KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI - GHANA

COLLEGE OF HEALTH SCIENCE SCHOOL OF PUBLIC HEALTH

ASSOCIATION BETWEEN EXPOSURE TO ORGANOCHLORINE AND PYRETHROID PESTICIDES AND NEUROBEHAVIORAL OUTCOMES AMONG CHILDREN UNDER FIVE YEARS OLD IN OFFINSO NORTH DISTRICT OF ASHANTI REGION, GHANA.

BY

FELIX BONNEY

OCTOBER, 2019

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A THESIS SUBMITTED TO THE SCHOOL OF PUBLIC HEALTH, COLLEGE OF HEALTH SCIENCES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN ENVIRONMENT AND PUBLIC HEALTH

BY FELIX BONNEY (BSc Chemistry) OCTOBER, 2019

DECLARATION

I, Felix Bonney hereby declare that, except for references to other people's work which have been duly acknowledged, this write-up, submitted to the department of public health, school of research and graduate studies, KNUST, Kumasi, is the result of my own original research work and that this thesis has not been submitted to any institution by any student elsewhere for the award of any degree.

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(ACADEMIC SUPERVISOR)

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DR. E. APPIAH – BREMPONG DATE

(HEAD OF DEPARTMENT)

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LIST OF ABBREVIATIONS

AChE	- Acetylcholinesterase
ASEBA	- Achenbach System of Empirically Based Assessment
ADHD	- Attention deficit/hyperactivity disorder
CBCL	- Child Behavior Checklist
ChE	- Cholinesterase
DAP	- Dialkyl phosphate
DDE	- Dichlorodiphenyldichloroethylene
DDT	- Dichlorodiphenyltricholoroethane
DEDTP	- Diethyldithiophosphate
DEP	- Diethylphosphate
DETP	- Diethylthiophosphate
DMAP	- Dimethyl alkylphosphate
DMDTP	- Dimethyldithiophosphate
DMP	- Dimethylphosphate
DMTP	- Dimethylthiophosphate
EPA	- Environmental Protection Agency
FAO	- Food and Agriculture Organisation
GAEC	- Ghana Atomic Energy Commission
GDP	- Gross Domestic Product
HCH	- Hexachlorocyclohexane
IQ	- Intelligence quotient
IPM	- Integrated Pest Management
WHO	- World Health Organisation
OC	- Organochlorines
OCPs	- Organochlorine Pesticides
ONFAS	- Offinso North District Farm Health Study

OP	- Organophosphates
PCBs	- Polychlorinated biphenyl
PCDD/Fs	- Polychlorinated dibenzodioxins/furans
PDD	- Pervasive Developmental Disorder
PON	- Paraoxonase
POPs	- Persistent Organic Pollutants
SD	- Standard deviation
α-HCH	- Alpha Hexachlorocyclohexane
β-HCH	- Beta Hexachlorocyclohexane
γ-HCH	- Gamma Hexachlorocyclohexane
δ-HCH	- Delta Hexachlorocyclohexane

ABSTRACT

Pesticide use for agricultural purposes in Ghana has increased over the years with its related health threats to farmers, people living around farming communities as well as the environment due to unsafe handling practices and application.

This cross-sectional study conducted at three farming communities in Offinso North District of Ashanti Region, Ghana was aimed at assessing the association between exposure to organochlorine and pyrethroid pesticides and neurobehavioral outcomes defined as attention, rule-breaking, social behavior and aggressiveness. The target population was children under five years old. Questionnaire data on exposure practices and child behaviors as well as urine samples were taken for analyses, from 170 children whose parents were vegetable farmers.

Majority of the children, 61.18% had contact with pesticide contaminated surfaces at home, 69.41% places thumbs/ fingers in the mouth and 84.29% places contaminated objects in the mouth. In the farm, 55.29% of the children had contact with pesticide contaminated surfaces, 61.76% places thumbs/ fingers in the mouth and 54.12% places contaminated objects in their mouths. There were also significant levels of pesticide residues of five organochlorines and one pyrethroid in their urines. The mean residual concentration of gamma-HCH found was 3.09µg/l (SD = 0.77µg/l); beta-HCH = 2.89µg/l (SD = 0.64µg/l); delta-HCH = 2.42µg/l (SD = 0.43µg/l); heptachlor = 3.55µg/l (SD = 0.90µg/l); lambda cyhalothrin = 3.11µg/l (SD = 0.69µg/l). There was no significant association found between urinary pesticide residues and neurobehavioral outcomes. However, there were significant inverse associations between exposure indicators at home, in the farm or both and Attention, Rule-Breaking, Social behavior and Aggressiveness with the association being strongest among children aged between 3 to 4 years.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

The agricultural sector accounts for a comparatively small portion of the world's economy, but remains central to the survival of many people. In 2015, an estimated 1.6 billion people out of the world's population of 7.3 billion were directly engaged in agriculture. However, in middle and low-income nations, where majority of the people are farmers, the sector accounts for a greater share of national income and employment. For instance, agriculture accounts for 18 and 54 percent of India's national income and employment respectively (World Bank 2016).

In Ghana, the Agriculture sector employs over 50% of the total workforce (FAO, 2013). In spite of this, its contribution to the total gross domestic product (GDP) continues to decline, with its share reducing from 23.0% in 2012 to 22.0% in 2013 (Ghana Statistical Service. April, 2014). This reduced share can be attributed to low productivity and crop yield due to loss to weeds and/or pests (Horna D, *et al.*, 2008).

To reduce the impact of weeds and/or pests, farmers resort to the use of pesticides. Several hundred pesticides of different chemical nature are applied to crops by farmers in Ghana and according to the Environmental Protection Agency (EPA) of Ghana, a total of about 540 registered pesticides brands were purchased in 2015 and used in the country (Ghana EPA, 2016). These pesticides provide unquestionable benefits for increasing agricultural production and as a result, many farmers use them without adequate information about the hazards associated with the chemical (Ntow *et al*, 2006).

More than three million people worldwide suffer from pesticide poisoning, and more than 20,000 people die every year. Most of these fatalities occur in developing countries (WHO, 2011; Hough, 2013), where regulatory control of chemicals is inadequate with consequent increased exposure (Soni *et al*., 2011) leading to poor health outcomes such as abnormal nervous systems development, male sterility, or cancers (Koureas *et al*., 2012; PANAP A Factsheet Series, 2014; Gunnell *et al*., 2007; Horton *et al*., 2011). Because pesticides are toxic with potential to kill certain species of organisms they show a high danger to nature and therefore their use has gained attention not only because of its effect on human health but also on the ecosystem (Stoate, *et al.*, 2001, Berny, 2007, and Power, 2010).

Pesticides like organochlorines are typical persistent organic pollutants (POPs) known for their resistance to degradation in the environment, high toxicity and bioaccumulation ability (Wong *et al*., 2005). At high doses, adverse health effects such as cancer, birth and neurological disorders occur and also, these pesticides affect other neurochemical targets including secondary growth hormones (Mrema *et al.*, 2013; Liu *et al.*, 2016). According to Gonzalez-Alzaga, *et al* (2015), postnatal exposure of children to pesticide can negatively affect neuropsychological performance whiles prenatal exposure has an association with speed performance. A cohort study conducted by Bouchard *et al.,* in 2011, also revealed that the mean concentration of maternal and postnatal pesticide metabolites were associated with worse working memory, processing speed, verbal understanding and complete IQ in seven (7) years old children of low-income families living in agricultural region of the Salinas Valley, California.

In this study, pesticide exposure experience in relation to neurobehavioral outcomes (attention, rule-breaking, social behavior and aggressiveness) among under five years old children in Offinso North District of Ghana whose parents are farmers will be ascertained.

1.2 Problem statement

The average Ghanaian depends either directly or indirectly on agriculture for their livelihood. However, low yield of crops as well as post-harvest losses due to pests and/or weed are major concerns to productivity in the agricultural sector. Therefore, the use of pesticides has become a 'necessary' part of Ghana's agriculture, and it is estimated that more than 85% of Ghanaian farmers use chemical pesticides to control pests and diseases on vegetables (Dinham, 2003). Organochlorine and pyrethroid pesticides are widely used in Ghana for agricultural and public health purposes and residues are found in vegetable crops (Prah, 2013; Osei-Fosu, *et al.*, 2014) and in humans (Quansah R., *et al.* 2016). Associated with the increased pesticide levels in humans due to abuse, misuse, overuse and inappropriate handling are health problems (Asante and Ntow, 2009), with children likely to be more susceptible to these health hazards, which are mostly neurological (Eaton et al., 2008).

Recent studies have shown that, incidence of emotional and behavioral problems in early childhood have increased (Boyle et al. 2011) and that pesticides (Eaton *et al.*, 2008), and other environmental pollutants contribute greatly to the risk of neurodevelopmental problems such as attention deficit/hyperactivity disorder (ADHD) (Polanska *et al.*, 2012; Tarver *et al.*, 2014). ADHD is a mental disorder characterized by attention deficit, hyperactivity, and impulsivity, and is one of the most common emotional and behavioral problems in children with a global prevalence of around 5% (Polanczyk *et al.*, 2007).

Akumadan, Afrancho and Nkenkenso in the Offinso North District of Ghana, are well known vegetable farming communities with estimated farming population of Two thousand, Two Hundred and Ninety Five (2,295) (Ntow, *et al.*, 2009). Study by Ntow WJ, *et al.* (2008) on a small stream at Akumadan, the capital of Offinso North, revealed significant levels of endosulfan and chlorpyrifos in the stream. Also Quansah R., *et al.* 2016, in a cross-sectional study revealed an association between organochlorines and pyrethroids and symptoms of respiratory diseases among vegetable farmers in Offinso North District of Ghana. This suggests that farmers in Akumadan like all others extensively use pesticides.

There have been several studies in Offinso north and other farming districts/communities in Ghana on the knowledge of farmers, their exposure to pesticides and the potential health threats. However none of these studies was targeted on children of farmers or children living in these farming communities. The level of exposure of children to pesticides in Ghana and its associated neurodevelopment and behavioral outcomes is therefore not known.

1.3 Conceptual Framework

Figure 1.1 below illustrates the relationship between pesticide exposure at home and in the farm or both and neurobehavioral outcomes among children below five years of age. Box 1 at the bottom of the figure contains the behavioral outcomes of interest for the study defined as; Attention, Rule-Breaking, Social behavior and Aggressiveness.

Pesticides exposure after birth may occur through contact with pesticide contaminated surfaces, placing contaminated objects and pesticide containers in the mouth or putting fingers or thumbs in the mouth after playing in contaminated soil, at home and/ or on the farmland [Boxes 2, 3 and 4]. It may be as a result of infants spending time closer to the ground and in some cases, parents taking their babies along with them to the farm during the time of pesticide application exposing them to the mostly volatile chemical (Bradman *et al.,* 2007; Lu *et al.,* 2004; Weiss 2000).

Increased pesticide exposure likely increases the amount of residues in the body [Box 5], which eventually decreases the activity of the enzyme responsible for neurodevelopment; acetyl cholinesterase (Holland *et al*., 2006), resulting in behavioral disorders such as attention disorder, hyperactivity, over or under aggressiveness and conduct problems, autism, speech disorders including lower intelligence quotients (IQ) [Box 1].

Smoking and drinking habit of parents can independently result in behavioral disorders in the under five year old children. While smoking exposes the children to toxic gases that could result in behavioral problems, parents who are drunkards, recklessly and carelessly deal with pesticides and its containers in a way that exposes their children to the chemicals. They also tend to be careless in the handling of the children creating a situation of less supervision and high exposure to likely pesticide contaminated surfaces. The level of education of the parent is likely to have a strong determining relation on the outcome. Parents with higher education have better understanding of the health hazards of pesticides on human. They tend to make better choices as to where to mix the pesticides and how to better dispose-off, its containers to avoid or reduce the exposure of the child to pesticides both in the home and on the farm. The age of the child and breastfeeding habits also can have strong effects on the outcome of interest. Older children are likely to have less contact with pesticide contaminated surfaces and soil than very young ones who mostly crawl on the ground. Also, breastfeeding mothers who get exposed to pesticide are likely to pass the residue or metabolites to their children through breast milk and this may have strong association with behavioral outcomes [Box 6].

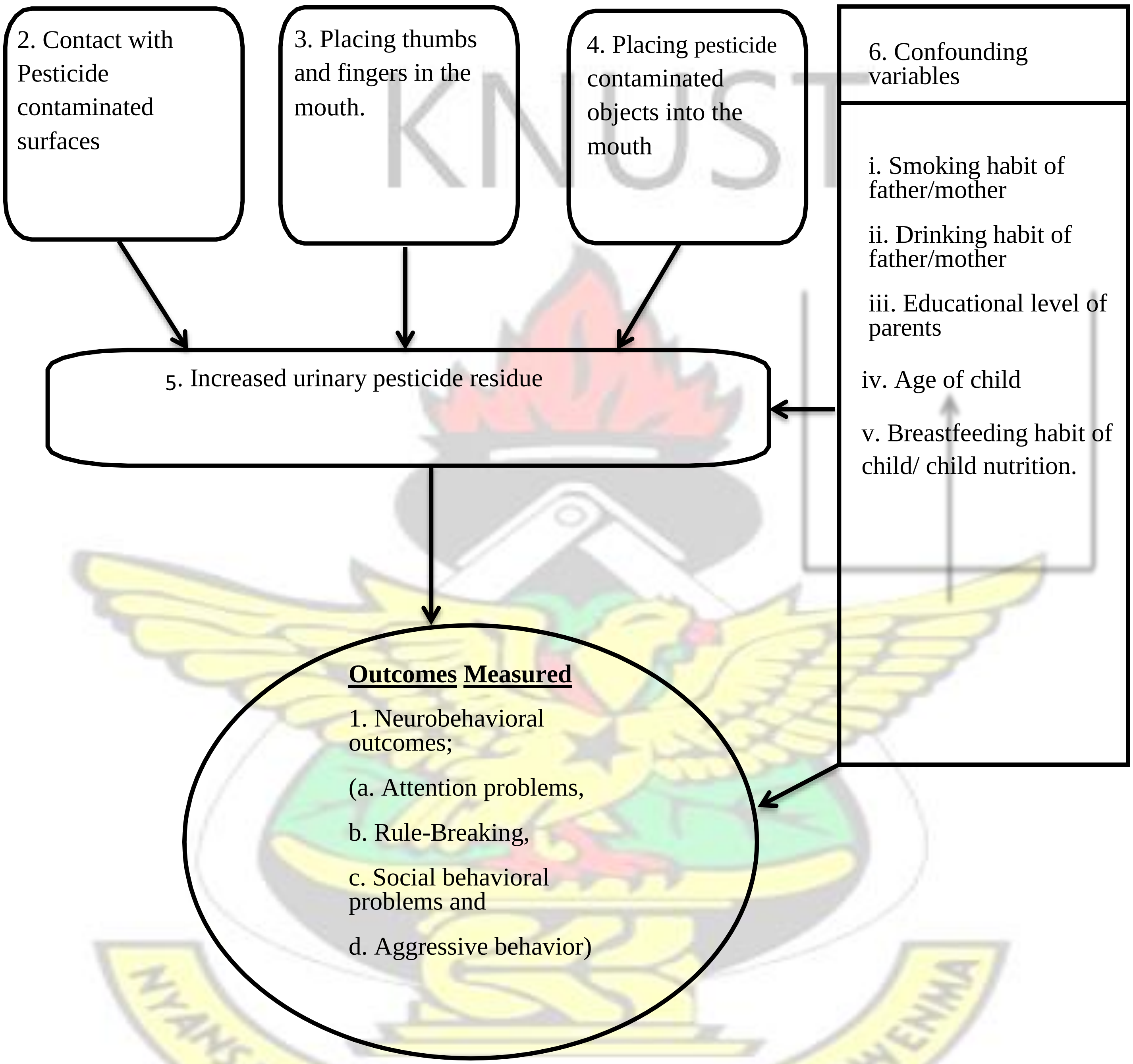

Figure 1.1: Conceptual frame work of the relationship between pesticides exposure at home, farm or both and neurobehavioral outcomes among children under five years of age.

1.4 Justification

Pesticides are substances that destroy, expel, or attack pests, which have a negative impact on agricultural productivity and profits. More than 90% of about 2,295 vegetable farmers in Akumadan, Nkenkenso and Afrancho in Offinso North district of Ghana use a variety of pesticides at various stages of cultivation, including organochlorines and pyrethroids (Ntow et al., 2009).

Studies have shown that exposure to pesticides can cause severe acute and chronic effects on human health with crop farm workers and their families at the greatest risk. Pesticide exposure potential for children from agricultural families may be higher than other non-farming pediatric populations, because concentrated pesticide formulations are used in large quantities near their homes. However, in Ghana, only few studies have examined the exposure of this potentially vulnerable population of children living with farmers, farm laborers or near farmlands. The study of proximal populations on agriculture largely follows erroneous events, without a specific focus on children. More so, there are no studies that assessed neurobehavioral outcomes related to pesticide exposure in children in the general population in Ghana.

This research work will therefore focus on characterizing children's pesticide exposure and its associated behavioral effect in a predominantly vegetable farming setting. The study will assess the relationship between exposure to organochlorines and pyrethroids and behavioral outcomes among children less than five (5) years old children in Offinso North District, Ghana. Findings from this work could inform the Ghana Government and policy formulators on whether or not existing policies regarding pesticide use and children health need review. There is the need to also quantify the burden of behavioral and attention disorder due to pesticides since such information may be important for long term strategic measures to minimize exposure and improve the safety of children.

1.5 Objectives of the Study

1.5.1 Main Objective:

The main objective of this study is to provide understanding of pesticide exposure experience in relation to behavioral outcomes defined as attention, rule-breaking, social behavior and aggressiveness in children below the age of five years.

1.5.2 Specific Objectives:

i. To ascertain the means of pesticide exposure at home, in the farm or both and their prevalence among children under five (5) years.

ii. To determine the levels of Organochlorine (OC) and pyrethroid pesticide residues in urine of children under five years.

iii. To determine the mean behavioral scores in children under five (5) years.

iv. To determine the association between indicators of pesticide exposure (i and ii) and mean behavioral outcomes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Scope of the Review

The literature review focused on the scientific evidence of the association between pesticides and neurodevelopmental disorders, with emphasis on organochlorines and pyrethroids. The scope of the review covers what pesticides are, history and uses of pesticides, classification of pesticides into; organochlorine pesticides, pyrethroids, organophosphate, etc., impact of pesticides, application and effects of pesticides on health, registration and regulatory requirements regarding pesticide use in Ghana, pesticides and children including ways of exposure and neurodevelopment of children.

Journals, books, online articles were all obtained from various offline and online sources such as IPCS INCHEM, Google Scholar, HINARI, Pubmed, Elsevier, Science Direct, Online Wiley library, Oxford Journals, and other data bases.

2.2 Definition of Pesticides

The US 'National Institute of Environmental Sciences' describes pesticides as any substances used to kill, expel, or control certain forms of plant or animal life that are considered pests. Pesticides include weed herbicides for destroying unwanted vegetation, insecticides to control various insects; fungicides are used to prevent mold and fungi, disinfectants to prevent the spread of bacteria and compounds used to control rats and mice.

According to the World Health Organization (WHO), pesticides are any substances used to destroy pests, including unwanted plant or animal species, during the production, storage, transportation, distribution and processing of food, agricultural products or animal feed, or which may be given to animals for the purpose of controlling ectoparasites. This term includes substances intended to be used as regulators of plant growth, defoliants, desiccants, fruit thinners, and substances used in plants before or after harvest to protect products from damage during storage and transportation. The term however, usually, does not include fertilizers, plant and animal nutrition, nutritional supplements and veterinary medicines (WHO, 1990).

2.3 History and uses of pesticides

Agriculture was practiced around 10,000 years ago in Mesopotamia, where edible seeds were originally harvested by hunter-gatherer populations. This was followed by planting cereals such as wheat, barley and peas, because the population was more established with agriculture as a way of life. Similarly, rice and millet were grown in China, while sorghum was planted in Africa around 7,500 years ago in the Sahel. In West Africa and perhaps New Guinea and Ethiopia, local crops were independently localized. Three regions in North and South America also localized corn, cashews, potatoes, and sunflowers (Kislev M.E *et al.*, 2004). It was clear that farmers will suffer crop losses resulting from pests and diseases that cause large yield losses, with the possibility of starvation as the population of the world becomes even greater. Currently, even with lots of progress made in agricultural sciences, losses resulting from pests and diseases ranges between 10 – 90% with an average of 35 – 40% for all potential fibre and food crops (Marcel Dekker, 2002). As a result of these losses, there was a big incentive to find ways to deal with problems caused by pests and diseases and this brought the idea of finding chemicals to control the pest and diseases.

The use of proven insecticides was first made about 4,500 years ago by Sumerians who used sulfur compounds to control insects and mites. About 3,200 years ago, the Chinese used mercury and arsenic compounds to control insects, while the early Romans used ordinary salt to control weeds and sulfur to control insects. In 1800, pyrethrin was found to have insecticidal properties. Another anti-insect material developed in the 1800s was the Paris green, a combination of arsenic and copper salts. A mixture of Bordeaux, a combination of lime and copper sulfate was also used to control the growth of fungi (Hodgson, 2004).

The use of inorganic chemicals as pesticides have been in existence since ancient times (Smith and Secoy., 1975, 1976), and in fact, mixture of copper-sulfate and lime (Bordeaux mixture) are still used in the treatment of various fungal diseases. However, it was only in the 1900s that it was discovered that the compounds have properties of pesticides. In the 1920s, petroleum oils were introduced as chemicals used to control red spider mites and insects. In the 1940s, chlorinated hydrocarbon insecticides such as DDT and phenoxy herbicides were formulated to eradicate Japanese rice plants and were later used as component of Agent Orange to destroy large areas of forests in jungle warfare. These chemicals have brought great benefits to farmers by increasing agricultural production even after the Second World War (Margaret et al., 2004).

Until the 1940s, synthetic substances like sodium chlorate and sulfuric acid, or chemicals from natural sources were still used widely to control pest. However, some pesticides were byproducts of gas production from coal or other industrial processes. Thus, early organic substances such as nitrophenol, chlorophenol, creosote, naphthalene and petroleum were being used to control insect pests and fungal growths, while ammonium sulfate and sodium arsenate were used as herbicides. The disadvantages of many of these products were high abuse by farmers and applicators, lack of selectivity and phytotoxicity (CropLife, 2002). Interest in synthetic pesticides grew in the 1940s with the discovery of the effects of DDT, BHC, aldrin, dieldrin, endrin, chlordane, parathion, and captan. These products though very effective were inexpensive, with DDT being the most widely used because of its broadband effects (Delaplane

K.S., 2000). DDT was widespread, appeared to have low toxicity in mammals and reduces insect-borne diseases such as malaria, yellow fever and typhus; and in 1949, Dr. Paul Müller received a Nobel Prize in medicine for discovering the insecticidal properties of DDT.

In the 1970s and 1980s, the world's best-selling family of herbicides, glyphosate, sulfonylurea and imidazolinone (imi) herbicides, dinitroanilines and aryloxyphenoxypropionate (fop) and cyclohexanedione (dim) were found. For insecticides, third generation pyrethroids were synthesized whiles avermectins, benzoylureas and Bt (Bacillus thuringiensis) were administered as spray treatments. This same period also witnessed the emergence of triazole, morpholine, imidazole, pyrimidine and dicarboxamide families used as fungicides. Because many of the agrochemicals introduced at the time had a single mechanism of action that made it more selective, resilience problems emerged and management strategies were introduced to combat these negative effects (Carson R and Houghton M., 2002).

In the 1990s, there were attempts by researchers to find new pesticides from existing ones that have greater selectivity and better ecological and toxicological profiles. New agrochemical families such as triazolopyrimidine, triketone and isoxazole herbicides, strobilurine and azolone fungicides and chloronicotinyl, spinosyn, fibrol and diacylhydrazine insecticides were all discovered and launched. These new chemical insecticides and fungicides have enabled better management of resistance and increased selectivity. During this period, old chemical products were also purified according to usage patterns, introducing newer, more user-friendly and more environmentally friendly formulations (CropLife, 2002). An integrated pest management system that uses all available pest management techniques to prevent the development of pest populations and reduce pesticide use was introduced to intervene and bring pesticide use to levels that are economically justified (OECD / FAO, 1999).

At present, the pest control toolbox has developed and includes the use of genetically modified crop plants that are able to produce their own insecticides or to be resistant to broad-spectrum herbicides or pests. These include plants that are resistant to herbicides such as soybeans, corn, cotton, canola, and other corn and cotton varieties that are resistant to corn borer and bollworm respectively (CropLife, 2002). In addition, the use of the Integrated Pest Management (IPM) system, which controls the development of pest populations and also reduces the use of agrochemicals, has become wider more popular and accepted. These changes have changed the nature of pest control and can reduce and / or change the rate at which agrochemicals are used.

2.4 Classification of pesticides

Pesticides are either extracted from plants or synthesized by man hence are described as synthetic (Barr & Needham, 2002). Pesticides are not only used for agricultural purposes, but also for professions and industries such as horticulture, veterinary medicine, public health, wood preservation, textiles, protection of building materials, etc., as well as for household use at home or in the garden (Mamane et al., 2015). Pesticides are classified in several ways based on their hazard levels, target organisms, and effects. They could also be categorized depending on their chemical properties into organochlorine pesticides (OC), organophosphate pesticides (OP), carbamates, dithiocarbamates, pyrethroids, phenoxyl, triazine, amide, and coumadin compounds (Ye et al., 2013).

2.4.1 Organochlorine Pesticides (OCPs)

Organochlorines are organic insecticides with carbon, hydrogen and chlorine as their main structural constituents. Due to the large weight of chlorine, OC pesticides disintegrate very slowly stays long in the environment and accumulates in the adipose tissue of animals and other organisms exposed after application (Swackhamer, Hites., 1988; Quintana *et al*., 2004).

The mode of action of OCPs and other similar pesticides is to target systems and enzymes in the pest organism to achieve its intended purpose to either kill or incapacitate the organism. Some of these systems and enzymes in the pests are similar to that of humans hence posing great risks to human health and the environment (Long, 2012).

Most organochlorine pesticides have endocrine disrupting effects and have adverse toxicity on hormonal (Lemaire *et al*., 2004) and central nervous systems resulting in hyper-aggressive state in brain or over excitation, convulsions, tremor, hyper-reflexia, lack of coordination and mental confusion (Barr *et al*., 2006; Costa, 2006).

2.4.2 Pyrethroids

Pyrethroids are synthetic organic pesticides manufactured from chrysanthemum flowers and are often used as domestic and commercial insecticides. Pyrethroid has been used for more than 50 years. They are preferred over other insecticides because they are less toxic to wildlife and does not persist in the environment for long and therefore considered relatively safe for humans. Their ability to incapacitate insect pests even at low doses is very high (WHO, 2005, Adelsbach and Tjeerdema, 2003).

Pyrethrin compounds are mainly used to control human fleas, mosquitoes, cockroaches, beetles and flies. Some pyrethrin compounds are used to control insects in horticultural plants, while others are used to store cereals and in poultry farms and on dogs and cats to control fleas and ticks. Natural pyrethrin quickly penetrates the body and attacks the nervous system of insects on contact. However, few minutes after application, insects cannot move or fly, but may not die

(IPCS, 1990; 1990d). Natural pyrethrin is rapidly detoxified by enzymes in insects. So some pests will recover. To slow down the action of the enzyme to provide lethal doses, organophosphate, carbamate or synergists are often added to the pyrethrin.

2.4.3 Organophosphate pesticides (OPs)

Organophosphates also known as phosphate esters are a class of organophosphorus compounds used for the control and destruction of insects on agricultural land, and in some cases for domestic or industrial applications. Organophosphates act as acetyl cholinesterase (AChE) retarders, which make the breakdown of acetylcholine impossible, thereby increasing both its concentration and period of action in the human body (Maria et al, 2013).

Organophosphate pesticide exposure is poisonous to humans and animals life (Levine, 2007; Tadeo et al., 2008). The neurological consequences of OP exposure as a child grows can be damaging even at low concentrations because neurotransmitters, including acetylcholine, play important roles in the brain architecture as well as the entire cellular development (Barr et al., 2006). Excessive exposure to humans causes excessive excitation of muscarinic and nicotinic receptors in the nervous system which leads to excessive accumulation of these neurotransmitters in cholinergic synapses due to phosphorylation of active cholinesterase molecules. This effect can cause various symptoms, including saliva, nausea, vomiting, tearing, seizures, and ultimately death (Costa, 2006).

2.4.4 Carbamates

These pesticides are also in insecticide category and have similar effects as the organophosphorus pesticides. Their actions can however easily be reversed and their effects are not as severe as the OP pesticides. Carbaryl (Sevin) and propoxur (Baygon) are the most popular of carbamate pesticides. ldicarb and methomyl are part of the very usual types employed in agriculture (Barr & Needham, 2002). A number of carbamates have also been measured in biological media such as serum and plasma. Carbamates are hydrolyzed to its main metabolite 1naphthol which is what is mostly measured in biological analysis.

2.5 Effects of pesticides on the environment and on human health

Pesticides are applied with the intention of controlling or eliminating pests, weeds, fungi and other undesirable plant and animal species in the agricultural system and in public health programme. However, when they are applied, it becomes difficult to define specific boundaries within which their effects are felt. Due to high water solubility and volatility of most of them, other living organisms which are not targets, the environment in which they are used and the applicators themselves are also affected. Excessive use and abuse of pesticides damage the health of applicators, consumers and the environment (Kriengkrai, 2006). The use of large quantities of pesticides has an impact on the atmosphere, water, soil and ecosystems, causing elimination of plants and animals and pollution of the environment. Many countries in Africa are reported to have stocked pesticide (aldrin, chlordane, DDT, dieldrin and heptachlor) in certain places that have been turned into landfills (Kriengkrai, 2006).

Another main impact of pesticide is to human health whether contact with the chemical is direct or indirect (Andersson H et al., 2014). Direct exposure to pesticides may occur in many ways. Individuals may directly be exposed to pesticides through manufacturing, formulation, mixing, application, suicides and mass poisoning. It has been established that, pesticide exposures cause health and disability problems such as attention deficit/ hyperactivity disorder in children, congenital abnormalities, cancer, chronic neurotoxic effects, infertility, miscarriages and the Parkinson's disease (Andersson et al., 2014).

Indirectly, pesticides impact humans through consumption of food contaminated with the chemical, contact with people directly involved with the manufacturing, formulation, mixing and application as well as exposure to pesticide residues in the air, water, soil, sediment, food materials, plants and animals (Kriengkrai, 2006). Studies on residues of pesticides support the identification and control of safe levels of pesticides in food, not only for commercial purposes, but also to ensure that, impact on human health is minimal. For this reason, maximum levels of residue (MRL) are established to ensure conformed agricultural practices (Juan et al., 2008). The maximum residual limit is the maximum concentration of pesticide residue (expressed in mg / kg) which is authorized in or on food items and feed for animals. Primarily, the MRL serves as a checklist to maintain good agricultural practices and promote international trade in products treated with pesticides. MRL is not a safety margin and exposure to pesticide residues above the MRL does not automatically imply health risks (Ellis, 2005).

The introduction of chemicals into farming activities has positively affected production but has also increased the pesticide residues and concentrations in food and in the environment, with negative health impacts associated with it (Richter, 2002). The impacts of chemical pesticides on health may vary depending on the exposure categories. People with direct exposure, indirectly exposed community members and users of food products with residues and various risks of pesticides are often classified based on how long the effect is felt; whether short-term (such as diarrhea, abdominal pain, headaches, nausea, vomiting, etc.) or long-term (such as skin disease, cancer, depression, neurological deficits, diabetes, genetic disorders or even death).

Studies suggest that, pesticide applicators who are directly exposed to the chemical over a long period of time suffer from depression (Beseler et al., 2008). Various researches were also conducted to analyze the effects of pesticide exposure on neurological outcomes. There is evidence that, the time spent on the farm during farm work is inversely proportional to neurological performance and it was established that exposure to pesticide was a relevant risk factor (Kamel et al., 2003). The results however are mixed depending on the type of pesticide being analyzed; whiles some suggest that symptoms of neurological disorders are associated only with cumulative exposure, especially for some fumigants and insecticides (Kamel et al., 2005), other pesticides such as triallates have no effects (Sathiakumar et al., 2004).

With people indirectly exposed to pesticides such as children and spouses of pesticide applicators, studies suggest that both acute high-intensity and cumulative pesticide exposure may contribute to functional cognitive deficits (Abdel et al., 2008; Rauh et al ., 2011), depression (Hong et al., 2009), Parkinson's disease (Yesavage, et al., 2004), and neurobehavioral performance (Bouchard et al., 2011; Eskenazi et al., 2010; Harari et al., 2010) especially among young populations (Eckerman et al., 2007).

2.6 Pesticides and behavioral problems in children

All pesticides are somehow toxic and risky for infants and children. The seriousness of the risk however depends on the type of active ingredients used in making the pesticide, the concentrations and also how much or long a child is exposed to the chemical. Babies and children are more sensitive to the toxic effects of pesticides than adults because of their immature organs, brain and other body systems which continue to develop after birth. Due to the immature body systems, the baby's liver and kidneys are not able to detoxify or remove the toxic substances present in the pesticides like those of adults. Children are also likely to be exposed to pesticides more than adults because they have faster breath rate and have relatively larger skin surface than their weight. Children also often spend more time closer to the ground, touching contaminated surfaces, soils and other materials on which pesticides might have been applied.

Children often eat and drink more than adults, which can cause high-dose pesticide residues per kilogram of body weight provided what they drink or eat are contaminated. Babies crawling on treated carpets and grasses may have greater possibility to carry pesticide residues on their skin or breathe in pesticide infested dusts. Children are also more likely to place fingers, toys, and other objects into their mouths. It is therefore important to reduce children's exposure to pesticides (npic.orst.edu).

Brender and his colleagues in a study conducted in 2010 found that, women who used pesticides at home or in their farms were twice as likely to have children with neural tube defects as compared to women without this reported exposure (Brender et al., 2010). Research has shown that certain pesticides residues in mothers can be transferred to unborn babies through the umbilical cord and also to breastfeeding babies through the breast milk (Pohl, HR, et al., 2000). Subsequent observations have led researchers to the conclusion that, chronic low doses of certain pesticides could endanger the health and development of children (Weiss et al., April 2004). The World Health Organization (WHO) reports that more than 30% of the world's total disease burden of children is caused by environmental factors including pesticides (WHO, 2006).

2.6.1 Organochlorines and behavioural problems in children

Evidences have it that prenatal and postnatal exposures to OC chemicals disrupt normal development of the fetus or developing child. Organochlorines and polychlorinated biphenyls disrupt the normal functioning of dopaminergic organs, as evident in alterations in dopamine levels in the brains of laboratory animals (Seegal et al., 2002); this is a potential mechanism by which OC pesticide can influence behaviors related to attention, because cellular dopamine degradation is associated with attention deficit disorders such as ADHD (Faraone SV, Biederman J., 1998). In another study, postnatal exposure to DDT resulted in sensitivity to other organic pollutants, and behavioral changes upon further exposure through meals (Johansson *et al*., 1995). Prenatal exposure to the organochlorine chemical chlordane and DDT at varying levels is linked with changes in behavioral patterns in both sexes of rats that lasted into adulthood (Cassidy *et* al., 1994; Palanzaa et al., 1999).

Organochlorine insecticide exposure is strongly related with neurodevelopmental health outcomes in humans. Exposures to the chemicals has been linked to decreased psychomotor and mental functions, including pictorial and auditory working memory, response time, attention, impulsivity and verbal skills in children (Vreugdenhil et al., 2004; Jacobson JL, Jacobson SW., 2003; Stewart et al., 2003; Stewart et al., 2005; Stewart et al., 2006). In short, it can be said that children born in rural areas where organochlorine pesticides are used have lower scores in several neurological behavioral tests than children who were born in non-agricultural communities (Jurewicz J, Hanke W., 2008, Ribas-Fito et al. , 2006; Roberts EM. et al., 2007).

2.6.2 Organophosphates and ADHD in children

The association of organophosphate pesticide with attention related problems may result from several biological processes. Basically, the main effect of organophosphate, especially those associated with acute poisoning is the inhibition of acetylcholinesterase (Sultatos LG, 1994), and disruptions in cholinergic signaling (Coccini et al., 2009). Even at lower doses, some organophosphates affect other neurochemical targets, rather than inhibiting acetylcholinesterase.

It also affects some neurotransmitters and secondary systems, including developmental factors (Verma et al., 2009; Slotkin TA, Seidler FJ, 2007). Prolonged effect on a number of neural systems can result from developmental exposure and that can cause ADHD behavior, such as: Lack of attention and cognitive deficits, similar to the effects of nicotine on development (Heath

CJ, Picciotto MR, 2009; Slotkin TA, 2004).
Five separate studies were conducted in the United States by the following; Rauh et al in 2006, Eskenazi et al in 2007, Sanchez et al in 2008, Bouchard et al in 2010 and Marks et al in 2010 with the aim of examining the association between organophosphate pesticides and attentionrelated outcomes among young children. Rauh VA et al., 2006 found that chlorpyrifos levels in maternal serum were significantly associated with maternal reports about pervasive developmental disorder symptoms (PDD) and attention problems such as attention-deficit hyperactivity disorder (ADHD). A birth cohort study conducted on children of farmworkers living in the Salinas Valley of California to assess mental and psychomotor development found significant associations between prenatal organophosphate metabolite and PDD at one year. The study also found out that dialkyl phosphate (DAP) exposures were adversely associated with maternal report of attention problems as well as direct and psychometrical observations at 3.5 and 5 years. The associations were however more profound in the 5 years old children than in 3.5 years old and stronger in boys than in girls. (Eskenazi et al., 2007; Marks et al., 2010).

Bouchard et al., 2010 reported a correlation between childhood organophosphate metabolite and ADHD among a population of U.S children aged between 8 to 15 years in a cross-sectional study. It was concluded that, children with higher urinary levels of dimethyl phosphate (DMAP) had greater possibility of being diagnosed with attention deficit hyperactivity disorder (ADHD) with children having higher levels than the median of detectable levels having twice the chance of having the disorder compared with their counterparts with undetectable levels. Another crosssectional study of Latin American children living in agricultural communities (Sánchez et al., 2008) has reported an adverse relationship between the urinary DAP of children and attention errors using the Wisconsin Card Sorting Test.

The results of studies reviewed show that children's exposure to organophosphate pesticides can cause PDD symptoms (Timofeeva et al., 2008; Rauh VA et al., 2006) and attention-deficit hyperactivity disorders (Rauh VA et al., 2006) or attention problems (Sánchez et al., 2008; Marks et al., 2010).

2.6.3 Pyrethroids and ADHD in children

Pyrethroids are usually considered safer than other insecticides. Studies conducted on rodents however suggest that, exposures during early-life and during the periods of puberty have effects on neurobehavioral functioning (Farag et al. 2007; Shafer et al. 2005; Sinha et al. 2006). A research by Quirós-Alcalá et al in 2014, reported of the widespread and increasing use of pyrethroid in the US but found no significant correlation between postnatal exposure and maternal report of ADHD. However, a recent study by Wagner-Schuman et al., 2015, found an association between increased pyrethroid pesticide exposure and ADHD which may be more pronounced for hyperactive-impulsive symptoms compared to inattention and more prevalent in boys than in girls.

2.7 Routes of exposure of pesticides

Due to the wide range use of pesticides in agriculture and public health, there are many ways through which exposure to the chemical can occur. These include inhalation of contaminated air, through contaminated food and water or through the skin by absorption (Nicolopoulou- Stamati P. and Pitsos M., 2001; Bouman *et al*., 2002; Shomar *et al*., 2005; Curwin et al., 2005, 2007; Lu et al., 2004, 2008; Munoz-Quezada et al., 2012; Naeher et al., 2010; Rodriguez et al., 2006; Valcke et al., 2006; Vida and Moretto, 2007). Though living or working close to sites where pesticides are applied increases the chance of exposure, the chemical due to their volatile nature are normally moved away from where they were/ are applied by air and water currents, causing contamination of water bodies, air and the soil (Fenske *et al*., 2002).

In children, prenatal and postnatal exposures may also occur and fetuses and children be at risk *in utero* as well as through breast feeding (Fitzgerald *et al*,. 2001; Hagmar *et al*., 2001; Mwevura *et al*., 2002; Jurewicz J, Hanke W. 2008) or directly feeding on contaminated food, water or soils. After application on floors, carpets and fabrics, children who play on the ground and show oral behaviors may be exposed through their skin and digestive track (Whyatt et al., 2003; ATSDR, 2003). In addition, Para occupational exposure, such as contact with someone who has been occupationally exposed to pesticides, or coming into contact with contaminated items used by an occupationally exposed person such as clothing, has been found to be an important exposure pathway in children or spouses of farmers or farmworkers (Vida and Moretto, 2007; Rodriguez et al., 2006; Valcke et al., 2006; Curl et al., 2002; Fenske et al., 2002).

2.8 Metabolism of pesticides

Microorganisms play important roles in the metabolism of organochlorine pesticides. However, traces of a number of them in their pure state are usually reported over a long period after exposure because they do not degrade easily, and accumulate biologically in fat-rich tissues (Swackhamer D, Hites RA., 1988). Detection of the various forms of OCPs is done by either measuring directly or indirectly the metabolite or the specific compound itself in the particular media being investigated. For instance the metabolite of Aldrin, dieldrin is mostly measured with chlordane and heptachlor also monitored as metabolites. Dichlorodiphenyltrichloroethane is measured sometimes as its biodegraded product or metabolite called DDE-Dichlorodiphenyldichloroethylene (Barr & Needham, 2002).

When organophosphate pesticide enters the human body, they are mostly metabolized into a more toxic form which bounds with AChE to release its pesticide-specific metabolite. With time, OP bounded AChE becomes non-correctable whiles pesticides not bonded with AChE are enzymatically or spontaneously hydrolyzed to form specific or non-specific DAP metabolite respectively through paraoxonase (PON). These metabolites or residues with half-life ranging between 24 – 48 hours are usually excreted into the urine or sequestered in lipid stores in the body (Bakke and Price, 1976, Ntow J, *et al*., 2009).

Pyrethroid pesticides are quickly circulated through the body immediately after injection and stored up in fatty tissues, stomach, liver, intestines and the nervous system (WHO, 2005). They are then broken down by means of hydrolyses and oxidation and bonded to amino acids, sugars or sulphate. This biological process which typically occurs in the liver through actions of enzymes such as hydrolases and cytochrome P_{450} monooxygenase, converts the lipophilic chemical into a hydrophilic compound so that it is removed through the urine or feces (WHO, 2005; ATSDR, 2003; Soderlund et al., 2002; Koureas et al., 2012). The metabolism of pyrethroids is generally considered to reduce their toxicity (WHO, 2005; Soderlund et al., 2002). In a study by Ray and Fry in 2006 however, it was hypothesized that, metabolism of pyrethroid may actually bio activate the chemical, creating more toxic metabolite than the parent pesticide (Ray, D. E. and Fry, J. R., 2006). Pyrethroid residues or metabolites have half-life varying from two hours to few days before excretion usually through the urine (Kaneko and Miyamoto, 2001).

2.9 Biomarkers of exposure of pesticides

Any measurement that reflects a relationship between a biological system and an agent of the environment such as pesticide through physical, biological or chemical mean is a biomarker (Van Damme *et al*., 1995). Generally, exposure to pesticides can be assessed through questionnaires administration to have knowledge about types of pesticides used and behavioral patterns that predisposes one to any of the chemicals or by biomonitoring (Sudakin and Stone, 2011; CDC., 2009; Fortin et al., 2008; Barr et al., 2006; Wessels et al., 2003; Duggan et al., 2003).

There are a number of studies in which residues and/or metabolites of organochlorine, organophosphate and other classes of pesticides were measured in body fluids after exposures (Barr et al., 2006; CDC, 2009; Duggan et al., 2003; Sudakin and Stone, 2011; Wessels et al., 2003). Also, residues of γ-HCH, β-HCH, α-HCH, and lambda cyhalothrin were measured in urine after exposure to organochlorine and pyrethroid (Quansah R *et al*., 2016; Coye MJ *et al*., 1986), whiles DDE, a metabolite DDT (Suzuki G *et al*., 2005), dieldrin, hexachlorobenzene, hexachlorocyclohexane, and heptachlor epoxide (Fitzgerald *et al*,. 2001; Hagmar *et al*., 2001; Mwevura *et al*., 2002; Jurewicz J, Hanke W. 2008) were measured in breast milk.

Metabolized and non-metabolized pyrethroids have also been quantified in body fluids such as urine, blood plasma and even breast milk of occupationally exposed women (WHO, 2005; Koureas et al., 2012; Chen, et al., 1991). It is however very difficult to tell which specific pyrethroid pesticide can be linked with an observed health outcome when using body fluids such as urine or blood. This is because; many different pyrethroid compounds can be metabolized into same metabolite such as 3-phenoxybenzoic acid (3BPA) (Oulhote, Y. and Bouchard, M., 2013).

Since organochlorine pesticides primarily bind with acetylcholinesterase and inhibits its activity, measurement of OP exposure can usually assessed by AChE activity screening. Furthermore, OP pesticide measurement can be done through monitoring specific and non-specific urinary metabolites, and measurement of the parent pesticide in blood (Barr et al., 2006; CDC, 2009, 2012). It is however worthy to note that, research shows that, the levels of OPs found in blood are typically three times lower than the levels found in urine due to quick degradation. This therefore complicates their measurement in blood and makes urinary residue or metabolite measurement more preferable to blood (Barr et al., 1999).

2.10 Registration and regulatory requirements regarding pesticide use in Ghana

The potential impacts of pesticide on human health, particularly the developing fetus and child as well as on the environment are of great concern to society and policy regulators. Also due to growing concerns about pesticide residues in food, many countries have introduced legislation regarding the manufacture, importation, the use and disposal of the chemical, to protect consumers from the hazards that it may pose. Today, most nations and governments pay much attention to pesticide registration requirements and in addition propose that even registered pesticides follow a re-registration process that complies with current guidelines, regulatory mechanisms and safety profiles.

The 1996 Pesticides Control and Management Act (Law 528) made Ghana's Environmental Protection Agency (EPA) the mandated agency, legally responsible for regulating the use of pesticides. Section one (1) of the act states that "No person shall import, manufacture, distribute, advertise, sell or use any pesticide in Ghana unless the pesticide has been registered by the Environmental Protection Agency in accordance with this act" In that respect, Ghana EPA has sole authority and is responsible for registering all imported, exported, manufactured, distributed and sold or used pesticides in Ghana.

Packaging and Labeling must comply with specified EPA standards and records must be kept and made available for review on request. The act prescribed punishments and penalties for various crimes (EPA Act 490., 1994). The agency also publishes the list of pesticides that are registered every year, their classifications, those that are tentatively cleared, suspended or prohibited pesticides and changes in the classification of pesticides (EPA Act 490, 1994).

2.11 Conclusion

The use of pesticides in agriculture to improve crop and animal productions started some thousands of years ago in Asia. As the farming population increases, the usage of pesticides has become widespread with its accompanying health effect on man and the environment. Literature has revealed that, studies on pesticides were mostly carried out in developed countries such as the Americas and parts of Asia. In recent times however, developing countries like Ghana, where the contribution of agriculture to GDP has been found to be high, interest in pesticide related research has increased. Studies by Ntow et al., 2009 and Quansah et al., 2016 revealed a substantial increase in the use of various varieties of pesticides in Akumadan, a predominantly vegetable farming community in Ghana with its associated poor handling practices.

It has generally been established in the above literature that, children are particularly at greater risk of being exposed and affected by pesticide because of their immature body system, high breathing rate and generally hand to mouth behaviors. Exposure has been assessed by using questionnaires and in some cases the use of body fluids such as blood, urine and breast milk (Bergkvist et al., 2012; Ntow et al., 2009). It was however established that, biomonitoring through urine has proven to be more effective than blood. In Ghana, the Environmental Protection Agency by an act of parliament (EPA Act 490., 1994) is the mandated organization that sees to the regulation and use of pesticides; The Ghana EPA has sole authority and is responsible for registering all imported, exported, manufactured, distributed and sold or used pesticides in Ghana.

There is currently no documented work that associated pesticide exposure and behavioral outcomes in under-five year old children in Ghana or any part of Africa. This makes this study the first of its kind to be conducted in predominantly farming communities that extensively use pesticides in Ghana.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design and Current Study

The study reported here is derived from the Offinso North district Farm Health Study (ONFAHS). The ONFAHS was collaboration among researchers from the school of Public

Health, University of Ghana, Kwame Nkrumah University of Science and Technology, Kumasi, University of Cape Coast, University of Development Studies and the University of Western Canada. It is a population-based cross-sectional study among farmers and their families residing in the Offinso north district of the Ashanti region of Ghana. In Phase 1 of ONFAHS, questionnaire data was collected from 930 individuals including children under five and their parents from 310 households between May and July. In Phase 2, urine samples were collected from a sub-population of parents and their children.

3.2 Study Location

Offinso North District is about 95km north –west of Kumasi, the capital of the Ashanti region. The district lies between longitudes 1^o 60 W and 1^o 45 E and latitudes 7^o 20 N and 6^o 50 S. The district was divided into three (3) geographical zones and each zone comprised of a major farming community and surrounding villages. The major farming communities are Akumadan (the biggest), Nkenkensu and Afrancho. These farming communities are on the KumasiTechiman main road and Afrancho is on the left of Akumadan whiles Nkenkensu is on the right. Offinso North district is a hetero-ethnic inhabited area with a population of over fifty-six thousand (56,000) of which about 70% are directly involved in farming (Ghana Statistical Service, 2010; MOFA). The major vegetables cultivated in the district are pepper, garden eggs, okra and tomatoes (Ntow et al., 1998, Osafo and Frempong, 1998). Other crops cultivated are maize, cassava, plantain and cocoa. There is no statistics on the amount of pesticides Offinso north consumes annually, but evidence has it that vegetable farmers in the Offinso north district utilized pesticides more than any other farming community in Ghana (Ntow et al 2007). The natural vegetation of the district is semi-deciduous forest type, most of which have been lost to extensive farming and logging activities. The soil type is mainly coarse grain sandstone and deep red clay loam. The district also lies in the transitional zone of Ghana with two rainfall seasons; with a mean annual rainfall level ranging between of 700mm and 1200mm. The major season begins from April to July. A short dry season occurs in August and the minor season is from September to October. Extension officers provide technical support to farmers in the district.

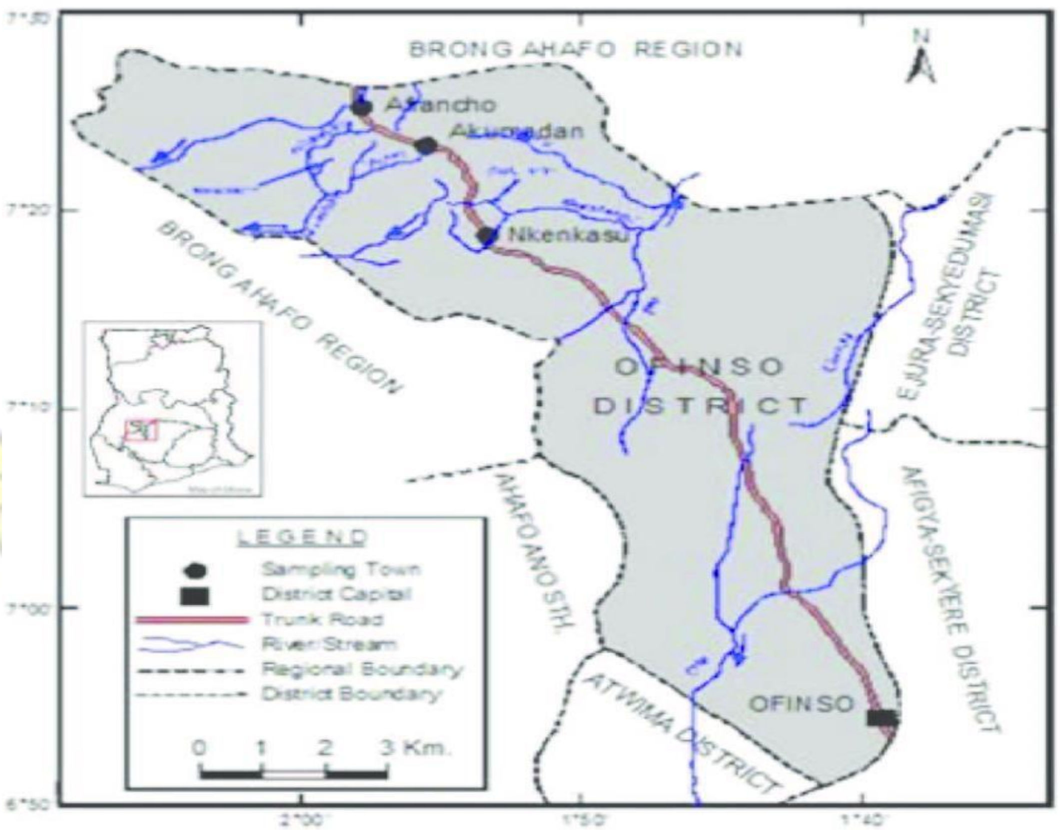

Fig. 3.1: A map of the study location

3.3 Source/ study Population

The source population for the study includes all children living in Afrancho, Nkenkensu and Akumadan, in the Offinso North District of Ashanti Region. The current study was limited to 170 under-five year old children who had complete data on urine and came from a household that met the eligibility criteria (see section 3.3.1 and 3.3.2) of the main study. In the main study, a household is defined as a home that has a man and/or a woman who are parents of a child below five years old.

3.3.1 Inclusion Criteria

The inclusion/ eligibility criteria for any household selected into the study included (i) a man and/ or a woman who was above 18 years, (ii) at least the man or the woman or both is/are farmer(s) (iii) the man or the woman or both is/are permanent residents in the study area, (iv) both the man and/ or the woman had a biological child who is below the age of five years, (v) the man and/or the woman was willing to follow the study protocol and complete the study questionnaire.

3.3.2 Exclusion Criteria

The exclusion criteria included (i) none of the household member is a farmer, (ii) a man and/or a woman in a household was below 18 years (iii) a man and/or a woman was/were non- permanent residents in the study area, (iv) no child in the household is below five years and (v) a child below the age of five had severe illness such as dysentery and typhoid fever.

3.4 Study Variables

3.4.1 Independent variables of Interest

The independent variables of interest were (i) urinary residual levels of five (5) organochlorine pesticides (gamma-HCH, beta-HCH, delta-HCH, heptachlor and aldrin) and one (1) pyrethroid pesticide (lambda cyhalothrin); and (ii) three (3) pesticide exposure indicators in the farm, at home or both. Pesticide exposure indicators in the farm and/or at home were measured as (i) contact with pesticide contaminated surfaces, (ii) putting contaminated thumbs/ fingers in the mouth and (iii) placing pesticide contaminated objects in the mouth (food and non-food items). Pesticide contaminated surface is defined as equipment such as nose mask, goggles and knapsack spraying machine, contact with empty pesticide containers, parents carrying children in contaminated cloth after farm work, etc.

3.4.2 Dependent Variables

The main dependent variables reported in the questionnaire included four neurobehavioral outcomes defined as; Attention, Rule-Breaking, Social behavior and Aggressiveness. The definition for these outcomes is consistent with the 'Manual for Achenbach System of Empirically Based Assessment (ASEBA) Pre-school Forms and Profiles' (Achenbach, T.M., and Rescorla, L.A., 2000) and is attached (Appendix C).

3.4.3 Confounding variables

Confounding factors include; socio-economic conditions; cooking fuel type; smoking habit of parents; drinking habit of parents; educational level of parents; age of child and breastfeeding/ nutrition and is based on literature (Smith et al., 2000).

3.5 Sample size calculation and sampling procedure

3.5.1 Sample size calculation

Yamane's formula (1967) which assumes a 95% confidence and error of 5% was used to determine the number of children sampled.

$$n = \frac{N}{1 + N\,(e)^2}$$

Where: 'n' is the sample size, 'N' is the population size; and 'e' is the error margin of 5%.

The sample size was based on the proportion of children in Offinso north district. Data on the population of under-five year old children was not available; hence the 2010 population and housing census report in the Ashanti region was relied on.

According to the 2010 census report, slightly more than three-quarters (78.8%) of all households in the Offinso North District engage in agriculture, particularly crop farming (99.3%). The estimated vegetable farming population in Akumadan, Afrancho and Nkenkenso is 2300 (Ntow et al., 2009). Estimated percentage of children under-five years is approximately 11.6% (Offinso North District Population Report., 2010).the estimated population of children under-five years born to vegetable farmers is therefore 266 (ie 11.6% of 2300). Using Yamane's equation;

$$n = \frac{266}{1+266\,(0.05)^2} = 159.76 \approx 160$$

Adjusting for non-response rate, the size of the study population was increased to **170** children. Using random number generator, the **170** children were selected from the original list of 310.

3.5.1 Sampling procedure

The Offinso north district was divided into 3 geographical zones. The original ONFAHS used convenient sampling to select 310 households including 310 children less than five years old. A list of all the 310 children was developed alphabetically and using a random number generator, 170 of them were selected for this study.

3.6 Pretest of data collection tools

To ensure validity and clarity, pretesting of sample questionnaire was done in Akumadan, the capital and largest town in Offinso North District. Agricultural extension officers and the leaders of various farmer groups in Akumadan who engage in vegetable farming and have children whose age falls within the required age for the study were used for the pretesting. In all, 20 questionnaires were pretested one month before the actual administration.

3.7 Data collection procedure

As indicated in section 3.1 above, this study involved two (2) stages. Stage one (1) was in three (3) phases: stakeholder consultation, training of extensive officers to help in the collection of data, enrollment of study participants and questionnaire data collection. Stage two (2) involved biological sample (urine) collection. To start with, a meeting was held with opinion leaders in the Offinso north district and these include; with the director of agriculture services, the agriculture extension officers, and the District Chief Executive and farmers representatives. The project was discussed and the research team was introduced.

Prior to enrollment into the study, a written consent form (Appendix A) was read to the parent(s) of the children in their local language and any questions raised by them were answered. The parents who agreed to be part after the consent had been explained and their questions answered were made to sign the consent form before they were enrolled in the study. In all three hundred (310) households were enrolled. Each parent of a participating household was interviewed with a structured questionnaire (Appendix B and D) to ascertain relevant information on certain practices and symptoms which are indicators of pesticide exposure. The 'Child Behavior Checklist' (Appendix C) for pre- school children was used to ascertain neurobehavioral outcomes and symptoms of behavioral problems. Information was also collected on the age and gender of children, age of parents; level of parents' education, smoking habit of parent, drinking habit of parent, type of cooking fuel, ethnic origin, mouthing behavior of child, child hygiene practices, contact with pesticide containers and equipment among others. The questionnaire and checklist are attached.

Parents assisted in the urine collection from the children on the day after the interview. They were provided with clean water and soap for hand washing before handing out to them sterile plastic urine containers for urine collection. They were instructed to collect only midstream urine into the plastic urine containers. 20ml of the urine were then drawn into sterile sample tubes and stored in cool boxes containing ice packs (at 4 – 8 ^{o}C) and transported to the Ghana Atomic Energy Commission (GAEC) Laboratory for storage at -20^{o}C.

3.8 Quality control

To ensure reliability of the data, and encourage participation of farmers, the Agricultural extension officers at Offinso North district were recruited to help in the collection of data. Data abstraction forms and questionnaires were critically examined at the end of each day. Data handled by the extension officers were also cross checked for consistency and completeness. The extension officers also double checked data gathered by the principal investigator all in aim to achieve accuracy.

Participants were made to wash their hands with soap and water before urine sample collection. Urine samples were packed in cool boxes containing ice packs to maintain the integrity of samples sent to laboratory for analysis.

3.9 Data Processing and Analyses

Means, were reported for continuous variables and proportions computed for categorical variables. Attention and behavioral effects of organochlorines and pyrethroids were assessed by considering the mean scores for each of the four (4) behavioral outcomes; attention, rulebreaking, social behavior and aggressiveness as dependent variables, based on ASEBA. Urinary pesticide residual levels (μg/l) and postnatal behaviors that predispose children to the pesticide were the independent variables.

Univariate and multiple linear regression analysis were conducted to compare the relationship between indicators of pesticide exposure, pesticide residue in urine and behavioral outcome score. Normality was tested with a histogram of the residuals whiles equality of variance and linearity were tested with a scatter plot. Since the study was cross – sectional, independence of the random error assumption was met. Adjusting for confounders, all data analyses was undertaken using SPSS® v.16 (IBM, Armonk, USA and Excel v14 (Microsoft, Redmond, USA) spreadsheet.

3.10 Privacy and Security of Records

3.10.1 Protection of subjects' privacy

Parents did not have to answer any survey questions that they felt was an invasion of their privacy. Also, participants did not have to participate in any particular aspects of the study that they found invasive.

3.10.2 Provision to prematurely end participation in the study

A parent of a child could opt to be interviewed in a location of their choice to increase privacy. In cases of adverse event or situation of distress, subject's participation in the study was to be concluded. However there was no such an event or distress that warranted premature conclusion.

3.10.3 Record storage and protection

All research records, data and specimens were protected against inappropriate use or disclosure, or malicious or accidental loss or destruction. Data extracted for this study was locked with access restricted only to me as the principal investigator and also on copying study-related materials on a secured laptop. There was routine electronic back up and encryption of digital data. Security software (firewall, anti-virus, anti-intrusion) were installed and regularly updated on all devices used in the project.

3.10.4 Destruction of data and specimens at the conclusion of study

Specimens of urine and other samples as well as identifiers on their storage containers were destroyed after laboratory analyses. Study survey forms (hard copy) were destroyed at the conclusion of the study.

3.11 Ethical consideration

Ethical clearance was obtained from the ethics committee of Kwame Nkrumah University of Science and Technology and Komfo Anokye Teaching Hospital, Kumasi. Clearance was also sought from the Offinso north district assembly. Discussions were held with chiefs, the directors of crops, assembly members and other opinion leaders in the three strata zones within the study area and the purpose of the study explained to them.

Questionnaires and observational guide had no space for names of respondents and/or children. Also, informed consent was obtained from the participants and only individuals who had consented to be included in the study were used. The benefits, risks and procedures of the study were read to individuals and they were at liberty to ask questions, seek clarifications or withdraw from the study unconditionally.

3.12 Study limitation

As indicated above, the original ONFAHS used convenient sampling to select 310 households from which 170 children under five years old were randomly selected for this study. The use of convenient sampling means that, the findings cannot be generalized to the large population of children under – five in Offinso North District.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-Demographic characteristics

Table 1 below illustrates the socio-demographic characteristics of the study population. Majority of the children (74.12%) were aged between three (3) and four (4) years. They were mostly males (72.35%), were not in school (57.65%), and were from other settler ethnic groups (80.00%) and whose parents had no formal education – mothers (71.34%) and fathers (72.19%). Also, majority of the children, over 80% had parents who never smoked nor drank alcohol but utilizes charcoal and/or wood (92.94%) as the main source of cooking fuel at home.

Table 4.1: Characteristics of study population of children under five (5) years of age, (n = 170)

Characteristics of study population	Frequency	Percentage (%)
Age of child in years		
≤ 1	21	12.35
1 – 2	23	13.53
3 – 4	126	74.12
Gender of child		
Male	123	72.35
Female	47	27.65
Level of education of child		
Crèche	11	6.47
Kindergarten	59	34.70
Lower primary	2	1.18
No education	98	57.65
Ethnic origin of parents		
Akan	25	14.71
Ewe	3	1.76
Hausa	6	3.53
Others*	136	80.00

Highest level of father's education	122	72.19
Have not been to school before	40	23.67
JSS/Middle school/primary school	7	4.14
SSS/Secondary /vocational or technical training		
Mother's smoking habit		
Current smoker	1	0.59
Past smoker	4	2.37
Never smoked	164	97.04
Father's smoking habit		
Current smoker	6	3.59
Past smoker	14	8.38
Never smoked	147	88.02
Mother's drinking habit		
Current drinker	12	7.06
Past drinker	4	2.35
Never drank	154	90.59
Father's drinking habit		
Current drinker	6	3.53
Past drinker	20	11.76
Never drank	144	84.71
Types of cooking fuel used		
Clean fuel (LPG)	5	2.94
Biomass fuel (Charcoal and/or wood)	158	92.94
Combination of fuels	7	4.12

4.2 Determinants of interest

4.2.1 Objective 1: Means of pesticide exposure at home, in the farm or both and their prevalence

At home, 61.18% of the study population had contacts with pesticide contaminated surfaces, 69.41% puts thumbs or fingers in the mouth and 85.29% places contaminated objects in the mouth.

In the farm, 55.29% had contact with pesticide contaminated surfaces, 61.76% puts thumbs or fingers in their mouths and 54.12% places contaminated objects in the mouth.

Majority of children, 52.35% had contacts with pesticide contaminated surfaces both at home and in the farm. Also, 60.00% and 53.53% of the population under study puts thumps/fingers and contaminated objects in their mouth respectively.

Table 4.2.1: Prevalence of exposure indicators at home, in the farm and at home and/or in the farm, by age of child; (n = 170)

Exposure indicators	All ages, n (%)	≤ 2, n (%)	3 – 4, n (%)
At home			
Contact with pesticide contaminated surfaces			
No	66 (38.82)	21 (47.73)	45 (35.71)
Yes	104 (61.18)	23 (52.27)	81 (64.29)
The child places thumbs/fingers in the mouth			
No	52 (30.59)	7 (15.91)	45 (35.71)
Yes	118 (69.41)	37 (84.09)	81 (64.29)
The child places contaminated objects in the mouth			
No	25 (14.71)	6 (13.64)	19 (15.08)
Yes	145 (85.29)	38 (86.36)	107 (84.92)
In the farm			
Contact with pesticide contaminated surfaces			
No	76 (44.71)	14 (31.82)	62 (49.21)
Yes	94 (55.29)	30 (68.18)	64 (50.79)
The child places thumbs/fingers in the mouth			
No	65 (38.24)	9 (20.45)	56 (44.44)
Yes	105 (61.76)	35 (79.55)	70 (55.56)
The child places contaminated objects in the mouth			
No	78 (45.88)	16 (36.36)	62 (49.21)
Yes	92 (54.12)	28 (63.64)	64 (50.79)
At home and/or in the farm			
Contact with pesticide contaminated surfaces			
No	81 (47.65)	21 (47.73)	60 (47.62)
Yes	89 (52.35)	23 (52.27)	66 (52.38)
The child places thumbs/fingers in the mouth			
No	68 (40.00)	10 (22.73)	58 (46.03)
Yes	102 (60.00)	34 (77.27)	68 (53.97)

The child places contaminated objects in the mouth			
No	79 (46.47)	16 (36.36)	63 (50.00)
Yes	91 (53.53)	28 (63.64)	63 (50.00)

4.2.2 Objective 2: Urinary residual levels of Organochlorine and pyrethroid pesticides

Residues of five (5) organochlorine insecticides and one (1) pyrethroid insecticide were identified and extracted from the urine of the 170 children of vegetable farmers in Akumadan, Afrancho and Nkenkenso using hexane solvent. Apart from aldrin whose mean residual concentration was very low (0.02µg/l), concentrations far above the level of detection (LOD) were found for all the other four (4) organochlorine and the pyrethroid pesticides in urine.

Table 4.2.2a below illustrates the mean, standard deviations, minimum, maximum as well as median urine residual levels among all the under-five year old children. The mean concentration of gamma-HCH was found to be 3.09µg/l with standard deviation of 0.77µg/l; beta-HCH = 2.89µg/l (SD = 0.64µg/l); delta-HCH = 2.42µg/l (SD = 0.43µg/l); heptachlor = 3.55µg/l (SD = 0.90µg/l); lambda cyhalothrin = 3.11µg/l (SD = 0.69µg/l).

Table 4.2.2a: Concentration of pesticides in urine of children aged 0 to 4 born to vegetable farmers in Offinso North District of Ashanti Region; (n = 170).

Pesticide	LOD (µg/L Urine)	Min. (µg/L Urine)	LQ (µg/L Urine)	UQ (µg/L Urine)	Mean (µg/L Urine)	Median (µg/L Urine)	SD (µg/L Urine)	Max. (µg/L Urine)	Lower 95% CL for mean	Upper 95% CL for mean
Organochlorines										
Gamma-HCH	0.01	2.00	2.50	3.35	3.09	3.00	0.77	5.60	2.98	3.20
Beta-HCH	0.01	1.60	2.40	3.20	2.89	2.60	0.64	4.80	2.80	2.98
Delta-HCH	0.01	2.00	2.10	2.50	2.42	2.30	0.43	4.50	2.36	2.49

Heptachlor	0.01	2.00	3.10	4.10	3.55	3.30	0.90	5.60	3.42	3.78
Aldrin	0.01	0.001	0.015	0.025	0.02	0.019	0.007	0.04	0.0186	0.0205
Pyrethroid										
Lambda-Cyhalothrin	0.01	2.00	2.50	3.40	3.11	3.20	0.69	5.20	3.01	3.21

LOD = limit of detection; LQ = lower quartile; UQ = upper quartile; SD = standard deviation; CL = confidence level; Min = minimum; Max = maximum.

Table 4.2.2b shows residual levels among children aged between 0 to 2 years. Residual level/ concentration of heptachlor was highest with mean value of 3.52µg/l (SD = 0.99µg/l), followed by gamma-HCH = 3.08µg/l (SD = 0.73µg/l), while the concentration of aldrin was lowest; 0.019µg/l (SD = 0.007µg/l). The rest were lambda cyhalothrin = 3.04µg/l (SD = 0.73µg/l); betaHCH = 2.73µg/l (SD = 0.61µg/l); and delta-HCH = 2.40µg/l (SD = 0.36µg/l).

Table 4.2.2b: Concentration of pesticides in urine of children aged ≤ 2 born to vegetable farmers in Offinso North District of Ashanti Region; (n = 44).

	(µg/L Urine)	(µg/L Urine)	(µg/L Urine)	UQ (µg/L Urine)	Mean (µg/L Urine)	Median (µg/L Urine)	SD (µg/L Urine)	Max. (µg/L Urine)	Low 95% CL for mea
Organochlorines									
Gamma-HCH	0.01	2.00	2.50	3.30	3.08	3.00	0.73	5.60	2.88
Beta-HCH	0.01	2.10	2.50	3.30	2.93	2.70	0.61	4.50	2.76
Delta-HCH	0.01	2.00	2.10	2.50	2.40	2.30	0.36	3.30	2.30
Heptachlor	0.01	2.00	2.80	4.10	3.52	3.30	0.99	5.60	3.24
Aldrin	0.01	0.001	0.014	0.025	0.019	0.017	0.007	0.04	0.01
Pyrethroid									
Lambda-Cyhalothrin	0.01	2.00	2.40	3.50	3.04	3.10	0.73	4.60	2.84

LOD = limit of detection; LQ = lower quartile; UQ = upper quartile; SD = standard deviation; CL = confidence level; Min = minimum; Max = maximum.

Table 4.2.2c shows the urinary pesticide residual concentrations in 3 to 4 years old children. Heptachlor had the highest mean concentration of 3.56µg/l (SD = 0.87µg/l) followed by the pyrethroid, lambda cyhalothrin = 3.14µg/l (SD = 0.68µg/l); gamma-HCH = 3.09µg/l (SD = 0.77µg/l); beta-HCH = 2.87µg/l (SD = 0.66µg/l) and delta-HCH = 2.43µg/l (SD = 0.46µg/l). Again, the concentration of aldrin in the urine was almost insignificant; 0.02µg/l (SD = 0.007µg/l).

Table 4.2.2c: Concentration of pesticides in urine of children aged 3 – 4 born to vegetable farmers in Offinso North District of Ashanti Region; (n = 126).

Pesticide	LOD (µg/L Urine)	Min. (µg/L Urine)	LQ (µg/L Urine)	UQ (µg/L Urine)	Mean (µg/L Urine)	Median (µg/L Urine)	SD (µg/L Urine)	Max. (µg/L Urine)	Lower 95% CL for mean	Upper 95% CL for mean
Organochlorines										
Gamma-HCH	0.01	2.00	2.50	3.40	3.09	3.00	0.77	5.60	2.96	3.23
Beta-HCH	0.01	1.60	2.40	3.20	2.87	2.60	0.66	4.80	2.76	2.98
Delta-HCH	0.01	2.00	2.10	2.60	2.43	2.30	0.46	4.50	2.35	2.51
Heptachlor	0.01	2.00	3.10	4.05	3.56	3.30	0.87	5.60	3.41	3.71
Aldrin	0.01	0.001	0.015	0.025	0.02	0.02	0.007	0.04	0.0187	0.0210
Pyrethroid										
Lambda-Cyhalothrin	0.01	2.00	2.60	3.40	3.14	3.20	0.68	5.20	3.03	3.26

LOD = limit of detection; LQ = lower quartile; UQ = upper quartile; SD = standard deviation; CL = confidence level; Min = minimum; Max = maximum.

4.3 Objective 3: Mean behavioral scores in children

The Child Behavior Checklist (CBCL) for children below the ages of five (5) (Achenbach and Reschorla., 2000), (Appendix C) was used to assess four indicators of behavioral outcomes namely; Attention, Rule-Breaking, Social behavior and Aggressiveness as shown in table 4.3 below. The sum of scores of all items that describe a particular outcome was found for each child and averages calculated. The mean score for aggressiveness (10.70) was highest with attention being the least with mean score of 7.86. The scores for rule-breaking and social behaviors were 8.01 and 8.85 respectively.

Table 4.3: Mean behavioral outcomes of children of vegetable farmers in Offinso North district of the Ashanti region of Ghana; (n = 170).

Behavior	Mean	Median	Lower Quartile	Upper Quartile	Standard deviation	Min	Max
Attention	7.86	8.00	6.00	10.00	3.38	0.00	18.00
Rule-Breaking	8.01	8.50	5.00	11.00	4.62	0.00	17.00
Social Behavior	8.85	10.00	6.00	12.00	5.01	0.00	23.00
Aggressiveness	10.70	11.50	8.00	15.00	5.40	0.00	24.00

4.4 Objective 4: Association between indicators of pesticide exposure at home, on the farm or both and mean behavioral outcomes.

4.4.1. Pesticide Exposure indicators and Attention

At home, contact with pesticide contaminated surfaces had significant inverse association with attention among all age groups (ie 0 to 4 years) [β = -0.17 (95% CI = -0.32 to -0.02)]. In the stratified population however, there was no significant association found among 0 – 2 years old but there was significant association found among 3 – 4 years old [β = -0.29 (95% CI = -0.11 to -0.48)]. The child places thumbs/ fingers in the mouth were not associated with attention. Also, child placing contaminated objects in the mouth and attention were not associated in the general study population but was significantly associated among children who were 0 – 2 years [0.36 (0.01 to 0.71)].

In the farm, contact with pesticide contaminated surfaces had significant inverse association with attention among 3 – 4 years old children [-0.33 (-0.52 to -0.14)] and also in the general population [-0.19 (-0.34 to -0.04)]. Placing thumbs/ fingers in the mouth was also inversely associated with attention [-0.20 (-0.35 to -0.14)] among the general population and among the 3 – 4 years old [-0.32 (-0.50 to -0.05)]. The child placing contaminated objects in the mouth in the farm was inversely associated with attention as follows; all ages [-0.18 (-0.33 to -0.03)]; 3 – 4 years old [-0.26 (-0.43 to -0.08)]. The association was however insignificant in 0 – 2 years old children [-0.04 (-0.34 to 0.25)].

At home and in the farm, contact with pesticide contaminated surfaces was significantly but inversely associated with attention among the general population of under five years old children [-0.17 (-0.32 to -0.02)] and among 3 – 4 years old [-0.29 (-0.48 to -0.11)]. The child placing thumbs/ fingers in the mouth was also significantly associated with attention as follows; all ages [-0.18 (-0.33 to -0.03)] and 3 – 4 years old [-0.30 (-0.49 to -0.11)]. Child placing contaminated objects in the mouth was inversely associated with attention [-0.20 (-0.34 to 0.05)] among all ages and among 3 – 4 years old children [-0.28 (-0.46 to -0.10)]. There was no significant association between the exposure indicators at home and in the farm and attention in ≤ 2 years old children.

Table 4.4.1: Association between pesticide exposure indicators; at home, in the farm or both and attention of under 5 year old children of vegetable farmers in Offinso North district of Ashanti region of Ghana; (n = 170).

ATTENTION

Exposure indicators	**All ages**		**0 – 2 years**		**3 – 4 years**	
	Crude β(95% CI) *p* - value	**Adjusted* β(95% CI) *p* - value**	**Crude β(95% CI) *p* - value**	**Adjusted* β(95% CI) *p* - value**	**Crude β(95% CI) *p* - value**	**Adjusted* β(95% CI) *p* – value**
At home Contact with pesticide contaminated surfaces	The child places contaminated objects in the mouth - 0.20 (-0.32 to -0.09)	-0.21 (-0.32 to -0.09) 0.0004	-0.19 (-0.34 to -0.04) 0.012	0.02 (-0.31 to 0.35) 0.914	0.22 (-0.12 to 0.56) 0.210	0.551 -0.04 (-0.34 to 0.25)
The child places thumbs/fingers in the mouth	0.0006	-0.21 (-0.33 to -0.10) 0.0002	-0.20 (-0.35 to -0.14) 0.009	0.08 (-0.17 to 0.30) 0.578	-0.25 (-0.58 to 0.08) 0.138	0.777 -0.28 (-0.41 to -0.14)
The child places contaminated objects in the mouth	-0.15 (-0.27 to -0.03) 0.013	-0.17 (-0.32 to -0.02) 0.023	-0.18 (-0.33 to -0.03) 0.016	0.0005 (-0.27 to 0.27) 0.997	0.36 (0.01 to 0.71) 0.043	< 0.0001 -0.16 (-0.30 to -0.02)
In the farm Contact with pesticide contaminated surfaces	-0.11 (-0.27 to 0.04) 0.160	-0.10 (-0.24 to 0.04) 0.017	-0.02 (-0.24 to 0.20) 0.872	-0.08 (-0.30 to 0.14) 0.483	-0.02 (-0.31 to 0.27) 0.902	0.022 -0.15 (-0.33 to 0.02)
The child places thumbs/fingers in the mouth	-0.19 (-0.30 to -0.08) 0.001	-0.09 (-0.26 to 0.07) 0.265	-0.21 (-0.49 to 0.08) 0.152		-0.14 (-0.58 to 0.31)	0.089 -0.29 (-0.43 to -0.16)

< 0.0001	< 0.0001	-0.29 (-0.11 to -0.48) 0.002	0.017	-0.33 (-0.52 to -0.14) 0.0008	-0.32 (-0.50 to -0.05) 0.0006	0.005
-0.29 (-0.42 to -0.16)	-0.28 (-0.41 to -0.14) < 0.0001	-0.12 (-0.30 to 0.05)	-0.09 (-0.25 to 0.07) 0.288		-0.26 (-0.43 to -0.08)	
At home and in the farm						
Contact with pesticide contaminated surfaces	-0.20 (-0.32 to -0.09) 0.0006	-0.17 (-0.32 to -0.02) 0.023	-0.02 (-0.24 to 0.20) 0.872	0.22 (-0.12 to 0.56) 0.210	-0.27 (-0.41 to -0.14) < 0.0001	-0.29 (-0.48 to -0.11) 0.0020
The child places thumbs/fingers in the mouth	-0.20 (-0.31 to -0.08) 0.0007	-0.18 (-0.33 to -0.03) 0.017	-0.02 (-0.28 to 0.25) 0.910	-0.14 (-0.47 to 0.20) 0.433	-0.28 (-0.41 to -0.14) < 0.0001	-0.30 (-0.49 to -0.11) 0.002
The child places contaminated objects in the mouth	-0.22 (-0.34 to -0.11) 0.0001	-0.20 (-0.34 to -0.05) 0.009	-0.08 (-0.30 to 0.14) 0.483	-0.04 (-0.34 to 0.25) 0.777	-0.29 (-0.42 to -0.15) < 0.0001	-0.28 (-0.46 to -0.10) 0.002

β = Regression coefficient

*β = Adjusted regression coefficient

Adjusted for cooking fuel type, smoking habit of parents, drinking habit of parents, educational level of parents, age of child and whether child still breastfeeds.

4.4.2. Pesticide Exposure indicators and Rule-breaking

At home, there was significant inverse association between contact with pesticide contaminated surfaces and rule-breaking among the general study population of children below five years of age [β = -0.27 (95% CI = -0.41 to -0.13)], but more significant among 3 – 4 years old children [-0.43 (-0.60 to -0.25)]. Also, the child places thumbs/ fingers in the mouth was inversely associated with rule-breaking [-0.15 (-0.28 to -0.02)]. However, the child placing contaminated objects in the mouth was not associated with rule-breaking.

In the farm, contact with pesticide contaminated surfaces had significant inverse association with rule-breaking among 3 – 4 years old children [-0.40 (-0.57 to -0.22)] and also in the general population [-0.18 (-0.32 to -0.04)]. Placing thumbs/ fingers in the mouth was also inversely associated with rule-breaking [-0.19 (-0.33 to -0.05)] among the general population. The child placing contaminated objects in the mouth in the farm was inversely associated with rule-breaking as follows; all ages [-0.24 (-0.38 to -0.10)]; 3 – 4 years old [-0.34 (-0.51 to -0.18)].

At home and in the farm, contact with pesticide contaminated surfaces was inversely and significantly associated with rule-breaking among the general population of under five years old children [-0.23 (-0.37 to -0.09)] and among 3 – 4 years old [-0.36 (-0.53 to -0.19)]. The child placing thumbs/ fingers in the mouth was also significantly associated with rule-breaking as follows; all ages [-0.20 (-0.33 to -0.06)] and 3 – 4 years old [-0.41 (-0.59 to -0.24)]. Child placing contaminated objects in the mouth and rule-breaking were inversely and significantly associated among the general population [-0.26 (-0.40 to -0.12)] and in the 3 – 4 years old children [-0.37 (-0.54 to -0.21)]. There was generally no significant association between any of the exposure indicators and rule-breaking among ≤ 2 years old children.

Table 4.4.2: Association between pesticide exposure indicators; at home, in the farm or both and rule-breaking of under 5 year old children of vegetable farmers in Offinso North district of Ashanti region of Ghana; (n = 170).

RULE-BREAKING

Exposure indicators	All ages **Crude** **β(95% CI)** ***p* - value**	**Adjusted*** **β(95% CI)** ***p* - value**	0 – 2 years **Crude** **β(95% CI)** ***p* - value**	**Adjusted*** **β(95% CI)** ***p* - value**	3 – 4 years **Crude** **β(95% CI)** ***p* - value**	**Adjusted*** **β(95% CI)** ***p* – value**
At home		The child places thumbs/fingers in the mouth	-0.30 (-0.41 to -0.19)			
Contact with pesticide contaminated surfaces	-0.37 (-0.48 to -0.26)		0.010	0.373	0.278	0.983
			< 0.0001			
	< 0.0001					
		The child places contaminated	-0.35 (-0.46 to -0.24) -0.19 (-0.33 to -0.05)	0.13 (-0.10 to 0.36)	-0.18 (-0.47 to 0.11)	-0.45 (-0.58 to -0.33)
The child places thumbs/fingers in the mouth	-0.29 (-0.40 to -0.18)	objects in the mouth	0.007	0.283	0.223	< 0.0001
		< 0.0001 -0.27 (-0.41 to -0.13)				
	< 0.0001		-0.24 (-0.38 to -0.10)		0.20 (-0.19 to 0.58)	
				0.01 (-0.25 to 0.27)		-0.30 (-0.42 to -0.17)
The child places contaminated	-0.09 (-0.24 to 0.06)	0.0002	0.001		0.314	
				0.938		< 0.0001
objects in the mouth	0.225					
		-0.15 (-0.28 to -0.02)	-0.15 (0.36 to 0.06)	-0.23 (-0.44 to -0.01)	0.26 (-0.02 to 0.55)	-0.16 (-0.32 to 0.01)
In the farm						
Contact with pesticide contaminated surfaces	-0.28 (-0.39 to -0.17)	0.023	0.166	0.038	0.070	0.059
	< 0.0001	-0.08 (-0.23 to 0.08)	-0.30 (-0.56 to -0.04)		0.39 (0.02 to 0.77)	-0.41 (-0.54 to -0.29)
		0.338	0.026		0.037	< 0.0001
		-0.18 (-0.32 to -0.04)	0.15 (-0.18 to 0.47)	0.17 (-0.14 to 0.47)	-0.003 (-0.28 to 0.27)	

-0.40 (-0.52 to -0.27)		< 0.0001	-0.18 (-0.35 to -0.02)	0.066	-0.38 (-0.55 to -0.21)	< 0.0001
		-0.43 (-0.60 to -0.25)				
< 0.0001			0.028	-0.40 (-0.57 to -0.22)	< 0.0001	
		< 0.0001				
-0.40 (-0.52 to -0.27)			-0.17 (-0.35 to 0.01)		-0.34 (-0.51 to -0.18)	
				< 0.0001		
At home and in the farm						
Contact with pesticide contaminated surfaces	-0.32 (-0.43 to -0.22)	-0.23 (-0.37 to -0.09)	-0.15 (-0.36 to 0.06)	0.17 (-0.14 to 0.47)	-0.39 (-0.52 to -0.26)	-0.36 (-0.53 to -0.19)
	< 0.0001	0.001	0.166	0.278	< 0.0001	< 0.0001
The child places thumbs/fingers in the mouth	-0.31 (-0.41 to -0.20)	-0.20 (-0.33 to -0.06)	-0.007 (-0.26 to 0.24)	0.22 (-0.10 to 0.54)	-0.40 (-0.53 to -0.28)	-0.41 (-0.59 to -0.24)
	< 0.0001	0.005	0.954	0.175	< 0.0001	< 0.0001
The child places contaminated objects in the mouth	-0.36 (-0.46 to -0.25)	-0.26 (-0.40 to -0.12)	-0.23 (-0.44 to -0.01)	-0.003 (-0.28 to 0.27)	-0.41 (-0.53 to -0.28)	-0.37 (-0.54 to -0.21)
	< 0.0001	0.0002	0.038	0.098	< 0.0001	< 0.0001

β = Regression coefficient

*β = Adjusted regression coefficient

Adjusted for cooking fuel type, smoking habit of parents, drinking habit of parents, educational level of parents, age of child and whether child still breastfeeds.

4.4.3. Pesticide Exposure indicators and Social behavior

At home, contact with pesticide contaminated surfaces had significant inverse association with social behavior among the general study population of children below five years of age [β = 0.31 (95% CI = -0.41 to -0.21)], the association was however more significant among 3 – 4 years old children [-0.42 (-0.58 to -0.25)]. Child places thumbs/ fingers in the mouth was also inversely associated with social behavior [-0.13 (-0.26 to -0.01)]. However, the child placing contaminated objects in the mouth was not associated with social behavior neither among the general study population nor among 3 – 4 years old children but had significant positive association among 0 – 2 years old [0.52 (0.13 to 0.90)].

In the farm, contact with pesticide contaminated surfaces had significant inverse association with social behavior among 3 – 4 years old children [-0.39 (-0.56 to -0.22)] and also in the general population [-0.22 (-0.35 to -0.09)]. Placing thumbs/ fingers in the mouth was also inversely associated with social behavior [-0.21 (0.34 to -0.08)] among the general population. The child placing contaminated objects in the mouth in the farm was inversely associated with social behavior as follows; all ages [-0.24 (-0.37 to -0.10)] and 3 – 4 years old [-0.40 (-0.24 to -0.56)].

At home and in the farm, contact with pesticide contaminated surfaces had significant inverse association with social behavior [-0.23 (-0.36 to -0.09)] among the general population of under five years old children and among 3 – 5 years old [-0.35 (-0.51 to -0.19)]. The child placing thumbs/ fingers in the mouth was also significantly associated with social behavior [-0.19 (-0.20 to -0.33)] for all ages, among 0 – 2 years old children [-0.18 (-0.32 to -0.10)] and among 3 – 4 years old [-0.34 (-0.63 to -0.24)]. Child placing contaminated objects in the mouth and social behavior were inversely and significantly associated among the general population [-0.25 (-0.38 to -0.12)] and in the 3 – 4 years old children [-0.42 (-0.57 to -0.26)].

4.4.3: Association between pesticide exposure indicators; at home, in the farm or both and social behavior of under 5 year old children of vegetable farmers in Offinso North district of Ashanti region of Ghana; (n = 170).

SOCIAL BEHAVIOR

Exposure indicators	**All ages** Crude β(95% CI) *p* - value	Adjusted* β(95% CI) *p* - value	**0 – 2 years** Crude β(95% CI) *p* - value	Adjusted* β(95% CI) *p* - value	**3 – 4 years** Crude β(95% CI) *p* - value	Adjusted* β(95% CI) *p* - value
At home			-0.22 (-0.35 to -0.09)			
Contact with pesticide contaminated surfaces	-0.31 (-0.41 to -0.21)			0.917	0.24 (-0.05 to 0.53)	0.19 (-0.08 to 0.45)
	< 0.0001		0.0008	0.37 (0.03 to 0.70)	0.108	0.169
		The child places contaminated	-0.29 (-0.39 to -0.18)	0.032		
The child places thumbs/fingers in the mouth	-0.22 (-0.32 to -0.11)	objects in the mouth	-0.21 (0.34 to -0.08)		0.06 (-0.24 to 0.36)	-0.40 (-0.52 to -0.28)
	< 0.0001	< 0.0001 -0.27 (-0.40 to -0.13)	0.002	0.08 (-0.14 to 0.30)	0.677	< 0.0001
The child places contaminated objects in the mouth	0.03 (-0.12 to 0.17)			0.477	0.52 (0.13 to 0.90)	-0.27 (-0.39 to -0.15)
	0.698	< 0.0001	-0.24 (-0.37 to -0.10)	0.13 (-0.13 to 0.37)	0.009	< 0.0001
In the farm		-0.13 (-0.26 to -0.01)	0.0004			
Contact with pesticide contaminated surfaces	-0.24 (-0.14 to -0.34)	0.037		0.328	0.05 (-0.20 to 0.31)	-0.06 (-0.22 to 0.10)
	< 0.0001	0.03 (-0.13 to 0.18)	-0.07 (-0.26 to 0.13)	-0.04 (-0.25 to 0.16)	0.677	0.447
The child places thumbs/fingers in the mouth	-0.26 (-0.36 to -0.16)	0.719	0.524		0.34 (-0.01 to 0.69)	-0.35 (-0.47 to -0.23)
	< 0.0001		-0.01 (-0.29 to 0.26)	0.672	0.056	< 0.0001

-0.36 (-0.48 to -0.24)		< 0.0001	-0.22 (-0.38 to -0.06)	0.431	< 0.0001	< 0.0001
< 0.0001		-0.42 (-0.58 to -0.25)	0.008	-0.39 (-0.56 to -0.22)	-0.41 (-0.57 to -0.24)	-0.40 (-0.24 to -0.56)
-0.37 (-0.49 to -0.25)		< 0.0001	-0.07 (-0.25 to 0.11)			< 0.0001
At home and in the farm						
Contact with pesticide contaminated surfaces	-0.26 (-0.36 to -0.16)	-0.23 (-0.36 to -0.09)	-0.07 (-0.26 to 0.13)	0.24 (-0.05 to 0.53)	-0.33 (-0.45 to -0.21)	-0.35 (-0.51 to -0.19)
	< 0.0001	0.0008	0.524	0.108	< 0.0001	< 0.0001
The child places thumbs/fingers in the mouth	-0.24 (-0.32 to -0.13)	-0.19 (-0.20 to -0.33)	-0.20 (-031 to -0.11)	-0.18 (-0.32 to -0.10)	-0.43 (-0.67 to -0.22)	-0.34 (-0.63 to -0.24)
		0.004		0.323		< 0.0001
The child places contaminated objects in the mouth	-0.29 (-0.40 to -0.19)	-0.25 (-0.38 to -0.12)	-0.04 (-0.25 to 0.16)	0.19 (-0.08 to 0.45)	-0.38 (-0.50 to -0.26)	-0.42 (-0.57 to -0.26)
	< 0.0001	0.0002	0.672	0.169	< 0.0001	< 0.0001

β = Regression coefficient

*β = Adjusted regression coefficient

Adjusted for cooking fuel type, smoking habit of parents, drinking habit of parents, educational level of parents, age of child and whether child still breastfeeds.

4.4.4. Pesticide Exposure indicators and Aggressiveness

At home, contact with pesticide contaminated surfaces had significant inverse association with aggressiveness among the general study population of children below five years of age [β = -0.20 (95% CI = -0.32 to -0.09)], but more significant among 3 – 4 years old children [-0.37 (0.52 to -0.22)]. Child places thumbs/ fingers in the mouth was generally also inversely associated with aggressiveness [-0.15 (-0.26 to -0.03)] but more pronounced among 3 – 4 years old children [-0.24 (-0.39 to -0.10)]. Child placing contaminated objects in the mouth was positively associated with aggressiveness among 0 – 2 years old children [0.36 (0.01 to 0.71)] but its overall impact on the general study population was insignificant.

In the farm, contact with pesticide contaminated surfaces had significant inverse association with aggressiveness among 3 – 4 years old children [-0.31 (-0.16 to -0.47)] and also in the general population [-0.11 (-0.15 to -0.27)]. Placing thumbs/ fingers in the mouth was also inversely associated with aggressiveness [-0.16 (-0.28 to -0.03)] among the general population. The child placing contaminated objects in the mouth in the farm was inversely associated with aggressiveness as follows; all ages [-0.18 (-0.30 to -0.05)] and 3 – 4 years old [-0.31 (-0.45 to -0.16)].

At home and in the farm, contact with pesticide contaminated surfaces had significant inverse association with aggressiveness [-0.17 (-0.28 to -0.04)] among the general population of under five years old children and among 3 – 4 years old [-0.31 (-0.46 to -0.16)] but positively associated among 0 – 2 years old [0.36 (0.09 to 0.65)]. Child places thumbs/ fingers in the mouth was also inversely associated with aggressiveness [-0.17 (-0.29 to -0.05)] for all ages and among 3 – 4 years old [-0.39 (-0.53 to -0.23)]. Child placing contaminated objects in the mouth and aggressiveness were inversely and significantly associated among the general population [0.19 (-0.31 to -0.06)] and in the 3 – 4 years old children [-0.32 (-0.47 to -0.18)].

4.4.4: Association between pesticide exposure indicators; at home, in the farm or both and aggressiveness of under 5 year old children of vegetable farmers in Offinso North district of Ashanti region of Ghana; (n = 170).

AGGRESSIVENESS

Exposure indicators	**All ages** Crude β(95% CI) *p* - value	Adjusted* β(95% CI) *p* - value	**0 – 2 years** Crude β(95% CI) *p* - value	Adjusted* β(95% CI) *p* - value	**3 – 4 years** Crude β(95% CI) *p* - value	Adjusted* β(95% CI) *p* – value
At home Contact with pesticide contaminated surfaces	-0.27 (-0.36 to -0.17) < 0.0001	< 0.0001 -0.24 (-0.33 to -0.15)	0.017 -0.16 (-0.28 to -0.03)	0.063 0.09 (-0.11 to 0.28)	0.37 (0.09 to 0.65) 0.010	0.18 (-0.07 to 0.43) 0.158
The child places thumbs/fingers in the mouth	-0.22 (-0.32 to -0.12) < 0.0001	< 0.0001 -0.20 (-0.32 to -0.09)	0.012 -0.18 (-0.30 to -0.05)	0.403 0.14 (-0.10 to 0.38)	0.04 (-0.23 to 0.32) 0.770	-0.34 (-0.45 to -0.23) < 0.0001
The child places contaminated objects in the mouth	0.03 (-0.10 to 0.16) 0.620	0.001 -0.15 (-0.26 to -0.03)	0.005 -0.05 (-0.23 to 0.14)	0.244 -0.10 (-0.29 to 0.09)	0.36 (0.01 to 0.71) 0.043	-0.27 (-0.37 to -0.16) < 0.0001
In the farm Contact with pesticide contaminated surfaces	-0.20 (-0.29 to -0.03) < 0.0001	0.013 0.01 (-0.13 to 0.16)	0.611 -0.01 (-0.26 to 0.24)	0.299	0.17 (-0.08 to 0.41) 0.178	-0.03 (-0.18 to 0.11) 0.645
The child places thumbs/fingers in the mouth	-0.21 (-0.31 to -0.12)	0.843 -0.11 (-0.15 to -0.27)	0.917 0.28 (-0.02 to 0.58)		0.40 (0.06 to 0.73) 0.020	-0.28 (-0.39 to -0.18) < 0.0001
The child places contaminated objects in the mouth						

-0.30 (-0.40 to -0.19)	-0.29 (-0.39 to -0.18)	< 0.0001	0.0009	-0.31 (-0.16 to -0.47)	-0.34 (-0.19 to -0.48)	< 0.0001
< 0.0001	< 0.0001		-0.09 (-0.25 to 0.07)	< 0.0001	< 0.0001	
	-0.37 (-0.52 to -0.22)	-0.24 (-0.39 to -0.10)	0.288		-0.31 (-0.45 to -0.16)	
At home and in the farm						
Contact with pesticide contaminated surfaces	-0.22 (-0.31 to -0.13)	-0.17 (-0.28 to -0.04)	-0.05 (-0.23 to 0.14)	0.36 (0.09 to 0.65)	-0.28 (-0.39 to -0.17)	-0.31 (-0.46 to -0.16)
	< 0.0001	0.0093	0.611	0.0098	< 0.0001	< 0.0001
The child places thumbs/fingers in the mouth	-0.22 (-0.31 to -0.13)	-0.17 (-0.29 to -0.05)	0.14 (-0.09 to 0.36)	0.28 (-0.01 to 0.57)	-0.31 (-0.42 to -0.21)	-0.39 (-0.53 to -0.23)
	< 0.0001	0.006	0.241	0.055	< 0.0001	< 0.0001
The child places contaminated objects in the mouth	-0.24 (-0.34 to -0.15)	-0.19 (-0.31 to -0.06)	-0.10 (-0.29 to 0.09)	0.18 (-0.07 to 0.43)	-0.29 (-0.40 to -0.18)	-0.32 (-0.47 to -0.18)
	< 0.0001	0.003	0.299	0.158	< 0.0001	< 0.0001

β = Regression coefficient

*β = Adjusted regression coefficient

Adjusted for cooking fuel type, smoking habit of parents, drinking habit of parents, educational level of parents, age of child and whether child still breastfeeds.

4.5 Objective 4: Association between urinary pesticide concentration and mean behavioral outcomes.

Adjusting for potential confounders, values from the regression coefficients show insignificant associations between residue concentrations of gamma-HCH, beta-HCH, delta-HCH, heptachlor, aldrin and lambda cyhalothrin and attention. However, gamma-HCH, beta-HCH and delta-HCH shows positive insignificant associations; [0.13 (-0.81 to 1.07)CI], [0.46 (-0.70 to 1.63)CI] and [0.26 (-1.48 to 2.00)CI] respectively whiles heptachlor, Aldrin and lambda cylalothrin had inverse insignificant associations; [-0.32 (-1.20 to 0.57)], [-33.78 (-124.77 to 57.21)] and [-0.52 (-1.52 to 0.48)] respectively for all ages. Among children ≤ 2 years old, delta-HCH [0.27 (-6.73 to 7.27)] was the only pesticide that had positive insignificant association whiles gamma-HCH, beta-HCH, heptachlor, Aldrin and lambda cyhalothrin were inverse. Adlrin [-46.77 (-234.66 to 141.12)] however had the greatest insignificant association. The insignificant associations between attention and the six pesticide residues among 4 – 5 year old children are as follows; gamma-HCH [0.35 (-0.82 to 1.52)], beta-HCH [0.27 (-1.04 to

1. 58)], delta-HCH [0.92 (-0.75 to 2.59)], heptachlor [0.56 (-0.62 to 1.732)], aldrin [-20.93 (141.80 to 99.93)] and lambda cyhalothrin [-0.88 (-4.70 to 0.23)].

The association between gamma-HCH, beta-HCH, delta-HCH, heptachlor, aldrin as well as lambda cyhlothrin and rule-breaking behavior were insignificant across all the age groups of the study population. The insignificant associations among all ages (0 – 4) are as follows; gammaHCH [-0.12 (-1.67 to 1.43)], beta-HCH [1.02 (-0.80 to 2.82)], delta-HCH [1.10 (-1.62 to 3.82)], heptachlor [-0.07 (-1.33 to 1.20)], Aldrin [-85.40 (-220.25 to 49.46)] and lambda cyhalothrin [-

1.06 (-2.51 to 0.39)]. In children ≤ 2 years old, the insignificant associations are as follows; gamma-HCH [0.01 (-3.43 to 3.45)], beta-HCH [0.75 (-3.69 to 5.18)], delta-HCH [-1.87 (-12.95 to 9.20)], heptachlor [-0.81 (-3.29 to 1.66)], Aldrin [-83.38 (-359.10 to 192.34)] and lambda cyhalothrin [-1.51 (-5.09 to 2.07)] whiles the associations in 3 – 4 years old are as follows; gamma-HCH [-0.35 (-2.36 to 1.65)], beta-HCH [0.62 (-1.48 to 2.73)], delta-HCH [1.65 (-1.19 to 4.49)], heptachlor [0.60 (-0.98 to 2.18)], Aldrin [-58.75 (-235.17 to 117.66)] and lambda cyhalothrin [-1.20 (-2.91 to 0.52)].

All the six pesticides found in the urine of the children were insignificantly associated with social behavior. The insignificant associations are as follows; among all ages (0 – 4), gammaHCH [0.11 (-1.63 to 1.85)], beta- HCH [1.11 (-1.15 to 3.36)], delta-HCH [-0.04 (-3.07 to 2.98)], heptachlor [-0.24 (-1.65 to 1.16)], aldrin [-112.05 (-262.17 to 38.07)] and lambda cyhalothrin [-0.73 (-2.35 to 0.90)]. Among ≤ 2 years old, gamma-HCH [-0.71 (-4.79 to 3.37)], beta- HCH [0.01 (-5.32 to 5.30)], delta-HCH [-4.28 (-17.22 to 8.66)], heptachlor [-1.11 (-4.11 to 1.90)], addrin [-106.39 (-434.65 to 221.87)] and lambda cyhalothrin [-1.27 (-5.59 to 3.04)] and in the 3 – 4 year old children, gamma-HCH [0.60 (-1.52 – 2.72)], beta- HCH [1.34 (-1.05 to 3.74)], delta-HCH [0.97 (-2.04 to 3.98], heptachlor [0.35 (-1.34 to 2.04)], aldrin [-100.55 (-287.65 to 86.55)] and lambda cyhalothrin [-0.91 (-2.74 to 0.91)].

With aggressiveness, the urinary pesticide residue concentrations of all five organochlorine and the pyrethroid pesticides are insignificantly associated. In children less or two years of age, the associations are as follows; gamma-HCH [-0.49 (-2.43 to 1.45)], beta-HCH [0.57 (-1.74 to 2.88)], delta-HCH [2.15 (-1.25 to 5.56)], heptachlor [-0.50 (-2.08 to 1.08)], Aldrin [-38.84 (209.50 to 131.81)] and lambda cyhalothrin [-1.24 (-3.06 to 0.57)]. The insignificant association between aggressiveness and the pesticide concentrations in ≤ 2 years old children are as follows; gamma-HCH [0.16 (-3.98 to 4.30)], beta-HCH [0.67 (-4.71 to 6.05)], delta-HCH [3.08 (-16.47 to 10.32)], heptachlor [-1.46 (-4.48 to 1.55)], Aldrin [21.15 (-316.40 to 358.70] and lambda cyhalothrin [-1.97 (-6.29 to 2.34)] whiles in 3 – 4 years old children the associations are as follows; gamma-HCH [-0.09 (-2.69 to 2.51], beta-HCH [0.46 (-2.36 to 3.29)], delta-HCH [2.75 (-0.92 to 6.41)], heptachlor [0.37 (-1.70 to 2.43)], Aldrin [-72.85 (-302.17 to 156.47)] and lambda cyhalothrin [-1.26 (-3.49 to 0.98)].

Table 4.5: Crude and adjusted regression coefficient of the association between urinary residue concentration (µ/l) of organochlorine and pyrethroid insecticides and behavioral outcomes of children of vegetable farmers in Offinso north district of Ashanti region; (n = 170)

ATTENTION

Pesticides	**All ages**		**0 – 2 years**		**3 – 4 years**	
	Crude β(95% CI)	**Adjusted* β(95% CI)**	**Crude β(95% CI)**	**Adjusted* β(95% CI)**	**Crude β(95% CI)**	**Adjusted* β(95% CI)**
Organochlorines γ – HCH	-0.05 (-0.90 to 0.80)	0.13 (-0.81 to 1.07)	-0.44 (-2.18 to 1.31)	-0.71 (-3.61 to 2.18)	0.10 (-0.92 to 1.11)	0.35 (-0.82 to 1.52)
p – values	0.909	0.778	0.608	0.599	0.851	0.548
β – HCH	0.17 (-0.75 to 1.09)	0.46 (-0.70 to 1.63)	0.21 (-2.03 to 2.44)	-0.77 (-4.05 to 2.50)	0.06 (-0.95 to 1.06)	0.27 (-1.04 to 1.58)
p – values	0.715	0.427	0.848	0.619	0.913	0.677
δ – HCH	0.52 (-1.12 to 2.15)	0.26 (-1.48 to 2.00)	1.16 (-5.06 to 7.37)	0.27 (-6.73 to 7.27)	0.68 (-0.92 to 2.27)	0.92 (-0.75 to 2.59)
p – values	0.531	0.767	0.701	0.934	0.398	0.272
Heptachlor	-0.08 (-0.81 to 0.66)	-0.32 (-1.20 to 0.57)	-0.25 (-1.78 to 1.28)	-0.94 (-2.50 to 0.62)	0.01 (-0.85 to 0.87)	0.56 (-0.62 to 1.732)
p – values	0.831	0.474	0.738	0.212	0.986	0.343
Aldrin	-69.39 (-156.3 to 17.57)	-33.78 (-124.8 to 57.2)	-92.44 (-262.74 to 77.86)	-46.77 (-234.7 to 141.12)	-59.62 (-164.09 to 44.84)	-20.93 (-141.80 to 99.93)
p – values	0.116	0.460	0.272	0.600	0.258	0.727
Pyrethroid						
Lambda Cyhalothrin	-0.14 (-1.04 to 0.76)	-0.52 (-1.52 to 0.48)	0.36 (-1.48 to 2.19)	-0.34 (-2.87 to 2.19)	-0.50 (-1.55 to 0.54)	-0.88 (-4.70 to 0.23)
p – values	0.750	0.300	0.690	0.776	0.336	0.119

Table 4.4 continued

*β = Adjusted for cooking fuel type, smoking habit of parents, drinking habit of parents, educational level of parents, age of child and whether child still breastfeeds.

RULE-BREAKING

Pesticides	**All ages**		**0 – 2 years**		**3 – 4 years**	
	Crude β(95% CI)	**Adjusted* β(95% CI)**	**Crude β(95% CI)**	**Adjusted* β(95% CI)**	**Crude β(95% CI)**	**Adjusted* β(95% CI)**
Organochlorines						
γ – HCH *p* – values	-0.50 (-1.61 to 0.62) 0.380	-0.12 (-1.67 to 1.43) 0.878	-0.71 (-2.82 to 1.39) 0.491	0.01 (-3.43 to 3.45) 0.993	-0.41 (-1.81 to 0.98) 0.555	-0.35 (-2.36 to 1.65) 0.723
β – HCH *p* – values	0.35 (-0.90 to 1.60) 0.579	1.02 (-0.80 to 2.82) 0.968	0.13 (-2.55 to 2.80) 0.923	0.75 (-3.69 to 5.18) 0.727	0.41 (-1.05 to 1.87) 0.578	0.62 (-1.48 to 2.73) 0.554
δ – HCH *p* – values	0.31 (-1.93 to 2.56) 0.781	1.10 (-1.62 to 3.82) 0.422	-3.90(-12.07 to 4.26) 0.332	-1.87 (-12.95 to 9.20) 0.721	0.89 (-1.39 to 3.18) 0.435	1.65 (-1.19 to 4.49) 0.247
Heptachlor *p* – values	-0.35 (-1.29 to 0.59) 0.465	-0.07 (-1.33 to 1.20) 0.915	-0.66 (-2.65 to 1.34) 0.503	-0.81 (-3.29 to 1.66) 0.494	-0.21 (-1.30 to 0.88) 0.701	0.60 (-0.98 to 2.18) 0.450
Aldrin *p* – values	-76.03 (-190.5 to 38.4) 0.191	-85.40(-220.3 to 49.5) 0.211	-89.54 (-302.0 to 123.0) 0.395	-83.38 (-359.0 to 192.3) 0.532	-67.10 (-209.1 to 74.9) 0.349	-58.75 (-235.2 to 117.7) 0.506

Table 4.4 continued

Pyrethroid						
Lambda Cyhalothrin	-0.68 (-1.83 to 0.47)	-1.06 (-2.51 to 0.39)	-0.68 (-2.97 to 1.61)	-1.51 (-5.09 to 2.07)	-0.72 (-2.09 to 0.65)	-1.20 (-2.91 to 0.52)
p – values	0.242	0.149	0.547	0.386	0.300	0.166

*β = Adjusted for cooking fuel type, smoking habit of parents, drinking habit of parents, educational level of parents, age of child and whether child still breastfeeds.

SOCIAL BEHAVIOR

	All ages		0 – 2 years		3 – 4 years	
Pesticides	**Crude β(95% CI)**	**Adjusted* β(95% CI)**	**Crude β(95% CI)**	**Adjusted* β(95% CI)**	**Crude β(95% CI)**	**Adjusted* β(95% CI)**
Organochlorines						
γ – HCH	-0.49 (-1.79 to 1.81)	0.11 (-1.63 to 1.85)	-1.33 (-3.84 to 1.16)	-0.71 (-4.79 to 3.37)	0.04 (-1.57 – 1.64)	0.60 (-1.52 – 2.72)
p – values	0.456	0.898	0.282	0.717	0.962	0.571
β – HCH	-0.02 (-1.52 to 1.47)	1.11 (-1.15 to 3.36)	-0.93 (-4.04 to 2.18)	-0.01 (-5.32 to 5.30)	0.45 (-1.33 to 2.23)	1.34 (-1.05 to 3.74)
p – values	0.974	0.226	0.545	0.998	0.617	0.265
δ – HCH	-0.47 (-3.01 to 2.07)	-0.04 (-3.07 to 2.98)	-7.09 (-16.25 to 2.079)	-4.28 (-17.22 to 8.66)	0.46 (-2.09 to 3.01)	0.97 (-2.04 to 3.98)
p – values	0.714	0.976	0.123	0.488	0.719	0.520
Heptachlor	-0.91 (-1.97 to 0.15)	-0.24 (-1.65 to 1.16)	-0.98 (-3.31 to 1.34)	-1.11 (-4.11 to 1.90)	-0.87 (-2.08 to 0.34)	0.35 (-1.34 to 2.04)
p – values	0.091	0.585	0.392	0.446	0.156	0.681

Table 4.4 continued

Aldrin	-84.98 (-216.6 to 46.6)	-112.05 (-262 to 38.1)	-95.92 (-345.3 to 153.5)	-106.39 (-434 to 221.9)	-77.48 (-238.9 to 83.90)	-100.55 (-287.7 to 86.6)
p – values	0.203	0.141	0.437	0.503	0.341	0.285
Pyrethroid						
Lambda Cyhalothrin	-0.71 (-2.03 to 0.61)	-0.73 (-2.35 to 0.90)	-0.75 (-3.43 to 1.93)	-1.27 (-5.59 to 3.04)	-0.70 (-2.26 to 0.85)	-0.91 (-2.74 to 0.91)
p – values	0.291	0.376	0.572	0.541	0.369	0.319

*β = Adjusted for cooking fuel type, smoking habit of parents, drinking habit of parents, educational level of parents, age of child and whether child still breastfeeds.

AGGRESSIVENESS

Pesticides	**All ages**		**0 – 2 years**		**3 – 4 years**	
	Crude β(95% CI)	**Adjusted* β(95% CI)**	**Crude β(95% CI)**	**Adjusted* β(95% CI)**	**Crude β(95% CI)**	**Adjusted* β(95% CI)**
Organochlorines						
γ – HCH	-0.98 (-2.43 to 0.48)	-0.49 (-2.43 to 1.45)	-1.53 (-4.01 to 0.95)	0.16 (-3.98 to 4.30)	-0.61 (-2.52 to 1.31)	-0.09 (-2.69 to 2.51)
p – values	0.186	0.617	0.214	0.936	0.530	0.944
β – HCH	0.01 (-1.65 to 1.67)	0.57 (-1.74 to 2.88)	-1.08 (-4.20 to 2.04)	0.67 (-4.71 to 6.05)	0.61 (-1.47 to 2.69)	0.46 (-2.36 to 3.29)
p – values	0.990	0.623	0.483	0.796	0.559	0.742

Table 4.4 continued

δ – HCH	1.04 (-1.79 to 3.87)	2.15 (-1.25 to 5.56)	-7.02 (-16.50 to 2.46)	-3.08 (-16.47 to 10.32)	2.09 (-0.84 to 5.03)	2.75 (-0.92 to 6.41)
p – values	0.467	0.211	0.139	0.628	0.159	0.138
Heptachlor	-0.82 (-2.04 to 0.40)	-0.50 (-2.08 to 1.08)	-1.84 (-4.11 to 0.42)	-1.46 (-4.48 to 1.55)	-0.32 (-1.82 to 1.17)	0.37 (-1.70 to 2.43)
p – values	0.186	0.531	0.106	0.317	0.668	0.720
Aldrin	-61.15 (-211 to 88.70)	-38.84 (-209.5 to 132)	26.89 (-226 to 280)	21.15 (-316. to 358.7)	-122.25 (-316.2 to 71.7)	-72.85 (-302.2 to 156.5)
p – values	0.420	0.651	0.829	0.896	0.213	0.526
Pyrethroid						
Lambda Cyhalothrin	-0.56 (-2.06 to 0.94)	-1.24 (-3.06 to 0.57)	-1.32 (-3.98 to 1.34)	-1.97 (-6.29 to 2.34)	-0.13 (-2.02 to 1.77)	-1.26 (-3.49 to 0.98)
p – values	0.463	0.494	0.317	0.348	0.895	0.264

*β = Adjusted for cooking fuel type, smoking habit of parents, drinking habit of parents, educational level of parents, age of child and whether child still breastfeed.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Main Findings

This study looked at the association between pesticide exposure and neurobehavioral outcomes defined as Attention, Rule-breaking, Social behavior and Aggressiveness among under five years old children of farmers in Akumadan, Afrancho and Nkenkenso in Offinso North district of Ashanti region, Ghana. It assessed urinary residual levels of the pesticides as well as questionnaire data on exposure indicators at home, in the farm or both. 61.18%, 55.29% and 52.35% of the entire study population had contacts with pesticide contaminated surfaces at home, on farm and at home and/or on farm respectively. While 52.27%, 68.18% and 52.27% of children aged between 0 – 2 had contact with contaminated surfaces at home, on farm and both respectively, 64.29%, 50.79% and 52.38% of the older age group; 3 – 4 years old had same exposure experiences respectively. Also the proportions of children that put their thumbs in the mouth and also put pesticide contaminated objects in their mouths were high.

Urinary pesticide residues levels found in the general study population were as follows; gammaHCH = 3.09μg/l with standard deviation (SD) of 0.77μg/l; beta-HCH = 2.86 (SD = 0.64μg/l); delta-HCH = 2.42μg/l (SD = 0.43μg/l); heptachlor = 3.55μg/l (SD = 0.90μg/l); lambda cyhalothrin = 3.11μg/l (SD = 0.69μg/l) and aldrin = 0.02 (SD = 0.007μg/l). In the ≤ 2 years children, the mean concentrations were as follows; gamma-HCH = 3.08μg/l (SD = 0.73μg/l); beta-HCH = 2.93 (SD = 0.61μg/l); delta-HCH = 2.40μg/l (SD = 0.36μg/l); heptachlor = 3.52μg/l (SD = 0.99μg/l); lambda cyhalothrin = 3.04μg/l (SD = 0.73μg/l) and aldrin = 0.02 (SD = 0.007μg/l). Also, in the older children aged between 3 to 4 years, the mean concentrations were as follows; gamma-HCH = 3.09μg/l (SD = 0.77μg/l); beta-HCH = 2.87 (SD = 0.66μg/l); deltaHCH = 2.43μg/l (SD = 0.46μg/l); heptachlor = 3.56μg/l (SD = 0.87μg/l); lambda cyhalothrin =

3.14µg/l (SD = 0.68µg/l) and aldrin = 0.02 (SD = 0.007µg/l). The mean behavioral scores of the children were found as follows; Attention – 7.86, Rule-breaking – 8.01, Social behavior – 8.85 and Aggressiveness – 10.70.

The study found no significant association between urinary residual concentrations of any of the pesticides and the behavioral outcomes. However, apart from placing contaminated objects in the mouth at home, which was insignificant and in some cases positively associated with the outcomes of interest, there were strong significant inverse associations between behaviors/ practices at home, in the farm or both as indicators of exposure and all the behavioral outcomes. These inverse associations were stronger and more prevalent among 3 – 4 years old children with rule-breaking, social behavior and aggressiveness being the most affected behavioral outcomes.

5.2 Validity of Method

This study is the first in Ghana that is aimed at assessing the association of pesticide exposure on behavioral outcomes among under-five year old children in vegetable farming communities. Two methods were used to assess pesticide exposure among children: a) urinary organochlorine and pyrethroid residual levels; b) behavior/ practices at home, farm or both that exposes children to the chemicals. The Child Behavior Checklist for children below 5 years (Achenbach and Reschorla., 2000) was used to assess the behavioral outcomes of the children and the mean score calculated.

The study population was derived from a large source population of over 930 individuals from 310 households which included all children under five years old living in Afrancho, Nkenkensu and Akumadan of Offinso-North District in the Ashanti Region whose parents were vegetable farmers. The large source population size with very high participation rate greatly minimized selection bias. Data on questionnaire also helped in adjusting for potential confounders in the analyses. The questionnaires were administered by well-educated agricultural extension officers who functioned as research assistants. Prior to the collection of the data, an extensive orientation and training was held to explain the study and need for accuracy in data gathering. Non-study participants were also accounted for in reaching the final sample study population of 170 and this enhanced precision on the estimates.

Urine samples were collected from children with the help of parents. Both parents and children were made to wash their hands well with soap and clean water before handling the sterile urine sample tubes to prevent contamination. Samples were kept and transported at a very low temperature and this helped maintain the integrity of urine samples. Laboratory analyses were done at well-established and standard laboratories in Ghana and with the help of well-trained laboratory technologists and this gave credence to the results obtained.

5.3 Comparing this study with previous literature

A systematic search from literature in Pubmed, Elsevier, IPCS INCHEM, HINARI, Oxford Journals, and other data bases did not reveal any study on this topic in Ghana. However, in closely related studies conducted in other developed countries, there were inconsistencies in results, using urine or blood samples to assess exposure to pesticides and by using methodologies like behavior/ practices as indicators. In the overall results however, behaviors/ practices using questionnaires were found to be more strongly associated with neurodevelopmental problems than bio monitoring. For instance, a birth cohort study by the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) in USA on 396 children at six (6) months old, 395 children who were 12 months old and 372 children at 24 months old found no association between maternal and child DAP metabolite in urine and attention problems using the ‘Child Behavior Checklist’ (Eskenazi et al., 2007). CHAMACOS later found out in study on the same children that prenatal DAP was not associated with reported symptoms of attention deficit at age 3.5 but significantly associated at 5 years with attention and ADHD (Marks et al., 2010). Interestingly, whiles some studies could not establish an association between urinary pesticide residues of children with neurodevelopmental outcomes (Bouchard et al., 2011; Eskenazi et al., 2007; Guodong et al., 2012; Lizardi et al., 2008; Lu et al., 2009; Ruckart et al., 2004), others reported strong relationships between postnatal exposure among children living in farming communities with same neurodevelopmental outcomes (Abdel Rasoul et al., 2008; Rohlman et al., 2005).

This study was undertaken in predominantly vegetable farming communities in Offinso-North district of Ghana where organochlorine and pyrethroid pesticides are extensively used (Ntow et al., 2006). The survey unlike Lee et al., 2007 and Sagiv et al., 2008; Stewart et al., 2003; 2005; 2006; Grandjean et al., 2001 and Vreugden-hil., 2004, did not use a cohort of children. The study also only relied on primary data of children but not secondary data such as health records of children from hospitals. However, the study found significant associations between behavior and practices that exposes children to pesticides and behavioral outcomes like found in previous literature but did not find same between urinary organochlorine and pyrethroid metabolite and behavior. Like the studies mentioned above, the association between exposures and behavioral outcomes were found to be stronger in more than three (3) years old children than in the younger ones.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

The results from this study show that majority of children under-five living in Akumadan, Nkenkenso and Afrancho in the Ashanti region of Ghana whose parents are farmers are exposed to pesticides at home, on the farm and at home and/or on the farm. There were also significant amounts of pesticide residues found in the urine of these children. Whiles the study shows significant inverse associations between exposure defined as behavior and practices at home, on the farm and at home and/or in the farm that predisposes children to organochlorine and pyrethroid pesticides and neurobehavioral outcomes, urinary pesticides levels to a large extent was on the contrary not associated with neurobehavioral outcomes. The findings hence confirm the previous literature on the effects of pesticides on human behavior following postnatal exposure. It also supports the previous reports that, effects of pesticides are more visible in older children due to longer exposure time. Pesticides therefore remain a serious public health problem because of their extensive use in agriculture and the vulnerability of children. The survey is therefore relevant to Ghana and other developing countries that rely largely on farming as an economic booster.

6.2. Recommendations

Considering the fact from the survey that, organochlorines and pyrethroid pesticides have adverse health effect on human especially children, there is the need for farmers to be sensitized about the potential effects of pesticides on children's health. In addition to this, the following recommendations are made;

1. Farmers in Offinso-North district and other farming districts in the country must be educated on the proper management/ storage of pesticides and their containers so that children do not come into contact with them.

2. Parents and teachers should educate and guide children to properly wash their hands before eating and also avoid picking items from the floor into the mouth.
3. There is the need to discourage farmers from taking their children along with them to the farm but instead, send them to school to avoid them being exposed.
4. Parents and the health ministry should periodically check the health of children living in the study area and other communities where pesticides are commonly used so that effects of pesticides on children health could be detected for early correction.
5. Government and policy makers must ensure strict adherence to safety use of pesticides at all times.

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APPENDIXES

APPENDIX A: PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

INFORMATION LEAFLET

Title of Research:
Association between exposure to organochlorine and pyrethroid pesticides and neurobehavioral outcomes among children under five (5) years old in Offinso North district of Ashanti Region, Ghana

Name(s) and affiliation(s) of researcher(s):
My name is Felix Bonney, a Master of Science, Environment and Public Health student of KNUST-African Institute of Sanitation and Waste Management, Accra, Ghana.

Background:
Dear participant, I am undertaking a study of pesticide exposure, behavioural disorder and symptoms of attention deficit hyperactivity disorder in under five year old children. This study will therefore focus on the association between pesticide exposure and neurodevelopmental disorders among children under five years in three farming communities in Offinso north district of Ghana.

Purpose(s) of research:
The purpose of this research is to find out the prevalence of neurodevelopmental disorders among children less than five years and assess the association between pesticide exposure and the developmental outcomes.

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research:

The study will involve answering questions from a structured questionnaire. Urine sample will be taken from children for laboratory analysis. In all, questionnaire will be administered to 300 households after which 170 children will randomly be selected for urine sample collection. This is purely an academic research which forms part of my work for the award of a Master of Science, environment and public health degree. I would be very grateful to have you as part of this study.

Risk(s):
This study will not cause any discomfort to the participants.

Benefit(s):
The goal of this study is to find the association between exposure of pesticide and developmental disorders and identify practices that expose children to pesticides. It is hoped that results obtained from this study will be used by policy makers and the community in particular to either improve upon existing safety measures or to enforce existing ones with the objective of better protecting the children of farming families from incidences of these exposure and neurodevelopmental disorders.

Confidentiality:
I would like to assure you that whatever information provided will be handled with strict confidentiality and will be used purely for the research purposes. Your responses will not be shared with anybody who is not part of the research team. Data analysis will be done at the aggregate level to ensure anonymity. No name will be recorded and data collected cannot be linked to you in anyway.

Voluntariness:
Participation in this study is voluntary and parents of the children can choose not to answer any particular question or all questions. You are at liberty to withdraw from the study at any time. However, it is encouraged that you participate since your opinion is important in determining the outcome of the study.

Alternatives to participation:
Your non-participation in this study will not mean you cannot benefit from the outcomes of the research and any interventions that may follow.

Withdrawal from the research:
You may choose to withdraw from the research at any time without having to explain yourself. You may also choose not to answer any question you find uncomfortable or private.

Consequence of Withdrawal:
There will be no consequence, loss of benefit or care to you if you choose to withdraw from the study.

Costs/Compensation:
For any inconvenience caused you, I will compensate you with a bar of soap worth GH¢10.00 to show my appreciation for your participation.

Contacts:

If you have any question concerning this study, please do not hesitate to contact me on 0246181816/0205480361 or felixbonney84@yahoo.com. You can also contact my supervisor, Dr Reginald Quansah on 0272620401 or yaw121@yahoo.co.uk.

Further, if you have any concern about the conduct of this study, your welfare or your rights as a research participant, you may contact:

The Office of the Chairman Committee on Human Research and Publication Ethics Kumasi
Tel:0322063248 or 020 5453785

CONSENT FORM

Statement of person obtaining informed consent:

I have fully explained this research to ________________________________ and have given sufficient information about the study, including that on procedures, risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: ____________________ NAME: ______________________________

Statement of person giving consent:

I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

NAME:____

DATE: ___________ SIGNATURE/THUMB PRINT: __________________

Statement of person witnessing consent (Process for Non-Literate Participants):

I ________________________________ (Name of Witness) certify that information given to (Name of Participant), in the local language, is a true reflection of what l have read from the study Participant Information Leaflet, attached.

WITNESS' SIGNATURE (maintain if participant is non-literate): ____________________

MOTHER'S SIGNATURE (maintain if participant is under 18 years): ________________

MOTHER'S NAME: ____

FATHER'S SIGNATURE (maintain if participant is under 18 years): _________________

FATHER'S NAME: ____

APPENDIX B: QUESTIONNAIRE

I am a MSc. Environment and Public Health student of Kwame Nkrumah University of Science and Technology, Africa Institute of Sanitation and Waste Management. I am conducting a study on the association between pesticide exposure and neurodevelopmental outcomes in children under five years old. The questionnaire seeks to collect information on the demographics, pesticide use and hopes to assess the relationship between pesticides exposure and behavioral outcomes among the under five year old children of vegetable farmers in Offinso north district.

All information will be treated with maximum confidentiality.

SECTION 1: CHILD DEMOGRAPHICS:				
S/N	Questions	Codes	Coding Categories (Circle)	Answers
1	Age at last birthday	Age		……………………………
2	Gender	Sex	1. Male 2. Female	
3	Level of education	edu	1. Crèche 2. Kindergarten 3. Lower primary 4. No education	

4	On the average how many hours does the child spend per week on the farm	ahof		…………………………
5	What types of crop do parents cultivate?	tocc	1. 2. Vegetables Maize 3. Cassava 4. Others Specify …………………..	
SECTION 2: GUARDIAN'S DEMOGRAPHIC INFORMATION				
6	Age of Guardian			……………………………
7	Gender of guardian		1. 2. Male Female	
8	Relationship to the child			……………………………
9	Level of education of the guardian		1. Primary school leaver 2. Junior high school leaver 3. Senior high school lever 4. Tertiary school leaver	
			5. Other (Specify) ……………………	
10	Average income of the household			……………………………
11	Specific role on the farm			……………………………
SECTION 3 PESTICIDES EXPOSURE **1. Pesticides Handling**				
12	Do you use pesticides in your farms?	upif	1. 2. Yes No	
13	If yes how long have you been using pesticides (in years)	lopu		……………………………
14	How often does the child come in to contact with pesticides?	ccp	1. Everyday 2. Once a week 3. Once a month	
15	What activity makes you come into contact with pesticides?	amup	1. Transportation of the pesticides 2. Mixing	

			3. Spraying 4. Storage	
16	Where are the pesticides stored before and after use?	psba	1. Home 2. Farm	
17	Do children have contact with stored pesticides?	chcp	1. Yes No 2.	
18	If yes, how often do they come into contact with these stored pesticides?	hosp	1. Everyday 2. Once a week 3. Once a month 4. Other Specif ‧…………………	
19	Where are pesticides mixed before application?	pmba	1. Home 2. Farm 3. both	
20	Are there spillages during the mixing process?	spill	1. Yes No 2.	
21	If yes, how often do these spillages occur?	hoso	1. Not often 2. Often 3. Very often	
22	Do children have contact with such spillages?	chss	1. Yes No 2.	
23	If yes, how often do the children come into contact with spillages?	hocs	1. Not often 2. Often 3. Very often	
24	Do pesticides come with specific instructions on how to use?	pcsi	1. Yes No 2.	
25	If yes, in what form?	iwf	1. MSDS 2. Label on container 3. Others y Specif …………………….	
26	Are you aware of any adverse health effects of pesticide?	awep	1. Yes No 2.	
27	If yes to the above what are Preventive measure put in place?	wpmi	1. Utility gloves 2. Goggles 3. Safety boots 4. Others Specify ………………….	
28	How often do you use personal protection when coming in to contact with pesticides?	uppc	1. Regularly 2. Sometimes 3. Never	
29	If no to question 28 any reason for not using any preventive measure?	ntqr	1. Discomfort 2. I don't have them	
			3. Others Specify ………………..	

2. Proximity of child to contaminated /exposure surfaces				
30	Does the child come into contact with the used protective attire/equipment?	ccue	0. No 1. Yes	
31	Does the child come into contact with contaminated surfaces such as used containers or dresses?	cccs	0. No Yes 1.	
32	Does the child play on the ground in the home?	cpgh	0. No Yes 1.	
33	Does the child ingest soil materials at home or farm?	Cish/f	0. No Yes 1.	
SECTION 4: SOCIOCULTURAL FACTORS				
34	How many people live in the Home?	hmph		………………………………
35	Does any member of the family smoke in the home?	dmfh	0. No Yes 1.	
36	Is the child in the home when this person is smoking?	chwps	0. No Yes 1.	

APPENDIX C: CHILD BEHAVIOR CHECKLIST FOR < 5 YEARS OLD CHILDREN

Below is a list of items that describes children. For each of the item that describes the child **now or within the past two months**, please tick the **2** if the item is **very true or often true** of the child. Tick **1** if the item is **somewhat or sometimes** true of the child. If item is **not true** of the child, tick the **0**.

0 = Not True (as far as you know) **1 = somewhat or Sometimes True** **2 = Very True or Often True**

S/N	ITEM	0	1	2
1	Aches and pains without medical cause (do not include stomach or headaches)			
2	Acts too young for age			
3	Afraid to try new things			
4	Avoids looking others in the eye			
5	Cannot concentrate or pay attention for long			
6	Can't sit still, restless or hyperactive			
7	Can't stand having things out of place			
8	Can't stand waiting; wants everything now			
9	Chews on things that are not edible			
10	Clings to adults or too dependent			
11	Constantly seeks help			
12	Constipated, doesn't move bowels (when not sick)			
13	Cries a lot			
14	Cruel to animals			
15	Defiant			
16	Demands must be met immediately			

17	Destroys his/her own things			
18	Destroys things belonging to his/her family or other children			
19	Diarrhea or loose bowels (when not sick)			
20	Disobedient			
21	Disturbed by any change in routine			
22	Doesn’t want to sleep alone			
23	Doesn’t answer when people speak to him/her			
24	Doesn’t eat well (describe):			
25	Doesn’t get along with other children			
26	Doesn’t know how to have fun; acts like a little adult			
27	Doesn’t seem to feel guilty after misbehaving			
28	Doesn’t want to go out of home			
29	Easily frustrated			
30	Easily jealous			
31	Eats or drinks things that are not food; don’t include sweets (describe):			
32	Fears certain animals, situations or places (describe):			
33	Feelings are easily hurt			
34	Gets hurt a lot, accident-prone			
35	Gets in many fights			
36	Gets into everything			
37	Gets too upset when separated from parents			
38	Has trouble getting to sleep			
39	Headaches (without medical cause)			
40	Hits others			
41	Holds his/her breath			
42	Hurts animals or people without meaning to			
43	Looks unhappy without good reason			
44	Angry moods			
45	Nausea, feels sick (without medical cause)			
46	Nervous movements or twitching (describe):			
47	Nervous, high-strung or tense			
48	Nightmares			
49	Overeating			
50	Overtired			
51	Shows panic for no good reason			
52	Painful bowel movements (without medical cause)			
53	Physically attacks people			
54	Picks nose, skin or other parts of body (describe):			
55	Plays with own sex parts too much			

56	Poorly coordinated or clumsy			
57	Problems with eyes (without medical cause) (describe):			
58	Punishment doesn't change his/her behavior			
59	Quickly shifts from one activity to another			
60	Rashes or other skin problems (without medical cause)			
61	Refuses to eat			
62	Refuses to play active games			
63	Repeatedly rocks head or body			
64	Resists going to bed at night			
65	Resists toilet training (describe)			
66	Screams a lot			
67	Seems unresponsive to affection			
68	Self-conscious or easily embarrassed			
69	Selfish			
70	Shows little affection towards people			
71	Shows little interest in things around him/her			
72	Shows too little fear of being hurt			
73	Too shy or timid			
74	Sleeps less than most kids during day and /or night (describe):			
75	Smears or plays with bowel movements			
76	Speech problem (describe):			
77	Stares into space or seems preoccupied			
78	Stomachaches or cramps (without medical cause)			
79	Rapid shifts between sadness and excitement			
80	Strange behavior (describe):			
81	Stubborn, sullen or irritable			
82	Sudden changes in mood or feelings			
83	Sulks a lot			
84	Talks or cries out in sleep			
85	Hot tempered			
86	Too concerned with neatness or cleanliness			
87	Too fearful or anxious			
88	Uncooperative			
89	Underactive, slow moving, or lacks energy			
90	Unhappy, sad, or depressed			
91	Unusually loud			
92	Upset by new people or situations (describe):			
93	Vomiting, throwing up (without medical cause)			
94	Wakes up often at night			
95	Wanders away			

96	Wants a lot of attention			
97	Whining			
98	Withdrawn, doesn't get involved with others			
99	Worries			

Does the child have any disability or illness (either physical or mental)? ☐ No ☐ Yes – Please describe

……
……
……

LANGUAGE DEVELOPMENT SURVEY FOR AGES 18 – 35 MONTHS

The Language Development Survey assesses children's word combination and vocabulary. By carefully completing the language development survey, you can help me obtain an accurate picture of the child's developing language. Please be sure to answer all items.

I. Was the child born earlier than the usual 9 months after conception?
☐ No Yes – ☐ how many weeks early? ______ weeks early.

II. How much did the child weigh at birth? __________ kilograms.

III. How many ear infections did the child have before age 24 months?
☐ 0-2 ☐ 3-5 ☐ 6-8 ☐ 9 or more

IV. Is any language apart from the child's mother tongue spoken in the child's home?
☐ No ☐ Yes – how many other languages? ____________

V. Has anyone in the child's family been slow in learning to talk?
☐ No ☐ Yes – relationship with child ________________.

VI. Are you worried about the child's language development?
☐ No ☐ Yes – why?

VII. Does the child spontaneously say words in any language? (not just imitates or understands words)?
☐ No ☐ Yes

VIII. Does the child combine two or more words into phrases?
☐ No ☐ Yes

ATTENTION PROBLEMS

2 Acts too young for age
5 Cannot concentrate or pay attention for long
6 Can't sit still, restless or hyperactive
10 Clings to adults or too dependent
11 Constantly seeks help

21 Disturbed by any change in routine
46 Nervous movements or twitching
59 Quickly shifts from one activity to another
77 Stares into space or seems preoccupied
95 Wanders away

SOCIAL BEHAVIOR PROBLEMS

4 Avoids looking others in the eye
23 Doesn't answer when people speak to him/her
25 Doesn't get along with other children
26 Doesn't know how to have fun; acts like a little adult
27 Doesn't seem to feel guilty after misbehaving
62 Refuses to play active games
67 Seems unresponsive to affection
70 Shows little affection towards people
71 Shows little interest in things around him/her
81 Stubborn, sullen or irritable
88 Uncooperative
89 Underactive, slow moving, or lacks energy
98 Withdrawn, doesn't get involved with others

AGGRESSIVE BEHAVIOR

15 Defiant
16 Demands must be met immediately
20 Disobedient
29 Easily frustrated
30 Easily jealous
35 Gets in many fights
40 Hits others
44 Angry moods
58 Punishment doesn't change his/her behavior
66 Screams a lot
69 Selfish
82 Sudden changes in mood or feelings
85 Hot tempered
91 Unusually loud
97 Whining

RULE-BREAKING/ DESTRUCTIVE BEHAVIOR

9 Chews on things that are not edible
14 Cruel to animals
17 Destroys his/her own things
18 Destroys things belonging to his/her family or other children
31 Eats or drinks things that are not food; don't include sweets
36 Gets into everything
42 Hurts animals or people without meaning to
59 Quickly shifts from one activity to another
63 Repeatedly rocks head or body
75 Smears or plays with bowel movements

APPENDIX D: VARIABLES FOR ANALYSIS Mouthing behavior *On the farm*

1. When the child accompanies the parent to the farm, how often does the child place thumb/fingers in the mouth?
   a. Never b. Rarely c. Sometimes d. Most of the time e. Always

2. When the child accompanies the parent to the farm, how often does the child put nonfood items in the mouth?
    a. Never b. Rarely c. Sometimes d. Most of the time e. Always
3. When the child is on the farm, how often does the child eats soil on the floor in the farm?
    a. Never b. Rarely c. Sometimes d. Most of the time e. Always ***At home***
4. When the child is at home, how often does the child eats soil on the floor at home?
    a. Never b. Rarely c. Sometimes d. Most of the time e. Always
5. When the child is at home, how often does the child places thumb/fingers in the mouth?
    a. Never b. Rarely c. Sometimes d. Most of the time e. Always
6. When the child is at home, how often does the child pick non-food items from the floor into the mouth?
    a. Never b. Rarely c. Sometimes d. Most of the time e. Always

Food handling practices *On the farm*

7. When the child accompanies the parent to the farm, how often does your child eat food dropped on floor in the farm?
   a. Never b. Rarely c. Sometimes d. Most of the time e. Always
8. When the child accompanies the parent to the farm, how often does the child eat food with fingers?
   a. Never b. Rarely c. Sometimes d. Most of the time e. Always

At home

9. When the child is at home, how often does the child eat food dropped on floor?
   a. Never b. Rarely c. Sometimes d. Most of the time e. Always
10. When the child is at home, how often does the child eat food with fingers at home?
    a. Never b. Rarely c. Sometimes d. Most of the time e. Always

Contact with contaminated pesticides containers/equipment

On the farm

11. When the child accompanies the parent to the farm, how often does the child come into contact with empty pesticide containers/pesticide containers?
    a. Never b. Rarely c. Sometimes d. Most of the time e. Always
12. When the child accompanies the parent to the farm, how often does the child come into contact with pesticide contaminated equipment (e.g. nose mask, googles, etc)? a. Never b. Rarely c. Sometimes d. Most of the time e. Always
13. When the child accompanies the parent to the farm, how often does the parent hold or carry the baby when he has not changed into clean clothes?
    a. Never b. Rarely c. Sometimes d. Most of the time e. Always

At home

14. When the child is at home, how often does the child come into contact with pesticide contaminated equipment (e.g. nose mask, googles, etc)?
    a. Never b. Rarely c. Sometimes d. Most of the time e. Always

15. When the child is at home, how often does the father hold or carry the baby when he has not changed into clean clothes?

    a. Never   b. Rarely   c. Sometimes d. Most of the time   e. Always

16. When the child is at home, how does the mother hold or carry the baby when she has not changed into clean clothes?

    a. Never   b. Rarely   c. Sometimes d. Most of the time   e. Always