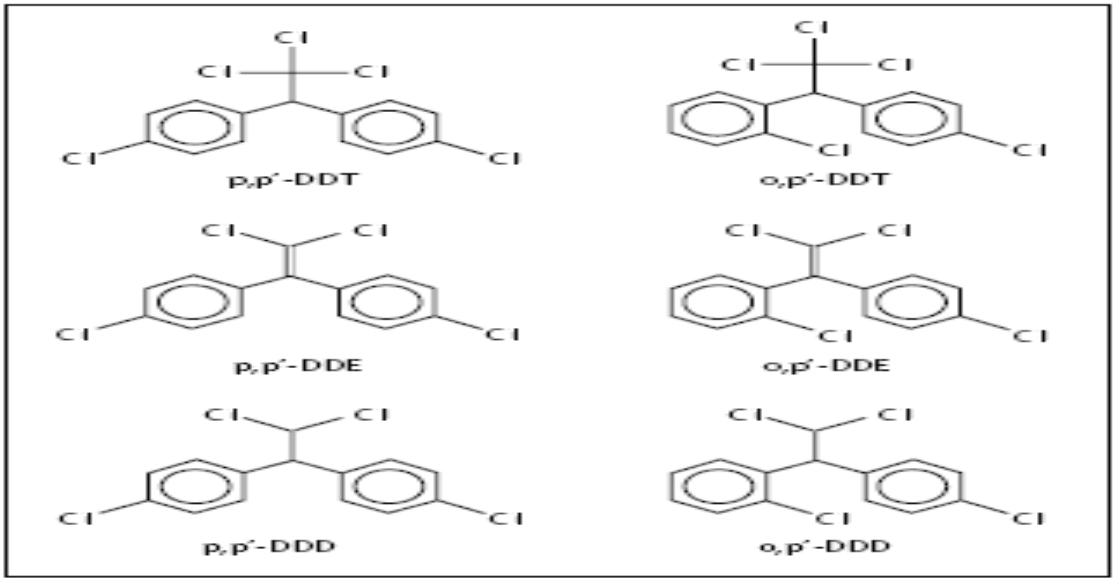

Figure 2.1: Chemical Structures of Isomers of DDT, DDE, and DDD

It is available in several different forms: aerosols, dustable powders, emulsifiable concentrates, granules and wettable powders. DDT is very soluble in fats and most organic solvents and practically insoluble in water (IPCS, 1976; 1979;1989). DDT is persistent in the environment, with a reported half life of between 20-30 years (http://spijker.nl.eu.org/research).

2.1.1. Properties:

Technical grade DDT is a white amorphous powder that melts over the range of 80–94^{o}C. Some physical and chemical properties of *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT, *o,p'*-DDE, and *o,p'*-DDD are listed in Table1(ATSDR, 2003)

Property	***p,p*'-DDT/ o,p'-DDT**	***p,p*'-DDE/o,p'-DDE**	***p,p*'-DDD / o,p'-DDD**
Molecular Weight	354.49	318.03	320.05
Melting Point	109^{o}C / 74^{o}C	89^{o}C / no	109–110^{o}C / 76-78^{o}C
Boiling Point	Decompos-185^{o}C / no	336^{o}C / no	350^{o}C / no
Physical State	Solid	Crystalline solid	Solid
Solubility: In water	0.025 mg/L at 25^{o}C / 0.085 mg/L at 25^{o}C	0.12 mg/L at 25^{o}C / 0.14 mg/L at 25^{o}C	0.090 mg/L at 25^{o}C / 0.1 mg/L at 25^{o}C
Solubility: In organic solvents	Slightly soluble in hydroxylic and polar solvents, very soluble in aromatic and chlorinated solvents	Lipids and most organic solvents	No data
Vapor Pressure	1.60x10^{-7} at 20^{o}C , torr	6.0x10^{-6} at 2^{o}C , torr	1.35x10^{-6} at 25^{o}C , torr

DDT is dehydrochlorinated to form DDE at temperatures above the melting point, especially in the presence of catalysts or light. Solutions in organic solvents are dehydrochlorinated by alkali or organic bases. Otherwise, DDT formulations are highly stable (IPCS, 1976). The compound is also relatively resistant to breakdown by the enzymes found in soil and higher organisms, and DDE is even more resistant (IPCS, 1979).

2.1.2. TOXICOLOGICAL EFFECTS

2.1.2.1. Acute Toxicity

DDT is moderately to slightly toxic to studied mammalian species via the oral route. Reported oral LD50s range from 113 to 1300 mg/kg depending on the species studied (IPCS, 1966; ATSDR, 2003).

DDT in single doses has caused acute effects on the different body systems in experimental animals such as decreased thyroid function (Goldman, 1981), increased blood levels of liver-produced enzymes (Garcia and Mourelle, 1984; Kitchin and Brown, 1988), tremors and leg paralysis (Hietanen and Vainio, 1976; Hong *et al*., 1986; Takayama *et al*., 1999), convulsions (Kashyap *et al*., 1977; Matin *et al*., 1981), aspartate and glutamine were increased (Hudson *et al*., 1985), acetylcholine and norepinephrine were decreased (Hrdina *et al*., 1973), and synthesis of dopamine in dopaminergic neurons was depressed (Leung *et al.,* (2003). Acute exposure of low to moderate doses in humans have resulted in nausea, diarrhea, increased liver enzyme activity, irritation (of the eyes, nose or throat), disturbed gait, malaise and excitability; while tremors and convulsions have been reported at higher doses (Kamrin, 1997).

2.1.2.2. Chronic Toxicity

2.1.2.2.1. Systemic and Neurological Effects

DDT has caused chronic effects on the different body systems in experimental animals. These effects differ in severity with the dose given, the exposure period and the species tested. Among the reported chronic effects

from exposure to DDT ranging from 28 days to 130 month included increased liver weight (Cecil, 1973; Rogan and Chen, 2005), liver damage (Jonsson *et al*., 1981; Takayama *et al*., 1999), increased liver enzyme activity (Kitchin and Brown, 1988; Holloway *et al*., 2005), hepatocytes histopathologic alterations, kidney damage (Ramalingam, 1987), adrenal atrophy, (Chowdhury *et al*., 1990), decreased brain lipid (Sanyal *et al*., 1986). In humans DDT was associated with a permanent decline in neurobehavioural functioning and an increase of neuropsychological and psychiatric symptoms (van Wendel de Joode, et *al*., 2001).

2.1.2.2.2. Immunological Effects

Currently, there is considerable evidence that DDT has adverse effects on the immune system in animals (Rehana and Rao, 1992; Banerjee, 1997; Misumi *et al*., 2005).

In humans *p*,*p´*-DDE has been shown to modulate immune responses whereby IgG levels decreased with increasing *p*,*p´*-DDE levels (Vine *et al*., 2001; Cooper *et al*., 2004).

2.1.2.2.3. Developmental Effects

Gladen *et al*., (2000) reported increased height and weight in boys at puberty who were exposed to DDE *in-utero*. Karmaus *et al*., (2002) found an inverse association between mother's serum DDE levels and height in the girls. Prenatal exposure to *p,p*'-DDE was associated with a delay in mental and psychomotor development (Ribas-Fitó *et al*., 2003).

In animal studies, prenatal and neonatal exposure to DDT and/or its metabolites caused adverse developmental effects. These effects include slowed embryo development (Fabro *et al*., 1984; Alm *et al.,* 1996; Greenlee *et al*., 1999), impaired male sexual development (You *et al*., 1998), decreased prostate weight (Loeffler and Peterson, 1999, You et al., 1999), and impaired learning performance in maze tests (Extension toxicology network, 1996).

DDT exposure to neonates has also been found to increase the susceptibility in adults to short-acting pesticides that have similar neurotoxic action. Johansson *et al*., (1995) found that adult exposure of short-acting pesticide such as bioallethrin to mice neonatally exposed to DDT resulted in irreversible changes to the muscarinic acetylcholine receptor and behavioral disturbances with additional changes two months following exposure.

2.1.2.2.4. Reproductive Effects

There is evidence that DDT causes reproductive effects in different test animals. These effects include thinning of the eggshell and difficulties in hatching in fish-eating bird (Bowerman *et al*., 1995: Turusov *et al*., 2002), reduced fertility (Jonsson *et al*., 1976; Cheek *et al*., 2001), reduced the sperm count (Bayley *et al*., 2001), reduced seminal vesicle and ventral prostate weight (Kelce *et al*., 1995; Loeffler and Peterson, 1999; You *et al*., 1999), reduced testicular weight (Ben-Rhouma *et al*., 2001), reduction in sex organ size and plasma testosterone concentrations (Guillette *et al.,* 1996), premature puberty and irregularities in the estrus cycle (Ottoboni *et al*., 1977; Parent et al., 2005), increased abortion and stillbirth (Khanjani and Sim, 2006).

In humans, DDT exposure has been associated with spontaneous abortion and birth defects (Saxena *et al*., 1981; Korrick *et al*., 2001; Longnecker *et al*., 2003; Mendola *et al*., 2005), DDT reduced duration of lactation and increased incidence of preterm births (Chen and Rogan, 2003). In another study mothers with the highest levels of DDE breastfed for only 3 months on average, compared with mothers with the lowest DDE levels who breastfed for an average of 7.5 months (Gladen and Rogen, 1995).

A large human study has shown associations of maternal *p,p´*-DDE with preterm birth and decreased birth weight (Longnecker *et al*., 2001). Preterm birth may have been the result of DDE blocking progesterone binding to its receptors (Klotz, 1997). Paternal occupational DDT exposure has also been associated with birth defects (Salazar-García *et al*., 2004). Elevated DDE levels in the mother's blood serum correlated with increased incidence of cryptorchidis, hypospadias and polythelia in male children (Longnecker *et al*., 2002).

The report of decreased fertility and increased frequency of stillbirths and birth defects in workers exposed to pesticides in cotton fields supports the hypothesis of a possible role of DDT exposure. In a study in India, a group of men who worked with DDT was found to have decreased fertility and significant increase in stillbirths, neonatal deaths and congenital defects among their children, and Israel men with unexplained fertility problem were found to have high blood levels of DDT (World Federation of Public Health Associations (WFPHA), 2000). Infertility, increased numbers of abnormal sperm and stillbirths

were all with exposure to DDT metabolites (Cocco, *et al* 2005; Charlier, 2005; De Jager *et al*., 2006). Children that were adopted from developing countries and then living in Europe were found to develop early puberty (Virdis et al., 1998; Krstevka-Konstantinova *et al*., 2001).

2.1.2.2.5. Teratogenic Effects

Literature search in the possible effects of DDT towards fetus did not result in much except that (Agency for Toxic Substances and Diseases Registry, 1994) reported abnormal tail development in rats exposed to 10 mg/kg/day of DDT.

2.1.2.2.6. Mutagenic Effects

In animals' studies, mutagenc effect of DDT is contradictory. In some studies chromosomal aberrations have been reported (Kelly-Garvert and Legator 1973; Larsen and Jalal, 1974), but not in others (Legator *et al*., 1973; Palmer *et al*., 1973; Wallace *et al*., 1976).

In human, evidence of mutagenic effects of DDT and related compounds is not conclusive. In some studies chromosomal aberrations and sister chromatid exchanges have been reported in blood cells from subjects occupationally exposed to DDT (Rabello *et al*., 1975; Rupa *et al*., 1988; Rupa *et al*., 1991) and in human lymphocytes exposed *in vitro* to DDT (Lessa *et al*., 1976). In another study no association was found between DDT and the incidence of micronuclei in peripheral lymphocytes (Vine *et al*., 2001). In a recent study concluded by Yanez *et al*., (2004) the association between

DDT and DNA damage in peripheral blood mononuclear cells *in-vitro* and *in-vivo* has been found.

2.1.2.2.7. Carcinogenic Effects

The evidence regarding the carcinogenicity of DDT is equivocal (WHO, 2004). Carcinogenicity has been demonstrated in animals. It has been shown to cause increased tumor production mainly in the liver and lung, but thyroid tumors, adrenal neoplasms, leukemia were also noted in test animals such as rats, mice and hamsters in some studies (Rossi *et al*., 1977; NTP, 1978; Cabral *et al*., 1982; Rossi *et al*., 1983; Takayama *et al*., 1999), but not in others (Adamson and Sieber, 1979, 1983; Tanaka *et al*., 1987). The carcinogenic doses ranged from 0.4 mg/kg/day to 95 mg/kg/day and the exposure duration was between 78 weeks and 130 months.

Although carcinogenicity has been demonstrated in animals, evidence in human is mixed. Some studies have raised serious concerns about adverse health outcomes in humans such as pancreatic cancer (Garabrant *et al*., 1992; Porta *et al*., 1999; 2000). In other studies, no significant associations were seen between DDT exposure and cancer (Baris *et al*., 1998; Cocco *et al*., 1998; Sturgeon *et al*., 1998).

Many epidemiological studies have investigated the association between breast cancer and levels of DDT and DDT-derived compounds in blood or adipose tissue from humans. Some studies have suggested a positive association (Falck *et al*., 1992; Wolff *et al*., 1993; Romieu.*et al*., 2000, Demers

et al., 2000), while others do not support such an association (Lopez-Carrillo *et al*., 1997; Dorgan *et al*., 1999; Helzlsouer *et al*., 1999; Mendonca *et al*., 1999; Zheng *et al*., 1999, 2000).

2.1.3. Toxicokinetics

DDT, DDE, and DDD are all absorbed through exposures from inhalation, oral, as well as dermal. However, absorption through the oral route is considered to be most significant in human. DDT, DDE, and DDD are preferential absorbed by the intestinal lymphatic system (Turner and Shanks, 1980; Noguchi *et al*., 1985; O' Driscoll *et al.*, 1991), and to some extent they are absorbed into the portal blood (Palin *et al*., 1982). Thus, these chemicals are distributed throughout the lymph system as well as to all body tissues. Ultimately regardless of its route of exposure DDT, DDE and DDD are stored in proportion to the lipid content of the tissue. Metabolism of DDT in humans appears similar to that seen in rats, mice, and hamsters, except that not all intermediate metabolites detected in animals have been identified in humans. Excretion of DDT in the form of its metabolites (e.g., DDA and its conjugates) is largely via the urine, regardless of its route of exposure, but DDT excretion may occur via feces, and breast milk (Takei *et al*., 1983; Lunden and Noren 1998). Some experiments have suggested that fecal excretion may be the major route of elimination at high doses (Gold and Brunk, 1982; IPCS, 1984).

2.1.3.1. Absorption

2.1.3.1.1. Inhalation Exposure

Absorption through the lung is considered to be a minor route of entry for DDT. This is thought to be limited absorption probably due to the large particle size of DDT (crystalline) that prevents it from entering deeper, smaller spaces of the lung. Thus, DDT is deposited on the upper respiratory tract mucosa and then eventually swallowed because of the action of the mucociliary apparatus (IPCS, 1999).

2.1.3.1.2. Dermal Exposure

Dermal absorption of DDT in humans and animals is considered to be limited (IPCS, 1999).

2.1.3.1.3. Oral Exposure

In humans, evidence of absorption of DDT, DDE and DDD following ingestion has been demonstrated by measuring the concentrations of these chemicals in serum and adipose tissue, as well as from measurement of DDA in the urine. Development of toxicity following accidental or suicidal ingestion of DDT is an indirect evident of its absorption.

DDT appeared in the serum and reached peak serum concentrations 3 hours after ingestion in subjects chronically exposed to approximately 0.3 mg/kg/day oral doses, serum levels of DDT remained elevated and returned to near pre-dose values 24 hours after each dose (Morgan and Roan, 1971).