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Evaluation of Occupational and Vegetable Dietary Exposures to Current-Use Agricultural Pesticides in Ghana

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Additional information is available at the end of the chapter

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1. Introduction

Crop pests and diseases pose a major challenge to world food security as a result of the severe loss of food they cause. Crop pests and diseases can account for between 40 to 70% losses of crops in the field [1]. In order to ensure food security, a large number of pesticides have been used in agriculture to combat insect pests, diseases, and weeds in the field. Towards the mid-twentieth century, many chlorinated pesticides such as dichlorodiphenyltrichloroethane (DDT), aldrin, dieldrin, hexachlorocyclohexane (HCH e.g. lindane (γ -HCH)), hexachlorobenzene (HCB), toxaphene, mirex and chlordane were commercially produced in some developed countries and were widely used for decades [2]. Many of these pesticides, DDT in particular, were hailed for their efficacy against a broad range of crop pests and diseases.

Residues of DDT and other chlorinated pesticides can however persist in various compartments of the environment for long periods without losing their toxicity, thereby causing damage to ecosystems. They are also volatile and can move off-site via air-drift and surface runoff to contaminate remote areas where they have not been used [3]. Another important characteristic of these pesticides is that they are lipid soluble, which, together with persistence, allows them to bioaccumulate along the food chain [4, 5] and biomagnify in higher trophic level organisms [6, 7] including humans [8, 9]. Many of these chlorinated pesticides are highly toxic and have produced adverse health effects such as cancer, neurological damage, reproductive system deformities, birth defects, and damage to the immune system in humans and other animals [10].

Due to their adverse human health and ecological impacts, the use of these chlorinated pesticides have been severely restricted or banned in many countries [11]. There have also

been international efforts to phase out the use of persistent chlorinated pesticides. One such effort is the Stockholm Convention on persistent organic pollutants (POPs), which seeks to eliminate the use of POPs including nine chlorinated pesticides namely DDT, HCB, chlordane, toxaphene, dieldrin, aldrin, endrin, heptachlor and mirex. Ghana has ratified the Stockholm Convention on POPs and consequently has banned the use of DDT, aldrin, dieldrin, endrin, chlordane, HCH, heptachlor, HCB, toxaphene and mirex. The organophosphate, carbamate and pyrethroid groups of pesticides, unlike the chlorinated pesticides, degrade more rapidly in the environment and are therefore preferred to the chlorinated pesticides. Consequently, they are currently the most widely used agricultural pesticides in those countries where chlorinated pesticides are banned. However, the organophosphate and carbamate pesticides are also toxic. For example, 3 million cases of acute and severe pesticide poisoning in humans are reported to have occurred worldwide with some 220,000 deaths [12], of which organophosphate and carbamate pesticides were largely responsible [13]. Majority of the poisoning cases and deaths occurred in developing countries compared to developed countries, where far greater quantities of pesticides are used [14].

In Ghana, organophosphate pesticides such as parathion, methyl-parathion, methamidophos, monocrotophos, and phosphamidon have been banned. However, this ban may not be effective due to high illiteracy rate among Ghanaian farmers, most of who often do not understand the labels that come with majority of pesticides they use in crop production [15]. They are also ill-trained in the proper handling and application of pesticides and as a result often ignore safety measures that ensure protection of human health and the environment. Thus, the use large quantities of pesticides above recommended doses at very short intervals has been reported [16]. Intensive pesticide application for prolonged periods, especially during the growing season when most of the use occurs, can expose farmers to high levels of pesticides (through skin contact and inhalation) and may result in poisoning and other sicknesses.

It is now known that certain organophosphate and carbamate pesticides inhibit cholinesterase (ChE) activity and are the class of pesticides most commonly implicated in pesticide poisonings [17, 18]. The mode of action of carbamate pesticides is very similar to that of the organophosphate pesticides but they differ in action in that the inhibitory effect of carbamates on ChE is brief while that of organophosphate may last longer. These ChE inhibiting pesticides act by binding to the esteratic site of ChE [19] and inhibiting the enzyme acetylcholinesterase (AChE, EC 3.1.1.7) at nerve endings. AChE is an important enzyme found in humans and other animals and belongs to a group of hydrolases classified as B-type esterases [20]. It plays an important role in neuron-transmission at cholinergic synapses by the rapid hydrolysis of the neurotransmitter acetylcholine to choline and acetate. Inhibition of AChE retards the hydrolysis of acetylcholine to choline and acetate, leading to the accumulation of acetylcholine. Symptoms include weakness, dizziness, nausea, and in extreme cases cardiac arrhythmias, epileptic seizures and even death [1].

There have been media reports of acute pesticide poisoning cases due to consumption of contaminated vegetables in certain parts of Ghana. Unlike acute toxicity effects, which may be detected easily by observation, chronic effects due to long-term exposure to low doses of pesticides may be more subtle and difficult to detect. Human exposure to organophosphate and carbamate group of pesticides can be evaluated based on blood ChE activity levels [20] or pesticide residues levels in food [21, 22]. This chapter evaluates the occupational and vegetable dietary exposures to current-use agricultural pesticides in Ghana.

2. Methodology

2.1. Study area

The study of cholinesterase activity in farmers was undertaken in two vegetable production areas in Ghana, the Offinso district in the Ashanti region and the Tono Irrigation Project in the Kassena - Nankana district of the Upper East region. The main criteria for selection of the areas were the relatively high activity of vegetable cultivation and heavy use of pesticides in the region [16, 23]. Residues of agricultural pesticides have been detected in surface waters, crops and human fluids at Akumadan in the Offinso district [23]. Pesticide residues have also been detected in vegetables from Kumasi in the Ashanti region [24, 25].

The target population was vegetable farmers from Akumadan and the Tono Irrigation Project of either gender, 18 years or above, who applied pesticides during the spray season. The selection of the subjects at each study site was random. For control, it is ideal to monitor changes in blood ChE activity of the study population during the peak and off-peak agricultural seasons so that each farmer could serve as his own control. This was, however, difficult to achieve since the farmers worked all through the year. A period of at least 30 days must separate the baseline determination from the farmer's last exposure to a cholinesterase-inhibiting compound [26]. About 2 months prior to the study, farmers at the Tono Irrigation Project were not farming (and for that matter were not spraying pesticides) due to damage of the water pumping system. Farmers from the Tono Irrigation Project who had not handled pesticides in at least the preceding 2 months were therefore selected without conscious bias as a control group. The control subjects matched the study population (farmers at Akumadan) very well in occupation, nutrition and socio-economic status. The mean whole-blood ChE levels obtained for the population at Tono Irrigation Project, therefore, served as a reference. In similar studies on farm workers in the Republic of South Africa, literature values have been used as a reference [27].

Surveys, formal and informal interviews, and observations were also conducted to obtain additional information for this study. The sample size was determined in order to achieve 95% confidence limits of 5% maximum error of the estimate, when the prevalence is 96% [28, 29]. A sample size of 60 farmers was therefore required for the study. However, for the anticipation of no-responses, the sample size was increased to 100 farmers. All farmers who participated in the study gave Prior Informed Consent after receiving oral and/or written information on the project. The Ethics Review Committee of the Health Research Unit of the

Ghana Health Service approved the study. The study of organophosphate pesticide contamination in vegetables was also conducted in Kumasi in the Ashanti region.

2.2. Blood sample collection, preparation and ChE analysis

At about 12 noon, blood samples of farmers were drawn with heparin-treated syringes and needles with the assistance of health workers and transferred into 5-ml disposable plastic tubes already containing EDTA anti-coagulant. Farmers at Akumadan had sprayed pesticides earlier in the day before sampling their blood while farmers at Tono Irrigation Project had not been spraying pesticides for at least two months preceding the day of sampling. The blood samples were placed in an ice-cooled chest and stored at -20°C at the nearest hospital within 1 h before they were transported to the laboratory. All blood samples were analyzed within 36 hours after sampling.

A Gallenkamp UV-Visible Spectrophotometer was used for the measurement of ChE activity. The instrument was calibrated for wavelength and absorbance in accordance with the manufacturer's instructions. All the reagents used in the study were of analytical grade and used without any further purification. Acetylthiocholine iodide (ATC) and 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) were purchased from the Sigma-Aldrich chemical company, Germany.

A 10 mmol/L DTNB reagent was prepared by mixing 61 ml of 100 mmol/L Na_2HPO_4 and 39 ml of 100 mmol/L NaH_2PO_4 . The pH of the resulting buffer solution was adjusted to 7, and 39.6 mg of 5,5'-dithiobis-2-nitrobenzoic acid dissolved in 10 ml of the 100 mmol/L buffer solution. The reagent was stored in a dark bottle at 4°C . An ATC substrate (75 mmol/L) was also prepared by mixing 5 ml of 100 mmol NaH_2PO_4 and 95 ml of 100 mmol Na_2HPO_4 , and the pH of the resulting buffer solution adjusted to 8 using the sodium dibasic salt or monobasic salt before dissolving 21.7 mg of ATC in 10 ml of the 100 mmol/L buffer solution.

Whole blood ChE activity for each farmer was assayed in triplicate following procedures described by Ellman *et al.* [30]. Briefly, 0.5 ml of blood sample was diluted to 10 ml with a freshly prepared 100 mmol/L sodium phosphate buffer (pH = 8). To 1 ml of the diluted blood were added 30 ml of 100 mmol/L sodium phosphate buffer (pH = 8) and 1 ml of DTNB. The resulting solution was mixed thoroughly and incubated at 30°C for 10 minutes. The spectrophotometric zero was set with 3 ml of this solution transferred into a cuvette using a pipette, after which 133 μl of ATC solution was added. The rate of reaction was monitored in kinetic mode by a spectrophotometer at 412 nm for 6 min at 30°C . The enzyme activity was expressed as μmoles of thiocholine hydrolysed $\text{min}^{-1} \text{ml}^{-1}$ of whole blood using an extinction coefficient of $13,600 \text{ cm}^{-1} \text{M}^{-1}$.

A structured questionnaire in English was administered to gather information from the farmers. The principal investigator translated the questionnaire into local languages so that farmers could understand and respond appropriately, taking care to retain their original meaning. The questionnaire consisted of both open and closed questions. Four groups of data were gathered: (1) personal, (2) farm details and work history, (3) pesticide use

practices and management, and (4) pesticide-exposure symptoms farmers experienced during the preceding week.

2.3. Statistical analysis

SPSS software (SPSS software, version 12.0.1 for Windows, SPSS Inc, Chicago, Illinois, USA) was used for all statistical analyses. The results of the ChE activities were analysed for normal distribution (Kolmogorov-Smirnov Test) and homogeneity of variance (Levene's Test). Statistical analysis of the results was evaluated using ANOVA and Dunnett's Test. A level of probability below 0.05 was considered statistically significant for all analysis. The independent *t*-test was used for bivariate comparisons between means. Categorical variables were compared by the Chi-squared test. Multiple linear regression analysis was carried out to evaluate the contribution of demographic characteristics and exposure to ChE activities. For the comparison of symptoms according to exposure, we used the odds ratio for the prevalence as a measure of association. A level of probability below 0.05 was considered statistically significant for all analysis [31].

2.4. Vegetable sampling, preparation and organophosphate pesticide analysis

Fifty (50) samples each of tomatoes, eggplant, and pepper were collected randomly from Kumasi Central Market. Samples collected were immediately wrapped in aluminum foil, placed in an ice-chest kept at 4 °C and sent to the laboratory for analysis. In the laboratory, similar vegetable items were wholly bulked together and ground in a waring blender to obtain a homogenous composite. About 2 g portions of homogenized samples were weighed in 10 mL screw-capped tubes, 5 mL of acetonitrile added and agitated in a rotatory shaker for 15 min, after which the acetonitrile layer was allowed to separate and then removed by decantation. The extraction step was repeated twice for each extract and the combined acetonitrile phase was centrifuged for 2 min at 3000 rpm. The supernatants were then carefully transferred into graduated vials and concentrated to about 2 mL using a Buchi rotary evaporator operating at a temperature of 30 °C under reduced pressure [32, 33]. Internal standards were added and the extracts made up to 2 mL with acetonitrile before GC injection. A Shimadzu Gas Chromatograph, GC-9A, equipped with a flame photometric detector, CR-7A integrator, DB-1 (methyl polysiloxane) and DB-17 (50% phenyl, 50% methyl polysiloxane) wide-bore capillary columns (30 m x 0.53 mm ID x 1.5 µm film thickness) were used for the analyses.

The gas chromatographic analysis was performed under the following conditions: detector temperature was 250 °C, injector temperature was 250 °C and column temperature was 200 °C (isothermal). Carrier gas was helium at a flow rate of 17 ml min⁻¹. One microliter (splitless) of sample was injected into the GC in the phosphorus mode [34]. Untreated samples were fortified at 0.001 mg kg⁻¹ by adding standard pesticide solutions. The samples were allowed to equilibrate for 30 min prior to extraction. After extraction and solvent evaporation, the samples were analyzed according to the proposed method. The recovery values were calculated from calibration curves constructed from the concentration and peak

areas of the chromatograms obtained with standard organophosphate pesticides. Detection limits of the method were found by determining the lowest concentrations of the residues in each of the matrices that could be reproducibly measured at the operating conditions of the GC using a signal-to-noise ratio of three.

Blank analyses were also performed in order to check interferences from the sample containers and reagents. All analyses were carried out in triplicates and the mean concentrations of pesticide residues were calculated based on the number of samples that tested positive to each pesticide residue. Analytical grade acetonitrile (99.5%) and ethylacetate (99.8%) were purchased from the British Drug House (BDH). Organophosphorus pesticide standards (methyl-chlorpyrifos, ethyl-chlorpyrifos, dichlorvos, dimethoate, monocrotophos, omethioate, methyl-parathion and ethyl-parathion) were purchased from Ehrenstorfer GmbH (Augsburg, Germany) in sealed vials. Glassware were washed with detergent, rinsed with distilled water, followed by acetone and then heated to 180 °C for 2 h. Standard solutions were prepared in ethylacetate and stored at 4 °C in a refrigerator.

2.5. Assessment of vegetable dietary exposure to organophosphate pesticide

To assess vegetable dietary exposure to organophosphate pesticides, Estimated Average Daily Intake (EADI, $\text{mgkg}^{-1}\text{day}^{-1}$) of detected pesticides were obtained from the product of the mean concentrations (mgkg^{-1}) of pesticides in vegetables and the daily vegetable consumption rates (kgday^{-1}). In the estimation of the average daily intake of pesticides, the consumption rates of tomatoes (13.56 kgyr^{-1}), eggplant (17.27 kgyr^{-1}) and pepper (0.12 kgyr^{-1}) in Ghana [35] were used. Hazard indices of detected organophosphate pesticides in vegetables were then derived by dividing the EADIs by their corresponding acceptable daily intake (ADI, $\text{mgkg}^{-1}\text{day}^{-1}$) values [36]. If hazard index (HI) is greater than 1, it indicates that lifetime consumption of vegetable containing the measured level of organophosphate pesticide residues could pose health risks [25].

$$\text{Estimated Average Daily Intake (EADI, } \text{mgkg}^{-1}\text{day}^{-1}\text{)} = C_c \times V_c \quad (1)$$

Where:

- C_c = mean concentration of pesticides in vegetable (mgkg^{-1})
- V_c = daily vegetable consumption rate (kgday^{-1})

$$\text{Hazard index (HI)} = \frac{\text{EADI (} \text{mgkg}^{-1}\text{day}^{-1}\text{)}}{\text{Acceptable daily intake (ADI, } \text{mgkg}^{-1}\text{day}^{-1}\text{)}} \quad (2)$$

3. Results

The demographic characteristics and the whole blood-ChE activities determined for the exposed farmers at Akumadan and the control farmers are shown in Table 1.

Characteristic	Farmers	
	Akumadan (N = 63)	Control (N = 58)
Mean age \pm SD (yrs)	33.9 \pm 8.5	32.3 \pm 9.8
Sex	"no value"	"no value"
No. male (%)	52	66
No. female (%)	48	34
Years of contact with pesticide (mean \pm SD)	17.1 \pm 5.3	14.2 \pm 6.1
ChE activity (mean \pm SD, $\mu\text{mol min}^{-1} \text{ml}^{-1}$)	3.59 \pm 2.93	7.27 \pm 1.71
% Falling below a level at 70% of normal reference mean ^a	73.0	13.8

^a Normal reference mean: 7.27 $\mu\text{mol min}^{-1} \text{ml}^{-1}$ of whole blood

Table 1. Demographic characteristics and the whole blood ChE activities determined for the exposed farmers at Akumadan and control farmers [16].

There were no statistically significant differences between the exposed and control groups regarding age and sex. All farmers (both exposed and control) were in the age range where ChE values do not change significantly with age [37]. The percentage of exposed vegetable farmers with a reduction of 30% in whole blood ChE activity was about 73%. The mean whole blood ChE activity of farmers at Akumadan (3.59 $\mu\text{molmin}^{-1}\text{ml}^{-1}$ blood) was 50.6% less than that of farmers considered unexposed from Tono Irrigation Project (7.27 $\mu\text{molmin}^{-1}\text{ml}^{-1}$ blood), and the difference was highly significant ($p < 0.05$) (Figure 1). Levels of cholinesterase were thus significantly lower in exposed farmers than in controls.

Farmers recalled some of the commonly observed symptoms of cholinesterase-inhibiting pesticides they had experienced within the week preceding the interview. Table 2 presents the prevalence of the commonest symptoms described by farmers.

Thirty-one (49.2%) subjects of the exposed farmers had experienced symptoms. The mean number of symptoms experienced by vegetable farmers at Akumadan was 2.5. Nineteen (32.8%) subjects of the control group of farmers from Tono Irrigation Project had experienced symptoms while the average number of symptoms they admitted to was 1.9. The difference between the number of poisoning symptoms between the exposed and control groups was not significant ($p > 0.05$). Among the exposed farmers, body weakness was the commonest symptom. One farmer had experienced unconsciousness. About 35% of the subjects from the control group had not experienced any poisoning symptom. The prevalence of weakness was found to be significantly ($p > 0.05$) higher in exposed farmers than in controls.

The frequencies of vegetable farmers' practices leading to potential exposure to pesticides at Akumadan are provided in Table 3. More than 40% of farmers at Akumadan wore no or only partial protective clothing and used knapsack sprayer to spray pesticide either against the wind or without considering the wind direction. A higher proportion (61.3%) of farmers returned to their farm within 48 h after spraying pesticide.

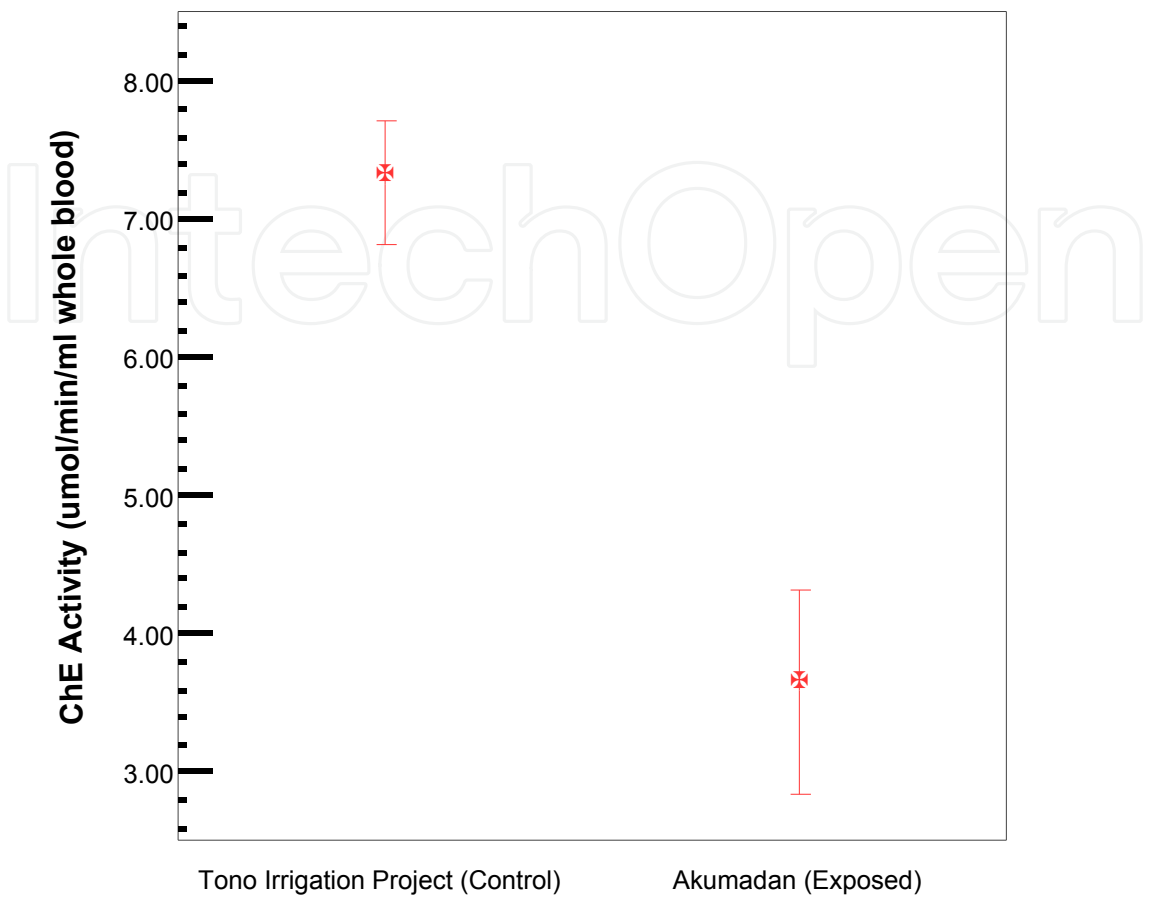


Figure 1. Whole blood ChE activities (mean \pm 95% CI) of farmers

Symptom	Prevalence (%)	
	Expose Farmers (N = 62)	Control Farmers (N = 58)
Vomiting	3.2	1.7
Weakness	49.2*	20.7*
Headache	31.7	32.8
Itching	3.2	5.2
Stomach pain	6.3	5.2
Unconsciousness	2.5	0
No symptoms	4.8*	34.5*

* significant difference between exposed and control groups at $p = 0.05$

Table 2. Prevalence of self-reported symptoms in exposed and control farmers [16]

Practice	Frequency (%)
Method of pesticide application	
By hand	5.8
Knapsack sprayer	66.7
Motorised	28.5
Direction of spraying of pesticide	"no value"
With the wind	44.4
Against the wind	9.5
Perpendicular	3.2
Do not consider wind direction	42.9
Kind of protective cover	"no value"
No or only partial protective covering ^a	85.7
Full protective covering ^b	14.3
Farmer re-entry periods	"no value"
Less than 48 h	61.3
From 48 to 72 h	27.4
More than 72 h	11.3

^a Short trousers/short sleeves or T-shirt; short trousers/long sleeves; short sleeves or T-shirt/long trousers; long trousers/long sleeves.

^b Long trousers, long sleeves, mask and gloves.

Table 3. Frequencies of vegetable farmers' practices leading to potential exposure to pesticides at Akumadan, Ghana ($N = 63$).

Concentrations of organophosphate pesticides in vegetables are shown in Figure 2

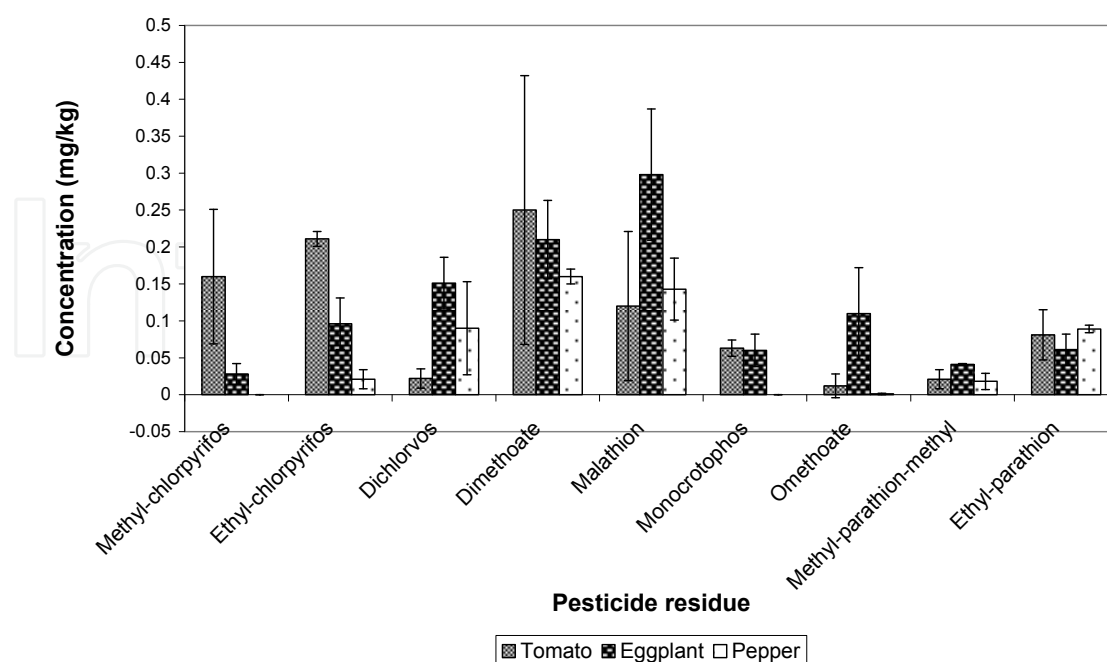


Figure 2. Concentrations of organophosphate pesticides residues in tomato, eggplant and pepper from Kumasi, Ghana. Source: Darko & Akoto, 2008.

Ethyl-chlorpyrifos was detected in 42% of tomato samples analyzed at a mean concentration of $0.211 \pm 0.010 \text{ mgkg}^{-1}$; in 10% of eggplant samples analyzed at a mean concentration of $0.096 \pm 0.035 \text{ mgkg}^{-1}$; and in 16% of pepper samples at a mean of $0.021 \pm 0.013 \text{ mgkg}^{-1}$. The mean concentration of ethyl-chlorpyrifos in tomato was below the WHO maximum residue limit (MRL) of 0.5 mgkg^{-1} [38]. Methyl-chlorpyrifos occurred in 30% of tomatoes and 8% of eggplant at mean concentrations of 0.160 ± 0.091 and $0.028 \pm 0.014 \text{ mgkg}^{-1}$, respectively. It was however not detected in any of the pepper samples analyzed. The mean levels of methyl-chlorpyrifos were below the WHO MRLs (0.1 mgkg^{-1} for eggplant and 0.5 mgkg^{-1} for pepper). Dichlorvos was the most frequently detected residue in all the samples analyzed. It occurred in 48% of tomato, 42% of eggplant and 26% of pepper samples with mean concentrations of 0.022 ± 0.013 , 0.151 ± 0.035 and $0.090 \pm 0.063 \text{ mgkg}^{-1}$, respectively. Malathion occurred at mean concentrations of $0.120 \pm 0.101 \text{ mgkg}^{-1}$ in tomato, $0.298 \pm 0.089 \text{ mgkg}^{-1}$ in eggplant, and $0.143 \pm 0.042 \text{ mgkg}^{-1}$ in pepper. The mean levels of malathion in tomatoes and pepper exceeded the WHO MRLs of 0.1 mg kg^{-1} . Monocrotophos was detected in two samples each of tomatoes and eggplant at mean concentrations of 0.063 ± 0.022 and $0.060 \pm 0.022 \text{ mgkg}^{-1}$, respectively. It was however, not detected in any of the pepper samples analyzed. Methyl-parathion was detected in five samples each of tomatoes and eggplant and in eight of pepper samples. The average concentrations of methyl-parathion were $0.021 \pm 0.013 \text{ mgkg}^{-1}$ in tomatoes, $0.041 \pm 0.001 \text{ mgkg}^{-1}$ in eggplant and $0.018 \pm 0.011 \text{ mgkg}^{-1}$ in pepper samples. Ethyl-parathion was detected at mean concentrations of $0.081 \pm 0.034 \text{ mgkg}^{-1}$ in 16% of tomato samples, $0.061 \pm 0.021 \text{ mgkg}^{-1}$ in 10% of eggplant and $0.089 \pm 0.005 \text{ mgkg}^{-1}$ in 12% of pepper samples. In a similar study in China [39], lower levels of ethyl-parathion (0.006 mgkg^{-1}) and methyl-parathion (0.003 mgkg^{-1}) were recorded in vegetables. Levels of ethyl-parathion were higher than its metabolite, methyl-parathion in all the vegetable types. Similarly, levels of ethyl-chlorpyrifos and dimethoate were higher than their metabolites, methyl-chlorpyrifos and omethoate, respectively. This may be an indication that the residues detected are as a result of current and/or continuous usage of the pesticides. No MRL is yet established for dichlorvos, monocrotophos, omethioate, methyl-parathion and ethyl-parathion in vegetables (tomato, eggplant and pepper) analyzed.

The estimated average daily intake (EADI) values and WHO/FAO acceptable daily intake values for the measured organophosphate pesticide residues in vegetables are presented in Table 4.

The calculated hazard indices for the pesticides in the vegetables analyzed are shown in Figure 3.

Hazard indices of methyl-chlorpyrifos, ethyl-chlorpyrifos and omethioate in tomatoes exceeded 1. Similarly, the hazard indices of methyl-chlorpyrifos, ethyl-chlorpyrifos, dichlorvos, monocrotophos and omethioate in eggplant were greater than 1. However, calculated hazard indices for all pesticide residues were less than 1 in pepper.

Organophosphate pesticide residue	WHO/FAO ADI (mgkg ⁻¹ day ⁻¹)	EADI (mgkg ⁻¹ day ⁻¹)		
		Tomato	Eggplant	Pepper
Methyl-chlorpyrifos	0.001	0.00592	0.00132	0
Ethyl-chlorpyrifos	0.001	0.00781	0.00451	0.00001
Dichlorvos	0.004	0.00081	0.00709	0.00003
Dimethoate	0.01	0.00925	0.00987	0.00005
Malathion	0.02	0.00444	0.01401	0.00004
Monocrotophos	0.0006	0.00233	0.00282	0
Omethoate	0.0003	0.00044	0.00517	0.00001
Methyl-parathion-methyl	0.02	0.00078	0.00193	0.00001
Ethyl-parathion	0.005	0.00299	0.00287	0.00003

Table 4. Estimated average daily intake (EADI) and WHO/FAO acceptable daily intake (ADI) values for the measured organophosphate pesticide residues in vegetables[25].

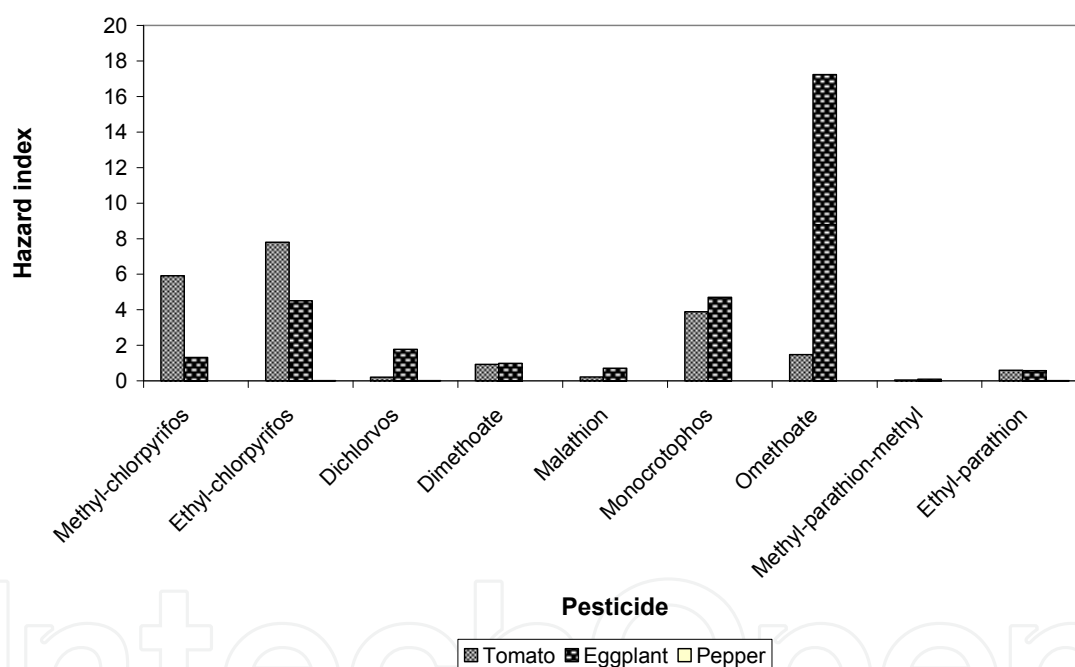


Figure 3. Hazard indices of organophosphate pesticide residues in vegetables from Kumasi, Ghana.

4. Discussion

The study has demonstrated significantly lower blood ChE activity in farmers at Akumadan compared to controls (farmers at Tono Irrigation Project). About 73% of all exposed farmers had blood ChE activity levels at or below 70% of the normal reference mean (that is, a reduction of 30% in ChE activity). A reduction of 30% in ChE activity has been recommended as a Health-based Biological Limit by a WHO study group [40]; as a Biological Exposure Index by American Conference of Governmental Industrial Hygienists; and as a Biological Tolerance Value (by the German Commission for the investigation of

Health Hazards of Chemical Compounds in the Work Area [41]. ChE values of 70% or less for whole blood could produce relatively mild and non-specific symptoms such as nausea, vomiting, headache, dizziness and weakness. Severe poisoning resulting in greater depression of ChE activity may result in unconsciousness, respiratory failure, and death [42].

In occupational settings, an abnormal reduction in ChE activity is usually a result of absorption of an anti-ChE compound. As a result, exposure of farmers to organophosphate or carbamate pesticides could be the main cause for significant reduction of ChE activity [43-45]. As other chemicals, including other pesticides, have demonstrated some estrogenic potency in cell systems [46] and as different detergents have inhibited the cholinesterase activity in mussels [47], the specificity of blood ChE as a marker for exposure to organophosphates and carbamates could be questioned. These observations may therefore limit the use of the marker in some instances, but in other situations, it might even be an advantage with a wider specificity with respect to chemical exposures affecting the biomarker [43].

Akumadan is known to be a highly contaminated site with pesticides, including anti-cholinesterase substances [23]. Use of pesticides such as carbofuran (a carbamate pesticide under the trade name Furadan 3G) and chlorpyrifos (an organophosphate pesticide under the trade name Durban 4E) in the study area has been reported [16, 31]. Carbofuran and chlorpyrifos are anti-ChE pesticides [48, 49] and we therefore speculate that carbofuran and chlorpyrifos are among the pesticides responsible for blood ChE inhibition at Akumadan.

Lowered ChE activity is a physiological effect of exposure to organophosphate pesticides, which may have chronic health effects. McConnel *et al.* [50] reports a study, which suggests that adults chronically exposed to organophosphate pesticides at work, have deficits in sustained attention and speed of information processing. In women, inhibition of blood ChE activity is of great public health concern, particularly in pregnant women. A study by Abu-Qare and Abou-Donia [51] has shown that exposure of pregnant women to organophosphate pesticides such as methyl-parathion and diazinon could result in adverse effects on their fetuses because these chemicals are easily transferred into their fetuses' nervous system, putting them at high risk of exposure to pesticides. Carbamate exposure is generally considered to be reversible and ChE inhibition is likely to undergo spontaneous reactivation [52]. The inhibitory effects of organophosphate exposure are also reversible, depending on new enzyme synthesis [53] with reactivation periods of 1-2 h for dimethyl phosphates but much slower for diethyl phosphates.

The observation of lowered ChE activity in farmers at Akumadan is probably due to unsafe working habits and their desire to have rapid knockdown of pests and increased income. Akumadan lies in the agricultural zone of the Ashanti Region of Ghana. The community at Akumadan is noted for the cultivation of various vegetables such as pepper, eggplants, onions, tomatoes and okra, which require frequent pesticide applications. Both anecdotal evidence and available data on farmers' use of pesticides at Akumadan indicate that they do not typically utilize recommended doses nor do they follow the chemical industry's

recommended practices for safe storage, handling, and application for various reasons [16]. On the one hand, most farmers at Akumadan have little or no formal education. While their traditional farming knowledge is adequate for them to benefit from the trade, effective and safe chemical pest management requires knowledge that goes beyond traditional farming practices [54]. A wide and changing array of pesticides are available to farmers, but little extension is available to guide farmers in their use, and most have not received training for safe storage, handling, and application. Most farmers rely on the recommendations of chemical dealers or their own experience in deciding how to use pesticides. Majority (66.7%) of the farmers represented in this study used knapsack sprayers to apply toxic pesticides but none took effective self-protection measures when working in the field with these materials.

Other practices that could have contributed to the high pesticide exposures and risk of pesticide poisoning symptoms include the lack of use of personal protective clothing, method of application of pesticide, short re-entry intervals and spraying pesticides against the wind direction (Table 3). Yassin *et al.* [55] have reported studies, which have found that occupational exposure to pesticides occurs from skin absorption and through inhalation. Wearing of protective clothing and gloves during pesticide spraying in the cultivation of vegetables could prevent dermal uptake. However, about 85% of the farmers at Akumadan did not use full personal protection. Some farmers (5.8%) even used bare hands to mix pesticides in a container.

Although the World Health Organization (WHO) recommends farmer protection during spraying, Ghanaian farmers do not generally observe this practice especially as very few can afford or have available protective clothing. Wearing of protective clothing and gloves is highly recommended when farmers handle dermal-absorbable pesticides. In a situation where majority of farmers use the knapsack to spray pesticides (or use brush, broom or leaves tied together to splash pesticides from a bucket), they are clearly exposed to the pesticide. The use of the knapsack sprayer in itself presents some danger to the user, since it is prone to leakage, especially as the sprayer ages [16]. Where farmers spray pesticide along rows back and forth or even against the wind or perpendicular to the wind direction, the wind may blow the chemicals into their faces and other parts of their bodies. This poor spraying practice presents great potential for exposure of farmers to agrochemicals from both skin contact and inhalation. About 95% of farmers who had been exposed to pesticides in vegetable farming at Akumadan had fallen ill. The frequent poisoning symptoms are reported as weakness (49.2%), headache and/or dizziness (31.7%) (Table 2). These symptoms are considered common manifestations of cholinesterase inhibition [55] although they could also be associated with other pesticide poisoning and common diseases such as influenza. Specific symptoms such as hypersalivation, lacrimation, bronchosecretion and miosis were however not reported.

A major limitation of a cross-sectional study like this one is that the generated information cannot be generalized for all population of vegetable farmers. The results only show what

was happening at the time of the study in the study areas. Furthermore, the criterion used for classifying study participants into exposed and a control group makes it difficult to reach significant differences, as they were all farmers with either direct or indirect exposure to pesticides. Determination of blood AChE inhibition also presents other limitations such as a high interindividual variation of its normal hydrolytic activity [56]. Thus, ideally, changes in blood ChE activity of the study population should have been monitored during the peak and off-peak spraying seasons, in which case each farmer could serve as his own control.

Organophosphate pesticide residues detected in vegetables were methyl-chlorpyrifos, ethyl-chlorpyrifos, dichlorvos, dimethoate, malathion, monocrotophos, omethoate, methyl-parathion-methyl and ethyl-parathion (Figure 2). Dichlorvos was the most frequently detected pesticide residue in all the samples. Although parathion, methyl-parathion, and monocrotophos have been banned in Ghana, they were detected in vegetables analyzed. Organophosphate pesticides such as methyl-chlorpyrifos, ethyl-chlorpyrifos, methyl-parathion, ethyl-parathion and monocrotophos are identified as ChE-inhibiting pesticides [48, 49] and thus consumption of these vegetables can lead to reduced levels of ChE activity. The levels of ethyl-parathion were higher than the levels of its metabolite, methyl-parathion in all the vegetable types. Similarly, the levels of ethyl-chlorpyrifos and methyl-chlorpyrifos were higher than their metabolites, dimethoate and omethoate, respectively. These observations may be an indication that the residues detected are as result of current usage of these banned pesticides.

The calculated hazard indices of methyl-chlorpyrifos, ethyl-chlorpyrifos and omethoate from daily consumption of tomato exceeded 1 while the health indices of methyl-chlorpyrifos, ethyl-chlorpyrifos, dichlorvos, monocrotophos and omethoate from daily consumption of eggplant also exceeded 1. Thus, the daily consumption of tomatoes and eggplant may present significant health risks to the consuming population. The daily consumption of pepper, however, does not present significant health risk as the associated HIs for the detected pesticides were all less than 1.

5. Conclusion

High depressed ChE activity has been observed in farmers at Akumadan which could be due to occupational exposure to current-use pesticides. There is however the need for pre-exposure baseline measurements of ChE for the exposed farmers at Akumadan in order to arrive at more definite conclusions. Further studies are needed to assess the implications of long-term exposure to these pesticides among the Ghanaian population. Additional step in future research would, therefore, be to assay urinary metabolites of pesticides in occupationally exposed subjects and in the general population of Ghana, since these metabolites are much more sensitive indicators of exposure than cholinesterase activity. The results also showed that vegetable dietary exposure to organophosphate pesticides might also present significant health risks. Clearly, there is the need for control and continuous monitoring of pesticides use at the local and national levels to reduce their adverse impact on human health and ecosystems.

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