African Journal of Agricultural Research

Full Length Research Paper

Butyrylcholinesterase activity among farm male pesticide handlers in Naivasha, Kenya

Lucy A. Mambo^{1*}, Eliud M. Njagi² and George Orinda²

¹Department of Public Health, Homegrown (Kenya) Limited, Nairobi, Kenya. ²Department of Biochemistry and Biotechnology, Kenyatta University, Nairobi, Kenya.

Accepted 13 August, 2013

To investigate the incidence of pesticide poisoning using serum cholinesterase activity patterns in a horticultural farm, 616 people comprising of 496 pesticide handlers (test group) and a control group of 120 persons participated in the study. A semi-structured questionnaire was used to obtain demographic information, while the activities of serum cholinesterase, serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase and bilirubin were estimated using standard commercial kits and absorbance measured using kinetic colorimetric tests. All the pesticide handlers (100%) were males, with majority (80.3%) aged 20 to 35 years old. Of the test population, 6% had significant cholinesterase enzyme depressions with no symptoms of exposure recorded. Significant difference (p<0.05) was observed in baseline cholinesterase activity between the test and control groups with a calculated intra-personal variation of 5.75%. Between the test and control groups, no correlation was observed on the baseline cholinesterase activity ($r^2 = 0.003$). Difference in cholinesterase activity was not significant (p>0.05) between the test and control groups based on years of handling pesticides. Use of pesticides in successive spray seasons significantly inhibited cholinesterase activity among the spray team, supervisors and harvesters (p<0.05). Higher cholinesterase activity was observed in the 31 to 40 age group with significant changes in cholinesterase activity (p<0.05) observed among those aged below 40 years. The study indicates that cholinesterase activity can be used effectively as an indicator of exposure to pesticides.

Key words: Cholinesterase activity, pesticide handlers, Naivasha, monitoring program.

INTRODUCTION

The horticultural sub sector in Kenya is the fastest growing industry within the agricultural sector, and is generally regarded as a success story. It has undergone dramatic growth over the years with several players getting involved in export and sale to local markets. Currently, horticulture is the leading export and top foreign exchange earner, bringing in Sh73.7 billion in 2008 (Nyambura-Mwaura, 2010; Waitathu, 2009). The Central Bureau of Statistics, which is a Department in the Ministry of Finance and Planning in the Kenyan

Government estimates that horticulture accounts for approximately 23% of the Gross Domestic Product and employs approximately 4.5 million people countrywide directly in production, processing, and marketing, while another 3.5 million people benefit indirectly through trade and other activities (KHC, 2010). Kenya has one of the most successful cut flower industries in Africa and the largest proportion of flower exports is supplied to Europe. It has surpassed Colombia and Israel as the largest supplier to the European Union, accounting for 58% of all

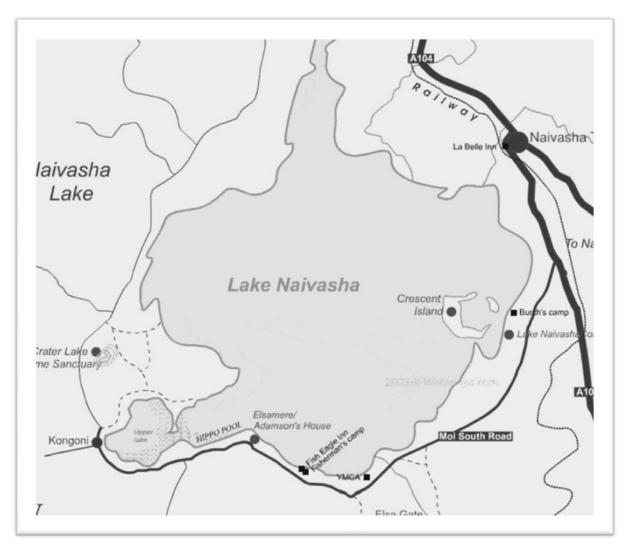


Figure 1. A map of Naivasha showing Lake Naivasha, the main source of water for horticulture in this region.

cut flower exports to the region (COLEAP, 2000; Hennock, 2002).

Managing pests and diseases is probably the most difficult aspect of growing crops at all stages. As such, sustainable agriculture requires an integrated approach that involves the use of pesticides (Lewis et al., 1981). With the use of pesticides, employees face the ever present risk of exposure and subsequent illness (USEPA, 2005). To ensure their health and safety, it is important that safe-use practices are established and monitored in order to minimize exposure to pesticides (USEPA, 2005). Also, data pertaining to exposure levels and patterns is required to set up surveillance programs to monitor the health of employees. Although previous studies have found pesticides as a major health and environmental problem in Kenya (Mbakaya et al., 1994; Ohayo-Mitoko et al., 1999; Ohayo-Mitoko et al., 2000), the impact of pesticide exposure on cholinesterase activity as a monitoring biomarker under controlled conditions has not been investigated and was the centrepiece of this study among pesticide handlers in a horticultural farm in Kenya.

MATERIALS AND METHODS

Study area

The study was carried out in Kingfisher farm, Naivasha, Rift Valley Province, Kenya (Figure 1). Since the 1980s the area is dominated by agriculture, mainly irrigated horticulture involving flowers, vegetables and fruits for both export and domestic market. The farms ship more than 88 million tons of cut flowers a year, worth some \$264 million (KFC, 1996). To maximize yields; pesticides are used extensively by farmers.

Study population

616 persons comprising of 496 pesticide handlers (test group), all males, ranging in age from 19 to 54 years and 120 controls taken

from departments that did not handle pesticides were incorporated into the study.

Study design

This was a descriptive exposure assessment study where the cholinesterase enzyme was used as a biomarker. All persons in the test group were subjected to a one month period free from pesticides by deployment in other general duties such as fencing, maintenance and construction. After the rest period, serum samples were collected for baseline studies, followed by periodic tests. Direct exposure defined as contact with pesticides during measuring, mixing and application was measured after the spray season. This included detailed observations on any skin contact and procedural operations. For the assessment of the effects of pesticide exposure, cholinesterase activity was determined in serum samples taken after a mandatory one month rest from handling of pesticides and comparisons made on pre-spray and post - spray season. Indirect exposure through potential water and food contamination (indirect exposure) was not studied as it was assumed that exposure through these roots was equal for both the study and control groups. Serum samples from the control group provided normal ranges for validation of the test kit The baseline test basically involved collection of two serum samples within a minimum of 24 h apart and a maximum of 72 h. The average of the two serum samples was taken as a working baseline. The periodic tests were scheduled at a month's intervals and involved both clinical examination and cholinesterase testing. Changes in an individual's cholinesterase levels were determined by calculating the percentage change from baseline, which was calculated using the formula below:

(Baseline Result - Periodic Result)

Baseline Result × 100 = percent Depression

Liver function tests were performed for all persons with cholinesterase activity below 4500 U/L. Additionally; questionnaires were used to establish individual lifestyles and behaviour before serum collection. Written consent was obtained from all persons.

Analytical methods

All testing was carried out in an accredited laboratory using competent analysts, and all measurements reported in International Units (IU). The cholinesterase test method was validated and population expected values calculated using the control group. Serum cholinesterase activity was measured colorimetrically according to method of Knedel and Bottger (1967), using the FLUITEST CHE kit manufactured by BIOCON, Germany and the absorbance of cholinesterase activity measured on Humalyzer 2000 Chemistry Analyzer.

Enzymatic patterns of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (AIP) and bilirubin wereused to differentiate cholinesterase depression due to excessive alcohol consumption and pesticide exposure. The activities of SGOT, SGPT, AIP and bilirubin were estimated colorimetrically using the Human kit manufactured by Human Diagnostics, Germany and the absorbance of the parameters recorded on Humalyzer 2000 chemistry analyzer.

Quality control

Lyophilised control serum Precinorm U (Lot 181528 Ver.1; Roche

Diagnostics GmbH, Mannheim) was used for simultaneous control of precision and accuracy. The day-to-day reproducibility was assessed from repeated parallel determinations of the control for all test assays. The control test value for cholinesterase was 5570 \pm 330 U/L, 0.778 \pm 0.062 mg/dl for direct bilirubin, 1.12 \pm 0.07 mg/dl for total bilirubin, 150 \pm 9 U/L for alkaline phosphatase and 46.2 \pm 2.8 U/L for both SGOT and SGPT.

Questionnaire development, validation and administration

The attributes investigated using a questionnaire included; demographics, attitudes, use of PPE, knowledge on pesticide use and effects, job history and occupational exposures, reproduction, illness, long-term prescriptions, febrile illness within the past 3 months, subjective symptoms, work practices, work conditions, other risk factors and health data. The questionnaire was first field pre-tested. Intra-observer variability testing was done twice as a training tool as well as to insure homogeneity between observers. The social and chronic disease indicators that should not vary over time were also tested for reliability by interviewing respondents with different observers two days in a row. The lowest score was 98%. The questionnaire was self-administered by both the test and control groups. On presentation to the medical facility for check-up before commencing work, all persons recruited into the study were required to complete the questionnaire. Those unable to complete the various sections on their own were assisted by the trained data collectors through face to face interviews.

Data management and statistical analysis

Uncertainties of measurement were established during method validation and mean cholinesterase activity was expressed as means ± standarddeviation and stratified by gender, tribe and designation while demographic variables from the questionnaire were categorized by age, gender, tribe, use of PPE, lifestylehabits and healthconditions. Dixons Q test was used to identify outliers and the t-test to compare mean serum cholinesterase activity in pesticide-exposed workers and the control group with level of significances as 5% (p < 0.05). Analysis of Variance (ANOVA) was used for comparison among groups followed by group wise comparisons by Bonferroni test with levels of significance also at 5% (p< 0.05). Linear regression was used for studying various relationships among continuous variables and percentiles to compute ranges of expected values for cholinesterase test. The p values for comparison of calculated expected values to those established by the kit manufacturer were derived from the z-statistic values calculated using Graphpad software. Data checks were inbuilt during data entry and validation checks were conducted continuously. Electronic back-up copies were made for analysis and future verification.

RESULTS AND DISCUSSION

Demographic profile of respondents

The test group was entirely male, aged 19-55 years with majority (80.3%) of the group aged 19-35 years old. More than half (50.60%) had worked with pesticides for more than three years. The control group (n = 120) comprised 86 (71.67%) men and 34 (28.33%) women. The youngest person in the control group was 22 years and the oldest was 54 years, with a mean age of 34.38 ± 6.66 years. All persons in the control group were believed

Table 1. Demographic profile of the respondents by age, gender, years of handling pesticides and use of PPE.

| Characteristics | Test group | (spray team) | Contr | ol group |
|-------------------------------------|-------------|------------------|------------|--------------|
| Age | N (%) | Mean ± SD | N (%) | Mean ± SD |
| Mean age | 496 (100) | 31.54 ± 6.47 | 120 (100) | 34.18 ± 6.87 |
| 19 – 30 | 297 (59.88) | | 18 | |
| 31 – 40 | 166 (33.47) | | 49 | |
| 41 – 55 | 33 (6.65) | | 19 | |
| Gender | | | | |
| Male | 496 (100) | | 86 (71.67) | |
| Female | _* | | 34 (28.33) | |
| Years of pesticide handling | | | | |
| 1 | 45 (9.07) | | _* | |
| 2 | 71 (14.31) | | _* | |
| 3 | 129 (26.01) | | _* | |
| 4 | 62 (12.5) | _* | | |
| 5 | 80 (16.13) | | _* | |
| >5 | 109 (21.98) | | _* | |
| Use of PPE | 496 (100) | | _* | |
| Access to information on pesticides | 496 (100) | | | |

^{*}Missing values were not applicable.

not to drink alcohol or smoke cigarettes based on their response to the questions in the administered questionnaire (Table 1).

In this study, 100% of those recruited in the spray team attended at least one lecture on pesticide use, protection, storage risk factors and symptoms of poisoning. Written material, informal talks and classroom lectures were the main sources of information for the pesticide handlers. Various aspects of classification and safe pesticide use were taught throughout the year. In addition, the study population recorded 100% adequate use of PPE and impromptu observations during the course of the study confirmed this despite reported cases of depressed butyrylcholinesterase activities.

Intra-personal cholinesterase activity variability

Intra-personal variation was based on the means of baseline test observations taken 48 to 72 h apart (Table 2). The purpose of this analysis was to determine variability independent of pesticide exposure in the case of the test group. Intra-personal percent depression was calculated from the observed intra-personal variation of actual levels and the average level. Analysis of cholinesterase activity at baseline indicates that there is no significant arithmetic change in cholinesterase activity between the baseline tests in each individual (p>0.05) with intra-personal variation at 0.8% for the control group and 5.75% for the test group (Table 2).

The butyrylcholinesterase patterns in this study illustrated two points. First, that normal cholinesterase baseline values vary widely among individuals and secondly that intra-personal variation is not as significant as interpersonal variation. In this study, although intrapersonal variability among the control and test groups was not significant, greater variability was observed among the pesticide handlers than in the control group (Table 2).

Incidence of pesticide poisoning

Thirty two (6.45%) pesticide handlers qualified for work removal status (Table 3) on the basis butyrylcholinesterase activity following retest at various levels. By definition, all subjects with cholinesterase depression (either increasing steadily on successive tests above baseline or more than 20% below a baseline on follow-up test) were classified as cases. Typically, all persons withdrawn from pesticide handling were asymptomatic despite the fact that some had depressions >40%; indicating that morbidity was not associated with depressed cholinesterase levels. In addition, accidental exposures were not reported in the course of the study. The lack of symptoms despite cholinesterase depression could be explained by the fact that cholinesterase activity may not have been sufficiently depressed to cause appreciable illness or that the pesticide handlers did fully understand the symptoms associated with pesticide

Table 2. Butyrylcholinesterase activity at two baseline tests for control and test groups.

| Parameter | Number | Mean | SD | Minimum | Maximum | |
|---------------------------------|--------|---------|-------------------|---------|---------|--|
| Control group | | | | | | |
| Baseline (1) CHE activity (U/L) | 120 | 7141.22 | 1521.05 | 4494 | 12001 | |
| Baseline (2) CHE activity (U/I) | 120 | 7132.20 | 1503.05 | 4666 | 12235 | |
| Difference | 120 | 9.02 | 18 | 176 | 234 | |
| % depression ^a | 120 | 0.13 | 1.18 ^b | 3.92 | 1.95 | |
| Test group | | | | | | |
| Baseline (1) CHE activity (U/I) | 496 | 6693.92 | 1180.30 | 4765 | 16234 | |
| Baseline (2) CHE activity (U/I) | 496 | 6705.43 | 1248.21 | 4297 | 16825 | |
| Difference | 496 | 11.51 | 67.91 | 468 | 591 | |
| % depression ^a | 496 | 0.17 | 5.75 ^b | 9.82 | 3.64 | |

(Baseline (1) — Baseline (2))

Baseline (1) ×100

Table 3. Biochemical profiles of persons from the test group eligible for work-removal status.

| Parameter | No. | Mean ± SD | Minimum | Maximum |
|-----------------------------------|-----------------|----------------------|---------|-------------------|
| Cholinesterase activity | | | | |
| Mean baseline CHE (U/L) | 32 | 6310.97 ± 1805.56 | 4765 | 11368 |
| Mean CHE after spray season (U/L) | 31 | 5170.92 ± 934.30 | 3173 | 7013 |
| Mean CHE after rest period (U/L) | 29 | 6064.74 ± 934.30 | 3530 | 12680 |
| Liver function tests | | | | |
| Alkaline phosphatase (U/L) | 33 ^a | 109 ± 35.30 | 11 | 207 ^b |
| SGPT (U/L) | 30 ^a | 17.79 ± 12.55 | 0.8 | 41 ^b |
| SGOT (U/L) | 26 ^a | 17.31 ± 9.78 | 3 | 45 ^b |
| Total Bilirubin (µmol/L) | 33 ^a | 19.20 ± 15.04 | 2.6 | 22.7 ^b |
| Direct Bilirubin (µmol/L) | 32 ^a | 4.88 ± 33.81 | 1.1 | 5.1 ^b |

^aThe actual number of tests analysed were 34 in each case. However, some values were out of quantifiable range and therefore omitted when calculating the means; ^bValues were above the expected upper limit.

poisoning. It is possible that lack of self reported symptoms could have been due to fear of investigations in the part of the sprayers.

To differentiate cholinesterase activity depression due to pesticide exposure from alcohol usage, liver function tests were used (Table 3). Liver function testing (LFTs) is used to evaluate various functions of the liver, as metabolism, storage, filtration, and exertion. Certain conditions, medication or alcohol may affect the functioning of the liver and will be demonstrated as abnormalities in certain parameters measured with this test. Some common parameters included in LFTs are alkaline phosphatase, SGOT, SGPT, and bilirubin (Schmidt and Schmidt, 1986). In alcohol related cholinesterase depression, SGOT, SGPT, alkaline phosphatase and Bilirubin are elevated differentially whereas in pesticide related cholinesterase depression,

these parameters are usually within the normal range or one parameter may be elevated by itself. The interpretation of elevated LFTs depends upon the entire clinical evaluation of a patient and must therefore be used with caution (Schmidt and Schmidt, 1986). The serum levels of SGOT, SGPT, alkaline phosphatase, total bilirubin and direct bilirubin in the study subjects eligible for work-removal status were mostly normal and elevated parameters did not suggest liver cirrhosis. It was therefore assumed that the cholinesterase depression was as a result of exposure to pesticides.

Baseline cholinesterase activity

The mean baseline cholinesterase activity in the whole cohort (n=496) was 6791.30 ± 1386.25 U/L. The control

^aPercent depression calculated as variation.

^bValue denotes calculated percentage intra-personal

Table 4. Baseline butyrylcholinesterase activity by duration of handling pesticides.

| Duration of pesticide handling (years) | No. of persons | Average cholinesterase activity (U/L) Mean ± SD | Minimum | Maximum | p value |
|---|----------------|--|---------|---------|---------|
| 1 | 45 | 6815.98 ± 1665.43 | 5040 | 14003 | 0.12* |
| 2 | 71 | 7437.51 ± 1811.84 | 4531 | 15391 | |
| 3 | 129 | 6826.78 ± 1523.70 | 4864 | 16530 | |
| 4 | 62 | 6575.10 ± 1018.11 | 4976 | 10748 | |
| 5 | 80 | 6371.29 ± 1097.51 | 4941 | 10889 | |
| >5 | 109 | 6749.90 ± 983.76 | 4917 | 9894 | |

^{*}p value not statistically significant when mean cholinesterase activity was compared among sprayers based on years of handling pesticides by ANOVA.

group baseline cholinesterase activity was normally distributed with a mean cholinesterase activity of 7081.60 ± 1543.75 U/L. The mean cholinesterase activity level of the test group was 6728.35 ± 1438.30 U/L, which was slightly lower than that of the control group. It was speculated that the lower baseline cholinesterase activity among the test group was as a result of pesticide exposure. In earlier studies, pesticide exposure has been reported to decrease cholinesterase activity (Bodgen et al., 1975; Quinones et al., 1976; Richards et al., 1983; Wicker et al., 1979). Similarly, the occupational health of workers in pesticide factories was examined in a Taiwan study that focused on absorption by inhalation of organophosphate among pesticide production workers and decreased cholinesterase activity was demonstrated (Wu et al., 1989). The test group baseline cholinesterase activity patterns differed significantly (p<0.05) from those of the control group (Table 5). Higher cholinesterase activity was observed in the 31 to 40 age groups for both the test and control subjects (Table 5). Significant difference in baseline cholinesterase activity was not demonstrated among the various age groups (p>0.05).

Slightly more than half of the spray team (50.81%) had worked with pesticides for more than three years (Table 4). Although there was difference in mean baseline cholinesterase activity among persons who had handled pesticides for less than three years (7011.71 ± 1661.66 U/L) when compared to those who had worked with pesticides for more than three years (6590.97 ± 1039.13 U/L), statistically, this difference was not significant (p>0.05). It was speculated that lower cholinesterase activity was associated with a higher frequency of pesticide exposure and attributed this to the cumulative effect of repeated exposure, but it could also be explained simply by the fact that people who have used pesticides more often have had more opportunity to develop a lowered cholinesterase activity. The greatest adverse effect among people who were exposed was an abnormal cholinesterase level, which confirms earlier studies on the effect of pesticides on the body, that people who are exposed to pesticides are more likely to have abnormal cholinesterase levels because use of pesticides has been found to be positively associated with pesticide poisoning (Coye et al., 1987; Edmiston and Maddy 1987; Faria et al., 2004; NIPC, 2004; Saunders et al., 1987; Xue et al., 1987).

Seasonal cholinesterase activity

To assess the seasonal variations in enzymatic activities, serum samples were taken before and after the spray season and results of cholinesterase activity compared to the baselines. Such activity was stratified by designation (Table 7) and age (Table 8). Significant difference (p<0.05) in serum cholinesterase activity was observed among pesticide handlers in the spray team, harvesters and supervisors. The bag fillers, pool, chemical mixers and pump operators did not show significant difference (p>0.05) in cholinesterase activity even when the post spray and after rest cholinesterase activity was compared (Table 7). The greatest variation in cholinesterase activity was observed among the supervisors, closely followed by the spray team.

Changes in enzymatic activity were significant (p<0.05) between the pre and post spray season and within successive spray seasons within the age groups of those aged less than 30 years and those aged 31 - 40 (Table 8). However, there was no significant difference (p>0.05) in seasonal cholinesterase activity among the pesticide handlers aged over 40 years even when post spray and after rest cholinesterase activity was compared to baseline and on successive periods. A general expectation is that frailty is usually associated with increase in age and therefore one might expect that those over 40 years would be more vulnerable to pesticide poisoning. However, in the case of this study the lack of change in cholinesterase activity for those over 40 years could simply be that several years experience in handling of pesticides may lead to extra carefulness hence less possibility of poisoning. Although age has been documented as one of the risk factors that should be considered in interpretation of cholinesterase activity (Brock and Brock, 1990; Lu, 2005), some researchers have observed no significant correlation of age to cholinesterase activity (Jors et al., 2006). The

Table 5. Baseline butyrylcholinesterase activity in control and test group males by age.

| | | Control group | | n ¹ value | | Test group | n ² value | p ³ value | | | |
|-----------|-----|--------------------------------------|------|----------------------|----------------------|------------|--------------------------------------|----------------------|-------|----------------------|---------------------|
| Age range | No. | Mean CHE activity (U/L) Mean ± SD | Min | Max | p' value (p<0.05) | No. | Mean CHE activity (U/L) Mean ± SD | Min | Max | p² value (p<0.05) | (p<0.05) |
| 19 – 30 | 19 | 6882.37±1254.22 | 4718 | 9331 | 0.342 ^a | 297 | 6686.26±1298.96 | 5011 | 13733 | 0.097 ^b | 0.0152 ^c |
| 31 – 40 | 48 | 7468.42±1450.41 | 4580 | 12118 | | 166 | 6972.20±1670.03 | 4531 | 16530 | | |
| 41 – 55 | 19 | 7437.90±1850.19 | 4888 | 11006 | | 33 | 6623.67±1180.49 | 5210 | 10174 | | |

^ap¹ difference in cholinesterase activity among the control group based on age was not significant by ANOVA; ^bp² difference in cholinesterase activity among the control group based on age was not significant by ANOVA; ^cp³ difference in cholinesterase activity between the test and control groups based on age was significant by t-test.

Table 6. Butyrylcholinesterase activity by season and designation.

| Designation | No. of persons | Baseline CHE activity Mean ± SD (1 st and 2 nd baseline tests) | Post-spray CHE activity (U/L) Mean ± SD | t ¹ | After rest CHE activity (U/L) Mean ± SD | t² | t³ |
|------------------------|----------------|--|--|---------------------|---|---------------------|---------------------|
| Sprayers | 144 | 6782.58±1027.43 | | | | | |
| 1 st season | | | 6573.95±1366.47 | 34.112 ^a | 6775.40±1467.86 | 1.174 | 32.938 ^c |
| 2 nd season | | | 6750.46±1045.93 | 5.252 | 6837.44±1119.03 | 8.970 ^b | 14.222 ^c |
| 3 rd season | | | 6859.66±1238.81 | 12.603 ^a | 6717.47±1027.71 | 10.646 ^b | 23.249 ^c |
| Mixers | 2 | 6653.00 ± 732.56 | | | | | |
| 1 st season | | | 6468.50±239.71 | 1.004 | 6697.00±414.36 | 0.239 | 1.243 |
| 2 nd season | | | 6708.50±132.23 | 0.302 | 7165.14±366.12 | 2.787 | 2.485 |
| 3 rd season | | | 6725.01±203.11 | 0.392 | 6617.47±1027.71 | 0.192 | 0.585 |
| Supervisors | 11 | 6447.78±1107.12 | | | | | |
| 1 st season | | | 5961.55±715.37 | 54.704 ^a | 6303.36±1071.86 | 38.456 ^b | 38.456 ^c |
| 2 nd season | | | 6789.80±950.13 | 38.480 ^a | 6580.90±761.20 | 23.503 ^b | 23.503 ^c |
| 3 rd season | | | 6572.30±571.41 | 14.009 ^a | 6509.40±902.04 | 7.707 | 7.077 |
| Pumpoperators | 9 | 6958.56±1364.36 | | | | | |
| 1 st season | | | 6776.75 ± 1365.42 | 2.128 | 6456.75 ± 696.74 | 5.874 | 3.746 |
| 2 nd season | | | 6714.50 ± 1102.19 | 2.857 | 6868.00 ± 930.75 | 1.060 | 1.797 |
| 3 rd season | | | 6824.86±781.69 | 1.565 | 7024.71±1389.05 | 0.774 | 2.339 |

Table 6. Contd.

| Pool | 12 | 6770.75±1214.62 | | | | | |
|------------------------|----|-----------------|-----------------|--------------------|-----------------|---------------------|---------------------|
| 1 st season | | | 6663.12±1457.84 | 0.586 | 6967.75±1606.39 | 1.073 | 1.659 |
| 2 nd season | | | 7245.63±1251.45 | 2.586 | 7481.43±1450.82 | 3.870 | 1.284 |
| 3 rd season | | | 7185.14±1586.22 | 2.257 | 7421.14±1712.59 | 3.542 | 1.285 |
| Harvesters | 10 | 6983.89±1234.15 | | | | | |
| 1 st season | | | 6912.25±1491.86 | 3.855 | 7229.75±987.93 | 13.230 ^b | 17.085° |
| 2 nd season | | | 7141.88±1834.16 | 8.502 ^a | 6832.88±1888.00 | 8.126 ^b | 16.628 ^c |
| 3 rd season | | | 6977.75±1210.62 | 0.330 | 6825.63±1374.49 | 8.516 ^b | 8.186 ^c |
| Bag fillers | 11 | 7062.25±1110.50 | | | | | |
| 1 st season | | | 8684.67±2762.74 | 4.417 | 8202.40±2712.62 | 3.104 | 1.313 |
| 2 nd season | | | 8555.50±3448.04 | 4.066 | 7947.00±2191.72 | 2.409 | 1.657 |
| 3 rd season | | | 6904.75±1257.21 | 0.429 | 6855.00±943.49 | 0.564 | 0.135 |

^at¹represents significant comparison of post-spray CHE activity to baseline by bonferroni test after ANOVA; ^bt² represents significant comparison of after rest CHE activity to baseline by bonferroni test after ANOVA; ^ct³ represents significant comparison between post spray and after rest CHE activity by bonferroni test after ANOVA. The baseline value is an average of the 1st and 2nd baseline tests.

Table 7. Butyrylcholinesterase activity by season and age.

| Age range | No. of persons | Mean baseline CHE activity (U/I) Mean ± SD (1 st and 2 nd baseline tests) | Mean post-spray CHE activity (U/I) Mean ± SD | t ¹ | Mean after rest CHE activity (U/I) Mean ± SD | t² | t³ |
|------------------------|----------------|---|--|---------------------|--|---------------------|---------------------|
| <u><</u> 30 | 75 | 6714.04±1115.37 | | | | | |
| 1 st season | | | 6614.49±1319.58 | 6.029 | 6659.4±1187.66 | 3.309 | 2.720 |
| 2 nd season | | | 6594.70±1341.99 | 7.228 | 6873.98±1552.04 | 9.687 ^b | 16.915 ^c |
| 3 rd season | | | 6888.75±1418.79 | 10.582 ^a | 6887.55±1246.90 | 10.509 ^b | 0.073 |
| 31 – 40 | 72 | 7000.47±1340.14 | | | | | |
| 1 st season | | | 6759.39±1050.96 | 6.455 | 6857.26±1437.77 | 3.835 | 2.621 |
| 2 nd season | | | 6714.75±1346.91 | 7.650 ^a | 7035.26±1349.91 | 0.932 | 8.582 ^c |
| 3 rd season | | | 6867.28±1302.43 | 3.566 | 7132.78±1148.01 | 3.543 | 7.109 |
| > 40 | 19 | 6730.22±1153.11 | | | | | |

Table 7. Contd.

| 1 st season | 6616.77±1423.48 | 1.207 | 6933.19±1577.56 | 2.160 | 3.368 |
|------------------------|-----------------|-------|-----------------|-------|-------|
| 2 nd season | 6773.81±1192.53 | 0.464 | 6924.34±1564.99 | 2.066 | 1.602 |
| 3 rd season | 6726.71±1337.17 | 0.037 | 7162.94±1319.35 | 4.605 | 4.643 |

at¹represents significant comparison of post-spray CHE activity to baseline by bonferroni test after ANOVA; bt² represents significant comparison of after rest CHE activity to baseline by bonferroni test after ANOVA; t² represents significant comparison between post spray and after rest CHE activity by bonferroni test after ANOVA. The baseline value is an average of the 1st and 2nd baseline tests.

Table 8. Classification of pesticides used in the study (WHO. 1994-1995).

| Brand name of pesticide | Active ingredient | Action | g/L or g/Kg | Unit of measurement | WHO class | WHO signal | LD 50 | Aquatic | Avian | Bee |
|-------------------------|---------------------|--------|-------------|---------------------|-----------|------------|------------|---------|-------|-------|
| Orthene 75 SC | Acephate | I | 750 | g/Kg | Ш | Caution | 1749 | XXX | xxxxx | XXXXX |
| Orthene Pellets | Acephate | 1 | 970 | g/Kg | Ш | Caution | 1749 | | | |
| ACE 750 WSP | Acephate | 1 | 750 | g/Kg | Ш | Caution | 866 | XXX | XXXXX | XXXXX |
| Pyrinex 48 EC | Chlorpyrifos | 1 | 480 | g/l | II | Warning | 135 | XXXX | XXXX | XXXX |
| Reldan 40 EC | Chlorpyrifos Methyl | 1 | 400 | g/l | IV | Caution | >3000 | XXXXX | XXXXX | XXXXX |
| Danadim 40 EC | Dimethoate | I/M | 400 | g/L | II | Warning | >800 | Х | XXXXX | XXXXX |
| Vyadate 10 G | Oxamyl | N | 100 | g/L | lb | Danger | 6 | XXXXX | XXX | XXXXX |
| Pirimor 50 DG | Pirimicarb | Ţ | 500 | 50%ww | 11 | Warning | 500 - 5000 | Х | 0 | 0 |

significant difference in cholinesterase activity among the different age groups led to the assumption that age was indeed a significant confounder of cholinesterase activity in this study.

Conclusion

In terms of the magnitude of potential exposures to the individual, it is clear that occupational exposures should be of greatest concern, and that exposure especially among supervisors, sprayers and harvesters must be carefully monitored to prevent serious disease.

ACKNOWLEDGEMENT

This project was fully sponsored by Home grown

(K) Limited.

Abbreviations: KFC, Kenya flower council; **KHC,** Kenya horticultural council; **NIPC,** National Pesticide Information Centre.

REFERENCES

Bodgen JD, Quinones MA, El Nakah A (1975). Pesticide exposure among migrant workers in southern New Jersey. Bull.Environ. Contam. Toxicol. 13:513-517.

Brock A, Brock V (1990). Plasma cholinesterase activity in a healthy population group with no occupational exposure to known cholinesterase inhibitors; Relative influence of some factors related to normal inter and intra – individual variation. Scand. J. Clin. Lab. Invest. 50:401–408.

COLEACP (2000). European imports of flowers and ornamental plants 1994 – 2000 http://www.coleacp.org/ (Accessed 8 January 2010).

Coye MJ, Barnett PG, Midtling JE, Velasco AR, Romero P, Clements CL and Rose TG (1987). Clinical confirmation of organophosphate poisoning by serial cholinesterase analyses. Arch. Intern. Med. 147:438-442.

Edmiston S, Maddy KT (1987). Summary of illnesses and injuries reported in California by physicians in 1986 as potentially related to pesticides. J. Vet. Hum. Toxicol. 29:391-397.

Faria NM, Facchini LA, Fassa AG and Tomasi E (2004). Rural work and pesticide poisoning. Cadernos deSaude Publica. 20(5):298-308.

Hennock M (2002). Kenya's flower farms flourish', BBC News 14 February 2002 http://news.bbc.co.uk/1/hi/business/1820515.stm (Accessed 14 May 2010).

Jors E, Morant R, Aguilar G, Huici O, Lander F, Bælum J and Konradsen F (2006). Occupational pesticide intoxication among farmers in Bolivia: a cross-sectional study. Environ. Health J. 5:10.

Kenya Flower Council (KCF) (1996). Kenya flower council code of practice edition 3, December 1996 [online]. [Accessed 10 May 2009]. Available

- at: http://www.pan-uk.org/pestnews/Issue/pn39/pn39p15.htm.
- Knedel M, Bottger RA (1967). Kinetic method for determination of the activity of pseudocholinesterase. Klin. Wochenschr. 45:325-327.
- Lewis PJ, Lowing RK, Gompertz D (1981). Automated discrete kinetic method for erythrocyte acetyl cholinesterase and plasma cholinesterase. Clin. Chem. J. 27:926.
- Lu L (2005). Risk factors to pesticide exposure and associated health symptoms among cut-flower farmers. Int. J. Environ. Health Res. 15(3):161-169.
- Mbakaya CFL, Ohayo-Mitoko GJA, Ngowi AVF, Mbabazi R, Simwa JM, Maeda DN, Stephens J, Hakuza H (1994). The status of pesticide usage in East Africa. Afric.J. Health Sci. 1:37–41.
- Nyambura-Mwaura H (2010). 'Vegetables save Kenya horticulture earnings in 2009', Reuters, 19/March/2010 [online]. Available at: http://af.reuters.com/article/investingNews/idAFJOE62I0CU20100319 (Accessed 14 May 2010).
- Ohayo-Mitoko GJ, Kromhout H, Karumba PN, Boleij, JS (1999). Identification of determinants of pesticide exposure among Kenyan agricultural workers using empirical modeling. Ann. Occup. Hyg. 43:519–525.
- Ohayo-Mitoko GJ, Kromhout H, Simwa JM, Boleij JS, Heederik D (2000). Self-reported symptoms and inhibition of acetylcholinesterase activity among Kenyan agricultural workers. J. Occup. Environ. Med. 57:195-200.
- Quinones MA, Bogden JD, Louria DB, El Nakah A (1976). Depressed cholinesterase activities among farmworkers in New Jersey. J. Sci. Tot. Environ. 6:155-159.
- Richards DM, Kraus JF, Kurtz P, Borhani NO (1983). A controlled field trial of physiological responses to organophosphate residues in farm workers, Environ. Path. Toxicol. J. 2:493-512.

- Saunders LD, Ames RG, Knaak JB, Jackson RJ (1987). Outbreak of Omite-CR-induced dermatitis among orange pickers in Tulare County, California.J. Occup. Med. 29:409-413.
- Schmidt E, Schmidt FW (1986). Fundamentals of diagnostic enzymology. 5th ed. Boehringer Mannheim GmbH. Diagnostica. United States Environmental Protection Agency (USEPA) (2005). How to comply with the worker protection standard for agricultural pesticides: what employers need to know" [online]. Accessed October 18, 2008. http://www.epa.gov/agriculture/agctr.html.
- Waitathu N (2009). Kenya's horticulture business in wilt', New Agriculturist, November 2009 [online]. Available at: http://www.new-ag.info/developments/devItem.php?a=1029 (Accessed 8 January 2010)
- Wicker GW, Williams WA, Guthrie FE (1979). Exposure of field workers to organophosphorus insecticides: sweet corn and peaches. Arch. Environ. Contam. Toxicol. 8:175-182.
- Wu YQ, Wang JD, Chen JS, Chung SC, Hwang SY (1989).
 Occupational risk of decreased plasma cholinesterase among pesticide production workers in Taiwan. Am. J. Ind. Med. 16:659-666.
- Xue SZ (1987). Health effects of pesticides: a review of epidemiologic research from the perspective of developing nations. Am. J. Ind. Med. 32:269-279.