#### FARM WORKERS' HEALTH AND PESTICIDE RESIDUE ANALYSIS OF THREE FARMS IN ETHIOPIA

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ABSTRACT: Cross sectional survey on the health status of the farm workers, engaged with pesticide use, at three agricultural farms; one in Debre zeit and two in Meki, Ethiopia, was conducted. A low prevalence of respiratory symptoms, chronic cough = 2.4%, in the farm workers as well as, chronic cough = 2.7%, in the control group was observed. The values obtained for the measured indices such as forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) in sprayers and non-sprayers, respectively, after short and long duration of employment were significantly lower than the predicted values (p<0.05 and p<0.01). Systolic, diastolic blood pressure and arterial pulse rate between the controls and the studied subjects were not significant (p>0.05). Soil and water samples were analyzed for diazinon and total endosulfan ( $\alpha$ -,  $\beta$ -endosulfan and endosulfan sulfate), using high-performance liquid chromatography (HPLC) with UV detection to evaluate the pesticide residue levels accumulated in the farms during regular pesticide applications. Mean concentrations of β-endosulfan in duplicate soil samples detected in two farms were 0.54 mg/kg and 0.40 mg/kg.  $\alpha$ endosulfan and endosulfan sulphate were not detected in the soil samples. Furthermore, there were no detectable residues of pesticides observed in water samples.

**Key words/phrases:** *Degradation products; Farm workers; Health effects; Pesticide residues; Ventilatory capacity.* 

#### **INTRODUCTION**

Agricultural farm workers are of high-risk groups to pesticide exposure and consequently to pesticide poisonings (Rosenberg and O'malley, 1997). Pesticide poisoning has long been a major public health problem in the developing countries. The easy access to pesticides has also been reported in its use for suicidal intentions (Kishi and Ladou, 2001; Van der Hoek *et al.*, 1998).

Exposure to pesticides under conditions of inappropriate use or insufficient personal protection can cause a range of health effects. Acute poisonings are the most likely to occur and have been identified as a major health

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consequence in the developing world (Hurtig *et al.*, 2003). Chronic health effects due to pesticide exposure include reproductive effects, cancer, peripheral neuropathies, neurobehavioural disorders, impaired immune functions and skin disorders (WHO, 1990).

The presence of pesticides in surface water, ground water, soils and sediments, in different parts of the world, have been documented and reported. The presence of such residues has raised concerns due to the risk to human health through consumption of contaminated water or food, the potential for environmental pollution and damage to biota. This consequently has led to the need for monitoring levels of pesticide residue in food and the environment (Akerblom, 1995).

Endosulfan and Diazinon are amongst the commonly used pesticides in Ethiopia; endosulfan is registered and approved for use on pests of cotton, maize, sorghum, tobacco while diazinon is approved for pests of cereals, vegetables, oilseeds, maize and sorghum (Anonymous, 2000). Enormous information is available on the transport and degradation of these pesticides under different environmental conditions, especially in temperate regions.  $\alpha$ endosulfan was detected in snow samples at concentrations ranging between 0.5 and 1.0 ng/l in Canadian Arctic explained through long range atmospheric transport (Gregor and Gummer, 1989). Endosulfan has been shown to dissipate through volatilization from cotton fields and transported in irrigation and runoff waters in New South Wales (Kennedy et al., 2001). Field studies conducted to assess movement of endosulfan applied on vegetables in the United States revealed formation of endosulfan sulphate and the relative persistence of beta-isomers. Similarly, conservation practices reduced run off and sediment loss and its movement from site of application to surface water run-off (Antonious and Byers, 1997).

The fate of pesticides in tropical soils is not as well understood as that of soils from temperate regions due to limited research in tropical soils or the difficulty in obtaining adequate experimental details for a full interpretation of the results obtained (Racke *et al.*, 1997). The concentration of diazinon in soil and stem samples of rice plants in Guilan province, Iran showed seasonal variations (as high as 55 ng/ml in June and as low as 2 ng/ml in September) (Ghassempour *et al.*, 2002). Field studies to understand the fate of endosulfan in cotton soil in Northern India revealed degradation of the parent compound into endosulfan diol and endosulfan sulphate. Endosulfan diol dissipated almost completely within 28 days (Kathpal *et al.*, 1997).

Laboratory studies on soils in Thailand showed significant correlation between endosulfan adsorption and soil organic matter, cation exchange capacity and clay content, respectively. Degradation of endosulfan was higher in Phrabat soil type collected from 0-20 cm depth (Parkpian *et al.*, 1998).

Existing information on the environmental fate or residue analysis of these or other pesticides is scant in Ethiopia. The widespread use of such pesticides in Ethiopia, in relation to the current attention given to the farming sector, necessitates the need for screening and monitoring pesticides.

The present study was therefore undertaken with the aim of assessing the health status of male and female farm workers exposed to pesticides in three farms in Ethiopia. Surveys such as this one have been focusing solely on the male individuals involved in spraying, neglecting the female groups. The second part of the study is concerned with pesticide residue analysis, with the objective of determining the amount of pesticides possibly present in the soil and water matrices of the three agricultural farms.

### MATERIALS AND METHODS

## Description of the study sites and pesticides used

The study areas are located in Debre Zeit and Meki, located 45 km and 130 km, respectively, away from the capital of Ethiopia, Addis Ababa. The study sites comprised of two farms in Meki, hereafter referred to as Farm A and Farm T (situated 8°N07.612', 38°E50.030' at an altitude of 1653 m and 8°N05.957', 38°E46.656' at 1668 m, respectively) and one in Debre Zeit, referred to as Farm G (10°N02.507', 52°E28.320' at 1965 m) (Figure 1). All the three farms were engaged in growing different kinds of vegetables such as tomatoes, onions, cabbages, pepper and the like mainly for their respective local markets and Addis Ababa. The fields were irrigated with water drawn from River Meki in Farm A, while the other two farms made use of boreholes. A variety of insecticides and fungicides were used to prevent and control pest-induced diseases, as necessary. Both isomers of endosulfan; *viz.*,  $\alpha$  and  $\beta$ , along with diazinon were the major types of pesticides used heavily, for at least a decade.

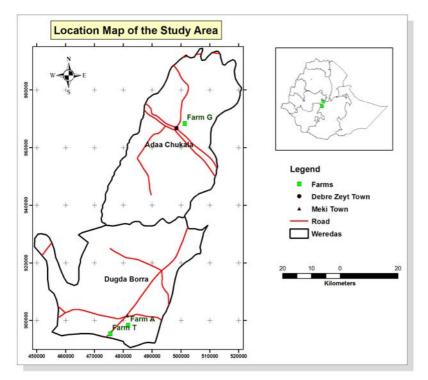


Fig. 1. Location of the study areas

# Subjects

A cross-sectional survey was conducted to assess the health status of 89 farm workers. Specific male individuals were regularly assigned to pesticide spraying activities while other male workers performed irrigation, hoeing and even planting. Females were mainly involved in planting, weeding and harvesting. Seventy four individuals, with no known occupational exposure to pesticides, were used as comparable controls. The controls were selected from local plastic and nail-producing factories. The aim of the study was explained to the study group and individuals willing to participate in the study were included.

# Methods

## **Respiratory symptoms**

A respiratory-symptom questionnaire based on the British Medical Research Council questionnaire (1960) was used along with additional questions regarding occupational, family history and smoking habits during an interview with each subject. Subjects who reported of having suffered from respiratory illnesses, such as tuberculosis, in the past or at present, were excluded from the study.

The following definitions were used based on the British Medical Research Council (1960):

**Chronic cough** - cough in the morning or during the day for more than three months per year

Chronic wheeze - chest wheezing or whistling most days or nights

**Chronic sputum** - sputum in the morning or during the day or night for more than 3 months per year

**Breathlessness** - shortness of breath upon walking with other people of one's own age on level ground

## Ventilatory capacity and blood pressure measurements

Ventilatory capacity measurements of farm workers and controls were performed using a spirometer (VCT-P<sub>1</sub> Mijnhardt, Holland). Spirometric measurements of the forced vital capacity (FVC), forced expiratory volume in one second (FEV<sub>1</sub>), the ratio of FEV<sub>1</sub> to FVC (FEV<sub>1</sub>/FVC %), peak expiratory flow rate (PEFR), maximum mid-expiratory flow rate (MMFR), maximum flow rates at 50% and the last 25% of the vital capacity (FEF<sub>50</sub>, FEF<sub>25</sub>) were taken. Each subject performed at least three attempts and the best value of the three acceptable tests was used as the result of the test (Cotes, 1979). The measured ventilatory capacity values were compared with the predicted values reported by Yoseph Assen and Yalemtsehay Mekonnen, (1985) for healthy Ethiopian population. Blood pressures and pulse rates were measured using a digital blood pressure monitor (Model 1063CVS, China) after five minutes of rest.

## Statistical analysis

Ventilatory capacity values that were not normally distributed were transformed using arc sine transformation. The results of ventilatory capacity measurements in farm workers for comparison to the predicted values were analyzed using the paired t-test. Analysis of variance was used to test the difference between means for ventilatory capacity, blood pressure and pulse rate in the study groups. The level of significance was tested at p-value of 0.05. Analyses were carried out using SPSS statistical software.

# Sampling, sample preparation and clean-up

# **Sampling procedures**

Sample collection was conducted in the month of November 2003 for all farms. Soil samples were collected from onion, tomato and basil plots after pesticide application (nine days after application in Farm A, a week after in Farm T and two months later in Farm G, respectively). Test plots were selected on the basis of recently sprayed plots. A detailed account of the sampling procedure was followed as described by Akerblom (1995). Composite samples consisting of ten soil cores (25 cm x 10 cm i.d.) were collected so as to ensure representative sampling. The soil samples were further partitioned into smaller fractions from which portions were taken to form triplicates. These triplicate samples were wrapped with methanolrinsed aluminium foil and put in polyethylene bags. The samples in this form were transported to the analytical laboratory, of the Department of Chemistry, Addis Ababa University and stored below -18°C until further treatment.

Surface water was collected from furrows that were being irrigated, two weeks after spraying in Farm A and, a week later, in Farm T. Water samples were kept refrigerated at 4°C until used for extraction.

# **Reagents and chemicals**

Standards of  $\alpha$ -endosulfan (96%),  $\beta$ -endosulfan (99%), endosulfan sulfate (97.5%) and diazinon (97.5%) were purchased from Ehrenstorfer Reference Compound Laboratories (Augsburg, Germany). Stock solutions of the standards (100 mg/l) were prepared in methanol. Working solutions were prepared by diluting the stock solutions as required.

Solvents used, cyclohexane and hexane (BDH, England), were distilled before use. HPLC-grade methanol and acetonitrile (Sigma-Aldrich, Germany) were used during analysis. Anhydrous sodium sulphate and sodium chloride were heated at 130°C for 4 hours before use. Florisil (60-100 mesh) was also activated prior to use.

# Chromatographic system

Pesticide residue analyses were performed with a high-performance liquid chromatography system (LKB Model 2150 HPLC pump, Stockholm, Sweden), a Gilson 118/119 UV/VIS variable wavelength detector (Middleton, USA), a loop injector VICI AG Valco, Schenkon, Switzerland) fitted with a 20  $\mu$ l loop and a Kipp and Zonen Partille, Holland) chart

recorder. Separation was achieved using a 4  $\mu$ m Genesis C<sub>18</sub> column (Jones Chromatography, 25 cm x 2.1 mm i.d.,UK). A 25  $\mu$ l syringe (Hamilton Co., USA) and a 100  $\mu$ l SGE syringe (Scantec Lab., Australia) were also utilized for injection of the extracts.

## Sample processing

Soil pH (water to soil ratio of 1:2.5) and moisture content were measured as per the procedures for soil and plant analysis (Sahlemedhin Sertsu and Taye Bekele, 2000). Particle size analysis was determined using the hydrometer method and organic carbon content was determined using the dichromate method of Walkley and Black (Sahlemedhin Sertsu and Taye Bekele, 2000).

## Extraction of soil and water samples

Samples were extracted following the procedures described for extraction of soil and water for pesticide residues by Akerblom (1995). Soil samples were first air-dried and sieved through a 2-mm mesh. 14 ml of 0.2 M ammonium chloride solution was added to 20 g of soil and allowed to stand for 15 minutes. Samples were then agitated; first, by vigorous manual shaking for an hour and later on a shaker (Gallenkamp, England) for 2 hours in 100 ml of cyclohexane/acetone (1:1, v/v). The mixture was allowed to stand overnight. Then, the organic phase was separated and dried with 10 g of anhydrous sodium sulphate. The dried extract was then transferred through Whatman No.1 filter paper into a 100 ml evaporation flask along with 20 ml acetone rinse of the sodium sulphate. The solvent was then removed by a rotavapor (Buchi Rotavapor Model 461, Switzerland), carried out at 30°C to an approximate volume of 10 ml. The concentrated extracts were transferred to a 4 ml vial using 2 ml acetone and refrigerated at 4°C until clean-up.

Water samples were well shaken before extraction. A 500 ml of water sample was transferred into a 1 l separatory funnel to which 50 g of sodium chloride was added and thoroughly shaken. This was folowed by addition of 120 ml of ethyl acetate and the separatory funnel was stoppered and shaken for 2-3 minutes releasing the pressure intermittently. The layers were allowed to separate for 10 minutes and then the organic layer was collected separately. Extraction was performed for two more times in the same manner, i.e., using 60 ml ethyl acetate each time. The combined ethyl acetate extracts were dried with 15 g sodium sulphate. The remaining sodium sulphate was washed with additional 20 ml ethyl acetate and constituted to about 5 ml. The processed extract was finally stored at  $4^{\circ}C$ .

# **Extract clean-up**

Sample clean-up was performed as per the procedures of United States Environmental Protection Agency, U.S. EPA Method 3620B (U.S. EPA, 1996) with slight modifications on the amount of eluents used. Twenty grams of florisil was packed into a chromatographic column followed by anhydrous sodium sulphate (1-2 cm) and the column was pre-eluted with 30 ml of hexane. The 10 ml extract was added to the top of the column along with 2 ml rinse of hexane, before exposure of the sodium sulphate to air. The column was first eluted with 50 ml of ethyl ether/hexane (6/94, v/v) and then followed by 100 ml of ethyl ether/hexane (15/85, v/v). The same procedures were followed for the clean-up of organochlorine as well as organophosphorus pesticides, except in the use of the third elution of 100 ml of ethyl ether/hexane (50/50, v/v), in the case of diazinon. Each eluate was collected separately, concentrated to dryness using a rotavapor and the final residue was leached with 10 ml of methanol and refrigerated until the final chromatographic analysis.

## Sample analysis

The mobile phase for endosulfan analysis comprised of acetonitrile:water (70:30, v/v), at a flow rate of 0.2 ml/min and maximum absorbance set at 214 nm (Siddique *et al.*, 2003). A mixture of methanol:water (80:20, v/v) was used as a mobile phase for analysis of diazinon at a wavelength of 220 nm (Farran *et al.*, 1988). The column was always flushed with acetonitrile:water (30:70, v/v) after each analysis. The mobile phase was degassed, prior to use in the flow system, by ultrasonic agitation (Decon, USA).

External standardization technique was performed for qualitative purposes. Analytes were identified by comparing their retention times with that of reference standards. The use of a constant flow rate of the mobile phase throughout analysis allowed the reliability of retention time for identification of analytes of interest. Further confirmation of analytes was obtained by spiking samples with known standards. Quantitative determinations of the analyte concentration were based on peak height measurements.

## Limit of detection

The limit of detection was determined by adjusting the recorder to give a baseline noise level at 10 mV and then injecting increasing amount of standards. The concentration giving a peak two times the noise level was

taken as the limit of detection (Akerblom, 1995).

#### RESULTS

### **Population characteristics**

The number of males was proportionally greater than that of females in the study groups as well as controls. Forty six (51.7%) of the farm workers were sprayers who were responsible for mixing, loading and applying the formulated pesticides to plants. Forty three (48.3%) of the farm workers were categorized as non-sprayers. These non-sprayers performed various tasks such as planting, weeding, irrigation and harvesting. Though this group of individuals did not spray pesticides, the male non-sprayers would mix and pour pesticides into knapsack sprayers. Individuals in the control group were employed as labourers in the factories they worked at. The mean age of the male and female farm workers was found to be 22 and 20 years (range: 18-40 years), their mean height was 1.68 and 1.57 cm (range: 1.42-1.80 cm), their median duration of employment was 1 year (range: 0.08-10 years) and their mean level of education was 7.4 and 7.5 years, respectively.

#### **Respiratory symptoms**

The prevalence of respiratory symptoms in the 89 farm workers and 74 controls are presented in Table 1. A very low prevalence of respiratory symptoms was observed in both groups. Two sprayers (2.2%) and two individuals (2.7%) from the control group replied having a chronic cough.

Respiratory symptoms	Farm workers	Control group (
	(n=89)	n=74)
Chronic cough	2 (2.2%)	2(2.7)
Chronic phlegm	0	0
Breathlessness	0	0
Wheezing	0	0
Cough/phlegm	0	0

Table 1. Prevalence of respiratory symptoms in farm workers and the control group<sup>a</sup>

<sup>a</sup>Data are presented as number of respondents (percentage).

### Ventilatory capacity and cardiovascular measurements

### Ventilatory capacity

Interpretation of the test indices was limited to FVC,  $FEV_1$  and  $FEV_1/FVC\%$  since these are the basic parameters used in interpreting spirometric results (Balmes and Scannell, 1997). The observed mean (SD) values of FVC,  $FEV_1$ ,  $FEV_1/FVC\%$  and PEFR obtained for sprayers was

3.03 (1.10), 2.44 (0.72), 87.09 (11.01) and 3.80 (1.09), respectively, followed by 3.16 (0.82), 2.87 (0.90), 97.91 (11.17) and 5.40 (2.26), for nonsprayers, respectively; while the controls showed values of 3.40 (0.78), 2.94 (0.66), 96.05 (12.29) and 4.95 (1.88), respectively. Table 2 presents the measured and predicted FVC, FEV<sub>1</sub>, FEV<sub>1</sub>/FVC% and PEFR in farm workers based on their job category and duration of employment. Significantly lower observed values compared to predicted values were recorded in sprayers and non-sprayers for FVC (p<0.01 and p<0.05), FEV<sub>1</sub> (p<0.01 and p<0.05) and PEFR (p<0.01 and p<0.05) despite the length of their employment period. On the other hand, FEV<sub>1</sub>/FVC% was found to be not significantly different in non-sprayers working for short as well as longer periods. However, FEV<sub>1</sub>/FVC% was shown to be significantly higher than predicted (p<0.01) in the sprayers irrespective of the duration of employment.

Job category	Employment (yrs)	FVC (L)	FEV <sub>1</sub> (L)	FEV <sub>1</sub> /FVC%	PEFR (L/m)
	(915)	(E)	(Ľ)		(L/ III)
Non-sprayers	<1	3.14 <sup>d</sup> (1.15)	2.55°(0.74)	94.62 (11.05)	4.40 <sup>d</sup> (1.53)
		3.82 <sup>b</sup> (0.63)	3.30 <sup>b</sup> (0.62)	85.96 <sup>b</sup> (3.09)	10.62 <sup>b</sup> (1.69)
	>1	2.80°(1.01)	2.19°(0.68)	93.98 (14.6)	4.75°(1.29)
		3.49 <sup>b</sup> (0.50)	3.00 <sup>b</sup> (0.50)	85.6 <sup>b</sup> (2.75)	11.48 <sup>b</sup> (1.48)
Sprayers	<1	3.15 <sup>d</sup> (0.71)	2.89 <sup>d</sup> (0.53)	100.57 <sup>d</sup> (5.73)	5.17 <sup>d</sup> (1.48)
		4.74 <sup>b</sup> (0.41)	4.14 <sup>b</sup> (0.33)	87.42 <sup>b</sup> (1.48)	9.32 <sup>b</sup> (0.44)
	>1	3.72 <sup>d</sup> (0.72)	3.02 <sup>d</sup> (0.77)	90.80 <sup>d</sup> (15.64)	4.55 <sup>d</sup> (2.05)
		4.49 <sup>b</sup> (0.59)	3.97 <sup>b</sup> (0.41)	88.50 <sup>b</sup> (2.58)	9.04 <sup>b</sup> (0.53)

Table 2. Ventilatory capacity in farm workers by job category and duration of employment<sup>a</sup>

<sup>a</sup>Ventilatory capacity are presented as mean (SD)

<sup>b</sup>predicted values of Yoseph Agonafir and Yalemtsehay Mekonnen (1985)

<sup>c</sup>p<0.05, <sup>d</sup>p<0.01

Comparison of ventilatory capacity between controls and farm workers, as a mean percentage of predicted pulmonary function, is given in Table 3. A higher  $FEV_1$  percent predicted was obtained in the controls, though the difference was not significant. Significantly higher  $FEV_1/FVC\%$  was obtained in the controls (p<0.05).

Ventilatory capacity	sprayers	non-sprayers	controls
FVC% pred.	74.2 (18.6)	82.40 (27.3)	75.7 (18.9)
FEV <sub>1</sub> % pred.	72.9 (17.8)	78.2 (23.7)	82.8 (30.9)
FEV <sub>1</sub> /FVC%	109.49 (15.2)	101.5 (13.0)	116.94 <sup>b</sup> (13.4)
PEFR % pred.	53.9 (21.1)	36.1 (13.1)	54.9 <sup>b</sup> (25.8)

Table 3. Comparison of ventilatory capacity between farm workers and controls<sup>a</sup>

<sup>a</sup>Data presented as mean (SD)

<sup>b</sup>p<0.01

#### **Cardiovascular measurements**

The mean (SD) values of systolic, diastolic blood pressure and pulse rates of sprayers and non-sprayers were 118.9 (9.45), 79.3 (5.80), and 68.4 (6.72) and 117.4 (5.60), 78.2 (4.30), 69.7 (7.75), respectively. On the other hand, the means (SD) of systolic, diastolic blood pressure and pulse rates of the controls were found to be 120.7 (10.3), 81.8 (6.7) and 70.4 (5.3), respectively. The sprayers and non-sprayers had slightly lower values than the controls, though the difference was not significant (p>0.05). No significant differences were found in systolic, diastolic blood pressure and pulse rates among non-sprayers employed for a short as well as longer duration. The same result was also obtained for sprayers as well (Table 4). One sprayer and one non-sprayer had diastolic blood pressure below and equal to 90 while one individual from the control group had a systolic blood pressure greater than 140. Neither bradycardia nor tachycardia was found to be prevalent in the farm workers as well as the control group.

Job category	Employment (yrs)	Вр		Pulse
	Q /	Systole	Diastole	
Non-sprayers	<1	117.4 (4.84)	77.6 (6.89)	69.6 (7.60)
	>1	116.6 (4.97)	77.3 (4.69)	70.7 (6.64)
Sprayers	<1	119.2 (7.60)	79.2 (4.93)	68.5 (5.55)
	>1	118.4 (9.38)	78.6 (9.0)	67.2 (5.72)

Table 4. Blood pressure (Bp) and pulse rate in farm workers by job category and duration of employment.

Data presented as mean (standard deviation) \**p*<0.05,\*\**p*<0.01

\**p*<0.05,\*\**p*<0.01

### Pesticide residue analysis

#### **Soil characteristics**

A list of the characteristics of the soils from the study area is shown in Table 5. The soil samples under investigation were found to have low organic carbon content. In addition, pH of the soils ranged from neutral (7.2) to

slightly alkaline (7.5 and 7.7). Sand comprised the dominant component of the soils in all the farms.

Farm	Locality	pН	Moisture	Composition, %					Texture
	-	•	%	oc	om	sand	silt	clay	
А	Meki	7.2	5.5	1.03	1.77	50	24	26	Sandy clay loam
Т	Meki	7.7	1.9	0.97	1.67	65	21	14	Loamy sand
G	Debre zeit	7.5	4.1	1.46	2.51	39	33	28	Sandy loam

Table 5. Selected physico-chemical properties of the soils on the farms.

oc- organic carbon, om-organic matter

#### **Retention time and linearity**

HPLC analysis of samples showed retention times of the  $\alpha$ -,  $\beta$ -isomers and the sulfate metabolite as well as that of diazinon to be 24.16, 19.16, 12.16 and 8.41 minutes, respectively. Linearity of the detector response was checked with five standard solutions of concentration in the range of 0.1-0.6 ppm (Figure 2). The correlation coefficients (r<sup>2</sup>) obtained were 0.996, 0.993 and 0.995 for  $\alpha$ -,  $\beta$ -endosulfan and endosulfan sulphate, respectively. The limit of detection was determined to be 0.1 mg/kg.

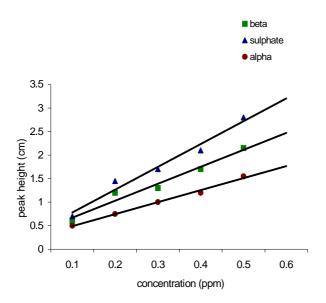


Fig. 2. Linearity of the detector response.

#### **Residues of endosulfan**

Endosulfan residues were detected in the soils of farm A and G (Figure 3). The mean concentrations of replicate analysis of  $\beta$ -endosulfan detected in Farm G were 0.54 mg/kg and 0.40 mg/kg in Farm A.  $\alpha$ -endosulfan and its principal metabolite, endosulfan sulphate, were not detected in the soils, and therefore, it was not possible to evaluate the total residues of endosulfan (comprising  $\alpha$ - and  $\beta$ -endosulfan and endosulfan sulfate).

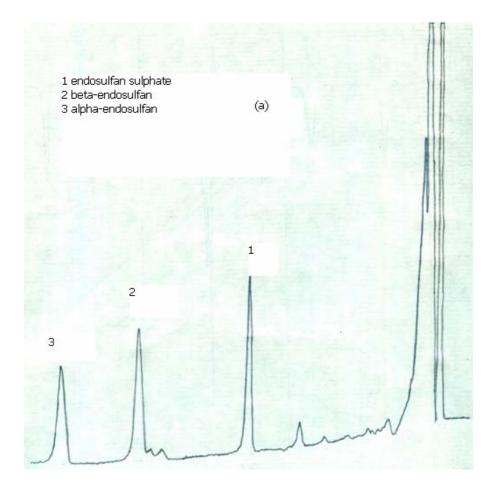


Fig. 3. Examples of chromatograms showing a) 10 ppm standard. Reversed-phase HPLC conditions-Column: Genesis RP C18, 25 cm x 2.1 mm, 4  $\mu$ m; mobile phase 70% acetonitrile - 30% water; flow rate:0.2 mL min-1; UV detection at 214 nm.

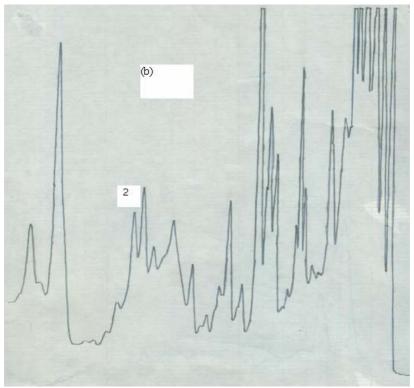


Fig. 4. Examples of chromatograms showing b) beta-endosulfan in soils. Reversed-phase HPLC conditions- Column: Genesis RP C18, 25 cm x 2.1 mm, 4  $\mu$ m; mobile phase 70% acetonitrile - 30% water; flow rate:0.2 mL min-1; UV detection at 214 nm.

#### DISCUSSION

### **Respiratory symptoms**

The findings of the lower respiratory symptoms, among the farm workers, are different from that of the commonly reported results of cross-sectional health studies. For example, Yalemtsehay Mekonnen and Tadesse Agonafir (2002) reported higher prevalence of cough (9.4%) in farm workers in the Rift Valley region, in contrast to the prevalence of chronic cough (2.2%) in the present study. These same authors also reported the prevalence of phlegm (9.4%), wheezing (8.9%) and breathlessness (21.2%) in farm workers as opposed to a prevalence of 5.3%, 8.4%, 10.6% and 16.8%, respectively, in the control group. The study of Kibruyisfa Lakew and Yalemtsehay Mekonnen (1998) also reported the prevalence of wheezing (13.9%), and cough (7.2%) among farm workers. A similar study of Croatian vineyard and orchard workers revealed a high prevalence of

chronic cough (27.6%), breathlessness (74.1%) and chest tightness (27.7%) compared to values of 15.7%, 5.2% and 0%, respectively, in the control group (Zuskin *et al.*, 1997). However, differences exist in the smoking habits and duration of employment of the subjects in the present study and the above mentioned studies. For instance, the mean length of employment for farm workers in the study of Kibruyisfa Lakew and Yalemtsehay Mekonnen (1998) was 8.5 years with a reported smoking prevalence of 22.1%, as well. In the Croatian study, 52.9% of vineyard workers were smokers and had been employed at the farms for an average of 14 years. Therefore, this fact could be one of the reasons for the observed lower prevalences in the present study.

In spite of this, the work of Gamsky *et al.* (1992) seems to be in agreement with the results of this study. The above authors reported the prevalence of chronic cough, chronic phlegm and persistent wheeze as being low; 1.6%, 5.1% and 2.8%, respectively, among the population of Hispanic farm workers compared to other populations of the same ethnic group. In this study as well, 47% of the population comprised of smokers, a factor which could perhaps account for the difference in the observed prevalence between their study and the present study. Similarly, in a case-controlled study of pesticide applicators and controls in Arkansas, USA, the prevalence of self-reported asthma and respiratory symptoms were found to be not different in the two groups (4-6%), with higher rates recorded among smokers (Jones *et al.*, 2003).

In addition, the pesticides that were used in the present study were chemicals that belonged to different groups including organochlorines, organophosphates, dithiocarbamate fungicides and so on. However, most of the pesticides applied in the farms in the study of Yalemtsehay Mekonnen and Tadesse Agonafir (2002), and Kibruyisfa Lakew and Yalemtsehay Mekonnen (1998) were organophosphate pesticides. It is believed that organophosphate and carbamate compounds can bring about respiratory symptoms through cholinesterase inhibition (Hoppin *et al.*, 2002). Therefore, it is also possible that the prevalence of respiratory symptoms could have been observed had the farms been restricted to the use of specific groups of pesticides.

## Ventilatory capacity and cardiovascular measurements

Reduced FVC and  $FEV_1$  values were obtained in all farm workers in spite of the length of exposure. Yalemtsehay Mekonnen and Tadesse Agonafir (2002) have likewise reported lower FVC and  $FEV_1$  values among non-

smoker supervisors and non-smoker sprayers. Zuskin et al. (1997) have similarly reported reduced FVC values in vineyard workers, differences being significant for workers employed for more than 10 years. Reduced lung function values were also obtained in workers employed for a short duration of period. Information from the occupational history of farm workers in this study revealed that 28% of sprayers had previously been employed in other farms, with their job description being sprayers. In addition, 20% of sprayers and 23% of non-sprayers had been working in other farms before the present job or had participated in agricultural activities. Moreover, farm workers in this study had low socio-economic status, which could have further affected lung function status, as evidenced in the lower lung function values in workers, irrespective of the duration of employment period. Studies have shown that poor economic or educational level can have an impact on respiratory health and function (Steinberg and Becklake, 1986; Higgins et al., 1977). A reduction in FVC and FEV<sub>1</sub>, in the absence of a significant change or increased FEV<sub>1</sub>/FVC% is consistent with that of a restrictive ventilatory defect (Balmes and Scannell, 1997) and the result obtained may give some indication of the pulmonary function status of the farm workers. However, it should be noted that pulmonary function testing is just one aspect of the evaluation of respiratory health and further confirmatory tests are recommended for diagnosis. The pulmonary function indices (FEV<sub>1</sub>% predicted and PEFR percent predicted) were observed to be higher in the control group, though the difference was significant for FEV<sub>1</sub>/FVC%. The probable reason for this could be the higher number of ex-smokers and smokers in the control group.

The absence of significant differences in blood pressure and pulse rate measurements between the farm workers and the controls and among the farm workers suggests that there was no abnormality at this level. The possible explanation could be the exposure of workers to a variety of pesticides in the farm, ranging from slightly to moderately toxic types. Cardiovascular effects such as tachycardia and hypertension, bradycardia after intoxication and hypotension are known to occur with organophosphate and carbamate insecticides (Benowitz, 1997). It is therefore possible to extrapolate that these effects could have been pronounced had the farm workers been regularly exposed to the above groups of pesticides.

### **Residues of endosulfan**

The amount of  $\beta$ -endosulfan determined in this study is not comparable to

other similar studies. The highest concentration of total endosulfan residues detected in soil samples from cashew nut plantations was reported to be 0.01 µg/g by Ramesh and Vijayalakshmi (2002), sprayed three months before sample collection -similar to a period of sample collection of two months after the last spray in Farm G. Szeto and Price (1991) detected β-endosulfan at a concentration of 0.42 µg/kg and 0.45 µg/kg in loamy sand and silt loam soils, respectively, in vegetable farms in Canada. The farms in which the residues were detected by the above authors had been involved in farming for a period of at least 20 years. However, farms in the present study had not been applying pesticides for such a long period, even though concentrations detected were much higher. The discrepancy between these results could possibly be attributed to sample size and other factors such as vegetation cover. The number of samples collected in both studies (Ramesh and Vijavalakshmi, 2002; Szeto and Price, 1991) is much greater than what has been collected in the present study. In addition, Ramesh and Vijavalakshmi (2002) described the area from where soil samples were collected as being covered by the tree canopy of the cashew plant, whereby fewer residues could have been deposited on the soil. This argument is substantiated by the finding that the authors reported detecting higher levels of total endosulfan in leaf samples (Ramesh and Vijayalakshmi, 2002).

β-endosulfan has been reported to be more persistent than α-endosulfan (Antonious *et al.*, 1998; Kathpal *et al.*, 1997), while the latter dissipates more rapidly. The dissipation of α-endosulfan in this study could be attributed to volatilization. Similar findings have been disclosed by Szeto and Price (1991) who reported the dissipation of α-endosulfan from loamy sand and silt loam soils, organic carbon content of which was in the range of 1.0-6.5%. The soils of farms in the present study had organic carbon content less than 2%, results similar to that of Szeto and Price (1991).

Volatilization is a major pathway of pesticide loss, the rate of which is higher than other routes such as chemical degradation, runoff or leaching (Van der Werf, 1996). The higher volatilization rate of  $\alpha$ -endosulfan is attributed to its relatively high vapour pressure, its high Henry's law constant and low water solubility (Antonious *et al.*, 1998).  $\alpha$ -endosulfan is known to possess a higher vapour pressure (6.0 x 10<sup>-3</sup> Pa at 25°C) and Henry's law constant (10.23 Pa m<sup>3</sup>/mol) as compared to  $\beta$ -endosulfan (3.0 x 10<sup>-3</sup> Pa at 25°C, 1.94 Pa m<sup>3</sup>/mol), characteristics which favour its higher rate of disappearance. The prevailing environmental conditions such as temperature, relative humidity could have influenced volatilization as well. Experimental evidence for the relative volatilization of  $\alpha$ -endosulfan has been documented from solid surfaces as well as aqueous systems (Singh *et al.*, 1991; Cotham and Bidleman, 1989). Furthermore, adsorption of  $\alpha$ -endosulfan in soil has been correlated with organic matter, cation exchange capacity and clay content of the soil (Parkpian *et al.*, 1998). The soils under investigation had a low percentage of organic matter and a higher percentage of the sand component probably leading to weaker adsorption.

Numerous studies have demonstrated that endosulfan sulfate is the most common degradation product, formation of which occurs by oxidation of the sulfite group through micro-organisms. However, the findings of this study are quite different from that of others in that endosulfan sulphate was not detected. One possible explanation can be attributed to the insignificant effect of microbial action-specifically those groups capable of oxidizing endosulfan. Guerin and Kennedy (1992) studied the dissipation of endosulfan in aqueous systems and reported that no endosulfan sulfate had been formed in aerobic media that was maintained sterile, suggesting that endosulfan sulfate would not be formed in aerated waters in the absence of microbial activity.

According to Peterson and Batley (1993), endosulfan sulfate is more likely to be formed in soils and sediments where the microbial population is high. Similarly, Shalini-Singh and Dureja (2000) reported the degradation of  $\alpha$ -,  $\beta$ - isomers and endosulfan sulphate as being higher in non-sterilized soils as compared to sterilized soils under laboratory biodegradation studies.

The other possible reason could be the depth of soil from which samples were taken. Soil cores were taken at a depth of 25 cm, which is quite deep compared to that reported by other authors. For instance, Kennedy *et al.* (2001) reported that a great majority of endosulfan residues were present in the surface layer of 6 cm, with concentrations declining much beyond 8 to 10-cm soil profile. Similarly, Kathpal and his colleagues (1997) found that endosulfan and its breakdown products (endosulfan diol and endosulfan sulfate) remained in the upper 10-cm layer of soils. In particular, endosulfan sulfate remained restricted to the top 5-cm layer. In addition, the authors reported that the amount of endosulfan present in the lower layer was much lower than in the top 5-cm layer. Thus, the depth from which soil cores were taken in the present study could possibly have led to the results obtained.

The presence of pesticides in runoff from agricultural lands draining into water bodies is of particular concern for those pesticides such as endosulfan that are toxic to aquatic organisms. However, no significant residues of pesticides were detected in water samples. Endosulfan and diazinon have log  $K_{ow}$  values of 4.79 and 3.30, respectively, (Tomlin, 1997). Compounds with high  $K_{ow}$  values tend to partition strongly into the organic matter of soils (Connell, 1997). Peterson and Batley (1993) have shown that a large proportion of endosulfan would associate with sediments in aquatic systems. In addition to this, the compounds have low solubilities 0.33 mg/l and 60 mg/l for endosulfan and diazinon, respectively, in water. It is, therefore, possible to assume that the above mentioned properties would favour the adherence of these hydrophobic pesticides to the soil system rather than to water. Castilho *et al.* (2000) have reported findings of highest concentrations of DDT in sediments of Atoya river, Chinandega, Nicaragua while pesticides such as lindane were mainly found in water samples from rivers. It should be noted that DDT is a hydrophobic pesticide with log  $K_{ow}$ value of 6.36 compared to the corresponding value of 2.67 for lindane, which seems to be in agreement to that of the earlier explanation.

No detectable residues of diazinon were found in soil samples of Farm T. Studies have shown that adsorption of diazinon in soils with low organic matter less than 2% is related to the silt and clay content of the soil (Arienzo et al., 1994). The low percentage of silt and clay fraction as well as the organic matter of the soils of farm T could possibly have resulted in less adsorption of diazinon. Szeto and Price (1991) reported not detecting residues of diazinon in loamy sand soils, with organic carbon between 1 and 1.8%, largely dominated by the sand fraction while the silt and clay content was minimal, similar to that in the present study. Pesticides that are not strongly adsorbed to soil are less likely to be persistent because they are more exposed to the forces of degradation and volatilization. Derivatives of this group of pesticides are more susceptible to hydrolysis than the other groups of organophosphate pesticides. Diazinon is known to hydrolyze in an alkaline and acidic media. In alkaline conditions, diethylphosphoric acid and 2-isopropyl-4-methyl-6-oxypyrimidine are formed (Gruzdyev, 1988). It is, therefore, possible to assume that diazinon could have undergone hydrolysis in the alkaline soils in light of its weak adsorption to soils and low sorption coefficient value.

#### CONCLUSION

The present study indicated that farm workers with occupational pesticide exposure had lower lung function values, irrespective of the duration of employment. However, respiratory symptoms amongst the workers were low and comparable to those of controls with no pesticide exposure. Further work ought to be carried out to conduct follow-up studies and effect of longterm exposure. More importantly, regular medical check-ups should also be encouraged.

One of the parent isomers of the pesticide endosulfan,  $\beta$ -endosulfan, was detected in the soils of the farms while no detectable levels of diazinon were recorded.  $\beta$ -endosulfan is known to be persistent in the environment. Further investigation could be suggested to be carried out to better understand the fate of this pesticide and its degradation products in the context of Ethiopian weather and soil type conditions. The absence of detectable residues of diazinon and endosulfan sulphate may differ seasonally which could be an indication of the need for regular monitoring of the pesticide levels.

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