UNIVERSITI PUTRA MALAYSIA

RELATIONSHIP BETWEEN LEARNING ABILITY AND GENOTOXICITY FROM ORGANOPHOSPHATE PESTICIDE EXPOSURE AMONG PRIMARY SCHOOL CHILDREN IN TANJUNG KARANG, SELANGOR, MALAYSIA

NURUL HUSNA BINTI MISWON

FPSK(M) 2016 66

RELATIONSHIP BETWEEN LEARNING ABILITY AND GENOTOXICITY FROM ORGANOPHOSPHATE PESTICIDE EXPOSURE AMONG PRIMARY SCHOOL CHILDREN IN TANJUNG KARANG, SELANGOR, MALAYSIA

By

NURUL HUSNA BINTI MISWON

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Master of Science

October 2016

COPYRIGHT

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

RELATIONSHIP BETWEEN LEARNING ABILITY AND GENOTOXICITY FROM ORGANOPHOSPHATE PESTICIDE EXPOSURE AMONG PRIMARY SCHOOL CHILDREN IN TANJUNG KARANG, SELANGOR, MALAYSIA

By

NURUL HUSNA MISWON

October 2016

Chair: Prof. Zailina Hashim, PhD
Faculty: Medicine and Health Sciences

Introduction: Organophosphate pesticides (OPs) mainly malathion are used extensively in agriculture throughout the world and has been linked to neurodevelopmental deficit and genotoxic effect. The effects are particularly profound in children because at this phase they are active, their organ and systems especially their cognitive functions are developing. Depression of blood cholinesterase is used as a biomarker of the OPs toxicity. **Objective:** The aim of the study was to determine the relationship of organophosphate pesticides exposure with learning ability and genotoxicity. **Methodology:** A comparative cross-sectional study was conducted among 150 primary school children. They were selected as a study group as they went to school and lived near paddy field. Another 100 primary school children who were located further away from agriculture site served as the comparative group. The questionnaires were completed by their parents or caretakers. The children's capillary blood was collected using finger prick. The blood cholinesterase levels were determined using cholinesterase test kit LOVIBOND 412870 AF287 and the McCarthy Scales of Children's Abilities (MSCA) was used to determine the learning ability. Two assays were used to determine the chromosome breakage and DNA damage namely the micronuclei and comet assay. Buccal cells were collected from children for genotoxic effect. **Result:** There were significant differences ($p<0.05$) in blood cholinesterase, all scales in MSCA, micronuclei frequency and comet tail length between the study and the comparative group. There were significant relationships between blood cholinesterase and learning ability in all scales in MSCA ($p<0.05$). For genotoxicity assessment, only comet tail length showed significant relationship with blood cholinesterase ($p<0.001$). Other variable significantly influenced the blood cholinesterase and genotoxicity was distance from the house to paddy field ($p<0.05$). Blood cholinesterase was the most significant influencing factor on the all scales in the learning ability ($p<0.05$). **Conclusion**: Blood cholinesterase which indicates organophosphate pesticides exposure had significant relationship with the learning ability scales and genotoxicity in the study group.

***Keyword*:** Organophosphate, learning ability, cholinesterase, genotoxicity

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

HUBUNGAN KEUPAYAAN BELAJAR DAN KETOKSIKAN GENETIK DARI PENDEDAHAN RACUN PEROSAK ORGANOFOSFAT DALAM KALANGAN KANAK-KANAK SEKOLAH RENDAH DI TANJUNG KARANG, SELANGOR, MALAYSIA.

Oleh

NURUL HUSNA MISWON

Oktober 2016

Pengerusi: Prof. Zailina Hashim, PhD
Fakulti: Perubatan dan Sains Kesihatan

Pengenalan: Racun perosak organofosfat (OPs) terutama malathion digunakan secara meluas dalam bidang pertanian di seluruh dunia dan telah dikaitkan dengan kerencatan tumbesaran neuro dan kesan genotoksisiti. Kesan pendedahan akan menjejaskan kanak-kanak kerana mereka aktif, organ dan sistem terutamanya fungsi kognitif masih di peringkat pembesaran. Paras kolinesterase dalam darah yang rendah akan menjadi penanda biologi ketoksikan OP. **Tujuan:** Tujuan kajian ini adalah untuk mengetahui kaitan antara pendedahan racun perosak organofosfat dengan keupayaan belajar dan genotoksisiti. **Kaedah:** Satu kajian keratan rentas perbandingan telah dijalankan dalam kalangan 150 orang kanak-kanak sekolah rendah. Mereka dipilih sebagai kumpulan kajian kerana mereka bersekolah dan tinggal berhampiran sawah padi. Selebihnya, 100 orang kanak-kanak yang tinggal jauh dari kawasan pertanian dipilih sebagai kumpulan perbandingan. Borang kaji selidik telah dilengkapkan oleh ibubapa atau penjaga mereka. Persampelan darah kapilari kanak0kanak adalah melalui teknik tusukan jari. Analisis paras kolinesteras telah menggunakan kit ujian kolinesteras LOVIBOND 412870 AF287 dan ujian keupayaan belajar menggunakan McCarthy Scales of Children's Abilities (MSCA). Dua kaedah asai telah digunakan untuk menunjukkan kerosakan kromosom dan DNA iaitu mikronukleus dan komet asai. Sel bukal telah diambil daripada kanak-kanak untuk mengukur kesan genotoksisiti. **Keputusan:** Keputusan daripada kajian menunjukkan terdapat perbezaan yang signifikan ($p<0.05$) dalam paras kolinesterase, semua skala ujian MSCA, bilangan kekerapan mikronukleus dan panjang ekor komet di antara kumpulan kajian dengan kumpulan perbandingan. Keputusan menunjukkan terdapat hubungan yang signifikan antara paras kolinesteras dalam darah dan keupayaan belajar menggunakan MSCA ($p<0.05$). Untuk penilaian genotoksisiti, hanya panjang ekor komet menunjukkan hubungan dengan kolinesteras dalam darah ($p<0.001$). Faktor lain yang mempengaruhi paras kolinesteras dalam darah dan genotoksisiti adalah jarak di antara rumah dengan sawah padi ($p<0.05$). Paras kolinesteras dalam darah mempengaruhi kesemua skala keupayaan

belajar ($p<0.05$). **Kesimpulan:** Paras kolinesteras dalam darah menunjukkan pendedahan racun perosak organofosfat mempunyai hubungan yang signifikan dengan skala keupayaan belajar dan kesan genotoksisiti dalam kalangan kumpulan kajian.

***Kata Kunci*:** organofosfat, keupayaan belajar, kolinesterase, genotoksisiti

ACKNOWLEDGEMENT

Praise to Allah SWT for all His blessings this project can be completed on time. Appreciations are also extended to the Ministry of Education, Department of Education, State of Selangor and Headmasters of Sekolah Kebangsaan Sawah Sempadan, Sekolah Kebangsaan Dato' Manan, Sekolah Kebangsaan Sri Tiram, Sekolah Kebangsaan Sg Sireh, Sekolah Kebangsaan Tanjung Karang and Sekolah Kebangsaan Batu 9 for their permission in carrying out the project in their schools.

A very special thanks to my parents, husband and family for their support in term of moral and financial as well as their blessings during my project progress. My gratitude is also expressed to parents and children who were involved in this study.

Special gratitude to Nur Naqibah Lokhman, Dr. Vivien How and Pn. Nani Menon for their help and advices. My appreciation to all my lecturers in the Department of Environmental and Occupational Health and friends who were involved directly and indirectly throughout my project. Thank you to all.

I certify that a Thesis Examination Committee has met on (date of viva voce) to conduct the final examination of Nurul Husna binti Miswon on her thesis entitled "Organophosphate Pesticide Exposure, Learning Ability and Genotoxicity among Primary School Children in Tanjung Karang, Selangor" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science (Environmental Health).

Members of the Thesis Examination Committee were as follows:

Associate Professor Dr. Juliana binti Jalaludin, PhD
Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia
(Chairman)

Associate Professor Dr. Abdah binti Md Akim, PhD
Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia
(Internal Examiner)

Associated Professor Dr. Zaleha Md Isa, PhD
Faculty of Health,
Universiti Kebangsaan Malaysia,
Malaysia
(External Examiner)

(Prof. Dr. Nor Aini Ab. Shukor, PhD)
Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia
Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the Master of Science. The members of the Supervisory Committee were as follows:

Zailina Hashim, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Hayati Kadir @ Shahar, MD
Senior lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

(ROBIAH BINTI YUNUS, PhD)
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: _____

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____	Signature:_____
Name of Chairman of Supervisory Committee:_____	Name of Member of Supervisory Committee_____

TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		ii
ACKNOWLEDGEMENT		iv
APPROVAL		v
DECLARATION		vii
LIST OF TABLES		xi
LIST OF FIGURES		xii
LIST OF ABBREVIATIONS		xiii
CHAPTER		
1	**INTRODUCTION**	1
2	**LITERATURE REVIEW**	
	2.1 Pesticides	12
	2.2 Health effects of organophosphate (OP)	13
	2.3 Structure of organophosphate (OP) pesticides	14
	2.4 Mechanism of organophosphate (OP) toxicity	15
	2.5 Environmental pesticides exposure	16
	2.6 Pesticides and learning ability among children	17
	2.7 Pesticides and genotoxicity among children	19
	2.8 Micronuclei frequency	21
	2.9 Comet tail length	23
3	**METHODOLOGY**	
	3.1 Study location	25
	3.2 Study design	27
	3.3 Sampling	
	3.3.1 Study population	27
	3.3.2 Sampling population	27
	3.3.3 Sample unit	27
	3.3.4 Sampling frame	27
	3.3.5 Sample size	28
	3.3.6 Sampling method	29
	3.4 Study Instrumentation	
	3.4.1 Questionnaire	31
	3.4.2 Cholinesterase test kit	31
	3.4.3 McCarthy Scales of Children's Abilities	31
	3.4.4 Genotoxicity assessment (MN Assay)	35
	3.4.5 Genotoxicity assessment (Comet Assay)	35
	3.5 Data collection procedure	36
	3.6 Data analysis	37
	3.7 Quality control	
	3.7.1 Questionnaire	37
	3.7.2 Cholinesterase test kit	38

3.7.3 McCarthy Scales of Children's Abilities 38
3.7.4 Micronucleus and comet assay 38
3.8 Ethical approval 38

4 RESULT

4.1 Socio-demographic information 39
4.2 Reported signs and symptoms 40
4.3 Blood cholinesterase of study groups 42
4.4 Learning ability scales of the study groups 42
4.4.1 General Cognitive Index 43
4.5 Genotoxicity of the study groups 44
4.6 Relationship between blood cholinesterase with learning ability and genotoxicity of the study groups 44
4.7 Predictors of the blood cholinesterase 45
4.8 Predictors of all scales in learning ability of the study group 48
4.9 Predictors of genotoxicity of the study group 57

5 DISCUSSION

5.1 Socio-demographic information 62
5.2 Blood cholinesterase among the study group 63
5.3 Blood cholinesterase and learning ability of the study groups 64
5.4 Genotoxicity of the study groups 67
5.5 Predictors of blood cholinesterase and genotoxicity of the study group 71
5.6 Predictors of learning ability of the study group 72

6 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Conclusion 75
6.2 Study limitation 75
6.3 Recommendations 75

REFERENCES 77
APPENDICES 95
BIODATA OF STUDENT 96
LIST OF PUBLICATIONS 97

LIST OF TABLES

Table		Page
3.1	Percentage and Category of Blood Cholinesterase Activity.	31
3.2	The Tests in Verbal Scale (V).	33
3.3	The Tests for Perceptual-Performance Scale (P).	33
3.4	The Tests for Quantitative Scale (Q).	34
3.5	The Motor Scale Tests.	34
3.6	Data Analysis	37
4.1	Distribution of Socio-Demographic Information for Both Groups	39
4.2	Distribution of Reported Signs and Symptoms	40
4.3	Comparison of Blood Cholinesterase between Two Groups.	42
4.4	Comparison of Blood Cholinesterase Levels between Groups.	42
4.5	Comparison of Learning Ability Scales between Two Groups.	43
4.6	Distribution of General Cognitive Index (GCI) Scores.	43
4.7	Comparison of Genotoxicity between Two Groups .	44
4.8	Relationship between Blood Cholinesterase Levels With All Scales in Learning Ability and Genotoxicity of the Study Groups.	45
4.9	Predictors of Blood Cholinesterase Level of the Study Group	46
4.10	Predictors of Verbal Scale of the Study Group	49
4.11	Predictors of Perceptual Scale of the Study Group	50
4.12	Predictors of Quantitative Scale of the Study Group	52
4.13	Predictors of General Cognitive Index Scale of the Study Group	53
4.14	Predictors of Memory Scale of the Study Group	55
4.15	Predictors of Motor Scale of the Study Group	56
4.16	Predictors of Micronuclei Frequency of the Study Group	58
4.17	Predictors of Comet Tail Length of the Study Group	60

LIST OF FIGURES

Figure		Page
1.1	Conceptual Framework.	7
2.1	US Insecticide Use in 2001 in millions of acres.	12
2.2	General structure of organophosphates (OPs) .	14
2.3	Model of organophosphate exposure and genotoxicity effect.	20
3.1	Study location.	26
3.2	Sampling method	30
3.3	Procedure for Comet Assay	36

LIST OF ABBREVIATIONS

Ach	Acetylcholine
AcHE	Acetylcholinesterase enzyme
MSCA	McCarthy Scales of Children's Abilities
OPs	Organophosphates
SOP	Standard Operating Procedure
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background

Agriculture is one major important factor and the best contributor to overall economic growth and modernization in Malaysia. Agriculture growth and productivity increases are crucial to sustain economic development. In agriculture, pesticides are used in order to protect the plantation from any pests and to improve plant growth. Globally, the first use of synthetic pesticides was in 1940 and the consumption is increasing until now (Abdul Rani, 2002).

The use of pesticides in agriculture has been one of the most important factors leading to increased yields and reduced product prices. Pesticides can save up 40% of crop losses, thus economically and socially justifying their use. Consequently, the sustainability of such a system depends on additional energy, artificial provided by farmers through common agriculture practice such as fertilization, irrigation and pesticides treatment. It has been estimated that currently, there are about 800 active ingredients used worldwide as pesticides. In 1991, 2.53 million metric tons of such substances were applied all over the world (USEPA, 2010).

In the year 2001, 2.26 million tons of active ingredients were used. Pesticides use was 25% of the world production in developing countries where 99% of deaths were due to pesticides. Pesticides consist of insecticides, herbicides and fungicides. Insecticides are mostly used in developing countries while fungicide or herbicide in developed countries (WHO, 2001). Insecticides used for killing insects such as organochlorines, organophosphate and carbamates.

Pesticides are the most important method in self-poisoning in the developing world. There are million cases of pesticides poisoning, nearly 220,000 fatal. It is occurring world-wide every year (Eddleston et al., 2002). While data on the acute toxicity of many of these chemicals are plentiful, knowledge on their delayed effects is much more limited. The International Agency for Cancer Research (IARC) has reviewed the potential carcinogen to laboratory animals. Associations with cancer have been reported in human studies for chemicals such as phenoxy acid herbicide, 2,4,5-trichlorophenoxyacetic (2,4,5-T), lindane, methoxychlor, toxaphene and several organophosphates (IARC, 2015).

Based on USEPA (2010), insecticides are chemicals used to control insects by killing or preventing them from engaging in behaviours deemed undesirable or destructive. They are classified based on their structure and mode of action. Many insecticides act

upon the nervous system of the insect (e.g., Cholinesterase (ChE) inhibition) while others act as growth regulators or endotoxins.

Insecticides are commonly used in agricultural, public health, and industrial applications, as well as household and commercial uses (e.g., control of roaches and termites). The most commonly used insecticides are organophosphates, pyrethroids and carbamates (USEPA, 2010).

Based on study "Pesticide Use in Malaysia" by Abdul Rani (2002), the pesticide industry is one of the most important support industries in Malaysian agriculture. The economic benefits of pesticides use in producing high crop yields and the role of pesticides in the control of disease-borne pests are undeniable. Equally the adverse effects of elevated pesticide residues in water, soil, and crops to man, domestic animals, wildlife, and the environment are well recognized and documented.

In a tropical country like Malaysia, crops such as rice and vegetables are particularly susceptible to the negative impacts of pesticides use (Fuad et al., 2012). This is attributed to the often indiscriminate and intensive use of pesticides associated with these crops. Other crops, including palm oil and rubber, also require intensive use of pesticides particularly herbicides and insecticides. The organophosphate pesticides affect the nervous system by disrupting the enzyme that regulates acetylcholine, a neurotransmitter and most organophosphates are insecticides (USEPA, 2010).

Blood cholinesterase can serve as a useful indicator in organophosphate poisonings and the level of activity in blood also are very well documented (Ryhanen, 1982). Organophosphate and carbamate are powerful inhibitors of acetylcholinesterase mainly found in blood. The inhibition of acetylcholinesterase in the nervous system leads to an accumulation of the neurotransmitter acetylcholine at synapses and myoneutral junction (El-Naggar et al., 2008).

According to the World Health Organization (WHO, 2001) around three million children under the age of five die due to environmental-related diseases every year. Children are more susceptible to chemicals than adult and estimates that 50% of life pesticides exposure occurs during the first five years of life. Children take in more pesticides relatively to body weight than adults and have developing organ systems that are more vulnerable and less able to detoxify toxic chemicals (Lizardi et al., 2008).

Human especially children who are vulnerable show that pesticides poisoning can lead to poor performance on test involving intellectual functioning, academic skills, abstraction, flexibility of thought, and motor skills also memory disturbance and inability to focus attention reaction time, and manual dexterity, besides, reduced perceptual speed (Lizardi et al., 2008).

Genotoxicity potential is a primary risk factor for long-term effects such as carcinogenic and reproductive toxicology. The majority of pesticides have been tested in a wide variety of mutagenicity assays covering gene mutation, chromosomal alteration and DNA damage (Bolognesi & Merlo, 1995; IARC, 1987). Genetic damage at the chromosomal level entails an alteration in either chromosome number or chromosome structure, and such alteration can be measured as Chromosomal aberrations (CA), micronuclei (MN) frequency or comet tail length. Children may be more sensitive than adults to genotoxicity agents, and the genetic damage occurring at a younger age may affect the lifetime risk of delayed adverse health outcome (Landrigan et al, 2003).

1.2 Problem statement

Organophosphate and carbamate are used in large quantities around the world as agricultural insecticides. Toxic exposure to these chemicals is a serious global public health problem, with more than three million poisoning and 200,000 deaths reported each year (El-Naggar et al., 2008). The widespread use of organophosphate (OP) pesticides in paddy fields has led to frequent exposure in adults and children. Even though the use of pesticide is to kill specific pests, the exposure may cause adverse health effects particularly in children (Lu et al., 2008). These pesticides can be absorbed rapidly via all routes such as respiratory, gastrointestinal, ocular and dermal. The onset of symptoms is quickest after inhalation. Oral ingestion is often accidental by children (El-Naggar et al., 2008).

Organophosphate (OP) pesticides are a group of chemical compounds used for control and elimination of insects in agriculture, and in some instances for residential or industrial applications. OP pesticides are widely used in agriculture. They are well-known acute neurotoxicants, which inhibit acetylcholinesterase, resulting in the buildup of acetylcholine in neuronal junctions (Eskenazi et al., 1999).

They act as acetylcholinesterase (AChE) inhibitors, which prevent breakdown of the neurotransmitter acetylcholine, increasing both its concentration and duration of action in the body (Munoz-Quezada et al., 2013). OP pesticides may cause synaptic dysregulation and disrupt the establishment of neuronal architecture (Bigbee et al., 1999). Recent studies have reported that level of chronic in utero and child OP pesticide exposures are associated with poorer cognitive and behavioural problems, for example are attentiveness problems in children (Bouchard et al., 2010).

Exposure to OP pesticides can be toxic to humans and animals (Levine, 2007; Tadeo et al., 2008). During development, neurologic effects of OP exposure, even at low levels, may be detrimental because neurotransmitter, including acetylcholine, play essential roles in the cellular and architectural development of the brain (Barr et al., 2006). Excessive exposure in humans results in overexcitation of muscarinic and nicotinic receptors of the nervous system, inducing an over-accumulation of this

neurotransmitter in the cholinergic synapse due to phosphorylation of the active cholinesterase molecule site. This effect can result in a variety of symptoms including salivation, nausea, vomiting, lacrimation, seizures and ultimately death (Costa, 2006).

Humans may be exposed to OP pesticides through a variety of pathways including working on or living in close proximity to a farm that applies OP pesticides, home or industrial use of OP pesticides, inhalation or non-dietary ingestion of OP pesticide-laden dust, and consumption of produce containing OP pesticide residues (Curwin et al., 2005). For children, dietary exposure is believed to be the predominant exposure pathway (Whyatt et al., 2003). In addition, occupational exposure example is exposure caused by contact with an occupationally exposed person or items that the person has come in contact with, such as clothing or surface, continues to be an important exposure pathway in children or spouse of farmers or farmworks (Vida and Moretto, 2007).

Continued stimulation must take into account the clinical signs and symptoms of organophosphate poisoning, including muscarinic, nicotinic and central nervous system effects. The effects particularly affect children because they are all active at this phase and their organs and systems especially their cognitive functions are in developing stage (El-Naggar et al., 2008).

Hence, school children studying near paddy fields tend to be exposed to insecticides and may affect their learning ability in the long term. The exposure of pesticides such as organophosphate insecticides may affect their nervous systems and contribute to neurological problems among children and affects their learning ability (Landrigan et al., 1999).

The number of studies evaluating the effect of environmental exposure to genotoxicity agents in children has rapidly grown in the last ten years (Neri et al, 2005; Suk et al, 2003). Among the several adverse health effects that have been studied in children exposed to environmental hazards, genetic damage has received a particular interest, especially after recent publication of epidemiologic study showing that high frequency of chromosome damage predicts cancer in healthy adults (Bonassi et al, 2004).

Recent studies indicated that pesticide exposure is widespread and presents potential risks to humans, especially to susceptible populations such as pregnant women and children (Whyatt et al., 2004; Eskenazi et al., 2004). The potential adverse effects of pesticides exposure to children's health including reproductive outcomes, childhood cancers, neurobehavioral toxicity, and endocrine distruption have been well established (Garry, 2004). Oxidative stress has been frequently proposed as an important mechanism that could link pesticides exposure to a number of health outcomes (Bagchi et al., 1995; Banerjee et al., 2001; Halliwell, 2002; Muniz et al., 2008).

1.3 Study justification

Residents of Tanjung Karang are actively doing agricultural activities. Due to this agricultural activities, they used pesticides widely in their agricultural work especially for organophosphate (OP) mainly malathion. Organophosphate is neurologic at high dose and has well-known neurotoxicity properties, with the primary mechanism of action involving inhibition of acetylcholinesterase at high doses (Kousba et al., 2004).

The data collection was carried out in end of January until early February 2015. Usually, in January, the workers start with a new plantation that the water started to flow to the blocks of the paddy field. All the seeds were being planted and fertilizer was being added to initiate growth of the paddy. In the end of January to February, insecticide was sprayed to control the growth of weed or attacked from any unwanted insects or pests.

The association between pesticides exposure and neurobehavioral as well as neurodevelopmental effects is an area of increasing concern. Postnatally, children are at greater risk from OP toxicity than adults because the brain is rapidly developing, the dose of pesticides per body weight is likely to be larger in children and they have a reduced capacity for detoxifying xenobiotics. In children, OP exposure has been associated with behavioural problems. OP exposure has been associated with behavioural problems, poorer short-term memory and motor skills, and longer reaction time (London et al., 2012).

Children are at higher risk of pesticides toxicity than adults because the developing brain is more susceptible to neurotoxins and the dose of pesticides per body weight is likely to be higher in children (Weiss et al., 2004). Young children may be highly exposed to these pesticides because they are prone to explore their environment, combined with their proximity to potentially contaminated floors, surfaces, and air. Characteristics of young children, such as high intake of food, water, and air per unit of body weight, may also increase their exposures (Eskenazi et al., 2007).

Children are the future generation to develop our country, hence their health are important especially in their learning ability that affect their learning process. The level of cholinesterase in blood can be either high or low and some control measures can be done to decrease the exposure towards the children.

A study by How et al. (2014) stated that the assessment of genotoxicity showed that the reduced blood cholinesterase level from organophosphate pesticides exposure is significantly associated with an increase in chromosome breakage and DNA strand breaks. Farm children's early chronic OP exposure causing cholinesterase inhibition is likely to establish genotoxicity risks that may lead to cancerous cell development if not repaired properly. From the subtle effects observed in this study, it can still be

unequivocally stated that a reasonable genotoxicity risk from OP pesticides exposure during the early life of farm children might not cause a direct correlation of cancer incidence, but it is sufficient to postulate a high risk of cancer development in their adulthood.

The researcher used two types of assays to indicate genotoxicity because the researcher needs two type of findings which were micronucleus assay is specifically test on chromosome damage while comet assay is directly very sensitive test in DNA damage (Sestili et al., 2006). In addition, the used of two types of assays were to strengthen this study findings regarding genotoxicity among children. Finally, these assays are non-invasive which suitable for children and the cost was reasonably economical (Blasiak and Trzeciak, 1998).

1.4 Conceptual framework

Figure 1.1 shows the conceptual framework of the study that aim to determine the relationship between blood cholinesterase level and the primary school children's learning ability and genotoxicity.

Agriculture is the main source of pesticides use in the country. Pesticides especially organophosphate (OP) is transferred from agriculture to the atmosphere and deposited into the terrestrial environment. The emission resulted in the increase of episodic exposure of pesticides into the environment. The affected groups are children, adults and the elderly. In this research, the focus group was children who are most vulnerable.

Children may be exposed to pesticides through inhalation, ingestion and direct contact which affect blood cholinesterase level. This finally may affect the learning ability and the genotoxicity such as micro nucleus frequency and comet tail length. Blood cholinesterase levels were measured using Model Lovibond 412670 AF267 test kit, the learning ability of these children was measured using McCarthy Scales of Children's Ability (MSCA) while genotoxicity was measured using micronucleus assay and comet assay.

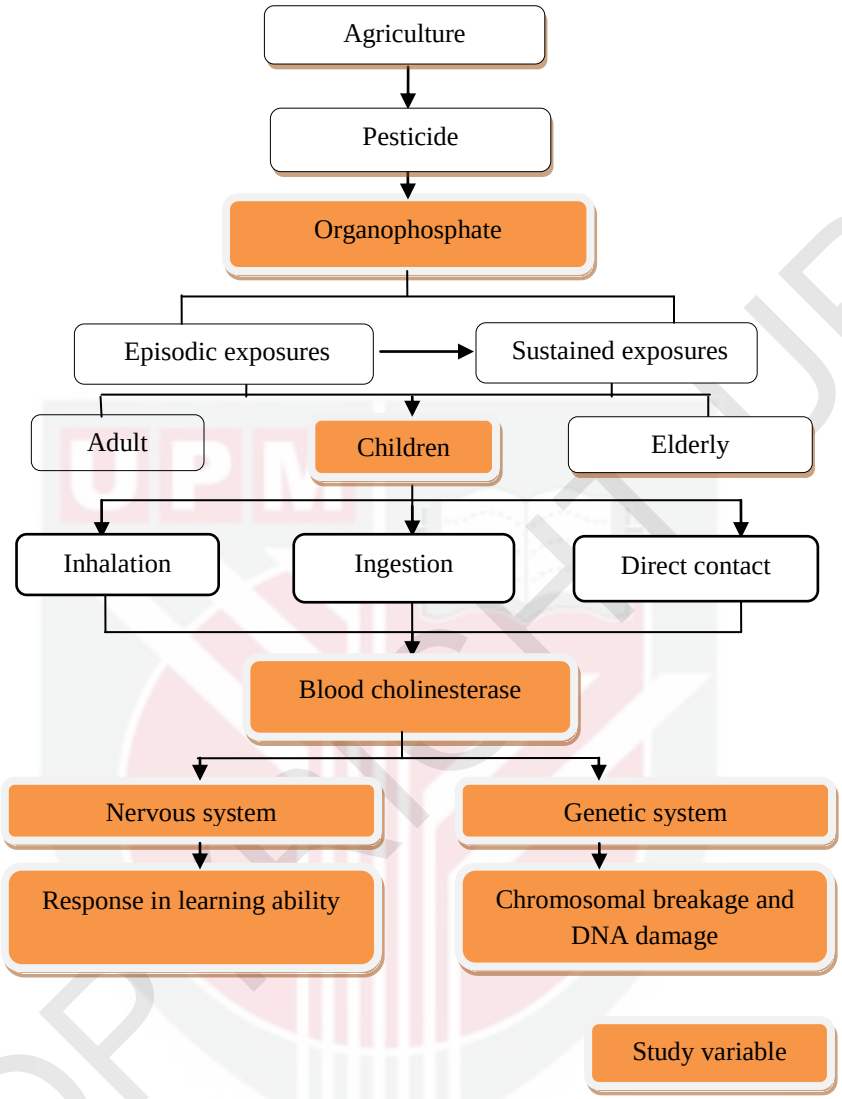

Figure 1.1: Conceptual Framework

1.5 Research objective

1.5.1 General objective

To determine the relationship between organophosphate pesticides exposure and the learning ability and genotoxicity among primary school children in Tanjung Karang, Selangor.

1.5.2 Specific objectives

1. To determine the socio-demographic characteristics of the respondents.
2. To determine the reported signs and symptoms of the respondents.
3. To determine the correlation between blood cholinesterase levels and learning ability of the study group.
4. To determine the correlation between blood cholinesterase level and genotoxicity (micronucleus frequency and comet tail length) of the study group.
5. To compare blood cholinesterase levels and learning ability between the study and comparative groups.
6. To compare total micronucleus and comet tail length between the study and comparative groups.
7. To determine variables that may influence the blood cholinesterase levels in the area of the study group.
8. To determine variables that may influence the learning ability scales (MSCA) and genotoxicity of the study group.

1.6 Research hypothesis

1. There is a significant correlation between blood cholinesterase levels and learning ability of the study group.
2. There is a significant correlation between blood cholinesterase levels and genotoxicity of the study group.
3. The blood cholinesterase levels and learning ability among study group (high exposed group) is significantly lower than the comparative group (low exposed group).
4. The micronucleus frequency and comet tail length among study group significantly have higher impairment than the comparative group.
5. The distance from house to paddy field among study group significantly influenced the blood cholinesterase levels of the study group.
6. Blood cholinesterase levels significantly influenced the learning ability scale and micronucleus frequency
7. Distance from house to paddy field significantly influenced the comet tail length of the study group.

1.7 Definition of term

1.7.1 Conceptual definition

i) Organophosphate pesticides

Organophosphate pesticides are chemical used to control insects by killing or preventing them from engaging in behaviours deemed undesired or destructive. They are classified based on their structure and mode of action (US EPA, 2010).

ii) Primary school children

Primary school children are children aged 7 to 12 years old who go to school to develop knowledge and understanding (Mangeot et al., 2001).

iii) Blood cholinesterase

Cholinesterase is a family of enzymes that catalyze the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation (El-Naggar et al., 2008).

iv) Learning ability

Learning ability is ability of learning as a relatively permanent change in behaviour including both observable activity and internal process such as thinking, attitudes and emotions based on an individual's interactional experience with the environment. Learning behaviours may exist for a lifetime, but they may also not appear throughout an individual's life. Learning might not manifests itself in observable behaviour until sometime after the educational programme has taken place (Burns, 1995).

v) Genotoxicity

Genotoxicity is a word in genetics defined as a destructive effect on a cell's genetic material (DNA, RNA) affecting its integrity. Genotoxicity leads to mutations in various cells and other bodily systems. Mutations can lead to a host of other problems, from cancer to a wide variety of different diseases. Mutations can come in many different forms; genetic information can be duplicated, deleted, or inserted (Shaily, 2012).

1.8.2 Operational definition

i) Organophosphate pesticides (OPs)

OP will inhibit the cholinesterase in the synapse, then, determine the exposure of pesticides in body through monitoring of the cholinesterase. The major used of OP were malathion, parathion and chlopyrifos. Blood cholinesterase level was used to determine the exposure of the pesticide in the school children.

ii) Primary school children

Primary school children involved were children between the age group of 7 to 8.5 years who were suitable for the use of McCarthy Scale of Children Ability (MSCA). The children who study and live 1km radius from paddy field area were selected as study group while children study and live more than 1km radius from paddy field as comparative group.

iii) Blood cholinesterase

Blood cholinesterase activity level in individual respondents of primary school children were measured using cholinesterase test kit Model LOVIBOND 412670 AF267 by taking 0.05 ml of children's blood through finger prick technique. The levels of blood cholinesterase are classified as normal (75-100%), over exposure (74.9-50.0%), serious over exposure (49.9-25.0%) and very serious and dangerous over exposure (24.9-0.0%).

iv) Learning ability

McCarthy Scale of Children's Abilities (MSCA) was used to measure the learning ability of the primary school children. The study group answered 18 core subtests to create six Index Scores which are verbal, perceptual, quantitative, general cognitive index, memory and motor. The classification of general cognitive index are very superior($\geq$130), superior(120-129), bright normal(110-119), average(90-109), dull normal (80-89), borderline(70-79) and mentally retarded($\leq$69) (McCarthy, 1972).

v) Genotoxicity

Genotoxicity was a primary risk factor for long-term effects such as carcinogenic and reproductive toxicology. The majority of pesticides had been tested in a wide variety of mutagenicity assays covering gene mutation, chromosomal alteration and DNA damage (Bolognesi & Merlo, 1995; IARC, 1987). Genotoxicity was measured by micronucleus assay and comet assay.

REFERENCES

Abdul Rani A. (2002). Pesticide use in Malaysia. ISBN:987-0-415-23917-2. doi: 10.1201/9780203165584.ch7. Retrieved from http://www.crcnetbase.com/doi/abs/10.1201/9780203165584.ch7

Abou-Donia, M. (2003). Organophosphorus ester-induced chronic neurotoxicity. *Archive of Environmental Health,*58(8):484-97

Alarcon, W.A., Calvert, G.M., Blondel, J.M., Mehler, L.N., Sievert, J., Propeck, M, et al. (2005). Acute illnesses associated with pesticide exposure at schools. *The Journal of the American Medical Association,* 294(4):455-65

Aldridge, J.E., Levin E.D., Seidler, F.J., Slotkin T.A. (2005a). Developmental exposure of rats to chlorpyrifos leads to behavioural alterations in adulthood, involving serotonergic mechanisms and resembling animal models of depression. *Environmental Health Perspectives, 113:*527-531

Ali, M.A., Oh, J.H., Kim, P.J. (2008). Evaluation of silicate iron slag amendment on reducing methane emission from flood water rice farming. *Agriculture, Ecosystems & Environment,* 128(1-2):21-26. Doi:10.1016/j.agee.2008.04.014

Ali, N., En, V.D., Dirtu, A.C., Neels, H., Covaci, A. (2012). Assessment of human exposure to indoor organic contaminants via dust ingestion in Pakistan. *Indoor Air, 22(3):* 200-211

Ambali, S.F., Aliyu, M.B. (2012). Short-term sensorimotor and cognitive changes induced by acute chlorpyrifos exposure in Wistar rats ameliorative effect of vitamin E. *Pharmacologia,* 3:31-38

Ambali, S.F., Oriejie, C., AbuBakar, W.O., Shittu, M., Kawu, M.U. (2011). Ameliorative effect of vitamin C on alteraions in thyroid hormones concentrations induced by subchronic coadministration of chlopyrifos and lead in Wistar rats. *Journal of Thyroid Research.* Doi:10.4061/2011/214924

Aprea, C., Colosio, C., Mammone, T., Minoia, C., Maroni, M. (2002). Biological monitoring of pesticide exposure: a review of analytical methods. *Journal Chromatography B: Analytical Technologies Biomedical Life Sciences,* 769:191-219

Ashby, J., Tinwell, H., Lefevre, P.A., Browne, M.A. (1995). The single cell gel electrophoresis assay for induced DNA damage (comet assay): measurement of tail length and moment. *Mutagenesis,* 10(2): 85-90. Doi:10.1093/mutage/10.2.85

Bagchi, D., Bagchi, M., Hassoun, E.A., Stohs, S.J. (1995). Invitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. *Toxicology,* 104:129-140

Bagchi, M., Zafra, S., Bagchi, D. (2006). DNA damage, gene expression and carcinogenesis by organophosphate and carbamtes. In: Gupta, R.C (Ed.), Toxicology of organophosphate and carbamate compounds. *Elsevier Academic Press*, New York, pp, 533-548

Baker, E.L., Warren, M., Zack, M., Dobbin, R.D., Miles, J.W., Miller, S., Alderman, L., Teeters, W.R. (1978). Epidemic malathion poisoning in Pakistan malaria workers. *Lancet*,1(8054):31-4

Bakir, F., Damluji, S.F., Amin-Zaki, L., Murtadha, M., Khalidi, A., Al-Rawi, N.Y., Tikriti, S… Doherty, R.A. (1973). Methylmercury poisoning in Iraq. *Science*, 181(4096):230-41

Banerjee, B.D., Seth, V., Ahmed, R.S. (2001). Pesticide-induced oxidative stress: perspective and trens. *Review of Environmental Health,* 16:1-40

Barr, D.B., Bradman, A., Freeman, N., Whyatt, R.M., Wang, R.Y., Nacher, L et al. (2006). Studying the relation between pesticide pesticide exposure and human development. Human developmental neurotoxicology. Nueva York: Taylor & Francis Group. p.253-85

Bearer, C.F. (1995). How are children different from adult? *Environmental Health Perspective*, 103:7-12

Bhali, J.A., Khan, Q.M., Haq, M.A., Khalid, Z.M., Nasim, A. (2006). Cytogenetic analysis of Pakistani individuals occupationally exposed to pesticides in a pesticide production industry. *Mutagenesis*, 21:143-148

Bigbee, J. W., Sharma, K. V., Gupta, J. J., & Dupree, J. L. (1999). Morphogenic role for acetylcholinesterase in axonal outgrowth during neural development. *Environmental Health Perspectives*, *107*(Suppl 1), 81–87.

Blasiak, J., trzeciak, A. (1998). Single cell gel electrophoresis (Comet Assay) as a tool for environmental biomonitoring. An example of pesticides. *Polish Journal of Environmental Studies*, 7(4):189-194

Bolognesi, C., Merlo, F. (1995). Biomonitoring of human populations exposed to pesticides. *Encyclopedia of Environmental Control Technology*, 28:673-737

Bonassi S., Znaor A., Norppa H., Hagmar L. (2004). Chromosomal aberrations and risk of cancer in humans: an epidemiological perspective. *Cytogenic and Genome Research*. 104:376-382

Bonassi, S., Coskun, E., Ceppi, M., Lando, C., Bolognesi, C., Burgaz, S., Holland, N…Giri, S. (2011). The human micronucleus project on exfoliated buccal cells (HUMN(XL)): the role of life-style, host factors, occupational exposures, health status, and assay protocol. *Mutation Research*, 728:88-97

Bortoli, G.M., Azevedo, M.B., Silva, L.B. (2009). Cytogenetic biomonitoring of Brazilian workers exposed to pesticides: Micronucleus analysis in buccal epithelial cells of soybean growers. *Mutation Research*, 675:1-4

Bouchard, M.F., Bellinger, D.C., Wright, R.O., Weiss, M.G. (2010). Attention-deficit/hyperactivity disorder and urinary metabolites of organophosphate pesticides. *Pediatrics*, 125(6): 1270-1277. Doi: 10.1542/peads.2009-3058

Bozina, N., Bradamante, V., Lovric, M. (2009). Genetic polymorphism of metabolic enxyme P450 (CYP) as a susceptibility factor for drug response, toxicity, and cancer risk. *Archives of Industrial Hygiene and Toxicology* , 60:217-242

Brunner, M., Nagy, G., Wilhelm, O. (2011). A tutorial on hierarchically structured constructs. *Journal of Personality Assessment*, 80(4): 796-846

Bull, S., Fletcher, K., Boobis, A.R., Battershill, J.M. (2006). Evidence for genotoxicity of pesticides in pesticide applicators: a review. *Mutagenesis*, 21:93-103

Burns, R.B (1995). The adult learner at work: a comprehensive guide to the context, psychology and methods of learning for the workplace. NSW, Australia: Business and Professional Publishing

Cammerer, Z., Elhajouji, A., Kirsch-Volders, M., Suter, W. (2007). Comparison of the peripheral blood micronucleus test using flow cytometry in rat and mouse exposed to aneugens after single-dose application. *Mutagenesis*, 22:129-3

Celik, A., Cavas, T., Ergene-Gozukara, S. (2003). Cytogenetic biomonitoring in petrol station attendants: micronucleus test in exfoliated buccal cells. *Mutagenesis*, 18:417-421

Chakraborty, T., Das, U., Poddar, S., Sengupta, B., De, M. (2006). Micronuclei and chrosomal aberrations as biomarkers: a study in an arsenic exposed population in West Bengal, India. *Bulletin Environmental Contamination Toxicology*, 76:970-976

Choi, S.M., Yoo, S.D., Lee, B.M. (2004). Toxicological characteristics of endocrine-disrupting chemicals: developmental toxicity, carcinogenicity, and mutagenicity. *Journal of Toxicology Environmental Health A Critical Review*, 7(1):1-24

Costa, C., Teixeira, J.P., Silva, S., Roma-Torres, J., Coelho, P., Gaspar, J. (2006). Cytogenetic and molecular biomonitoring of a Portuguese population exposed to pesticide. *Mutagenesis*, 21:343-50

Costa, L.G. (2006). Current issues in organophosphate toxicology. *Clin Chim Acta*, 366(1-2): 1-13

Crumpton, T.L., Seidler, F.J., Slotkin, T.A. (2000). Is oxidative stress involved in the developmental neurotoxicity of chlorpyrifos? *Brain Reearch Development,* 121:189-195

Curl, C.I., Fenske, R., Kissel, J.C., Shirai, J.H., Moate, T.F., Griffith, W., Coronado, G., Thompson, B. (2002). Evaluation of take-home organophospharus pesticide exposure among agricultural workers and their children. *Environmental Health Perspectives,* 110(12):A787-72

Curwin, B., Hein, M., Sanderson, W., Nishioka, M., Reynolds, S., Ward, E. (2005). Pesticide contamination inside farm and nonfarm homes. *Journal of Occupational and Environmental Hygiene,* 2(7): 357-67

Curwin, B., Hein, M., Sanderson, W., Striley, C., Heederik, D., Kromhout. (2007). Urinary pesticide concentrations among children, mothers and fathers licing in farm and non-farm households in Loa. *The Annals of Occupational Hygiene,* 51(1): 53-65

Dallaire, R., Muckle, G., Rouget, F., Kadhel, P., Bataille, H., Guldner, L,...Cordier, S. (2012). Cognitive, visual, and motor development of 7-month-old Guadeloupean infants exposed to chlordecone. *Environmental Research, 118,* 79-85. doi:10.1016/j.envres.2012.07.006

Dalvie, M.A., Sosan, M.B., Africa, A., Cairncross, E., London, L. (2014). Environmental monitoring of pesticide residues from farms at a neighbouring primary and pre-school in the Western Cape in South Africa. *Science of the Total Environment,* 466-467:1078-1084.Doi: 10.1016/j.scitotenv.2013.07.099

Daniels, J.L., Olshan, A.F., Savitz, D.A. (1997). Pesticides and childhood cancers. *Environmental Health Perspectives,* 105:1068–1077.

Davis, C.D. (2003). Use of exfoliated cells from target tissues to predict responses to bioactive food components. *Journal of Nutrition,* 133:1769-1772

Dori, A., Cohen, J., Silverman, W.F., Pollack, Y., Soreq, H. (2005). Functional manipulations of acetylcholinesterase splice variants highlight alternative splicing contributions to murine neocorticol development. *Cerebral Cortex,* 15(4): 419-430

Eckerman, D.A., Gimenes, L.S., De-Souza, R.C., Galvao, P.R., Sarcinelli, P.N., Chrisman, J.R. (2007). Age related effects of pesticide exposure on neurobehavioral performance of adolescent farm waorkers in Brazil. *Neurotoxicology and Teratology,* 29:164-75

Eddleston, M., Karalliedde, L., Buckley, N., Fernando, R., Hutchinson, G., Isbister, G., Konradsen, F… Smit, L. (2002). Pesticide poisoning in the developing world-a minimum pesticides list. *Lancet,* 360(9340): 1163-1167

Elersek . T, Filipic. M. (2011). Organophosphorous Pesticide – Mechanisms of their Tocixity, Pesticide – The Impacts of Pesticide exposure, Stoytcheva (Ed.), ISBN:978-953-307-531-0, *InTech*, Available from: http://www.intechopen.com/books/pesticide-the-impacts-of-pesticides-esposure /organophosphorus-pesticides-mechanism-of-their-toxicity

El-Naggar, A.E., Abdalla, M.S., El-Sebaey, A.S., Badawy, S.M., (2008). Clinical findings and cholinesterase levels in children of organophosphates and carbamates poisoning. *European Journal of Pediatrics, 168:951-956.* Doi:10.1007/s00431-008-0866-z

Eskenazi, B., Bradman, A., & Castorina, R. (1999). Exposures of children to organophosphate pesticides and their potential adverse health effects. *Environmental Health Perspectives, 107*(SUPPL. 3), 409-419.

Eskenazi, B., Harley, K., Bradman, A., Weltzien, E., Jewell, N.P., Barr, D.B., Furlong, C.E., Holland, N.T. (2004). Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. *Environmental Health Perspectives,* 112:1116-1124

Eskenazi, B., Marks, A.R., Bradman, A., Harley, K.,Barr, D.B., Johnson, C… Jewel, N.P. (2007). Organophosphate Pesticide Exposure and neurodevelopment in young Mexican-American children. *Environmental Health Perspectives,* 115(5):792-798, doi:10.1289/ehp.9828

Faustman, E.M., Silbernagel, S.M., Fenske, R.A., Burbacher, T.M., Ponce, R.A. (2000). Mechanisms underlying children's susceptibility to environmental toxicants. *Environmental Health Perspectives,* 108(3):13-21

Fenech, M., Kirsch-Volders, M., Natarajan, A. T., Surralles, J., Crott, J. W., Parry, J., et al. (2011). Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. *Mutagenesis* 26: 125–132. doi: 10.1093/mutage/geq052

Fenech, M., Morley, A.A. (1986). Cytokinesis-block micronucleus method in human lymphocytes: effect of in vivo ageing and low dose X-irradation. *Mutation Research/Fundamental and Molecular Mechanism of Mutagenesis,* 161(2):193-198

Fenske, R. A., Bradman, A., Whyatt, R. M., Wolff, M. S., & Barr, D. B. (2005). Lessons learned for the assessment of children's pesticide exposure: Critical sampling and analytical issues for future studies. *Environmental Health Perspectives, 113*(10), 1455-1462.

Fenske, R.A., Lu, C., Barr, D., Needham, L. (2002). Children's exposure to chlorpyrifos and parathion in an agricultural community in central Washington State. *Environmental Health Perspectives,* 110(5):549-53

Fenske, R.A., Lu, C., Simcox, N.J., Loewenherz, C., Touchstone, J., Moate, T.F et al. (2000). Strategies for assessing children's organophosphorus pesticide exposures in agricultural communities. *Journal of Exposure Annals Environmental Epidemiological,* 10(6):662-71

Fiedler, N., Rohitrattana, J., Siriwong, W., Suttiwan, P., Strickland, P.O., Ryan, P.B., Rohlman, D.S., Panuwet, P., Barr, D.B., Robson, M.G. (2015). Neurobehavioral effects of exposure to organophosphates and pyrethroid pesticides among Thai children. *Neurotoxicology*, 48: 90-99

Fuad M.J., Junaidi, A.B., Habibah, A., Hamzah, J., Toriman, M.E., Lyndon, N., Er, A.C., Selvadurai, S., Azina, A.M. (2012). The impact of pesticide on paddy farmers and ecosystem. *Advances in Natural and Applied Sciences*, *6(1*), 65-70.

Fucic, A., Markucic, D., Mijic, A., Jazbec, A.M. (2000). Estimation of genome damage after exposure to ionising radiation and ultrasound used in industry, *Environmental Molecular Mutagenesis,* 36:47-51

Gaikwad, A.s., Karunamoorthy, P., Kondhalkar, S.J., Ambikapathy, M., Beerappa, R. (2015). Assessment of haematological, biochemical effects and genotoxicity among pesticide sprayers in grape garden. *Journal of Occupational Medicine and Toxicology*. 10:11. Doi: 10.1186/s12995-015-0049-6

Garry, V.F. (2004). Pesticides and children. *Toxicology Applied Pharmacology,* 198:152-163

Gass, C.S., Curiel, R.E. (2011). Test anxiety in relation to measures of cognitive and ontellectual functioning. *Archive Clinical Neuropsychology,* 26(5):396-404

GenPharmTox. (2015). Micronucleus Assay. Retrieved from file:///C:/Users/user-PC/Downloads/AssaySheetMikronucleusAssay.pdf

Ginsberg, G., Hattis, D., Sonawane, B. (2004). Incorporating pharmacokinetic differences between children and adults in assessing children's risk to environmental toxicants. *Toxicology and Applied Pharmacology*, 198:164-183

Giri, S., Giri, A., Sharma, G.D., Prasad, S.B. (2002). Mutagenic effects of carbosulfan, a carbamate pesticide. *Mutation Research.* 519:75-82

Google Earth. (2016). Sekolah Kebangsaan Sawah Sempadan, Tanjung Karang, Selangor. Retrieved from https://www.google.com/earth/

Green, M.A., Heumann, M.A., Wehr, H.M., Foster, L.R., Williams, L.P, Polder, J.A., Morgan, C.L…Witt, J.M. (1987). An outbreak of watermelon-borne pesticide toxicity. *American Journal of Public Health,* 77(11):1431-1434

Hagmar, L., Bonassi, S., Stromberg, U., Bragger, A., Knudsen, L., Norppa, H, et al. (1998). Chromosomal aberrations in lymphocytes predict human cancer- a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). *Cancer Research*, 58:4117-4121

Halliwell, B. (2002). Effect of diet on cancer development: is oxidative DNA damage a biomarker? *Free Radical Biological Medicine*, 32:968-974

Halliwell, B. (2007). Oxidative stress and cancer: have we moved forward? *Biochemical Journal*, 401:1-11

Hamzah, N.A.,Hashim, Z., Miswon, N,H., Baguma, D. (2015). Blood cholinesterase level and cognitive functioning among primary school children near paddy field in Tanjung Karang, Selangor. *Autralian Journal of Basic and Applied Sciences*, 9(22):49-55

Handal, A. J., Lozoff, B., Breilh, J., & Harlow, S. D. (2007). Effect of community of residence on neurobehavioral development in infants and young children in a flower-growing region of ecuador. *Environmental Health Perspectives*, *115*(1), 128-133.

Hernandez, A.F., Lopez, O., Rodrigo, L., Gil, F., Pena, G., Serrano, J.L., Parron, T., Alvarez, J.C., Lorante, J.A., Pla, A. (2005). Changes in erythrocyte enzymes in humans long-term exposed to pesticides influence of several markers of individual susceptibility. *Toxicology Letters*, 159:13-21

Hohmann, C.F. (2003). A morphogenetic role for acetylcholine in mouse cerebral neocortex. *Neuroscience Biobehavioral Revision*, 27(4):351-363

Hoppin, J.A., Umbach, D.M., London, S.J., Lynch, C.F., Alavanja, M.C.., Sandler, D.P. (2006). Pesticides associated with wheeze among commercial pesticide applicators in the Agricultural Health Study. *American Journal of Epidemiology*, 163(12):1129-37

How, V., Hashim, Z., Ismail, P., Md Said, Salmiah., Omar, Dzolkhifli, O., Mohd Tamrin, S.B. (2014). Exploring cancer development in adulthood: cholinesterase depression and genotoxic effect from chronic exposure to organophosphate pesticide among rural farm children. *Journal of Agromedicine*, 19: 35-43. Doi: 10.1080/1059924X.2013.866917

Hundekari, I.A., Surkayar, A.N., Rathi, D.B. (2013). Acute organo-phosphorus pesticide poisoning in North Karnataka India: oxidative damage, haemoglobin level and total leukocytes. *African Health Sciences*, 13: 129-136

Icenogle, L.M., Christopher, N.C., Blackwelder, W.P., Caldwell, D.P., Qiao, D., Seidler, F.J. (2004). Behavioural alterations in adolescent and adult rats

caused by a brief subtoxic exposure to chlorpyrifos during neurulation. *Neurotoxicology*, 26:95-101

International Agency for Research on Cancer (IARC). (1987). IARC Monorgaphs on the evaluation of carcinogenic risks to humans. *Genetic and Related Effects*, 1(42)

International Agency for Research on Cancer (IARC). (2015). Evaluation of five organophosphate insecticides and herbicides. Retrieved from https://www.iarc.fr/en/media-centre/iarcnews/pdf/MonographVolume112.pdf

Jackson, C.E. (2011). Cholinergic system. *Encyclopedia of Clinical Neuropsychology, 562-564*. Doi: 10.1007/978-0-387-79948-3_1113

Jenkins, J. (2014). How siblings interact tied to young child's development. *Pediatric.Research*

Joksic, G., Vidakovic, A., Spasojevic-Tisma, V. (1997). Cytogenetic monitoring of pesticide sprayers. *Environmental Research,* 75:113-8

Jones, D.C., Miller, G.W. (2008). The effects of environmental neurotoxicants on the dopaminergic system: a possible role in drug addiction. *Biochemical and Pharmacology*, 76:569-581

Jones, K., Everard, M., Harding, A.H. (2014). Investigation of gastrointestinal effects of organophosphate and carbamte pesticide residues on young children. *International Journal of Hygiene and Environmental Health*, 217: 392-398

Jose, R., Suarez-Lopez., John, H., Himes., David, R., Jacobs., Bruce, H., Alexander., Megan, R., Gunnar. (2013). Acetylcholinesterase activity and neurodevelopment in boys and girls. *Pediatrics,*132(6): 1649-1658. Doi: 10.1542/peds.2013-0108

Kamel, F., Hoppin, J.A., (2004). Association of pesticide exposure with neurologic dysfunction and disease. *Environmental Health Perspectives, 112(9):* 950-958. doi:10.1289/ehp.7135

Kanaya, T., Ceci, S. (2012). The impact of the Flynn on LD diagnoses in special education. *Journal of Learning Disability*, 45(4):319-26

Kapka-Skrzypczak, L., Cyranka, M., Skrzypczak, M., Kruszewski, M. (2011). Biomonitoring and biomarkers of organophosphate pesticides exposure-state of the art. *Annals of Agricultual and Environmental Medicine.* 18(2):294-303

Kausar, A., Giri, S., Roy, P., Giri, A. (2014). Changes in buccal micronucleus cytome parameters associated with smokeless tobacco and pesticide exposure among

female tea garden workers of Assam, India. *International Journal of Hygiene and Environmental Health*, 217:169-175

Kehrer, J.P. (1993). Free radicals as mediators of tissue injury and disease. *Critical Review Toxicology*, 23:21-48

Khan, M.F., Aslam, M., Naqvi, S.N.H., Tabassum, R., Qadri S.S. (2005). Determination of pesticides exposure of farmers of Multan, Pakistan with acetylcholinesterase inhabitation measurement at pre, post and during spray season. *Journal Basic Application Sciences, 2(1)*, 61-63

Kliesch, U., Adler, I.D. (1987). Micronucleus test in bone marrow of mice treated with 1-nitropropane, 2-nitropropane and cisplatin. *Mutation Research*, 192:181-184

Koch, D., Lu, C., Fisker-Anderson, J., Jolley, L., Fenske, R.A. (2002). Temporal association of children's pesticide exposure and agricultural spraying: a report of a longitudinal biological monitoring study. *Environmental Health Perspectives*, 110(8): 829-33

Kofman, O., Berger, A., Massarwa, A., Friedman, A., & Jaffar, A. A. (2006). Motor inhibition and learning impairments in school-aged children following exposure to organophosphate pesticides in infancy. *Pediatric Research, 60*(1), 88-92.

Kousba, A.A., Sultatos, L.G., Poet, T.S., Timchalk, C. (2004). Comparison of chlorpyrifos-oxon and paraoxon acetylcholinesterase inhibition dynamics:potential role of a peripheral binding site. *Toxicological Sciences, 80(2)*, 239-248

Kumar, L.P., Panneerselvam, N. (2008). Toxic effects of pesticides: A review on cytogenetic biomonitoring studies. *Medicine and Biology*, 15(2):46-50

Landrigan JK., Kimmel CA., Correa A., Eskenazi B. (2003). Children's health and the environment: public health issues and challenges for risk assessment. *Environmental Health Perspectives*. 112:257-265

Landrigan, P.J. (2001). Pesticides and polychlorinated biphenyl (PCBs): an analysis of the evidence that impair children's neurobehavioral development. *Molecular Genetic Metabolism*, 73:11-7

Landrigan, P.J., Claudio, L., Markowitz, S.B., Berkowitz, G.S., Brenner, B.L., Romero, H., Wetmur, J.G… Wolff, M.S. (1999). Pesticides and inner-city children: exposures, risks, and prevention. *Environmental Health Perspectives*, 107(3): 431-7

Lebailly, P., Vigreux, C., Lechevrel, C., Ledemeney, D., Godard, T., Sichel, F, et al. (1998). DNA damage in mononuclear leukocytes of farmers measured using

the alkaline comet assay: modifications of DNA damage levels after a one-day field spraying period with selected pesticides. *Cancer Epidemiological Biomarkers Preview*, 7(10): 929-40.

Lemeshow, S., Hosmer, D.W., Klar, J., K.Lwanga, S. (1990). Aduquacy of sample size in health studies. England: John Wiley $ Sons Ltd.

Levin, E.D., Addy, N., Baruah, A., Elias, A., Cannelle-Christopher, N., Seidler, F.J., Slotlin, T.A. (2002). Prenatal chlopyrifos exposure in rats causes persistent behavioural alternations. *Neurotoxicology and Teratology*, 24:733-741

Levin, E.D., Addy, N., Nakajima, A., Christopher, N.C., Seidler, F.J., Slotkin, T.A. (2001). Persistent behavioural consequences of neonatal chlorpyrifos exposure in rats. *Development Brain Research*, 130(1) :83-89

Levine, M.J. (2007). Pesticides: a toxic time bomb in our midst. United States of America, USA: Praeger

Lizardi, P. S., O'Rourke, M. K., & Morris, R. J. (2008). The effects of organophosphate pesticide exposure on hispanic children's cognitive and behavioural functioning. *Journal of Paediatric Psychology*, 33(1), 91-101. doi:10.1093/jpepsy/jsm047

Lodovici, M., Casalini, C., Dolara, P. (1997). Oxidative liver DNA damage in rats treated with pesticide mixtures. *Toxicology*, 117: 55-60

London, L., Beseler, C., Bouchard, M.F., Bellinger, D.C., Colosio, C., Grandjean, P., Harari, R., Kootbodien, T., Kromhout, H… Stallones, L. (2012). Neurobehavioral and neurodevelopmental effects of pesticide exposures. *Neurotoxicology*, 33(4): 887-96. Doi: 10.1016/j.neuro.2012.01.004

Lu, C., Barr, D. B., Pearson, M. A., & Waller, L. A. (2008). Dietary Intake and Its Contribution to Longitudinal Organophosphorus Pesticide Exposure in Urban/Suburban Children. *Environmental Health Perspectives*, 116(4):537–542. http://doi.org/10.1289/ehp.10912

Lu, C., Kedan, G., Fisker-Anderson, J., Kissel, J., Fenske, R. (2004). Multipathway organophosphorus pesticide exposures of preschool children living in agricultural and non-agricultural communities. *Environmental Research*, 96(3):283-9

Lu, C., Richard, A.F., Nancy, J.S., Kalman, D. (2000). Pesticide exposure of children in an agricultural community: evidence of household proximity to farmland and take home exposure pathways. *Environmental Research Section A*, 84:290-302. Doi: 10.1006/enrs.2000.4076

Magallona, E.D. (1989). Effects of insecticides in rice ecosystems in Southeast Asia. In: Bourdeau, P., Haines, J.A., Klein,W, et al., eds. *Ecotoxicology and Climate*, 265-297

Majer, B.J., Laky, B., Knasmuller, S., Kassie, F. (2001). Use of micronucleus assay with exfoliated epithelial cells as biomarker for monitoring individuals at elevated risk of genetic damage and in chemorevention trials. *Mutation Research*, 489:147-172

Mangeot, S.D., Miller, L.J., McIntosh, D.N., Clarke, J.M., Simon, J., Hagerman, R.J., Goldson, E. (2001). Sensory modulation dysfunction in children with attention-deficit-hyperactive disorder. *Development Medicine and Child Neurology, 43(6),* 399-406

Marks, A.R., Harley, K., Bradman, A., Kogut, K., Barr, D.B., Johnson, C. (2010). Organophosphate pesticide exposure and attention in young Mexian-American children: the CHAMACOS study. *Environmental Health Perspectives*, 118:1768-1774

Maroni, M., Colosio, C., Ferioli, A., Fait, A. (2000). Biological monitoring of pesticide exposure: a review introduction. *Toxicology*, 143:1-118

Maroni, M., Fait, A. (1993). Health effects in man from long-term exposure to pesticide: A review of the 1975-1991 literature. *Toxicology*, 78(1-3):1-180

Mason, H.J. (2000). The recovery of plasma cholinesterase and erythrocyte acetylcholisterase activity in workers after over-exposure to dichlorvos. *Occupational Medicine, 50(5),* 343-7

Mateuca, R., Lombaert, N., Aka, P. V., Decordier, I., and Kirsch-Volders, M. (2006). Chromosomal changes: induction, detection methods and applicability in human biomonitoring. *Biochimie,* 88: 1515–1531. doi: 10.1016/j.biochi.2006.07.004

Matthews, G. (2006). Pesticides: Health, Safety and the Environment. Oxford: Blackwell Publishing.

McBride, D.L. (2012). Prenatal pesticide exposure linked to lower IQ in children. *Journal of Pediatric Nursing*, 27, 85-87. doi:10.1016/j.pedn.2011.11.003

McCarthy, D. (1972). *McCarthy Scales of Children's Abilities.* US, America: The Psychological Corporation.

Mills, K., Kegley, S. (2006). Air monitoring for chlorpyrifos in Lindsay, California: pesticide action network North America, p,12-13. Retrieved from http://www.panna. irg/campaigns/docsDrift/Lindsay-CP_7_18_06.pdf.

Miswon, N.H., Zailina, H., How, V., Chokeli, R. (2015). Blood cholinesterase level and learning ability of primary school children in an agricultural village, Tanjung Karang, Malaysia. *British Journal of Medicine and Medical Research,* 8(1): 52-60. Doi:10.9734?BJMMR/2015/16804

Mitchell, A., Steffenson, N., Hogan, H., Coleman, R. (1995). Acute organophosphate pesticide poisoning in children. *MCN: The American Journal of Maternal/Child Nursing,* 20:261-268

Mourad, P. D., Kliot, M., Mesiwala, A., Patterson, R., & Jarvik, J. G. (2005). Systems and methods for making noninvasive physiological assessments. *U.S. Patent No. 6,875,176*. Washington, DC: U.S. Patent and Trademark Office.

Muniz, J.F., McCauley, L., Scherer, J., Lasarev, M., Koshy, M., Kow, Y.W., Nazar-Stewart, V., Kisby, G.E. (2008). Biomarkers of oxidative stress and DNA damage in agricultural workers: a pilot study. *Toxicology Applied Pharmacology,* 227: 97-107

Munoz-Quezada, M.T., Lucero, B.A., Barr, D.B., Steenland, K., Levy, K., Ryan, P.B., Iglesias, V… Vega, C. (2013). Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: A systematic review. *Neurotoxicology,* 39:158-168

Naeher, L., Tulve, N., Egeghy, P., Barr, D., Adetona, O., Fortmann, R. (2010). Organophosphorus and pyrethroid insecticide urinary metabolite concentrations in young children living in southeastern United State city. *Science Total Environment,* 408:1145-53

Needham, L.L., Sexton, K. (2000). Assessing children's exposure to hazardous environmental chemicals: an overview of selected research challenges and complexities. *Journal of Exposure Annals Environment Epidemiological,* 10:611-29

Neri. M., Bonassi. S., Knudsen, L.E., Sram R., Holland N., Ugolini D. (2005). Children's exposure to environmental pollutants and biomarkers of genetic damage, overview and critical issues. *Mutation Research,* Doi:10.1016/j.mrrev.2005.04.001

Nigg, H. N., & Knaak, J. B. (2000). Blood cholinesterases as human biomarkers of organophosphorus pesticide exposure. In *Reviews of Environmental Contamination and Toxicology* (pp. 29-111). Springer New York.

Olive PL and Banáth JP. (2006). The comet assay: a method to measure DNA damage in individual cells. *Nature Publishing Group.* doi:10.1038/nprot.2006.5

Ostling, O., and Johanson, K.J. (1984) Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. *Biochemical and Biophysical Research Communication*, 123: 291-8.

Panemangalore, M., Dowla, H.A., Byers, M.E. (1999). Occupational exposure to agricultural chemicals: effect on the activities of some enzymes in the blood of farm workers. *International Archive Occupational and Environmental Health*, 72:84-88

Popescu, N.C. (1999). Sister chromatid exchange formation in mammalian cells is modulated by deoxyribonucleotide pool imbalance. *Somatic Cell Molecular Genetic,* 25(2):101-8

Qiao, D., Seidler, F.J., Abreu-Villaca, Y., Tate, C.A., Cousins, M.M., Slotkin, T.A. (2004). Chlopyrifos exposure during neurulation: cholinergic synaptic dysfunction and cellular alterations in brain regions at adolescent and adulthood. *Developmental Brain Research,* 148:43-52

Quandt, S.A., Arcury, T.A., Rao, P., Snively, B.M., Camann, D.E., Doran, A.M, et al. (2004). Agricultural and residential pesticides in wipe samples from farmworker family residences in North Carolina and Virginia. *Environmental Health Perspectives,* 112(3):382-7

Quirós-Alcalá, L., Bradman, A., Nishioka, M., Harnly, M. E., Hubbard, A., McKone, T. E., … Eskenazi, B. (2011). Pesticides in house dust from urban and farmworker households in California: an observational measurement study. *Environmental Health*, *10*, 19. http://doi.org/10.1186/1476-069X-10-19

Rauh, V., Arunajadai, S., Horton, M., Perera, F., Hoepner, L., Barr, D.B., Whyatt, R. (2011). Seven-year neurodevelopment scores and prenatal exposure to chlorpyrifos, a common agriculture pesticide. *Environmental Health Perspectives, 119(8),* 1196-1201. doi:10.1289/ehp.1003160

Rauh, V., Perera, F.P., Horton, M.K., Whyatt, R.M., Bansal, R., Hao, X. (2012). Brain anomalies in children exposed prenatally to a common organophosphate pesticide. *Proceeding of the National Academy of Sciences USA,* 109:7871-7876

Rauh, V.A., Garcia, W.E., Whyatt, R.M., Horton, M.K., Barr, D.B., Louis, E.D. (2015). Prenatal exposure to the organophosphate pesticide chlorpyrifos and childhood tremor. *Neurotoxicology,* 51:80-86

Rauh, V.A., Garfinkel, R., Perera, F.P., Andrews, H.F., Hoepner, L., Barr, D.B. (2006). Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. *Pediatrics,* 118:1845-1859

Rehner, T.A., kolbo, J.R., Trump, R., Smith, C., Reid, D. (2000). Depression among victims of South Mississippi's methylparathion disaster. *Health Social Work*, 25(1):33-40. Doi:10.1093/hsw/25.1.33

Restrepo, M., Muñoz, N., Day, N., Parra, J. E., Hernandez, C., Blettner, M., & Giraldo, A.. (1990). Birth defects among children born to a population occupationally

exposed to pesticides in Colombia. *Scandinavian Journal of Work, Environment and Health*, 16(4): 239–246.

Reuter, S., Gupta, S.C., Chaturvedi, W.M., Aggarwal, B.B. (2010). Oxidative stress, inflammation, and cancer: how are they linked? *Free Radical Biological Medicine,* 49:603-616

Rodriquez, T., Youglove, L., Lu, C., Funez, A., Weppner, S., Barr, D. (2006). Biological monitoring of pesticide exposures among applicators and their children in Nicaragua. *International Journal of Occupational and Environmental Health,* 12(4):312-20

Rohlman, D.S., Arcury, T.A., Quandt, S.A., Lasarev, M., Rothlein, J., Travers, R… (2005). Neurobehavioral performance in preschool children from agricultural and non-agricultural communities in Oregon and North Carolina. *Neurotoxicology*, 26:589-98

Rohlman, D.S., Bodner, T., Arcury, T.A., Quandt, S.A., McCauley, L. (2007). Developing methods for assessing neurotoxic effects in hispanic non-english speaking children. *Neurotoxicology,* 28:240-4

Rohlman, D.S., Lucchini, R., Anger, W.K., Bellinger, D.C., Van Thriel, C. (2008). Neurobehavioral testing in human risk assessment. *Neurotoxicology,* 29:556-67

Rojas, A., Ojeda, M.E., Barraza, X. (2000). Congenital malformations and pesticide exposure. Revista Medica de Chile. 128(4): 399-404

Ruckart, P.Z., Kakolewski, K., Bove, F.J., Kaye, W.E. (2003). Long-term neurobehavioral health effects of methyl parathion exposure in children in Mississippi and Ohio. *Environmental Health Perspectives*, 112:46-51

Ryhanen, R.J.J. (1982). Pseudocholinesterase activity in some human body fluids. *Genetic Pharmacology, 14(4),* 459-460. doi: 10.1016/0306-3623(83)90030-7.

Salvador, M., Bordin, D.L., Andreazza, A.C., Silva, J.D., Henriques, J., Erdtmann, B. (2008). Determination of oxidative stress markers and serum cholinesterase among pesticide sprayers in southern Brazil. *Toxicology Environmental Chemical,* 4:809-814

Sanborn, M., Kerr, K.J., Sanin, L.H., Cole, D.C., Bassil, K.L., Vakil, C. (2007). Non-cancer health effects of pesticides. *Canadian Family Physician,* 53:1712-1720

San-Miguel-Montes, L.E., Allen, D.N., Puente, A.E., Neblina, C. (2010). Validity of the WISC-IV Spanish for a clinically referred sample of Hispanic children. *Psychology Assess,* 22(2):465-9

Santi, A., Menezes, C., Duarte, M.M., Leitemperger, J., Lopes, T., Loro, V.L. (2011). Oxidative stress biomarkers and acetylcholinesterase activity in human erythrocytes exposed to clomazone (in vitro). *Interdisciplinary Toxicology*, 4:149-153

Savage, E.P., Keefe, T.J., Mounce, L.M., Heaton, R.K., Lewis, J.A., Burcar, B.J. (1988). Chronic neurological sequelae of acute organophosphate pesticide poisoning. *Archive of Environmental Health, 43:38-45*

Scarpato, R., Migliore, L., Angotzi, G., Fedi, A., Miligi, L., Loprieno, N. (1996). Cytogenetic monitoring of a group of Italian floriculturists: no evidence of DNA damage related to pesticide exposure. *Mutation Research*, 367:73-82

Schmid, W. (1975). The micronucleus test. *Mutation Research/Environmental Mutagenesis and Related Subjects*, 31(1):9-15

Schwartz, D.A and LoGerfo, J.P. (1988). Congenital limb reduction defects in the agricultural setting. *American Journal of Public Health*, 78(6): 654-658. doi: 10.2105/AJPH.78.6.654

Sedelnikova, O.A., Nakamura, A., Kovalchuk, O., Koturbash, I., Mitchell, S. A., Marino, S. A., et al. (2007). DNA double-strand breaks form in bystander cells after microbeam irradiation of threedimensional human tissue models. *Cancer Research.* 67: 4295–4302. doi: 10.1158/0008-5472.CAN-06-4442

Sestili, P., Martinelli, C., Stocchi, V. (2006). The fast halo assay: An improved method to quantify genomic DNA strand breakage at the single-cell level. *Mutation Research*, 607:205-14

Shah, Z.H., O'Dell, K.M.C., Miller, S.C.M., An, X., Jacobs, H.T. (1997). Metazoan nuclear genes for mitoribosomal protein S12. *Genetic*, 204(1-2): 55-62. Doi: 10.1016/S0378-1119(97)00521-0

Shaily, U.S. (2012). Importance of Genotoxicity & S2A guidelines for genotoxicity testing for pharmaceuticals. *IOSR Journal of Pharmacy and Biological Sciences (IOSRJPBS)*. 2278-3008:1:2. 43-54

Sigma-Aldrich (2014). Comet Assay. Available from: http://www.sigmaaldrich.com/life-science/cell-biology/cancer-research/learning-center/cancer-research-protocols/comet-assay.html

Simcox, N.J., Fenske, R.A., Wolz, S.A., Lee, I.C., Kalman, D.A. (1995). Pesticides in household dust and soil:exposure pathways for children of agriculture families. *Environmental Health Perspectives*, 103(12), 1126-1134

Singh, N.P, et al. (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Res*earch, 175: 184-91.

Singh, V. K., Reddy, M. K., Kesavachandran, C., Rastogi, S. K., & Siddiqui, M. K. J. (2007). Biomonitoring of organochlorines, glutathione, lipid peroxidation and cholinesterase activity among pesticide sprayers in mango orchards. *Clinica Chimica Acta*, *377*(1), 268-272.

Slotkin, T.A. (2004). Cholinergic systems in brain development and disruption by neurotoxicants: nicotine, environmental tobacco smoke, organophosphates. *Toxicology Application Pharmacology*, 198:132-151

Slotkin, T.A., Seidler, F.J. (2005). The alterations in CNS serotonergic mechanisms caused by neonatal chlopyrifos exposure are permanent. *Developmental Brain Research*, 158:115-119

Smith, A.H., Hopenhayn-Rich, C., Warner, M., Biggs, M.L., Moore, L., Smoth, M.T. (1993). Rationale for selecting exfoliated bladder cell micronuclei as potential biomarkers for arsenic genotoxicity. *Journal of Toxicology and Environmental Health*, 40:223-234

Suarez-Lopez, J.R., Jacobs, D.R., Himes, J.H., Alexander, B.H. (2013). Acetylcholinesterase activity, cohabitation with floricultural workers, and blood pressure in Ecuadorian children. *Environmental Health Perspectives*, 121(5): 619-624

Suk WA., Murray K., Avakian MD. (2003). Environmental hazards to children's health in the modern world. *Mutation Research*. 544:235-242

Sumi, Y., Oode, Y., Tanaka, H. (2008). Chinese dumpling scare hits Japan- a case of methamidophos food poisoning. *The Journal of Toxicological Sciences*, 33(4):485-486

Tadeo, J., Sanchez-Brunete, C., Gonzalez, L. (2008). Pesticides: classification and properties. New York, NY: CRC Press. p:1-34

Tapia, L.R., Parron, T.P., Santed, F.S. (2005). Neuropsychological effects of long-term exposure to organophosphate pesticides. *Neurotoxicology and Teratology*, *27*, 259-266. doi: 10.1016/j.ntt.2004.12.002

Thomas, P., Fenech, M. (2011). Buccal micronucleus cytome assay. *Methods Molecule Biology*, 682:235-248

Tian, Y., Ishikawa, H., Yamauchi, T., Yokoyama, K. (2005). Teratogenicity and developmental toxicity of chlorpyrifos. Maternal exposure during organogenesis in mice. *Reproductive Toxicology*, 20:267-270

Tice, R.R. (1995). The single cell gel/comet assay: A microgel electrophoretic technique for the detection of DNA damage and repair in individual cells. In:

Phillips DH, Venitt S, editors. *Environmental Mutagenesis.* Oxford: Bios Scientific Publishers. p 315–339

Tourism Malaysia. (2016). Map of Selangor. Retrieved from http://www.tourismmalaysia.or.jp/map/pdf/map_Selangor.pdf

Trivegen. (2014). Comet Assay. Retrieved from http://www.trevigen.com/cat/1/3/0/CometAssay/

USEPA (U.S. Environmental Protection Agency). (2010). Reregistration Eligibility Dicision (RED) for Malathion. Available from http://www.epa.gov/pesticide/reregistration/status_op.htm

USEPA (U.S. Environmental Protection Agency). (2015). Introduction to pesticide drift. Available from: http://www.epa.gov/reducing-pesticide-drift/introduction-pesticide-drift

Valavanidis, A., Vlachogianni, T., Fiotakis, C. (2009). 8-hydroxy-2'-deoxyguanosine (8-OhdG): a critical biomarker of oxidative stress and carcinogenesis. *Journal of Environmental Science Health Part C Environmental Carcinogenesis and Ecotoxicology Reviews.* 27:120-139

Valcke, M., Samuel, O., Bouchard, M., Dumas, P., Belleville, D., Trembly, C. (2006). Biological monitoring of exposure to organophosphate pesticides in children living in peri-urban areas of the Province of Quebec, Canada. *International Achieve Occupational and Environmental Health,* 79:568-77

Vida, P., Moretto, A. (2007). Pesticide exposure pathways among children of agricultural workers. *Journal of Public Health,* 15:289-99

Vrhovac, V.G., Zeljezic, D. (2000). Evaluatoin of DNA damage in workers occupationally exposed to pesticides using single-cell gel electrophoresis (SCGE) assay pesticide genotoxicity revealed by comet assay. *Mutation Research.* 469:179-285

Ward, M.H., Lubin, J., Giglierano, J., Colt, J.S., Wolter, C., Bekiroglu, N, et al. (2006). Proximity to crops and residential exposure to agricultural herbicides in Iowa. *Environmental Health Perspectives,* 114(6): 893-7

Waris, G., Ahsan, H. (2006). Reactive oxygen species: role in the development of cancer and various chronic conditions. *Journal of Carcinogenesis,* 5:14-21

Wechsler, D. (2003). Wechsler Intelligence Scale for Children: Administration and Scoring Manual, 4th Ed. Harcourt Assessment, San Antonio, TX(USA).

Weiss, B., Amler, S., Amler, R.W. (2004). Pesticides. *Pediatrics.* doi:113:1030–1036.

Weppner, S., Elgethun, K., Lu, C., Hebert, V., Yost, M.G. Fenske, R.A. (2006). The Washington aerial spray drift: children's exposure to methanidophos in an agricultural community following fixed-wing aircraft applications. *Journal of Exposure Science Environmental Epidemiological,* 16(5):387-96

WHO (2001) Public health impact of pesticide used in agriculture. Geneva:*World Health Organization 51:* p 86

Whyatt, R.M., Barr, D.B., Camann, D.E., Kinney, P.L., Barr, J.R., Andrews, H.F et al. (2003). Contemporary-use pesticides in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and newborns. *Environmental Health Perspectives,* 111(5): 749-56

Whyatt, R.M., Rauh, V., Barr, D.B., Camann, D.E., Andrews, H.F., Garfinkel, R., Hoepner, L.A., Diaz, D., Dietrich, J., Reyes, A., Tang, D., Kinney, P.L., Perera, F.P. (2004). Prenatal insecticide exposures and birth weight and length among an urban minority cohort. *Enviromental Health Perspectives,* 112:1125-1132

Wong, R.H., Kuo, C.Y., Hsu, M.L., Wang, T.Y., Chang, P.I., Wu, T.H., Huang, S. (2005). Increased levels of 8-hydroxy-2'-deoxyguanosine attributable to carcinogenic metal exposure among schoolchildren. *Environmental Health Perspectives,* 113: 1386-1390

Zahm, S.H., Ward, M.H. (1998). Pesticides and childhood cancer. *Environmental Health Perspectives,* 106:893–908.

Zailina, H., Junaidah, R., Josephine, Y., Jamal, H.H., (2008). The influence of low blood lead concentrations on the cognitive and physical development of primary school children in Malaysia. *Asia-Pasific Journal of Public Health, 20(4),* 317-326. doi:10.1177/1010539508322697

Zailina, H., Junidah, R., Saliza, M.E., Shamsul, B.S., Jamal,H.H. (2011). Low blood lead concentrations and cognitive development of primary school children from three areas in Malaysia. *Journal of Environmental Science and Engineering, 5,* 493-499