**SOUTHWESTERN NIGERIA**

**BY**

**SURAJUDEEN ADEBAYO, YAQUB**

**BMLS (Ekpoma), M.Sc. (Ibadan)**

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EXPOSED TO DICHLORVOS ORGANOPHOSPHATE PESTICIDE IN IBARAPA COMMUNITY, SOUTHWESTERNNIGERIA" was carried out by Surajudeen Adebayo, YAQUB under my direct supervision in the Department of Chemical Pathology, University of Ibadan, Ibadan.

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Supervisor

O.G. Arinola (Ph.D; Cert Immunology, Switzerland)

Professor of Immunology

Department of Chemical Pathology,

University of Ibadan.

organophosphate chemicals because of non-persistence in the environment. Dichlorvos organophosphate pesticide (DOP) is indiscriminately used by farm workers in spite of its associated acute and chronic adverse effects on nervous system. Although, information on the adverse effects of DOP on nervous system of farm workers is well documented, information on its effects on their immune status which can be drawn from inflammatory markers is scarce. This study was designed to determine changes in inflammatory markers in DOP-exposed farm workers.

Ethical approval (UI/EC/11/0107) and informed consent were obtained as appropriate. Knowledge, attitudes, practices of farm workers towards pesticide use and associated toxicity symptoms were obtained using structured questionnaire. Farm workers (FW) consisting of 60 pesticide applicators (PA) and 60 farmers exposed to DOP for ten to fifteen years were randomly selected into this case-control study. Sixty apparently healthy adults without occupational exposure to DOP served as controls. Blood sample was collected and serum obtained by centrifugation. Serum activity of acetylcholinesterase (AChE) was determined by HPLC, differential leucocyte count was determined using thin film microscopy, serum levels of immunoglobulin E (IgE), C-reactive protein (CRP), interferon gamma (IFN-γ), interleukins (IL) 4 and 10 were determined using ELISA. Activities of myeloperoxidase, NADPH oxidase (Nox) and catalase were determined by spectrophotometry. Type-1 hypersensitivity was carried out using skin prick test with environmental allergens and neutrophil-lymphocyte ratio (NLR) was calculated. Data were analysed using ANOVA, Student's t-test and Pearson moment correlation coefficient (PMCC) at $\alpha_{0.05}$

Lymphocyte counts (56.0±8.0; 56.0±8.0; 56.0±4.0 against 41.0±5.0), eosinophil (2.0±1.0; 3.0±1.0; 2.0±1.0 against 1.0±0.0), levels of IgE (327.4±169.3; 320.7±171.4; 334.2±168.4 against 229.3±178.4 IU/mL), CRP (10.1±8.8; 12.6±10.4; 7.6±5.7 against 7.2±6.6 mg/L), IFN-γ (104.8±9.5; 128.4±16.8; 81.2±7.9 vs 65.1±5.6 pg/mL), IL-4 (214.1±16.3; 249.7±27.8; 178.4±16.0 against 87.2±48.3 pg/mL), activities of myeloperoxidase (12.3±9.3; 10.5±7.3; 14.1±10.8 against 7.7±5.6 U/mL), Nox (7.9±5.4; 7.3±5.7; 8.6±5.1 against 4.8±3.9 U/mL), the diameter of skin reaction to grass (3.7±0.7; 3.6±0.9; 3.7±1.0 against 3.0±0.0 mm) and mold (3.6±0.7; 3.5±0.9; 3.6±0.9 against 3.0±0.0 mm) allergens were higher in FW, PA and farmers compared with control, respectively. Serum activities of AChE (7.3±0.9; 6.6±0.9; 7.9±0.6

IU/mL), myeloperoxidase (10.5±7.3 against 14.1±10.8 U/mL) and catalase (2.5±1.1 against 3.3±1.6 U/mg protein) but significantly raised counts of monocyte (2.0±1.0 against 1.0±0.0) and eosinophil (3.0±1.0 vs 2.0±1.0), serum levels of CRP (12.6±10.4 against 7.6±5.7 mg/L), IFN-γ (128.4±16.8 against 81.2±7.9 pg/mL), IL-4 (249.7±27.8 vs 178.4±16.0 pg/mL) and IL-10 (116.4±9.9 vs 91.1±11.1 pg/mL) were found in PA compared with farmers.

Long term exposure to dichlorvos organophosphate pesticide increases most of the inflammatory markers in farm workers especially among pesticide applicators.

**Keywords**: Inflammatory markers, Occupational exposure, Pesticide applicators

**Word count**: 478

To the memories of my grand father and my mother for giving me balanced education.

To the sacrifice of my family for putting up with the inconveniences of separation resulting from my preoccupation with this study and

To the efforts of the poor farm workers who ensure availability of food for our teeming population.

beseech His blessings upon the noble soul of prophet Muhammed (peace be upon him) and his companions.

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APC = Antigen Presenting Cell

ATP = Adenosine triphosphate

ATSDR = Agency for Toxic Substances and Disease Registry

BChE = Butyrlcholinesterase

BHC = Beneze Hexachloride (BHC),

CD = Cluster of Differentiation

CDC= Center for Disease Control

CE = Carboxylesterase

ChE = Cholinesterase

CPHO = Chaiyaphum Provincial Health Office

CYP = Cytochrome P

CYP = Cytochrome P450

DAG = Diacylglycerol

DDT = Dichlorodiphenyltrichloroethane

DDVP = Dimethy 2, 2-dichlorovinyl phosphate

DEDTP = Diethyl dithiophosphate

DEP = Diethyl phosphate

DETP = Diethyl thiophosphate

DMDTP = Dimethyl dithiophosphate

DMP = Dimethyl phosphate

DMTP = Dimethyl thiophosphate

DNA = Deoxyribonucleic acid

DOP = Dichlorvos Organophosphate Pesticide

ELISA = Enzyme linked immunosorbent assay

ERK = Extracellular signal-regulated kinases

FAO = Food and Agricultural Organization

FcR = Fc Receptor

GABA = Gamma-aminobutyric acid

GDP = Gross Domestic Product

IARC = International Agency for Research on Cancer

ICAM = Intracelullar adhesion molecule

Ig = Immunoglobulin

IGRs = Insect Growth Regulators

IL = Interleukin

IPCS = International Programme on Chemical Safety

IPM = Integrated Pest Management

ISO = International Standard Organization

LC50 = Lethal Concentration 50 percent

LD50 = Lethal Dose 50 percent

LGL = Large granular lymphocyte

LPS = Lipopolysaccharide

MALT = Mucosa Associated Lymphoid Tissue

MAPK = Mitogen-activated protein kinases

MDP = Muramyl dipeptide

MHC = Major Histocompatibility Complex

mRNA = messenger Ribonucleic acid

NADPH = Nicotinamide adenine dinucleotide phosphate

NK = Natural killer

NLR = Neutrophil Lymphocyte Ratio

NOS = Nitric oxide synthetase

NOx = NADPH Oxidase

NPC = National Population Commission

NPIC = National Pesticide Information Center

NRC = National Research Council

NTE = Neuropathy Target Esterase

OCs = Organochlorines

OPs = Organophosphate Pesticides

PKC = Protein kinase-C

PMN = Polymorphonuclear

RNS = Reactive Nitrogen Species

ROS = Reactive Oxygen Species

Th = T-helper

TLR = Toll-like receptor

TWBC = Total White Blood Cell

ULV = Ultra-Low Volume

USEPA = United States Environmental Protection Agency

WHO = World Health Organization

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## 1.1 Background of the study

Population pressure in most developing countries including Nigeria has necessitated a search for better methods to increase food production involving the use of modern agricultural technology and pesticides (Anetor *et al.,* 2001); which is unguided.

Pesticides are chemicals used in agriculture to protect crops against destructive pests both in the field and during storage (Blasco *et al.*, 2003; Leong *et al.,* 2007; Pesticide Action Network, 2017). Their use in modern agriculture improves yield and quality of farm produce, reduces pest epidemics, saves cost and energy, increases income of farmers and generates revenue for government as well as guarantes save global food supply (NASS, 2017). However, these pesticides are absorbed, metabolized and eliminated from the body differently. They also have different mechanisms of action given rise to varyinghuman toxicity (Gupta, 2006; Vale and Lotti, 2015). Yet, the application is unregulated.

Previous studies showed that pesticides such as persistent organic pollutants (POPs) especially, organochlorine (OC) pesticides are relatively safe to use but persist in the environment and bioaccumulate in the food chain. They had been banned because they pose serious adverse effects to exposed workers (Szmedra, 1994). They were replaced with pesticides such as organophosphate pesticides (OPs) which are acutely toxic but do not persist in the environment beyond a few months (Pesticide Action Network, 2017). However, their use need to be controlled.

Dichlorvos, chlorpyrifos and dimethoate are OPs which are frequently sprayed in cocoa, cashew, orange and mango plantations of South-Western Nigeria, a tree crop belt of the country (Sosan *et al*., 2009). Of all, dichlorvos, a class 1b highly hazardous OP according to International Standard Organization (ISO) with trade names Siege, Snipers, DDforce, Didwell is the commonest and the most widely used in this region. Although, the chemical structures of OPs vary considerably, they have a similar physiological mode of action which is inhibition of cholinesterase (ChE) activity. Inhibition of ChE activity results in accumulation of acetylcholine and eventually alteration of cholinergic activities (Vale and Lotti, 2015). Thus, OPs control pests by exerting neurotoxic effects on the pests that attack crops. However, they affect both the

exactadverse effects during chronic occupational exposures are yet to be explicitely understood (Kamanyire and Karalliedde, 2004). However, large amount of experimental data have implicated pesticides in the induction of immunosuppression, hypersensitivity and autoimmunity (Germolec and Lusster, 1994; Sobhana *et al.,*2006)but evidence that OPs may severely impair immune functions in humans is presently scarce.

Turner (1994) had reported direct immunotoxicity of the cells, tissues and organs of the immune system, inhibition or induction of important enzymes in components of the immune systemor cytokinesthat modulate functions of the immune cells. Neurotoxicity, effects of nutrition and metabolism on the cells and organs of immune system have been reported as indirect immunotoxicity. Although, there are controversial reports on pesticide immunotoxicity in human, the featutres of immunotoxicities resulting from OPs exposure in human varies but dependent on the extent of exposure and toxicity of the OP involved (Galloway and Handy, 2003; Sarwar, 2015).

Inspite of lack of convincing human data, a potential risk for the immune system cannot be ruled out, especially during chronic occupational exposure to pesticides. Emerging evidences show that inflammation is of vital function in the pathophysiology of DOP toxicity in humans and since it precedes immunosuppression, hypersensitivity and autoimmunity (Luster *et al*., 1992; Galloway and Handy, 2003; Banks and Lein, 2012).However, inflammation is yet to be accorded its rightful position among the important mechanisms in DOP toxicity. In this regard, assessing inflammatory makers (as a measure of inflammatory response) and by convention, innate and adaptive immune responses in farm workers exposed to DOP could provide additional but vital information that might be useful in the overall assessment of occupational exposure to OPs.

Agriculture is a basic source of income and subsistence among many Nigerians. Though, agriculture contributes about 40% to Gross Domestic Product (GDP) in Nigeria (Awolola, 1991; Emeka, 2007; Are *et al*., 2010). Its tendency to expose the immune system of farm workers to pesticide toxicity is usually not accorded the deserved attention. Hence, farm workers face increased risk of mortality from short

have funded most researches into improving food production but not the health of farm workers responsible for the production.

Abandonement of traditional farming practices such as multiple or mixed cropping or intercropping as methods of pest control has encouraged pests. In order to boost food production, Green Revolution an attempt by Nigerian government facilitated significant use of modern technology such as pesticides, fertilizers and sophisticated farm implements. The magnitude and distribution of the adverse effects on the immune status of occupationally exposed workers that resulted from adoption of this modern agricultural technology is unknown especially in Nigeria. Having in mind that safe working environment will promote physical, mental and social well being of the farm workers resulting in effective work output.

This study therefore assessed inflammatory markers of male farm workers exposed to Dichlorvos Organophosphate pesticide (DOP) in Ibarapa community, Southwestern Nigeria and also identified the cohort of farm workers that is more at risk of inflammatory based disorders.

## 1.2 JUSTIFICATION OF THE STUDY

Nervous, endocrine and immune systems act in concert to coordinate body metabolism (Paul, 1999). Most researches into health impacts of pesticides on exposed workers have concentrated on the damage to the nervous system (Galloway and Handy, 2003). They have satisfactorily reported mechanisms of neurotoxic effects of acute and chronic exposure to OPs (Kamanyire and Karalliedde, 2004; Edem *et al.,* 2012; Vale and Lotti, 2015). However, there is less emphasis on the immune system, which is another system vulnerable to injury from acute and chronic exposure to OPs especially DOP.

Previous experimental studies on human cell cultures, laboratory animals and wildlife provide strong evidence that many pesticides are immunotoxic (Turner, 1994; Owoeye *et al.*, 2012; Chandra *et al*., 2017). There is increasing evidence that OPs exert immunotoxic effects on human too (Luster *et al*., 1992; Galloway and Handy, 2003; Hertz-Picciotto *et al*., 2018). Previous reports showed that some compounds that block

inflammatory effects are solube in nature. It is possible that dichlorvos or its metabolites either bind directly to receptor on immune cell or directly to the immune cell itself thereby inhibiting or enhancing its activity (Hermanowiz, 1984; Luster *et al*., 1992; Ashade *et al*., 2011). The alterations observed in inflammatory markers due to interference from DOP residues or its metabolites may enhance or reduce immune responses which may be accompanied by increased risk of allergy, autoimmunity, cancer and susceptibility to infection in farm workers exposed to DOP compared to non-exposed. Though, not conclusive, the weight of evidence gives ground for concern. The skin and mucous membranes in addition to acting as physical barriers to the penetration of foreign substances during occupational exposure are provided with active mechanisms for trapping them (Salimonu, 2016). Environmental triggers are not limited to microorganisms as intact dichlorvos residues may trigger local inflammatory response in the skin and mucus membranes and if not resolved may bind or interact with other components of the immune sytem resulting in systemic inflammation which may predispose the body to inflammatory based disorders.

Despite the fact that the developing nations consume 20% of the world's total pesticide use (Zyoud *et al*., 2010), studies on farm workers exposed to various pesticides have concentrated in developed countries where industrial chemicals have been a bigger worry than pesticides (Rose, 1992; NASS, 2017). Presently, DOP is the most active, widely and uncontrolablly used OP in Nigeria but there is paucity of information on its effect on the immune status of farm workers exposed in the agrarian area of Ibarapa community in Oyo State, Nigeria. Therefore, evaluation ofinflammatory markers in male farm workers exposed to DOP may be of advantage in the overall assessment of occupational health.

## 1.3 HYPOTHESIS

$H_o$:

There are no differences between the inflammatory markers of male farm workers exposed to dichlorvos organophosphate pesticide compared with the controls.

## 1.4 AIM OF THE STUDY

To determine inflammatory markers in male farm workers (PA and farmers) exposed to DOP and identify the group of farm workers at higher risk of inflammatory based disorders.

## 1.5 OBJECTIVES OF THE STUDY

a) To determine serum cholinesterase (ChE) activity in DOP-exposed male farm workers compared with the non-exposed participants.

b) To determine cellular inflammatory markers: Neutrophil, lymphocyte, monocyte, and eosinophil counts and Neutrophil-Lymphocyte ratio in DOP-exposed male farm workers compared with the non-exposed participants.

c) To determine humoral inflammatory markers: serum levels of immunoglobulin E (IgE), C-reactive protein (CRP), Tumour Necrosis Factor-alpha (TNF-α), Interferon-gamma (IFN-γ), Interleukin4 (IL-4), Interleukin-10 (IL-10) and skin prick test to eight environmental allergens in DOP-exposed male farm workers compared with the non-exposed participants.

d) To determine serum levels of enzymatic mediators of inflammation: Myeloperoxidase (MPO), NADPH oxidase (Nox), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in DOP-exposed male farm workers compared with the non-exposed participants.

e) To identify the group of male farm workers that has higher risk of inflammatory disorders due to DOP exposure.

## 2.1 Agriculture

Agriculture is the art and science of growing plants and other crops and raising animals for food, other human needs, or economic gain (Bareja, 2019). It is the systematic raising of useful plants and livestock under the management of man (Rimando, 2004).Agriculture in Nigeria has greatly improved in the past few years because of the advent of modern technology and other necessary infrastructures (Olajide *et al.,* 2012). Although, initially, Nigerian farmers only practice subsistence farming making food available for their family with less available for marketing (Awolola, 1991).Slash-and-burn land-clearing methods and crop rotation were early agricultural techniques. Steady improvements in tools and methods, mechanization, selective breeding and hybridizationas well as use of pecticides over the centuries increased agricultural output (Are *et al.,* 2010).

Growth in agricultural output was rising as farmers are stepping away from subsistence agriculture and embracing mechanized farming (Emeka, 2007). The Nigerian soil and climatic condition is very suitable for the production of wide varieties of crops. There are over a hundred different food and cash crops produced by farmers in Nigeria on yearly basis which includes yam, maize, millet, sorghum, beans, potatoes, rice, onions, garbage, carrot, pear, cocoa, cashew, mango, palm oil, cocoa yam, okra, vegetables and very many others (Olajide *et al.,* 2012).Agriculture in Nigeria contributes merely about 20 percent of the Nigeria total GDP, trailing behind petroleum which is the major Nigerian domestic produce. In 1990, out of ninety-one million hectares of Nigerian total land mass, eight-two million were actually cultivable only forty-two percent of it was use for agricultural purposes. It is presently believed that the sector of agriculture in Nigeria is a potential source of revenue that is yet underdeveloped and unexplored (Emeka, 2007).

Figure 2.1a: Photograph ofa typical farm at Fedegbo village, Igboora, Ibarapa community taken on 21/03/2012.

Figure 2.1b: Photograph ofa typical farm at Dagilegbo village, Eruwa, Ibarapa community taken on 03/04/2012.

human health and development (Gbaruko*et al.,* 2009). Individuals are exposed to occupational toxicants at the workplace through inhalation, dermal absorption and oral routes (Gbaruko*et al.,* 2009; Wesseling *et al.,* 2011). While there is some debate as to the levels and types of exposure that precipitate health problems, there is mounting evidence that many toxicants may be linked to negative health outcomes (Galloway and Handy 2003; Costa, 2018). While a very large number of occupational toxicants are potentially harmful to health, the most commonly studied ones can be divided into three major categories: heavy metals, organics, and pesticides (Gbaruko*et al.,* 2009).

### 2.2.1 Pesticides

Pesticides are substances that are used to prevent, destroy, repell or mitigate pests (Costa, 2018). There is a steady increase globally in the useof pesticidesin order to make food available for the teaming population of the world (US EPA, 2017). Meanwhile,the available hecters of land for agricultural purposes is reducingdue to industrialization (Zyoud *et al.,* 2010). The consequencesof which has been an increase in prevalence of adversehealth effects in humans particularly and environmental pollution in general (Kamrin, 1997; Ekeleme *et al.,* 2008; Hashim and Khan, 2010; Sarwar 2015).Other factors include continous use of pesticides such as OC that have been banned for long or restricted in the United States in developing countries like Nigeria, weakly enforced regulations guiding pesticides use and farm workers lack of adequate training and equipment to handle pesticides safely in Nigeria (Repetto and Baliga, 1996; Emeka, 2007; Clune *et al*., 2012).


Figure 2.2: Photograph of containers of various pesticides at Ola Olorun agro chemical retail store, Igbole, Igboora taken on 22/03/2012.

world. Although the history of pest control likely began with the first human who swatted mosquito or picked off a tick. However, during the emergence of organized agriculture when pests started to attackplants grown for food and threatened man's survivalthat the battle for the control of theplanet earth began (Eric and Gordon, 2007; Soltaninejad and Shadnia, 2014). Sulphur element dust was first used as pesticide to protect crops. Arsenic, mercury and lead were used as pesticide around 1500 followed bythe use of of tobacco extract nicotine sulfate around 1700. Pyrethrum and rotenone naturally extractedfrom chrysanthemums and rotenonic roots of tropical vegetables respectively were introduced in the 19$^{th}$ century (Miller, 2002). Paul Müller introduced Dichlorodiphenyltrichloroethane (DDT) as a highly effective pesticide around 1950 (Ritter, 2009). However, DDT an organochlorine was observed to be persistent in the environment and bioaccumulate in the food chain and was replaced by organophosphates and carbamates around 1970s.Pyrethrin became dominating pesticide on the discovery of acute toxicity of organophosphates and carbamates especially in the United States of American (Ritter, 2009; Pesicide Action Network, 2001). Meanwhile, Nigeria and other developing countries are still using organochlorines and organophosphates despite the side effects (Pesticide Action Network, 2017).

Around 20th century, World's population grew exponentially and more food was needed to feed everyone. The World Wars I and II occurred, and many scientific efforts were directed to the creation and dispersal of weaponized chemical agentswhicheventually led to the making of synthetic pesticides (Miller, 2002). Specifically, an upusurge in the use of pesticidesfollowed the second world war. Among the pesticidesintroduced were Dichlorodiphenyltrichloroethane, beneze hexachloride and 2,4 dinitrophenylhydrazine (Benbrook, 1991). DDT was commonly used because of its broad-spectrum activity against insect pests of agriculture. Due to their inexpensive and effectiveness against pesticides, they were used to control pesticides in cash crops (Eric and Gordon, 2007; Damalas, 2015).

Following prolonged use of pesticides, a quite number of pestsdeveloped genetic resistance to pesticides resulting inharmful effects on the non-target plants and animals and detection of pesticide residuesin variousareas of the environment. Scientists found

Carson was published in 1962 shook the confidence of public on the use pesticides. Carson painted a grim picture of environmental consequences of careless pesticide use. Though, there were severe critics of the quality of her report, Carson, more than anyone before pointed out the risks of careless pesticides use (Carson, 2012). This resulted in the introduction of environmental friendly natural pesticides derived from plants and insect growth regulators collectively referred to as biorational (Knutson *et al*., 1990; Benbrook, 1991).

In the 1960s, researchers initiated integrated pest management (IPM)aiming at keeping pests at reduced less significant levelthrough theuse of crop rotation methods that discourage pests but encourage beneficial predators or parasites that attack pests (Benbrook, 1991). Actually, the goal was not to eradicate the use of pesticides and this not even desiredin most cases because eliminating a pest may lead toloss of some predators or parasites beneficial to some pestssurvival. IPM does not replace theuse of pesticides butto improve on the effective use or decrease the totalpesticide use to reasonable level. Even with implementation of IPM, frequent uses of pesticides at appropriate times is the only solution tomanage emergent pest epidermics (Benbrook, 1991). Presently, Scientists have been able to make specific pesticides directed at specific pest, such as algicides, bactericides, fungicides, and insecticides. Most of these newer products are available to Nigerian farmers. While in many ways these were safer than the general poisons farm workers used before, the new pesticides also tended to be much more powerful, and sometimes misused due to lack of formal training in handling them (Clune *et al.*, 2012).

### 2.2.3 Prevalence of pesticides use

Though globally, systematic estimates of overall exposure are not available, evidence indicates that hundreds of millions of farm workers, farm households, and consumers are probably exposed to dangerous levels of pesticides (Repetto and Baliga, 1996; Colosio *et al*., 2013). Almost half of the world's 6.5 billion people live in rural areas, mostly in farm households. Approximately, out of 2 billion workers in the world in 1990; 1.1 billion (over half) are farm workers, the largest occupationally exposed group of which 95

Calvert *et al*. (2004) reported ayearly occurrence of eighteenper hundred thousand of health related sickness in workers occupational exposed to pesticides in United State of America. Also, according to WHO estimation, almost twenty thousandexposed farmworkers experience death due to occupational exposure to OPs annually. The report showed highest incidence from developing nation (Miller, 2004). WHO further estimated three million OPs intoxications per year with estimated number of casualities and deaths put at three thousand per year (Damalas, 2015). Ahmed *et al.* (2002) reported cases of severe acute poisoning due to pesticides exposure in Multan, Pakistan and occupational pesticides poisoning in hospitalized patients. According to annual health service report of 2005 from Chaiyaphum Provincial Health Office (CPHO) in Thailand, the prevalence of toxicityin farm workers exposed to pesticides was 8.4%. Kachaiyaphum *et al.* (2010) also from Thailand reported overall toxicity prevalence of 32.0% (11.1% unsafe and 20.9% risky). However, Anetor et al. (2001) reported 27% risky prevalence and 8% unsafe prevalence of organophosphate poisoning in Nigerian male occupationally exposed farm workers. Also, Sosan *et al.* (2009) in a study conducted on cocoa farmers in Southwestern Nigerian reprted a prevalence of risky and unsafe exposure of 28% (8.0% unsafe and 20% risky).

Pesticide use in Nigeria has been on the increase ever since its introduction in early fifties for cocoa production. Nigerian cocoa production is still dependent on pesticides to attain acceptable levels of crop production. There was a significant increased production of cocoa in 1960sfollowingthe recommended use of Lindane in 1957 against mirid (Gerard, 1967; Sosan *et al*., 2009). To this end, the increase in the use of pesticides in the cocoa belt of Nigeria became phenomenal and earlier studies have shown that input subsidies helped greatly in boosting pesticide use (Awolola, 1991). The initiation of Green Revolution in the late 1970sand operation feed the nation later also increased the prevalence of pesticides use in Nigeria (FAO, 2005; Sosan *et al.,* 2009). This led to a steady rise in pesticides import to the countryin 2001 to 2003 from around thirteen million dollars totwenty million dollars. Insecticidesonly accounted for up to 32% (Sosan *et al.,* 2009; US EPA, 2017). The increased availability of pesticides resulted to unguided and mishandling of these chemical and various adverse effects (Sosan *et al.*,

pyrethroids, 12% biopesticides and 6% organochlorines. Approximately, Nigeria uses about one hundred and thirty metric tons of pesticides annually (Ikemefuna, 1998; Asogwa and Dongo, 2009). In 1991 only, cocoa pesticides accounted for about 31% of the total agro-chemical market of which fungicides accounted for 65% and insecticides 35% (Ikemefuna, 1998).

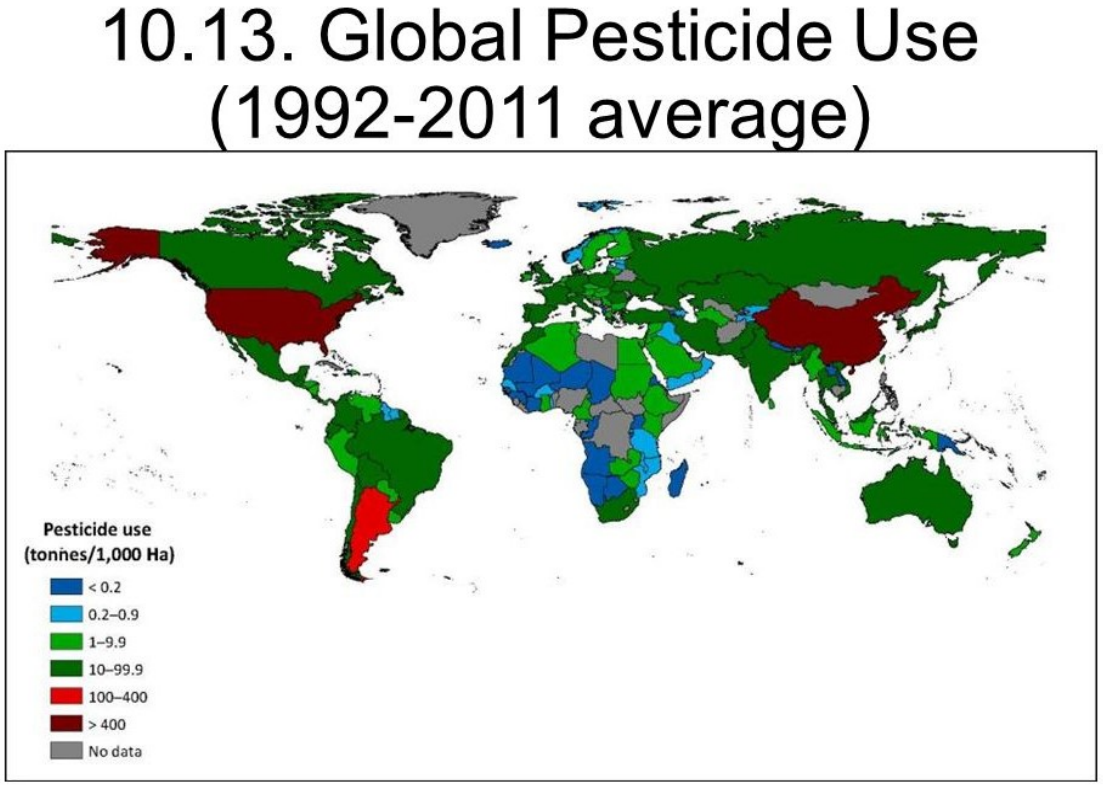



Figure 2.3: A map showing prevalence of pesticide (WHO, 2014)

1. Chemical nature
2. Target organism
3. Toxicity
4. Mode of action

#### 2.2.4.1. Classification based on chemical nature

Pesticides are categorized into two groups based on their chemical nature intoorganic and inorganic pesticides (Gilden *et al*., 2010). Organic pesticides are based on chemicals having carbon as the basis of their molecular structure. The chemicals in organic pesticides are more complex and not readily dissolve in universal solvent. Inorganic pesticides appear like crystals of salt in shape, environmentally stable and readily dissolve in water. Sulfur and lime were the earliest inorganic pesticides used (Miller, 2002). The vast majority of modern pesticides contain organic chemicals. There have been hundreds of pesticides developed based on organic chemicals, often with oxygen, phosphorus, or sulfur in their molecules, in addition to their basic carbon structure (USEPA, 2005; WHO, 2009).

Organic pesticides can be subdivided into two additional groups: the natural organics and the synthetic organics. The natural organic pesticides (sometimes called organics) are derivable from natural plants sources including rotenone and pyrethrum among others while synthetic organic pesticides (usually called synthetics) are manufactured from chemicals synthesis withDOP and cypermethrin as examples among other ones (Kamrin, 1997; WHO, 2009).

#### 2.2.4.2 Classification based on target organism

Most pesticides may be classified according to the pests they kill. Major subclasses of pesticides include insecticides, herbicides and fungicides among others (Table 2.1). Prominent insecticide chemical families include organochlorines (OCs), organophosphates (OPs), carbamates and pyrethroids (Xue *et al.,* 2000; Kamrin, 1997; Collins, 2006)

| | |
|---|---|
| Algicides or Algaecides | Algae |
| Avicides | Birds |
| Fungicides | Fungi and Oomycetes |
| Bactericides | Bacteria |
| Insecticides | insects |
| Rodenticides | Rodents |
| Virucides | Virus |
| Nematicides | Nematodes |
| Molluscicides | Snails |
| Herbicides | Weeds |
| Piscicides | Fish |

been created by a national or international government-related or sponsored organization. The toxicity can be grouped into toxicity class I, II, III and IV respectively (WHO, 2001). WHO hazard classification of toxicity of formulated chemical product shows that pesticide which belongs to WHO class Ia is associated with extrem hazard, class 1b high hazard, class11 moderate hazard, class111 slight hazard and class 1V not likely hazardous under controlled usage (WHO, 2009). Almost ninety percent of restricted pesticidesare of categories 1a/ 1b/11 (WHO, 2014).

#### 2.2.4.4 Classification based on mode of action

Pesticides can also be classified by how they enter the target organism or where they act. Based on mode of action, they can be classified into four distinctive groups namely, stomach poisoning, contact poisoning, fumigants or systemic acting.

Stomach pesticides enter insects' body through the mouth and digestive tract. They are usually acquired during feeding. Contact pesticides enter the body of pests via their epidermis upon contact and causes death by poisoning. Fumigating pesticides are usually in gaseous form, they enter the body of pests via their respiratory system and cause death by poisoning (Adams and Robert, 2005). Systemic pesticides are present in the body fluid of a host organism following consumption, any pest that feed on this becomes poisoned and died of pesticide toxicity (Kamrin, 1997).

#### 2.2.4.5 Naming of pesticides

A pesticide is given three names. The active ingredient contained in the pesticide is written as the common name on a label on the pesticide container,each producer gives a brand or trade name which is written conspicously on the label and thestructure of the active ingredient as the chemical or scientific name (Kamrin, 1997; WHO, 2009).

### 2.2.5 Organophosphate pesticides (OPs)

Organophosphate pesticides (OPs) are phosphate esters, amides or thiol derivatives of

phosphate atom with a double bond to either sulphur or oxygen, two ethyl or methyl organic side chains and a leaving group which is specific for a given pesticide or individual organophosphate (US EPA, 1996). All OPs share some common chemical properties as shown in figure 2.4 (NPIC, 2009; Costa, 2018).

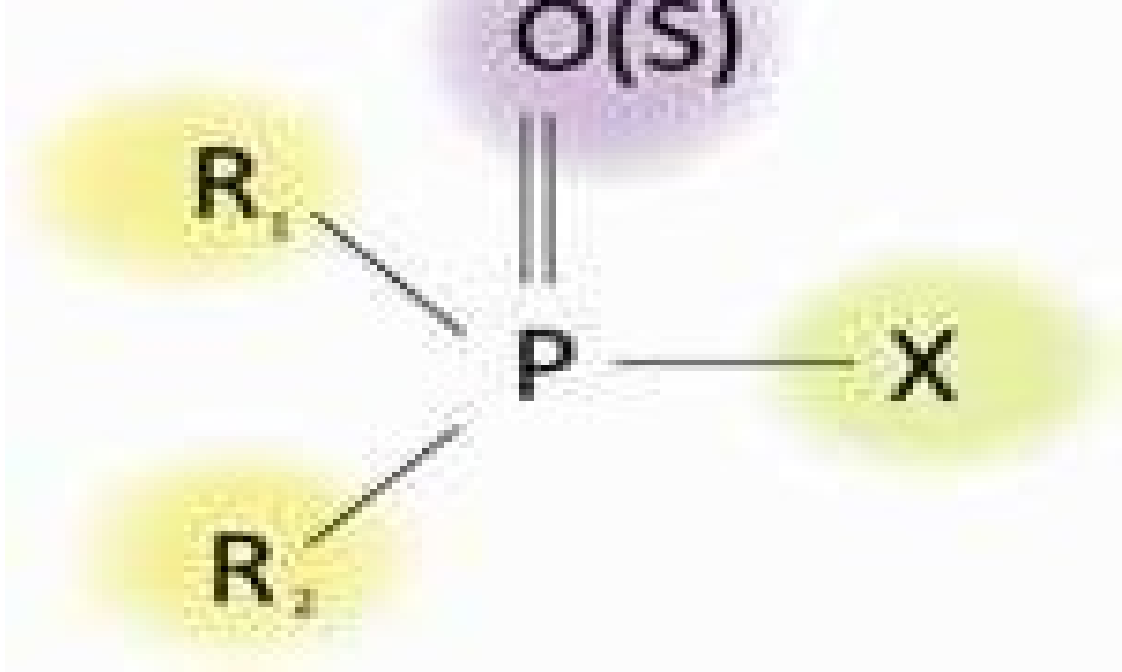



Figure 2.4: Common structure of organophosphates (Kwong, 2002).

X denotes leaving or specific group

R1 and R2 denote side groups

Schrader, a German Chemist (Costa, 2006). OP was later developed to severe toxic agents and used as nerve gas but changed to less toxic substances in the mid of 19th century (Gupta, 2006). However, the use of OPs rose rapidly towards the end of that centuryat the time the use of organochlorine pesticides like DDT was banned as a result of long persistence and bioaccumulation in the environment.

OPs form a large family of approximately fifty thousand chemical agents (Kamanyire and Karalliedde, 2004). Altogether, over 100,000 OP compounds have been screened for their insecticidal properties, of which over 100 have been developed for commercial use (Pesticides Trust, 1996; Pesticide use data, 2017). OPs are also widely used because they are cheaper than the newer alternatives (Soltaninejad and Shadnia, 2014). So with the switch from organochlorines to OPs, it can be assumed that the consumer has benefited at the expense of the pesticide operator (Soltaninejad and Shadnia, 2014). The OPs in use among farm workers in Southwestern Nigeria contain dichlorvos, chlorpyrifos and dimethoate as their active ingridients (ATSDR, 1997).

#### 2.2.5.1 Mechanism of action of OPs

OPs share a common mechanism of toxicity, through inhibitory effects on cholinesterase enzymes (Roberts and Reigart, 2013). In Figure 2.4, X which denotes the leaving or specific group is substituted through nucleophilic replacement by the oxonal serine of ChE enzyme active site. The extent of depression of ChE enzyme by any OP is largely dependent on the kind of specific group contained such that high probablity of a leaving group resulting in high affinity of the OP to ChE enzyme. The oxon phosphorus=oxygen structural type is the active form that link to the active site of ChE. (Figure 2.5). Meanwhile, majority of novel OP contain the thio Phosphorus=Sulphur linkage whichmust first be converted to an oxon group by CYP enzymes to allow OP act as ChE inhibitor(Kamanyire and Karalliedde, 2004; Gupta, 2006).

Most of the adverse effects that follow OPs exposure have been associated with the inhibition of ChEs (Coggon, 2002). However, Monnet-Tschudi *et al* (2000) has claimed that onlycholinesterase inhibition may not be responsible for the spectrum of

Karalliedde, 2004; Costa, 2018). Other effects of OPs that have been experimented include inactivation of beta esterases by phosphorylation.and interference with innate and adaptive immune response among others.

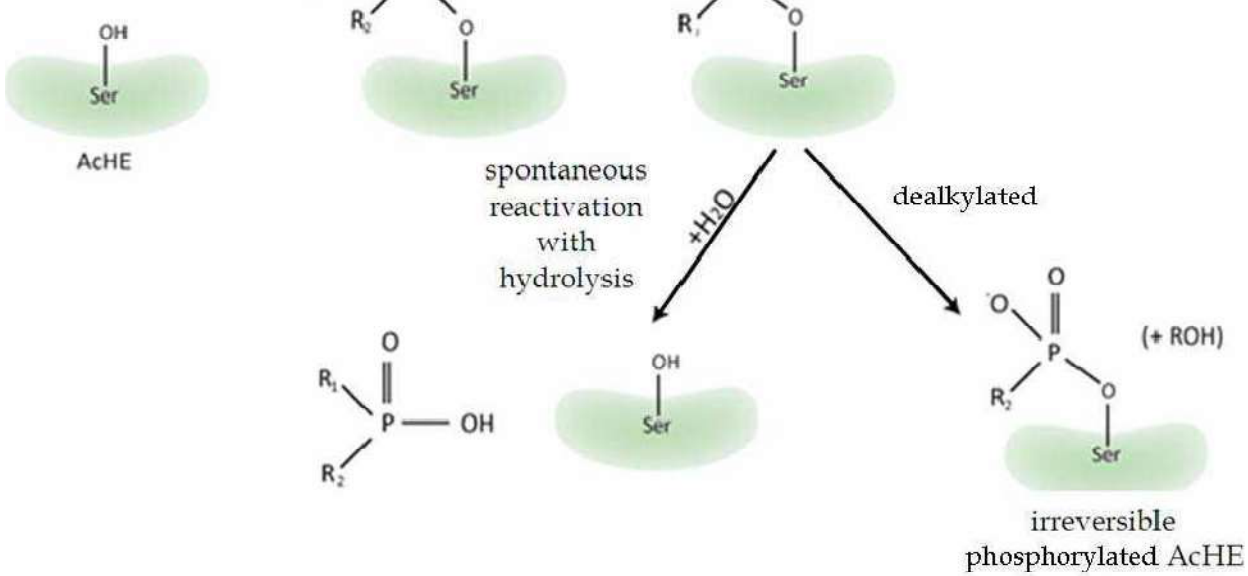



Figure 2.5: The Oxon structure of OP binds to the active site of ChE (Kwong, 2002)

dermal, mucosalsurfaces, gastrointestinal and respiratory tracts (Karalliedde *et al.,* 2003; Costa, 2018).

The liver metabolises xenobiotics majorly although minor metabolism occurs in the lung and intestine. OPs metabolism which comprisesphase I and phase IIoccurs the same way withmost of xenobiotics.Absorbed OP is activated by the metabolic enzymes in phase Ithroughattachment of functional groups forming an intermediate metabolite which undergoesphase II reactions. The phase II enzymes conjugate various hydrophilic groups such as glucuronides, sulphates, glutamate, glycine with the intermediate metabolite enabling its excretion from the body. Detoxification reactions take place exclusively in phase II metabolism (Kwong, 2002).

Oxidation and hydrolysis are involved in Phase I of OP metabolism (Figure 2.7). Oxidation activates OP thio to form active inhibitors of ChE through oxidative desulphuration where CYP enzymes catalyzes replacement of the sulphur atom in the OP thio structure with one atom of oxygen to form an unstable intermediate OP oxon metabolite. This reaction is a key step for most of neurotoxicity effectedby OPs because OP oxon metabolites are strong inhibitors of ChE (Kwong, 2002).Hydrolysis catalyzed by paraoxonase (esterase A) or carboxylesterase detoxifies OP oxon metabolite through cleavage to dialkylphosphate and the specific group. (Kwong, 2002).

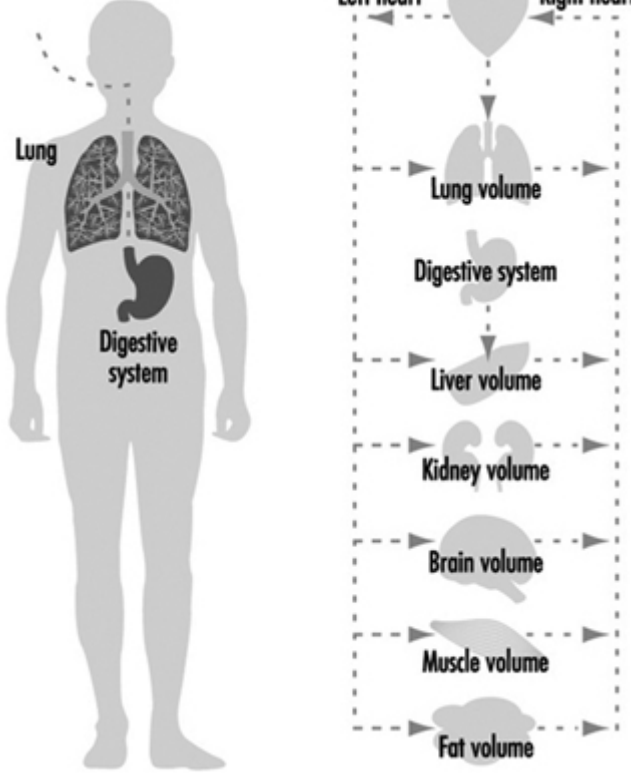



Figure 2.6: Absorption and distribution of DOP (Gupta, 2006).

an unstable intermediate with CYP enzymes. However, it should be noted that oxidative desulphuration activates OPs whreaseither dealkylation or oxidative cleavage detoxifies them. Thus, the two reactions compete and the equilibrium between them becomes very important for the final toxicity of the OPs (Gupta, 2006).

Most of the studies onthe metabolism of model OPs showedcytochromes 1A1, 3A4, 2B6 and 2C19 as the most essential enzymes. Cytochromes 1A1, 3A4, 2B6 hadbeen shown to have the highest affinity for oxidative desulphuration while cytochrome 2C19 have been the most efficient in oxidative cleavage (Kwong, 2002). This varied polymorphic forms in the numerous human cytochrome enzymes implies that whether a person will be susceptibile or not to toxicity of OPs is dependent on which isoforms of cytochrome enzymesexpressed and probably forms the basis for using paraoxonase-1 as a biomarker of susceptibility of OPs (Buratti *et al.,* 2007).

The kidneys eliminate toxicants including pesticides and their metaboliteswith waste products of metabolism and maintenance of homeostasis (De Alwas *et al*., 2008). Pesticides in most cases are lipophilicbut are rendered polar during metabolism in the liver to readily excretable form from the body primarily through the kidneysand bile in terms of the overall excretion (Krieger, 2011).

Cellular transporter proteins facilitate excretion of these compounds into bile or the systemic circulation. Transporters and enzyme activities are influenced by endogenous factors such as circadian rhythms, hormones, cytokines, disease states, genetic factors, sex, ethnicity, age, and nutritional status, as well as by exogenous drugs or chemicals. Bile is the major excretory route for hepatic metabolites. Compounds excreted in bile may undergo enterohepatic circulation, being reabsorbed in the small intestine and re-entering the portal circulation (Krieger, 2011).

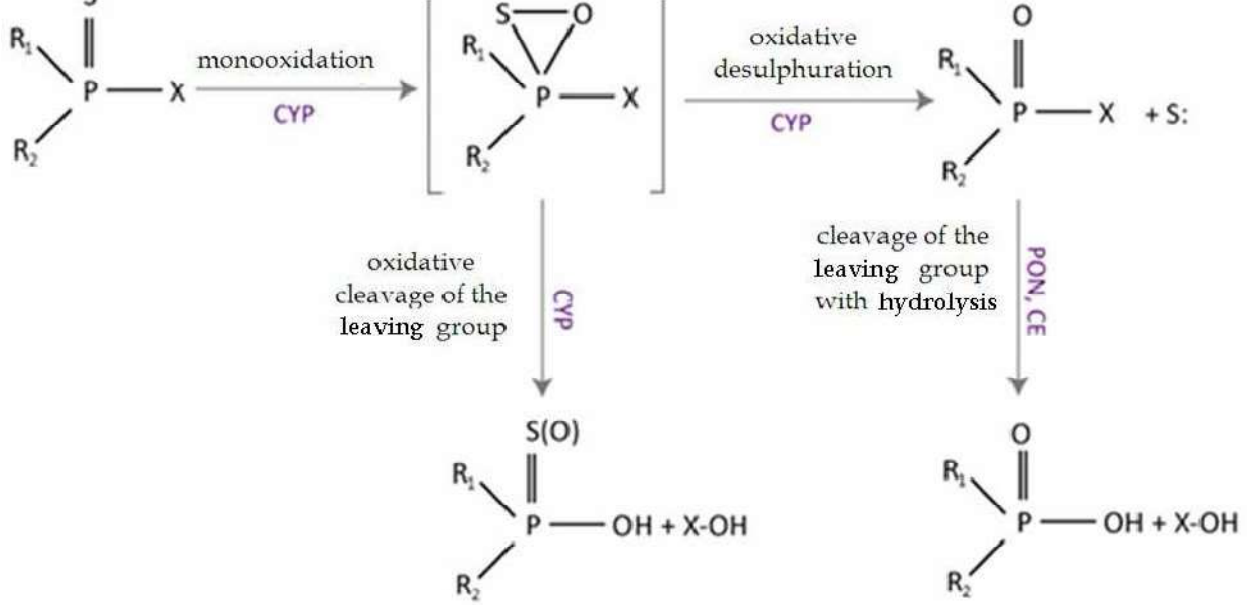



Figure 2.7: OP metabolism (Kwong, 2002).

CYP denotes cytochrome P450

PON denotes paraoxonase

CE denotes carboxylesterase

P

effects (Kamrin, 1997; Mew, *et al.*, 2017) with OPs being the most dangerous (Kamanyire and Karalliede, 2004). Acute toxicity of pesticides are adverse effects resulting from a point exposure or a short time repeated exposures. (Clyde *et al.*, 2011).Generally, OPs possessacute toxicity but with varied level of toxic effects.

Chronic toxicity refers to the non-immediate toxic effects of persistent chronic exposure to small doses of pesticides. Years may pass before signs and symptoms develop (Gbaruko*et al.,* 2009; Colosio *et al*., 2013).

The adverse effects of OPs is dependent on the chemical structure,mode of absorption and dose absorbed, metabolism by exposed individual among others (Kwong, 2002). However, the most studiedadverse effects of OPs are the neurotoxic effectsdue to inhibition of the primary target ChE enzymesin acute poisoning. Chronic poisoning probably due to potential secondary targets enzymes and thus adverse effects besidesneurotoxicity especially human immunotoxicity have not been adequately research into but are also very essential inevaluating and assessing associated risks.

**2.2.5.3.1 Neurotoxic effect of OPs**

The primary effect of exposure to OPs is neurotoxicity. The mechanism involves inhibition of the enzyme AChE by OPs causing accumulation of enormous acetylcholine in the synapses whichinturn responsible fortoxicity of the neuronsmanifested as paralysis of the muscles of the whole body (Gupta, 2006).OPs inhibit AChE by binding covalently toAChE active site with very slow spontaneous hydrolysis or irreversible in chronic exposure which result in delayed adverse effects (Costa, 2018).

AChEis the enzyme that regulates activities of synapses in the central and peripheral nervous systemsby converting neurotransmitter acetylcholineinto choline and acetate. The brain and spinal cordforms the central nervous system while the somatic nerves and autonomic nerves which consist of sympathetic and parasympathetic parts constitute the peripheral nervous system.

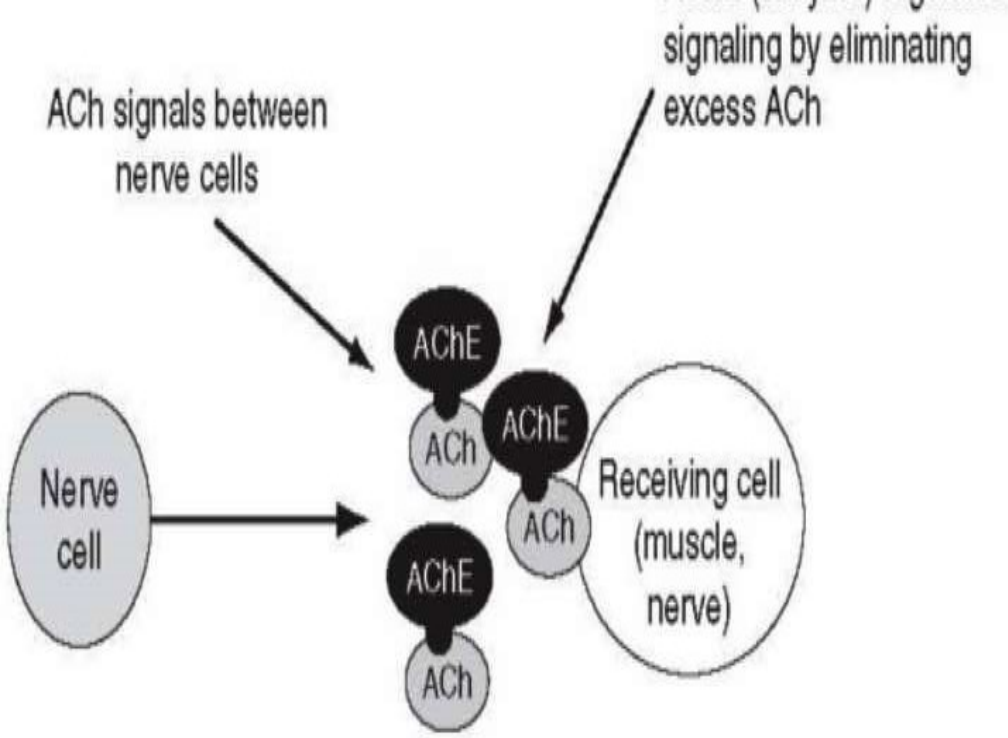
Inaccessibility of the central nervous system to experiment has made studies of mechanisms of OPs poisoning a bit difficult compared with the peripheral nervous

acute poisoning due to OPs exposure can be grouped based on the site of acetylcholine accumulation (Mew *et al*., 2017).

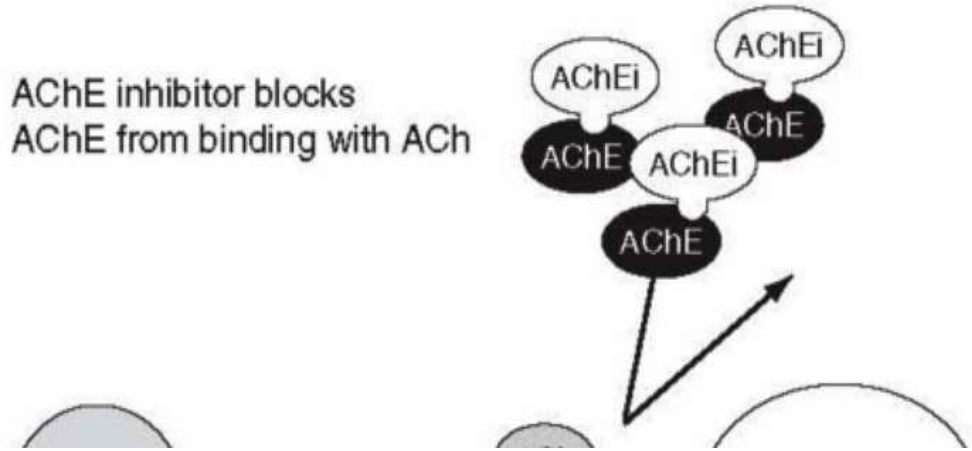
Some OPs cause delayed symptoms in addition toacute symptoms. Difficulties in breathing and muscle weakness often occurwithin 1 - 4 days following exposure while peripheral muscle weakness appear within 7 - 21 days. The cause of the delayed symptoms has been attributed to inhibition of another enzyme neuropathy target esterase (NTE) whichof similar class of serine esterases like AChE, however, itselementary functionhas not been well established (Kamanyire and Karalliedde, 2004).

Exposure to OPs causes a sequential triphasic illness in exposed individuals. In most cases, the earliest cholinergic phase may only be observed. This cholinergic phase proceeds to the intermediate syndrome in approximately 20% of exposed individuals. The acute cholinergic phase and the intermediate syndrome are both associated with a high risk of mortality and exposed subjects are best managed in an intensive care unit unless the poisoning is very mild. Organophosphate-induced delayed polyneuropathy is the final phase which does not carry the risk of death. It sets in within 7 – 21 days after exposure to an OP and may not progress to either the cholinergic phase or the intermediate syndrome (Kamanriye and Karalliedde, 2004).

The inactivation leads to accumulation of acetylcholine at muscarinic and nicotinic sites as well as the central nervous system leading to acute cholinergic phase (Karalliedde and Henry, 2001). The intermediate syndrome characterized by weakness of muscles, nerve palsies, breathing problem and respiratory failure followed acute cholinergic stage few days after (Senanayake and Karalliedde, 1987; Benson *et al.,* 1992). Organophosphate-induced delayed polyneuropathyis associated predominantlywith neuropathy target esterase (NTE) (Owoeye *et al*., 2012; Sarwar, 2015).




Normal nerve signaling



Although the primary mechanism of neurotoxic effect of OPs has been extensively reported but there is paucity of information onthe secondary mode of action and outcomes of chronic OPs exposure on human tissues and organs apart from the targeted nervous system.However, some studies have reported many secondary targets for OPs that may block an array of metabolic processes which include inhibition of carboxylases (Sarwar, 2015). The inhibition of other enzymes includingcytochrome enzymes(Hodgson, 2003),lipases, DAG-lipase, lysyl oxidase (Casida and Quistad, 2004) among others have been reported.

Some OPs have been reported to activate signalling pathways of protein kinase -C (PKC) indirectly while others have been reported toactivate Ca2+/cAMPin experimental animals (Hodgson, 2003).

**2.2.5.3.3 Immunotoxic effects of OPs**

Large amount of experimental data have implicated pesticides in the induction of immunosuppression, hypersensitivity and autoimmunity (Germolec and Lusster, 1994; Sobhana *et al*., 2006)but evidence that OPs may severely impair immune functions in humans is presently scarce. However, Luster *et al*. (1993) have reported thatexposure tooccupational and environmental pollutantsgenrally interfere with immune response in humans. There are controversial reports on pesticide immunotoxicity in human, the featutres of immunotoxicities resulting from OPs exposure in human varies but dependent on the extent of exposure and toxicity of the pesticide involved (Galloway and Handy, 2003). Inspite of lack of convincing human data, a potential risk for the immune system cannot be ruled out, especially during chronic occupational exposure to pesticides whichmay enhance the immune response leading to allergy or autoimmunity, or result in immunosuppression that may induce cancer susceptibility and high risk of infections (IPCS, 1996; Colosio *et al*., 2013).

In experimental immunotoxicology, basic mode of actions of pesticides on cells and organs of the immune system are studied to recognisespecific parts that are affected directly or indirectly (Luster *et al*., 1992; Ashade *et al*., 2011).Turner (1994) had reported direct immunotoxicity of the cells, tissues and organs of the immune system,

However, human immune system is physiologically diverse and to selecteright biomarkers and analytical methods for evaluation and assessment may be very tasking (Galloway and Handy, 2003). In most cases, the observed outcomes are very slight and not conclusive to ascertain risks to human health. Therefore, immunotoxic effects observed under experimental conditions are not convincing enough to adgorge toxicity to human health. This is highly challenging to be able rate the contributions of pesticide exposure to the burden of immune-related diseases.

Existing data on experimental studies suggest a tier approach whichshould be based on three steps. The first approach excludeor point out the modulation of the immune system followed by refining the outcomes of the first phase to provide essential information on the mode of action and pattern of the observed effects whilst the last stage probe more to establish immunotoxic effects of a pesticide (Luster *et al*., 1992). A similar tier approach shown in Table 2.2.is advocated in human studies also. (IPCS, 1996; Chandra *et al.*, 2017). According to previous recommendations,Tier 2 should be done on all subjects with abnormal findings in tier 1 and Tier 3 should be carried outonpeople with abnormal results in tier 2.

| Tier | Group | Parameters |
|---|---|---|
| 1 | Cellular | Complete and differential blood cells count<br>lymphocyte subpopulations:<br>CD3: T-Cells<br>CD4: T-Helper<br>CD8: T-Suppressor/Cytotoxic<br>CD20: B-Cells<br>CD16: Natural killer<br>CD57: Natural killerCD3/HLA |
| | Blood and serum | Erythrocyte Sedimentation Rate (ESR)<br>C-reactive protein<br>Immunoglobulins (IgGs, IgAs, IgE, IgM)<br>IgE to specific allergens<br>Complement factors<br>Rheumatoid factor<br>Non-organ-specific antibody: Anti-nuclear antibody |
| 2 | | Anti-thyroglobulin antibody |
| | Cellular | lymphocyte subpopulations:<br>CD25-DR: T-cell activated<br>CD57: Natural killer<br>CD3/HLA-DR: T-Cell activated |
| | Serum | Immunoglobulin subclasses (IgA1, IgA2, IgG1–4)<br>Sera level of selected cytokines |
| | | Proliferative response to:<br>Phytohemoagglutinin, |

| | | |
|---|---|---|
| | | Polyclonal immunoglobulin production after pokeweed stimulation |
| | | Immunization to a vaccination (B-hepatitis) |
| | | In vitro cytokine production (spontaneous and stimulated) |
| | | NK activity |
| 3 | Miscellaneous | RNA-messenger for cytokines |
| | | Cytokine soluble receptors |
| | | Surface soluble markers |
| | | Other cytokine production |

progression of malignancies andelucidating the underlying mechanisms is an essential area of research. However, available informationas regardsthe potencyof OPs to cause cancers in exposed humansare from epidemiological reports.In such studies, chronic occupational exposed workershave been reported to have high risk of lymphomas and different types of leukaemia (IARC, 1987; ATSDR, 1997).

Most of the cancer causing chemicas interact with multiple stage of carcinodenesis by initiating, promoting and progressingcancer stages via mutating and causing DNA damage inducing neoplastic cell transformation (Sarwar, 2015).

#### 2.2.5.3.5 Other effects of OPs

Other studies have reported that chronic exposure to very small doses of OPs may result in cumulative poisoning which may produce sub-clinical effects initially but render the individual susceptible to further toxic insults (Fajewonyomi, 1995; Clune *et al.*, 2012).

There is conflicting evidence concerning the teratogenic effects of OPs in animals (Gadoth and Fisher, 1978; Karalliedde *et al.,* 1988). Data on the effects of occupational exposure to OPs on pregnant women and their foetuses are not available (Minton and Murray, 1988). Furthermore, hepatotoxicity (Edem *et al.,* 2012), nephrotoxicity (Azmi *et al.,* 2009), and oxidative damage (Prakasam *et al.,* 2001; Bhat and Bhat, 2016) as a result of exposure to OPs have been reported.

Previous research reported high occurrence of infections of upper respiratory tract in OP exposed farm workerswho also demonstrated depressed serum and RBC ChE activity (Karalliedde and Senanayake, 1989; Hantson *et al*., 1996; Mew *et al.*, 2017).

### 2.2.6 Dichlorvos organophosphate pesticide (DOP)

Dichlorvos (Dimethyl 2, 2-dichlorovinyl phosphate) belongs to organophosphate family of insecticide pesticides. It is a group 1b chemical, an agent that is highly hazardous (WHO, 2009). It is also classified as a group IIb chemical, an agent that is carcinogenic to humans (WHO, 2014).

agrarian community of Oyo state, Nigeria under different trade names such as DDforce, Siege, Snipers, Didwell among others. The main release route is through emission during use and eventual inhalation by exposed farm workers. Dichlorvos has a half life of 7hours (Chen *et al.,* 1984; Ashade *et al*., 2011), persist in the environment with potential risk for farmers who re-enter sprayed areas within that period. The half-life values for hydrolysis of dichlorvos reduceswithan increase in temperature but enhance in basic conditions. The products of hydrolysis of dichlorvos are dimethylphosphate and dichloroacetyaldehyde. Dichlorvos is considered to be biodegradable in aerobic and anaerobic conditions thus making its environmental elimination by hydrolysis and biodegradation possible and thereforehas low bioaccumulation level (Abd-Allah, 1995; Costa, 2018).


Figure 2.9: Photograph of containers showing trade name of DOP taken on 22/03/2012 at Ola Olorun agro chemical retail store, Igbole, Igboora.

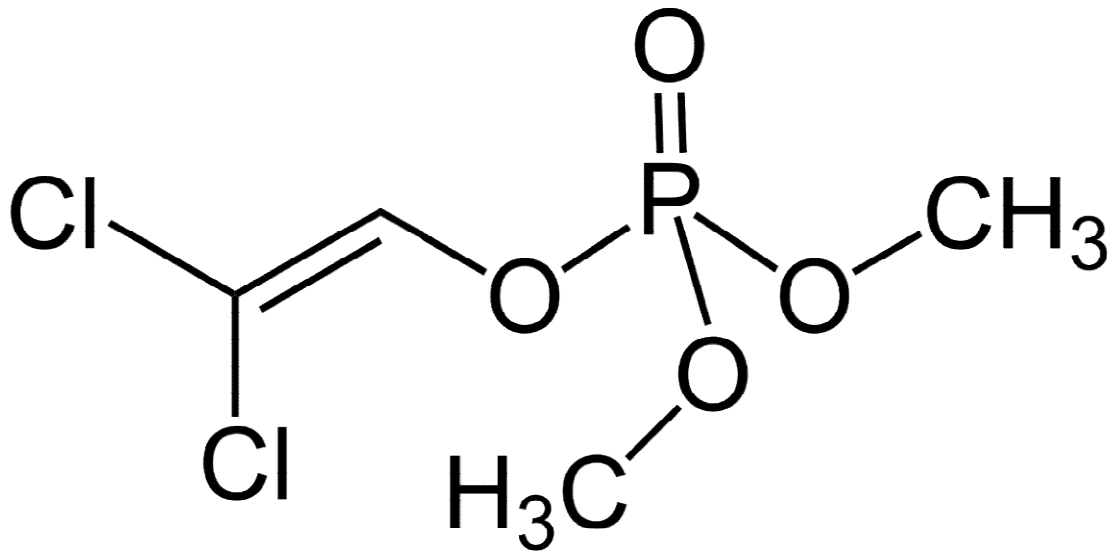



Figure 2.10: Structure of dichlorvos (Kwong, 2002).

| | |
|---|---|
| Chemical name | Dimethyl 2,2-dichlorovinyl phosphate (DDVP) |
| Synonyms | Dichlorvos;2,2-dichlorovinyl dimethylphosphate |
| Trade name | Siege, snipers, DDForce, Didwell |
| Molecular formular | $C_4H_7Cl_2O_4P$ |
| Appearance | Colourless to amber liquid |
| Melting point | $< -60^0C$ |
| Boiling point | $140^0C$ at (2.7kPa) |
| Vapour pressure | 1.6 Pa at $(20^0)C$ |
| Solubility in water | 8g/L at $(20^0)C$, miscible with alcohol and toluene |

The central phosphorous atom of dichlorvos has less electrons making it vulnerable to nucleophilic attack much more than the carbon atom of either methyl group making phosphorylation of AChE than alkylation to 4-nitrobenzyl pyridine a common reaction. The activity of dichlorvos in the methylation of DNA is reduced by the presence of type-A esterase in mammalian tissues such as plasma and liver(Aldridge and Johson, 1977; ATSDR, 1997; Costa, 2018). In experimental studies, phosphorylation of AChE and other esterases is of significance than methylation of DNA purine basesbeing negligibly account for less carcinogenic effects of OPs generally (Wooder *et al.,* 1977; Wright *et al.,* 1979; Chandra *et al*., 2017).

**2.2.6.2 Absorption, metabolism and excretion of DOP**

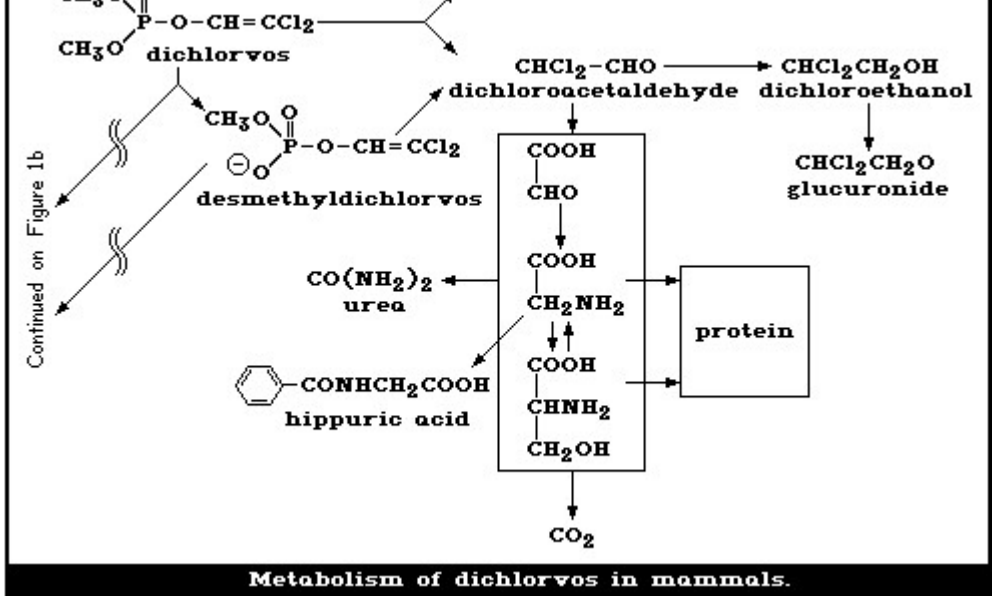
Previous studies in human volunteer have established the absorption ofinhaled dichlorvos (Hutson and Hoadley, 1972b; Das *et al.,* 1983; Costa, 2006), via the oral route in experimental animals (Mohammad *et al,* 1989; Stanton *et al.,* 1979; ATSDR, 1997) and dermal absorption also in experimental animals (Durham *et al.,* 1957; Vale and Lotti, 2015).

In an experimental study, dichorvos inhaled was reported to accumulate much more in the lung than the trachea (Blair *et al*., 1975; ATSDR, 1997) and oral administration of dichlorvos with absorption via gastrointestinal tract and metabolism in the liver gives an undectectable concentration in the tissues because of rapid clearance (Gaines, 1969; Costa *et al*., 2013). The results of human and experimental studies in mice, rats, Syrian hamsters, pigs, goats and female cows using isotope-labelled dichorvos in various administration routes indicated a similarmetabolic pathways for dichlorvos (IPCS, 1996; Vale and Lotti, 2015).

The metabolism of dichlorvos occurs in the main route through hydrolysis of a bond between phosphate and vinyl catalyzed by type-A esterase forming dimethylphosphate which is excreted in urine and dichloroacetaldehydewhich is further metabolized to dichloroethanol immediately (Wright *et al.,* 1979; Costa *et al.*, 2013). Dichlorvos

excretion in urine occurs (Wright *et al.,* 1979; Costa *et al.*, 2013).

Thus, dichloroethanol glucuronide is the final products through the first path way via dichloroacetyldehyde and dichloroethanol while hippuric acid, urea and carbon (IV) oxide are the final products of the second path way via dichloromation to dichloroacetyldehyde (Hutson and Hoadley, 1972b; ATSDR, 1997). Metabolites of dichlorvos are excreted in the breathr, urine, faeces and carcass (Casida *et al.,* 1962; Hutson and Hoadley, 1972b; Costa *et al.*, 2005).



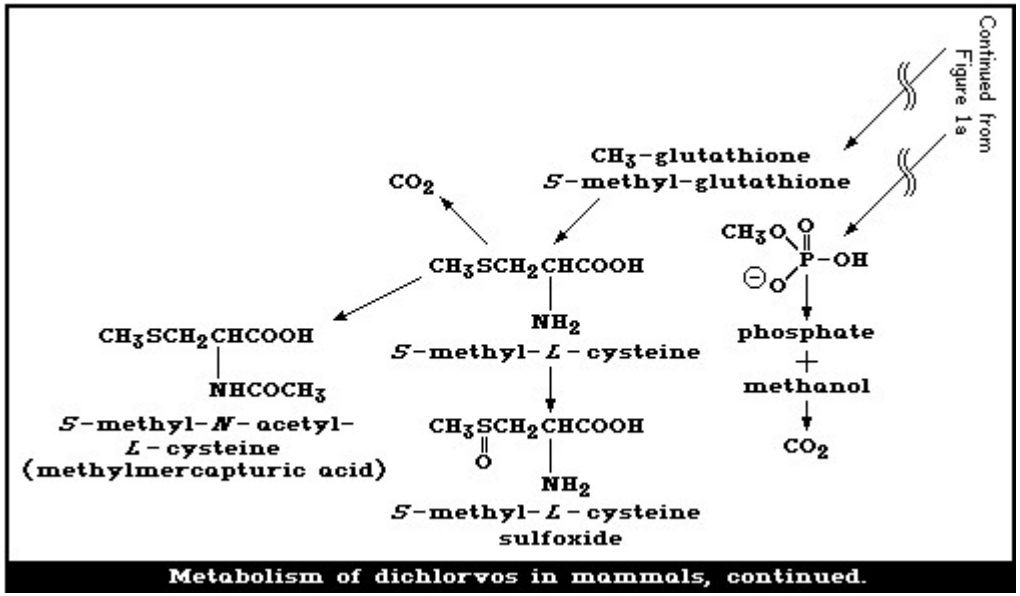




Figure 2.11: Metabolism of dichlorvos (Costa, 2018)

Bisby and Simpson (1975) reported acute effects of DOP such as dizziness, dysphea and delibility due to severe anaemia as well asdepressed serum cholinesterase activity, anticholinergic like symptomsand axonal degeneration neuropathy (Cervoni *et al.,* 1969; Wadia *et al.,* 1985; Mew *et al*., 2017). However, long term chronic effects through oral administration, inhalation and dermal contact also produced a depressed plasma cholinesterase activity and RBC cholinesterase activity of applicators with detection of dimethylphosphate in their urine (Stein *et al.,* 1966; Sasinovich, 1970; Menz *et al.,* 1974; Das *et al.*, 1983; Colosio *et al*., 2013)

Studies investigating neurotoxicity of dichlorvos in experimental animals reported reduction in brain and platelet NTE activities (IPCS, 1996). Dichlorvos moderately sensitize the skin (Fujita, 1985) but severely irritate it (Arimatsu *et al.,* 1977). However, other studies observed no reproductiveor teratogenic abnormality but suggest developmental toxicity (Schwetz *et al*., 1979; Dambaska and Maslinska, 1982; Chandra *et al*., 2017).

**2.2.7 Occupational exposure to OPs and Routes of exposure**

Human beings can be exposed through environmental contamination or occupational use of pesticides. Acute toxicity of most of these pesticides have been adequately studied however available knowledge on delayed effects in occupational exposure is much more limited (Fajewonyomi, 1995; Damalas, 2015).

Exposure to pesticides is among the most essential risksof occupation in farm workers ofdeveloping nations. Occupational exposure to pesticides is essential to the identification of the hazardous use of pesticide and in establising methods of safe handling of pesticides because worldwide misuse of pesticide in various ways in agriculturehas been associated with numerous health disorders and contamination of the environment (Gbaruko*et al.,* 2009). The misuse of extremely, highly and moderately hazardous pesticidesplus a totally absent or a weak legislation in the use of pesticides have been reported as the major causes of the higher occurrence of pesticide toxic effects in developing nations (Konradsen *et al.,* 2003). Other factors that have been reported to be associated with the intoxication scenario include dearth of

safety precautions and environmental hazards on the label,using faulty spraying equipment or its inadequate maintenance and inavailablity of personal protective gadgets during handling of pesticides are other factors (Sosan *et al*., 2009). Also, the common use of inappropriate equipment to measure and dispense pesticides in developing nations is common.

It becomes important to communicate clearly the potential hazards of unsafe pesticide use to the farm workersdue to the reported adverse health effects and their latencies. There is also need to re-orientate them regarding the reportedunawareness of the toxicity of pesticides by some farm workers and the wrong perceptions by the others. Fortunately, most of the farm workers have advocated for proper trainings and guided information on safe pesticide use and are therefore likely to embrace such intervention that most researchers usually emphasizedwhich aim at reducingthe risks (Oluwole and Cheke, 2009). The erroneous perceptions of pesticides and associated hazard can decrease the ability of farm workers to protect themselves against these hazards. Thus, identification of the magnitude of the problem through assessing farm workers safe handlingbecomes important in developing a successful educational agricultural programe (Wesseling *et al.,* 2011). This can be achieved through agricultural extension scheme whichis a mainmode of communication between research experts and farm workers. This provides a better link between research institutes and farm workers where many development in agricultural technologies including use of pesticides are communicated and modified accordingly. Apart from this, interventions which include decisions on methods of pest control to adopt that will equip farm workers and that may result in safe and effective pesticide use. In spite of apparent homogeneity from the previous studies, a number of farm workers have different needs, problems and production practices which requires considerations. Therefore for a successful agricultural extension intervention,cosiderations must be given to every person in any particular cohort.

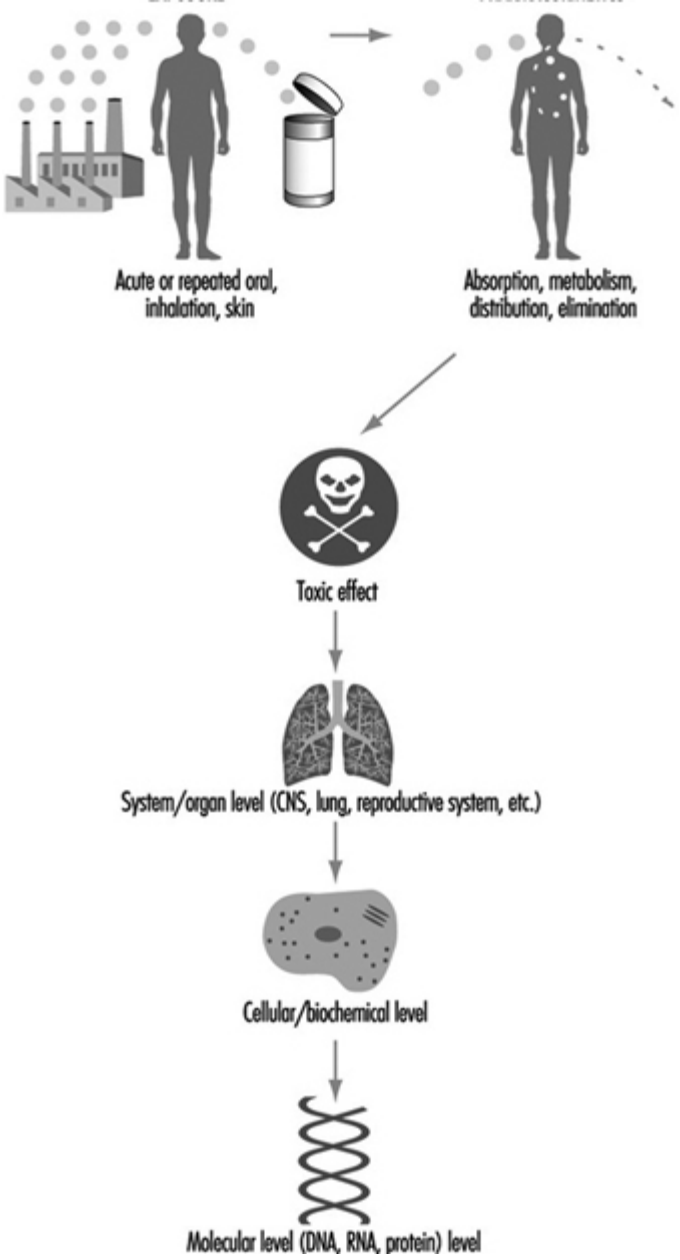



Fig. 2.12: Routes of exposure to OPs (WHO, 2001)

The superficial surface area of skin of a human adult is approximately 1.75 $m^2$. It is almost the main route of exposure in accidental acute exposure. The pesticidewhich may be in vapor, particle or dropletformulation can splash or spillon the skin or clothing of the pesticide applicators especially during the windy weather. Splashing of pesticides on eyes, mixing and loadingof pesticides, touching pesticide treated plants or soil, rubbing of forehead or face with gloves or hands contaminated with pesticidescan also lead to skin exposure (Ekeleme *et al*., 2008).

Skin absorption is the commonest route of exposure because pesticides may spill on the body during mixing, loading and spraying. Absorption will continue to take place through the skin at different rates (Adams and Robert, 2005). The scalp of the head,canal of the ear and the genitals are mostly particularly vulnerable (Hashmi and Khan, 2010).

**2.2.7.2 Inhalation or respiratory exposure**

Some pesticides vapours and residues may be taken inthrough breathing sufficientlyto pose high toxic effects to nostrils, throats and the lungs. Inhaled pesticides viathe lungs get to the blood stream (Adams and Robert, 2005). DOP is inhaled by farm workers occupationally exposed to it.Lungs are exposed to pesticide residues through inhaledvapours of powders. Working with wet powders can be hazardous because the powder may be inhaled during mixing operations and usually contains concentrated pesticide active ingredient (Damalas, 2015).

Many pesticides vapourised when exposed to air and air currents as a result of wind can reduce level of vapour substantially.Increase in temperature increaseslevel of vapour of most pesticides (Adams and Robert, 2005). It is therefore advocated to apply pesticides when air temperature is less than 30°C and use respirator if need be. Sources of inhalational exposure include exposure to dusts, powders or other dry formulations duringmixing and loading, drift during or after spraying and use of poorly fitted or inappropriate respirators (Damalas, 2015).

Oral exposure occurs if hands are not washed before smoking, chewing or eating after applying pesticides oraccidental pesticide splashed into mouth. Drift on lips or in mouth, carelesselly storing pesticides in drinking containers or accidentally applying pesticides to food can result in oral exposure to pesticide (Adams and Robert, 2005).

**2.2.7.4 Measures to reduce occupational exposure**

Occupatinal exposure to pesticides can be reduced by applying the pesticides following recommendations late evening or early morning. This can also be achieved by delaying harvest ofpesticide applied vegetables for commercial purposes or for the purpose of consumption tillone week following application in the wet season but longer waiting interval of almost two weeks should be observed during the dry season (GIFAP 1983; US EPA, 2017).

Before measuring and mixing pesticides, as a form of measure to reduce occupational exposure, it is essential to always read the label, calibrate the sprayer with water.It is equally important to cross-check all the functioning parts of the sprayer for optimum performance, cleaning the sprayer's knapsack and seting the pressure gauge at lower level. Wearing of personal protective equipment (PPE) including eye goggles, hand gloves, overalls, boots, and respirators correctly and consistently (Figure 2.13), ensuringproper ventilation and avoidingdermal contamination and splashing during measuring and mixing of pesticides are other measures to reduce occupational exposure to pesticides (Kamara *et al*, 2004).

Adequate training is a prerequisite for applying pesticides to reduce occupational exposure. Part of the training is to avoid eating, drinking or smoking while operating a sprayer on the farm. Other ones are to raise the nozzle of the sprayer at knee height or specifically fifty centimeters above the ground during application, avoiding double sprayingof an area and ensuring gaps are not left within. Cautions must be taken not to allow children, animals, farmers and non-workers not wearing PPE to visit the areas sprayed with pesticides within one day of spray. It is also important to avoidusingcontaminated hands for eating, drinking or smoking. Hands must be washed immediatelly and thoroughly with soap after handling pesticides and reminiscence of

Figure 2.13: Correct protective clothing (Ekeleme *et al.,* 2008)

a pesticide to humans is not easy, since humans cannot be used as test subjects. Because of this, other animals such as rats are used (Clyde *et al.*, 2011). In experimental studies, the fact that a particular chemical is toxic to experimental animal does not mean it will be toxic to humans. Hence, experimental toxicity research are just guidelines for estimatingthe extent of toxicity of a chemical and comparable with the others. Chemicals that are toxic in a sigle exposure or dose are known to possess acute toxicity while those that are toxic on repeated exposure or small continuous doses are said to be chronically toxic (Clyde *et al*., 2011).

**2.2.7.5.1 Assessment of acute toxicity**

Acute toxicity can be measured in terms of acute oral, dermal or inhalation toxicity (Clyde *et al.*, 2011). Minton and Murray (1988) have divided OPs into three groups. The first most toxic group which includes chlorfenvinphos, has an LD50 in the range 1-30 mg/kg. The LD50 range for the second group which includes dichlorvos, is 30-50 mg/kg, and the least toxic group which includes malathion, has a range of 60-1,300 mg/kg (Minton and Murray, 1988). The $LD_{50}$ is the dose per unit of body weight, such as milligrams per kilogram (mg/kg) that kills half of a cohort of experimental animals in acute exposre (NPDS, 2010). A pesticide with an $LD_{50}$ of 10mg/kg is much more toxic than a pesticide with an $LD_{50}$ of 1000mg/kg (NPDS, 2010).

**2.2.7.5.2 Assessment of chronic toxicity**

Measurement of human exposure to OPs can be done directly and/or indirectly. Direct measurement involves determination of pesticide level in the media through which exposure occurs. These techniques according to Krieger(2011) provide a direct and calculable measure of human exposure under actual conditions. Most often, however, measurement is not possible and in these situations indirect methods are employed. Many of dose-related steps in the environmental health paradigm occur at inaccessible sites in the body (for instance liver, kidney, heart, brain, nerves). Biological makers (biomarkers) are the indicators of these significant but inaccessible events that can be measured in accessible human tissues (for example, blood) and excreta (for example,

origin and a biological system (WHO, 2009). Kamanyire and Karalliede (2004) reported that a biomarker was originally defined as the concentration of toxic agents, or their metabolites in biological samples. They, however showed that the concept of biomarker has been further expanded to include those biological changes resulting from interaction between chemicals and their cellular or molecular targets, as well as genetically determined polymorphisms of enzymes involved in Phase I and II of metabolism, which are responsible for the differential susceptibility of individuals to chemical agents. The use of biological markers in the evaluation of disease risk has increased markedly in the last decades. Identification ofcausal associations and makingbetter quantitative calculations of those associations at different relevant levels of exposure can be done using biomarkers (WHO, 2009). Molecular genetic tecniquesadvancement have led to a rise in use and relying in most biomarkers (Vainio, 2001).

Biomarkers include intact pesticide residue itself or its metabolites detected in urine or blood. Variations in the number, structural or functional cellular or biochemical components and detectable changes in genetic material and dead cells represent biological events detected. Molecular and cellular biology recent advances make the measurement of biomarkers of exposure, effect or susceptibility in humans possible. Blood and urine are the most frequently used matrices for exposure assessment using biomonitoring. The chemicals measured in these samples include the intact pesticide residues, their metabolites and the environmental degradates (Arcury *et al*., 2007). Organophosphate pesticides can be assessed through the use of biomarkers. The relationship between toxic levels of chemicals in the body and their toxic response is rather complex because it depends on several factors, including toxicokinetic and genetic aspects. When mixtures of pesticides are used for a longer duration, monitoring the adverse health effects of exposure in farm workers may become complicated. Preventing toxic effects of pesticides requires specific and sensitive detection of these substances or their metabolites. Thus, biomonitoring is often used for assessing farm workers health risks due to pesticide exposure. These biomarkers are emloyedas indicators ofsublte effects of exposure to pesticides priorto the onset of clinical adverse

#### 2.2.7.5.2.1 Biomarkers of exposure

Biomarkers of exposure are employed for detection and measurement of intact pesticides or their metabolites either in tissues, excreta orbreathfor assessing exposed populations to a particular toxicant compared to an appropriate referencein order to evaluate extent of exposure and potential health risk.Information on the potential,absorbed and biologically effective dose of pesticide are readily provided which can predict a risk of occurrence of disorders but cannot indicate adverse effect (WHO, 1996). Thus, information on exposure level becomes a first step in the processes of riskevaluation processes (Vianio, 2001).

Human exposure to pesticides occurs through multiple media and routes Environmental biomonitoring of exposure to pesticides determines the potential dose which accounts for all external media and routes in order to calculate accurately individual exposures. Estimating the amount of pesticide that is absorbed into the body integrating all pathways of exposure via measurements of the pesticide or its metabolite acconuts for the internal dose while the amount of a pesticide that came in contact with a particular tissue and impair celluar functionsaccounts for the biologically effective dose(Damalas, 2015).

Chromatography such as High Performance Liquid Chromatography (HPLC) with various detection systems ranging from ultrviolent absorbance to mass spectrometric analysers have been used to measure differet biomarkers in serum and urine among others (WHO, 1996).

For instance, in the metabolism of OPs, the chemical structure of the leaving group is specific to each organophosphate pesticide, the detection and quantification of the leaving group in urine is a specific biomarker of exposure of absorbed dose of the parent pesticide. Usually, most OPs are metabolized to yield six metabolite dialkylphosphates (DAP) includingdimethyl phosphate, dimethyl thiophosphate, dimethyl dithiophosphate, diethyl phosphate, diethyl thiophosphate and diethyl dithiophosphate(CDC, 2009). However, the interpretation of urinary dialkylphosphates is also complicated because the hydrolysis of a specific

only in the urine (CDC, 2009).

**2.2.7.5.2.2 Biomarkers of effects**

Biomarker of effect is the detection andmeasurement ofalterations of biochemical, physiological, behavioural or otherwise in individual exposed to pesticide and may be associated with adverse health effects(WHO, 2009). Documentation of preclinical alterations elicited by exposed potential, absorbed and biologically effective dose which might reflect an early stage in the development of an abnormality that precede structural or functional damage and thereforepredictive of eventual disorderis made possible with the use of biomarker of effects (Vianio, 2001). Thesebiomarkers are specific for target tissues and are used in daily practice to assist in clinical diagnosis although an ideal biomarker of effect for prevention of adverse effect is the one that measures alterations that is still reversible. Nevertheless, in epidemiological studies, certain biomarkers which measure irreversible effects may stillprovide the opportunity for early clinical intervention and thus become very useful. An example of biomarkers of effect arecholinesterase enzymes.

Cholinesterases are serine hydrolases which are traditionally classified into two main types, corresponding to two distinct genes: true or specific acetylcholinesterase (AChE) [E.C. 3.1.1.7], and pseudo or nonspecific butyrylcholinesterase or pseudocholinesterase (BChE) [E.C. 3.1.1.8] (Galehr and Plattner, 1927; Augustinsson, 1948; Rappaport *et al.*, 1959; Bauer*et al.,* 1974; Vale and Lotti, 2015). The major role of AChE is to catalyze the hydrolysis of acetycholine (ACh) in cholinergic synapses, whereas the function of BChE is less clearly defined though it can partially compensate for the absence of AChE in the nervous system and it has the power of splitting ACh (Goedde and Altland, 1968; Xie *et al.*, 2000; Duysen *et al.*, 2001). This distinction has been made on the basis of substrate preference (Brimjoin and Hammond, 1988). The human serum is a rich source of butyrylcholinesterase whereas human red blood cell membranes are a source of acetylcholinesterase (Skater, 1973). Therefore, BChE is important for hydrolyzing ACh in the circulation (Roberts and Reigart, 2013).

Measurements of cholinesterase activities have been used as primary biomarkers of effect in occupational medical emergenciesespecially in suspected cases of accidental

and prevalence of health related symptoms of chronic toxicity(Al-Sarar*et al*., 2009; Mew *et al*., 2017).

#### 2.2.7.5.2.3 Biomarkers of susceptibility

Biomarkers of susceptibility are biomarkers that reflectlinks in genetics or acquired susceptibility to chemicals which mayinduce or reducerisk of a person to developadverse reactions (WHO, 2009). The susceptibility of individual caused by polymorphic key enzymes involved in metabolism of pesticides are recognized and have been shown to play significant function in the risk evaluation and mayprovidereasons for the identification and protection of individuals sensitive (Costa *et al*., 2005).

Cytochrome P450 enzymes, glutathione S-transferases and the esterases are involved bioactivation and detoxification of pesticides. Individual susceptibility due to the deficiency of these enzymes has been shown to behelpful in the overall assessment of exposure to pesticides because at the same level of exposure, it determines whether health related symptoms or even intoxication will occur or not (Costa*et al*., 2005).

## 2.3. Surveillance in occupational health

Surveillance may be defined as close observation of suspicious people. It was initiallyused in public health in a bid to control communicable diseases but its principleshave been extended to non-infectious chronic diseases such as cardiovascular diseases and cancers (Krieger, 2011).

Surveillance is one of the basic activity in occupational health practice. Hazard surveillance and health surveillance are the two main divisions. The main concern of hazard surveillance is dangers at the variuos workplaces while health surveillance relates with the actual health of workers at their workplaces. The twoof them are indispensable in the practice of occupational health and one complementing the other (Krieger, 2011).

of exposure to levels of hazards responsible for diseases and injuries (Wagner, 1997; WHO, 2014). It involves establishing a specific exposue to health outcome which would result in steps to reduce exposure to such hazard in indicated workplaces to eventually reduce the adverse health outcome and possibly disease burden that may arise. It may be conducted as periodic national occupational exposure surveys usually based on sampling representation of specificprocesses or work places. Taking record of dangerous happenings in occurrence log books or using computer software packages containing exposure database for specific occupational groups is another way of evaluating hazard surveillance.For instance, exposure to needle or sharppricks among health care workers (Sarwar, 2015).

Mostillnesses due to occupational exposure take time to develop and thus,taking survey of hazards get rid of the need to wait for occurrence of diseases before taking preventive measures.Moreso, the process of identifying a single hazard is easier than detectinga disease because diseaseswith long latent periods may also have multifactorial causes and therefore making diagnosis complex. In essence, the main concern of hazard surveillance is to ensurethat ultimate attention is paid to preventable causes of diseases. Though, in any hazad surveillance, to monitor individual hazards is easier to implement but practicability of exposure to a single hazad is rare thus integrated exposure databases and surveillance systems for combined exposures for improve health and safety at workpotentially offer a greater promise because not every exposure results in disoders. This has a considerable advantage and offers the opportunity of monitoring trends or observing emerging patterns in exposure to hazard at workplaces since workers cannot be barred from engaging in their chosen carrier. The information gathered can be used to project or predict future disease burdens where prevention is inadequate (Wagner, 1997; Colosio *et al*., 2013).

### 2.3.2 Health surveillance

Health Surveillance can be defined in various ways. Classically it has been understood to comprise those strategies and methods to detect and assess systematically the adverse effects of work on the health of workers. It has however also been used to

review, clinical assessment, medical examination, special investigations and determination of immune status among others. It is essential in detectingadverse health effects emanating from occupational exposures at earlier stage so that prompt appropriate preventive measures can be instituted.The second aspect is the review of the health status of groups of workers by public health. Findings from health surveillance suggest individuals at increased risk,rule out significant hazardous exposure, the adequacy of control measures in place, baseline medical data of workers, benchmarks for preventive action, and opportunities to provide health education. Above all, it makes the quantification of incidence and prevalence of occupational disease available for critical descions(Mew *et al*., 2017)

Occupational health surveillance at times requires pre-employment medical examinations to provide baseline information for health insurance. Additionally, specific occupational groups may require national regulations stipulating pre-medical and periodic medical examinationsor statutory periodic medical examination. Finally, there may becases requiringmaintenance of records of workers for the period of engagement and thirty years after and provision of accessibility of the workers to their records on request should be granted (Krieger, 2011)

#### 2.3.2.1 Biological monitoring

Occupational health surveillance sometimes require biological monitoring as a screening procedure. Actually, detection of the presence and quantitation of toxicants or their metabolites in biological samples indicate exposure rather than detecting an early adverse health effects being the essence of biological monitoring. Thus, biological monitoring is more of hazard surveillance than health surveillance in accordance with earlier definitions (Vianio, 2001)

#### 2.3.2.2 Biological effect monitoring

Some early indicators ofadverse health effects such as detectable changes in biochemical parameters in the body fluids or excretions of exposed workers are termed

Risk assessment of occupational exposure requires an improved understanding of disease mechanisms and clarification of the roles of identified molecular biomarkers becausemany diseases associated with occupational and environmental exposure are of multifactorial causes. An integrated approach that combines evaluation of several factors is advocated (Vianio, 2001; Clune *et al.*, 2012).

### 2.4.Immune response

The cells and molecules responsible for immunity constitute the immune system and their collective and coordinated response to the introduction of foreign substances is the immune response (Abass *et al*., 2017). The immune system that comprises of many different cells, tissues and organslocated in various parts of the bodyis a remarkably versatile defense system. The cells and molecules of this system interplay dynamically like that of endocrine and nervous systems (Paul, 2012). Mechanisms of resistance to infections are also involved in individual's response to non-infectious foreign substances including pesticides (Abass *et al.*, 2017). Furthermore, mechanisms that normally protect individuals from infections and eliminate foreign substances are themselves capable of causing tissue injury and disease in some situations (Abass *et al*., 2017). Some previous studies in pesticide exposed workers showed activation of a particular immune system mechanism but majority also showed various immunosuppressive effects (Newcombe, 1992a; Barnett and Rodgers, 1994; Sobhana *et al.*, 2006). Other studies have described modulation by both enhancement and suppression of immune function (Luster *et al.*, 1992; Tarkowski *et al.*, 2004; Corsini *et al.*, 2008). However, healthy individuals protect themselves against foreign invaders by means of many different mechanisms (Table 2.4). Some of these protective mechanisms comprise of innate (natural or native) and adaptive (specific) immunity (Akira *et al.*, 2001; Abass *et al*., 2017).

The first line of defense against infection or injury is provided by innate immunity whose most of the components are present before the onset of exposure exposure to a particular toxicant or infections.They are cellular and molecular components which constitute a set of mechanisms that resist diseases which are non-specific to a particular antigen, toxicant

are highly responsive to and rapidly stimulated by foreign invaders (Abass *et al.*, 2017). Adaptive immunity response occurs within five to six days. A memory response is formed with exposure to the same antigen at another other time, stronger and usually more effective in neutralizing and mopping the antigen or toxicant. Lymphocytes, antibodies and the other molecules they produce are the major agents of adaptive immunity. In the first exposure of the body to an antigen, innate immunity gives the first line of defense simply because adaptive immune responses need some time to set. A healthy individual readily clears most of the microorganisms and toxicants encountered during a few days by the innate immune system defense mechanisms before the activation of the adaptive immune system (Hoebe *et al.*, 2004).

| Components | | |
|---|---|---|
| Physical and chemical barriers | Skin, Mucosal epithelia; Secretions (e.g defensins) | Cutaneous and Mucosal i/s; Secreted antibodies |
| Blood proteins | Complement, Inflammatory mediators | Antibodies |
| Cells | Phagocytes (macrophages, Neutrophils), NK cells | B and T lymphocytes |
| Characteristics | | |
| Specificity for foreign agent | Relatively low | High |
| Diversity | Limited | Large |
| Specialization | Relatively stereotypic | Highly specialized |
| Memory | No | Yes |

defensive barriers (Kimbrell and Beutler, 2001).

#### 2.4.1.1 Anatomic barriers

The first line of defense against foreign invaders in an organism is anatomic and physical barriers that ensure prevention of the entrance of toxicants and pathogens. The surface of mucous membranes and the skn are effecient barriers to the entrance of most antigens and environmental toxicants. The skin consists of a thicker layer dermis with a water proofing protein named keratin. Blood vessels, hair follicles, sebaceous and sweat glands are contained in the connective tissues that make up the dermis. The sebaceous glands produce sebum, an oily secretion associated with the hair follicles. The epidermis is a thin outer layer of the skin which contains many layers of epithelial cells tightly packed. Toxicants such as pesticides and infectious agents enter the body through the cuts in the skin as a result of wounds, abrasion or scratches (Beutler, 2004). Farm workers are occupationally exposed to pesticides through direct contact with chemicals, contact with pesticide residue on treated crops or equipment, drift, and entering treated fields (Wesselling *et al.,* 2011). Pesticides from any of these contacts penetrate through mouth, skin or inhalation indicating the unsafe usage of pesticides especially in developing countries (Wesseling *et al.*, 1997). Since dermal exposure is the main exposure route for farm workers, OPs are particularly capable of being absorbed through the skin even if cotton clothing is worn (Damalas, 2015).

The mucous membranes that lined the conjunctivae, alimentary, respiratory and urogenital tracts consist of a thinner outer layer epidermis which contains many layers of epithelial cells tightly packed and outer epithelial layer with an underlying layer of connective tissues have several nonspecific defense mechanisms to ensure prevention of the entranceof many antigens into the body (Kimbrell and Beutler, 2001).

The skin with its large surface area is an essential anatomic barrier to the exterior environment ininnate defenses. The epidermisconsists of keratinocytes which express class II MHC molecules and secrete several cytokines that induce local inflammatory reaction. Keratinocytes may also function as antigen presenting cells. A type of dendritic cell (DC) known as Langerhans cells are scattered along the epithelial cell

histocompatibility complex molecules. The outer layer also consist of intraepidermal lymphocytewhich is the same to the intraepithelial lymphocytes of mucosa associated lymphoid tissues because they are almost cytotoxic cells with T-cell receptors which have restricted diversity for antigens. Antigens that enter through the skin are trapped bythese well situated intraepidermal T cells. It is the opinion of some immunologists that these cells have a major function in combating foreign invaders that come into the body through dermal route. Scattered CD4 and CD8 T cells and macrophages that were either previously activated cells or memory cells were contained in the dermis (Medzhitow and Janeway, 2000).

Following an insult such as exposure to DOP, cytokines are synthezised localy at the site of injuryto activate cellular inflammatory response essential for normal host defense. Acute phase response is improved by releasing a quantity of cytokines leading to stimulation of growth factors and macrophages regulated through a reduction in the proinflammatory mediators and through releasing of antiiflammatory mediators to maintain homeostasis. Malaise and fever may occur as manifestation of immune response. If inflammatory balance is not maintainedand inflammation continued into systemic, a significant systemic reaction occurs which leads to end-organ dysfunction (Kaplan, 2017).

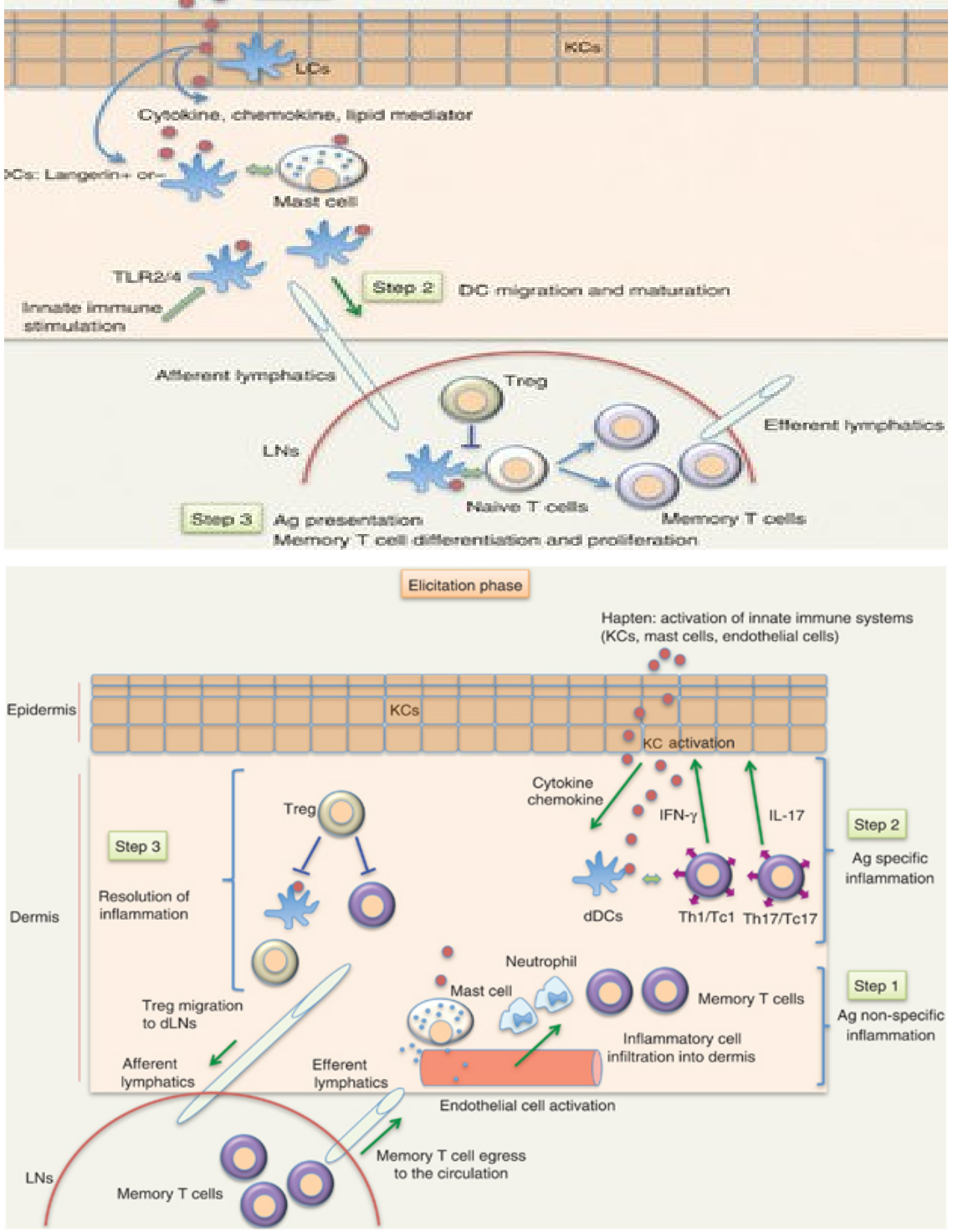



Figure 2.14: A schematic view of the sensitization and elicitation phase of inflammation (Liu, 2001)

lysozyme, interferon and complement factors.Lysozyme is an enzyme produced by lysosome. Interferons produced by activated immunocomptent cells have the cability to bind to neighbouring cells and many other functions.Complement is a collection of proteins in the serum that move round in an inactive form which can be converted to active forms by many specific and nonspecific immunologic mechanisms thereby facilitating antigen clearance from the system. Complement may also function as an effector.Reactions between complement molecules or their fragments and cellular receptors triggers activation of cells of the non-specific and specific immune response (Medzhitow and Janeway, 2000). Innate immunity is able to recognize pesticides because they are unique molecules and are not naturally present in humans. They are recognized by soluble molecules with pattern recognitive ability such as lysozyme and complement components described earlier (Beutler, 2004).

**2.4.1.3 Phagocytosis**

Phagocytosis is another essential mechanism in innate defense. It is ingestion of extracellular particulate material as a form of endocytosis. Specialized cells like neutrophils, blood monocytes and tissue macrophages conducted phagocytosis. Though, other types of cells are capable of conducting one or other form of endocytosis such as pinocytosis and receptor-mediated endocytosis. Pinocytosis involves cellular uptake of fluids and molecules within the fluid from the surrounding medium whileextracellular molecule is internalized after binding by specific cellular receptorsin receptor mediated endocytosis (Kimbrell and Beutler, 2001).

Phagocytes ingest and digest exogenous antigens including pesticide residues and endogenous matterincludingcellular debris among others. Phagocytosis starts by the process ofchemotaxis. Followed by antigen adherence to the phagocyte cell membrane through induction of pseudopodia that extend around the attached material such as pesticide residue.Joining of the pseudopodia encloses the ingested particle within a phagosomewhich fuses with a lysosome in forming a structure called phagolysosome. Lysozyme contained in the lysosomes and other numerous hydrolytic enzymes act on the particlesingested and ellimnate them through exocytosis (Beutler, 2004).

immunity(Abass *et al.*, 2017).

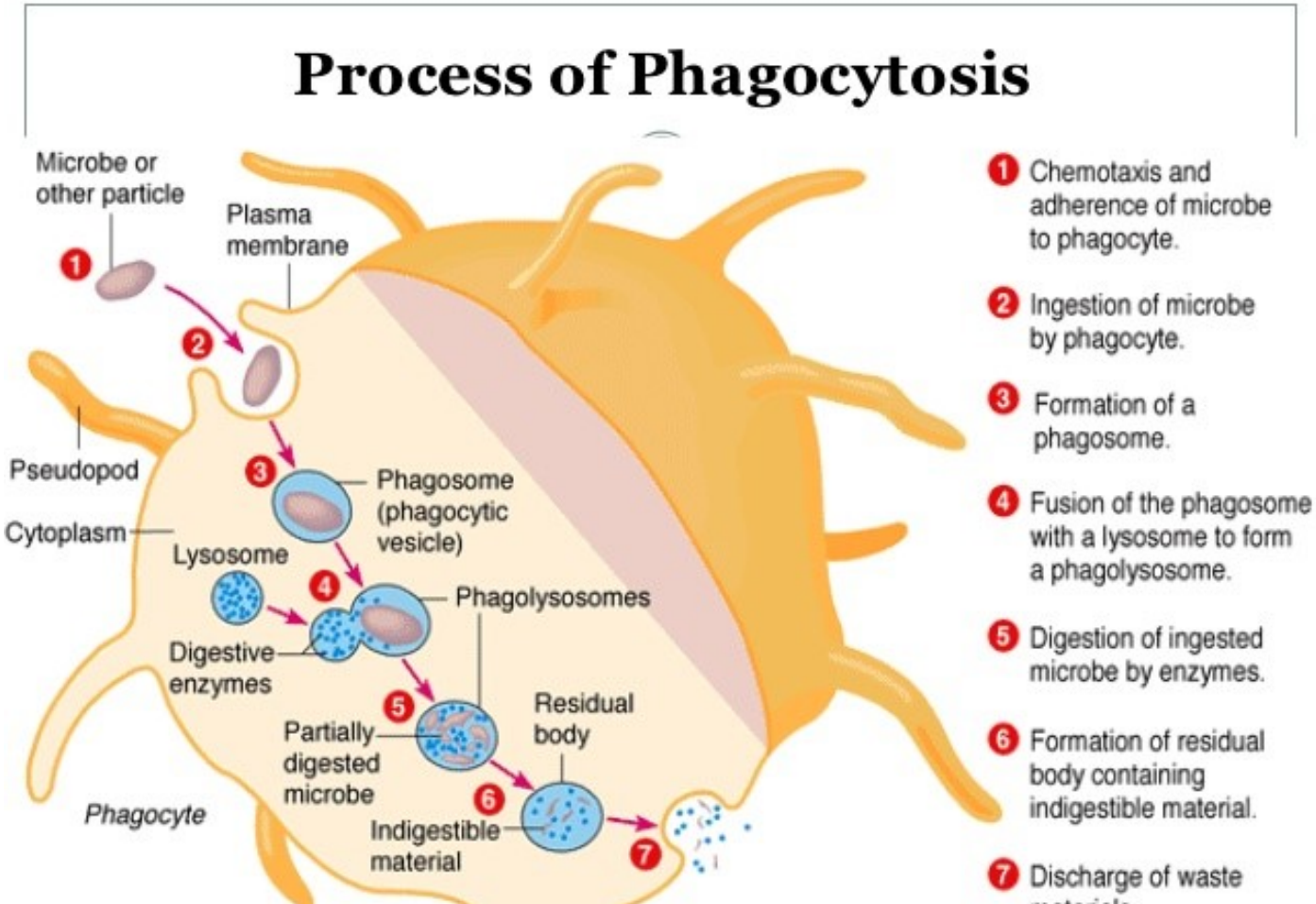


Figure 2. 15: Stages of phagocytosis (Liu, 2001)

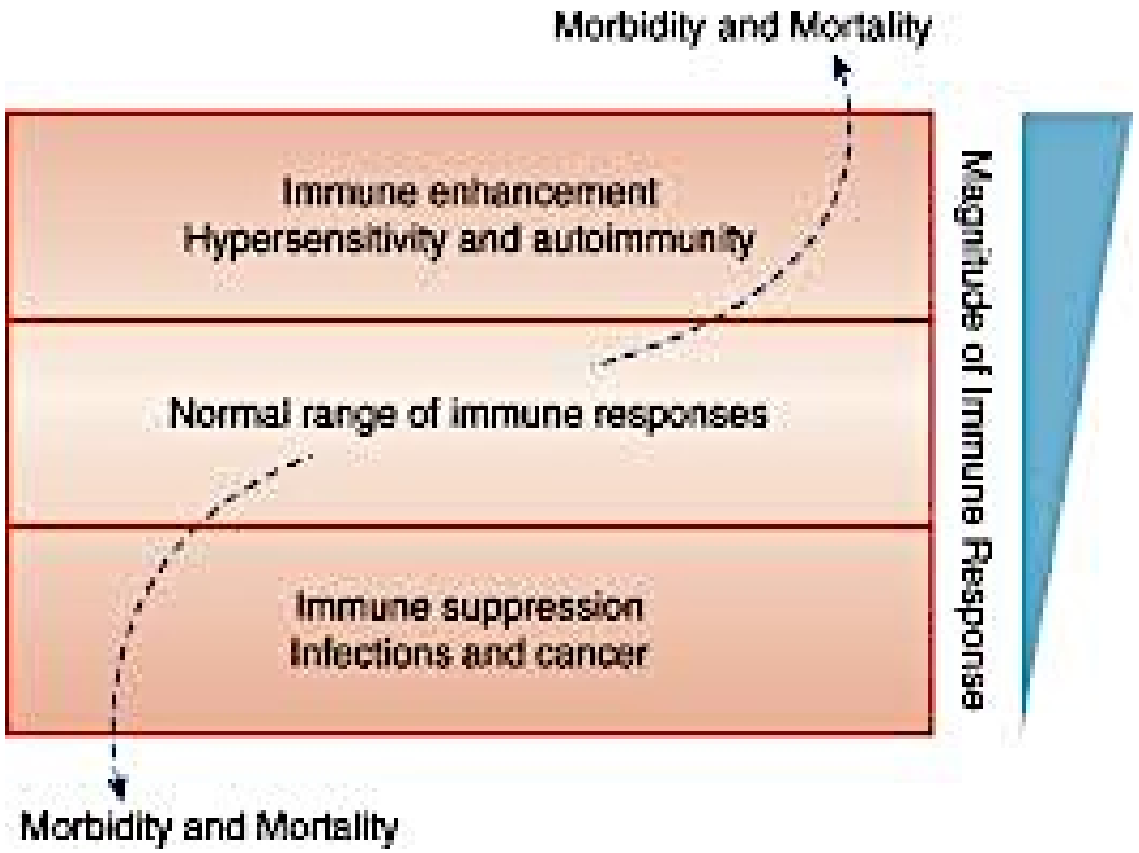


Figure 2.16: Magnitude of immune response (Kaplan, 2017)

#### 2.4.1.4 Inflammation

Inflammation is a physiological response of a body to a harmful stimulus such as entrance of a chemical like pesticide residue or physical agent or infection or tissue injury in order to reestablish homeostatic environment. It requires coordination of many cells and mediators whose involvementis dependent on the type of the initiating stimulus (Lawrence and Gilroy, 2007; Medzhitov, 2008). Acute inflammation supposed to be self-limiting or responsive to appropriate therapy. However, a chronic inflammatory process may result if acute inflammation is inadequate or ineffective or incomplete or therapy fails leading to inflammatory disease (Kaplan, 2017). Innate response to classical infection requires the normal acute and systemicinflammatory response.

The four cardinal signs of acute inflammation are classical features of the localized inflammatory response and had been described by the Roman physician Celsus as redness (rubor), swelling (tumor), heat (calor) and pain (dolor). A fifth sign ofloss of function (functio laesa)was added by Galen. However, these cardinal signs of acute inflammation is a reflection of the three main happenings in any response to inflammation. The first is vasodilation which is an enlongation of the diameters of neigbouringcapillary bood vessels causing redness (erythema) and a raised temperature in tissues. The second is a capillary permeability increament allowing flow of its

Although, inflammation is a protective response intended to get rid of the initiating cause as well as results of the initial insult. However, overwhelming injury with excessive inflammatory response, loss of control of local inflammation and release of proinflammatory mediators to the circulation lead to systemic inflammation and subsequent systemic inflammation based disoders. Also, failure to neutralize and remove the initial stimulus or even the clearance of apoptotic inflammatory cells from the inflamed tissue make the inflammatory process to persist and a state of chronic inflammation or auto-immunity may arise with different agents such as T-lymphocytes being recruited and the development of lymphoid infiltrates in the tissue (Lawrence and Gilroy, 2007).

Many kinds of mediators play various functions in the inflammatory response. Chemokines produce have chemoattractantactions and thus activate molecules during leukocyte extravasation. Plasma enzyme mediators increase vascular permeability. Plasmin breaks clots of fibrin into chemotactic substances and thus activates complementpath ways. Numerous products of activated complement path way serve as chemotactic, opsonins and anaphylatoxic molecules for neutrophils and monocytes. IL-1, IL-6 and TNF-α are three cytokinesthat mediate many of the features of localized and systemic inflammation. Additionally, stimulation of tissue macrophages and mast cells degranulation result in production ofvarious mediators of inflammation. Some of these activateacute phase response that includes fever, leukocytosis and production of acute phase protein and corticosteroids (Medzhitov, 2008). Serum levels of most of these mediators can be measured as markers of inflammation in occupationally exposed workers (Roitt and Delves, 1998; Bellingan *et al.,* 2002; Huynh *et al.*, 2002; Serhan 2007;Lawrence and Gilroy 2007; Medzhitov, 2008; Banks and Lein, 2012).

The inflammatory cascade that occurs following exposure to DOP can be summarized as thus. Exposure to DOP residue leads to the activation of the inflammatory cascade. Initially, a proinflammatory activation of innate defense occurs but almost immediately thereafter a counter reaction suppressing anti-inflammatory response occurs too.Tissue

ribonucleic acid (mRNA) that activates the generation of IL-6, IL-8 and interferon gamma as proinflammatory cytokines (Kaplan, 2017).Other cytokines, especially IL-6, activate production of CRP (Kaplan, 2017). A state of non-balanced dominated by inflammation is a result of cascades of reactions which is restored by production ofIL-4 and IL-10 that decrease the generation of TNF-α, IL-1, IL-6 and IL-8 (Kaplan, 2017)

Thus, the inflammatory state that accompanies OP exposure does not completely fit into the classical definition of acute or chronic inflammation, in that it is not accompanied by infection and no massive tissue injury takes occur. Additionally, the magnitude of activation of inflammatory response is not as pronounced. This indicates that OP exposure has unique inflammatory features especially as its causes are far from being fully understood. It is assumed that exposure to OP residues provoke inflammatory response locally at the site of contact (dermal or inhalation or oral). Tissue damage and/or homeostatic imbalance of one or several physiological systems have been identified as the basis of the inflammatory process which progresses to a dysregulation implicated in hypersensitivity, immunosuppression or autoimmunity (Medzhitov, 2008). This pathology seems to originate from alterations of the immune system, particularly as regards patterns of cytokine expression, resulting in a condition known as systemic low grade inflammation.

With this, emerging evidences indicate that inflammation plays a vital role in the pathophysiology of DOP toxicity, although; inflammation is yet to be accorded its rightful position among the important mechanism. Excessive acumulation of DOP particles during cumulative exposure may trigger an imbalance between cellular and molecular components of inflammation or a molecular component of a pesticide residue may activate an inflammatory response via interaction with receptors on the cell surfaces. Mobilization of components of the innate immunityand specific adaptive immune response to clear the pesticide residue irritationare the final result of inflammation. This altered inflammatory homeostasis may favour pro-inflammation and the attendant inflammatory disease may ensue. Prominent among the inflammatory cytokines produced are TNF-α, IFN-γ, IL-4 and IL-10 (Banks and Lein, 2012).

against the spread of infection or tissue injury. It is stimuulated by factors released from injured cells. This arrest further tissue destructions and promotes healing of any damaged tissue afterof clearance of pathogens or cell debris. Some of the molecules produced during inflammation also sensitise pain receptors, cause localized vasodilatation of blood vessels and attract phagocytes, especially neutrophils and macrophages, which then trigger other parts of the immune system. Failure to initiate a response allows uncontrolled proliferation of invading agents and severe tissue injury (Wittmann *et al.*, 2012).

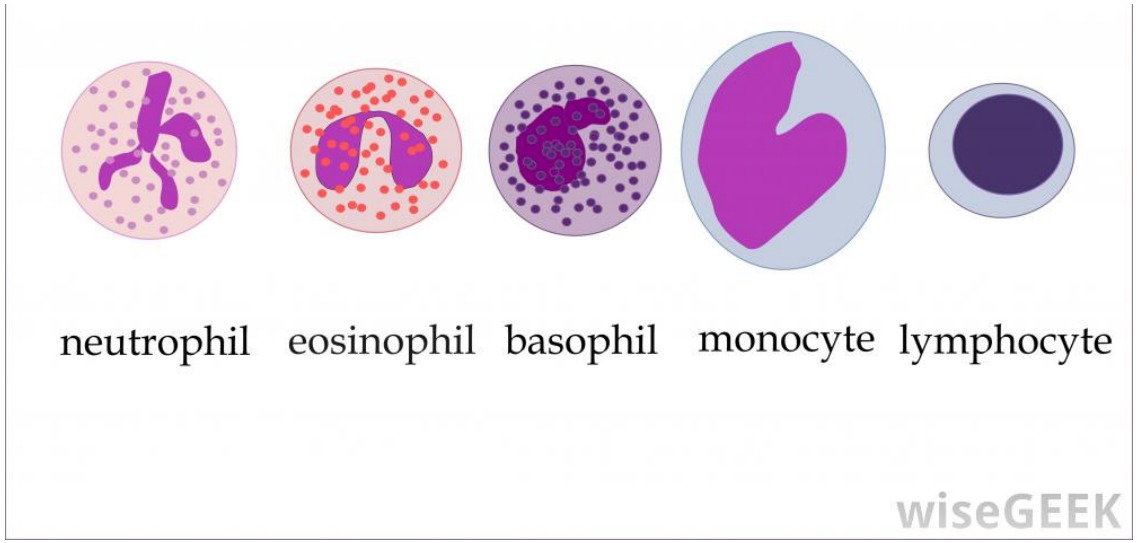
### 2.5 Inflammatory markers

Inflammation is a protective response that involves the interaction of a number of cellular and molecular components. These interacting elements must be coordinated and controlled in order to deliever appropriate response to irritation or infection (Kaplan, 2017). Hence, measurement of serum levels of interacting cellular and molecular components are used as markers of inflammatory response in occupationally exposed farm workers.

#### 2.5.1 Cellular inflammatory markers

All cells participating in immune responses originate from the bone marrow as pluripotent stem cells in form of myeloid series producing monocytes, neutrophils, eosinophil and basophils and lymphoid series producing lymphocytes in immunological pathways.

Monocytes that circulate in the blood and macrophagesfound in the tissues make up mononuclear phagocytic system whereas neutrophils, eosinophils and basophils based on cellular morphology and characteristics of cytoplasmic stainingare categorised as granulocytes(Paul, 2012). The neutrophils have multilobed nuclei and granulated cytoplasms which are stained by acidic and basic dyes. They are usually referred to as polymorphonuclear leukocytes (PMNs) because of thier multilobed nuclei. The eosinophilshave bilobed nuclei and granulated cytoplasms that are stained by the acidic dye eosin red from where they coined out the name. The basophilshave lobed nuclei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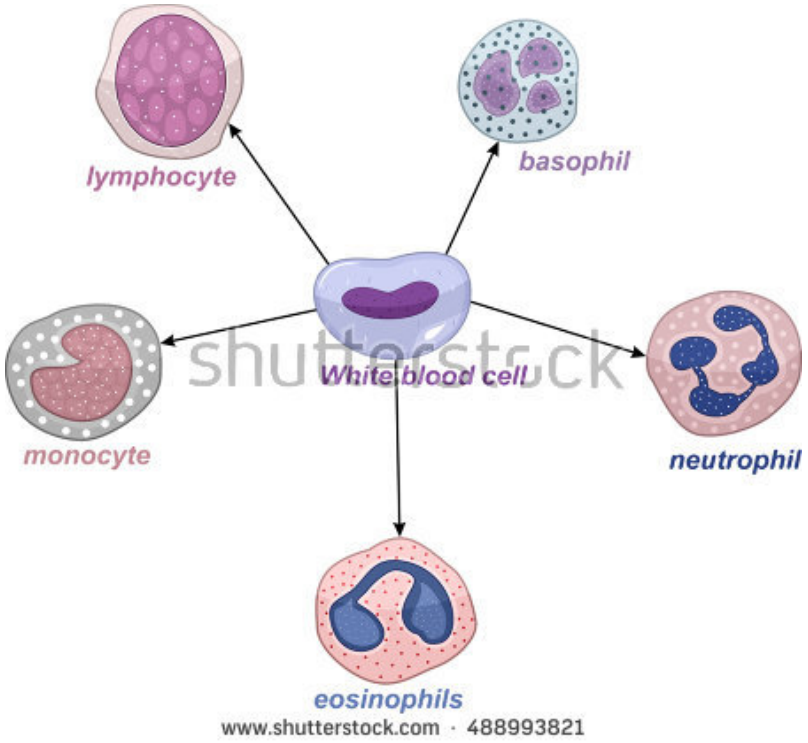
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White blood cell
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neutrophil
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#### 2.5.1.1 Monocytes/macrophages

Monocytes move in the blood stream for around eight hours. They become enlarged five to ten folds. Followed by migration to tissuespaces and differentiationinto specific tissue macrophages through increament in number and complexity of intracellular organelles. They also acquire more ability to phagocytose, produce higher levels of hydrolytic enzymes and initiate secretionof varieties of soluble factors (Medzhitow and Janeway, 2000).

Macrophages are scattered throughout the body. Those who reside in particular tissues become fixed macrophages. They are named according to their tissue location where they perform different functions. Such includeliver Kupffer cells, lung Alveolar macrophages, Langerhans cells in the skin, Histiocytes in connective tissues, Mesangial cells in the kidney, Osteoclasts in the bone and Microglial cells in the brain. The remainingmotile are known as wandering or free macrophages which migratethroughout the tissues by amoeboid movement. Macrophages have a centralized function in the defense against irritation and infection in the host. They are usually in a resting state but get activated by a variety of stimuli including phagocytosis of particulate antigens such as pesticide residue. Their activities are further enhanced in the presence of cytokines secreted by activated TH cells. Thus, during immune response macrophages and TH cells facilitate the activation of each other (Abbas *et al*., 1999; Beutler, 2004). Activated macrophages exhibit higher phagocytic activity, produce more inflammatory mediators and cytotoxic ptoteins and hence more effective than resting ones in getting rid of foreign antigens. Higher ability to activate T cells as well as expression ofraised levels of MHC class II moleculesconferred in the activated macrophages the ability to function more efficiently as antigen presenting cells. (Liu, 2001).

circulating leucocytes and are more numerous than other granulocytes, eosinophils (1%–3%) or basophils (1%) (Ward *et al.*, 2000).There are used to be more than usual number of neutrophils as an immune response to several kinds of injuryor infections resulting in transient leukocytosis. They are the first cells to reach site of inflammatory response throughextravasation involving adheranceand diapedesis. Chemotactic factors generated during inflammatory reactions include components of the complement pathway and blood-clotting system and several cytokines produced by activated macrophages and TH cellspromoteneutrophil accumulation at the site inflammation.

Neutrophils are active at phagocytosis like macrophages. They have similar phagocytic activity as macrophagesexcept that the toxic substances and lytic enzymes contained in neutrophils are enclosed within primary and secondary granules both of which join with phagosomes. The ingested particles are then processed and eliminated almost in the same way like that of macrophages. Additionally, neutrophilsmake use ofboth pathways of oxygen-dependent and oxygen-independent to produce anti-toxic compoundsand thusare more efficient compared to macrophages in the elimination of ingested antigens. Neutrophils thereforedemonstratehigher respiratory burst compare to macrophages. Consequently, they produce more ROS and RNS. Neutrophils products such as collagenase chemotactic factors secreted extracellularly also modulate inflammatory process (Ward *et al.*, 2000).

#### 2.5.1.3 Eosinophils

Eosinophils like neutrophilsand macrophages are mobile phagocytes but with significantly less important phagocytic role compare to neutrophils.They have been known toplay significant role in immunological defense against parasitic organisms and in response to some allergenic sensitization. The secreted contents of eosinophilic granules may attack large particules particularly parasitic worms which cannot be phagocytosed. Eosinophils are potential phagocytes ingesting antigen-antibody complexes (Ward *et al.*, 2000).

#### 2.5.1.4 Basophils/mast cells

tissue fixed in numerous organs' connective tissues and mucosa of the digestive, genitourinary and respiratory tracts as well as the skin. When activated, basophils and mast cells release histamine which initiates the inflammatory response. Additionally, IgE-coated mast cells and basophils degranulate on further exposure to the same allergens to produce the classical type-1 hypersensitivity reaction or allergy such as asthma. DOP is a possible allergen that can trigger such reaction (Paul, 2012).

**2.5.1.5 Lymphocytes**

Adaptive immunity and innate immunity are interdependent. For instance,the phagocytic cells important ininnate immune responses are responsible for the activation of the adative immune responses while various soluble factors produced by theadaptive immune responsesdo augment the activity of the phagocytic cells of innate defense.Thus, the two arms of the immune systemwork closely together to get ridof foreign invader through the carefully regulated interplay(Abass *et al.*, 1996).

Lymphocytes are the major effector cells of the adaptive immune responses. They account for 20%–40% of the leucocyte in the body and 99% of the lymph cells. Lymphocytes circulate continuously in the blood and lymph and at times migrate into tissue spaces and lymphoid organs.They coordinate and integrate the different arms of the immune system effectively and are subdivided into B, T and natural killer cell sub population based ontheir functions and componets of their cell-membrane. Peripheral blood normally contains 70-90% T cells, 5-10% B cells and approximately 1-10% natural killer cells.

A Natural killer cell (NK cells**)** is a large granular lymphocyte that lacks the kind of surface markers found in T or B cell whereas T and B lymphocytes are morphologically similar at rest. They are small, motile, nonphagocytic cells. Naiveor unprimed B and T lymphocytes referred to as small lymphocytes are resting cells that have not interacted with antigen in the G0 phase of the cell cycle. They are about 6 μm in diameter with barely discernible rimsof cytoplasms around the nuclei, highly packed with chromatin but with minute mitochondria and endoplasmic reticulum that is poorly developed and Golgi apparatus (Abbas *et al.*, 1996).

precursor small lymphocytes as they progress through the cell cycle. Lymphoblasts proliferation and eventual differentiationresult into effectorormemory cells. The effector cells are short lived from a few days to weeks and function in various ways to get rid antigens. Memory cells resemble small lymphocytes but may have or may not have certain cell membrane molecules. They are responsible for life-long immunity to many antigens.(Abbas *et al.*, 1996).

Plasma cells are the effector cells of the B cell lineage of the adaptive immune response that secret antibody. They have characteristic cytoplasms which containabundant endoplasmic reticulum and other organelles require for high turn over of protein. Cytotoxic T-lymphcytes and cytokine-secreting TH cells are the effector cells of the T-cell lineage. (Abbas *et al.*, 1996). The expression of membrane molecules recognized byparticular monoclonal antibodyassist in the categorization of different lineages or maturational stages of lymphocytes into Cluster of differentiation (CD)(Abbas *et al.,* 1996).

**2.5.1.6 Neutrophil-lymphocyte ratio (NLR)**

Primarily, white blood cells are responsible for protecting the body against exogenous and endogenous toxic substances in the blood and in the tissues (Han *et al.,* 2013). A lower level of TWBC count suggests immunosuppression while a higher level may indicate overwhelm of the immune system. Thus, individual with lower TWBC count either naturally or induced by factors such as exposure to chemicals tend to have compromised immunity and may be susceptible to infections. Previous studies have linked increases in illness and death from infectious diseases to pesticide exposure induced immunosuppression (Isakanderov, 1986; Faiziev, 1989; Bakhritdinov, 1991; Kovtyukh, 1995a; Colosio *et al.*, 2013). Volkova (1991) reported that pesticide exposure may also exacerbate pre-existing infections and may cause direct damage to organ systems, leaving open the role of immunosuppression.

The NLR is calculated as a simple ratio between the absolute neutrophil count and the absolute lymphocyte count, two different parts of the total white blood count (Gary *et al.*, 2013). Ohayo-mitoko (1999) reported reduced neutrophil count and raised

specific immune response to pesticide residues that elicit the immune process.

NLR has been reported to correlate with other inflammatory biomarkers and was proposed as predictive marker of morbidity and mortality in risk assessment of chronic disorders (Uthamalingam *et al*., 2011; Han *et al.*, 2013). However, the usefulness of NLR to determine inflammation in farm workers exposed to OPs has not been proven.

### 2.5.2 Humoral inflammatory markers

#### 2.5.2.1 Cytokines

Inflammatory, hematopoietic and lymphoid cells are involved in the development of an effective immune response. Cytokins mediate the complex interactions among these cells showing their importance in cellular communication. Cytokines are low molecular weight glycoproteins each consisting of a single chain (Abbas *et al.,* 2012). TH cells and macrophages majorly secret cytokins although other cells can secret too.Cytokines are effective at very low concentration (picogram per ml) due to high affinity binding to their specific receptors on the target cells. These receptors transmit signals to the cell nucleus (Arinola, 2016). They were originally recognized as molecules that mediated signaling between white blood cells and were therefore termed ‘interleukins’. This name persists for many cytokines and is abbreviated to IL, giving IL-1, IL-2, IL-3, IL-4 etc. However, the recognition, that these molecules functioned in the interaction of a much greater range of cells that participate in immune response lead to the more generic term ‘cytokines’ being adopted (Costa *et al.*, 2013).

##### 2.5.2.1.1 Tumor Necrosis Factor- alpha (TNF-α)

Human TNF is a 17.3 kDa protein. Its cDNA is 1585 base pairs in length and encodes a protein of 233 amino acids. It has been mapped to human chromosome 6p21.3, spans about 3 kilobasesand contains 4exons (Nedwin *et al.*, 1985; Salimonu, 2016).Tumor necrosis factor (TNF) is involved in systemic inflammationand stimulationofacute phase reaction. William Coley first observed its activity. He observed certain bacterial infections that developed necrosis in some cancer patients. He injected cancer patients with culture supernatants called Coley’s toxins derived from various bacterial cultures in

synthezised by macrophages. However, Pennica *et al.* (1984) revealed that LT and TNF are similar and they share 30% amino acid homology. Another name TNF-α was given to TNF while another name TNF-β was given to LT (Nedwin *et al.*, 1985; Steel and Whitehead, 1994; Kunkel and Butcher, 2002).

TNF-α is a pro-inflammatory cytokine whose levels are increased in various clinical conditions. It is produced primarily by macrophages, but lymphoid cells, mast cells, endothelial cells, cardiac myocytes, adipose tissue, fibroblasts, and neuronal tissue also produce it (Broudy *et al.*, 1986). Activation of macrophages by IFN-γ promotes TNF-α production. TNF-α and IFN-γ synergistically initiate a chronic inflammatory response (Kunkel and Butcher, 2002).

**2.5.2.1.2 Interferon gamma (IFN-γ)**

Interferons are soluble factors produced by most cells of vertebrate species in response to viral infection or other selected stimuli. They have potent anti-proliferative and immunomodulatory activities. There are three main types including interferon-alpha, interferon-beta and interferon-gamma. Interferon gamma (IFN-γ), a lymphokine also known as immune interferon has a molecular weight of between 20,000Da and 25,000Da and it is produced primarily by the Th1 subset of helper T-cell. It is produced during immune reactions by antigen, mitogen or lecithin-stimulated T-lymphocytes or by large granular leucocytes (LGL) with NK activity (Arinola, 2016).

IFN-γ secreted by activated TH cells is a potent activator of macrophages. It activates macrophages and increase bactericidal/tumoricidal capabilities of macrophages and augument their accessory cell functions. The activitation of macrophages by IFN-γ is accompanied by increased expression of the Fc receptors of immunoglobulin (FcR). This promotes phagocytosis of immune complexes and increases the capacity of the macrophages to lyse antibody-coated bacteria, parasites and tumour cells by ADCC. IFN-γ mantain the expression of class II MHC on the surface of macrophages as well as on other cell types (Arinola, 2016).

IFN-γ can either augument or suppress cellular and humoral immunity depending on the dose, time of administration and genetic make-up of the receipient. *In vivo*

macrophages and promotes the development of Th1 cells by stimulating IFN-γ production whereas IL-10 is produced by Th2 cells and inhibits the development of Th1 cells by limiting IFN-γ production.

**2.5.2.1.3 Interleukin-4 (IL-4)**

IL-4 is a 20kDa peptide produced by Th2 subset of helper T-cells and mast cells. It therefore enhances humoral immunity by increasing the number of Th2 cells. IL-4 synergises with IL-2 to stimulate B-cell growth. Although it is not a growth factor for resting B cells, IL-4 induces rapid increases in B cell surface expression of class II MHC antigens and Fc receptors for IgE. It induces IgE and IgG1 production and decreases IgG2 and IgG3 production by lipopolysaccharides-stimulated B cells. Its ability to promote immunoglobulin isotype switching towards IgE and IgG makes it an important cytokine in the aetiology of atopic allergies. IL-4 is mitogenic for T cells and supports the growth of mast cell lines. Furthermore, IL-4 is an activator of microphage cytocidal functions and induces the expression of cell surface class II MHC antigens on B cells and macrophages (Arinola, 2016).

**2.5.2.1.4 Interleukin-10 (IL-10)**

IL-10 is an essential pleiotropic immune regulator mainly secreted by macrophages, but also by Th1 and Th2 lymphocytes, dendritic cells, cytotoxic T cells, B lymphocytes, monocytes and mast cells. Some studies have shown that it can be produced even by human carcinoma cell lines (Akdis *et al.*, 2011).

Interleukin 10 (IL-10), first described in 1989 as cytokine synthesis inhibitory factor, is a key regulator of the inflammatory response. In humans, both $T_H1$ and $T_H2$ cells are capable of producing IL-10 however; the main source of T-cell derived IL-10 is Treg cells (Fiorentino *et al.*, 1989; Akdis *et al.*, 2011). It is also produced by B cells, monocytes and macrophages. Human IL-10 has a MW of 18 kDa and is secreted as a homodimer consisting of 2 subunits of 178 amino acids. It consists of 5 exons and 4 introns, spans about 4.7 kb on the gene of chromosome 1 (Fiorentino *et al.*, 1989).Variations of IL-10 gene exist but IL10.G and IL10.R are the frequently studied

IL-10 is a centrally operating anti-inflammatory cytokine (van Exel *et al.*, 2002). Its immunosuppressive effects protect humans from exaggerated inflammatory responses and autoimmune diseases. It has been shown that IL-10 inhibits the secretion of many cytokines including IL-6, IL-18, IL-12 and GM-CSF among others (de Waal *et al.*, 1991a, 1991b) by interfering with NF-kB activation pathway through the inhibition of IkB activation and inhibition of NF-kB DNA binding activity (Schottelius *et al.*, 1999).

IL-10 activity is mediated by the IL-10 receptor (IL-10R) which belongs to class II cytokine family of receptors. IL-10 inhibits the capacity of monocytes and macrophages to present antigen to T cells via an inhibitory effect on secretion of MHC class II, costimulatory molecules such as CD80 (B7.1) and CD86 (B7.2) and therefore downregulates the expression and production of IL-1, IL-6, IL-8, IL-12 and TNF-α among other pro-inflammatory cytokines (Kaplan, 2017). In B cells, IL-10 prevents apoptosis, enhances cell proliferation and has a role in immunoglobulin (Ig) class switch.

#### 2.5.2.2 Immunoglobulin E (IgE)

IgE has extremely low average serum concentration of approximately 0.3ng/ml compared with other immunoglobulin classes but with potent biological activity. It mediates the immediate type hypersensitivity reactions responsible for the symptoms of asthma, hives, anaphylaxisand hay fever. Prausnitz and Kustner in 1921 first demonstrated a serum component responsible for wheal and flare reaction analogous to hives by injection of an allergic individual serum intradermally to a non-allergic person. ThisP-K reaction forms the basis of analytical assay of IgE activity. The actual IgE identification was done by Ishizaka in 1966 whoprepared antiisotype antiserumfrom immunized rabbitssensitized with serum from an allergic individual (Frazer and Capra, 1999).

In the sensitization phase of first exposure to allergen such as pesticide residues, IgE binds to Fc receptors on basophils and mast cells. Binding of the same allergen to the IgE on the basophils and mast cells cause them todegranulate and release a variety of pharmacologically active mediators to the extracellular environment giving rise to allergic manifestations. Measuring IgE levels is also helpful in diagnosing helminth

response to inflammation. Those that increase are called positive acute phase proteins and those that decrease are referred to as negative acute phase proteins (Steel and Whitehead, 1994). Acute inflammation is associated with the production of pro-inflammatory cytokines. These cytokines stimulate the liver to produce acute phase proteins including CRP among others (Cals *et al*., 2010). In clinical terms, measurement of acute phase proteins is useful to assess the degree of inflammation in an individual and also to assess the response to therapy (Cals *et al*., 2010).

**2.5.2.3.1 CRP**

CRPis synthezised by the liver mainly as acute phase protein in response to tissue damage (Yudkin *et al.*, 1999). Its name was derived from its pattern recognition activity (Kindmark, 1972; Schultz and Arnold, 1990). C-reactive protein is an alpha globulin composed of 523 kilo dalton subunits and has been classified as a member of the pentraxin family (Moshage *et al.*, 1988). It is composed of five identical units which are non-covalently assembled as a cyclic pentamer (Dowling and Cook, 1972). The CRP gene is located on the first chromosome(1q21-q23). C-reactive protein binds to antigens, activates the complement pathways which facilitate the clearance of the antigen either by complement-mediated lysis or increased phagocytosis.

Its serum concentration increases in thousand folds during an acute phase response (Pepys and Baltz, 1983; Gabay and Kushner, 1999; Cals *et al*., 2010). The rise in CRP level is due to increase in the concentration of IL-6 in the plasma. Macrophages are the predominant producers of this CRP. In acute inflammation, CRP increases in thousand folds rising above upper normal limits within 6 hours with a peak at 48 hours. It has a constant half-life making the rate of production which reflect severity of the triggering cause determines its major serum level (Pradhan *et al*., 2001; Pepys and Hirschfield, 2003).

**2.5.2.4 Skin Prick Test (SPT)**

It is probably the more clinically informative method because it tests the functional integrity of the mast cell response in vivo. Although, problem with SPT have included

allergens is generally detected and assessed by SPT. SPT is a relatively cheap and quick test with opportunity to screen multiple allergens at a time. Type I hypersensitivity can also be assessed by determination of total IgE antibody level in the serum using high sensitive radioimmunosorbent assay (Barbee *et al*., 1981; Berger, 2002).

### 2.5.3 Enzymatic inflammatory markers

#### 2.5.3.1 Respiratory burst system

During phagocytosis, there is an increase in glucose and oxygen consumption by the cells whichis referred to as the respiratory burst (Morel *et al*., 1991). The consequence of the respiratory burst is that a number of oxygen-containing compounds are produced that eliminate the antigen being phagocytosed in a process referred to as oxygen-dependent intracellular process which could be oxygen-dependent myeloperoxidase-independent or oxygen-dependent myeloperoxidase-dependent intracellular(Leto and Geiszt, 2006). However, internerlized agent can also be eliminated by pre-formed substances released from granulaes or lysosomes when they are fuse with the phagosome which is referred to as oxygen-independent intracellular elimination (Lee *et al*., 2006).

In oxygen-dependent myeloperoxidase-independent mechanism, glucose is metabolized via the pentose monophosphate shunt and NADPH is formed. Cytochrome B which is part of the specific granule combines with the membrane-bound NADPH oxidase (NOx) and activates it. The reduction of oxygen to superoxide anion is catalyzed by activated Nox. Superoxide dismutase (SOD) catalyzes the conversion of superoxide anion to generate hydroxyl radicals, hydrogen peroxide ($H_2O_2$) and other powerful oxidizing agents (Wittmann*et al*., 2012).

In oxygen-dependent myeloperoxidase-dependent mechanism, the lysosome joins with the phagosome induces myeloperoxidase activity producinghypochlorite which is toxic to the ingested antigen from hydrogen peroxide and chloride ions. However, PMNs and macrophages protect themselves from the toxic ROS by detoxifying superoxide anion to hydrogen peroxide using SOD and conversion of hydrogen peroxide to water by catalase. Since, the oxygen dependent mechanisms are much more efficient in

In the mechanism of oxygen-independent elimination, activated macrophages synthesize numerous hydrolytic enzymes including lysozyme and cytotoxic cysteine-rich cationic peptidesdefensins that form ion-permeable channels with internalized antigen.

Nitric oxide-dependent elimination of agents requires the combination of activated macrophages with component of pesticide residues together with IFN-γto generate higherconcentration of nitric oxide synthetase (NOS) whichyielded L-citrulline and nitric oxide (NO) from oxidation of L-arginine (Leto and Geiszt, 2006).

Oxidative stress (OS) is thought to be the consequence of increased respiratory burst processes in the cellular defense of the body against overwhelmed irritation or infection through phagocytosis resulting in the increased production of prooxidant molecules referred to as reactive oxygen species (ROS). Damages caused by oxidative stress occurs primarily through ROS. ROS are free radicals possessing one or more unpaired electron in their outer orbit (Lee *et al*., 2006) which seek stability by adopting electrons from proteins, lipds and nucleic acidsresulting in damaging of individual cells and thus disease phenomena (Lee *et al*., 2006). Their production, however, multiplies several folds during exposure to toxic substances and pathological conditions (Morel *et al.*, 1991). Certain oxidative stress markers play important roles in the respiratory burst system. Among them are the enzymatic markers of inflammation which include MPO, NOx, CAT, SOD and GPx. These enzymes play important roles in respiratory burst mechanism of defense.

Evidences suggest oxidative stress involvement in the aetiology, pathogenesis, and development of DOP induced inflammatory disorders (Zhou *et al*., 2012). OS can be defined as an imbalance between the amount of oxidants and the intracellular and extracellular antioxidant protection systems (Halliwel, 2000).Several studies showed that oxidative stress occurs more frequently in people occupationally exposed to chemicals than non-exposed (Owoeye*et al.*, 2012). Free radicals in cells and ROS-derived lipid peroxides directly damage proteins, lipids and nucleic acids resulting in mitochondrial dysfunction. Experimental study showed that reactive oxygen species

and MPO is accompanied by a decrease in antioxidant enzymes activities. The mode of action involved in increasing oxidative stress and inflammation include higher generation of superoxide anion via the oxygen-dependent intracellular elimination pathway which causes deregulated production of cytokines such as IL-6 (Zhou *et al*., 2012). Binukumar (2010) reported that chronic subjection of rats to DOP causes microglial activation with the induction of NADPH oxidase and pro-inflammatory cytokines (TNF-α, IL-1β and IL-6).In DOP exposed farm workers, free radicals in cells and ROS-derived lipidperoxides directly damage proteins, lipids and nucleic acids resulting in mitochondrial dysfunction. These may provide an evidence of impaired mitochondrial bioenergetics which may lead to liver dysfunction after chronic exposure to dichlorvos (Binukumar *et al.*, 2010; Owoeye *et al.*, 2012).

**2.5.3.1.1 Myeloperoxidase (MPO)**

Myeloperoxidase was discovered as an enzyme present in the azurophilic granules of the neutrophils (Klebanoff, 1970). The MPO gene is situated on chromosome 17, has a size of 11 kb and is composed of 12 exons and 11 introns (Morishita *et al.*, 1987). The mature MPO, formed from promyeloperoxidase, has a molecular mass of 120–160 kDa and is composed of a pair of protomers each consisting a heavy (59 kDa) and a light subunit (13.5 kDa) linked together through a disulfide bond.

MPO is a peroxidase enzyme present abundantly in neutrophils and at a lower amount, in monocytes (Nichols and Bainton, 1973; Wittmann *et al*., 2012). It is not present in promonocytes (Kutter *et al.*, 1998) and is lost when monocytes mature into tissue macrophages (Van Furth *et al.*, 1970; Klebanoff, 2005). Neutrophil respiratory burst yields HOCl from $H_2O_2$ and $Cl^-$which combines with MPO. MPO-$H_2O_2$ product is a powerful oxidants with high physiological effects (Klebanoff, 1999).

Hydrogen peroxide alone and in conjunction with the amplification activity of myeloperoxidase (MPO) is responsible for elimination of antigen (Nauseef, 2001; Nauseef, 2004). MPO, which is abundantly present in phagocyte granules, catalyses the conversion of halides and pseudohalides such as $Cl^-$, $I^-$, $Br^-$, and $SCN^-$to form

Cyclooxygenase, lipoxygenase, cytochrome P450 enzymes, nitric-oxide synthase, xanthine oxidase, mitochondrial NADH-ubiquinone oxidoreductase and NADPH oxidase (Leto and Geiszt, 2006)are various oxidoreductases that catalyze the transfer of electrons from the electron donor to the electron acceptor to produce superoxide. Nox is a distinct professional, enzymatic source of superoxideproducer whereas the other oxidoreductive enzymes produce ROS only as by-products along with their specific catalytic pathways (Nauseef, 2004).

Cytotic organic and inorganic chemicals including pesticide residues and heavy mtals such as lead can activate Nox (Klebanoff, 2005). Biological factors including endogenous and exogenous inflammatory signaling can also activate both phagocytic and nonphagocytic Nox (Nauseef, 2001). Nox activity was first found in phagocytes and suggested to play a major role in inflammation as a non specific immunity. Secondary inflammatory mediators produce during inflammation and different cytokines, such as TNF-α, IL-1, IFN-γ (Li *et al*., 2002) are also capable of activating Nox.

**2.5.3.1.3 Catalase (CAT)**

Catalase is an enzyme common to almost all living cells that have contact with oxygen having largest turnover. It has 4 polypeptide chains, and four porphyrinhaeme groups that allow the enzyme to react with the hydrogen peroxide (Maehly and Chance, 1954). It was the first antioxidant enzyme to be characterized. It removes $H_2O_2$ found in the peroxisomes in most tissues and probably serves to remove peroxide generated by peroxisomal oxidase enzymes during respiratory burst (Morel *et al*., 1991). The highest activity of CAT occurs in the liver and erythrocytes but some CAT activities are present in all tissues (Zhou *et al*., 2012).It also catalyses the two stage conversion of $H_2O_2$ to water and Oxygen:

$$\text{CAT-Fe (iii)} + H_2O_2 \rightarrow \text{Compound 1}$$

$$\text{Compound 1} + H_2O_2 \rightarrow \text{CAT-Fe (iii)} + 2H_2O + O_2$$

It is almost impossible to saturate the enzyme in vivo because the reactions have

activated Nox. Superoxide dismutase (SOD) catalyzes the conversion of superoxide anion to generate oxygen, hydroxyl radicals, hydrogen peroxide ($H_2O_2$) and other powerful oxidizing agents(Wittmann *et al*., 2012).Although spontaneous dismutation may occur which involves the consumption of two protons.

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

There are 3 kinds of superoxide dismutases found in human tissues but specifically located and distributed in cellular compartments. These forms are Copper-Zinc SOD, Manganase SOD and Extracellular SOD.

Copper-Zinc superoxide dismutase is present in the cytosol and cellular organellar parts of human. Mn-SOD with a molecular mass of 40,000KDa is present in the mitochondria of virtually all cells. Extracellular superoxide dismutase was first discovered by Marlund in 1982 to contain Cu and Zn but different from the CuZn-superoxide dismutase andproduced by only few cell types (Liu, 2001).

**2.5.3.1.5Glutathione peroxidase (GPx)**

The oxidation of glutathione in the presence of $H_2O_2$or other peroxide is catalyzed by GPx.

$$ROOH + 2GSH \rightarrow GSSG + H_2O + ROH$$

The enzyme requires selenium at the active site. Its activity is dependent on the constant availability of reduced glutathione through the activity of another enzyme, glutathione reductase. Discrete genes encoded many GPx enzymes. However, the kidney produced majorly the plasma form. GPx is found virtually in all tissues with the highest concentrations found in the liver although predominates in the cytosol and mitochondria suggestingit as the major scavenger of $H_2O_2$ in the subcellular sections (Zhou *et al*., 2012).

# CHAPTER THREE

## 3.0 METHODOLOGY

### 3.1 Study area and population

The study participants were recruited from Ibarapa community in Oyo State, Nigeria. Ibarapa community is located at the Southern part of Oyo state with a land mass of over 3000 sq km. It lies on latitude $7^0$ $20^1$ – $50^1$ North and longitude $2^0$ $0^1$ – $30^1$ East. Vegetation pattern is that of the rain forest in the South and guinea savannah in the North. It is made up of Ibarapa East, Ibarapa Central and Ibarapa North local government areas consisting of seven towns and over five hundred villages and hamlets. It has a total population of three hundred and twenty thousand, seven hundred and eighteen (320,718) with male contributing one hundred and sixty-two thousand, six hundred and eight-one (162,681) (NPC, 2007). The inhabitants are predominantly farmers engaging in tree-crop agriculture such as cocoa and cashew.

### 3.1.1 Study design

A farmer (also called an agriculturer) is a person engaged in agriculture, raising living organisms for food or raw materials. The term usually applies to people who engage in raising field crops, orchards, vineyards, poultry, or other livestock. A farmer might own the farmed land or might work as a labourer on land owned by others, but in advanced economies, a farmer is usually a farm owner, while employees of the farm are farm workers (Bereja, 2010). In this study, farm workers refer to the farmers who own farm and also work on the farm, the employees of the farm who work as farmers or pesticide applicators. Migrant and seasonal farm workers include professional, hired or commercial pesticide applicators and farmers. A migrant farm worker is an individual whose principal employment is in agriculture on a seasonal basis and who for purposes of employment establishes a temporary home. The migration may be from farm to farm,

The study was both laboratory and field based. A cross section of 400 male agricultural farm workers (pesticide applicators and farmers) in Ibarapa community who have used pesticides for ten to fifteen years were asked to fill in a questionnaire on knowledge, attitudes, practice towards pesticide use, and associated toxicity symptoms. Preliminary investigations which include urinalysis, random blood glucose, HIV screening were carried out on the participants. Acetylcholinesterase assay, an index of exposure to DOP was also done. One hundred and twenty (120) participants consisting sixty (60) farmers and sixty (60) pesticide applicatorswith depressed serum acetylcholinesterase level were selected and enrolled into this study. Questionnaire administration and blood sample collections were done during dry season when DOP was used regularly. Most of the PA sprayed DOP for atleast three hours for more than three times in a month. Sixty (60) apparently healthy male administrative/office workers who are not occupationally exposed to DOP but reside within the community were recruited as controls. All the farm workers and controls were recruited after obtaining a written informed consent. Ten (10) ml of venous blood samples were collected from the study participants, separated and stored at $-20^0$c.

### 3.1.2 Selection of participants

**a. Inclusion criteria**

**Inclusion criteria for exposed participants**

Adult males aged 30 years and above from Ibarapa community

Use of DOP for 10 to 15 years

Ability to give informed consent

**Inclusion criteria for control**

Adult males aged 30 years and above from Ibarapa community

Not exposed to DOP occupationally

Ability to give informed consent

**b. Exclusion criteria for exposed and controls**

Regular home use of insecticides (for controls)

### 3.1.3 Ethical consideration and informed consent

This research work was done after obtaining an ethical approval from the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Committee. Each participant also signed a written informed consent.

### 3.1.4 Sample size determination

Sosan *et al.* (2009) in a study conducted on cocoa farm workers in South-western Nigerian reported a prevalence of toxicity due to unsafe exposure to organophosphate pesticides of 8.0%. Farm workers were classified to belong to unsafe group, therefore, the prevalence of toxicity due to unsafe exposure of farm workers to organophosphate pesticide is considered in this study.

Sample size $(S) = \frac{pq\,(Z\,\alpha)^2}{e^2}$

Where:

P= prevalence = 8.0% = 0.08

q =1-p =1-0.08

Z = Standard Deviation = 1.96

E = margin of error of 5% =5/100 =0.05

Therefore $S = \frac{(0.08)\,(0.92)\,(3.8416)}{0.0025} = 113$

The sample size for this study is 120 farm workers (60 pesticide applicators and 60 farmers)

### 3.2 Demographic indices and characteristics of farm workers

Information on demography, knowledge, attitudes, practices of farm workers towards pesticide use and associated toxicity symptoms were obtained using a structured questionnaire containing five sections filled by each participant. (Appendix II).

scale. The reading was recorded to the nearest 0.5kg.

### 3.3.2 Height

The height of each participant was measured in meters using (Standard steel) with subjects standing barefooted as upright as possible on a hard level ground against a vertical wall and without raising the heels from the ground with the feet kept together while the back and heel were aligned with a ruled bar against the vertical surface. The measurement was made by moving a sliding head piece to the vertex of the subject's head and the reading at that point was recorded to the nearest 0.1 metre.

### 3.3.3 Body Mass Index (BMI)

This was calculated as the ratio of the body weight to the square of the height in $kg/m^2$. BMI was considered normal if it fell between 18.5 and 24.9$kg/m^2$, overweight if between 25 and 29.9$kg/m^2$, obese if greater than 30$kg/m^2$.

### 3.3.4 Blood pressure

This was measured with the use of electronic sphygmomanometer following the calibration of the instrument with mercury sphygmomanometer to the nearest 2mmHg. Blood pressure was taken in a sitting position when the participants have rested for ten minutes and recorded to the nearest mmHg. Blood pressure was considered high if systolic pressure is equal or greater than 140 mmHg (≥140mmHg) and diastolic pressure is equal or greater than 90mmHg (≥90mmHg) (Charles Davis *et al.,* 2012)

## 3.4 Collection of blood samples

Ten (10) ml of venous blood sample was asceptically obtained by venepuncture from the study participants. This was done by applying a tourniquet 4-6 inches (10-15cm) above the intended puncture site to obstruct the return of venous blood to the heart and to the distended vein. The site of the puncture, the media cubital vein in the antecubital fossa was first cleansed with alcohol swab, blood was collected with new disposable pyrogen free needles and syringes after the skin had air-dried. Four (4) ml of blood was dispensed

### 3.5 Laboratory analyses

#### 3.5.1 Determination of Serum Cholinesterase (ChE) activity

Analysis of serum cholinesterase activity was done using Water model 626 HPLC pump with ion pac and ASII-HC anion–exchange column (Bird, 1989).

*Principle*

Serum Cholinestrase activity was determined from the rate at which acetic acid is being liberated when acetycholinesterase act on acetylcholine.

*Methodology* (Appendix III)

#### 3.5.2 Differential Leukocyte Counts

Differential Leukocyte Count (DLC) was done manually by thin film microscopy (Cheesbrough, 2000)

*Principle*

The manual differential white blood cell count is performed to determine the relative number of each type of white blood cell present in the blood. The differential staining allows one to identify the types of white blood cells on the smear.

*Methodology* (Appendix III)

#### Calculation of Neutrophil-Lymphocyte ratio (NLR)

The NLR was calculated as a simple ratio between the absolute neutrophil count and the absolute lymphocyte count, two different parts of the white blood count (Gary *et al.,* 2013).

#### 3.5.3 Determination of serum IgE

Enzyme linked Immunosorbent Assay was used to determine the serum levels of IgE as described by the manufacturer (Lienco Tech, USA).

*Principle*

This assay employes a quantitative sandwich enzyme immunoassay technique. A mouse

enzyme substrate is added. The colour development is stopped and the intensity of the colour which is directly proportional to the concentration of IgE in the standard or sample is measured.

*Methodology* (Appendix III)

### 3.5.4 Determination of serum levels of C-reactive protein (CRP)

The CRP concentration was determined using ELISA as described by the manufacturer (Lienco Tech, USA).

*Principle*

As described for IgE.

*Methodology* (Appendix III)

### 3.5.5 Determination of TNF-α, IFN-γ, IL-4 and IL-10 concentrations

The concentrations of TNF-α, IFN-γ, IL-4 and IL-10 were determined using standard sandwich ELISA as described by the manufacturer (Elabscience Biotechnology CO. Ltd).

*Principle*

As described for IgE.

*Methodology* (Appendix III)

### 3.5.6 Skin Prick Test (SPT)

Skin prick test reactivity to common environmental allergen was tested with extracts of cat, dog, grass, cockroach, mango, mite, mold and mouse (Greer Lenoir, USA). Small amounts of each extract are introduced on the forearm by superficial scratching.A histamine positive control and saline negative control was used to reduce false positives and negatives. SPT was done on the volar side of the lower arm using skin prick lancets. The wheal size was measured after 20 min. Skin prick reactivity was considered positive when the longest diameter of wheal size was greater than or equal to 3 mm (Berger, 2002).

carried out.

*Principle*

This method is based on the rate of decomposition of $H_2O_2$ by peroxidase, with guaicol as hydrogen donor. The produced tetraguaiacol was measured at 436 nm and at $25^0$C.

*Methodology* (Appendix III)

**3.5.7.2 Determination of NADPH oxidase (Nox) activity**

NOx was estimated using the method of Li *et al*. (2002) as previously carried out.

*Principle*

The NOx activity was evaluated by NADPH-dependent superoxide production using SOD-inhibitable cytochrome c reduction.This is based on a colorimetric assay that measures the reduction of cytochrome c by NADPH-Cytochrome c reductase in the presence of NADPH. The reduction of cytochrome c results in the formation of distinct bands in the absorption spectrum and the increase in absorbance at 550 nm is measured with time.

*Methodology* (Appendix III)

**3.5.7.3 Determination of catalase activity**

Catalase activity was determined using the method of Sinha (1971) as previously carried out.

*Principle*

This is based on the reduction of dichromate in acetic acid to chromic acetate when heated in the presence of $H_2O_2$ to form perchromic acid as an unstable intermediate whichis measured at 570 nm using a spectrophotometer.

*Methodology* (Appendix III)

**3.5.7.4 Determination of superoxide dismutase (SOD) activity**

The SOD activity was determined using colorimetric method as described by Misra and Fridovich (1972) as previously carried out.

*Principle*

#### 3.5.7.5 Determination of glutathione peroxidase (GPx) activity

Serum glutathione peroxidase activity was determined using the method of Rotruck et al. (1973) as previously carried out.

*Principle*

This method depends on determination of the rate of glutathione oxidation by $H_2O_2$ as catalyzed by the GPx present in the sample. The colour developed is read against a reagent blank at 412nm. The activity of GPx is expressed in terms of nmole of GSH oxidized/min/mg protein.

*Methodology* (Appendix III)

### 3.6 Statistical analysis

Statistical Package for Social Sciences (SPSS) software version 17.0 was used for data analysis. ANOVA, Student's t-test, Mann-Whitney *U* and Wilcoxon signed-rank test were used as appropriate to determine differences in the means of variables between the different groups. Chi-square test was used to test the significance of association between categorical variables. Pearson's correlation coefficient was used to examine the strength of linear relationships between quantitative variables. P-value less than 0.05 was considered as statistically significant. The results are expressed as Mean ± Standard Deviation (S.D) or median (interquartile range) as appropriate.

# CHAPTER FOUR

## 4.0 RESULTS

### General characteristics of the study participants.

### Route of contact and Prevalence of health related symptoms among the farm workers

Statistical analysis of the questionnaire administered to the farm workers are presented on tables 4.1 and 4.2. Table 4.1 showed that pesticide applicators were significantly exposed through inhalation compared with the farmers. Redness of eyes, skin rash, itching and chest pain were significantly higher while excessive sweating was significantly lower in pesticide applicators compared with the farmers.

### Perceptions of DOP use among the farm workers

As shown in Table 4.2, the perception of negative effects of chemical pesticides, adverse effects of long term exposure to pesticides, use of protective devices and adhering to instruction were significantly higher in pesticide applicators compared with the farmers. Similarly, perception of existence of pesticides residues in soil, ground water, air, fruits, seeds and leaves of vegetables were significantly higher inpesticide applicatos compared with the farmers. Also, storage of empty pesticide containers in specific store on the farms was significantly higher whereas storage of empty pesticide containers in the home was significantly lower in pesticide applicators compared with the farmers.

### Demographic, anthropometric and clinical indices

Table 4.3showeddemographic, anthropometric and clinical indices of the farm workers

When the farm workers were subdivided into pesticide applicators (PA) and farmers, it was observed that the mean BMI, SBP and DBP were significantly higher in PA and farmers compared with the controls. However, the mean BMI, SBP and DBP were similar between PA and farmers.

Table 4.1: Route of contact and Prevalence of health related symptoms among farm workers using DOP in Ibarapa community.

| | **P A (n=60)** | | **Farmers (n=60)** | | **$X^2$** | **p-value** |
|---|---|---|---|---|---|---|
| | **Yes** | **No** | **Yes** | **No** | | |
| ***Route of pesticide contact*** | | | | | | |
| **Dermal** | 40 | 20 | 34 | 26 | 1.268 | 0.100 |
| **Inhalation** | 56 | 04 | 40 | 20 | 13.332 | 0.001** |
| **Oral** | 04 | 56 | 02 | 58 | 0.103 | 0.100 |
| ***Symptoms*** | | | | | | |
| **Redness of eyes** | 60 | 00 | 33 | 27 | 34.838 | 0.001** |
| **Headache** | 20 | 40 | 21 | 39 | 0.036 | 0.100 |
| **Body weakness** | 30 | 30 | 36 | 24 | 1.210 | 0.100 |
| **Nausea and vomiting** | 16 | 44 | 10 | 50 | 1.766 | 0.100 |
| **Fever** | 40 | 20 | 42 | 18 | 0.154 | 0.100 |
| **Skin rash** | 50 | 10 | 20 | 40 | 30.858 | 0.001** |
| **Abdominal pain** | 50 | 10 | 44 | 16 | 1.766 | 0.100 |
| **Itching** | 44 | 16 | 24 | 36 | 13.574 | 0.001** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **sweating** | | | | | | |

PA = Pesticide Applicators

P-value: **Significantly different at P<0.01 level (2-tailed)

*Significantly different at *P*<0.05 (2-tailed)

Table 4.2: Perceptions of DOP use among Farm workers in Ibarapa community

| | **PA (n= 60)** | | **Farmers (n=60)** | | **$X^2$** | **p-value** |
|---|---|---|---|---|---|---|
| | **High** | **Low** | **High** | **Low** | | |
| **Negative effects of pesticides** | 53 | 07 | 31 | 29 | 19.206 | 0.001** |
| **Long term exposure may cause diseases** | 38 | 22 | 2733 | | 4.062 | 0.050* |
| **Short term exposure may cause death** | 51 | 09 | 49 | 11 | 0.240 | 0.100 |
| **Using PPE while applying pesticide can protect** | 58 | 02 | 31 | 29 | 31.708 | 0.001** |
| **Adhering to instruction can reduce risk** | 58 | 02 | 42 | 18 | 15.360 | 0.001** |
| ***Places where pesticide exist*** | | | | | | |
| **a. Soil** | 60 | 00 | 56 | 04 | 4.138 | 0.050* |
| **b. Ground water** | 56 | 04 | 40 | 20 | 13.332 | 0.001** |

| | | | | | | |
|---|---|---|---|---|---|---|
| ***containers*** | | | | | | |
| **a. Farm** | 53 | 07 | 43 | 17 | 5.208 | 0.050* |
| **b. Home** | 07 | 53 | 17 | 43 | 5.208 | 0.050* |

PA = Pesticide Applicators

P-value: **Significantly different at 0.01 level (2-tailed)

*Significantly different at $P<0.05$ (2-tailed)

Table 4.3: Demographic, anthropometric and clinical indices of study participants

| | **Controls (n = 60)** | **Farm workers (PA + F) (n=120)** | **Pesticide Applicators (n=60)** | **Farmers (n = 60)** |
|---|---|---|---|---|
| **Age (yrs)** | 46.0±10.0 | 47.0±7.0 | 46.0±15.0 | 47.0±18.0 |
| **BW (Kg)** | 59.9±11.2 | 61.5±10.9 | 62.1±10.4 | 61.6±10.4 |
| **Height (m)** | 1.7±0.1 | 1.6±0.1 | 1.6±0.1 | 1.6±0.1 |
| **BMI ($Kg/m^2$)** | 21.5±3.1 | 23.6±4.4[a] | 23.9±5.1[a] | 23.4±3.6[a] |
| **SBP (mmHg)** | 111.7±7.3 | 137.2±19.8[a] | 135.1±20.0[a] | 139.2±19.7[a] |
| **DBP (mmHg)** | 71.0±1.0 | 76.9±13.4[a] | 78.6±13.3[a] | 75.2±13.6[a] |

[a]Significantly different from controls at $P < 0.05$)

Values are presented as Mean ± Standard Deviation

BW = Body Weight

BMI = Body Mass Index

SBP = Systolic Blood Pressure

DBP = Diastolic Blood Pressure

PA = Pesticide Applicators

**ChE activity and inflammatory markers (cellular, humoral and enzymatic) in the study participants**

As shown in Table 4.4, 4.5 and 4.6 lymphocyte and eosinophil counts, serum levels of IgE, CRP, IFN-γ, IL-4, serum activities of MPO and NOxwere significantly higher whereas serum ChE and CAT activities, neutrophil counts and NLR were significantly lower in farm workers compared with the controls. The diameter of skin reaction to grass and mold allergens were higher in farm workers compared to controls as presented in Table 4.7. Other inflammatory markers were similar in both groups.

When the farm workers were sub-divided into pesticide applicators (PA) and farmers, it was observed that lymphocyte and eosinophil counts, serum level of IgE, CRP, IFN-γ, IL-4, activities of MPO, NOx, diameters of skin reaction to grass and mold allergens were significantly higher while serum activities of ChE, CAT, neutrophil counts and NLR were significantly lower in pesticide applicatorsor farmers compared with the control. However, pesticide applicators had significantly reduced serum activities of ChE, CATand significantly raised serum level of CRP, IFN-γ, IL-4, IL-10, monocyte and eosinophil counts compared with the farmers. Other inflammatory markers were similar in the groups.

Table 4.4: ChE activity and cellular inflammatory markers in the study participants

| | **Controls (n=60)** | **Farmworkers (PA+ F) (n=120)** | **Pesticide Applicators (n=60)** | **Farmers (n=60)** |
|---|---|---|---|---|
| **ChE (IU/ml)** | 9.4±1.0 | 7.3±0.9[a] | 6.6±0.9[a,b] | 7.9±0.6[a] |
| **Neutroph (%)** | 57.2±6.0 | 40.8±8.5[a] | 40.4±7.1[a] | 41.2±9.7[a] |
| **Lymphoc (%)** | 41.0±5.0 | 56.0±8.1[a] | 55.8±7.3[a] | 56.2±4.1[a] |
| **Monocyte (%)** | 1.0(0.0-2.0) | 1.0(0.0-2.0) | 1.5(1.0-2.0)[b] | 0.0(0.0-1.0) |
| **Eosinoph (%)** | 1.0(0.0-1.0) | 1.0(1.0-2.0)[a] | 2.0(1.0-3.0)[a,b] | 1.0(0.0-2.0)[a] |
| **NLR** | 1.4±0.3 | 0.8±0.3[a] | 0.8±0.3[a] | 0.8±0.3[a] |

[a]Significantly different from controls at $P < 0.05$

[b]Significantly different from farmers at $P < 0.05$

Values are presented as Mean ± Standard Deviation or as Median (Interquartile range)

ChE = serum Cholinesterase

NLR = Neutrophil-Lymphocyte Ratio

PA = Pesticide Applicators

Table 4.5: Humoral inflammatory markers in the study participants

| | **Controls (n = 60)** | **Farm workers (PA + F) (n= 120)** | **Pesticide Applicators (n = 60)** | **Farmers (n = 60)** |
|---|---|---|---|---|
| **IgE (IU/mL)** | 229.3±178.4 | 327.4±169.3[a] | 320.7±171.4[a] | 334.2±168.4[a] |
| **CRP (mg/L)** | 4.0(4.0-6.0) | 6.0(4.0-12.0)[a] | 6.0(4.5-24.0)[a,b] | 6.0(4.0-6.0)[a] |
| **TNF-α (pg/mL)** | 15.0(15.0-19.0) | 16.0(15.0-21.0) | 16.0(15.0-21.0) | 15.0(15.0-20.0) |
| **IFN-γ (pg/mL)** | 21.7(12.1-50.5) | 71.7(42.4-119.2)[a] | 94(50.4-119.7)[a,,b] | 62.6(39.9-100.0)[a] |
| **IL-4 (pg/mL)** | 58.7(15.2-154.2) | 165(103-246.5)[a] | 177(121-262.1)[a,b] | 137(87.6-233.9)[a] |
| **IL-10 (pg/mL)** | 95.0(37.0-157.0) | 75(48.0-144.0) | 104(52.0-148.0)[b] | 60(48.0-115.0) |

[a]Significantly different from controls at P<0.05

[b]Significantly different from farmersat P<0.05

Values are presented as Mean ± Standard Deviation or as Median (interquartile range)

IgE = Immunoglobulin E

CRP = C-reactive protein

TNF-α = Tumuor Necrosis Factor-alpha

IFN-γ = Interferon gamma

IL-4 = Interleukin-4

IL-10 = interleukin-10

Table 4.6: Enzymatic markers of inflammation in the study participants

| | **Controls (n =60)** | **Farm workers (PA + F) (n=120)** | **Pesticide Applicators (n=60)** | **Farmers (n=60)** |
|---|---|---|---|---|
| **MPO (U/mL)** | 4.8(4.4-8.8) | 9.2(4.8-18.5)[a] | 6.5(4.6-18.7)[a] | 10.3(7.4-17.8)[a] |
| **NOx (U/mL)** | 3.4(2.1-6.3) | 6.8(3.6-13.2)[a] | 4.9(3.2-13.6)[a] | 7.7(5.4-12.8)[a] |
| **CAT(µm/mgpr)** | 3.6(3.0-4.0) | 2.6(2.1-3.4)[a] | 2.3(1.8-2.8)[a,b] | 2.8(2.2-3.5)[a] |
| **SOD (U/mg pr)** | 36.6±5.1 | 33.6±5.0 | 32.2±4.9 | 35.3±4.8 |
| **GPx (U/mg pr)** | 95.0±23.8 | 91.1±33.5 | 90.3±26.3 | 91.9±39.6 |

[a]Significantly different from controls at P<0.05

[b]Significantly different from farmers at P<0.05

Values are presented as Mean ± Standard Deviation or as Median (Interquartile range)

MPO = Myeloperoxidase

NOx = NADPH oxidase

CAT = Catalase

SOD = Superoxide Dismutase

**Selected environmental allergens in the study participants**

Table 4.7 showed that farm workers, PA and farmers exposed to DOP had significantly increased diameters of skin reactions to grass and mold allergens compared to controls.Other allergens tested in FW, PA and farmers have similar diameters of skin reactions compared to controls.

Table 4.7: Selected environmental allergens in the study participants

| | **Controls (n = 60)** | **Farm workers (PA + F) (n= 120)** | **Pesticide Applicators (n = 60)** | **Farmers (n = 60)** |
|---|---|---|---|---|
| **Grass (mm)** | 3.0 ± 0.0 | 3.7 ±0.7[a] | 3. 6 ±0.9[a] | 3.7 ±1.0[a] |
| **Dog (mm)** | 2.6 ± 0.2 | 2.6 ± 0.1 | 2.5 ± 0.1 | 2.5 ± 0.1 |
| **Mite (mm)** | 3.0± 0.2 | 2.8 ± 0.1 | 2.7 ± 0.1 | 2.8 ± 0.1 |
| **Cockroach (mm)** | 2.7 ± 0.2 | 2.6 ± 0.1 | 2.6 ± 0.2 | 2.6 ± 0.1 |
| **Mango (mm)** | 2.8 ± 0.2 | 2.9 ± 0.2 | 2.8 ± 0.2 | 2.9 ± 0.2 |
| **Mouse (mm)** | 2.9 ± 0.2 | 2.9 ± 0.2 | 2.9 ± 0.2 | 2.9 ± 0.1 |
| **Cat (mm)** | 2.6 ± 0.2 | 2.6 ± 0.1 | 2.6 ± 0.1 | 2.5 ± 0.1 |
| **Mold (mm)** | 3.0 ± 0.0 | 3.6 ± 0.7[a] | 3.5 ± 0.9[a] | 3.6 ± 0.9[a] |

[a]Significantly different from controls at $P<0.05$

**Correlation between cholinesterase activity and characteristics of pesticide applicators and farmers**

Table 4.8 showed that BMI, body weight and age had significant positive correlation with serum ChE activity in pesticide applicators while duration of exposure had significant inverse correlation with ChE activity in pesticide applicators

Table 4.8: Correlation between cholinesterase activity and characteristics of pesticide applicators and farmers

| **Parameters** | **P A** | | **Farmers** | |
|---|---|---|---|---|
| | **ChE activity** | | **ChE activity** | |
| | r-value | p-value | r-value | p-value |
| Age (yrs) | 0.273 | 0.035* | 0.003 | 0.980 |
| BW (kg) | 0.287 | 0.026* | -0.120 | 0.360 |
| Height (m) | -0.097 | 0.460 | 0.040 | 0.759 |
| BMI($Kg/m^2$) | 0.362 | 0.004** | -0.232 | 0.074 |
| SBP(mmHg) | 0.022 | 0.869 | -0.034 | 0.799 |
| DPB(mmHg) | 0.180 | 0.169 | 0.005 | 0.970 |
| DOE (yrs) | -0.536 | 0.000** | -0.012 | 0.928 |

Values are reported as correlation coefficient

P-value: **Significantly correlated at P<0.01 level (2-tailed)

*Significantly correlated at *P*<0.05 (2-tailed)

P A= Pesticide Applicators

BW = Body Weight

BMI = Body Mass Index

SBP = Systolic Blood Pressure

DBP = Diastolic Blood Pressure

**Correlation of inflammatory markers with serum ChE activity in the pesticide applicators and farmers exposed to DOP**

Table 4.9 showed that eosinophil count in pesticide applicators and NLR in farmers had significant inverse correlation with ChE activity.

Table 4.10 showed that Serum IgE level had significant inverse correlation with serum ChE activity in pesticide applicators

Table 4.11 showed that activity of MPO in pesticide applicators had significant positive correlation with serum ChE activity

Table 4.9: Correlation between cholinesterase activity and cellular inflammatory markers in pesticide applicators and farmers

| **Parameters** | **P A** | | **Farmers** | |
|---|---|---|---|---|
| | **ChE activity** | | **ChE activity** | |
| | r-value | p-value | r-value | p-value |
| **Lympho(%)** | 0.127 | 0.335 | 0.344 | 0.007 |
| **NLR** | -0.127 | 0.334 | -0.269 | 0.038* |
| **Eosino (%)** | -0.397 | 0.002* | -0.081 | 0.539 |
| **Neutro (%)** | -0.111 | 0.400 | -0.205 | 0.116 |
| **Monoc (%)** | -0.024 | 0.853 | 0.200 | 0.125 |

Values are reported as correlation coefficient

P-value: *Significantly correlated at $P<0.05$ (2-tailed)

NLR = Neutrophil-Lymphocyte Ratio

Table 4.10: Correlation between cholinesterase activity and humoral inflammatory markers in pesticide applicators and farmers

| **Parameters** | **P A** | | **Farmers** | |
|---|---|---|---|---|
| | **ChE activity** | | **ChE activity** | |
| | r-value | p-value | r-value | p-value |
| **CRP(mg/L)** | 0.109 | 0.406 | -0.147 | 0.261 |
| **TNF-α(pg/ml)** | 0.127 | 0.332 | -0.101 | 0.443 |
| **IL-4 (pg/ml)** | -0.078 | 0.555 | 0.153 | 0.244 |
| **IgE (IU/ml)** | -0.425 | 0.001** | -0.030 | 0.823 |
| **IFN-(pg/ml)** | -0.100 | 0.449 | -0.061 | 0.641 |
| **IL-10(pg/ml)** | 0.072 | 0.584 | 0.138 | 0.292 |

Values are reported as correlation coefficient

P-value: **Significantly correlated at P<0.01 level (2-tailed)

*Significantly correlated at *P*<0.05 (2-tailed)

P A= Pesticide Applicators

IgE = Immunoglobulin

CRP = C-Reactive Protein

TNF-α = Tumuor Necrosis Factor-alpha

IFN-γ = Interferon gamma

IL-4 = Interleukin-4

IL-10 = interleukin-10

Table 4.11: Correlation between cholinesterase activity and enzymatic inflammatory markers in pesticide applicators and farmers

| **Parameters** | **PA** | | **Farmers** | |
|---|---|---|---|---|
| | **ChE activity** | | **ChE activity** | |
| | r-value | p-value | r-value | p-value |
| **MPO(U/ml)** | 0.255 | 0.049* | 0.064 | 0.625 |
| **NOx (U/ml)** | 0.237 | 0.068 | 0.075 | 0.568 |
| **SOD(U/mgPr)** | 0.151 | 0.251 | -0.244 | 0.060 |
| **GPx(U/mgPr)** | 0.203 | 0.120 | -0.219 | 0.093 |
| **CAT(μm/mgpr)** | -0.089 | 0.500 | 0.240 | 0.064 |

Values are reported as correlation coefficient

P-value: *Significantly correlated at $P<0.05$ (2-tailed)

P A= Pesticide Applicators

MPO = Myeloperoxidase

NOx = NADPH oxidase

SOD = Superoxide Dismutase

GPx = Glutathione Peroxidase.

CAT = Catalase

# CHAPTER FIVE

## 5.0 DISCUSSION

Pesticides use in modern agriculture has been reported to improve productions, to increaseincome of farmers, to generate revenue for government and to guaranteglobal save food supply (US EPA, 2017). However,critical attention must be paid to the rise in observedtoxicityof farm workers exposed (Rothlein *et al.*, 2006; Banks and Lein, 2012). Although acute exposure is detected and diagnosed easily, the effects resulting from chronic exposure to cumulative low doses of pesticides usually present difficulty during assessment (Wesseling *et al.*, 1997; Colosio *et al*., 2013). Healthy existence of humans indicates that the immune system has the potential capacity to cope successfully with the challenges of pesticide exposure. However, there is need to better understand the mechanism of toxicity of pesticide and how it modulates immune responses and the attendant cosequences. It is noteworthy that the same armoury in the same person is involved in defense against infectious animate or inanimate, natural or synthetic agent (pesticdes inclusive) (Arinola, 2017).

### 5.1 Characteristics of farm workers

**Route of contact and prevalence of health related symptoms among farm workers.**

It was observed in this study that applicators of DOP were more exposed through inhalation compared with the farmers. It was also observed that pesticide applicators experienced redness of eyes, skin rash, itching and chest pain more than farmers. But, more farmers than applicators experienced excessive sweating. This is in line with the reported worldwide prevalence of symptoms in occupationally exposed farm workers. It has been shown that skin itching, headache, coughing, sweating, dizziness, fatigue, vomiting, vision, respiratory and behavioral problems are common symptoms in farm workers (Strong *et al.*, 2004). Al-Sarar *et al.* (2009) also reported skin itching, headache and coughing as the most common symptoms in farm workers exposed to OPs. Sweating, difficult breathing, fatigue, blurring of vision, dizziness, changes in mood, sleeplessness, vomiting, forgetfulness or memory disorders were also reported. However,Del Prado-Lu (2007) reported that headache was the most frequent symptom, followed by easy fatigability and cough in cutflower

Considering perceptions of pesticide use, more pesticide applicators than farmers were aware of toxic effects of chemical pesticides on them and their family. Similarly, more applicators than farmers perceived that long term exposure to pesticides may cause diseases and short term exposure may cause death and that use of protective devices and adhering to instruction will reduce negative effect of exposure to dichlorvos organophosphate pesticide. More applicators than farmers also have high perceptions of places where pesticides residues exist and hence more applicators store empty pesticide containers on the farms rather than at home as compared with farmers. Despite the knowledge, more applicators manifested symptoms of exposure than farmers. This supports the dose-response explanation earlier reported (Gupta, 2006). Gupta report showed that dose, intensity, duration and frequency of exposure correlates with manifestation of symptoms and risk of toxicity. This observation in our study might be due to lack of use of personal protective equipment (PPE) such as face masks, gloves, goggles and overall which most of the farm workers that participated claimed unavailable (Wesseling *et al.,* 2011). Keifer *et al.* (1996) reported that farm workers do not consistently use PPE which can reduce exposure and this may lead to increased adverse effects.

5.2 Clinical and anthropometric indices

**Blood pressure**

In this study, the observed significantly higher blood pressure (although within normal range) in PA and farmers exposed to DOP compared with the controls shows that individuals who are occupationally exposed to DOP have increased risk of developing hypertension. Previous studies have shown that exposure to persistent organic pollutants (POPs) such as organochlorine pesticides (OC) increase the risk of chronic diseases such as hypertension. This has led to the banning of OC use. Experimental evidence suggests that dichlorodiphenyltrichloroethane (DDT) which is an OC can act on several arms of renin angiotensin system (RAS) to increase the risk of hypertension (Tomlin, 2006). Studies have also shown that environmental pollutants such as lead (Pb) inhibits the activity of sodium potassium adenosine triphosphatase (Na+ -K+ ATPase) which has an inverse association with blood pressure (Armitage *et al.,* 2005; Grun and Blumberge, 2006; Alwasel and Ashton, 2009; Klimentidis *et al.*, 2011). These mechanisms may also be applicable to DOP and thus

picciotto *et al.*, 2018). It is thought that repeated sympathetic and parasympathetic over-activity for prolonged period may produce clinically evident or subclinical conditions which clinical measurements may reveal for prompt attention. Regular check of blood pressure for farm workers using DOP is therefore advocated.

**Body Mass Index**

Hypertension and diabetes mellitus are closely related with obesity as risk factor. There is steady increase in this obesity throughout the world (Charles-Davies *et al.,* 2012). The role of environmental/occupational toxicants such as pesticides continues to garner increased attention; this has given rise to the concepts of obesogens (Holtcamp, 2012). This study found significantly higher BMI in PA and farmers exposed to DOP compared with controls. Studies have shown that there is a link between pesticides exposure, increased BMI and insulin resistance (Lim *et al.*, 2009; Valvi *et al.,* 2012; Lasram *et al.*, 2014). This might be responsible for our observed higher BMI (although still within normal range) in DOP applicators and farmers compared with the controls. A number of chemicals, dichlorvos inclusive have been shown to interact with the signaling pathways involved in weight regulation.

5.3 Index of DOP exposure

**Cholinesterase acitivity**

Nigg and Knaak, (2000) and Tapia *et al*. (2006) have reported a strong association between organophosphate pesticides exposure and inhibition of activities of cholinesterase enzymes and thus, the use of serum level of ChE activity as a biomarker of effect of exposure to these pesticides (Eason and O'Halloran, 2002; Joshaghani *et al.,* 2007a). The binding of DOP to ChE inhibits the activity of the enzyme resulting in accumulated acetylcholine level which stimulate nicotinic and muscarinic receptors with attendant toxic effects (Costa, 2018). Serum ChE activity significantly reduced in either PA or farmers compared with controls and in PA compared with farmers in this study. The observed lower serum ChE activity in both applicators and farmers exposed to DOP compared to controls is not a novel finding as it is in line with earlier findings of Anetor *et al.* (2001) and Reddy and Jagdish (2012). However, the significant depression in serum ChE activity in

Invariably, DOP applicators are likely to manifest or be more predisposed to disorders arising from toxicity of DOP than farmers. This is further alluded to by the observed significant inverse correlation between serum ChE activity and duration of exposure in PA exposed to DOP. The negative correlation might implie that the longer duration of exposure to DOP the more it correlates with reduced serum ChE activity and associatewith higher risk of toxicity. This study also observed that PA were significantly exposed through inhalation compared to farmers which invariably translated to significant toxic effect observed as depressed serum ChE activity. Hence, farmworkers require periodic check up of their serum ChE activity and should be encouraged to use personal protective equipment (PPE) correctly and consistently to reduce adverse health effects associated with exposure to DOP. Measurement of serum level of ChE activity reveals the adverse effects of exposure to DOP than measurement of serum level of dichlorvos residues or its metabolites which may only show extent of exposure and may not reveal subclinical or clinical damage.

5.4 Cellular markers of inflammation

**Neutrophil and Lymphocyte counts**

Although, the immune system has the capacity to cope successfully with exogenous immunogenic substances including DOP, alterations that may lead to serious immunological disorders may ensue if the immune system is overwhelmed. Primarily, white blood cells are responsible for protecting the body against exogenous and endogenous toxic substances in the blood and in the tissues (Han *et al.,* 2013). In this study, PA and farmers exposed to DOP were shown to have lower counts of neutrophils but raised counts of lymphocytes and eosinophils compared with the controls. There is consistency with previous studies in farm workers and other workers occupationally exposed to pesticides in different parts of the world (Desi, 1992). This low neutrophil count may reduce their resistantto infections and non infectious agents such as pesticide residues encountered through persistent dermal absorption and inhalation. Some previous studies linked increases in illness and death from infectious diseases to pesticide exposure (Isakanderov, 1986; Faiziev, 1989; Bakhritdinov, 1991; Kovtyukh, 1995a). Volkova (1991) also reported that pesticide exposure may also exacerbate pre-existing infections and may cause direct damage to pluripotent stem cells in the bone marrow which may result in deficiency of immunocomptent cells.

features include local rubor, dollor, tumour and calor. Underlying this microscopically is an invasion of the tissue by polymorphonuclear neutrophils, macrophages and later lymphocytes. The observed reduced circulating neutrophils in applicators and farmers compared with the controls may suggest overwhelming innate immune response through phagocytosis in these workers; neutrophils being the first to arrive at the site of injury offering sacrificial protection. It may also be hypothesized that neutrophil being involved in phagocytic function requires esterases to move about by chemotaxis which DOP suppresses by chemically binding to the membrane-bound protein with which cell to cell interaction occurs and thereby suppressing its function (Ohayo-mitoko, 1999).

The observed significant increase in lymphocyte count in this study corroborates this finding as the immune response moves towards adaptive by mobilizing lymphocytes. Lymphocyte, being the effector cell of the adaptive immune response, is raised in exposed farm workers compared to the controls and this might suggest efficient specific immune response to pesticide residues that elicit the immune process. This study provides basis for strong association between chronic exposure to DOP and chronic inflammation. When talking about migration of immune cells, neutrophils are early migrators, followed by macrophages/monocytes and later by lympocytes. Thus, when lymphocytes are increased, it indicates on going chronic inflammation.

**Neutrophil-Lymphocyte Ratio (NLR)**

An improvement on the use of absolute values of neutrophils and lymphocytes as cellular inflammatory markers is Neutrophil-Lymphocyte ratio (NLR). NLR has been reported to correlate with other inflammatory biomarkers and was proposed as a predictive marker of chronic inflammation in risk assessment of chronic disorders (Uthamalingam *et al.,* 2011; Han *et al.,* 2013). However, the usefulness of NLR to determine inflammation in farm workers exposed to DOP has not been proven to the best of our knowledge. It was observed in this study that PA and farmers exposed to DOP have significantly lower NLR when compared with the controls. This implies that the higher the exposure the lower the NLR value and the higher the risk of chronic inflammmation in farm workers exposed to DOP. Hence, NLR may be used in predicting risk of chronic inflammaton in these workers. This is further attested to by a negative correlation between serum ChE activity and NLR in PA and farmers exposed to DOP although significant in farmers only. The

In contrary, this study found significantly higher eosinophil count in farm workers exposed to DOP compared with the controls. This observation may suggest allergic sensitization in exposed workers. A mixture of findings indicating immunosuppression, hypersensistivity and /or autoimmunity have been reported as possible effects of OPs on the immune system (WRI, 1996; Banks and Lein, 2012). Earlier researchers reported that some biomarkers do indicate immmunotoxic potentials of pesticides in exposed farm workers but it was difficult to connect them with clinical symptoms and disorders. However, allergic reactions provide clear bases that pesticides may have some clinically observable effects on the immune system. Germolec and Luster (1994) reported that pesticide exposure induces allergic contact dermatitis, an inflammatory response that produces a rash. The results of health related symptoms including redness of eyes, skin rash, itching and chest pain obtained from this study through questionnaire support clinical manifestation in exposed farm workers thus establishing a link between pesticide exposure and clinical manifestation. Infact, these symptoms were significantly higher in PA when compared with the farmers supporting higher toxicity in PA exposed to DOP than farmers. Even, this study observed through questionnaire that PA inhaled DOP more than farmers and are likely to suffer more from adverse health effects of DOP than farmers.

Additionally, the pesticide applicators have increased eosinophil and monocyte count compared to the farmers. This indicates that eosinophilia and monocytosis might be features of persistent exposure to DOP and might be distinguishing cellular inflammatory features of PA from farmers. Previous studies indicated siginificantmonocytic counts in chemical-induced stress (Al-Sarar *et al,* 2009) and or monocytic leukemia (Demeras *et al*, 1993).

5.4 Humoral markers of inflammation

**Immunoglobulin E (IgE)**

Furthermore, higher serum level of total IgE found in farm workers compared with the controls supports allergic sensitization of farm workers by DOP residues. Initially, inhaled DOP residues may present asallergens which provoke the body to produce IgE leading to sensitization. Subsequent exposure to the same DOP allergens may result in anaphylactic reaction. Ogunbileje *et al.* (2010) ealier reported high serum level of IgE in Nigerian cement factory workers. Also, Olopade *et al.*

probably an immune response to protect the body. Allergic rhinitis and asthma are IgE antibody-mediated reactions in pulmonary allergy (Germolec and Luster, 1994). As observed in this study, most prevalent toxicity symptoms (redness of eyes, skin rash, itching and chest pain) that are significantly increased in PA compared with farmers support allergyplushigher IgE level and eosinophil count.

**Skin Prick Test (SPT)**

In clinical immunology, demonstration of elevated level of total IgE in individual requires further assessment to determine the allergen responsible for the elevation. The result of this study showed that FW, PA and farmers exposed to DOP had significantly increased diameters of skin reactions to grass and mold allergens among the environmental allergens tested compared to controls indicating that farm workers showed allergic skin sensitization. This implies that the method is appropriate to determine hypersensitivity type 1 status in FW exposed to DOP and thus supports high serum level of total IgE found in this study and previous studies (Berger, 2002; AAAAI, 2010).

**C-reactive Protein (CRP)**

This study found significantly raised serum CRP level in farm workers occupationally exposed to DOP compared with the controls and PA compared with the farmers. These observations were consistent with ealier findings and show that there is higher inflammatory response in farm workers compared with the controls generally and in PA more than farmers specifically. Inflammation is a first line response of the immune system to irritationsor infections. It is stimulated by factors released from exposed cells to establish a physical barrier against the spread of the irritations or infections (Coussens and Web, 2002; Libby, 2002; Van Hove *et al.*, 2008). Inhalation is the major route of exposure of farm workers to DOP as observed in ths study which is consistent with the report of Keifer *et al.*, (1996). Chronic inhalation of DOP may cause irritation of the alveoli characterized by localized inflammatory response. Molecules produce during inflammation localizes vasodilatation of blood vessels, and attract phagocytes (especially neutrophils and macrophages) (Wittmann *et al.,* 2012). This might have led to systemic inflammatory response where IL-1, IL-6 and TNF-α would have acted on the liver to increase the production of CRP. This may partly explain the observed increased level of CRP in this study. Also, the liver being the main organ involved in

a positive acute phase protein. Thus, determination of CRP level in farm workers exposed to DOPmay prove useful in establishing inflammation.

**Inflammatory cytokines**

Cytokine is essential in regulating immune responses in humans, however the homeostasis between pro- and anti-inflammatory cytokines are more important and the pathology that may ensue from dysregulation though poorly understood are the most important in delineating disorders associated with immune responses (Gangemi *et al.*, 2016). This study observed significant higher serum level of IFN-γ and IL-4 in farm workers exposed to DOP compared with the controls and also in PA compared with farmers. IL-10 was also significantly higher in PA compared with farmers. These findings show that both Th1 and Th2 responses respond to DOP triggers with a view to protecting the exposed workers. IL-12 is produced by macrophages and promotes the development of Th1 cells by stimulating IFN-γ production whereas IL-10 is produced by Th2 cells and inhibits the development of Th1 cells by limiting IFN-γ production. IL-10 plays a central role in regulating immune response. Its elevation in PA compared to farmers supports this role. Since inflammation is more pronounced in PA as ealier discussed, IL-10 production was increased to ameliorate the adverse effects of DOP in this group and in the functional capability of IL-10 as an anti-inflammatory cytokine. This study showed a balanced of proinflammatory and anti-inflammatory cytokines in order to protect exposed workers from on going chronic inflammation.

Though, experimental studies suggest an enhanced pro-inflammatory Th1 response through TNF-α or IFN-γ cytokine production with T-cell activation and cell mediated immune response in exposed animals (Chen *et al.*, 2014). However, human studies suggest that relative amounts of IL-4, IL-10 and IL-12 drive the differentiation of Th1 and Th2 cells and therefore enhance either cell-mediated or humoral immunity respectively. This is likely to have medical consequences because the host defense against certain injury or infection is either cell-mediated or humoral-mediated. Production of TNF-α and IFN-γ are necessary for the Th1 response and stimulation of phagocytosis to enhance clearance of pesticide residues. As a form of check, IL-10, a Th2 cytokine downregulates Th1 cytokine production resulting in a lower IFN-γ through IL-12. IL-10 is therefore suggested as a regulatorto counteract the potential harmfuleffects of proinflammatory response (Costa *et al.,* 2013).

5.5 Enzymatic markers of inflammation

Most reports on the adverse health effects of OP exposure have largely been associated to cholinesterase inhibition. However, there are recent findings challenging this view justifiably that only inhibition of cholinesterase itself cannot be responsible for the spectrumof the disorders. As discussed ealier, alterations of immune responses are important mechanisms. Oxidative stress through increased respiratory burst during phagocytosis has been suggested as another possible mechanism to explain adverse health effects of DOP exposure. Oxidative stress is defined as a disruption of the prooxidant-antioxidant balance in favour of the former leading to potential damages (Aly *et al.*, 2010). Oxidative stress is thought to be the consequence of increased respiratory burst processes in the cellular defense of the body against overwhelmed irritation or infection through phagocytosis resulting in the increased production of prooxidant molecules referred to as reactive oxygen species (ROS). The damage caused by oxidative stress occurs primarily through ROS. ROS are free radicals possessing one or more unpaired electron in their outer orbit which seek stability by adopting electrons from proteins, lipds and nucleic acids, lipids resulting to the damage of individual cells and thus disease phenomena. Their production, however, multiplies several folds during exposure to toxic substances and pathological conditions. Certain oxidative stress markers play important roles in the respiratory burst system. Among them are the enzymatic mediators of inflammation which include MPO, NOx, CAT, SOD and GPx. These enzymes play important roles in respiratory burst mechanism of defense.

**Myeloperoxidase (MPO), NADPH oxidase (NOx), Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx)**

In this study, serum activities of MPO and NOx were significantly higher whereas serum activity of CAT was significantly lower in farm workers compared with the controls. When the farm workers were sub-divided into pesticide applicators (PA) and farmers, it was observed that serum activities of MPO and NOx were still significantly higher while serum activity of CAT was still significantly

reduction in CAT activity which tend to counteract the effects of the prooxidants by converting hydrogen peroxide to water and oxygen. MPO is a peroxidase enzyme present abundantly in neutrophils (Klebanoff, 2005). In the mechanism of oxygen-dependent myeloperoxidase destruction, MPO produces hypochlorous acid from hydrogen peroxide and chloride anion during neutrophil respiratory burst. MPO-$H_2H_2$system products are powerful oxidants with great biological effects (Klebanoff, 1999). The observed significantly higher MPO activity found in farm workers compared with controls might indicate that respiratory burst process is an important process in host defence against DOP exposure in farm workers. But, in oxygen-dependent myeloperoxidase-independent mechanism, activated NOx uses oxygen to oxidize the NADPH resulting in the production of superoxide anion, a notorious ROS. In either case, the enzymes are elevated in farm workers compared with the controls suggesting respiratory burst system as an efficient host defense mechanism in these workers.Earlier studies have reported that ROS can be generated as a bye product of cellular metabolism or created by enzymes with the primary function of ROS generation (Rojkind *et al.*, 2002). ROS generation is increased in several folds in phagocytic cells during respiratory burst process in DOP exposed farm workers.

The significant reduction in CAT activity in farm workers compared with controls might be an indication of higher ROS generation in farm workers than controls. This observation of reduced CAT activity in farm workers exposed to DOP compared with controls was further alluded to by a significant reduction in PA compared with farmers probably indicating pesticide applicators were more exposed to DOP than farmers. Aly *et al.* (2010) also reported lower CAT activity in organophosphate exposed farm workers compared to controls.

This study observed no difference in the activities of SOD and GPx between farm workers and controls. This may be associated with diverse protective compensatory mechanisms in farm workers which might have restored the activities of these enzymes.

Taken together all the observations from this study, it could be concludedthat long term exposure to dichlorvos organophosphate pesticide increase the serum levels of most of the inflammatory markers in farm workers especially among pesticide applicators. Therefore, farm workers exposed to dichlorvos organophosphate pesticidewould benefit from consistent and correct use of protective personal equipment as well as routine clinical examinations to prevent chronic inflammation.

**Contribution to knowledge**

This study submits that long term exposure to dichlorvos organophosphate pesticide:

i. increasecellular inflammatory markers (lymphocytes and eosinophils) but reduce neutrophil count and neutrophil-lypmhocyte ratio in farm workers
ii. raise levels of humoral inflammatory markers (immunoglobulin E, C-reactive protein, interferon gamma, interleukin-4) and increase diameters of skin reaction to grass and mold allergens in farm workers
iii. increase activities of enzymatic inflammatory markers (myeloperoxidase and NADPH oxidase) but make no difference in activities of superoxide dismutase and glutathione peroxidase in farm workers.

**Recommendation**

Base on the observations from this study, the following recommendations could be made:

a. Government at all levels in Nigeria should enforce regulations on cautious use of organophosphate pesticide in agricultural practices
b. There should be improved campaign against excessive, uncontrolled and unprotected occupational exposure to organophosphate pesticides
c. Public enlightenment programs should be organized by Agricultural Extension Agents on the need for correct and consistent use of PPE by farm workers.
d. Farm workers that are occupationally exposed to organophosphate pesticides should be encouraged to go for regular periodic medical check-ups

**Further study**

Measurement of ESR, Complement factors, Rheumatoid factors, Allergen specific IgE, IL-1β, IL-6 and Immunoglobulin subclasses

Determination of lymphocyte subpopulations (CD4, CD8 etc) and

Certain autoantibodies (Anti-thyroglobulin antibody, Anti-nuclear antibody etc).

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**UNIVERSITY OF IBADAN.**

Good day sir, this questionnaire is being administered to evaluate some immunotoxicological indices and health problems in farmworkers exposed to OP pesticides. The information derived will be used to improve the health status of farmworkers and their families and will serve as evidence for the need to better management of pesticides in our society. Your kind cooperation in supplying correct information to the questions below will be highly appreciated. All information provided shall be kept very confidential.

Date: _____ Village:_____ ID No:_____

**SECTION ONE (DEMOGRAPHIC CHARACTERISTICS)**

Sex _____male [ ] female [ ]

Age (yrs) _____<34 [ ] 35-44 [ ] >50 [ ]

Marital status _____married [ ] single [ ] divorced [ ] separated [ ]

Level of education ____ illiterate [ ] primary [ ] secondary [ ] higher institution [ ]

Main occupation-------farmer [ ] applicator [ ] gardener [ ]

Spraying pesticides? Yes [ ] No [ ]

Type of farmworker----------seasonal [ ] permanent [ ]

**SECTION TWO (ANTHROPOMETRIC INDICES AND PRELIMINARY MEDICAL TEST)**

Weight _____ kg

Height _____ m

Body Mass Index (BMI) _____ $kg/m^2$

Body fat _____%

Blood pressure _____ mmHg

Fasting Blood Glucose or Random Blood Glucose _____ mg/dl

Urinalysis _____pH SG glucose protein blood bilirubin urobilinogen leucocyte nitrite ketone .

Frequency of pesticide-use per month? 1-3times [ ]>3times [ ]

Trade name of pesticide in-use? DDForce [ ] Termex [ ] Best [ ]

Active ingredient? Dichlorvos [ ] Chlorpyrifos [ ] Dimethoate [ ]

Formulations of pesticide in-use? Liquid [ ] Solid(gel,paste,granules,pellets,powder) [ ]

Packaging containers of pesticides in-use? Plastic [ ] metal [ ] glass [ ] paper bags [ ]

Equipment use in spraying? Hand sprayer [ ] ox-draw sprayer [ ]

Concentration of pesticide in-use?

Only one, not mixed? Always [ ] sometimes [ ] Never [ ]

Mixed with other pesticide*? Always [ ] sometimes [ ] Never [ ]

Mixed with herbs*? Always [ ] sometimes [ ] Never [ ]

Do you carefully read and understand all instructions? Always [ ] sometimes [ ] Never [ ]

Preparing pesticide: do you observe PPE?

Wearing long-sleeved shirt and trousers? Always [ ] sometimes [ ] Never [ ]

Wearing a hat? Always [ ] sometimes [ ] Never [ ]

Wearing a nasal mask? Always [ ] sometimes [ ] Never [ ]

Using special boots? Always [ ] sometimes [ ] Never [ ]

Using goggles? Always [ ] sometimes [ ] Never [ ]

Wearing gloves? Always [ ] sometimes [ ] Never [ ]

Do you follow directions on a label affixed to pesticide container? Always [ ] sometimes [ ]
Never [ ]

Practices while spraying pesticide?

Wearing long-sleeved shirt and trousers? Always [ ] sometimes [ ] Never [ ]

Wearing a hat? Always [ ] sometimes [ ] Never [ ]

Wearing a nasal mask? Always [ ] sometimes [ ] Never [ ]

Using special boots? Always [ ] sometimes [ ] Never [ ]

Using goggles? Always [ ] sometimes [ ] Never [ ]

Wearing gloves? Always [ ] sometimes [ ] Never [ ]

Chewing gum?* Always [ ] sometimes [ ] Never [ ]

Practices after spraying pesticide?

Immediately washing hands in clean water? Always [ ] sometimes [ ] Never [ ]

Immediately washing hands with soap or bath cream? Always [ ] sometimes [ ] Never [ ]

Immediately taking a shower? Always [ ] sometimes [ ] Never [ ]

Immediately taking a shower using soap or bath cream? Always [ ] sometimes [ ] Never [ ]

Returning to the field after spraying pesticide before re-entry period?* Always [ ] sometimes [ ] Never [ ]

Dealing with empty pesticide container?

Discard on the garbage dump? Always [ ] sometimes [ ]Never [ ]

Burying? Always [ ] sometimes [ ] Never [ ]

Burning?* Always [ ] sometimes [ ] Never [ ]

Storage water?* Always [ ] sometimes [ ] Never [ ]

Storage food stuff?* Always [ ] sometimes [ ] Never [ ]

Reaping crops during safe period after last spraying? Always [ ] sometimes [ ] Never [ ]

**SECTION FOUR (KNOWLEDGE OF PESTICIDE USE)**

Route of pesticide entry into the body?

Mouth? Yes [ ] No [ ]

Skin? Yes [ ] No [ ]

Inhalation? Yes [ ] No [ ]

Pesticide-related symptoms?

Sore eyes? Yes [ ] No [ ]

Headache/dizness? Yes [ ] No [ ]

Weakness? Yes [ ] No [ ]

Nausea and vomiting? Yes [ ] No [ ]

Fever? Yes [ ] No [ ]

Skin rash? Yes [ ] No [ ]

Excessive sweating? Yes [ ] No [ ]

Infertility/miscarriage? Yes [ ] No [ ]

33. Risk of getting disease due to long-term exposure to pesticide?

Immunological diseases? Yes [ ] No [ ]

Respiratory diseases? Yes [ ] No [ ]

Dermatological diseases? Yes [ ] No [ ]

Neurological diseases? Yes [ ] No [ ]

Cancers? Yes [ ] No [ ]

34. Places where pesticide residues exist?

Soil? Yes [ ] No [ ]

Ground water? Yes [ ] No [ ]

Fruits, seeds and leaves of vegetables? Yes [ ] No [ ]

Air? Yes [ ] No [ ]

35. Alternative pest control to chemical pesticides?

Biological control e.g., effective microorganism (EM)? Yes [ ] No [ ]

Natural pest control? Yes [ ] No [ ]

Traditional ways of controlling pests (by burning weeds or ploughing them)? Yes [ ] No [ ]

36. Checking spraying equipment conditions before using? Yes [ ] No [ ]

37. Do not spray against the wind? Yes [ ] No [ ]

38. Place to store empty pesticide containers?

In specific store on the farm? Yes [ ] No [ ]

In the home? Yes [ ] No [ ]

**SECTION FIVE (PERCEPTIONS OF PESTICIDE USE)**

39. Agricultural occupations are at risk of negative effects from chemical pesticides?

High [ ] moderate [ ] low [ ]

40. Agricultural family members are at risk of negative effects from chemical pesticides?

chemicals? High [ ] moderate [ ] low [ ]

44. Strictly adhering to chemical use instructions can reduce the risks of and dangers of chemical use? High [ ] moderate [ ] low [ ]

45. Using chemo-protective equipment while spraying increases costs?

High [ ] moderate [ ] low [ ]

46. Using chemo-protective equipment causes difficulty and feeling uncomfortable while working? High [ ] moderate [ ] low [ ]

***negative item**

## APPENDIX III PREPARATION OF REAGENTS AND PROCEDURES

**Reagents preparation and procedure for serum Cholinesterase (ChE)**

Equipment, materials and reagents

- Waters 616/626 HPLC
- Fume hood
- Precision pipette
- Extraction tubes
- Volumetric flasks

- Dilute HCl (0.1N)
- Reference standards of 1000ppm

Procedure

- 0.25ml of serum was pipetted into each of the extraction tube
- 10ml of ultrapure water was added and swirl
- 10ml of 0.1N HCl was added and mix
- The mixture was mounted on the digestion block primed at $80^0$C for 21/2 hours heating it to clear solution
- The mixture was removed and allow to cool to room temperature
- Each tube was made up to 50ml with ultrapure water and shake for 10mins
- Centrifuged at 3500rpm for 5mins to obtain supernatant used for the analysis
- Each sample is diluted 10 times
- Necessary information including weight of the sample, extraction volume, dilution factors, number of standards and their concentrations (0.0, 0.2, 0.4, 0.6 and 0.8ppm were fed on the HPLC software
- Dluted samples and standards were loaded on the autoanalyser cups
- Autoanalyser was set as per the speed, time and volume of sample taken by a suction
- The stationary phase (column) and the mobile phase (nitrogen gas) were set
- Fluorescent detector, time taken for each sample (0.45s), time used to rinse probe after each sample suction (0.5s) were set on the software
- The analysis was started running the working standards in ascending order first
- After runs, the software calculated the concentrations of ChE from the relationship between the working standard curve equation and the molecular weight intensity of the analyte
- The concentrations obtained were converted to U/ml using the conversion factor (2.793)

**Reagents preparation and procedure for Differential Leukocyte count (DLC)**

Reagents

Giemsa stain:

- Giemsa powder 3.8g

the stain and mixed well. The bottle was placed in a water bath at $56^{o}$ C for up to 2hrs mixing the content at intervals of 15mins

Procedure

- a thin blood film was prepared, air dried and fix in methanol
- 10% Giemsa stain was prepared from the stock solution fresh
- the slides were flooded with the stain solution and allowed to stain for 15 mins, rinsed and air dried
- Each slide is observed under 100x objective
- The differential leukocyte counts identify and count various types of white blood cells (neutrophil, lymphocyte, monocyte, eosinophil, basophil) and express the number of each type per 100 white cells (percentage).
- The five types of leukocytes observed in normal peripheral blood smear were counted and reported in percentage.

**Reagents preparation and procedure for IgE**

- Desired numbers of coated wells were selected into the holder.
- 20µl of standard, specimens, and controls were dispensed into appropriate wells.
- 100µl of Zero Buffer was dispensed into each well.
- The wells were thoroughly mixed for 10 seconds.
- Incubated at room temperature (18-$22^{0}$C) for 30 minutes.
- The incubation mixtures were removed by flicking plate content into a waste container.
- The microtiter wells were rinsed and flicked 5 times with washing buffer (1X). Wells were struck sharply onto absorbent paper to remove all residual water droplets.
- 150µl of Enzyme Conjugate Reagent was dispensed into each well and gently mix for 5 seconds.
- The mixture was incubated at room temperature for 30 minutes.
- The incubation mixture by was removed flicking plate contents into sink.
- The microtiter wells were rinsed and flicked 5 times with washing buffer (1X).
- The wells were struck sharply onto absorbent paper to remove all residual water droplets.
- 100µl TMB solution was dispensed into each well and gently mix for 5 seconds.

- A standard curve was constructed by plotting the mean absorbance obtained from each reference standard against its concentration in IU/ml on graph paper, with absorbance values on thevertical or Y axis and concentrations on the horizontal or X axis.
- The mean absorbance values for each specimen were used to determine the corresponding concentration of IgE in IU/ml from the standard curve.

**Reagents preparation and perodcedure for catalase activity estimation**

Reagents

- 5% Dichromate solution ($K_2Cr_2O_7$)

5g of $K_2Cr_2O_7$ was dissolved in 80 ml of distilled water and made up to 100 ml.

- 0.2 M $H_2O_2$

11.50 ml of 30% (w/w) $H_2O_2$ was diluted with distilled water and the solution made up to 500 ml.

- Dichromate/acetic acid solution

This reagent was prepared by mixing 5% solution of $K_2Cr_2O_7$ with glacial acetic acid (1:3 v/v).

- Phospahte buffer

3.58g of $Na_2HPO_4.12H_2O$ and 1.19g $NaH_2PO_4.2H_2O$ were dissolved in distilled water; the pH was adjusted to 7.0 and made up to 1 litre.

- Assay mixture

2 ml of $H_2O_2$ was mixed with 2.5 ml of phosphate buffer

Procedure

- 1 in 50 dilutions of the samples were done by adding 0.1 ml of sample to 4.9 ml of distilled water.
- Serial dilution of $H_2O_2$ was made by adding 0.95 ml, 0.90 ml, 0.85 ml, 0.80 ml, 0.70 ml, 0.60 ml, 0.50 ml of distilled water to 0.05 ml, 0.10 ml, 0.15 ml, 0.20 ml, 0.30 ml, 0.40 ml, 0.50 ml of $H_2O_2$ to yield 10, 20, 30, 40, 60, 80, 1nd 100 µmoles of $H_2O_2$ respectively.
- 2 ml of $H_2O_2$ and 2.5 ml of phosphate buffer (assay mixture) were dispensed into allthe test tubes
- 0.5 ml of the diluted sample/standard was dispensed into the tubes appropriately.
- 2ml of dichromate/acetic acid solution was dispensed into another set of test tubes and 1 ml

- The standard curve was plotted and concentrations of the sample were extrapolated from the curve.
- Concentrations were multiplied by the dilution factor
- Catalase activity was calculated as $H_2O_2$ consumed/mg protein

**Reagents preparation and procedure for superoxide dismutase (SOD) activity estimation**

Reagents

- 0.05 M Carbonate buffer (pH 10.2)

3.58g of $Na_2CO_3.10H_2O$ and 1.05g of $NaHCO_3$ were dissolved in 200 ml of distilled water. The pH was adjusted to 10.2 and then made up to 250 ml with distilled water.

- 0.3 mM Adrenaline

0.01g of adrenaline (epinephrine) was dissolved in 200 ml of distilled water. It was prepared fresh on the day of the analysis.

Procedure

- 1 in 10 dilutions (1:10) were done by adding 0.1 ml of sample to 0.9 ml of distilled water.
- 2.5 ml of the buffer was dispensed into all the test tubes
- 0.2 ml of sample was added to the tubes appropriately
- 0.3 ml of adrenaline was added to all the tubes and the solution mixed by inversion
- The absorbance was taken at 480 nm using a spectrophotometer and absorbance values were recorded every 30 seconds until 150 seconds i.e 0, 30, 60, 120 and 150 sec.
- The blank contained 2.5 ml of buffer, 0.3 ml of adrenaline and 0.2 ml of distilled water.
- Concentrations were multiplied by the dilution factor
- SOD activity was calculated as:

a. Change ($\Delta$) in absorbance = $A_{150}$-$A_0$/2.5
b. % inhibition (x) = 100 – 100 ($\Delta$Abs of sample/$\Delta$Abs of blank)
c. SOD activity (% inhibition) = x/50

**Reagents preparation and procedure for glutathione peroxidase (GPx)**

phosphate buffer

- Hydrogen peroxide ($H_2O_2$; 2.5mM): 28µL of hydrogen peroxide was dissolved in 100mL of distilled water
- Trichloroacetic acid (TCA; 10%): 2g of TCA was dissolved in 20mL of distilled water
- Dipotassium hydrogen orthophosphate ($K_2HPO_4$; 0.3mM): 5.23g of di-potassium hydrogen orthophosphate dissolved in 100mL of water
- 5'-5'-dithiobis-(2-dinitrobenzoic acid) DNTB: 0.04g of DNTB was dissolved in 100mL of phosphate buffer
- Phosphate buffer: 0.992g of $K_2HPO_4$ and 1.946g of $KH_2PO_4$ were dissolved in 200mL of distilled water and adjusted to pH of 7.4

Procedure

- 500µl of standards, samples and controls were dispensed into test tubes
- 500µl of phosphate buffer, 100µl of $NaN_3$, 200µl of GSH, 100µl of $H_2O_2$ and 600µl of distilled water were added to each tube
- The mixture was incubated at 37$^{o}$C for 3 minutes
- 0.5mL of TCA was added and centrifuged at 3000rpm for 5 minutes to obtain ImL of supernatant
- 2mL of phosphate buffer and 1mL of DNTB solution were added to the supernatant and the absorbance was read at 412nm against a blank
- GPx activity was calculated by plotting the standard curve

**Reagents preparation and procedure for myeloperoxidase (MPO) activity estimation**

Reagents

- 0.1M potassium phosphate buffer (pH 7.0)

0.53g of $KH_2PO_4$ and 1.06g of $K_2HPO_4$ were dissolved in distilled water, pH adjusted to 7.0 and made up to 100 ml.

- 0.018 M Guaiacol

22.3 mg of guaiacol was dissolved in 10 ml of distilled water and stored on ice. It was prepared fresh on the day of analysis.

- 2.80 ml of buffer, 0.05 ml of guaiacol and 0.05 ml of $H_2O_2$ were dispensed into test tubes for sample and blank.
- The mixture was equilibrated and the absorbance was monitored at 436 nm until constant.
- 0.1 ml of sample and 0.1 ml of distilled water were dispensed into test tubes for sample and blank respectively.
- The mixture was mixed by inversion and exactly at 1 minute, the absorbance was read at 436 nm.
- Volume activity (U/ml) =
  (Absorbance of test – Absorbance of blank) x 4 x total volume

micromolar extinction co-efficient of tetraguaiacol (25.5) x sample volume

**Reagents preparation and procedure for NADPH oxidase (NOX) activity estimation**

- Serum (final concentration 1 mg/mL) was distributed in 96-well flat-bottom culture plates (final volume 200 µL/well).
- Cytochrome c (500 µmol/L) and NADPH (100 µmol/L) were added in the presence or absence of SOD (200 U/mL) and incubated at room temperature for 30 min.
- Cytochrome c reduction was measured at 550-nm wavelength on a microplate reader.
- Superoxide production in nmol/mg protein was calculated from the difference between absorbance with and without SOD and extinction coefficient for change of ferricytochrome c to ferrocytochrome c, i.e., 21.0 mmol $\cdot L^{-1} \cdot cm^{-}$

**Procedure for tumor necrosis factor-alpha (TNF-α), interferon gamma (IFN-γ, interleukin-4 (IL-4), and interleukin-10 (IL-10) estimation**

- Samples were diluted in 10 fold by adding 20 µL of sample to 180 µL of sample diuent.
- 0.1 ml of the diluted samples and standards were dispensed appropriately into the microtitre wells appropriately.
- The plate was sealed and incubated at $37^0$C for 90 minutes.
- The cover was removed, content discarded and blotted unto paper towels.

blotted on absorbent paper on each episode of washing.

- 0.1 ml of ABC working solution was dispensed into each well and incubated at $37^0$C for 30 minutes.
- The plate was manually washed 5 times as described earlier.
- 90 µL of TMB colour developing agent was dispensed into all the wells and incubated at $37^0$C in dark for 20 minutes.
- 0.1 ml of stop solution was added and the colour changed to yellow immediately.
- Absorbance was read at 450 nm with a microplate reader within 30 minutes after adding the stop solution.
- A standard curve was plotted and the concentrations of unknown samples were determined from the curve.
- The concentrations obtained were multiplied by the dilution factor.

**Procedure for C-reactive protein (CRP)**

- Samples were diluted in 100 folds (5 µL of sample was added to 495 µL of sample diluents).
- 10 µL of the diluted samples/standards were dispensed into appropriate wells
- 100 µL of CRP enzyme conjugate was dispensed into all the wells
- The plate was gently shaken for 30 seconds and incubated at room temperature for 45 minutes.
- The plate contents were flicked into a waste container.
- The plate was rinsed and washed manually with distilled water 5 times.
- The wells were stricken sharply onto absorbent paper at each wash to remove residual water droplet.
- 100 µL of TMB solution was dispensed into each well and gently mixed for 5 seconds.
- The plate was incubated for 20 minutes and the reaction was stopped with 100 µL of stop solution.
- The mixture was gently mixed for 30 seconds to ensure change in colour from blue to yellow.