DETERMINATION OF LEVELS OF AFLATOXIN IN MAIZE SAMPLES: A CASE STUDY OF UASIN GISHU COUNTY

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University for award of a degree. No part of this thesis may be produced without the prior written permission of the author and University of Eldoret.

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Declaration by supervisors

This thesis has been submitted with our approval as University supervisors for examination with our approval as University supervisors.

Signature……..Date…….

Dr. Samuel Lutta

Signature……..Date…….

Prof. Fred Segor

DEDICATION

I dedicate this work to farmers so that they can follow the correct pre and post-harvest practices that minimize aflatoxin contamination in order to minimize any loss caused by fungal infection and to meet market standards.

ABSTRACT

The determination of levels of aflatoxins was done in Uasin Gishu County Kenya. Aflatoxins are compound produced by the two fungi; *aspergillus flavus* and *aspergillus parasiticus.* The main aflatoxins B_1, B_2, G_1 and G_2 have been detected in cereals. In Kenya outbreaks of maize poisoning was reported in Makueny County in 2004 and 2005. Twenty samples from every division were collected and pre and post-harvest farming practices were identified using questionnaire. At the market level, 15 samples per month were collected from various outlets for the 3 consecutive months; June, July and August. All samples were analyzed for the presents of aflatoxin using the TLC method, procedure used adopted from KEBS. Quantification of an aflatoxin was done using ELISA method. The four main types of aflatoxins B_1, B_2, G_1 and G_2 were detected an indication that the fungal *A. parasiticus* and *A. flavus* are present within the region. The results showed that in the whole county 49 out of 165 samples tested positive of aflatoxin, 36 from the farms and 13 from the market outlets. Ainabkoi division had aflatoxin mean level of 3.26 ppb as compared to other five divisions. Six samples tested positive of aflatoxin with the highest concentration of 21.90 ppb with minimum 4.40 ppb. 2 samples were above MTL. In Kesses division, among the twenty samples collected, 7 tested positive. The sample with the maximum aflatoxin concentration recorded was 11.20 ppb while the lowest was 1.60 ppb only 1 sample was above the MTL with divisional mean being 1.48 ppb. Moiben division had the highest number of positive samples (10 samples) which contributed to higher mean of 4.26 ppb. In Kapseret division, 8 samples tested positive and all samples which tested positive were below 10 ppb, the limit set by KEBS. The mean value recorded was 1.70 ppb with the highest concentration being 8.90 ppb and the lowest was 1.70 ppb. In soy division, only 3 samples tested positive with the mean of 0.68 ppb and all the 3 samples were within the recommended standard value. In Turbo division 4 samples tested positive but 2 samples had 10.60 ppb and 15.20 ppb which were above the limit recommended by KEBS. Divisional mean value was 1.91 ppb. From the samples collected from the market 13 out of 45 samples tested positive accounting for 27.2 %. Aflatoxin level over the three months found to be in order July > August > Junewith the respective values of 22.22 ppb, 17.60 ppb and 9.33 ppb. June had the sample with lowest concentration of 0.90 ppb while July had the sample with the highest concentration of 68.10 ppb. Ten factors causing aflatoxin contamination in maize was studied in the whole county and the data were analyzed using descriptive statistics the significant level within 0.5 to 1.5. Sorting, storage method, clean storage device, granary use and use of pesticide were within this range. ANOVA confirmed that there was significant difference in the test results and also in aflatoxin concentration; the F-ratio was 1.649 which was above 1 when confidence level of 95 % was used. For the post-hog test analysis considering the mean difference at the 0.05 and 0.1 levels confirmed that there was a relationship within the county in pre and post-harvest practices and also aflatoxin concentration. Levene's and t-test results agreed with some of the tested variables in aflatoxin contamination because p-values obtained were above the critical value of 0.05

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ABBREVIATIONS

AFL	Aflatoxin
AOAC	Association of Official Analytical Chemist
ANOVA	Analysis of Variance
BSA	Bovine Serum Albumin
C.P.C	Corn Product Company
ELAS	Enzyme-Linked immunoassay
ELISA	Enzyme-Linked immunosorbent assay
FAO	Food and Agricultural Organization
FDA	Food and Drug Administration
HRP	Horseraish peroxidase
HPLC	Higher Performance Liquid Chromatography
IR	Infrared Light
KEBS	Kenya Bureau of Standards
LD_{50}	Lethal dose
MTL	Maximum Tolerated Level
ODNR	Overseas Development Natural Resource Institute
ppb	Parts per billion
TLC	Thin Layer Chromatography
WHO	World Health Organization
UV	Ultraviolet light
PFDB	Pathogenic Fungi Database

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I am grateful to my grandmother Christine Rono and my parents for paying my school fees and meeting all my needs during my studies.

CHAPTER ONE

INTRODUCTION

1.1 Background

In 1960 in the United Kingdom, more than 100, 000 young turkeys died after eating peanut meal (John, 2008). Since the causal agent was unknown and the affected animals exhibited similar symptoms, the disease was labeled: turkey "X" disease (Blount.,1960). In 1961 a toxic compound was isolated from Brazilian groundnut meal used in turkey feed (Sargeant *et al.,* 1961). Since the compound was produced by *aspergillus flavus* and *aspergillus parasiticus* and the link was named aflatoxin. The term 'aflatoxin' is a composite word derived from 'A' for *aspergillus,* 'fla*'* for *flavus* and 'toxin' to refer to group of highly toxin metabolites produced by moulds of *aspergillus flavus* and *aspergillus parasiticus.* It is now known that aflatoxins are not one compound but a group of more than eighteen compounds.

Aflatoxins B_1, B_2, G_1 and G_2 have often been detected in groundnut, maize and other agricultural commodities (WHO, 1979) and aflatoxin M_1 and M_2 Has been detected in milk and dairy products from animal fed with contaminated feed of aflatoxin B_1 and B_2 which makes it to reach man indirectly (Iongh *et al.,* 1964). Nursing infant may expose to to aflatoxin in breast milk. Aflatoxins were detected in 90 of 264 breast-milk samples collected from nursing mothers in Africa (Zarba *et al.,* 1992). Occupation exposure to aflatoxins occurs by inhalation of dust generated during the handling and processing of contaminated crops and feeds. Farmers and other agricultural workers have the greatest risk of occupational exposure in workplace and storage areas or during unloading of raw materials used in animal feeds (Ghosh *et al.,* 1976, Autrup *et al.,* 1993)

Maize is the most important food crop in Kenya, over 85 % of population depends on it as their primary food source. Every year up to 1,600,000 hectares of maize is grown and 75 % of this comes from small holder farmers averaging to 2 hectares. Maize production for the last few years has been below the national consumption level of 34 million bags. Consequently Kenya has become a net importer of maize grain following decline in production due to changing weather among other factors.

In Kenya outbreaks of maize poisoning in Makueni County in 2004 and 2005 resulted in 395 cases of severe aflatoxin poisoning with 157 deaths. Since then little has been done to mitigate future outbreaks. On October 2nd 2008 there was alert over maize poisoning (Gatonye, 2008). The survey in Makueni showed local maize to contain extremely higher levels of aflatoxins almost 50 times over the safe levels of 20 ppb (Zhouhi *et al.,* 2007). Aflatoxins are among the moulds that can be found indoors and outdoors, they grow best in warm, damp and humid conditions. Everything that is animal or vegetable can get mouldy. While living things are alive the moulds attackers can be held at bay but as soon as they are dead moulding begins.

The test shows that aflatoxins are always present in cancer patients: it builds up due to body's inability to detoxify it in a reasonable time (IARC 2002). The aflatoxin reaches the liver and simply kills portion of it. After hefty does the liver is weakened for a long possible years. Hepatitis and liver cirrhosis case always reveal aflatoxins. The liver fights hard to detoxify aflatoxins and manages for two to three weeks, a portion of it succumbs so the toxic effects of a dose of aflatoxins which is not noticeable for several weeks (Alpert *et al.,* 1970).

Many countries set maximum limits for aflatoxin according to different consumer and commodities. The current total maximum tolerable level (MTL) in food for human consumption recommended by United States, Food and Drug Administration (FDA)

is 20 ppb. In European countries maize used as ingredients in food stuffs, the aflatoxin B_1 should not exceed 5.0 ppb. Aflatoxin levels of 20 ppb or more should not be consumed by humans, young poultry and swine (Grybauskas *et al.,* 2000, Larson. 2001).

Table 1.1 shows bodies and organizations and their set limits of aflatoxin contamination, any food staffs with aflatoxin above the set limits is not allowed to be consumed. FDA allows low levels because they are considered unavoidable contaminants (FAO, 2003).

Table 1.1: Bodies and Organizations and their maximum tolerable limits (MTL) for aflatoxin

BODY/ ORGANISATIONS	MTL of aflatoxin in ppb
FDA	20
WHO	20
United states	20
European Nations	5 of B1 type
KCPB	20
KEBS	10

1.2 Justification of the Study

The economic importance of aflatoxin is derived directly from crop and livestock loss due to aflatoxin and directly from the cost of regulatory programs designed to reduce risk to human and animal's health. For example the 2009 FAO report which reported that about 25 % of the world food crops are affected by mycotocins each year.

In Kenya most lives have been lost in Makueni County and this shows that the survey of aflatoxins is crucial not only in affected regions but countrywide. The findings of this study will be very important in recommending practices that can be adopted by farmers to reduce aflatoxin contamination in order to improve the quality of harvested maize in the country.

The agricultural section in the Vision 2030 emphasizes innovation, commercially oriented and modern agriculture which lead to good quality in agricultural production hence meeting the world market standards. This study will meet this vision by giving

recommendations on ways to reduce aflatoxin contamination hence reducing expenditure in farming (Srimam, 2007).

Most farmers from Uasin Gishu County believe that aflatoxin contamination is only applicable to the imported maize which is called “yellow maize” or maize from Ukambani region. This study therefore intended to help correct and give the correct pre and post-harvest practices in maize production. Uasin Gishu County was selected as an area of study because it is among the chief growing areas in Kenya.

1.3 Objectives

1.3.1 General Objective

The general objective of this study was to determine the levels of aflatoxin in maize samples in the six divisions of Uasin Gishu County in Kenya.

1.3.2 Specific Objectives

The specific objectives were:

(i) To establish post-harvest maize farmer practice in Uasin Gishu County as related to aflatoxin contamination.

(ii) To identify the different types of aflatoxins prevalent in Uasin Gishu County.

(iii) To determine the level of aflatoxins contamination in maize samples grown and sold in various markets in Uasin Gishu County.

(iv) To verify whether the aflatoxin levels of the samples were within the limit set by FDA, WHO and KEBS.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Aflatoxins are produced by the two fungi A. *flavus* and A.*parasiticus*. These fungiare closely related and grow as a saprophyte on plant debris of many crop plants left on and in the soil. They are distributed worldwide but more common in countries with tropical climate that have extreme ranges of rainfall, temperature and humidity. The optimum temperature for growth is 37 ^{o}C but the fungus readily grows between 12-48 ^{o}C. (Richard *et al*., 2003).

Members of the genus *aspergillus* are characterized by production of non-septate conidiophores which are quite distinct from hyphae and which are swollen at the top to form vesicles on which numerous specialized spore-producing cells known as *metulae* (biseriate). Difficulties are experienced in the determination of primary sterigimata because they are tiny and are obscured by spores. Colonies of *A.flavus* are green-yellow to yellow-green or green on Czapek's agar. They usually have *biseriate sterigmata*; reddish-brown and the *sclerotia* are often present, *conidia* are finely roughened, variable in size and oval to spherical in shape. Taxonomy of A. flavus has a broad link: kingdom- fungi, phylum- *Ascomycota*, order- E*urotiales*, class- E*uromycetes*, family-T*richocomaceaea*, genus- A*sperrgillus* and species- *flavus* (Fig 2.1).

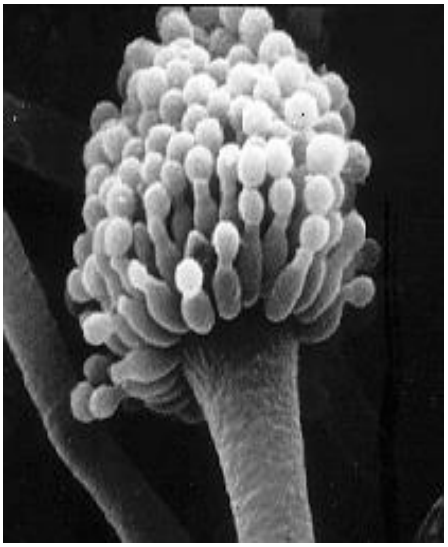


Figure 2.1: Terminal portion of conidiophores of *A. flavus* (Source- PFDB).

Colonies of *A. parasiticus* are dark to green on Czepak's agar and it remains green on age. *Sterigmata* are uniseriate, *sclerotia* are usually absent*; conidia* are coarsely echinulate, uniform in shape and size. A. parasiticus belongs to fungi kingdom, phylum- *Ascomycota*, order- E*urotiales*, class- E*uromycetes*, family- T*richocomaceaea*, genus- A*sperrgillus* and species- *parasiticus* (Fig.2.2) (Reddy *et al.*, 2008)

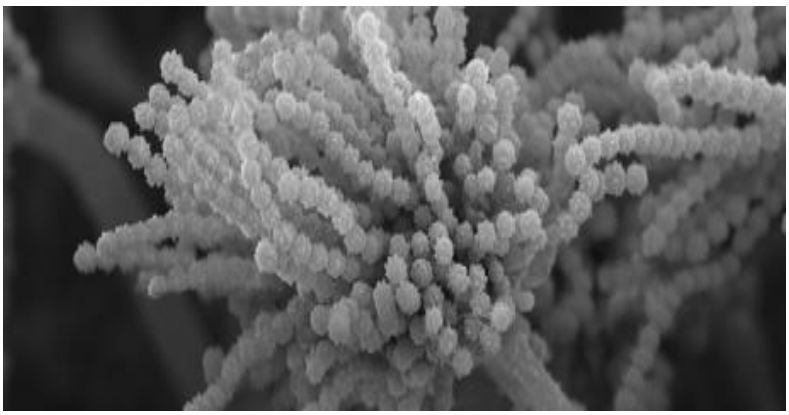


Figure 2.2: Terminal portion **of conidiophores of *A. parasiticus* (Source- PFDB).**

2.2 Types of Aflatoxins

The major types of aflatoxin mycotoxins are aflatoxin B which includes aflatoxin B_1 and B_2. Aflatoxin B_1 is the most common as well as the most toxic and carcinogenic. Aflatoxin G comprises aflatoxin G_1 and G_2. Aflatoxin M This group includes aflatoxin M_1 and M_2. These aflatoxins are metabolic products which are found in urine, milk and eggs produced by animals which have been given feeds with aflatoxin in it (Hell *et al.,* 2000). Aflatoxin G_1 is identical to B_1 except that a 5-valerolactone ring is substituted for the cyclopentanone ring. Aflatoxin B_2 and G_2 are dihydro addition product of the terminal dehydrofuran ring of B_1 and G_1 respectively. The unsaturated aflatoxin B_1 and G_1 is most likely responsible for some of the chemical reactivity of those molecules especially towards oxidizing agents (fig 2.1).

2.3 Physical Properties of Aflatoxin

Pure AFB_1 is pale-white to yellow crystalline odourless solid. Aflatoxins are soluble in methanol, chloroform, acetone and acetnitrile (Reddy, 2000). *A. flavus* typically produce AFB_1 and AFB_2 whereas *A. parasiticus* produce AFG_1 and AFG_2 as well as AFB_1, AFB_2, M_1 and M_2.The aflatoxins display potency of toxicity, carcinogenicity, mutagenicity in the order of $AFB_1>AFG_1>AFB_2>AFG_2$ as illustrated by their LD_{50} values for a day-old ducklings. The aflatoxins fluorescence is strong in ultraviolet light (365 nm). B_1 and B_2 produce a blue fluorescence where as G_1 and G_2 produce green fluorescence (FDA, 2002)

Table 2.1 shows eight types of aflatoxins, their molecular formular, molecular weight and their melting points. From the table it's evident that their melting points cannot be reached by boiling water.

Table 2.1: Types of aflatoxins, molecular formula, molecular weight and their melting points

Aflatoxin	molecular formula	molecular weight	M.Pt (^{o}C)
B_1	$C_{17}H_{12}O_6$	312.3	268-269
B_2	$C_{17}H_{14}O_6$	314.3	287-289
G_1	$C_{17}H_{14}O_7$	328.3	244-246
G_2	$C_{17}H_{14}O_7$	330.3	237-240
M_1	$C_{17}H_{12}O_7$	328.3	237-240
M_2	$C_{17}H_{14}O_7$	330	299
B_{2A}	$C_{17}H_{14}O_7$	330	240
G_{2A}	$C_{17}H_{14}O_8$	346	190

2.4 Chemical structures of Aflatoxins

The chemical structures of the six main aflatoxins are shown in the figure 2.3, in each group of aflatoxin they are related in their chemical structures. Alatoxins belongs to difurocoumarins group and from their structures they are classified into two, difurocoumarocyclopentanone and these comprises of B_1, B_2, M_1, M_2 B_{2A} and M_{2A} while difurocoumarolactone consists of G_1, G_2, G_{2A}, GM_1, GM_2 and GM_{2A}.

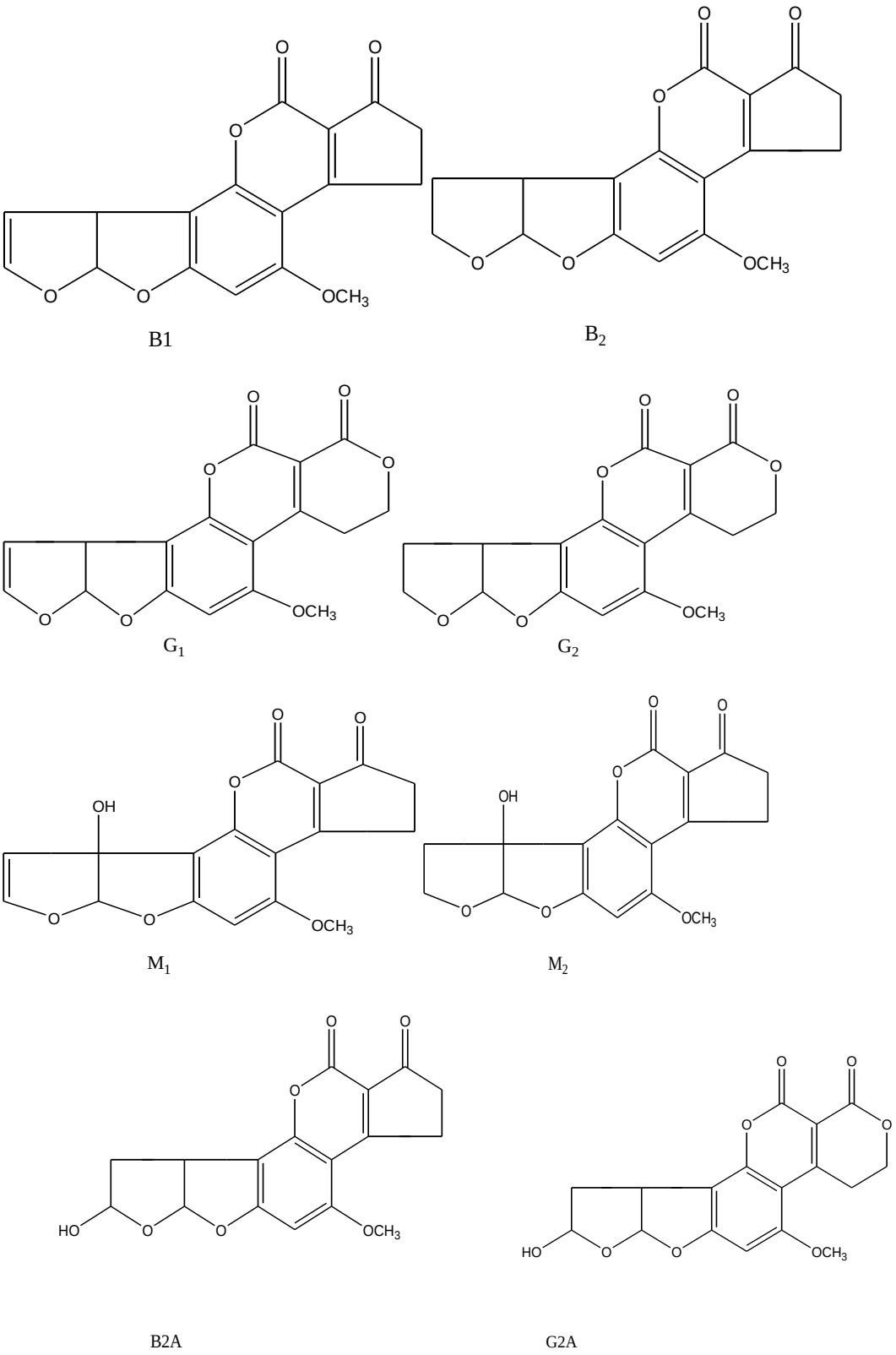


Figure 2.3: structure of the six main aflatoxins

2.5 Chemical Properties of Aflatoxins

The reactions of aflatoxin to various physical and chemical reagents have been studied extensively because of the possible application of such reactions to detoxification of aflatoxin contaminated material

2.5.1 Effect of Heat on Aflatoxins

Aflatoxins when in dry state are very stable to heat up to the melting point (Table 2.1). However, in the presence of moisture and at elevated temperatures there is destruction of aflatoxins over a period of time. Such destruction can either occurs with aflatoxins in oilseeds feed (Reddy *et al.*, 1976).

2.5.2 Reaction of Alkali with Aflatoxins

In alkali solution hydrolysis of the lactone moiety occurs. This hydrolysis appears to be reversible, since it has been shown that recyclization occurs following acidification of a basic solution containing aflatoxin. At higher temperature of approximately 100 ^{o}C, the ring opening followed by decarboxylation may proceed further, leading to lose of methoxy group from aromatic ring.

When a mixture of aflatoxin B_1 and ammonium hydroxide is stirred in room temperature for more than 21 hours, aflatoxin B_2 is obtained after acidification and chloroform extraction but the amount decrease when similar reactions of the mixture are stirred at room temperature for 8,10,11,14 and 18 days. Drying on a rotary at 60 ^{o}C yields a brown product which upon acetone extraction gives a mixture of substituted O-coumaric acid (Fig. 2.4) and aflatoxin B_1 as evidenced by infrared and ultraviolet spectroscopy. The brown residue is nontoxic in chick embryo bioassay. The brown solid is also insoluble in ethanol but soluble in dimethyl sulfoxide. The compound was tested for its LD_{50}.

In a study, aflatoxin B_1 was reacted with 17 M ammonium hydroxide, hydrolysis process opened the lactone ring of aflatoxin B_1 to form a substitute O-coumaric acid which absorbs light at 324 nm. Addition of base produces bathochromic shift of 360 nm. Acidification of an aflatoxin reacted for 21 hours with ammonium hydroxide results in almost complete recovery of unchanged toxin as determined by TLC, IR, UV and emission fluorescence (Vesonder *et al.*, 1975)

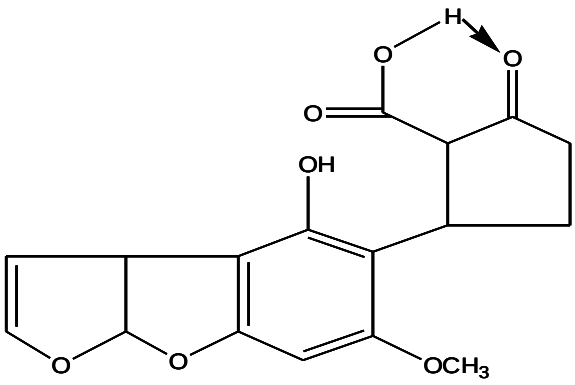


Figure 2.4: Chemical structure of O-coumaric acid

When mass spectral analysis is done, peaks at 330, 312, 297, 285 and 267 nm are observed. These peaks are in agreement with o-coumaric acid. When this series of reactions are monitored it indicates lactone ring opening in the presence of ammonia. Evaporation removes ammonia to give a mixture of the lactone and free acid. The free acid can then hydrogen bond to the ketone carbonyl of the cyclopentone ring of aflatoxin B_1 (Cooms *et al.*, 1966).

2.5.3 Reaction of Acids and Aflatoxins

In the presence of mineral acid, aflatoxin B_1 and G_1 are converted into aflatoxin B_{2A} and G_{2A} due to acid catalyzed addition of water across the double bond in the furan ring. In presence of acetic anhydride and hydrochloric acid the reaction proceds

further to give acetoxy derivatives. Similar adducts of aflatoxin B_1 and G_1 are formed with formic acid imonyl chloride.

2.6. AflatoxinDetoxification

Loss of fluorescence or change of R_f on TLC are the principal indications of aflatoxin detoxification. Various experimentnts have been conducted to identify the reagents which can be used to detoxify (Table 2.2).

Table 2.2: Time for disappearance of fluorescence of aflatoxin B1 and G1 after addition of 1 drop of the noted reagents to 0.03µg spots on a silica gel (Brinkman thin layer plates)

Reagents	Concentration	Time in sec.
NaOCl	5%	0^{α}.
NaOCl	2.5%	0
NaOCl	1.25%	0
NaOCl	0.63%	5
NaOCl	0.31%	10
$KMnO_4$	1%	0
$KMnO_4$	0.1%	120
Phenol	92%	0
Phenol	9.2%	0
Phenol	0.92%	120
Chlorohydroquinone	10%	0
Chlorohydroquinone	1%	120
Resorcinol	10%	0
Resorcinol	1%	0
Na_2SO_3	0.2M	120
$NaBO_3$	0.1%	15
$NaBO_2$	0.2%	120
H_2O_2(3%)+HCL	2.0M	30-45
H_2O_2(3%)+HCL	0.2M	120
HCl	2.0M	120
NaOH	1.0M	30-45

Reactions were instantaneous when time is given as 0 seconds (William *et al.,* 1967)

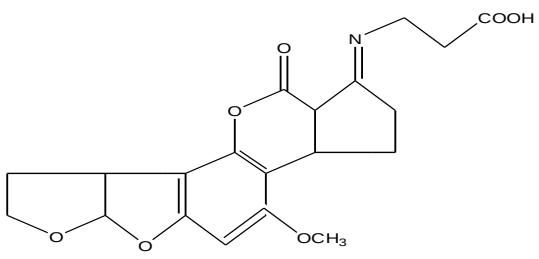
2.7 Synthesis of Aflatoxins

The aflatoxin total synthesis deals with synthesis of all groupS of aflatoxin. Organic synthesis of aflatoxins serves a different purpose. Traditionally it served to prove the structure of a complex biocompound in addition to evidence obtained from spectroscopy. Also synthesis will create the routes to be used in detoxification (Enric

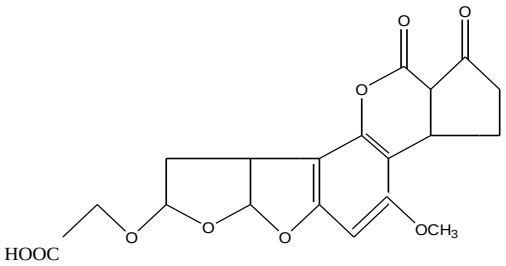
et al., 1991). Aflatoxins are not manufactured in commercial quantities but may be produced in small quantities for research purposes. Total annual production was reported to be less than 100 g (IARC, 2002). The use of dry, crystalline aflatoxin standard is very hazardous. All appropriate safety measures must be ensured that is employed. A coat, gloves, glasses and mask must be worn when working in designated areas. Equipment and working area must be decontaminated after use, Colleagues must be warned of the hazard (Hulghes, 2006).

2.8 Immunoassay for Aflatoxin

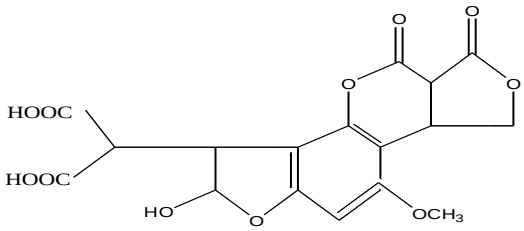
In order to obtain specific antibodies against an analyte with a small molecule, a complete manual of immunogens containing all or most of the target chemical structures should be prepared. A hapten of the analyte therefore needs to be designed and synthesized. A hapten is an antigen. An antigen is a substance that evokes the production of one or more antibodies, each antibody binds to specific antigen by the way of an interaction similar to lock and key. The immunizing hapten should therefore represent a near perfect mimic of the target molecule in structure, electronic and hydrophobic properties and therefore type of aflatoxin has its own haptene (Fig.2.5) (Goodrow *et al.*, 1995). It should have an attachment arm of the length of 8-6 carbons with an active group for instance -NH_2-COOH which can easily be used for conjugation with the carrier protein. For aflatoxin all haptens have been derived from current standards (Peiwu, 2009).



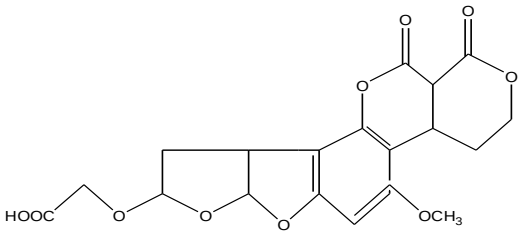
Target fof AFB1



Target fof AFB2



Target forAFG1



Target for AFG2

Figure 2.5: Chemical Structures of haptenes

The hapten used should have a cross reaction with the main aflatoxin. The higher density of hapten on the carrier protein is not essential for sensitivity and specifity of the antibody. Because of the lack of specifity of antibodies within the aflatoxin family the antibodies within low cross reactivity towards non-targeted compound is still needed.

2.9 Factors Affecting Aflatoxin Contamination in Maize

2.9.1 Harvesting Time

Leaving maize to dry in the field for more than three weeks before shelling has been found to be among the most important factors associated with higher aflatoxin levels in sampled maize. It has been found that delayed harvest enhance pre-harvest aflatoxin contamination (FAO 1999, Bankole, 2003).

2.9.2 Sorting of Maize after Harvesting

Sorting out infected, damaged and discoloured maize kernels as well as cleaning before storage is associated with low aflatoxin levels. These practices help to reduce the fungal inoculate load and infected with aflatoxin substrates (Hell *et al.,* 2000, Galuez *et al.,* 2003).

2.9.3 Use of Modern Granaries

Use of improved granaries is also related to reduction in aflatoxin contamination. Improved storage structures are recommended by Kenya Agricultural Research Institute (KARI). These may be better in terms of maintaining the quality of maize grain during storage compared to traditional granaries (Kaaya, 2005).

2.9.4 Use of Pesticide

Use of synthetic pesticides like actellic super and malation (2 %) is another practice that significantly reduces aflatoxin development. These pesticides control insect pests that have a direct effect on moulds and aflatoxin contamination. From previous studies it shows that actellic super does not have any direct effect on *A. flavus* development in maize grain thus reducing aflatoxin levels (El-Kady, 1993).

2.9.5 Drying of Maize on a Bare Ground

When maize grain is dried on a bare ground it can be contaminated with soil moulds and foreign matter and also the grains will pick moisture creating appropriate environment for moulding, improper drying is therefore associated to aflatoxins contamination (Odogola *et al.,* 1991).

2.9.6 Conversion Method Used

The process of shelling maize by traditional method of thrashing using a stick inflicts physical or mechanical damage to the grain making them prone to fungal invasion including *A.flavus* and *A. parasiticus.* A shelling machine which is in good working condition reduces grain cracks (Thite *et al.*, 1985, Bankole., 2003).

2.9.7 Storage Method

Heaping maize on the floor during storage or use of polypropene bags in storage of maize leads to infestations by insects, rodents, moulds and moisture pick-up which are factors that promote aflatoxin contamination (Udoh *et al.*, 2000).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

The results presented in this thesis were obtained from both the farms and market. From the market, 15 samples were collected in one day in a month for the 3 consecutive months, June, July and August. The total samples collected in 3 months were therefore 45. From farms it was done in the 6 divisions in the county. In each division 20 samples were collected making a total of 120 samples. First qualitative analysis was done using TLC method to sort the contaminated samples from those which were not. Aflatoxin quantification was done using ELISA method.

3.2 Study Area

Uasin Gishu county extends between longitude 34^{o} 50' and 35^{o} 37' East and 0^{o} 55' North covering a total area of 3,218 sq.km. (Fig.3.1). It is highly plateau with on altitude range of 1500 m to 2100 m above sea level. Rainfall is reliable and evenly distributed with annual average of 960 mm. Uasin Gishu county has six divisions namely; Ainabkoi, Kessess, Moiben, Kapseret, Soy and Turbo. Maize is grown in all the 6 divisions and it is normaly harvested in the month of November and December. Maize is then sold to Cereal Boards depots at Kitale, Moi's Bridge, Ziwa and Eldoret. The maize is also sold to millers such as Unga, Dolla and Corn Product Company (C.P.C) (PRSP 2005).

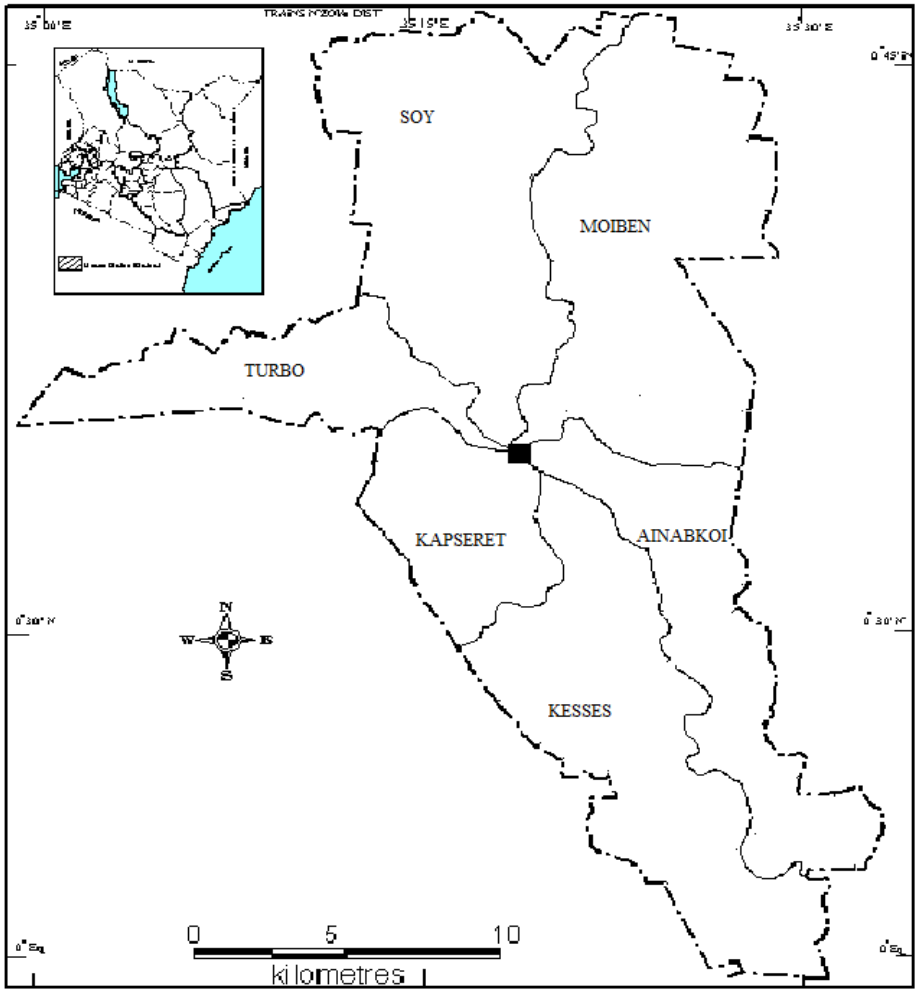


Figure 3.1: Map of Uasin Gishu County showing the six divisions; sampling areas (Source- L ocal Government/ Transitional Authority)

3.3 Sampling Maize for Aflatoxin Analysis.

A survey was conducted during the month of June 2009 to establish harvesting, drying, shelling and storage practice of maize used by farmers in the 6 divisions in of Uasin Gishu County. To obtain the required information, a questionnaire was used and farmers were given one day to complete the questionnaire. In each division, 20 farmers were identified approximately 10 km apart. The farmers who responded to questions on harvest practice of maize also provided the samples for aflatoxin analysis. For each maize sample, 10 maize cobs each (unshelled) or 1 kg shelled was

sampled from each of the farmers store following the method recommended as per Table 3.1 (Juan et al., 1995; FAO 1982). The unshelled maize samples were hand shelled to form sample lots which were analyzed for aflatoxins. Each sample was reduced to 500g- working sample for analysis (ICRITSAT 1987).

Table 3.1: Summary of Sampling Plans for Aflatoxin in Cereals Grains

Consignment Weight (tones)	Number of lots	Number of incremental Samples per lot	Weight of each incremental sample (grams)	Aggregate sample weight (kg)	Total number of laboratory samples per lot
≤1	1 lot	1 – 10	300	0.3 – 3	1
>1≤3	1 lot	20	300	6	1
>3≤10	1 lot	40	300	12	1
>10≤20	1 lot	100	300	30	3
>20≤50	2 lots	100	300	30	6
>50≤100	3 lots	100	300	30	9
>100	Lots of 50 tonnes	100	300	30	3 per tonnes

3.4 Sampling and Sample Preparation

3.4.1 Experimental Procedure

Twenty grams of the sample was weighed to nearest 0.01 g into 500 ml wide mouth flat bottom flask then mixed with 20 g of hyflo super-cel, 200 ml chloroform and 20 ml water. The flask was covered with aluminum foil and the contents shaken on mechanical shaker for 30 minutes. It was then removed and filtered through a Whatman filter No. 254 into a 100 ml measuring cylinder. The first 100 ml was collected and transferred into a around bottom flask and concentrated on a rotary evaporator at 40 ^{o}C to near dryness.

3.4.2 Analysis Using TLC

The TLC plate was prepared by punching holes at equal intervals of 10 mm using a needle. Using a micro-syringe, the extract and the mixed standard was spotted on the TLC plates using spotting protocol prepared. The volume of the standard was varied (2, 4, 8 and 10 μl) keeping the sample volume constant at 20 μl. The syringe was washed and rinsed properly after spotting the sample and the standard. The plate was dried in a stream of air or nitrogen gas to make it ready for development. The developing tank which comprised of chloroform, ethyl acetate, toluene and formic acid in the ratio of 30:25:35:10 was prepared to make 100 ml which formed 1 cm layer in the unsaturated tank. The plate was inserted into the tank and left until the mobile phase (solvent) reached the limit line. The plate was removed and dried on the hood or rack that had been fixed on the spray tray.

The qualitative analysis of aflatoxin was done using u.v light of 360 nm and 10 cm above the TLC plate. B_1 andB_2 produced Blue fluoresce while G_1 and G_2 aflatoxins produced green fluoresce. The R_f value of each type of aflatoxin originating from

extract corresponded with each type of aflatoxin from the standards. For any results with doubt the presence of alflatoxin was confirmed by spraying the chromatogram with 50% v/v sulfuric acid using a spay gun (wetting of chromatogram was not done rather a light spray was given) if the florescence of the spots turned yellow under u.v light then the whole process was repeated.

3.4.3 Preparation of Standards used in TLC

The standards B_1, B_2, G_1 and G_2 were crystalline pure solids, the stock solution was prepared from a stock of each aflatoxin containing 100 µg/ml solutions in chloroform and then mixed to make a concentration of about 10 µg/ml containing each type of aflatoxin. The flask was tightly wrapped using aluminium foil and stored at or below 0 ^{o}C. In order to avoid incorporation of water by condensation all the standards were brought to room temperature before use. The aluminium foil was not removed from the flask until the contents had reached room temperature.

3.5 Quantitative Analysis Using ELISA

The stat Fax 3200 is a laboratory instrument intended for in-vitro diagnostic use. It is a compact, microprocessor- controlled multi-purpose photometer system designed to read and calculate the results of the assays, which are read in micro titre plates (3200 stat Fax operator manual Rev. F).

3.5.1 Test Principle-Enzyme-Linked Immunosorbent Assay (ELISA)

Extract was analyzed using a competitive enzyme-linked immunosorbent assay (ELISA) Kit. This method has been reported to have aflatoxin recovery of 98 ± 10 % and lowest detection limit of 0.09 ppb with maize products.

Aflatoxins were extracted from the test samples with methanol: water (50:50) solvent, defatted with hexane and diluted to 10 % methanol content. A 50µl of this liquid sample extract and of those calibrated aflatoxin standard solution were incubated simultaneously with an aflatoxin-enzyme conjugate solution in wells of coated micro titre plates, the antibody binding sites are available on the coated micro plates, the amount of aflatoxin-enzyme conjugate bound is inversely proportional to the amount of free toxin in standard or sample extract solution.

After appropriate washing steps, the amount of aflatoxin-enzyme conjugate bound to the antibody was determined by incubation with substrate solution. The resultant colour could be evaluated visually or measured with spectrophotometer (ELISA reader). The intensity of the formed color is inversely proportional to the amount of aflatoxin in the sample extract solution. By including several dilutions of a well-calibrated aflatoxin standard in each assay, absorbance values of aflatoxin standard dilutions were measured with ELISA reader and used to construct standard curve. On the basis of this standard curve, the aflatoxin content of sample extract dilution was then quantitatively determined. The intensity of colour in both standard and test extract was determined by reading the absorbance at 450 nm using an ELISA reader (Uniskan 11 Labsystems, Finland). A standard curve of percentage inhibition against aflatoxin concentration of the samples was constructed.

3.6 Sample Preparation and Extraction

The sample was finely ground using a hammer mill. The ground sample was then thoroughly mixed using a high speed-laboratory blender.

3.6.1 ExtractionProcess

Ten grams of grounded sample was weighed to approximately 0.01 g on a piece of aluminum foil and transferred to 100 ml beaker. A 50 ml methanol: water 50 ml (50:50) mixture and 10 ml hexane was added and mixed thoroughly for 30 minutes using a magnetic stirrer. About 10 ml of the mixture was centrifuged at a speed of 1500 k for 10 minutes. About 3 ml of the lower methanol: water layer was mixed thoroughly on a vortex mixer model vm-1000. A 400 µl of the sample extract was pipetted into a mixture of 1600 µl PBS and 2000 µl methanol: water (10:90) in a mixing vial. It was then kept for ELISA analysis.

3.6.2 ElisaAnalysis

This step requires a number of ELISA micro-strips, enzyme conjugate and enzyme substrate. These were removed from the refrigerator and placed at room temperature for about 30 minutes before use.

3.6.3 Elisa Procedure

Aflatoxin standard was prepared in the concentrations of (0, 5.6 , 20 and 50 ppb) by serial dilution of the calibrated standard provided as follows: set of 5 disposable test tubes with labels N, S1, S2, S3 and S4 where S1-S4 were standards of various concentration (Table 3.2)

Table 3.2: Preparation of the blank **and the aflatoxin standards**

Tube Number	Volume of diluents (methanol:PBS,10:90)	Volume of aflatoxin solution to be added
N	1000 µl	10 µl of provided aflatoxin standard
S1	2000 µl	20 µl of solution in tube N
S2	1000 µl	500 µl of solution in tube S1
S3	1000 µl	500 µl of solution in tube S2
S4	1000 µl	Nothing

The enzyme (horse reddish peroxidase) conjugate was prepared by diluting the concentration supplied by Boratest chemist. A 10 µl enzyme conjugate was concentrated to 10 ml of PBS and then mixed gently on vortex. The ELISA micro-strips were washed two times with washing solution and partially dried by tapping on blotting paper. A 50 µl of each standard was transferred in duplicate wells and also each sample extract in designated wells as shown in table 3.3.A 50 µl of the diluted enzyme conjugate was then added to all wells. The ELISA micro-strips were incubated for 60 minutes at room temperature before they were emptied and washed three times using distilled water and dried. 100 µl of 2,2-azino –di-3-ethylbenzthiazone-6-sulfonate substrate solution was added to each well and left for colour to develop for 7-10 minutes. The colour was stopped by adding 100 µl of stopping solution (1M sulphuric acid) to each well to prevent the denaturing of the HRP enzyme.

Table 3.3: Titration format for ELISA micro-strips

	Standards	Samples	
	1	2	3
A	S4	P1	P5
B	S4	P1	P5
C	S3	P2	P6
D	S3	P2	P6
E	S2	P3	P7
F	S2	P3	P7
G	S1	P4	P8
H	S1	P4	P8

Key: S1-S4- Standard 1 to 4 (from tubes S1-S4).

P1-P8-Sample 1 to 8. These must be titrated at the same time with the standards.

3.6.4. Aflatoxin Quantification and Reporting

The result was red using ELISA reader; aflatoxin content in ppb was displayed on the screen and printed on paper. Alternatively, one may calculate the average absorbance values of each aflatoxin standard and samples extract dilution (B) and reagent blank (Bo). These values are then used to calculate percentage inhibition (B/Bo %) on Y-axis. The best fit line between points was determined there on a graph paper using suitable software. Aflatoxin contamination of each sample was determined by drawing a line of abscissa from its percentage inhibition value on the Y-axis to the curve and line of ordinate from intersect from the curve to the X-axis. The value obtained was then multiplied with the same dilution factor to determine the actual amount of aflatoxin.

In order to draw a precise conclusion from the results, several methods were used and these were descriptive statistics; significant level within 0.5 and 1.5 and confident level of 95 %. ANOVA test was used and the significant level was within 0.05 and 0.1, the F-ratio was considered to be above 1 and confident level of 95 %. Inferential statistics with p-values within 0.05 and 0.1 the confidence level used was 95 % (Miller, 2000). The statistical method used was STATA/IC. Version 12 and two sample test with equal variance.

CHAPTER FOUR

RESULTS

4.1 Introduction

From this study, the four main types of aflatoxins B_1, B_2, G_1 and G_2 were detected in Uasin Gishu County. This was an indication that the fungal *A. parasiticus* and *A. flavus* are present within the region. The results showed that in the whole county 49 out of 165 samples tested positive of aflatoxin, 36 from the farm and 13 from the market level.

4.2 Results from the 6 Divisions of Uasin Gishu County

Appendixes 2 to 7 and Table 4.1 shows the summary of pre and post-harvest practices done by farmers in each division in Uasin Gishu County. Appendix 2 illustrates the summaries for samples collected from the Ainabkoi division and the pre and post grain handling practices done by farmers within the division. From the results 85 % of the samples were lately harvested, that is, after three weeks. Most of the samples representing 85% were dried on bare ground while 15 % of the samples dried using other methods. Seventy five percent of the samples were collected from the farms which shelled their cobs to obtain maize grains and 25 % had their produce thrashed to obtain the grains. Most of the farm samples were sorted before being stored, 75 % of the samples sorted before storage while twenty five were not sorted. At the same time, most of the samples comprising 80 % were stored in bags as grains while 20 % were stored in cobs as they had not been shelled. Samples were either stored on the floor or in other modes, with 25 % percent of them being stored on the floor while the remaining was stored in other modes. Ninety percent of the samples were obtained from stores that were cleaned before storage process with 70 % of the samples being

stored in a granary. Ninety five percent of the farmers used pesticides for preservation (Table 4.1).

Appendix 3 shows the summaries for samples collected from the Kesses division. Ninety percent of the samples were lately harvested, that is, after three weeks. Fifteen percent of the samples were dried on bare ground with 85 % being dried by other methods. Eighty five percent of the samples were collected from the farms where shelling was done using machine to obtain maize grains with 15 % of them had their produce thrashed to obtain the grains. Most of the farm samples were sorted (75 %). Eighty five percent were stored in shelled form in bags as grains while 15 % were stored in cobs. Samples were either stored on the floor or in other modes, with 25 % of them being stored on the floor while the remaining were stored in other modes. Most of the samples representing 95 % were obtained from stores that were cleaned before storage process with 30 % of the samples being stored in granaries. Eighty five percent of the farmers used pesticides for grain preservation (Table 4.1).

Table 4.1: Percentages of pre and post-harvest practiced by farmers in each division

	AINAKOI	KESSES	MOIBEN	KAPSERET	SOY	TURBO
Late harvesting	90 %	90 %	90 %	90 %	85 %	85 %
Drying on bare Ground	85 %	15 %	25 %	15 %	25 %	10 %
Shelling using Machine	75 %	85 %	90 %	55 %	85 %	90 %
Sorted	55 %	75 %	70 %	45 %	55 %	85 %
Stored in bags	80 %	85 %	95 %	5 %	85 %	85 %
Stored on floor	45 %	25 %	30 %	30 %	25 %	35 %
Unclean storage	10 %	5 %	_	5 %	-	-
Not stored in a granary	30 %	75 %	40 %	50 %	55 %	55 %
Use of Pesticide	95 %	85 %	90 %	90 %	95 %	-

Appendix 4 shows the summaries for samples collected from the Moiben division. Ninety percent of the samples were lately harvested, only 10 % being timely harvested. Twenty five percent of the samples were dried on bare ground with samples 75 % being dried by other methods. Ninety percent of the samples were collected from the farms were shelling were done using machines to obtain maize grains with 10 % of them used thrashing method to obtain the grains. Most of the farm samples (70 %) were sorted before storage. Ninety five percent of the samples

were stored in bags as grains while 5 % were stored in cobs form. Thirty percent of the samples were stored on the floor while the remaining were stored in other modes. All the samples were obtained from stores that were cleaned before storage process with 60 % of the samples being stored in granaries and the remaining 40 % being stored in other modes. Ninety percent of the farmers used pesticides for preservation with the remainder not using pesticides during storage (Table 4.1).

Appendix 5 shows the case summaries for samples collected from the Kapseret division. Ninety percent of the samples were lately harvested with the remaining percentage being harvested on time. Fifteen percent of the samples were dried on bare ground with 85 % samples being dried by other methods. Fifty five percent of the samples were collected from the farms where shelling was done to obtain maize grains with 45 % of them had their produce thrashed to obtain the grains. Forty five percent of the farm samples were sorted out before storage while 55 % were not sorted. Most of the samples representing 85 % were shelled into grains before being stored with 15 % left unshelled or in cobs form. At the same time, most of the samples comprising 95 % were stored in bags as grains while only 5 % were stored in cobs. Most of the samples were stored in bags with 30 % stored on the floor with other modes being utilized. Ninety five percent of the samples were kept in stores that were cleaned before use with 50 % of the farmers using granaries for storage. Only 10 % of the farmers did not use pesticides with 90 % using the pesticides (Table 4.1).

Seventeen of the 20 samples from Soy division were harvested after three weeks showing that they were harvested late. The drying method least used by the farmers in this division was the use of a bare ground with 75 % of the farmers preferring using other methods to dry their produce. As can be seen from the appendix 6 most farmers, 85 % of them utilized the maize shelling machines with the remaining 15 % using other methods such as thrashing to yield out the maize grains. Forty five percent of the farmers did not sort out the maize before storage and 85 % storing their grains as opposed to maize cobs. Most farmers (75 %) opted not to leave their grains or cobs on

the floor as opposed to other modes. At the same time, all the farmers cleaned their storage space or device before use but in comparison a small number of farmers representing 45 % used granaries to store their produce. Ninety five percent of the farmers utilized pesticides to preserve their produce (Table 4.1).

Appendix 7 displays the case summaries for the samples from Turbo division. Most of the samples, 85 % from this division were harvested after three weeks showing that they were harvested late with only three samples being harvested on time. Only two samples were dried on the bare ground with other samples being dried using other methods. Ninety percent of the farmers utilized maize shelling machines and the remaining used other methods such as thrashing to yield out the maize grains. Eighty five percent of the farmers sorted out their maize before storage with another 85 % storing in grain form with the remaining 15 % stored their grains in cobs form. Most farmers opted not to leave the grains or cobs on the floor as opposed to other modes. All the farmers cleaned their storage space or device before use but in comparison a small number of farmers representing 45 % used granaries to store their produce. All the farmers in this divisions utilized pesticides to preserve their produce (Table 4.1).

From the 20 samples collected from the farms in Ainabkoi division, aflatoxin was detected in six samples with highest concentration was at 21.90 ppb and the lowest concentration at 2.20 ppb (Table 4.2), the mean concentration was at 3.26 ppb of the samples with aflatoxins. Type B_2 was the most prevalent in 3 samples, type G_1 in 2 samples, type G_2 and B_1 appeared in one sample each.

Table 4.2: Aflatoxin case summary for Ainabkoi Division

Sample	Test Result	Aflatoxin Type	Aflatoxin Concentration in (ppb)
A-9	Detected	B2	4.40
A-10	Detected	G2	21.90
A-15	Detected	B2	2.20
A-16	Detected	B1 & B2	8.70
A-17	Detected	G1	6.20
A-19	Detected	G1	21.80
Mean			3.26

Key: A- Ainabkoi

From the 20 samples collected from the farms in Kesses division, aflatoxin was detected in five samples with highest concentration of 11.20 ppb (Ke-14) which was the only sample above KEBS limit of 10.00 ppb (Table 4.3). The one with the lowest aflatoxin level was 1.60 ppb, the mean concentration was 1.48 ppb and the 3 type's aflatoxins were detected G_1, B_1 and G_2 all appearing in 2 samples each.

Table 4.3: Aflatoxin case summary for Kesses Division

Sample	Test Result	Aflatoxin type	Aflatoxin Concentration (ppb)
Ke-6	Detected	B1	2.60
Ke-8	Detected	G2	1.60
Ke-11	Detected	G1&G2	7.20
Ke-14	Detected	B1	11.20
Ke-18	Detected	G1	6.90
Mean			1.48

Key. Ke- Kesses

Table 4.4 illustrates the aflatoxin case summary for Moiben division, from the 20 samples collected from the farms 50 % samples tested positive for aflatoxin. The highest concentration was 23.30 ppb while the lowest level was 0.50 ppb with mean concentration being 4.26 ppb. Aflatoxin B_1 was the most prevalent in 5 samples, G_1 in 3 samples while G_2 appeared in 2 samples.

Table 4.4: Aflatoxin case summary for Moiben Division

Sample	Test Result	Aflatoxin type	Aflatoxin Concentration (ppb)
Mo-1	Detected	G_2	8.20
Mo-3	Detected	G_1	3.40
Mo-5	Detected	G_1	7.70
Mo-8	Detected	B_1	0.50
Mo-10	Detected	G_2	9.60
Mo-11	Detected	B_1	13.00
Mo-12	Detected	B_1	6.30
Mo-15	Detected	B_1	11.70
Mo-16	Detected	G_1	23.30
Mo-19	Detected	B_1	1.50
Mean			4.26

Key: Mo- Moiben

In Kapseret division eight samples representing 40 % of the samples were detected for the aflatoxins with the aflatoxin type B_1 being prevalent in 6 samples and G_2 in 5 samples. The highest concentration of aflatoxin was 8.90 ppb with the lowest concentration being 1.70 ppb with a mean concentration of 1.70 ppb.

Table 4.5: Aflatoxin case **summary for Kapseret Division**

Sample	Test Result	Aflatoxin type	Aflatoxin Concentration (ppb)
Ka-2	Detected	G_2& B_1	4.30
Ka-3	Detected	G_2	3.90
Ka-4	Detected	B_1	5.20
Ka-5	Detected	G_2& G_1	1.90
Ka-10	Detected	B_1	1.70
Ka-12	Detected	B_1	8.00
Ka-15	Detected	G_2& B_1	6.80
Ka-16	Detected	G_2& B_1	8.90
Mean			1.70

Key: Ka-Kapseret

Aflatoxins were detected in only three samples representing 15 %, aflatoxin type G_2 was the only type appearing at three samples. The highest concentration of aflatoxin was 6.00 ppb with the lowest level being 3.40 ppb with a mean concentration of 0.68 ppb which shows that all the samples met the market standards (Table 4.6).

Table 4.6: Aflatoxin case summary for Soy Division

Sample	Test Result	Aflatoxin type	Aflatoxin Concentration (ppb)
So-4	Detected	G2	6.00
So-5	Detected	G2	3.40
So-19	Detected	G2	4.20
Mean			0.68

Key: So-Soy

In Turbo division, aflatoxins were detected in four samples representing 20 % of the samples with the aflatoxin, type G_2 which appeared in the three samples while types B_1 and B_2 appeared in one sample each (Table 4.7). The highest concentration of aflatoxin was 15.20 ppb with the lowest level of 6.20 ppb, the division had a mean value of 1.91 ppb. T-1 sample which was from turbo division had aflatoxin concentration of 6.20 ppb, the sample was not sorted, stored in cobs form and in bags which must have produced optimum conditions for moulds to development, also no granary was used. T-5 sample tested positive of G_2 and B_2 aflatoxins with total concentration of 6.20 ppb, the sample was shelled and stored in bags and if the moisture was above 14 % then that was the main cause of aflatoxin contamination. T-7 sample had aflatoxin concentration of 15.20 ppb which was above MTL

recommended by KEBS of 10 ppb. This sample was shelled using a machine and stored in bags. T-14 sample tested positive of G_2 with a value of 10.60 ppb. The lot was shelled using a machine and stored in bags.

Table 4.7: Aflatoxin case summary for Turbo Division

Sample	Test Result	Aflatoxin type	Aflatoxin Concentration (ppb)
T-1	Detected	B1	6.20
T-5	Detected	G2 & B2	6.20
T-7	Detected	G2	15.20
T-14	Detected	G2	10.60
Mean			1.91

T-Turbo

From this research it is evident that Moiben division had the highest mean concentration levels of 4.26 ppb and also the one with the highest number of samples testing positive which was 10 samples representing 50 %. Ainabkoi division was the second with the mean of 3.26 ppb, Soy division was the lowest with the mean concentration of 0.68 ppb, it's the division with the least number of samples (3) testing positive of aflatoxins. Turbo division was 3rd with mean of 1.91 ppb. The sample with the maximum and minimun aflatoxin concentration was from Moiben division with 23.3 ppb and 0.5 ppb, respectively.

Table 4.8 shows a longitudinal study test result on the market samples collected for the months of June, July and August 2009. As it can be seen from the table, on June, aflatoxins were detected in four samples, while in July, five samples and in August, aflatoxin was detected in five samples.

The highest values of aflatoxins were found in market samples in of July at 68.10 ppb with the lowest concentration being in of June at 0.90 ppb. The month of July had the highest mean concentration of 7.31 ppb, August samples recorded 4.40 ppb while June showed the lowest mean of 1.87 ppb. In June, four samples tested positive for aflatoxins with maximum being 14.30 ppb and minimum values of 0.90 ppb with a standard deviation of 4.97 ppb. In of July the sample with the maximum aflatoxin concentration was 68.10 ppb with a minimum at 2.60 ppb and the standard deviation of 22.23 ppb. August had sample with a maximum value of 64.90 ppb and minimum of 2.50 ppb with a standard deviation of 16.09 ppb (Table 4.8).

Table 4.8: Aflatoxin case summary for market samples

June Samples			July Samples			August Samples		
Sample	Aflatoxin type	Aflatoxin Conc. (ppb)	Sample	Aflatoxin type	Aflatoxin Conc. (ppb)	Sample	Aflatoxin type	Aflatoxin Conc. (ppb)
M1-1	G_1	12.30	M2-1	G_1	63.50	M3-1	None	N/D
M1-3	None	N/D	M2-3	None	N/D	M3-3	B_2	7.40
M1-4	B_1	9.80	M2-4	None	N/D	M3-4	None	N/D
M1-6	None	N/D	M2-6	G_2	2.60	M3-6	None	N/D
M1-7	B_1 & G_2	0.90	M2-7	G_2	68.10	M3-7	None	N/D
M1-9	None	N/D	M2-9	None	N/D	M3-9	B_1	64.90
M1-10	None	N/D	M2-10	B_1& G_2	5.90	M3-10	B_1	2.50
M1-11	None	N/D	M2-11	None	N/D	M3-11	B_1	4.70
M1-12	None	N/D	M2-12	B_1& G_2	6.00	M3-12	B_1	8.50
M1-15	G_2	14.30	M2-15	None	N/D	M3-15	None	N/D
Mean		1.87			7.31			4.40

Table 4.9 results for descriptive statistics for the samples collected from farmers. With most variables having two choices either YES represented by 1 or NO represented by 2. Mean statistics of between 0.5-1.4 demonstrate an affirmative view of yes with statistic of over 1.5 representing a negative opinion. Five of the variables had mean statistic value over 1.5 showing that it had an alternate view concerning the issue involved. These were harvesting time, drying method used either over a cover or on bare ground, the conversion method used to obtain grains either by thrashing or using

a shelling machine, storage mode in cobs or in grain form and finally storage over fire.

Table 4.9: Descriptive statistics of pre and post- harvest practices done by farmers

Descriptive Statistics				
	Number of samples	Mean		Std. Deviation
	Statistics	Statistics	Std. Error	Statistics
Harvest Time	120	1.87	0.030	0.332
Drying Method	120	1.82	0.035	0.382
Conversion method	120	1.80	0.037	0.402
Sorting out	120	1.34	0.043	0.476
Storage Form	120	1.23	0.039	0.425
Storage Method	120	1.12	0.029	0.322
Storage format	120	1.68	0.043	0.467
Clean storage device	120	1.03	0.016	0.180
Granary use	120	1.50	0.046	0.502
Fire storage	120	1.98	0.012	0.129
Pesticide use	120	1.08	0.024	0.264
Test Result	180	1.73	0.033	0.446
Aflatoxin Concentration (ppb)	49	11.210	2.150	15.052

Table 4.10 illustrates the distribution of aflatoxins among the samples tested positive of aflatoxin.

Table 4.10: Test result at the farm **and market level**

Test Result							
		Frequency	%	Min. aflatoxin conc. (ppb)	Max conc. of afatoxins (ppb)	Mean of aflatoxin (ppb)	Std. deviation (ppb)
Farm Level	Positive	36	30.0	0.50	23.30	7.41	4.44
	Negative	84	70.0	-	-	-	-
Market Level	Detected	13	28.9	0.90	68.10	18.72	15.05
	Not Detected	32	71.1	-	-	-	-

From all the farm samples, 36 out of 120 tested positive of aflatoxin, this representing 30 % of all the farm samples. Concerning the market sample, 13 samples out of 45 tested positive of aflatoxin and this formed 21.7 % of the samples. In total, the number in which aflatoxin were detected represented 27.2 % of the samples with the remaining 72.8 % not having being detected. At the farm level aflatoxin mean concentration was 2.22 ppb while that of the market mean was 4.53 ppb with a standard deviation of 15.05 ppb. The maximum aflatoxin was 68.10 ppb which was collected at the market level in the month of July with the sample having a minimum concentration of 0.50 ppb.

Table 4.11 shows the test results from both market and farm levels. In total 49 samples out of 165 tested positive of aflatoxins representing 27.2 %.

Table 4.11: Results from the farm and market

Test Results					
		Frequency	Percent	Valid Percent	Cumulative percent
Valid	Positive	49	27.2	27.2	27.2
	Negative	116	72.8	72.8	100.0
	Total	165	100.0	100.0	

4.3 F-ratio test

The F-ratio is a measure of the ratio of the variation explained by the model and the variation explained by unsystematic factors. It is therefore a measure of the ratio of systematic variations to unsystematic variations. As such, it is the ratio of the experimental effect to the individual difference in the levels of aflatoxin. If its value is less than 1 then it must by definition, represent a non-significant effect which in real terms means that there is more unsystematic than systematic variance. The significant level falls between 0.05 and 0.1.

Table 4.12 illustrates the distribution of aflatoxins among the samples. The total mean aflatoxin level was 11.21 ppb with standard deviation of 15.05. The maximum aflatoxin was 68.10 ppb which was collected from July for the sample with the minimum concentration of 0.50 ppb.

Table 4.12: Distribution of aflatoxins in the tested samples

Descriptive statistics						
	N	Minimum	Maximum	Mean		Standard Deviation
	Statistics	Statistics	Statistics	Statistics	Std. error	Statistics
Aflatoxin conc.	49	0.50	68.10	11.21	2.15	15.05

Table 4.13 shows ANOVA analysis on the test results leading to aflatoxin contamination in Uasin Gishu County. The F-ratio from the table was 1.649 which was above 1, this shows that there was relationship between the six divisions in the county practiced by farmers.

Table 4.13: ANOVA analysis tables on the tested **samples**

ANOVA					
Test Result					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1.700	5.000	0.340	1.649	0.153
Within Groups	23.500	114.000	0.206		
Total	25.200	119.000			

F (5,114) = 1.649, p= 0.153.

There was a significant statistical difference in the test result between the different divisions in the Uasin Gishu County. The F-ratio was 2.355 which was above 1 which is significant (Table 4.14).

Table 4.14: ANOVA analysis of the aflatoxin level within the county

ANOVA					
Aflatoxin Concentration					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	365.955	5.000	73.191	2.355	0.064
Within Groups	932.295	30.000	31.076		
Total	1298.250	35.000			

Table 4:15 shows post-hoc analysis, when significance level was considered at 0.05, it shows that there was a significant statistical difference in practices as related to aflatoxin between the different divisions in the Uasin Gishu County between Ainabkoi and Kesses, Ainabkoi and Soy, Moiben and Kesses which had significant level of below 0.05.

4.15: Post-Hoc test analysis of pre and post-harvest practices at significance level of 0.05

Multiple Comparisons				
Aflatoxin Concentration LSD				
(I) Division	(J) Division	Mean Difference (I-J)	Std. Error	Sig.
Ainabkoi	Kesses	8.267	3.376	0.020
	Kapseret	9.079	3.011	0.005
	Soy	9.633	3.941	0.021
*. The mean difference is significant at the 0.05 level.				

F (5, 30) = 2.355, p= 0.064.

There was a significant statistical difference in concentration of aflatoxin between the different divisions in the Uasin Gishu County, between Ainabkoi and Kesses and Ainabkoi and Moiben divisions (Table 4.16).

4.16: Post Hoc tests analysis on aflatoxin concentration at significance level of 0.05

Multiple Comparisons				
Aflatoxin Concentration LSD				
(I) Division	(J) Division	Mean Difference (I-J)	Std. Error	Sig.
Ainabkoi	Kesses	8.267	3.376	0.020
	Kapseret	9.079	3.011	0.005
	Soy	9.633	3.942	0.021
*. The mean difference is significant at the 0.05 level.				

F (5, 30) = 2.355, p= 0.064.

Table 4.17 shows the test results together with aflatoxin levels for all samples at the farm and market places.

Table 4.17: Farm and **market samples with their mean and std. Deviation**

Group Statistics					
	Sample Collected	N	Mean	Std. Deviation	Std. Error Mean
Test Result	Farm	120	1.70	0.460	0.042
	Market	45	1.71	0.458	0.068
Aflatoxin Concentration	Farm	36	8.117	6.090	1.015
	Market	13	19.777	26.265	7.285

The statistics indicate the samples origin on the aflatoxin detection and aflatoxin concentration (Table 4.18). F-ratio was not within 0.05 and 0.1 shows that there was a significance difference between farm samples and market samples with statistics showing that market samples had higher aflatoxin concentration than farm samples.

Table 4.18: Farm and market samples using Levene's test and t-test

Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Test Result	Equal variances assumed	0.078	0.780	-0.138	163.000	0.890	-0.011	0.080
	Equal variances not assumed			-0.139	79.355	0.890	-0.011	0.080
Aflatoxin Conc.	Equal variances assumed	40.477	0.000	-2.525	47.000	0.015	-11.660	46.188
	Equal variances not assumed			-1.585	12.469	0.138	-11.660	7.355

T (47) = 40.477, p=0.015

The statistics indicate that both farm and market samples had similar means in regard to the test results showing that there was no difference between the two samples.

The statistics indicate the effect of harvest time on the aflatoxin detection and aflatoxin concentration. Table 4.19 shows that there was a significance difference between those samples harvested timely and lately with statistics showing that those that were harvested timely tested positive for aflatoxin than those which were harvested late.

Table 4.19: Timely and late harvest **in relation to aflatoxin concentration**

Group Statistics					
	Harvest Time	N	Mean	Std. Deviation	Std. Error Mean
Test Result	Timely	15	1.87	0.352	0.091
	Late	105	1.68	0.470	0.046
Aflatoxin Concentration	Timely	2	1.40	11.030	7.800
	Late	34	7.77	5.782	0.916

T-test results indicate that there was no statistically significant difference in harvest time in relation to the aflatoxin concentration in the samples (Table 4.20).

Table 4.20: Levene's test and t-test analysis in timely and late harvest in relation to aflatoxin concentration

Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Test Result	Equal variances assumed	17.491	0.000	1.508	118	0.134	0.190	0.126
	Equal variances not assumed			1.871	21.861	0.075	0.190	0.102
Aflatoxin Conc.	Equal variances assumed	1.653	0.207	1.426	34	0.163	6.229	4.367
	Equal variances not assumed			0.792	1.033	0.570	6.229	7.862

T (118) =1.871, p=0.075

The statistics indicate the effect of drying method on the aflatoxin detection and aflatoxin concentration (Table 4.21).

Table 4.21: Drying method in relation to aflatoxin concentration

Group Statistics					
	Drying Method	N	Mean	Std. Deviation	Std. Error Mean
Test Result	Bare ground	21	1.57	0.507	0.111
	Other method	99	1.73	0.448	0.045
Aflatoxin Concentration	Bare ground	9	9.48	6.129	2.043
	Other method	27	7.66	6.125	1.179

T-test results indicate that there was no statistically significant difference in drying method used in relation to the aflatoxin detection and aflatoxin concentration in the samples (Table 4.22).

Table 4.22: Levene's test **and t-test analysis in drying method in relation to aflatoxin concentration.**

Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Test Result	Equal variances assumed	4.258	0.041	-1.416	118	0.160	-.156	0.110
	Equal variances not assumed			-1.305	27.007	0.203	-.156	0.119
Aflatoxin Conc.	Equal variances assumed	0.007	0.935	0.770	34	0.447	1.81481	2.35796
	Equal variances not assumed			0.769	13.744	0.455	1.81481	2.35880

The statistics indicate the effect of conversion method on the aflatoxin detection and aflatoxin concentration with maize which was converted by thrashing having high concentration of aflatoxin (Table 4.23).

Table 4.23: Conversion method in relation to aflatoxin concentration

Group Statistics					
	Conversion method	N	Mean	Std. Deviation	Std. Error Mean
Test Result	Thrashing	24	1.83	0.381	0.078
	Sheller	96	1.67	0.474	0.048
Aflatoxin Conc.	Thrashing	4	4.40	3.151	1.575
	Sheller	32	8.58	6.238	1.103

T-test results indicate that there was no statistically significant difference in shelling method used in relation to aflatoxin detection and aflatoxin concentration in the samples (Table 4.24).

Table 4.24: levene's test and t-test analysis in conversion method in relation toaflatoxin concentration

Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Test Result	Equal variances assumed	16.338	0.000	1.597	118.000	0.113	0.167	0.104
	Equal variances not assumed			1.821	42.718	0.076	0.167	0.092
Aflatoxin Conc.	Equal variances assumed	1.353	0.253	-1.308	34	0.200	-4.181	3.198
	Equal variances not assumed			-2.174	6.509	0.069	-4.181	1.922

The statistics indicate that there was no effect of sorting on the aflatoxin detection and aflatoxin concentration (Table 4.25).

Table 4.25: Sorting maize in relation to aflatoxin concentration

Group Statistics					
	Sorting	N	Mean	Std. Deviation	Std. Error Mean
Test Result	Yes	79	1.71	.457	.051
	No	41	1.68	.471	.074
Aflatoxin Conc.	Yes	23	8.457	5.615	1.171
	No	13	7.515	7.055	1.957

T-test results indicate that there is no statistically significant difference in between the samples that were sorted or not in relation to aflatoxin detection and aflatoxin concentration in the sample (Table 4.26).

Table 4.26: levene's test and **t-test analysis in sorting of maize in relation to aflatoxin concentration**

Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Test Result	Equal variances assumed	0.326	0.569	0.292	118.000	0.771	0.026	0.089
	Equal variances not assumed			0.289	78.980	0.773	0.026	0.090
Aflatoxin Conc.	Equal variances assumed	1.020	0.320	0.440	34.000	0.663	0.941	2.138
	Equal variances not assumed			0.413	20.685	0.684	0.9411	2.280

The statistics indicate the effect of storage form on the aflatoxin detection and aflatoxin concentration. There was some significance difference between samples which were stored in grains or cobs form. This shows that there was a significance difference between these two samples with statistics showing that those that were

stored in shelled form had higher mean aflatoxin concentration of 8.94 ppb while those that were stored in the non-shelled form was 5.64 ppb (Table 4.27).

Table 4.27: storage form **in relation to aflatoxin concentration**

Group Statistics					
	Storage Form	N	Mean	Std. Deviation	Std. Error Mean
Test Result	Shelled	92	1.71	0.458	0.048
	Not shelled	28	1.68	0.476	0.090
Aflatoxin Conc.	Shelled	27	8.94	6.730	1.295
	Not shelled	9	5.64	2.425	0.808

T-test results indicate that there was no statistically significant difference in storage in relation to the aflatoxin detection in the samples (Table 4.28).

Table 4.28: levene's test and t-test analysis in storage of maize in relation to aflatoxin concentration

Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Test Result	Equal variances assumed	0.291	0.591	0.280	118.000	0.780	0.028	0.100
	Equal variances not assumed			0.275	43.356	0.785	0.028	0.102
Aflatoxin Conc.	Equal variances assumed	5.766	0.022	1.427	34.000	0.163	3.296	2.310
	Equal variances not assumed			2.159	33.622	0.038	3.296	1.527

The statistics indicate the effect of storage method on the aflatoxin detection and aflatoxin concentration with those stored in cobs having high concentration in aflatoxin (Table 4.29).

Table 4.29: storage method in relation to aflatoxin concentration

Group Statistics					
	Storage Method	N	Mean	Std. Deviation	Std. Error Mean
Test Result	In bags	106	1.67	0.473	0.046
	In cobs	14	1.93	0.267	0.071
Aflatoxin Conc.	In bags	35	8.22	6.142	1.038
	In cobs	1	4.20	0	0

T-test results indicate that there was no statistically significant difference in the different methods of storage whether in cobs and in bags in relation to the aflatoxin concentration in the samples (tables 4.30).

Table 4.30: Levene's test **and t-test analysis in storage method of maize in relation to aflatoxin concentration**

Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Test Result	Equal variances assumed	41.326	0.000	-2.002	118.000	0.048	-0.259	0.129
	Equal variances not assumed			-3.048	25.413	0.005	-0.259	0.085
Aflatoxin Conc.	Equal variances assumed	0.000	0.000	0.647	34.000	0.522	4.02857	6.229
	Equal variances not assumed			0.000	0.000	0.000	4.02857	0.000

In Table 4.31 the statistics indicate the effect of storage places on the aflatoxin detection and aflatoxin concentration.

Table 4.31: Storage format in relation to aflatoxin concentration

Group Statistics					
	Storage format	N	Mean	Std. Deviation	Std. Error Mean
Test Result	On floor	38	1.68	0.471	0.076
	Other format	82	1.71	0.458	0.051
Aflatoxin Conc.	On floor	12	7.34	6.360	1.836
	Other format	24	8.50	6.052	1.235

T-test results indicate that there was no statistically significant difference in the storage format in relation to the aflatoxin concentration in the samples (Table 4.32).

Table 4.32: Levene's test and t-test analysis in storage format of maize in relation to aflatoxin concentration

Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Test Result	Equal variances assumed	0.249	0.619	-0.255	118.000	0.799	-0.023	0.091
	Equal variances not assumed			-0.252	70.321	0.802	-0.023	0.092
Aflatoxin Conc.	Equal variances assumed	0.000	0.989	-0.534	34.000	0.597	-1.163	2.175
	Equal variances not assumed			-0.525	21.143	0.605	-1.163	2.213

From this research it was found that those grains stored in clean storage facilities had high aflatoxin concentration of 8.39 ppb while those stored in unclean device had mean of 3.50 ppb (Table 4.33).

Table 4.33: Clean storage device in relation to aflatoxin concentration

Group Statistics					
	Clean storage device	N	Mean	Std. Deviation	Std. Error Mean
Test Result	Yes	116	1.71	0.457	0.042
	No	4	1.50	0.577	0.289
Aflatoxin Conc.	Yes	34	8.39	6.158	1.056
	No	2	3.50	1.273	0.900

T-test results indicate that there was no statistically significant difference in whether storage facilities were cleaned in relation to the aflatoxin concentration in the samples (table 4.34).

Table 4.34: Levene's test **and t-test analysis in use of clean storage device in relation to aflatoxin concentration**

Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Test Result	Equal variances assumed	0.813	0.369	0.883	118.000	0.379	0.207	0.234
	Equal variances not assumed			0.709	3.131	0.527	0.207	0.292
Aflatoxin Conc.	Equal variances assumed	1.551	0.221	1.107	34.000	0.276	4.888	4.417
	Equal variances not assumed			3.523	5.343	0.015	4.888	1.388

The statistics indicate that there was significance difference between those samples stored in granary and the ones not. Samples not stored in the granary had higher mean levels of aflatoxins of 5.63 ppb while those that were stored in the granary had 1.06 ppb (Table 4.35).

Table 4.35: Granary use in relation to aflatoxin concentration

Group Statistics					
	Granary use	N	Mean	Std. Deviation	Std. Error Mean
Test Result	Yes	60	1.70	0.462	0.060
	No	60	1.70	0.462	0.060
Aflatoxin Conc.	Yes	18	1.06	6.035	1.423
	No	18	5.63	5.179	1.221

T-test results indicate that there was no statistically significant difference in the use of granary in relation to the aflatoxin detection in the samples (Table4.36).

Table 4.36: Levene's test and t-test analysis in use of granary in relation to aflatoxin concentration

Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Test Result	Equal variances assumed	0.000	1.000	0.000	118.000	1.000	0.000	0.084
	Equal variances not assumed			0.000	118.000	1.000	0.000	0.084
Aflatoxin Conc.	Equal variances assumed	0.777	0.384	2.655	34.000	0.012	4.978	1.875
	Equal variances not assumed			2.655	33.235	0.012	4.978	1.875

T(34),=2.655, p=0.012

From table 4.37 only two samples were stored over fire. The statistics indicate that there was an effect of use of fire to dry maize cobs on the aflatoxin detection and aflatoxin concentration.

Table 4.37: fire storage in relation to aflatoxin concentration

Group Statistics					
	Fire storage	N	Mean	Std. Deviation	Std. Error Mean
Test Result	Yes	2	1.50	0.707	0.500
	No	118	1.70	0.459	0.042
Aflatoxin Conc.	Yes	1	8.20	0.000	0.000
	No	35	8.11	6.179	1.044

T-test results indicate that there was no statistically significant difference in the use of fire to dry the maize in relation to the aflatoxin detection in the samples as per Table 4.38.

Table 4.38: levene's test and t-test analysis in use of top of fire in relation to aflatoxin concentration

Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Test Result	Equal variances assumed	0.390	0.534	-0.618	118.000	0.538	-0.203	0.329
	Equal variances not assumed			-0.405	1.014	0.754	-0.203	0.502
Aflatoxin Conc.	Equal variances assumed	.	.	0.014	34.000	0.989	0.086	6.267
	Equal variances not assumed			0.000	0.000	0.000	0.086	0.000

The statistics indicate the effect of use of pesticides on the aflatoxin detection and aflatoxin concentration. Maize grains preserved using pesticide had aflatoxin mean of 8.01 ppb while those ones not preserved using pesticide had aflatoxin mean of 9.00 ppb (Table 4.39).

Table 4.39: Pesticide use in relation to aflatoxin concentration

Group Statistics					
	Pesticide use	N	Mean	Std. Deviation	Std. Error Mean
Test Result	Yes	111	1.71	0.455	0.043
	No	9	1.56	0.527	0.176
Aflatoxin Conc.	Yes	32	8.01	5.842	1.033
	No	4	9.00	8.884	4.442

T-test results indicate that there was no statistically significant difference in the use of pesticides in relation to the aflatoxin detection in the sample (Table 4.40).

Table 4.40: levene's test and t-test analysis in pesticide use in relation to aflatoxin concentration

Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Test Result	Equal variances assumed	1.665	0.199	0.979	118.000	0.330	0.156	0.160
	Equal variances not assumed			0.863	8.994	0.410	0.156	0.181
Aflatoxin Conc.	Equal variances assumed	1.010	0.322	-0.304	34.000	0.763	0.094	3.273
	Equal variances not assumed			-0.218	3.332	0.840	0.094	4.561

CHAPTER FIVE

DISCUSIONS

Four types of *aflatoxin*s B_1, B_2, G_1 and G_2 were detected and this confirms that within this area of study the A. *flavus* and A. *parasiticus* are present because the area lies within the tropics (Sargeant *et al.,* 1961). From the results it is evident that Moiben division had the highest aflatoxin mean level of 4.26 ppb as compared to the other 5 division, this was contributed by the highest number of samples which tested positive of aflatoxin (10 samples). Ainabkoi was the second with aflatoxin level of 3.26 ppb, these was caused by two samples which had high levels of aflatoxin of above 20 ppb. Turbo division was 3rd with mean level 1.91 ppb. Kapseret division was 4th overall with mean of 1.70 ppb and it was second when the number of positive samples was used. Kesses division was 5th with one sample having aflatoxin concentration of 11.20 ppb which was slightly above 10 ppb the limit recommended by KEBS. Soy division had the lowest mean of 0.68 ppb and also the division with the least number of samples that tested positive.

The first sample from Ainabkoi that tested positive was A-9, it tested positive of aflatoxin B_2 with a concentration of 4.40 ppb. For any mycotoxin contamination particularly in grains, it occurs in pockets of high concentration which are not randomly distributed and therefore in unsorted lot, a grain contains enough aflatoxin to result in significant level of aflatoxins levels (Hell *et al.,* 2000). Sample A-10 tested positive of aflatoxin G_2 (21.90 ppb) although from the results, correct grain handling was reported it may be relative, the maize was stored in a polypropene bags which do not allow moisture loss, creating a conducive environment for mycotoxins development. Sample A-15 was not sorted while sample A-16 was stored on the floor

and these were the main factors which led to aflatoxin contamination (Galuez *et al.*, 2003).

Sample A-17 tested positive of aflatoxin G_2 and the lot was not stored in granary. Sample A-10 was the sample with the highest level of aflatoxin of 21.90 ppb which was above MTL of 20 ppb as recommended by FAO, WHO and KEBS (FAO/WHO, 2003). This sample was from the lot which was not sorted, not stored in a granary and also no pesticides had been applied (Table 4.7). This is not surprising since Ainabkoi division borders Kaptagat forest to the west side making it to fall under different ecological zone of being wet most of the time within the year as compared to the other divisions. The result of Ainabkoi division further indicates that 85 % of the samples were lately harvested, that is, after three weeks (Bankole, 2003). Most of the samples representing 85 % were dried on bare ground with the 15 % of them being dried using other methods. Seventy five percent of the samples were collected from the farms whose cobs were shelled to obtain maize grains. Most of the farm samples were sorted before storage representing 55 %.

With the storage mode, most of the samples comprising 80 % were stored in bags as grains while 20 % were stored in cobs as they had not been shelled. Samples were either stored on the floor or in other modes, with 45 % of them being stored on the floor while the remaining was stored using other modes (Appendix 2). Considering these factors it is clear why Ainabkoi division was second highest in aflatoxin mean.

In Kesses division, among the 20 collected samples, 7 tested positive of aflatoxin which formed 35 % the samples with highest aflatoxin concentration being 11.20 ppb (Ke-14) while the lowest was 1.60 ppb. Only 1 sample recorded a value above the

MTL with its mean of 1.48 ppb (Table 4.3). The sample Ke-11 was from the lot stored in bags and if storage was done when the grains were not fully dried then it could be the cause of this high level. Sample Ke-6 had aflatoxin level of 2.60 ppb and was dried on bare ground, not sorted, stored on the floor, storage device was not cleaned, granary was not used and finally no pesticide was used. Ke-8 was stored on floor and no granary was used. Sample Ke-18 had aflatoxin concentration of 6.90 ppb, as per the data collected apart from late harvest, all the other protocol was observed showing that it was not perfectly done. Considering the parameters contributing to aflatoxin contamination, 90 % of the samples were lately harvested. It was also found that 85 % of the samples were dried on bare ground and another 85 % were stored in shelled form in polypropene bag though cheap they do not allow drying to take place since they hold back the moisture which creates a conducive environment for moulds growth (Kaaya., 2005). Seventy percent of the samples were stored in other forms rather than the use the recommended granary.

Kapseret division as per Table 4.5 shows that 8 samples tested positive of aflatoxins representing 40 % of the samples collected in the division. All samples which tested positive were, however below 10 ppb which is the limit set by KEBS and The mean level was 1.70 ppb. The sample with the highest concentration had a value of 8.90 ppb while the lowest was 1.70 ppb. Sample Ka-2 tested positive of G_2 and B_1 types of aflatoxin had aflatoxin concentration of 4.30 ppb. This sample had been dried on the bare ground, not sorted and also no granary was used for storage. This was also applicable to sample Ka-3 which had aflatoxin concentration of 3.90 ppb. Sample Ka-4 with aflatoxin concentration of 5.20 ppb was stored in bags and if the moisture content was above 14 % then this must have led to development of moulds. Ka-5

sample tested positive of G_1 and G_2 with a value of 1.90 ppb. The conversion method used was thrashing to obtain the grain and was not stored in the granary. Ka-10 sample tested positive of B_1 aflatoxin and concentration of 1.70 ppb, the lot was not stored in a granary. Ka-15 and Ka-16 both tested positive of G_2 and B_1 aflatoxin type with levels of 6.80 ppb and 8.90 ppb respectively. From the entire division, 90 % of the samples were lately harvested while 85 % were dried using other methods and 85 % being shelled and stored in bags (Appendix 5).

Soy division is summarized by Tables 4.6 and. Only 3 samples tested positive of aflatoxin with a mean of 0.68 ppb. All the 3 samples were not found to be beyond the recommended standard value which shows that all the collected samples were fit for human consumption. Sample So-4 tested positive with aflatoxin concentration of 6.00 ppb, this sample was dried on the bare ground. So-19 was not sorted, stored on the floor and also not stored in the granary (Appendix6). Apart from late harvesting, most of the practices were done correctly. All farmers who gave maize samples harvested their maize late and because this region is somehow dry during the month of November maize must have been dry. Eighty five percent of the samples collected were shelled using machines, implying the maize grains did not crack and the fungi could not penetrate (Bankole, 2003). It was also found that 65 % of the farmers sorted their maize and most of them did not dry their maize on bare ground. All the farmers cleaned their storage structures and this was reflected by only 3 samples testing positive of aflatoxin (Table 4.1).

The results of Turbo division are shown in Tables 4.1 and 4.7. From the tables it was evident that only 4 samples tested positive of aflatoxin but two samples had 10.60 ppb

and 15.20 ppb which were above the limit recommended by KEBS. Eighty five percent of the samples were harvested late and only 2 samples out of 20 were dried on bare ground. It was also found that 90 % of the farmers utilized shelling machine and 85 % sorted their maize before storage. When all the factors were considered there was relation in grain handling in Turbo and Soy division and also these two regions tend to be in the same ecological zone.

From the statistical analysis it was found that both farm and market samples had relatively similar means in regards to the test results. The test results for the farm samples had amean of 1.70 ppb while the market samples had a mean of 1.71 ppb. This shows that there was a chain in aflatoxin contamination from the farm to the market.

From the post-hoc test analysis, using the confidence level of 95 % and considering the mean difference at the 0.05 level in the test results and aflatoxin concentration between different divisions in Uasin Gishu County, it was found that there was significant mean difference between Ainabkoi and Kesses, Ainabkoi and Moiben and Kapsaret and Soy (Table 4.15).

With descriptive statistics for the samples collected for the study, the significant mean value of between 0.5-1.5 demonstrated an affirmative view on the factors leading to aflatoxin contamination and these were; sorting, storage method, clean storage device, granary use and pesticide use. These confirmed that they contributed to aflatoxin contamination. The other five remaining factors had statistic mean of above 1.5 which

showed that there were an alternative contributor of aflatoxin contamination necessitating further research (Mill, 2000)

Drying methods used can be either on bare ground or by the use of other methods. T-test results indicate that there was no statistically significant difference in drying methods used in relation to aflatoxin detection in samples. This shows that although maize was dried on ground or using other methods, drying was not thorough to reduce the moisture content to below 14 % which stills leaves the grain in condition necessary for moulds growth. Because of this case, farmers should access apparatus used to monitor moisture in grains and be advised accordingly in the method of drying (Table 4.22).

Statistics indicated the effect of conversion method on the aflatoxin detection and concentration. For the conversion method used maize converted using thrashing had a mean of 4.40 ppb while the ones in which conversion was done using shelling machine had a mean of 8.58 ppb. From these results it was evident that those ones which were converted using thrashing had a lower mean, showing that although grains crack, the moisture content was likely to be below 14 % because this conversion is done when grains are needed for use and by that time they are dry. Those ones converted using a sheller had the higher mean, since this conversion was done immediately after harvesting to reduce the bulkiness of maize and because the grains are not yet dry, they crack easily and this makes the fungi to penetrate the grains easily hence high concentration of aflatoxin (Bankole,2003). This was also justified by the t-test and descriptive statistic (Table 4.24).

The statistics indicated that there was no effect of sorting maize in relation to aflatoxin detection and concentration. T-test results indicate that there was no statistical significant difference between the samples that were sorted or not in relation to aflatoxin detection and concentration in the sample. Even one contaminated grain is enough to give a high concentration in a 90 kg bag of maize so even if sorting is done and by accident few contaminated grains are left behind, the levels of aflatoxin will still be high, (Hell *et al.*,2000) and (Udoh *et al.,* 2000). Also during harvesting, manual workers are paid as per the quantity of bags and if one sorts his/her maize by removing the discoloured maize cobs it will lead to reduction in the quantity of maize hence reduction in the pay. This therefore is a clear route to contaminated maize.

The statistics indicate the effect of storage form on the aflatoxin detection and aflatoxin concentration. There was some significant difference between samples which were stored in grains or cobs form. Storage of maize in shelled form was negatively related to aflatoxin development as was established in Benin, West Africa (Hell *et al.,* 2000). The significant value (0.022) from Levene's test shows that there was a significant difference between those samples stored in grains and cobs form which also agreed with descriptive statistics, whose results showed that those that were stored in shelled form had higher mean of aflatoxin concentration at 8.94 ppb while those that were stored in the non-shelled form at 5.64 ppb (Table 4.27, table 4.28). The maize which was stored in shelled form, mostly in polypropene bags thereby not allowing moisture to escape and hence creating a conducive environment for fungi to develop although these bag does well if the grains are dry to the moisture content below 14%, if not, this resulted in the aflatoxin contamination to be high in

shelled maize. Farmers prefer polypropene bags over the sisal made because they are cheap (Udoh *et al.,* 2000).

The statistics indicate the effect of use of granary on the aflatoxin detection and aflatoxin concentration, it shows that there was a significance difference between those samples stored in granary and those ones that were not. The results also showed that those that were stored in the granary had low mean aflatoxin concentration of 1.06 ppb than those that were not with the value of 5.63 ppb (Table 4.35). Use of improved granaries leads to reduction in aflatoxin contamination (Kaaya *et al.,* 2006)

Further statistics indicated the effect of storage places on the aflatoxin detection and aflatoxin concentration. Those that were stored on the floor had aflatoxin concentration of 7.34 ppb while the ones stored in other forms had a concentration of 8.50 ppb. T-test results indicated that there was no statistically significant difference in the storage places in relation to the aflatoxin concentration in the samples. This was so because the other avenues of storage were not considered. Other forms can be methods which expose the maize to fungi growth like keeping them in a damp floor or even being on bags arranged outside and not sheltered well from rain.

Statistics indicated the effect of use of fire to dry maize cobs on the aflatoxin detection and aflatoxin concentration. T-test results indicated that there was no statistically significant difference in the use of fire to dry the maize in relation to the aflatoxin detection in the samples. Only two samples were stored over fire and this did not give the true effect of storing maize over fire. From this research it also

showed that this method was rarely used in this region because out of 120 collected samples, only 2 were dried over fire.

The statistics indicated the effect of use of pesticides on the aflatoxin detection and aflatoxin concentration. Those samples in which pesticides were applied had aflatoxin concentration of 8.00 ppb and those which were not applied had a level of 9.00 ppb. T-test results indicated that there was no statistically significant difference in the use of pesticides in relation to the aflatoxin detection in the samples. Two things which determine the effectiveness of a pesticide is correct application by making it to be evenly distributed and also the right quantity. A study done on use of pesticides was observed that actellic did not have any direct effect on *A. flavus* development in maize grain but insecticides were secondary agents in the aflatoxin contamination (El-Kady *et al*.,1993).

CHAPTER SIX

6.1 Conclusion

The results of the study involving all the six divisions of Uasin Gishu County revealed that there are 4 main aflatoxins types namely; B_1, B_2, G_1 and G_2 found in Uasin Gishu County. The concentration of aflatoxin ranged from 0.50 ppb to 23.30 ppb at the farm level and at the market level the range was 0.90 ppb in June and 68.10 ppb in July. At the farmers level, out of 120 collected samples in the whole County, 36 samples tested positive which forms 30%. Three samples had quantities of 21.80 ppb, 21.90 ppb and 23.30 ppb, the first 2 from Ainabkoi division and 1 from Moiben division above MTL which is a risk not only to our health but also to our economy.

The cereals get contaminated when incorrect pre and post harvesting practice is not followed and because the samples analyzed tested positive of aflatoxin it confirms that correct grain handling was not followed. For the 3 months studied June, July and August in the market, the levels varied depending on market price. This is because the cereals are not properly handled during transportation. The business people do their packing and transportation hurriedly in order to maximize on profits and during this time the probability of maize to be contaminated is high. Methods used allow moisture to pile up especially during drying where porous materials are used which allow moisture and microorganisms to penetrate through reaching the cereals. Also during drying process people ensuring that sun heat is distributed they use their legs to spread the maize after stepping on ground leading to aflatoxin contamination.

ANOVA results showed that there was relation between Kesses and Moiben, Soy and Kapseret and Turbo and Moiben divisions in the level of aflatoxin concentration, the

F- ratio were above 1. From descriptive statistics it was found that the practices; sorting, storage method, clean storage device, granary use and use of pesticide limit aflatoxin contamination in maize. Their significant mean were within 0.5 to 1.5. The post-Hoc test revealed that the only practice which was mostly practiced was use of pesticide but the other practices studied if done it were not perfectly.

6.2 Recommendations

To minimize aflatoxin contamination the following are suggestions;

1. Farmers should ensure their cereals are dry with the moisture content being below 14 % and drying should be within 24 to 48 hours after harvesting and not on the bare ground.
2. Farmers to sort their maize properly before storage to avoid aflatoxin contamination.
3. Farmers should not use discoloured maize to feed their animals because aflatoxin M1 and M2 will be in the animal products; milk and eggs.
4. Promote pre and post-harvest technologies that minimize aflatoxin contamination.
5. Information dissemination on the methods of control should be effective.
6. Do participatory evaluation of cultural practice to reduce aflatoxin contamination, demonstrate best-bet harvest and drying techniques.
7. Training for technology dissemination, have a laboratory in each district to monitor quality of cereals and advice farmers accordingly.

For further research:

1. Research on prevalence and concentration of aflatoxin on specific types.
2. The 3 months sampling was not enough, a research covering full year should be done.
3. Studies to be done to identify varieties which are tolerable to aflatoxin.

REFERENCES

Allergens, mycotoxins and poisonous Fungi A. parasiticus ad A. nomius produce a mycotoxin. http:/www.angelfire.com/wizard. 5th Nov. 2009

Alpert M., Serek A. and B., Rafage Polan. (1970) *Aflatoxin-induces hepatic injury in the Africa monkey.* Arch Environ. Heath,.

Autrup J., Schmidt J., Autrup H. (1993): "Exposure to aflatoxin B1 in animal-feed production plant workers". *Journal in Eviron Health Perspect* 99 195-197.

Battacone G., Nudda A., Pamelomba M., Nicolussi P., Pulina G. (2005), "Transfer of aflatoxin B1, from feed to milk and from milk to curd." *Journal on Dairy science* 88(9):3036-3069.

Bankole, Adebanjo (2003). "mycotoxins in food in West Africa, current situation and possibilities of controlling it." *African Journal Biol*. 2:254-263.

Blount W. 1961, *Turkey "X" disease*. Turkeys.

Cooms J., Crowthers P., Fevell A., Francis, B.J. (1966), *Nature (London*) 209-406..

El-kady., I.A., S.S., M., El Maraghy, Abedl Y., Hassn H. (1993), "Effect of four pesticides on aflatoxin production." *A Journal ofMicrobiol* 148:549-557.

Enric, Francisco, Jordi, B., Merce, Anna- Mara, Angel M. (1991), "Synthesis and mutagenicity of aflatoxin B1 Model 3a,8a-Dihydro-4,6-dimethoxyfuro[2,3-b]benzofuran and its 2,3-epoxy Dirivative*." Journal of Agric. Food chem*. 39: 1723-1728.

FAO (1982). *Mycotoxin surveillance:* A Guideline FAO Food and Nutrition paper 21. Rome.

FAO (1999). *Preventing Mycotoxin Contamination in Food Nutrition and Agricuture*. FAO Food and Nutrition Division 23, Rome.

FAO. *Worldwide regulations for mycotoxin in food and feed*. FAO and Nutrition paper 81 Rome Italy.

Food and Agricultural organization (FAO) and United Nations. (2003) "Evaluation of certain mycotoxin in Food-fifty-sixth Report of the joint FAO/WHO, Export Committee on Food Additive and World Health Organization". Geneva 2002.

Food and Drug administration (FDA) and United States (2002). *Food borne pathogenic microorganisms and Natural Toxins Handbook.* FDA/CFSAN Bad Bug Book aflatoxins, U.S Food & Drug Administration, Centre for food safety & Applied Nutrition

Galuez, F.C., MLDL Francisco, Villarno H., Luste A. and Resurreccion V., (2005) . "Manual sorting to eliminate aflatoxin from peanuts" *Journal of Food Protection* 661879-1884.

Gatonye gathura. "Alert over maize poisoning." Daily Nation 8th August 2008

Grybasuskas A., Thomison P. and Casel E., *Aflatoxins Maryland cooperative Extension fact sheet 444,P88/R2000*.

Goodrow M., Sanborn J., D.W., Stoutamire, Gee S., B.D., Hammock. (1995) *Strategies for immunoassay hapten designin J.O Nelson A.E Karuy r.B Wong (Editors).* Immunogens of Agrochemicals: Emerging Technologies. Amertican chemical Society symposium Series No. 586 American Chemical Society Washington D.C U.S.A : 119-133.

Hell K., Cardwell M., Setamou and Peohling H. (2000),"The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin." West Africa Journal of Stored Products365-382.

Hughes E., and Davies E. (2006), "Determinatiot of Aflatoxin in Foodstuffs". Campeded and Chorleywood research Association. 1-18.

International Agency for Research on Cancer (IARC). (2002). "*Aflatoxin in traditional Herbal medicine, Some Mycotoxins, Naphthalene and styrene*". IARC monographs on the Evaluation of carcinogenic Risks of Humans, vol. 82. Lyon France 171-366.

International Crops Reseatch Institute for Semi-Arid Tropics(ICRITSAT) (1987). *Aflatoxin contamination of groundnuts*.

John L. (2008) "Discovery of Aflatoxins and Significant Hitorical Features." Research Article Vol.27 NO.3-4.

JuanL., Carvajal M. and Ituarte B. (1995):, *Supervising programme of aflatoxin in Mexican corn Food Aditives*. Count 12297-312.

Kaaya A.,Warren H. (2005) "A Review of Past and Present Research on Aflatoxin in Uganda. *African Journal of Food Agriculture Nutrition and Development*. Peer Review Article No. 5

Kaaya A., Kyamuhangiire W., Kyamanywa S., (2006), "Factors Affecting Aflatoxin Contamination of Harvested Maize in the three Agro ecological Zones of Uganda." Journal of Applied Scinces 6(11):2401-2409

Larson E.(2001) "minimizing Aflatoxin in corn Mississippi".*State university Extension service,* Information sheet 1563,

Miller N., Jane C., Miller. (2000). Statistics and Chemometrics for Analytical Chemistry. 4th Ed. Pearson Education Limited. Edinburgh Gate. Published

Odogala, W.R., Henrksson R (1991).., "Post-harvest management and storage of maize." Technical systems for agricultural UNDP/OPS regional program on operations technology for small holders in East and Sourthern Africa

Peiwu L., Zhang Q. (2009). "Immunoassay for Aflatoxin." *Journal in trends in Analytical chemistry Vol.28 No. 9.*

Reddy S., Waliyar F. (1976), "Properties of aflatoxin and it producing Fungi." *Journal of international Research Institute for the Semi-Arid Tropics.* 1-4.

Republic of Kenya. (2005), *Uasin Gishu District PRSP consultation Report for the period 2001-2004PRSP* website: www. Treasury .go.Ke/prs.

Richard J., Payne G. (2003), "Mycotoxins in plant, animal and human system." Task force report No. 39. Council for Science and Technoly

Tutite J., Koh-Knox R., Strohsine F., Cantone and Baumen L., (1985) "effect of physical damage to corn Kernels on the development of *penicillium species* and *aspergillus glaucus* in storage phytopathology."

Udoh, J.M., Cardwell K., Ikotum T. (2000), "Storage structures and aflatoxin content of maize in five agroecological zones of Nigeria" *Journal in. stored products.* 36 187-201.

Srimam B., *Kenya vision 2030* 1st Edition of vision 2030 published by Government of Kenya 2007.

Ssebukyu E. (2002), "Fungi and aflatoxins in maize in Uganda." MSC Thesis, Department of Botonany, Makerere University Kampala, Uganda

WilliamT., Leonard S. (1967)., "possible reactions of afalatoxin detoxification" Vol. 15 No. 4

Vessonder, Alfred C., Robert J, (1975) “Ammonium hydroxide treatment of aflatoxin B1, some chemical characteristics and biological effects.” *Jounal of Agr.Food Chem. Vol 23. No. 2.*

World Health Organization (WHO) (1979). *Environment health criteria 11*: mycotoxins Geneva Switzerland World Health Organization.

Zarba A.,Wild C., Hall A., Montesano R., Hudson G. (1992) “Aflatoxin M1 in human breast milk from the Gambia, West Africa”. *Journal inCarcinogenesis* 13(5): 827-894.

Zhouhui J., Yu P., Wang B., Xie F. (2007), “Immunology methods.” *Chemistry Journal* 328:79

APPENDICES

Appendix I

QUESTIONNAIRE

I am a postgraduate student currently undertaking a research on **"DETERMINATION OF LEVELS OF AFLATOXIN IN MAIZE SAMPLES, A CASE STUDY OF UASIN GISHU COUNTY"**

You have been chosen as one of our respondents. Kindly respond to these questions by ticking or circling the appropriate question. Be honest as much as possible. The information will be treated with the highest level of confidentiality. There is no right or wrong answer to these questions.

INSTRUCTIONS: Please fill the responses in the spaces provided.

Q1. Did you leave your maize to dry in the field for more than three weeks?

{YES} {NO}

Q2. Did you dry your maize on bare ground?

{YES} {NO}

Q3. Did you shell your maize by?

(i) Thrashing {YES} {NO}

(ii) Using tractor Sheller {YES} {NO}

Q4. Did you sort your maize before storage?

{YES} {NO}

Q5. Is your maize stored in stored form?

{YES} {NO}

Q6. Is your maize stored in bags?

{YES} {NO}

Q7. Is your maize heaped on floor during storage?

{YES} {NO}

Q8. Did you clean your storage structure before storage?

{YES} {NO}

Q9. Do you use improved granary as a storage structure?

{YES} {NO}

Q10. Do you store your maize above fire place?

{YES} {NO}

Q11. Do you store you use synthetic pesticides (Actellic super or Malathion 2%)?

{YES} {NO}

Thank you.

Appendix II: Case summary for Ainabkoi Division

	Case summary Ainabkoi Division									
A1-1	Harvest Time	Drying Method	Conversion method	Sorting	Storage Form	Storage Method	Storage modes	Clean storage device	Granary use	Pesticide use
A1-2	Late	Other method	Sheller	Yes	Shelled	In cobs	On floor	Yes	Yes	Yes
A1-3	Late	Other method	Sheller	No	Shelled	In bags	On floor	Yes	Yes	Yes
A1-3	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
A1-4	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	No	Yes	Yes
A1-5	Timely	Other method	Thrashing	Yes	Not shelled	In cobs	On floor	Yes	Yes	Yes
A1-6	Late	Other method	Thrashing	Yes	Not shelled	In cobs	On floor	Yes	Yes	Yes
A1-7	Late	Bare ground	Sheller	Yes	Shelled	In bags	On floor	Yes	No	Yes
A1-8	Late	Bare ground	Sheller	No	Shelled	In bags	On floor	Yes	No	Yes
A1-9	Late	Other method	Sheller	No	Shelled	In bags	Other modes	No	Yes	Yes
A1-10	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
A1-11	Late	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	Yes	Yes
A1-12	Late	Other method	Thrashing	Yes	Not shelled	In cobs	On floor	Yes	No	Yes
A1-13	Late	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	Yes	Yes
A1-14	Timely	Other method	Thrashing	Yes	Not shelled	In cobs	On floor	Yes	Yes	Yes
A1-15	Late	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	Yes	Yes
A1-16	Late	Bare ground	Sheller	Yes	Shelled	In bags	On floor	Yes	Yes	Yes
A1-17	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	Yes
A1-18	Late	Other method	Thrashing	Yes	Shelled	In bags	Other modes	No	No	Yes
A1-19	Timely	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	No	No
A1-20	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes

APPENDIX III: Case summary for Kesses Division

	Case summary Kesses Division									
	Harvest Time	Drying Method	Conversion method	Sorting	Storage Form	Storage Method	Storage modes	Clean storage device	Granary use	Pesticide use
Ke2-1	Late	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	No	Yes
Ke2-2	Timely	Bare ground	Sheller	No	Shelled	In bags	On floor	Yes	No	No
Ke2-3	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	No
Ke2-4	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
Ke2-5	Timely	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	No	Yes
Ke2-6	Late	Bare ground	Thrashing	No	Not shelled	In bags	On floor	No	No	No
Ke2-7	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
Ke2-8	Late	Other method	Sheller	Yes	Not shelled	In bags	On floor	Yes	No	Yes
Ke2-9	Late	Bare ground	Thrashing	Yes	Shelled	In bags	On floor	Yes	Yes	Yes
Ke2-10	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
Ke2-11	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
Ke2-12	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	Yes
Ke2-13	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	Yes
Ke2-14	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	Yes
Ke2-15	Late	Other method	Thrashing	Yes	Not shelled	In cobs	On floor	Yes	No	Yes
Ke2-16	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	Yes
Ke2-17	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	Yes
Ke2-18	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
Ke2-19	Late	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	No	Yes
Ke2-20	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	Yes

Appendix IV: Case summary for Moiben Division

	Case summary Moiben Division									
	Harvest Time	Drying Method	Conversion method	Sorting	Storage Form	Storage Method	Storage modes	Clean storage device	Granary use	Pesticide use
Mo3-1	Late	Other method	Sheller	Yes	Not shelled	In bags	Other modes	Yes	Yes	No
Mo3-2	Late	Other method	Beating	No	Not shelled	In bags	Other modes	Yes	No	Yes
Mo3-3	Late	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	No	Yes
Mo3-4	Late	Other method	Beating	Yes	Not shelled	In cobs	On floor	Yes	Yes	Yes
Mo3-5	Late	Bare ground	Sheller	Yes	Shelled	In bags	On floor	Yes	Yes	Yes
Mo3-6	Late	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	No	No
Mo3-7	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	Yes
Mo3-8	Late	Other method	Sheller	No	Shelled	In bags	On floor	Yes	No	Yes
Mo3-9	Timely	Other method	Sheller	Yes	Not shelled	In bags	Other modes	Yes	Yes	Yes
Mo3-10	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
Mo3-11	Late	Bare ground	Sheller	No	Shelled	In bags	On floor	Yes	Yes	Yes
Mo3-12	Late	Other method	Sheller	Yes	Not shelled	In bags	Other modes	Yes	No	Yes
Mo3-13	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
Mo3-14	Timely	Bare ground	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
Mo3-15	Late	Bare ground	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	Yes
Mo3-16	Late	Bare ground	Sheller	Yes	Shelled	In bags	On floor	Yes	Yes	Yes
Mo3-17	Late	Other method	Sheller	No	Shelled	In bags	On floor	Yes	Yes	Yes
Mo3-18	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
Mo3-19	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	Yes
Mo3-20	Late	Other method	Sheller	Yes	Not shelled	In bags	Other modes	Yes	Yes	Yes

Appendix V: Case summary for Kapseret Divisiona

	Case summary Kapseret Division									
	Harvest Time	Drying Method	Conversion method	Sorting	Storage Form	Storage Method	Storage modes	Clean storage device	Granary use	Pesticide use
Ka4-1	Late	Bare ground	Sheller	No	Shelled	In bags	On floor	Yes	No	Yes
Ka4-2	Late	Bare ground	Sheller	No	Shelled	In bags	Other modes	Yes	No	Yes
Ka4-3	Late	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	No	Yes
Ka4-4	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
Ka4-5	Late	Other method	Beating	Yes	Shelled	In bags	Other modes	Yes	No	Yes
Ka4-6	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
Ka4-7	Timely	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	Yes	Yes
Ka4-8	Late	Other method	Beating	No	Shelled	In bags	On floor	Yes	No	No
Ka4-9	Late	Other method	Beating	No	Shelled	In bags	On floor	No	No	Yes
Ka4-10	Late	Other method	Sheller	Yes	Shelled	In bags	On floor	Yes	No	Yes
Ka4-11	Late	Other method	Beating	No	Shelled	In bags	Other modes	Yes	Yes	No
Ka4-12	Late	Bare ground	Sheller	No	Shelled	In bags	On floor	Yes	Yes	Yes
Ka4-13	Late	Other method	Beating	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
Ka4-14	Late	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	No	Yes
Ka4-15	Late	Other method	Sheller	Yes	Not shelled	In bags	Other modes	Yes	Yes	Yes
Ka4-16	Late	Other method	Beating	Yes	Not shelled	In bags	Other modes	Yes	No	Yes
Ka4-17	Late	Other method	Beating	No	Shelled	In bags	Other modes	Yes	Yes	Yes
Ka4-18	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
Ka4-19	Timely	Other method	Beating	Yes	Not shelled	In cobs	On floor	Yes	No	Yes
Ka4-20	Late	Other method	Beating	No	Shelled	In bags	Other modes	Yes	Yes	Yes

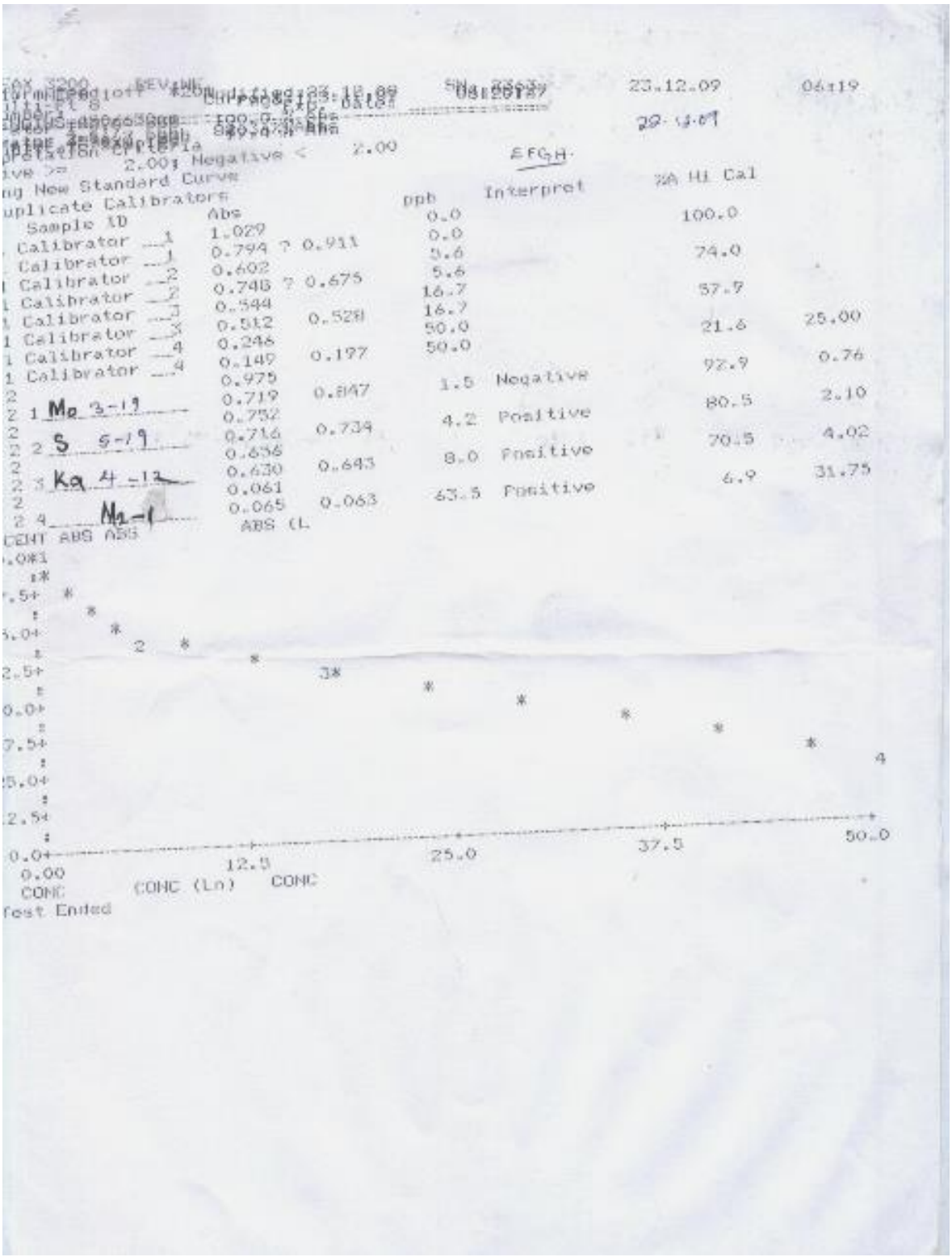
Appendix VI: Case summary for Soy Division

	Case summary Soy Division									
	Harvest Time	Drying Method	Conversion method	Sorting	Storage Form	Storage Method	Storage modes	Clean storage device	Granary use	Pesticide use
So5-1	Timely	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	Yes	Yes
So5-2	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
So5-3	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
So5-4	Late	Bare ground	Sheller	Yes	Not shelled	In bags	Other modes	Yes	Yes	Yes
So5-5	Late	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	No	No
So5-6	Late	Bare ground	Beating	No	Not shelled	In bags	On floor	Yes	Yes	Yes
So5-7	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	Yes
So5-8	Late	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	No	Yes
So5-9	Late	Bare ground	Sheller	No	Shelled	In bags	On floor	Yes	No	Yes
So5-10	Timely	Other method	Sheller	Yes	Not shelled	In cobs	On floor	Yes	No	Yes
So5-11	Late	Other method	Sheller	Yes	Not shelled	In bags	Other modes	Yes	No	Yes
So5-12	Late	Bare ground	Beating	Yes	Not shelled	In cobs	On floor	Yes	No	Yes
So5-13	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
So5-14	Late	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	Yes	Yes
So5-15	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	Yes
So5-16	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes
So5-17	Late	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	No	Yes
So5-18	Timely	Other method	Sheller	No	Not shelled	In bags	Other modes	Yes	No	Yes
So5-19	Late	Other method	Beating	No	Not shelled	In cobs	On floor	Yes	No	Yes
So5-20	Late	Bare ground	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes

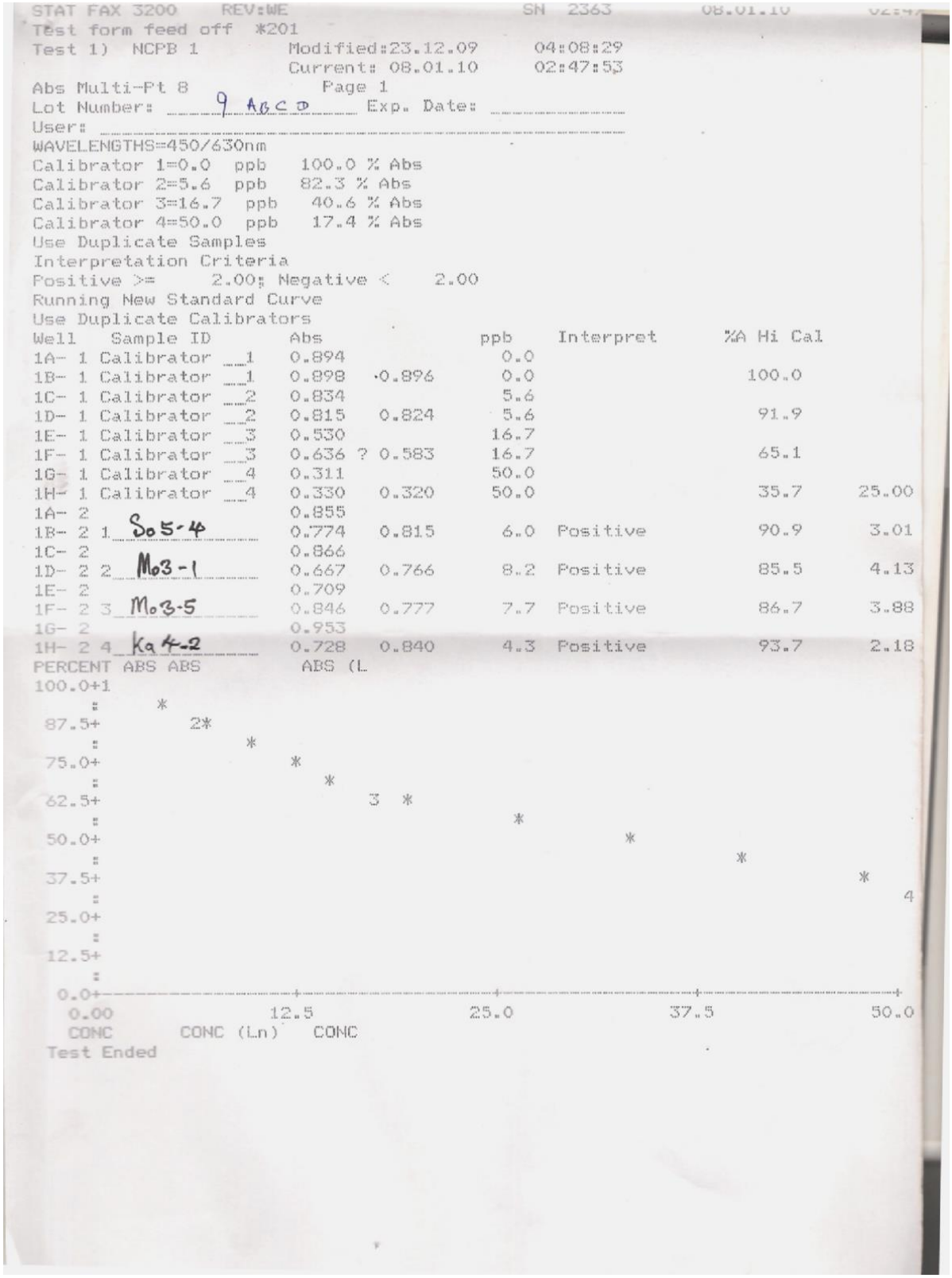
Appendix VII: Case summary for Turbo Division

	Case summary Turbo Division										
	Harvest Time	Drying Method	Conversion method	Sorting	Storage Form	Storage Method	Storage modes	Clean storage device	Granary use	Fire storage	Pesticide use
T6-1	Timely	Other method	Sheller	No	Not shelled	In bags	On floor	Yes	No	No	Yes
T6-2	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	No	Yes
T6-3	Late	Other method	Sheller	No	Shelled	In bags	Other modes	Yes	Yes	No	Yes
T6-4	Late	Other method	Beating	Yes	Not shelled	In cobs	On floor	Yes	No	No	Yes
T6-5	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	No	Yes
T6-6	Timely	Other method	Sheller	Yes	Shelled	In cobs	On floor	Yes	No	No	Yes
T6-7	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	No	Yes
T6-8	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	No	Yes
T6-9	Late	Bare ground	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	Yes	Yes
T6-10	Late	Other method	Sheller	Yes	Not shelled	In bags	Other modes	Yes	Yes	No	Yes
T6-11	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	No	Yes
T6-12	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	No	Yes
T6-13	Late	Other method	Sheller	Yes	Not shelled	In bags	On floor	Yes	No	No	Yes
T6-14	Late	Other method	Sheller	Yes	Shelled	In bags	On floor	Yes	Yes	No	Yes
T6-15	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	No	Yes
T6-16	Late	Other method	Beating	Yes	Not shelled	In cobs	On floor	Yes	No	No	Yes
T6-17	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	No	Yes
T6-18	Late	Bare ground	Sheller	No	Shelled	In bags	On floor	Yes	No	No	Yes
T6-19	Timely	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	Yes	No	Yes
T6-20	Late	Other method	Sheller	Yes	Shelled	In bags	Other modes	Yes	No	No	Yes

Appendix VIII: ELISA printout of samples Mo3-19, So5-19, Ka4-12 and M2-1



Appendix IX: ELISA printout of samples So5-4, Mo3-1, Mo3-5 and Ka4-2.



STAT FAX 3200 REV:WE SN 2363 08.01.10 02:47

Test form feed off *201

Test 1) NCPB 1 Modified:23.12.09 04:08:29
Current: 08.01.10 02:47:53

Abs Multi-Pt 8 Page 1

Lot Number: 9 ABCD Exp. Date: _____

User: _____

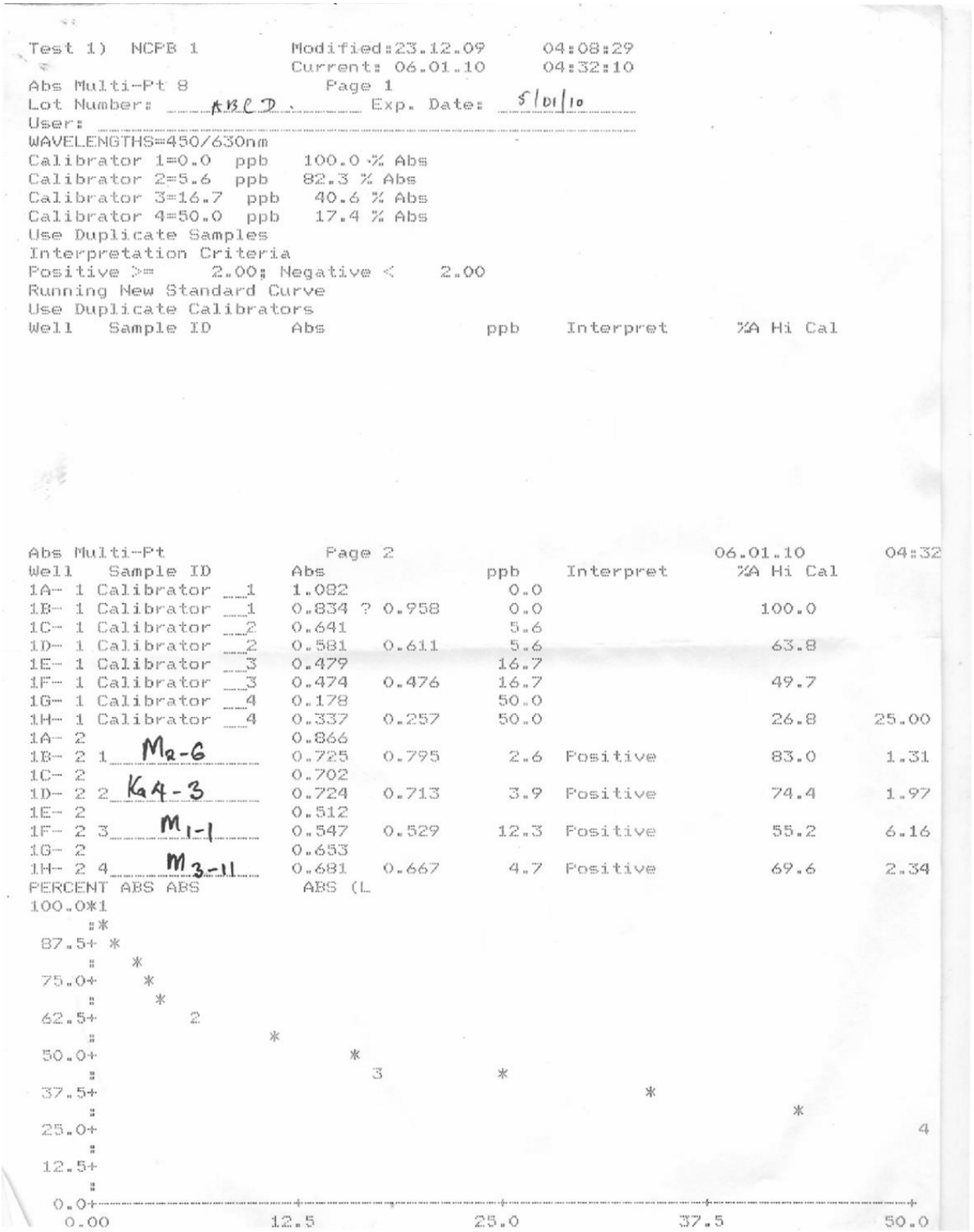
WAVELENGTHS=450/630nm
Calibrator 1=0.0 ppb 100.0 % Abs
Calibrator 2=5.6 ppb 82.3 % Abs
Calibrator 3=16.7 ppb 40.6 % Abs
Calibrator 4=50.0 ppb 17.4 % Abs
Use Duplicate Samples
Interpretation Criteria
Positive >= 2.00; Negative < 2.00
Running New Standard Curve
Use Duplicate Calibrators

Well	Sample ID		Abs		ppb	Interpret	%A Hi Cal	
1A- 1	Calibrator	1	0.894		0.0			
1B- 1	Calibrator	1	0.898	0.896	0.0		100.0	
1C- 1	Calibrator	2	0.834		5.6			
1D- 1	Calibrator	2	0.815	0.824	5.6		91.9	
1E- 1	Calibrator	3	0.530		16.7			
1F- 1	Calibrator	3	0.636 ?	0.583	16.7		65.1	
1G- 1	Calibrator	4	0.311		50.0			
1H- 1	Calibrator	4	0.330	0.320	50.0		35.7	25.00
1A- 2			0.855					
1B- 2	1 So5-4		0.774	0.815	6.0	Positive	90.9	3.01
1C- 2			0.866					
1D- 2	2 Mo3-1		0.667	0.766	8.2	Positive	85.5	4.13
1E- 2			0.709					
1F- 2	3 Mo3-5		0.846	0.777	7.7	Positive	86.7	3.88
1G- 2			0.953					
1H- 2	4 Ka4-2		0.728	0.840	4.3	Positive	93.7	2.18

PERCENT ABS ABS ABS (L

Test Ended

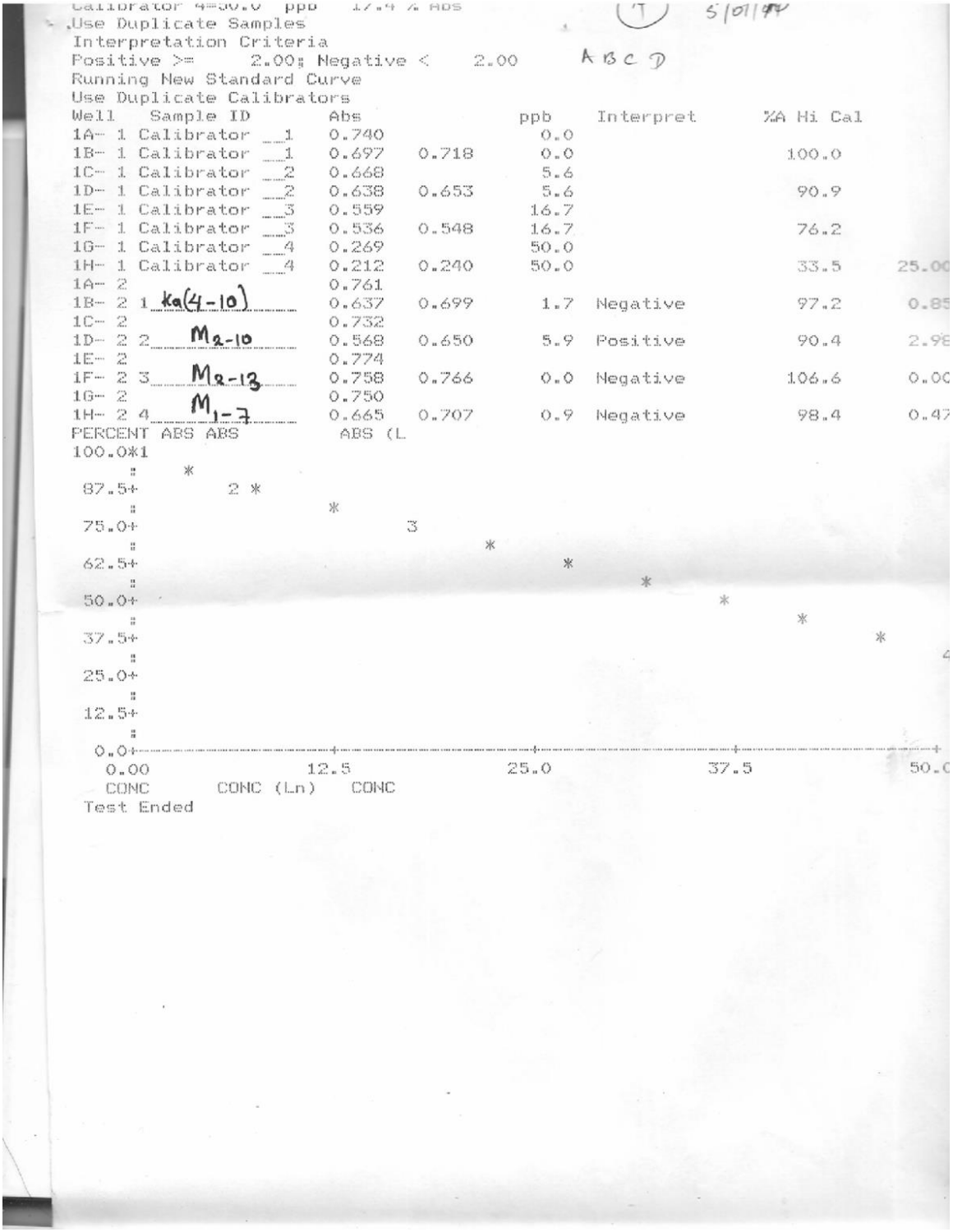
Appendix X: ELISA printout of samples M2-6, Ka4-3, M1-1 and M3-11.



```
Test 1)  NCPB 1          Modified:23.12.09      04:08:29
                         Current: 06.01.10      04:32:10
Abs Multi-Pt 8             Page 1
Lot Number: KBLD          Exp. Date: 5/01/10
User:
WAVELENGTHS=450/630nm
Calibrator 1=0.0  ppb   100.0 % Abs
Calibrator 2=5.6  ppb   82.3 % Abs
Calibrator 3=16.7  ppb   40.6 % Abs
Calibrator 4=50.0  ppb   17.4 % Abs
Use Duplicate Samples
Interpretation Criteria
Positive >=     2.00; Negative <     2.00
Running New Standard Curve
Use Duplicate Calibrators
Well   Sample ID        Abs              ppb     Interpret       %A Hi Cal
```

```
Abs Multi-Pt               Page 2                          06.01.10        04:32
Well   Sample ID        Abs                ppb     Interpret       %A Hi Cal
1A- 1 Calibrator __1    1.082               0.0
1B- 1 Calibrator __1    0.834 ? 0.958       0.0                       100.0
1C- 1 Calibrator __2    0.641               5.6
1D- 1 Calibrator __2    0.581   0.611       5.6                        63.8
1E- 1 Calibrator __3    0.479              16.7
1F- 1 Calibrator __3    0.474   0.476      16.7                        49.7
1G- 1 Calibrator __4    0.178              50.0
1H- 1 Calibrator __4    0.337   0.257      50.0                        26.8      25.00
1A- 2                   0.866
1B- 2 1 M2-6            0.725   0.795       2.6  Positive              83.0       1.31
1C- 2                   0.702
1D- 2 2 Ka4-3           0.724   0.713       3.9  Positive              74.4       1.97
1E- 2                   0.512
1F- 2 3 M1-1            0.547   0.529      12.3  Positive              55.2       6.16
1G- 2                   0.653
1H- 2 4 M3-11           0.681   0.667       4.7  Positive              69.6       2.34
PERCENT ABS ABS          ABS (L
100.0*1
    :*
 87.5+ *
    :   *
 75.0+    *
    :      *
 62.5+         2
    :                *
 50.0+                     *
    :                        3          *
 37.5+                                          *
    :                                                    *
 25.0+                                                                    4
    :
 12.5+
    :
  0.0+-----------+-----------+-----------+-----------+-----------+
    0.00                12.5                25.0                37.5                50.0
```

Appendix XI: ELISA printout of samples Ka4-10, M2-10, M2-13and M1-7.



Calibrator 4=50.0 ppb 17.4 % ABS (T) 5/01/94

Use Duplicate Samples

Interpretation Criteria

Positive >= 2.00; Negative < 2.00 A B C D

Running New Standard Curve

Use Duplicate Calibrators

Well	Sample ID	Abs		ppb	Interpret	%A Hi Cal	
1A- 1	Calibrator 1	0.740		0.0			
1B- 1	Calibrator 1	0.697	0.718	0.0		100.0	
1C- 1	Calibrator 2	0.668		5.6			
1D- 1	Calibrator 2	0.638	0.653	5.6		90.9	
1E- 1	Calibrator 3	0.559		16.7			
1F- 1	Calibrator 3	0.536	0.548	16.7		76.2	
1G- 1	Calibrator 4	0.269		50.0			
1H- 1	Calibrator 4	0.212	0.240	50.0		33.5	25.0
1A- 2		0.761					
1B- 2	1 Ka(4-10)	0.637	0.699	1.7	Negative	97.2	0.8
1C- 2		0.732					
1D- 2	2 M2-10	0.568	0.650	5.9	Positive	90.4	2.9
1E- 2		0.774					
1F- 2	3 M2-13	0.758	0.766	0.0	Negative	106.6	0.0
1G- 2		0.750					
1H- 2	4 M1-7	0.665	0.707	0.9	Negative	98.4	0.4

PERCENT ABS ABS ABS (L

100.0%1

87.5+ 2

75.0+ 3

62.5+

50.0+

37.5+

25.0+

12.5+

0.0+

0.00 12.5 25.0 37.5 50.0

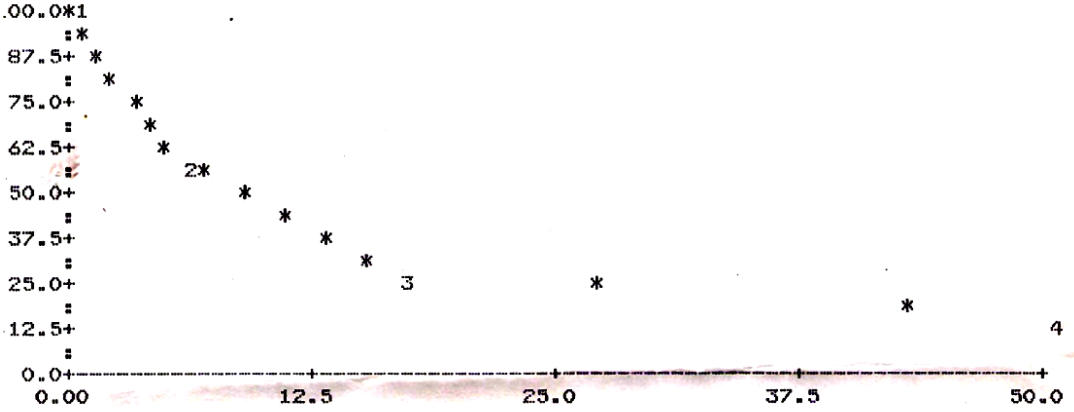
CONC CONC (Ln) CONC

Test Ended

Appendix XII: ELISA printout of samples Ka4-16, Mo3-12, Mo3-10 and A1-16.

STAT FAX 3200 REV:WE SN 2363 23.12.09 04:0

est form feed off *201
est 1) NCPB 1 Modified:15.12.09 07:12:11
Current: 23.12.09 04:08:29
bs Multi-Pt 8 3 Page 1
ot Number: _____ Exp. Date: 22.12.09.
ser: ABCD
AVELENGTHS=450/630nm
alibrator 1=0.0 ppb 100.0 % Abs
alibrator 2=5.6 ppb 60.0 % Abs
alibrator 3=16.7 ppb 29.1 % Abs
alibrator 4=50.0 ppb 16.1 % Abs
se Duplicate Samples
nterpretation Criteria
ositive >= 2.00; Negative < 2.00
ERCENT ABS ABS ABS (L



unning New Standard Curve
se Duplicate Calibrators

Well	Sample ID	Abs		ppb	Interpret	%A Hi Cal	
A- 1	Calibrator 1	1.313		0.0			
B- 1	Calibrator 1	1.129	1.221	0.0		100.0	
C- 1	Calibrator 2	1.083		5.6			
D- 1	Calibrator 2	0.927	1.005	5.6		82.3	
E- 1	Calibrator 3	0.512		16.7			
F- 1	Calibrator 3	0.482	0.497	16.7		40.6	
G- 1	Calibrator 4	0.201		50.0			
H- 1	Calibrator 4	0.225	0.213	50.0		17.4	25.00
A- 2		0.912					
B- 2	1 Ka4-16	0.794	0.853	8.9	Positive	69.8	4.46
C- 2		0.982					
D- 2	2 Mo3-12	0.961	0.971	6.3	Positive	79.5	3.16
E- 2		0.929					
F- 2	3 Mo 3-10	0.715	0.822	9.6	Positive	67.3	4.79
G- 2		0.698					
H- 2	4 A 1-16	1.027	0.862	8.7	Positive	70.6	4.36