MONITORING OF CARBOFURAN RESIDUES IN LAIKIPIA AND ISIOLO DISTRICTS IN KENYA FOR ECOLOGICAL RISK ASSESSMENT

By

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ABSTRACT

Granular carbofuran which was widely used to control agricultural pests in Isiolo and Laikipia districts was also misused by local farmers and pastoralists to kill predators leading to massive deaths of *Gyps* vultures through secondary poisoning. In support of conservation efforts, this study was conducted to monitor carbofuran usage and its environmental contamination in the two districts and to establish potential routes of exposure to *Gyps* vultures' species. A survey was conducted to establish the level of carbofuran use and misuse by administering questionnaires to the farmers, pastoralists and the conservationists. Soil, water and *zea mays* leaves samples from selected farms as well as lion carcass and vulture tissues were solvent extracted and residues analysed by HPLC and confirmed by GC–MS. Survey data which was analysed by SPSS statistical package indicated that 67.5% of the farmers use carbamate pesticides and 16.8% of the respondents reported that carbofuran was intentionally used as a poison to kill predators. The residues analysis results were subjected to MSTAT (ANOVA) statistical package and the mean concentration of 3-ketocarbofuran and 3-hydroxycarbofuran were found to be 0.199 ±0.016 and 0.087 ± 0.019 ppm, respectively, in vulture's crop content indicating carbofuran exposure. The vultures' feet and beak samples contained mean concentration ranges of 0.025-0.04 ppm (carbofuran), 0.185-0.0.24 ppm (3-keto-carbofuran), 0.59-0.0.73 ppm (3-hydroxycarbofuran). The mean concentration of the residues of carbofuran, 3-ketocarbofuran and 3-hydroxycarbofuran found in plant samples ranged from 0.099-0.269, 0.145-0.641 and 0.196-0.499 ppm respectively in the study area The water samples contained mean concentrations of carbofuran, 3-ketocarbofuran and 3-hydroxycarbofuran in the ranges of 0.011-0.592, 0.068-0.525 and 0.118-0.0.646 ppm in Isiolo and Laikipia respectively, while dried soil sample mean concentrations ranged between 0.146-0.179, 0.484-0.313 and 0.433-0.694 ppm respectively in both districts. There was significant regional and seasonal variation in mean levels of residue in plants, water and soil sample taken in the wet and dry seasons with higher mean concentration recorded in wet season for carbofuran. The levels showed extensive environmental contamination of the farms, rivers and plants around the conservancies. These mean levels of carbofuran residues obtained in the environmental matrices do not confirm them as potential routes of exposure but indicate the usage as well as level of contamination.

Levels of residues in lion tissue demonstrate exposure before death and could possibly be the cause of death. Data gathered forms a basis to create awareness about toxicity of carbofuran and discourage its misuse.

1.0 INTRODUCTION

1.1 Background Information

Carbofuran exists in the N-Methylcarbamate class of pesticide bearing a furan nucleus which belongs to an important group of heterocyclic compounds and has a chemical structure shown in Figure 1 (Hayes, 2001). The carbamates are further classified into two groups, the phenyl N-methyl carbamates (e.g. carbofuran, carbaryl and propoxur) and oxime carbamates (e.g. aldicarb and methomyl). The substituents on the phenyl ring can change the nature of the different molecules through hydrophobic, electronic and hydrogen bonding and influence their complexation with acetylcholinesterase (AChE) and change their corresponding inhibition activities (Hayes, 2001). It is among the insecticides which have replaced organochlorine pesticides in the recent years in many countries as the most important line of defence against agricultural pests and disease vectors. Organochlorine chemicals have markedly decreased in quantities over the years in many countries due to their persistence in the environment and associated negative effects on human health and non-target organisms (Lopez *et al.*, 2005). Although this newly introduced cholinesterase-inhibiting compound is less persistent in the environment and has low mamalian toxicity than chlorinated predecessors, it still poses risks to nontarget organisms and ecological systems (Hopkins and Scholz, 2006). The unintended impacts of this pesticide has been investigated for decades, but many important details regarding environmental exposure and misuse as a poison are still poorly understood or not investigated at all.

Carbofuran with an IUPAC name of (2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methylcarbamate) is an effective systemic and contact pesticide used both as insecticide and nematicide against a wide range of agricultural pests since it has a broad spectrum of activity. Globally, approximately 20,000 tonnes of carbofuran was consumed in 2002 (FAO, 2009) and the consumption has steadily been on the rise especially in Kenya where many farmers consider it as the best line of action against pests Carbofuran is synthesized when a cold solution of 16.4 parts 7-hydroxy-2, 3-dihydrobenzofuran in 14 parts of ether is treated with 5.8 parts methyl isocynate and 0.1 part triethylamine. The mixture is stirred at room temperature and a white crystalline product precipitated. Separation of the solid yields 17.5 parts of product whose melting point is 152° C-153°C (

pure) and 150°C-152°C for technical grade (Sittig, 1977). It is manufactured as a pesticide by FMC Corporation based in Philadelphia in the United States of America and distributed locally by Juanco Kenya Ltd under the trade name of Furadan 5 G (5% a.i). It is packed in quantities of 200 grams and 500 grams and sold freely over the counter in Agrovet shops in Kenya. A survey was done to find the level of usage in Laikipia and Isiolo where misuse had been extensively reported.

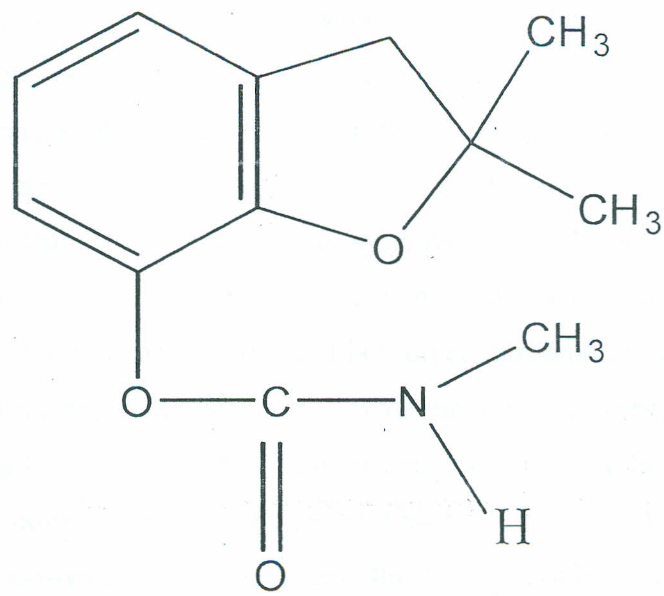

Figure 1. Chemical structure of carbofuran.

Carbofuran is reported to have low mammalian toxicity (LD_{50} 11 mg/kg in rats) but very toxic to invertebrates and birds and should therefore be handled with care (Hodgson *et al.,* 1991). However, acute administration of carbofuran, just like other carbamates, through accidental exposures can result in acute toxicities and fatalities even in human (Hayes, 1982). It has been used worldwide for control of pests in sugarcane, sugar beet, maize, rice and coffee and is very effective in controlling rice pests such as green leafhoppers, brown plant hoppers, stem borers and whorl maggots. Other pests, which are resistant to organophosphate insecticides e.g. white flies, leaf miners, ants, scale insects, cockroaches, wasps and aphids are also effectively controlled by carbofuran (Hodgson *et al.,* 1991). Carbofuran has rapid action against both nymphs and adults killing them within 20 minutes of application (Suett, 1986). This gives it an advantage over other pesticides. Like carbamates, it acts on the nervous system by inhibiting the formation of acetylcholinesterase, an enzyme that destroys acetylcholine on the synapses. This leads to accumulation of acetylcholine which results into continuous firing of the nerves hence convulsion and death (Emden, 1992).

The metabolism of carbofuran involves carbamylation and decarbamylation and oxidation or hydroxylation of the ring itself (Hodgson *et al.*, 1991) resulting into metabolites which can further degrade into other compounds as discussed in detail in section 2.4. It is the significant rate of decarbamylation of the inhibited enzyme that is responsible for slight reversibility of inhibition of the AChE in some carbamates (Hodgson *et al.,* 1991) as further explained in the mechanism of action in Figure 3. In organisms, *in vivo* metabolism of carbofuran occurs through the Phase I (oxidation, reduction and hydrolysis) and Phase II P_{450} systems and by conjugation with various substrates including glutathione, glucuronic acid, glutamic acid and glycine leading to more polar metabolites that are excreted. Like other carbamates, carbofuran is non-persistent and does not bioaccumulate in the environment. It is very hydrophilic (water solubility of 700 mg/L @ 20°C) and is slow to penetrate the cuticle and does not show significant contact toxicity (Lalah *et al.*, 1996). Once taken in, the knockdown effect is quick and usually non-reversible. In contrast, the more lipophylic carbamates such as carbaryl penetrate the cuticle easily and give rapid knockdown effect, which has some degree of reversibility. It is due to these and other desirable properties that carbofuran is widely used to control soil dwelling and foliar pests on agricultural crops (Helmut, 1990). In recent years, it has been used to replace organochlorine pesticides that are known to be highly persistent and bioaccumulative in the animal tissues.

In Kenya, carbofuran in the form of 5% technical Furadan granules is applied mainly as seed dressing at the rate of 0.5-4 kg a.i /ha for control of soil-dwelling and foliar-feeding insects. According to Pest Control Products Board of Kenya reports (PCPB, 2009), carbofuran is marketed in Kenya as Furadan 3G granules (for treatment of wheat and barley seeds using seed treatment equipments; restricted use). Furadan 5G (5% a.i for seed dressing in rice, banana, beans, vegetables, coffee applied manually) and Furadan 10G (applied by granular applicators in control of soil insects and nematodes and early foliar feeding insects in coffee, bananas, pineapples, pyrethrum nurseries and maize (G.O.K Report, 1992). On the other hand, Furadan 350ST liquid is also marketed and used in dressing barley seeds. Approximately more than 23 tonnes of granules and more than 15,000L of concentrate are imported annually (G.O.K Report, 1992). Being a systemic and contact pesticide it is possible for the carbofuran residues to remain on the plant leaves and also in the plant system, a possibility which could endanger the foraging birds (Mineau and Collins, 1988).

Previous studies on Ahero paddy fields indicated that in flooded soils most carbofuran residues tend to stay in the top 10 cm layer of the soil and water where it has been applied for the first three weeks after application (Lalah and Wandiga, 1996b) a fact that is supported by its high water solubility. It can also leach easily when it rains and the granules can be carried away from surfaces of the soil where they are applied. Therefore, the most likely route of exposure to fish, mammals and to birds would be through water a fact that had been confirmed by scientific studies (EXTOXNET, 2001a). In flooded soil in Ahero paddy, over 50% of the residues would have disappeared within the first 25 days after initial application at the rate of 20 ppm in soil and half-lives of dissipation of carbofuran were found to be 66 days in submerged soil with pure carbofuran, 96.3days in non-submerged soil with pure carbofuran and 115.5 days in non sub-merged soil with technical Furadan (Lalah *et al.,* 1996; Lalah and Wandiga, 1996b). This makes application of carbofuran in flooded soil a great risk to birds that sift waterlogged sediment in search of food (Mineau, 1993). There are cases that carbofuran granules have given rise to many instances of waterfowl mortality in flooded or partly flooded fields. It is, therefore, advisable that carbofuran should not be applied to flooded soils or soils subject to flooding since these soils are acidic. Problems have arisen primarily in acidic soils, presumably on account of longer half-life of the granules under those conditions (Mineau, 1993; Lalah *et al.,* 1996; Lalah and Wandiga, 1996b). Elsewhere, carbofuran is reportedly applied to soil at 5-50 ppm, but higher amount is required when nematicidal action is desired. It then persists in acidic soils but disappears up to 10 times faster from alkaline soils (Hassall, 1990). Degradation of carbofuran, therefore, depends on factors like soil properties which includes pH and the presence of organic matter. The ability of carbofuran to persist in acidic soil environment provides a potential route of exposure to the birds and other aquatic organisms in the flooded soils and non-flooded soils. This explains why soil and water samples needed to for determination of exposure levels.

Carbofuran is known to be highly toxic to birds and according to a report released by the Environmental Protection Agency in 1992, granular carbofuran was blamed for millions of birds' mortality per year in the United States (USEPA, 1992). It also has significant toxicological risks to human and wildlife and therefore monitoring its residues has become necessary for environmental protection. Monitoring involves comparing toxicity information and the amount of the pesticide a given organism may be exposed to in the environment. Based on this, ecological risk assessment can then be defined as the evaluation of the likelihood that adverse ecological effects may occur or are occurring because of exposure to one or more agents (EPA, 2008). It provides a critical element for environmental decision making by transforming scientific data into meaningful information, which can be interpreted for use in wildlife conservation and monitoring programme.

Agricultural farmers and pastoralists mostly misuse pesticides as poisons to kill the predator animals and the birds of prey particular the vultures in Africa (VEU, 2005). Some of the pesticides misused include aldicarb, parathion and carbofuran. This study aimed at establishing the concentration levels of carbofuran residues in various compartments, the cause of birds' deaths and the potential direct and indirect routes of exposure of carbofuran to the birds. Analysis of the soil, plants, water points, and animal tissues was done to establish the presence and concentration levels of carbofuran residues in the two affected districts.

1.2 Characteristics of Vultures and their importance in the Environment

The African white-backed vultures (*Gyps africanus*) considered in this study exist in the group of scavenger birds, which are birds of prey that are easily recognizable and have exceptional vision, sharp talons and hooked upper beak to tear apart food. They are found in the order of Falconiformes and Accipitridae family (ABC, 2007). The African white-backed vultures are the tropical species and are the most numerous of the African vultures' species which are the inhabitants of the African plains, savannahs and occasionally desert regions (ABC, 2007). They have a sharp vision to see carcass far away and once they land on the food their obvious black and white plumage makes them readily visible to other birds, which also join easily in the feast (ABC, 2007). They congregate in large numbers and it is for this reason that many are found dead and lying just next to poisoned or laced carcass. It is possible to see a number of birds feeding from one carcass, which explains how the corpse of an elephant can be stripped clean in just a matter of hours. Plate 1 shows a number of vultures that assembled to feed on a carcass in the wilderness and can explain how massive deaths of vultures can easily occur if the carcass were poisoned. Vultures' existence at the top of the food chain many times makes them get exposed and become vulnerable especially when they scavenge on carcasses that are poisoned.

They have been for many years persecuted and misunderstood and it is for this reason that they are continuously declining at a rapid rate (EPA, 2008). Their habitat and unique behavior are unknown or misunderstood and so is their reason for existence. Vultures world over are facing similar threats from habitat destruction, declining food availability, illegal collection for traditional use and more seriously secondary poisoning and this explains the concern and hence need for their conservation. Vultures and other avian scavengers are very important within an ecosystem because as scavengers they help prevent the spread of some diseases by eating the carcass of dead animals and sometimes the garbage. They can also alert the pastoralists about the dead stock in the field and in this way prevent the spread of disease outbreak (EPA, 2009). Besides, as part of the larger wildlife species they attract tourists and their conservation is important for preservation of our rich heritage for future generation. Their presence is so conspicuous in the environment that their reduction in population is a clear indicator of the serious damage to the environment (EXTOXNET, 2001b) The number of vultures species now endangered, threatened and vulnerable is now at a high level and the real risk of extinction exist for several other less common bird species in Kenya, a fact little realized by the public (EPA, 2008). They are victims of irresponsible pesticide use and currently this has led to their disappearance and the disappearance of other birds of prey species from around the world. Consequently, the study of secondary exposure of vultures to carbofuran was important in order to address the rapid decrease in their population and to help provide appropriate mitigation measures to this problem. It was on the strength of the information on carbofuran toxicity, its use and misuse in Laikipia and Isiolo Districts that it was alleged to have caused deaths of many vultures in these regions hence the need to establish the cause-effect relationship. Based on generated data appropriate mitigation measures would be proposed in order to save our vultures and other wildlife species from extinction. In this regard samples of poisoned predators and vultures were analyzed to determine possible causes of death.

Recent research studies showed the rapid decline of vultures over a three-year period in Laikipia district, Rift Valley Kenya, where raptors were observed to have declined by more than 40% per over the period from 2001-2003 and vultures including Bateleurs (*Terathopius ecaudatus*) accounted for most of the decline (Ogada and Keesing, 2009). Ogada and Keesing, 2009 further reported that vulture sightings declined by 77% during the same period. The rapid decline was attributed to consumption of poisoned baits from Furadan which the pastoralists are using increasingly to kill large predators that attack their livestock (Ogada and Keesing, 2009). Although other toxic pesticides such as dicofol, fensulfothion and diazinon (Frank *et al.*, 1991; Elliot *et al.*, 1996) and antibiotics such as diclofenac (Prakash, 2004) have been involved in bird poisoning cases in other countries such as India, the Kenyan cases have been found to be mainly due to Furadan misuse. Following the massive deaths in Isiolo Kenya as seen in Plates 2, 3 and 4 a study was proposed to generate enough scientific data which would help provide mitigation measures.

The Photograph in Plate 1 demonstrates that vultures move and feed together in large numbers such that if a carcass is poisoned then it is possible to see massive deaths in one instant a situation that would significantly reduce their population in a short time.

Plate 1: Shows evidence of vultures congregating around a carcass in the game reserve (Photo taken in Lewa Wildlife Conservancy).

Plate 2: A photograph of dead vultures in Isiolo District. Other evidences of poisoning are shown in plates 3 and 4 (*Courtesy of Lewa Wildlife Conservancy*)

Plate 5 in the Appendix II shows the African white-backed vultures' species that is most affected in the study regions. Apart from direct secondary poisoning as observed in Plates 2, 3 and 4, vultures can possibly get exposed through drinking and frequently taking bath in contaminated water. The presence of carbofuran in soil and water is also a potential risk of exposure and analysis of these environmental matrices was necessary.

1.3 Statement of the Problem

1.3.1 Carbofuran exposure and toxicity to birds in Kenya

There was a lot of concern from the environmentalists and conservationists about the possible fatal effects of carbofuran use on bird population especially the scavenging birds such as vultures in northern Kenya and areas around Mt. Kenya where carbofuran was extensively used. All Vultures' species in Kenya face great threat of extinction as a result of environmental pollution and indirect poisoning incidents. Numerous cases of vultures' deaths were reported in Isiolo District around Lewa Wildlife Conservancy in May, 2007 and it was strongly suspected that it could be due to exposure to carbofuran, an acetylcholinesterase inhibitor, and one of the most toxic carbamates in the market today sold in the two districts. Other cases of carbofuran have also been reported in various parts of the country over the years (Appendix I). This concern was also supported by the fact that carbofuran is very toxic to birds through inhalation and ingestion of granules or through secondary exposure but moderately toxic by dermal absorption (USEPA, 1992). Consequently, its use has been restricted and banned in many countries like USA and Canada (USEPA, 1992). Carbofuran, though restricted in Kenya, has continuously been used but with far-reaching eco-toxicological problems with a recent reported case where over twenty vultures died in Isiolo district in the year 2007 according to reports from Lewa wildlife conservancy. They fed on carcass of a camel that had been killed by lions was allegedly laced with a chemical suspected to be carbofuran by the pastoralists.

This was aimed at killing the predators that came to feed on the carcass as away of solving the problem of lion menace to domestic animals and avoiding a repeat of the same. Consequently, two lions died and about twenty vultures that had come to feed on the carcass died on the spot as can be seen in Plate 2.

It is important to note that globally vultures are not usually the target organism but their scavenging and feeding behaviour subject them to high risk of intentional and accidental poisoning (Vyas *et al.,* 2003). Studies conducted by American Birds Conservancy indicate that birds that consume sprayed insects, drink contaminated water or feed on poisoned carcass are susceptible to secondary exposure and possible poisoning. According to ecotoxicity studies, carbofuran has been documented in hundreds of avian mortality events sometimes involving large numbers of birds in each incident (ABC, 2007). This study, therefore, aimed at establishing if carbofuran was indeed the cause of huge mortalities of vultures witnessed in the area under study.

Plate 3: A photograph of a carcass of Lion allegedly poisoned by carbofuran (*courtesy of Mbirikani group Ranch)*

Other studies conducted by American Birds Conservancy indicate that a granule of carbofuran is enough to kill a small songbird and that there is no way carbofuran can be used either for intended or unintended purpose without killing birds (ABC, 2007). Other documented cases of carbofuran acute toxicity include fulvous whistling ducks with LD_{50} 0.238 mg/kg, Mallard 0.51 mg/kg, Northern Bobwhite 12.0 mg/kg and house sparrow with LD_{50} 1.3 mg/kg (ABC, 2007).These indicate clearly how dangerous carbofuran is even in small quantity and the fact that the whole ecosystem is threatened as lesser bird species are also under risk of exposure. In that respect, use of carbofuran either as a pesticide in agriculture or as a poison to solve the human–wildlife conflict is a serious problem that needs to be addressed.

Plate 4: A photograph of a carcass of a vulture (*courtesy of Mbirikani group Ranch).*

In Plates 2 and 4 the exposure could have occurred through eating contaminated carcass and gut content covered with contaminated blood from Lion' carcass. The vultures' carcass in Plates 2, 4, 9 and 10 (see Appendix I) were found dead next to the carcass of a lion as seen in Plate 3. The beaks and feet samples shown in Plate 6 in the Appendix II were cut off from these carcasses of vultures for Laboratory analysis to determine exposure. Crop content and upper gut that had not decomposed completely were also collected for analysis.

1.4 Justification of the Study

Concern has been expressed worldwide regarding pesticide residues in the environment and their effects on non-target organisms (Hayes, 1982). Carbofuran has killed mammals and fish and according to US Fish and Wildlife Biologists "there are no known circumstances under which carbofuran can be used without killing birds." In 1989, US EPA estimated that 1 to 2 million birds were killed each year in United States of America by carbofuran alone. According to the Ecological Incident Investigation System, carbofuran has been responsible for more avian deaths than any other pesticide in the wildlife history (ABC, 2007. It is the only toxic pesticide to birds left in the market today. Since its introduction in 1967, it has been responsible for the deaths of many birds including Bald and Golden Eagles, Red-tailed Hawks and migratory songbirds and other wildlife in the United States of America (Helmut and David 1996). In 2006, US Environmental Protection Agency cancelled the registration of carbofuran following overwhelming scientific evidence for the extreme toxicity of carbofuran released by environmentalists and wildlife conservationists (EPA, 2008).

According to a study conducted by Pricewaterhouse Coopers (2005) Human-wildlife conflict is one of the serious challenges facing the two districts under the study. Destruction of crops and killing of people and livestock by wildlife is a common occurrence. The reports cite lack of clear incentives and support for their wildlife conservation efforts. This has resulted to cases where carbofuran is used by pastoral communities to poison livestock predators, causing deaths of lions and vultures in Laikipia and Isiolo District. The pesticide is considered as one of the major contributing factors to the threatened eradication of the African white-backed vultures (*Gyps africanus*) species in the country through secondary poisoning. From ecological standpoint, the existence of toxic carbofuran residues in wildlife food chain could cause devastating effects not only on birds of prey but also on other smaller birds and non-target animals. Vultures are specifically targeted in this study because they are so conspicuous in the environment that their absence is a clear indicator of the damage to the environment. Besides, their presence at high position in the food chain makes them be greatly threatened with eradication from poisoning activities. Cases of poisoning by diclofenac have been recorded for the *Gyps* species in the Indian sub-continent and this caused rapid decrease in their population (Prakash, 2004). The Asian vulture crisis is a clear demonstration of how far this poisoning of vultures can go if not addressed in good time. If not monitored then soon we shall loose this natural heritage from our landscape and the future generations will only have an opportunity to see vultures it in pictures or housed in cages. Little attention has been accorded to the *Gyps* vultures' species and on that strength, the study was rightly justified.

In order to deal with this carbofuran exposure risk issue, scientific data needed to be established to support the allegation. Through a survey, sufficient data on carbofuran use and potential routes of exposure to the target and non-target organisms such as mammals and birds in Laikipia and Isiolo districts needed to be established. This required documenting the amount of carbofuran applied, application methods, the formulations used, application rates, the target group against which the pesticide is applied, possible non-target organisms, the catchment's characteristics i.e. plants and animals exposed as well as farms, soil and water bodies such as rivers, canals and ponds. Secondly, analysis was done for carbofuran residues in soil, water, plant, and animal tissue samples taken from the affected areas to generate data, which was a guide in identifying the quantities of carbofuran residues present in local environment and in predator organisms and subsequently the potential routes of exposure to birds. Major routes of exposure like ingestion and inhalation have been studied in other countries, however, little has been done about secondary poisoning (non-point sources of exposure) like taking carbofuran-contaminated water and feeding on poisoned carcass which then needed to be studied. The study was, therefore, further meant to establish the presence of its residues in water/soil in the affected environments, the extent of distribution in these compartments and its impact on the birds in the conservancies covering Isiolo/Laikipia district. Monitoring the residues and the break down products tend to help determine the accumulation and distribution and further predict the possible future hazards to non-target organism. If cause–effect relationship were to be demonstrated repeatedly in the findings then it would provide a strong evidence of causality. In that respect, ecological risk assessment was necessary to serve as a basis for recommendations on the continued restricted use or total ban of this pesticide. Such Studies have been done successfully in America, Canada and Britain with none in Africa.

Continued use and misuse of carbofuran in Kenya to poison wildlife directly and indirectly through secondary poisoning will subsequently result into extinction of many species if not checked. Ironically it is the banned toxic pesticides in the USA that continues to find its way in Kenya for legal and illegal and therefore there is need to monitor its residues in order to address its impact on wildlife conservation a concept that is new in the Kenya

1.5 General Objective of the Study

To assess the usage of carbofuran and determine the concentration levels of carbofuran residues and the possible routes of exposure of carbofuran and its degradation products as well as its potential risks to the endangered and threatened vultures species.

1.5.1 Specific Objectives

- To carry out a survey on carbofuran toxicity and carbofuran usage in Isiolo and Laikipia Districts in Kenya.
- To study seasonal and spatial variation in carbofuran residues concentrations in Isiolo and Laikipia districts.
- To analyze carbofuran residues and it's metabolites in selected water points, soils and plants in selected sites in the two districts to show environmental exposure.
- To analyze animal carcass and dead vultures' tissues sampled from the two districts to establish the cause of death.

1.5.2 Hypothesis

- The use of carbofuran and its misuse as poison in Laikipia and Isiolo districts is responsible for the deaths of vultures in this region.
- The farm soil, water points and plants in Isiolo and Laikipia districts are contaminated with carbofuran residues beyond permissible levels.
- Analysis of carbofuran residues and its metabolites in carcass, gut content, beaks and feet of vultures by HPLC and GC-MS provide useful forensic tool for evidence on carbofuran occurrence and poisoning of scavenger birds.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The area under investigation

Isiolo district is located in Eastern province and has a geographical coverage of 25, 605 km^2. It lies between longitudes 36° 50' and 39° 30'E and latitudes 0° 05'S and 2°N. The district is characterised by flat low-lying plains that rises to 300 m in the Merti plateau (Mati *et al.*, 2006). Isiolo District is dry and rainfall is generally scarce and unreliable and is distributed within the two seasons in the year with an average of 580.2 mm per year (Mati *et al.*, 2006). The soils are generally sandy though pockets of black cotton and red soils that can sustain agriculture exist in some areas (Republic of Kenya, 2002). According to the CBS (2002), the human density is generally sparse (average density of 4.4 people per km^2) but concentration of settlements is determined by proximity to market centres and water points. The major economic activity in the district is nomadic pastoralism where cattle, sheep, goats and camels are kept .However, crop farming is also done but to a small extent because less than 1% of the total land is considered arable (Republic of Kenya, 2002; Mati *et al.*, 2006). Agricultural activity is generally low and is mainly attributed to unreliable rainfall. Maize, beans, cowpeas and bananas are the primary food crops grown in this region (Republic of Kenya, 2002). The district is divided into three climatic zones namely semi-arid, arid and very arid zones with Lewa wildlife conservancy, the area under study falling under semi arid zone (Mati *et al.*, 2006).

Lewa wildlife conservancy (LWC) is a home to a wide variety of wildlife and covers an area of 250 km^2 (LWC, 2009). It was started as a cattle ranch in 1922 and was later turned into guarded sanctuary for the black Rhino (LWC, 2009). Currently, it is a wildlife conservancy that has gained a world-wide reputation for wildlife conservation. However, wildlife in the conservancy has continuously faced serious threat due to the unending human-wildlife conflict (LDP, 2009a). The agricultural farmers and pastoralists around the conservancies have resorted to other methods to avenge the destruction of their crops and the kills of their livestock by wildlife.

Laikipia district, another area of study, is the home to a number of wildlife conservancies and is equally faced with the same challenges. The district exists in a vast plateau located on Equator in the Rift valley province, and covers about 9000 Km^2, It lies within longitudes 36.00'E and 36.45'E and latitudes 1.00'N and 0.00'. It enjoys a cool, temperate climate with both rainy and dry seasons and is more fertile compared to Isiolo. (LDP, 2009a). The economic activities in this region include tourism, ranching and agriculture where grains are mainly grown (LDP, 2009b). The area is characterized by black cotton soil with some areas having clay and sandy soil (LDP, 2009a). Ol Ari Nyiro wildlife conservancy which covers over 360 Km^2 (approx 90, 000 acres) of land was specifically chosen as study area in Laikipia district. It lies between latitudes 0° 30'N and 0° 45'N and longitudes 36° 15'E and 36° 30'E It is surrounded by a series of man-made dams and springs feeding Makutan River which provide water to the wildlife and domestic stock. It is a private wildlife sanctuary situated on the extreme western edge of the Laikipia plateau (GMAC, 2009). Ol Ari Nyiro wildlife conservancy is partly located in Ngarua and Ol-Moran division which has a population of 65,545 and 11,069 respectively (GMAC, 2009). Dramatic increase in population in this region has led to man encroaching into wildlife habitat leaving predators with no areas to fetch their wild prey. This has resulted to the wildlife coming into contact with human and their livestock and crops a fact that has caused human-wildlife conflict in this area (GMAC, 2009).

2.2 Carbofuran toxicity in birds and forensic evidence.

According to a report given by American Bird Conservancy, Defender of Wildlife and Bird Conservation Alliance, carbofuran has been the greatest chemical threat to wild birds since the pesticides DDT and dieldrin were banned in the early 1970s following the discovery of their endocrine disruption effects (ABC, 2007). Many of the cholinesterase inhibiting products which replaced the persistent and bioacccumulatory products, such as Dichlorodiphenyltrichloroethane (DDT) and cyclodiene insecticides (aldrin and dieldrin), have proved to be extremely toxic to birds with LD_{50} values predicted to be below 1 mg/kg of some sensitive species (Mineau, 2001). This level of toxicity is almost unprecedented in the mammalian world where values below 10 mg/kg in a rat generally denote products of exceptionally acute toxicity (Mineau, 2001). Ecological risk assessment of carbofuran in the year 2005 by the Environmental Protection Agency (EPA) stated that there were no single legal uses of carbofuran that did not kill wild birds

(EPA, 2009). Helmut and David (1996) further confirmed that carbofuran is highly toxic to non- target organisms especially to the birds and a single granule contains sufficient amount of pesticide enough to kill a small bird. Deaths of birds are often observed in fields treated with carbofuran and many instances of mortality in actual use have been documented. Secondary poisoning of scavengers, insectivores, and raptors has also been observed (Helmut, 1990). Routine applications of carbofuran have resulted in deaths of non-target organisms in most of the cases observed. Birds and other wildlife foraging in fields on which the insecticides have been applied are susceptible to coming into contact with the granules. The principal route of exposure here appears to be the attractiveness of the granules as dietary grit (Knapton and Mineau, 1994). This is because carbofuran granules are left on the surface of the soil especially if they are not properly mixed within the furrows. The death of birds especially vultures could be as a result of direct ingestion, inhalation or feeding on baits that are laced with high concentrations of liquid, granular or powder formulations of highly toxic insecticides (Nimish *et al.,* 2005). According to Martin and Forsyth (1998) the potential for greater dermal toxicity coupled with a relatively high oral toxicity may result in an increased risk for birds moving on places and foods that have been sprayed with carbofuran.

In trying to establish the cause-effect relationship between insecticide residue and raptor mortality, it is important that the dead bird be recovered from the field and analyzed to confirm the presence of pesticide. If poisoning is suspected then biochemical and chemical analysis are conducted. For organophosphate and carbamate insecticides poisonings, the brain cholinesterase activity is measured to determine the mechanisms of death. Accurate diagnosis of carbamate-induced wildlife mortalities in pesticide exposure incidents using brain acetylcholinesterase (AChE) inhibition is often difficult. It may be confounded by unknown post-mortem carcass history prior to sample retrieval, during which inhibited brain AChE may spontaneously reactivate (Hill, 1989) making it sometimes unsuitable to used be for analysis. Brain AChE values reported from carbamate kills routinely have wide variable activities, lending considerable uncertainty to the diagnosis of exposure (Hill and Fleming, 1982). However, recent studies by Hunt *et al.* (1995) on new techniques of detecting carbofuran exposure by stabilizing and reactivating carbamates induced brain AChE has helped solve the uncertainty. Tony and

Reinhold (1991) reports that AChE activity is strongly influenced by exposure of birds to the carbofuran pesticide and as such measurement of AChE levels in blood and brain with a reduction of 50% is indicative of lethal dose. Other scientists therefore consider brain tissue as the best to analyse because it is less variable and changes more slowly than those parameters do in blood samples (Tony and David, 1991). But still the residue analyses of crop samples and in cases where it is not available then beak and feet should be performed to substantiate the diagnosis of carbofuran exposure resulting from brain reactivation data (Hunt *et al.,* 1995). Blood cholinesterase activity can also be measured but may not show significant depression unless blood samples are drawn and assayed immediately, owing to rapid cholinesterase re-generation (Amdur *et al*., 1991). But this again must be coupled with other tissues analyses to authenticate the data collected since blood is too sensitive to pesticide AChE activity which would rapidly return back to normal (Tony and David, 1991; Amdur *et al*., 1991).

Chemical residue analysis of the gastrointestinal tract or its contents is also performed to identify the insecticide responsible for the death (Hill and Fleming, 1982). This is because gastrointestinal tract is the site of entry for chemicals that are ingested and in many instances the gastrointestinal contents are analysed first because large amount of the residual unabsorbed poison may be present here (Amdur *et al*., 1991). Carbofuran kills so quickly that the compound is almost always present in the upper gastrointestinal tract. The bird's crop and its content would also be appropriate for analysis and efforts should be made to recover the carcass before they decompose. Other matrices for analysis includes the liver because poison is first carried to the liver before it enters the general circulation system (Amdur *et al.,* 1991). Urine may also be analysed as the kidney is the major organ of excretion for most poisons and high concentration of toxicants or their metabolites are often found in the urine. The main route of excretion is in the urine as conjugates of glutathione and so the presence of the metabolites in the urine is one of the ways one can get to know the pesticide the organism was exposed to just before death occurred (Ferguson *et al,* 1984). However, the analysis may be complicated because of normal chemical changes that occur during decomposition of the carcasses. As such the toxicological analysis should be started as soon as possible because the enzymatic and non enzymatic processes of decomposition and microbial metabolism may destroy a poison initially present at death. It may also produce substances or compounds with chemical and physical properties similar to those of commonly encountered poisons (Amdur *et al*., 1991). In general the presence of poison or and its metabolites in the body tissues is a clear show of the presence of the poison. It is important to note that wildlife forensic laboratories may analyze insecticide residues on feet if they are available (Frank *et al.*, 1991; Stroud and Adrian 1996). According to Vyas *et al.* (2003) diazinon and chlorpyrifos applied on the lawns were found on the feet of brown-headed cowbirds (*molothrus attar*) that had weathered for one month and this further justifies the use of the feet matrices in this study.

Since carbamate just like organophosphate pesticide kill through inhibition of the brain acetylcholinesterase activity, exposure to carbamate can be determined by recovering the brain tissue in order to analyse the acetylcholinesterase activity (Amdur *et al.,* 1991). Since inhibition by carbofuran is spontaneous and reversible, samples of the brain needs to be removed before too much autolysis has occurred and should then be kept frozen until analysis is done. Other conventional matrices for analysis include crop and gizzard content which should also be collected and frozen before analysis is done. In a related study done in Croatia, results showed that 15 Griffon vultures analysed, carbofuran was found in their livers, 11 vultures had carbofuran in their crops, 9 had it in their stomach content and 4 in their intestines (Slotta-Bachmayr *et al.,* 2004). However, scavenging, decomposition and pesticide degradation may render these conventional matrices unsuitable for analysis (Vyas, 1999; Vyas *et al.*, 2003).

In a forensic investigation done by Nimish *et al* (2005) to develop new carcass matrices that can be used to establish cause-effect relationship, carbofuran residues were found on the owl's feet which was in contact with a laced bait for 40 minutes. In that regard, if the conventionally analyzable matrices are not available due to decomposition and scavenging then the feet or claws can be used since they are retained intact even after decomposition of the birds' carcass (Nimish *et al.,* 2005). Since many carcasses retain intact feet, analysis of the weathered raptor feet was done as a matrix for determining carbofuran exposure to vultures besides beak samples In general the presence of carbofuran residues on the feet do not necessarily imply a lethal dermal exposure but serve as an evidence about the insecticide to which the bird was exposed and report the minimal insecticide concentration that was initially present on the foot (Stroud and Adrian 1996). However, depending on the insecticide's toxicity, its history on wildlife mortalities and the findings during field investigations, detection of certain insecticide from the feet can provide evidence of the cause of death (Nimish *et al.*, 2005). Persistence of carbofuran on the feet of avian carcasses has been proved by research and therefore their reliability, as matrices for identifying carbofuran exposure to birds cannot be doubted. A lot of care must then be taken to secure the feet within the shortest time possible after the death and its half-life (Allen *et al.*, 1996). In general, persistence of carbofuran in the environment increases under conditions of low moisture, low temperature, low pH and lack of suitable microbial degraders as these can indeed affect the half-life.

2.3 Environmental exposure and toxicity in non-target species

Concern about pesticide run-off into surface waters is regarded as a big problem in certain regions of heavy rainfall. Run-off will occur when a pesticide is used in a situation where there is a combination of sloping land nearby watercourse and appropriate rainfall condition (Hutson and Roberts, 1994). Approximately 90% of agricultural pesticide application never reaches it's target organism but is instead dispersed through air, soil and water (Moses *et al.*, 1993). It was further reported by Metcalf (1955) that spillage of pesticide into the environment affects local systems and diffuses contamination through agricultural run-off which then affects larger areas and is difficult to control. Surface water resources that are commonly used by the birds may be contaminated via direct contamination by improper disposal or accident, direct contamination through spray during application or run-off of surface and drainage water from fields where crops or soil are treated (Helmut, 1990). Flooding of fields is another way in which several birds' species have been killed by carbofuran granules. Studies conducted by Canadian wildlife services indicate that this problem has been very severe in the heavy acidic soils where carbofuran is known to have longer half-life. Exposure can be through sifting of the contaminated soils and also through drinking contaminated water. the vultures are known to drink a lot of water and bathe in it (ABC, 2007). It is possible that birds could be taking water, which is highly contaminated with the highly soluble insecticide resulting in poisoning during such episodes. In this regard, water samples were taken in this study to establish the presence and levels of carbofuran residues in the current study. Soil samples and plant samples from the affected areas were also analyzed to establish the level of carbofuran residue exposure in the affected environment and find out if this could be a source of contamination.

Secondary poisoning of eagles, hawks, vultures and other birds of prey by granular carbofuran has been documented where birds eat earthworms with carbofuran granules stuck on them (Mineau, 1993). Average residue concentration per worm was 84.7 ppm, a concentration that would result in a dose of 43.5 mg/kg for an average-sized American Robin (Mineau, 1993). Field studies went further to report cases of American Robins (*Turdus migratorius*) which died after feeding on earthworms that were sprayed with carbofuran. It is therefore likely that small mammals such as moles that regularly consume earthworms are very much at risk from use of granular formulations of carbofuran. Intentional misuse of carbofuran has also been reported in the United States of America (Vyas *et al.*, 2003). Large-scale poisoning of vultures in South African cornfields has been reported too (VUE, 2005). Other aspects of secondary exposure to birds include consumption of invertebrates notably earthworms because the granules can adhere to the invertebrates and contaminate their tissues (Mineau, 1993). Based on the residue levels found in the earthworms from cornfield, realistic hazard assessment indicates that with the least carbofuran application rate in the corn, a single earthworm can be lethal to the songbird consuming it (Mineau, 2003). Carbofuran has also been documented in hundreds of avian mortality events for example in Tishomingo National Wildlife Refuge, Oklahoma in 1976, where approximately 500 Canada geese died after feeding in a field treated with 0.5 lbs/acre liquid carbofuran (ABC, 2007). This level of use is only one-sixth of the application rate of some crops (e.g. 2.96 lbs/acre is used on grapes, according to 1997 average usage figures from USDA (ABC, 2007). In another related case in New Jersey in 1990, after carbofuran application in fruit orchards, approximately 100 carcasses were discovered, including those of blue jay, American robin, and dark-eyed junco. Laboratory analyses confirmed carbofuran as the cause of their death (ABC, 2007). In Saskatchewan, Canada, in the year 1986, forty-five California gulls were found dead after a landowner applied liquid carbofuran in a grain field. Gulls had crops full of grasshoppers; analysis of the grasshoppers showed 4.2-7.2 ppm carbofuran (ABC, 2007). Other examples include that in Linden (California) in 1990 where liquid carbofuran was applied by irrigation, with exposure via puddle water. Carcasses of 30 mourning doves, 100 American robins were recovered (ABC, 2007). In Colusa (California) in 1989, 1,985 dead ducks, approximately 97% northern pintails, and 3% green-winged teal were found in an area where carbofuran had been used. Carbofuran residues were found in the crop of the ducks and mud samples. The dead birds were not found in an agricultural field, but in an area that was routinely flown-over by airplanes moving between two local airstrips and rice fields (ABC, 2007). This shows that the birds were exposed either through ingestion or inhalation of carbofuran. Besides, many of the birds at times die offsite especially if not properly dozed (Mineau *et al.*, 1999). In Steven County, Oklahoma, in 1985, carcasses of 150-160 American widgeon and ten Canada geese were found in an alfalfa field treated with a flowable formulation of carbofuran (ABC, 2007). Exposure of birds can also be through consuming vegetation with carbofuran residues on the surface because it is used as a contact pesticide (Mineau, 2001).

In 1991, Virginia state monitoring project in United States of America documented wildlife mortalities in 10 of the 11 farm sites where carbofuran was in use (FAO, 2009). Following this monitoring effort, the state of Virginia banned all granular formulation of carbofuran for sale and use (Mineau *et al.*, 1999). As a result of widespread pressure from conservationists Canada also banned the use of carbofuran in 1998. The United States Fish and Wildlife Services maintains that the use of carbofuran poses unreasonable hazards to birds and more than one hundred species have been documented as having died from carbofuran poisoning (Elliot *et al.*, 1996).

It has been demonstrated under laboratory conditions that a single granule of carbofuran can be lethal to a small song bird (ABC, 2007). This, therefore, demonstrates that exposure need only to be minimal to cause impact. In a sample of 479 horned Larks (*Erenophilla alpestris*) found dead in a group of Utah cornfields treated with granular carbofuran the median of granules found in the birds was two (EXTONET, 2001a).

It must be emphasized that it is much more difficult to estimate the actual number of birds that die from pesticide poisoning in the field. Reason being, the difficulty in finding carcasses because of the high scavenging rate that is a common norm in the field (Mineau, 2003).

Table 1: Acute oral toxicity of technical–grade carbofuran to birds, in order from the most sensitive to the least sensitive species.

Species	*Sex*	*Age*	*LD_{50} (mg/kg)*	*95% confidence int*
Fulvous Whistling – Duck (*Dendrocygma bicolor*)				
Mallard (*Anas latyrhynchos*)	U	33 – 39 h	0.370	0.283 – 0.484
	U	6 – 8d	0.628	0.530 - 0.744
	U	27 – 33d	0.510	0.410 - 0.635
	F	3 – 4 mo	0. 397	0.315 - 0.500
	M/F	6 mo	0.415	0. 333 – 0.516
	M*	12mo	0.480	0.381 – 0 .604
	F*	12mo	0.510	0.410 – 0.635
Red - winged Blackbird (*Agelaius phoeniceus*)	U	adult	0.422	
Red – billed Quelea (*Quelea quelea*)	U	adult	0.422 – 0.562	
Americal Kestrel (*Falcom sparverius*)	M/F	1-4yr	0.6	05 – 1.0
House sparrow (*Passer domesticus*)	U	adult	1.33	
Rock Dove (*columba livia*)	U	adult	1.33	
Brown – headed Cowbird (*molothrus ater*)	U	adult	1.33	
Common Grackle (*quiscalus quiscula*)	U	adult	1.33 – 3 16	
Japanese Quail(*Cotumix coturnix*)	M	14d	1.9	1.7 – 2.1
	F	14d	1.7	1.3 – 1.9
Eastern Screech – Owl (*Otus asio*)	M/F	2 – 5yrs	1.9	1.4 – 2.1
Ring - necked Pheasant (*Phasianus colchicus*)	F	3mo	4.15	2.38 - 722
Northern Bobwhite (*colnus virginius*)	F	3mo	5.04	3.64-6.99
	M/F	16 – 20	12	7.0 – 19
	M/F	Wk 1-2yr	8.0	6.0 - 10
European starling (*sturnus vulgaris*)	U	adult	5.62	

M= Male U = Sex unknown F = Female h = hour, d = day, wk = week, mo = months, yrs = years (Mineau, 1993).

It is not unusual for birds to be more susceptible than mammal to cholinesterase inhibiting insecticides. However, the LD_{50} values given in Table 1, which are below 1 mg/kg for the two species of waterfowl tested, half of the song birds and one of the two birds of prey mean that carbofuran has one of the highest recorded toxicities to birds than any insecticide registered for use in Canada (Mineau, 1993).

In Africa, direct and secondary poisoning of vultures have been witnessed in South Africa where vultures species like cape vultures (*Gyps coprotheres*), Hooded vultures

(*Gyps Necrosyrtes monachus*) and Ruppells Vultures (*gyps rueppellii*) are listed in the Eskom Red Data Book of birds of South Africa, Lesotho, Swaziland as either vulnerable, endangered or regionally extinct (VUE, 2005). In East Africa, particularly in Kenya 187 African white-backed vultures and hyenas were found dead near Athi River in 2004 (EXTOXNET, 2001a). Carbofuran pesticide was suspected to have been used as a poison to kill the hyenas consequently vultures that fed on the poisoned carcasses died of secondary poisoning and were found lying next to the carcasses (EXTOXNET, 2001b). More African white-backed vultures and hyenas were found dead in Queen Elizabeth National park near Kasenyi village on the shores of Lake George in Uganda (EXTOXNET, 2001a). Carbofuran poisoning was also suspected. There are several other poisoning incidents in Kenya that sometimes go unnoticed and therefore undocumented (Seamus, 2008). International co-operation is, therefore, needed to address global pesticide use and misuse in order to offer adequate protection to birds (Helmut and David 1996). All the studies on the risks posed by carbofuran on the birds of prey have been carried out in United States of America and Canada but none in any African country yet. It is rightly justified that this project focuses on the studies to monitor the levels of carbofuran residues and its risks to the vultures in Isiolo and Laikipia districts and subsequently develop legal framework for provision of mitigation measures. The data generated here provided information on the potential routes of exposure of carbofuran to scavenging birds, which could then be used for ecological risk assessment and provision of appropriate mitigation measures.

It is worth noting that carbofuran as a pesticide has great impact on the wildlife and this matter has globally been discussed at various levels resulting into its ban and restriction in other countries, however, trade in such restricted pesticides remains considerably high in developing countries (Helmut, 1990). In 1990, over US $ 12 million worth of these pesticides including carbofuran that were banned or had their registration cancelled were stopped from US port destined for export (Helmut, 1990). However, the export for carbofuran by FMC Corporation of US has continued over the years. Most of the pesticide used in the tropics originates from developed countries and under current legislation nothing stops them from exporting banned or restricted pesticide (Bottrell, 1994). This explains why carbofuran a banned pesticide in the U.S.A still finds market in

Kenya. The U.S.A along with other developed countries has been accused of dumping banned toxic pesticides Parathion, aldicarb and carbofuran in developing countries (Emden, 1992).

2. 4 Metabolism, environmental degradation and dissipation

In order to understand and monitor carbofuran residues in the environment it is important that its sources, metabolism and degradation processes be understood. The dominant source of carbofuran emission to the environment is the application of the compound as an insecticide (Hassall, 1990). Carbofuran metabolises by hydrolysis, oxidation and conjugation, however, the rates of dissipation and metabolism in soil also is dependant on its rates of volatilization and chemical transformation to other metabolites. These rates are enhanced by adsorption/desorption processes in the soil matrix, sunlight intensity, extent of chemical reactions such as hydrolysis and oxidation/reduction reactions, and microbial activity in soil (Lalah *et al.,* 2001). Chemical transformation processes on pesticides in soil are influenced by soil characteristics such as temperature, clay content, pH, organic matter content, moisture content, presence of micro-organisms and the type of functional groups attached to the pesticide molecule (Lalah and Wandiga, 1996a). For example the amino and carbonyl groups in carbofuran can participate in hydrogen bonding with other O- or N atoms present in soil colloids (Lalah *et al.,* 2001). The presence also of a large moiety such as benzene ring with mobile π electrons in carbofuran molecules influence its polarization and therefore would increase the strength of their adsorption bonds hence degradation (Lalah *et al*., 2001).

In addition to the characteristic groups that are responsible for its insecticidal properties, other functional groups such as –COOH and -NH attached to the aliphatic and aromatic groups in carbofuran are likely to affect its interaction with dissolved organic matter, soil colloids and soil micro-organisms which could influence its interaction with soil matrix and its chemical degradation including hydrolysis (Lalah *et al*., 2001). The study further reported that pesticides such as carbofuran with functional groups such as -NHCOR and -NHR in their molecular structure tend to show higher adsorption to soil The amino groups in carbofuran can also adsorb to soil colloids as cations, after getting protonated depending on the soil pH and their individual pKa values. Soil pH is also one of the factors that affect chemical hydrolysis of carbofuran. This is expected to occur more rapidly in alkaline soil as compared to neutral or acidic soils (Lalah and Wandiga, 1996a). Carbofuran is more readily degraded in aquatic systems than in soil. Infact, it is hydrolysed both in flooded and non-flooded soils but slightly more rapidly under flooded conditions and it shows increased persistence when incorporated into non-flooded soils as a result of reduced hydrolysis and reduced losses due to volatilization and photochemical degradation (Lalah and Wandiga, 1996b). According to Tarwoski (2004) the Freundlich co-efficients (K_f) of carbofuran range from 0.1 to 30.3 (median 0.72) and the adsorption co-efficient K_{oc} values range from 9 to 62 millilitre per gram (median 30) an indication that carbofuran is highly mobile and can leach to the ground in many soils or reach the surface waters via run-off. Most chemical degradation reactions of carbofuran in soil are mediated through water which acts as a reactant or provides a reaction medium. Degradation in both soil and water involves hydroxylation and hydrolysis at C-3 carbon of the carbofuran molecule (EXTOXNET, 2001a) to give degradation products as shown in Figure 2.

Figure 2 shows the pathways of degradation of carbofuran in aerobic soils by Hydroxylation and oxidation at C-3 position to yield 3-hydroxycarbofuran and 3-ketocarbofuran which on further oxidation small amounts of doubly hydroxylated N-Hydroxymethylcarbofuran and 3-Hydroxy-N-hyroxymethylcarbofuran are formed (Hassall, 1990). Apart from the major degradation products (3-hydroxycarbofuran and 3-ketocarbofuran), carbofuran also breaks down through hydrolysis to form carbofuran phenol, 3-hydroxycarbofuran phenol and 3-ketocarbofuran phenol (Lalah *et al.*, 2001).

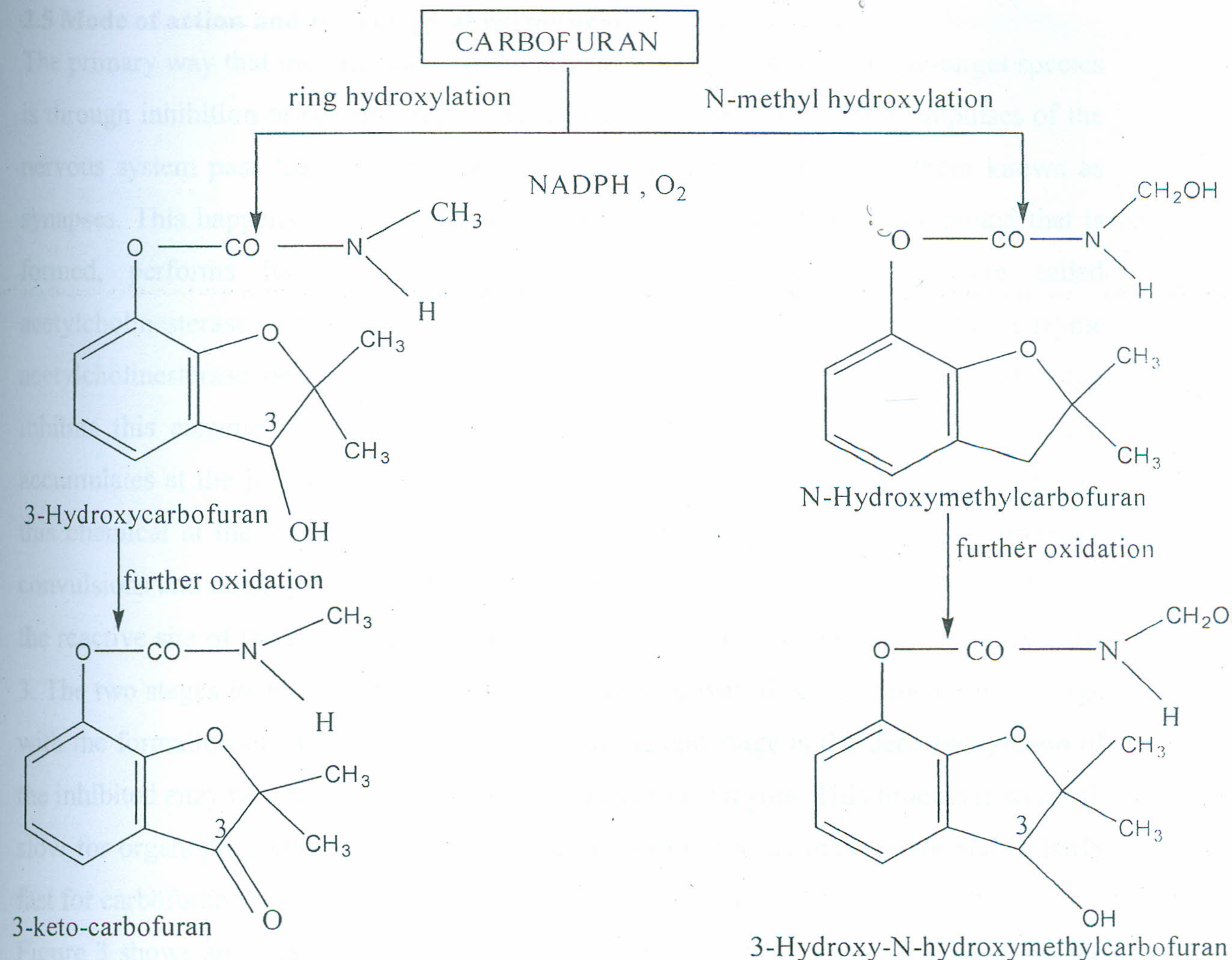

Figure 2 Oxidative metabolites of carbofuran (Hassall, 1990)

Carbofuran persists moderately in the soil with a half-life of a few weeks in tropical soil but this can be up to 378 days in temperate soil conditions. It is due to its rapid disappearance in tropical soils that farmers tend to apply higher amount of carbofuran than the recommended doses in order to achieve the desired effectiveness (Lalah *et al.*, 2001). As a result, large amounts of this pesticide can find its way into the environment. It is important to note that the rate of degradation of carbofuran is greatly influenced by pre-treatment and according to Hassall (1990), carbofuran persists longer in soils where it has been used continuously for along period.

2.5 Mode of action and toxicology of carbofuran

The primary way that insecticidal carbofuran works on both target and non-target species is through inhibition of the enzyme acetylcholinesterase (Emden, 1992). Impulses of the nervous system pass from nerve to nerve across minute gaps between them known as synapses. This happens with the aid of the chemical acetylcholine, a compound that is formed, performs its function and is then destroyed by the enzyme called acetylcholinesterase (AchE). The nerve transmission ends when the enzyme acetylcholinesterase breaks the acetylcholine into choline and acetic acid. Carbofuran inhibits this enzyme (acetylcholinesterase) so that acetylcholine is not destroyed but accumulates at the junction of the nerve cell and the receptor site. The accumulation of this chemical at the synapses causes the nerve to fire continuously leading to tremors, convulsions and death (Emden, 1992). During this process, carbamic acid esters attach to the reactive site of the AchE and undergo hydrolysis into two stages as shown in Figure. 3. The two stages include the first one which is the removal of X substituent (aryl group) with the formation of carbamylated enzyme. The second stage is the decarbamylation of the inhibited enzyme with the generation of a free active enzyme. This process is so much slow for organophosphate so that it is frequently referred to as irreversible and is fairly fast for carbofuran thus its reversibility (Amdur *et al.,* 1991).

Figure 3 shows an equation which represents a simple enzyme kinetics that explains the subject of acetylcholinesterase enzyme inhibition and its recovery. It is based on the concept of formation of intermediate unstable enzyme-inhibitor complex (Hassall, 1990; Michael, 1997). The first reaction represents formation of transient enzyme–inhibitor complex (not covalently bonded); the second represents carbamylation of the enzyme where the enzyme and the inhibitor molecule are covalently bonded. The third reaction is the decarbamylation of the enzyme (Amdur *et al.,* 1991). Out of the four rate constants, k_3 and k_4 are of interest concerning the mode of action of carbofuran as k_3 determines how rapidly the enzymes become blocked through carbamylation. The rate constant k_4 which is the rate limiting step determines how rapidly the blocked enzyme becomes unblocked by hydrolytic decarbamylation to form free enzymes (Hassall, 1990). This means that if the rate constant k_4 is higher than k_3 then the blocked enzymes can easily become free thus reducing the effect of carbofuran on the organism. This explains why carbamates are considered to have reversible action on organism and so if antidote is provided on time then the organism is likely to recover from its effects.

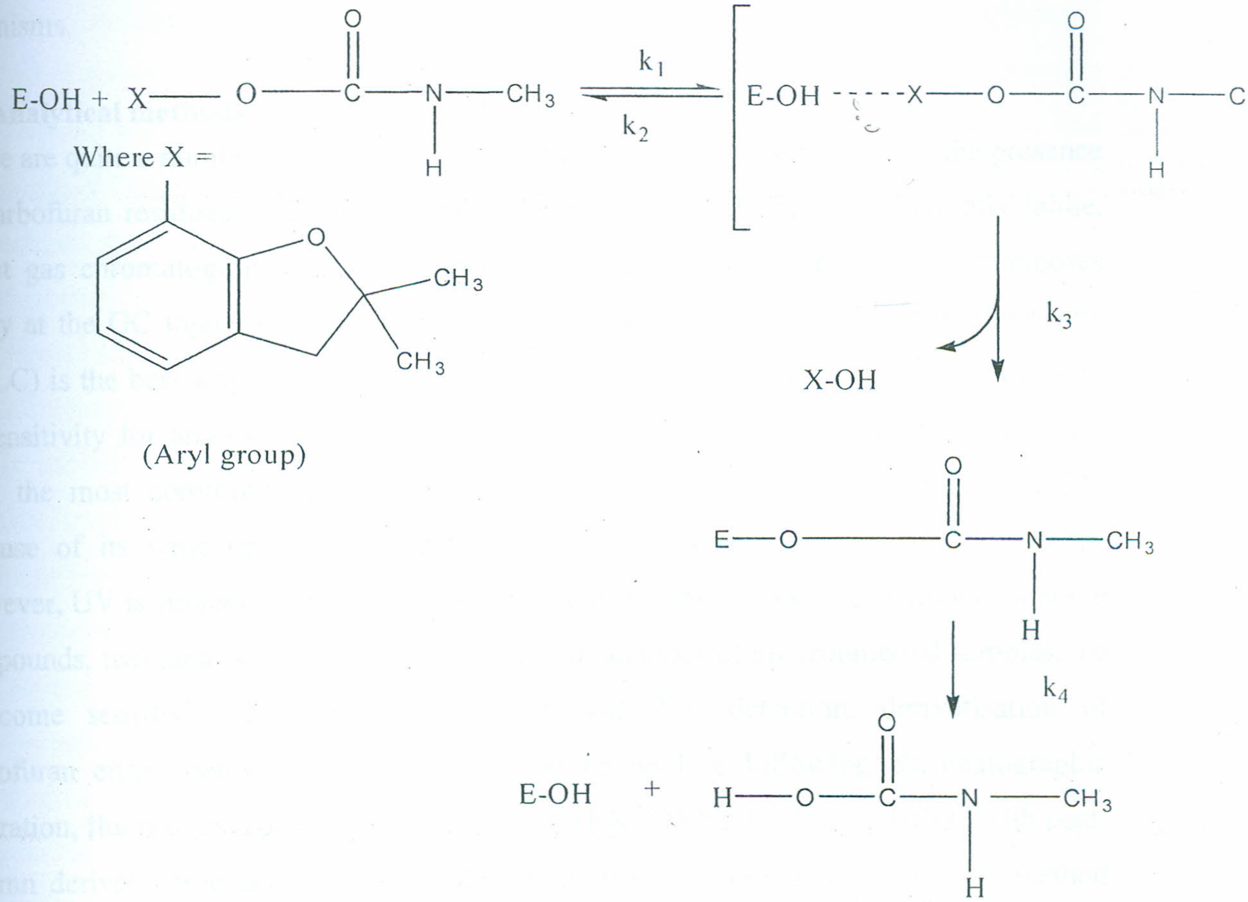

Figure 3: Action mechanism of carbofuran on acetylcholinesterase (AChE) (Amdur *et al.*, 1991).

Inhibition of the acetylcholinesterase is one of the matrices that can be used to determine the cause of death of birds. Accurate diagnosis of carbofuran-induced wildlife mortality in pesticide exposure incidents using brain cholinesterase is often difficult. This is because it is confounded by unknown post-mortem carcass history prior to sample retrieval, during which inhibited brain ChE may spontaneously reactivate (Hill, 1989) as explained by Figure 3. Brain ChE values reported from the carbofuran kills routinely have widely variable activities leading to considerable uncertainty to the diagnosis of exposure (Hill and Fleming, 1982). However, recent studies by Hunt *et al.* (1995) show that brain ChE can successfully be used to diagnose carbofuran-induced mortality. In doing this the brain ChE activity is measured and compared to normal brain ChE activity of the same species to determine the decrease in enzyme activity from normal levels. Measuring the brain ChE is just one of the ways of determining the cause of death of the organisms.

2.6 Analytical methods

There are quite a number of analytical methods currently in place to analyze the presence of carbofuran residues in environmental samples. Since carbofuran is thermally labile, direct gas chromatography is not generally applicable since it thermally decomposes easily at the GC injection port (EPA, 2008). High performance liquid chromatography (HPLC) is the best way to isolate carbofuran, yet conventional detectors lack specificity or sensitivity for analysis of environmental samples (EPA, 2008). UV absorbance has been the most commonly used detection method in HPLC determinations probably because of its wide applicability and consequent presence in most HPLC systems. However, UV is subject to interference from co-extractives and lacks sensitivity for some compounds, two factors that limit its usefulness in analysis of environmental samples. To overcome sensitivity limitation experienced with UV detection, derivatisation of carbofuran either before HPLC determination or on–line following chromatographic separation, fluorescence detection has been used (EXTOXNET, 2001b). HPLC with post-column derivatisation and fluorescent detection (US Environmental Protection method 531.1) is the prevalent technique for analysis of N-Methylcarbamate pesticides (UNEP, 1995). However, fluorescence detection has not been widely used in detection of carbofuran since it does not possess native fluorescence. However, for carbamates that fluoresce or can be made to fluoresce by derivatisation, fluorescence detection offers a degree of sensitivity and selectivity higher than UV. In this case carbofuran is readily reacted with o-phthalaldehyde and mecaptan after reverse phase separation and hydrolysis to form high fluorescence compounds (Chuang *et al.,* 1999; PL, 2008). The best way of analysis is then to couple both gas chromatography and liquid chromatography. In this regard, the HPLC then needs to be combined with particle Beam Mass Spectrometer as the detector to obtain positive identification and structural information (Vyas *et al.,* 1996). Studies conducted by Kawamoto and Makihata (2003) succeeded in analyzing thermally unstable trichlorfon and methyl dymron using GC/MS by temperature programmable inlet (TPI) on-column injection. Using this method it is possible to obtain a mass spectrum with specificity higher than the fluorescence spectrum obtained by the fluorescence detection method.

Carbofuran is unstable in alcohols and it is observed that HPLC analysis of carbofuran standard in acetonitrile has an analyte peak smaller than a secondary peak which grows in size (Lea and Mladen, 1999). It is expected that this secondary peak is for one of the four metabolites of carbofuran. However, carbofuran appears stable enough in acetonitrile but it undergoes slow decomposition a fact which must not be ignored (EXTOXNET, 2001a). It is important to ascertain the purity of all the chemicals before the analysis is done. All the reagents and the solvents used must be of the highest grade possible and free from the contaminants that may interfere with or distort the findings. As mentioned before HPLC-UV is the most suitable means of identification of carbofuran and its metabolites, however, positive results obtained must be confirmed by a second analytical procedure that identifies the particular analyte. Such additional testing is performed to establish an unequivocal identification of the analyte and presently it is the GC-MS that is used to do this (Amdur *et al.*, 1991).

In some cases qualitative identification of carbofuran may be sufficient enough to know the cause of death of vultures but there is need to get reliable concentration estimates for forensic interpretation. For quantitative analysis, the linearity, precision and specificity must be established. Linearity should be determined by use of at least three calibration whose concentrations bracket the anticipated concentrations in the samples. Precision, which statistically demonstrates the variance in the value obtained, is determined by multiple analyses of a specimen of known concentration. For a variety of reasons, occasionally a quantitative result will deviate spuriously from the true value. Therefore, replicate quantitative determinations should be performed on all samples at least in triplicate (Blanke, 1987). However, it is important to report that recent studies by Jaiswal *et al.* (2008) show that carbofuran can also be analysed by Thin Layer Chromatography (TLC) in biological samples which is very simple, cheap, rapid and can be performed in much less time than GLC and HPLC. Other recent work done by Abad *et al.* (1999) has come up with immunoassays (ELISA) as an analytical method for analysis of non-purified samples containing carbofuran. It is based on interaction of an analyte with antibody that recognises it with high affinity and specificity (Abad *et al.*, 1999).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Choice and description of the area of study

3.1.1 Sampling sites in Isiolo and Laikipia Districts.

The region around Lewa wildlife conservancy (LWC) in Isiolo was picked as an area of study. River Ngare Ndare and Ngare Sirgoi were sampled both upstream and down stream. Water from both rivers is used by farmers living adjacent to the river banks for purposes of irrigating their farms. It is expected that the pesticide residues find their way into the river from adjacent farms where application of pesticide is done. Other water points like ponds were also sampled both inside and outside the conservancy as identified in Figure 4. Soil samples were collected from the farms that border Lewa wildlife conservancy. These included the grazing areas and even areas where the carcass of both vultures and Lion were found. The specific sampling sites eg Manyangalo, Ngare Ndare forest, Loperua, Meru central, Ngare Ndare farms and Borana farms are all identified in Figure 4. Plant samples were similarly collected from areas around the conservancy and specific sites are covered in Figure 4. Vultures tissue were collected from the carcass found in the sites marked on map in Figure 4.

The survey that was conducted covered the inhabited areas around LWC. As mentioned earlier in section 2.1, the population here is sparse and therefore focused on the region where farmers and pastoralists were. This included regions like Manyangalo, Loperua Borana area and around the Ngare Ndare forest.

In Isiolo there were six water sampling sites. The two rivers Ngare Sirgoi and Ngare Ndare that run adjacent to the conservancy were sampled both upstream and down stream to establish the variation in the concentration levels of carbofuran. There were six sampling sites for soil and plants. Similarly there were four sites namely Loperua and Borana areas from which the vultures were collected around the LWC. The sampling sites in LWC lied within longitudes 36° 50' and 39° 30'E and latitudes 0° 05'S and 2°N. More description of the area of study is covered in section 2.1.

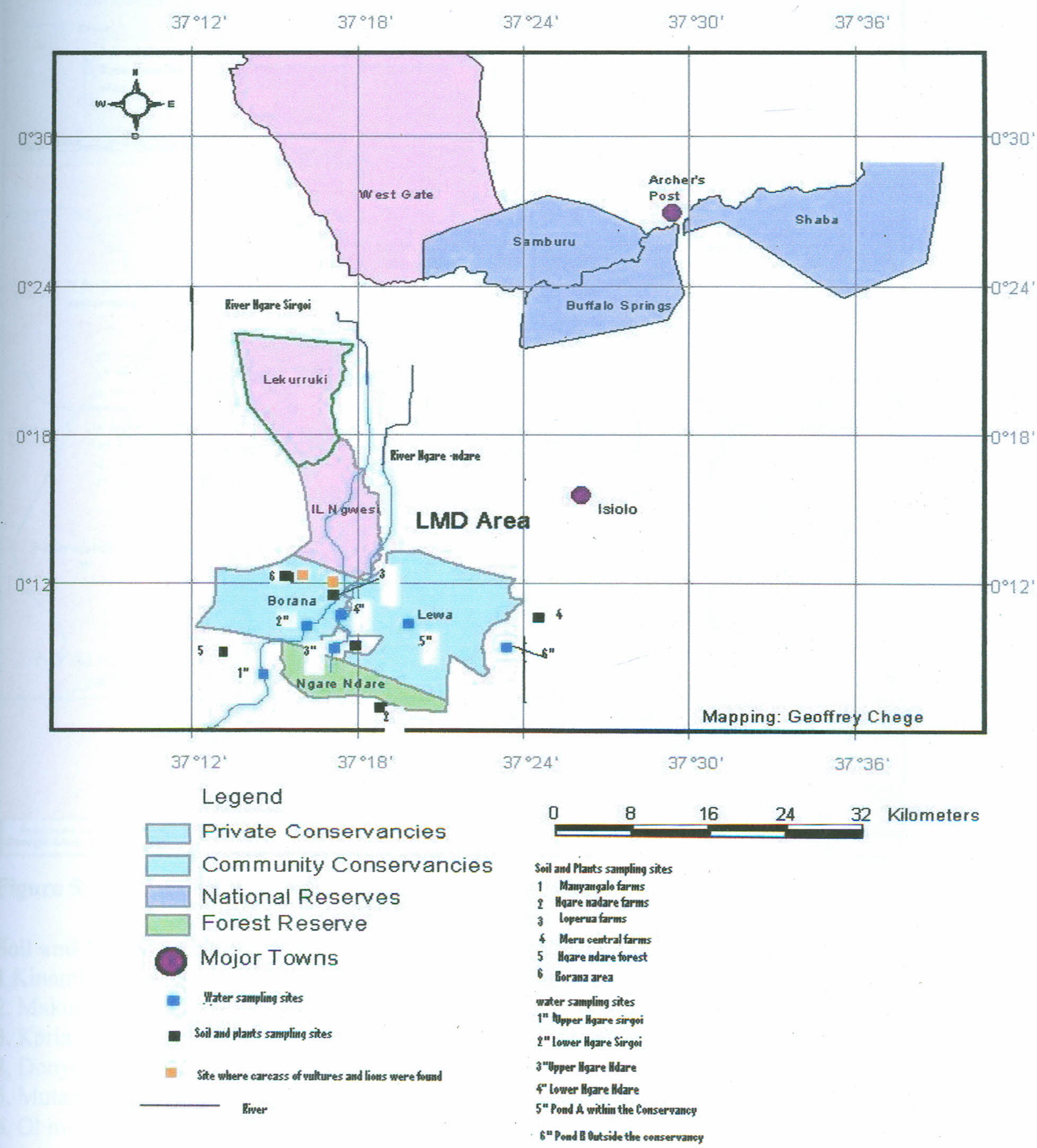

Figure 4: Map of Lewa wildlife conservancy showing sampling sites.

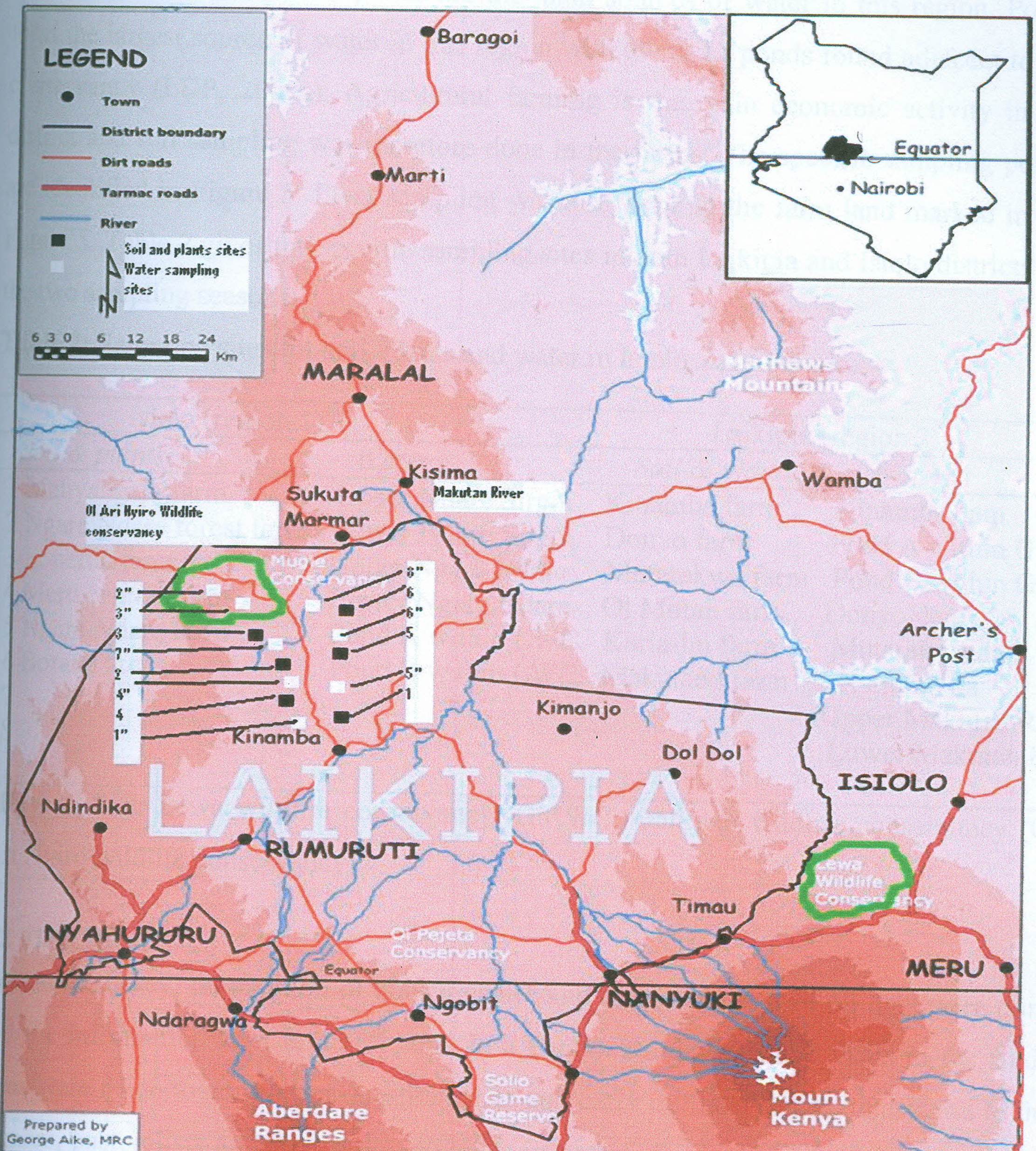

Figure 5: Map of Laikipia District showing sampling sites

Soil and Plants sampling Sites

1 Kinamba farms
2. Makutano farms
3. Karia-ni Farms
4. Donyo farms
5. Mutarakwa Farms
6. Ol moran farms

Water sampling sites

1" Kinamba pond
2" Pond A within GMWC the conservancy
3" Pond B within GMWC the conservancy
4" Donyo pond
5" Mutarakwa pond
6" Ol-moran pond
7" Upstream Makutan River
8" Downstream Makutan River

In Laikipia water sampling was done from eight different sites identified in the map in Figure 5. The rivers and the ponds are the main sources of water in this region. Ponds form the largest source of water in this region with about 12 ponds found adjacent to the conservancy (LDP, 2009a). Agricultural farming is the main economic activity in the district and soil sampling was therefore done in the farms. The specific sampling points are identified in Figure 5. Plant sampling was also done in the farm land marked in the Figure 5. Table 2 gives the specific sampling sites in both Laikipia and Isiolo districts for the two sampling seasons.

Table 2: Sampling sites for soil, plants and water in Isiolo and Laikipia.

Isiolo (region 1)		Laikipia (region 2)	
Soil & plants	*Water*	*Soil & plants*	*Water*
1 Manyangalo farm	Upper Ndare Sirgoi	Kinamba farm	Kinamba dam
2 Ngare Ndare forest farm	Lower Ndare Sirgoi	Donyo farm	Pond A within GMWC
3 Loperua farm	Upper Ngare Ndare	Mutarakwa farm	Pond B within GMWC
4 Meru central farm	Lower Ngare Ndare	Ol Moran farm	Donyo dam
5 Ngare Ndare farm	Pond A within LWC	Karia-ini farm	Mutarakwa dam
6 Borana area	Pond B within LWC	Makutano farm	Ol moran da
7			Upper Makutan River
8			Lower Makutan River

Key: LWC: Lewa wildlife conservancy, GMWC: Gallman wildlife conservancy (Ol Ari Nyiro wildlife conservancy)

3.2 Field survey

Literature survey, preparation of questionnaire and documentation of all data were done and the information kept in a soft copy in a Laptop computer. Field visits to the study areas were made to administer questionnaires and carry out face to face interviews to the farmers, pastoralists and the conservationists in both Laikipia and Isiolo and a copy the questionnaire is in the Appendix III. This was done with the help of an assistant from the local community who helped in the translation. The assistant was first educated on the proper use of the questionnaire before data collection. Visits were also made to the National Museum of Kenya and Pest Control Products Board of Kenya offices to get information regarding the use and information regarding pesticide regulation especially on carbofuran. The survey data was then subjected to statistical analysis using SPSS statistical package.

3.2.1 Survey Design

The survey was done once in areas around the two conservancies by use of snowball sampling design. Isiolo and Laikipia Districts had a population of 100,861 and 322,187 respectively with no information on the number of farmers and pastoralists who were the target of the study. Snowball sampling, a nonprobability method, was found to be appropriate since the desired characteristic was rare (Salganik and Heckathorn, 2004). It is commonly used when it is not easy to have access to sufficient respondents with the intended characteristics and when the sample is limited to a very small subgroup of the population (Heckathorn, 2002). In this method, farmers and pastoralists who use carbofuran in the farms and as poison were identified with the help of the research assistant and the personnel from the conservancies. Once they were interviewed they were asked for other referrals and this continued until fifty and thirty three questionnaires were filled in Laikipia and Isiolo respectively. The study areas were selected by use of purposive sampling design which depended on the reports mass deaths of scavenger birds and predators.

Fifty questionnaires were administered in Laikipia because it was densely populated compared to thirty three questionnaires in Isiolo which less is populated (4 people per km^2) as stated by Mati *et al.* (2006). A total of eighty three questionnaires were, therefore, administered in both areas under study and preference was given to areas where agricultural activities were prevalent. In Isiolo district the survey covered Manyangalo, Ndare forest, Borana and Loperua areas. In Laikipia district the survey covered Kinamba, Ng'arua, Ol Donyo Nyiro areas. The same design was used in sampling conservationists and stockists. Five and four conservationists were interviewed in Isiolo and Laikipia respectively and the responses summarized. Stockists were also interviewed from both districts and responses tabulated.. The survey involved the use of a semi-structured questionnaire with open and close-ended questions and a face-to-face interview which had an advantage of eliciting more information apart from what was included in the questionnaire.

3.2.2 Survey of pesticide use in the two districts

Survey on the use of carbofuran pesticide by the farmers in Laikipia and Isiolo District was done through administration of the questionnaires and face to face interviews to elicit more response on areas that were not clear on the questionnaire. This touched on the amount frequency and times of application in the year and methods of disposal of the un-used chemicals and used containers. Questionnaires also targeted Pest Control Products Board of Kenya as pesticide regulatory authority. This was done to establish data on the amount supplied and purchased in a year and which specific regions supplied with pesticide, labelling and legal aspects.

Questionnaires were also administered to the farmers and pastoralists where ten respondents, which included both farmers and pastoralists, were selected randomly from the four regions around Lewa wildlife conservancy. The same number was selected from six other regions around Ol Ari Nyiro wildlife conservancy in Laikipia district. Personnel from Kenya Wildlife services, Lewa wildlife Conservancy Ol Ari Nyiro Wildlife conservancy and Ornithology Department of National Museum of Kenya were also interviewed to assess frequency of the deaths of birds.

Other aspects covered by the survey through questionnaire included quality control checks i.e. any other carbamates used other than Carbofuran in the same areas, any other pesticides (either organochlorine or organophosphates) that are prevalently used in the two areas and pesticide combinations which may contain carbofuran in their formulations. Statistical package for social scientists (SPSS) was used to analyze the survey data.

3.3 Sample collection, preparation and experimental design

3.3.1 Soil sampling

Stratified random sampling method was used to collect the soil, plant and water samples. The samples were collected twice; one during the rainy season (October, 15^{th} - 26^{th} 2007) and another during the dry season (June, 9^{th} -20^{th} 2008). Soil samples were taken randomly from six different selected sites in the farms around LWC and Ol Ari Nyiro conservancy as shown in section 3.2 and Figure 4. The distance between sites was maintained at about 1-2 km kilometre. Since the area to be sampled was estimated to be 55 km^2, composite sampling design was used in order to get a representative sample for this region. An Auger was used to get a large scoop of soil up to a depth of 2 cm. From each site five soil samples of about 25 grams each were collected randomly within a radius of 20 m and combined to form one composite sample which weighed 125 grams. From each composite sample about 30 grams was taken for analysis. This was repeated for each site to get three replicates for each of the six sampling sites in both districts in the two sampling seasons. A total of 18 samples (540 grams) for rainy season and another 18 samples (540 grams) for dry season were gathered in Isiolo and Laikipia District respectively. This was done in accordance with the sampling design described above. Samples were wrapped in aluminium foil and transported in the icebox to the laboratory for analysis. In the laboratory soil samples were air dried for 60 hours under at 25° C to remove moisture before they were analysed. Sampling was done twice. The first was done between October 15th and 26th, 07 during the rainy season when carbofuran had just been applied in the farms and the second was done between June 9th and 20th, 08 during the dry season. Plate 7 in Appendix II shows the researcher in the filed collecting soil samples from Manyangalo farm in Isiolo district.

3.3.2 Plant (*Zea mays*)sampling

The plant samples were collected from different sites as shown in section 3.2.2. The distance between the sampling sites was maintained at between 1-2kms. In the rainy season, maize plants were collected from selected farms around LWC and Ol Ari Nyiro in Laikipia and Isiolo districts, respectively. In the dry season were dry maize plants were collected. Sampling was done in three replicates giving a total of 18 replicates from the six sites in the dry season and the same number in the rainy season. This was also done in Laikipia district.

About Five hundred (500) grams of maize samples were collected randomly from six different selected spots in the agricultural farms around both conservancies. This was done between October 15th and 26th, 07 and between June 9th and 20th, 08. The samples were also kept in the icebox and transported to the laboratory for analysis.

3.3.3 Water sampling

Water samples were collected from the two main rivers adjacent to LWC and the ponds. In Isiolo the rivers were sampled both upstream and down stream and a distance of 2 km was maintained between the sampling points. The ponds were similarly sampled in both regions. Sampling was done in three replicates giving a total of 18 samples from the six sites in the dry season and the same number in the rainy season. In Laikipia district the ponds both inside the conservancy and outside as well as River Makutan were sampled for analysis.

Two litres of water samples were collected randomly upstream and down stream from the two rivers adjacent to LWC and the farms where irrigation is commonly done i.e. river Ngare-Ndare and Ngare-Sirgoi. Another two litres was collected from the ponds in the study areas. In Laikipia district two litres of water samples were also collected from the six ponds (man-made dams), two of which were inside the conservancy and four were outside the conservancy i.e in the farms. Makutan River that flows adjacent to Ol Ari Nyiro conservancy was also sampled both upstream and downstream. The water samples were then kept in bottles and transported in an icebox containing icepacks at 0°and transported to the laboratory for analysis. The experiment was done using Randomized Complete Block Design (RCBD) 2 factorial where Regions were considered as the main treatment and seasons as sub treatment.

3.3.4 Vultures and Lion tissues

Tissues of vultures which included the beaks, feet, crop content and the gut were collected from Isiolo on October 15th, 2007 and from Laikipia on October 23rd, 2007. Other samples of beak, feet, blood and gut and muscles were received from Mbirikani and Athi River ranch. Although Athi River was outside the study area samples fro there were considered because cases of poisoning of wildlife seemed to be a common occurrence in nearly all the conservancies and ranches in Kenya. Apparent poisoning cases were also common in these areas during the study and therefore were good for comparison.

Twenty pieces of weathered feet weighing about 15 grams each sampled from the dead vultures as seen in Plate 6 in the Appendix II were collected for analysis. Vulture heads numbering 20 pieces each weighing about 17 grams were also collected as shown in Plates 6, 9 and 10. The samples were placed in a cool box where they were kept at 4°C or below (Nimish *et al.,* 2005) before transported to Maseno University Laboratory for analysis. Other 16 pieces of weathered feet and 14 pieces of the beaks were collected from Ol Ari Nyiro wildlife conservancy in Laikipia district. In all the cases the samples were kept under ice to maintain the temperatures at or below 4°C in order to reduce possibilities of carbofuran breaking down to its metabolites.

Other samples like the gut, muscles from the carcass of a lion and blood sample of vultures were received from Simon Thomsett of Athi River Ranch and Seamus Maclennan of Mbirikani group ranch. This was done since cases of carbofuran poisoning had been suspected in various parts of the country as shown in the map in the Appendix I.

3.4 Cleaning of reagents and apparatus before extraction.

Crystalline anhydrous sodium sulphate for drying samples and florisil for clean-up of the samples extract were obtained from Kobian (K) Ltd and were pre-extracted before use by using n-hexane in a Soxhlet apparatus for 8 hour in accordance with UNEP (1995). Similarly activated charcoal which was used for removal of lipids and colour from plants and animal tissues was also obtained from Kobian Kenya Ltd (Nairobi). It was also pre-extracted by use of n-hexane.

Glassware were all scrubbed with brushes in hot water and detergent. They were rinsed with tap, distilled water and finally with acetone. They were then dried in an oven at 150°C before being stored in dust free cupboard with a tight seal of aluminium foil. Thimble and filter papers used during extraction were also pre-extracted using 250 ml dichloromethane for 8 hours in a Soxhlet apparatus. Pesticide residue analysis-grade solvents including dichloromethane, acetone, methanol and HPLC water were obtained from Kobian Kenya Ltd (Nairobi). Pure analytical pesticide standard mixture containing carbofuran and its two metabolites 3-hydroxycarbofuran and 3-ketocarbofuran (10 mgl^{-1} in acetonitrile, purity > 99.9%) was obtained from the Institute of Ecological Chemistry, Helmholtz Zentrum, Munich, Germany.

3.5 Extraction

The analytical procedure used involved solvent extraction of homogenized samples, clean-up on a solid phase extraction column and analysis on reverse-phase column of HPLC with UV detector (EXTOXNET, 2001a). This procedure was chosen after review of previous methods available in literature (Lalah and Wandiga, 1996b; Yang *et al.*, 1996; Takino *et al.*, 2004; Vyas *et al.*, 2003).

3.5.1 Extraction of vultures' tissues and animal samples

During extraction, about 25 grams weathered feet collected from the dead birds/carcass and the birds' tissues were cut using a pair of scissor. Each foot (below the distal end of the *tarsome tatarsus*) as seen Plate 6 in the Appendix II was cut further into approximately 0.6 cm pieces using a pair of scissors then crashed and ground using a grinder to facilitate chemical extraction. The beaks, the birds' crop, the muscles from the carcass and the birds' gut were treated in the same way. Each sample was extracted three times with acetone and dichloromethane in the ratio of 1:1. A volume of 50 ml of acetone and 50 ml of dichloromethane was used. During the extraction the mixture was shaken for 15 minutes before it was left to settle for 5 minutes. The extract was then filtered using whatman No 1 filter paper The blood sample of 10 ml volume was extracted using acetone and dichloromethane in the ratio of 1:1 at a volume of 50 ml each. This was done three times. The extract was then combined and reduced to 5 ml in a rotary evaporator at about 20°C. The extraction was done according to Hunt *et al.* (1995) Vyas *et al.* (2003) method. The extracts were then taken for separation and clean-up using open glass column chromatography.

3.5.2 Extraction of soil samples

Soil samples were air-dried in the laboratory at room temperature until there was constant weight then 25 grams weighed. To achieve satisfactory recovery samples were further dried by mixing the sample separately with 20 g of anhydrous sodium sulphate to dehydrate the extract and then homogenized in a clean mortar with a pestle before being sieved through 2 mm mesh. The homogenized sample was placed in pre-cleaned thimbles and extracted in a Soxhlet extractor for 4 hours with 130 ml mixture of dichloromethane and acetone; (10:3 volume).The acetone/dichloromethane extract was concentrated in a rotary evaporator to about 5 ml at 20°C The extracts were then taken for separation and clean-up using open glass column chromatography. The extraction was according to method used by Lalah and Wandiga (1996a).

3.5.3 Extraction of plants samples

Five hundred (500) grams of air-dried plant samples were macerated homogenized with 2 g anhydrous sodium sulphate and extracted using Soxhlet apparatus with 150 ml solvent mixture of dichloromethane and acetone in the ratio 10:5. The Soxhlet extracts was concentrated in a rotary evaporator to about 5 ml at 20°C The extracts were then taken for separation and clean-up using open glass column chromatography. The extraction was done according to method used by Abad *et al.* (1999) and Lalah *et al.* (2003).

3.5.4 Extraction of water samples

The water sample (500 ml each) was partitioned with dichloromethane in a 1 litre separatory funnel, shaking with 100 ml dichloromethane for 15 minutes and left to settle for 5 minutes. The organic extract was drained and the procedure repeated using 60 ml and 50 ml dichloromethane respectively. The organic extracts were then pooled together and concentrated in a rotary evaporator to 5 ml at a reduced pressure and temperature of 20°C. Five grams of sodium sulphate was added to dehydrate the extracts and then filtered before taken for separation and clean-up. The extraction was according to method used by Kawamoto and Makihata (2003).

3.6 Separation and clean-up

The separation and clean up of sample extracts was performed using florisil in small glass column (Lalah and Wandiga, 1996a). Glass Teflon stopcocks were used as glass columns and plugged with glass wool at the bottom end, 4 g of pre-extracted florisil was added then 2 grams anhydrous sodium sulphate placed at the top. For plants and carcass sample extracts, 2 grams of activated charcoal was added at the top of the column for decolorizing the plant pigments and carcass lipids according to Lalah *et al.* (2003). In the extraction column, 10 ml of the dichloromethane was added to condition it. The extract (5 ml) was poured on top of the column and eluted with 10 ml dichloromethane, then 10 ml dichloromethane/acetone (95:5 by volume) and finally with 10 ml dichloromethane /acetone (10:90 volume) (Vyas *et al.*, 2003) It is important to note that this clean up method was found to be very efficient and to avoid loss of sample, the collection of the eluate was started at the same time as the sample was applied to the column. The eluates were pooled and evaporated to dryness using rotary evaporator before it was dissolved in 5 ml methanol for analysis by HPLC. This separation and clean-up was done according method used by Kawamoto and Makihata (2003), Lalah *et al.* (2003) and Abad *et al.* (1999).

3.7 HPLC and GC-MS Analysis

After clean-up procedure the extract was filtered through Gelman Acrodisc GHP filters (13 mm, 0.45 μm) in readiness for both GC-MS and HPLC analysis. The carbofuran residues were analyzed both qualitatively and quantitatively by GC-MS and HPLC respectively. The residues were analysed by using an HPLC of an Agilent 1100 series model made in Japan equipped with a UV/VIS detector at λ max = 254 nm A Supelco column (250 x 4.6 mm ODS 5μm) reverse phase C_{18} cartridge column was used with HPLC grade acetonitrile: water (4:1v/v) as the mobile phase at a flow rate of 1 ml min^{-1}. The calibration curve, the chromatograms for the standard and the samples obtained during the analysis are attached in the Appendix IV.

The G.C-MS conditions were; carrier gas N_2 at a flow rate of 1.0 ml/min, injection volume was 1 μl, an attenuation of the integrator was set to 0, chart speed was 0.5 cm/min, injector and detector temperatures were 280°C and 310°C respectively. The initial column temperature was 180°C programmed at a rate of 60C for 5min to 225°C and held for 5 minutes before increasing at (15°C/minute) to and holding at 280°C for 1 minute. The temperature program was found to give optimum separation based on the several trials that were done. In the Appendix IV are copies of the GC-MS chromatogram and the spectra which compared well with the one provided in the certificate of analysis and computer library. Apart from quantifying the carbofuran and 3-keto carbofuran levels, the components were also identified as shown in the spectra in the appendix.

Carbofuran residues (carbofuran, 3-ketocarbofuran and 3-hydroxycarbofuran) were identified by comparing their retention times with those of the standard. Quantification was done by extrapolation of sample peak areas to obtain corresponding concentrations using calibration standard curves. This was done by using the standard solutions (1μl) of concentrations ranging from 0.01 to 2 ppm which were injected into the HPLC. The peak areas of standard solution were plotted against their concentration. A line of best fit was then drawn through the points and the limits of detection taken at 3 times the detector noise level. Appendix V gives details for the calibration curve for 3-ketocarbofuran and carbofuran from HPLC and GC-MS analysis. The detection limit was found to be 0.01 ppm. For quantification and identification carbofuran, 3-ketocarbofuran and 3-

hydroxycarbofuran standards (10 mg/l in acetonitrile purity > 99.9%) were obtained from Dr Ehrenstorfer GmbH in Germany.

3.8 Quality control

The precision of the methods used in this study were established by the analysis of the samples in triplicate. The accuracy of the method was confirmed by running a spiked sample of plants, water, soil and animal tissues prior to sample analysis. This was further ensured by running blank solvents and standards (every six injections) between the injections. The agreement between measured and certified concentrations of individual analyte confirmed the accuracy of the method. This reference material was introduced on regular basis after running 10 samples as away of checking the procedure. Blanks solvents were run as an opportunity to evaluate and monitor the potential introduction of contaminants into the samples during processing. The blanks were also introduced on a regular basis in between the analysis of the samples. For recovery efficiency, 0.5 μg of carbofuran standard mixture was added to control samples of 500ml of water, 25 g of soil and 500 g of plants, respectively, for analysis following the same procedures described above. The recovery percentages in section 4.3 tested on all the environmental matrices analysed were within the acceptable range of above 70%. All the data were corrected according to the percentage recoveries obtained. Standard solutions of concentration ranging from 0.01 ppm to 1 ppm were prepared for carbofuran, 3-ketocarbofuran and 3-hydroxycarbofuran and 1 μl was injected in both HPLC and GC-MS. Peak areas were plotted against their concentration and a line of best fir was drawn and the limits of detection was taken. Detection limits were found to be 0.01 ppm for water and 0.01 ppm for animal tissues, plants and soil samples.

3.9 Statistical Analysis

Analysis of variance (ANOVA) was used to determine the variation between different seasons. Summary statistics (means, 95% confidence intervals, medians) were derived for each sample. Significance level was accepted at $p < 0.05$. Analysis of variance was done to determine the relationship between the concentration levels of carbofuran and the planting seasons and if there was also any relationship to the death of the vultures. The statistical analyses were by MSTAT and SPSS statistical software packages.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Survey results from the farmers and pastoralists

Literature survey revealed that the region under study fall within the tropical. Isiolo district is dry and falls under arid and semi arid region with low rainfall. The district is characterized by flat low-lying plains which has low agricultural productivity. Table 3 gives a summary of the characteristics of the catchment areas.

Table 3: Characteristics of the catchment area

	Isiolo	Laikipia
Climatic conditions	Rainfall is spread into the two seasons (aver 580.2mm) Classified as Arid and semi-arid	Cool temperate climate with both dry and rainy season (600mm -1200mm per year). Semi arid to high potential
Latitudes and Longitudes	Longitude36° 50'E and 39° 30'E and latitudes 0° 05'S and 2°N.	Longitude 36.00'E and 36.45'E; latitudes 1.00'N and 0.00'
Population and area	100,861 25,789 km^2	322,187 (Ngarua 65,545) 120, 500 km^2
Economic activities	Mainly pastoralism, tourism and arable farming.	Agriculture, pastoralism, ranching
Type of farming	Small scale mixed farming	Small mixed farming and large scale commercial farms
Size of farms	Average of 0.5 ha per individual;	Average of 3.5. -16 ha
Water sources	Rivers, ponds and boreholes.	Rivers, ponds and boreholes.
Crops grown	Maize, beans, cow peas. Bananas, cabbages etc	Maize, wheat, beans, cow peas. Bananas, cabbages etc
Livestock kept	Sheep, cattle, Goats ,camels and donkeys	Sheep, cattle, Goats ,camels and donkeys
Soil type	Black cotton soil/sandy/clay	Black cotton soil/sandy/clay
Carbofuran usage		
Estimated sales of carbofuran in a year.	110 kg/year	256 kg/year
Formulation used	Furadan 5G (5% active ingredient)	Furadan 5G (5% active ingredient)
Target organisms	Maize stalk borer, maize aphid, leaf miner, root nematodes, cutworms etc	Maize stalk borer, maize aphid, leaf miner, root nematodes, cutworms etc
Non-target organisms	Predator wildlife eg lions, elephants, jackal and scavenging birds eg vultures	Predator wildlife eg lions, elephants, jackal and scavenging birds eg vultures
Application rate	0.5-4 kg a.i /ha	0.5-4 kg a.i /ha

The survey also revealed that both regions are endowed with abundant wildlife and is a home to a number of sanctuaries and conservancies. However, due to increased pressure on land and water recourses by increased population there have been cases of human-wildlife conflict reported in both regions.

From randomly selected agrovet shops in Isiolo, Manyangalo centre in Isiolo district it was clear that carbofuran (Furadan) 5G formulation is preferred. An average of 110 kg/year of carbofuran is sold in Isiolo compared to an average of 256 kg/year in Laikipia District as shown in Table 3. The higher sales recorded in Laikipia could be due to more area under farming in Laikipia than it was in Isiolo. The rate of application of carbofuran is between 0.5-4 kg a.i/ha but this is not usually the case as many times application rate is done by approximation and in higher rates. This may explain the presence of carbofuran and its degradation products in soil, water and plant samples

The survey data obtained from both Isiolo and Laikipia show that maize, Beans, Vegetables (cabbages) and sweet potatoes are some of the main crops grown here. Maize is the most cultivated crop in both area and out of 68.7% respondents 26.5% of them grow maize in Isiolo as seen in Table 4. This is followed by beans which are grown as an intercrop as indicated in Figure 5 According to a related report based on the Laikipia agricultural activities in the (LDP, 2009b). Laikipia maize production covers an area of about 32,560 ha. As seen Table 3. The area under cultivation is big and explains why 46.9% of the respondents in Laikipia have large scale farming compared to Isiolo with the highest percentage of small scale farmers

Table 4: Tabulated responses from farmers and pastoralists on the usage of carbofuran

Region	*Isiolo*	*Laikipia*	*Total*
Isiolo Number of respondent	33	50	83
Percentage respondents	39.8	60.2	100
Crops grown			
Maize	26.5	42.2	68.7
Beans	7.2	9.7	16.9
Vegetables (Cabbages)	3.6	4.8	8.4
Potatoes	2.4	3.6	6.0
Estimated area under crop prod			
Less than 2ha	32.5	13.3	45.8
More than 2 ha	7.22	46.98	54.2
Common pests			
Insects	3.6	4.8	8.4
Wild animals	2.4	2.4	4.8
Wild animals and pest	33.7	53.0	86.7
Pesticides used			
Organophosphates	9.6	10.0	19.6
Carbamates	25.3	33.7	59.0
Both organophosphate. and carbamate	4.8	16.6	21.4
Most effective and preferred			
organophosphate	14.5	28.0	32.5
carbamate	25.3	42.2	67.5
Accessibility and availability of carbofuran			
Easily available	27.7	50.53	78.23
Not available	12.0	9.7	21.7
Are there cases of crop destruction /Kills by wildlife			
Yes there are cases	38.5	60.2	98.7
No there no cases	1	0.0	1.3
Action taken incase of destruction/kills			
Report to KWS/Conservationists	30.1	37.4	67.5
Not report	9.6	22.9	32.5
Compensation by the GOK/Private conservancies.			
Yes	0	0	0
No	39.7	60.3	83
Action taken to prevent further destruction/kills			
No action	13.2	21.7	34.9
Lay trap	13.2	12.0	25.2
Use poison	3.6	12.1	15.7
Keep vigil	9.6	14.6	24.2
Intentional use of Carbofuran as a poison			
Lay trap	13.2	12.0	25.2
Use poison	3.6	12.1	15.7
Keep vigil	9.6	14.6	24.2

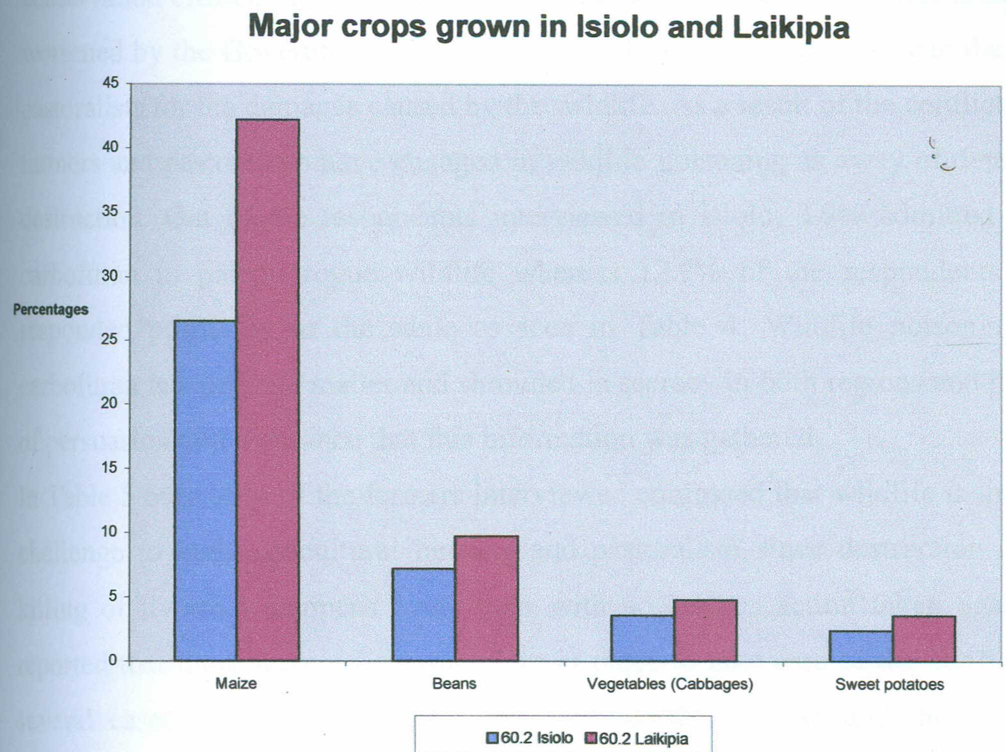

Figure 6. Percentages of crops mainly grown in both Isiolo and Laikipia Districts.

The difference in crop production is clearly shown in Figure 6 and also gives a picture of the crop type where carbofuran is mostly used. Carbofuran which is selling in the market under trade name of Furadan is easily purchased over the counter in all the outlet shops in Manyangalo centre and Isiolo town. Plate 8 in the Appendix II is a sample of Carbofuran (Furadan) in a 200 g tin that sells at Kshs 100 from the stockists. From the respondents interviewed, about 67.5%, as observed in Table 4, of farmers from the areas surveyed admitted using this pesticide to control both foliar feeding and soil dwelling pests in maize farms and horticultural farm since it is highly effective and works fairly fast. About 33.7% and 25.3% of the respondents in Laikipia and Isiolo respectively prefer to use carbofuran because it is cheap, effective and easily available as reported in Table 4. High agricultural productivity, relatively favourable climatic conditions and consequently increased use of carbofuran in the small and large farms explains why Laikipia recorded high levels of carbofuran and it s degradation products in soil, water and plant samples than Isiolo.

Crop destruction and kills of the livestock are the main causes of the human-wildlife conflict in both areas. The concern was further supported by all the conservationist respondents in both who agreed that human-wildlife conflict is indeed a challenge to the conservation efforts. From the interviews with the farmers and pastoralists this conflict is worsened by the Government's insensitivity and inability to compensate the farmers and pastoralists for the damages caused by the wildlife. As a result of the conflict a number of farmers and pastoralists have engaged in wildlife poisoning as away of deterring further destruction. Out of the respondents interviewed in Isiolo, 3.9% admitted having used carbofuran to poison rogue wildlife whereas 12.9% of the respondents interviewed responded positively to the same as seen in Table 4. Wildlife poisoning by use of carbofuran is a delicate matter and shrouded in secrecy in both regions and it is after a lot of persuasion and assurance that this information was gathered.

In Table 5 over 98% of the farmers interviewed confessed that wildlife is indeed a major challenge to both agricultural farming and pastoralism since destruction of crops and killing of livestock happens every year with no serious action taken against it. They reported that there were no measures put in place to take care of the conflict even after several cases were reported to the Kenya Wildlife Services and the private wildlife conservancies. It is on this basis that farmers and pastoralists in a bid to protect their crops and livestock use carbofuran as a poison which was then sprayed on the baits hoping that the predator animals would come and feed on it and consequently die. It was at this point that the vultures got exposed to secondary poisoning and also died in numbers. It is important to note that the presence of poisoned carcass in the field forms the death chambers for the vultures and other scavenging birds that eventually find their way here as was observed in Isiolo and Mbirikani where vultures died after feeding on poisoned Lion (see Plates 2, 4 and 10.) Vultures as a matter of fact are not the target species but their feeding habits, ability to spot carcass miles away and travel far distances looking for carcasses make them quite vulnerable. Besides, they are long-lived raptors with low reproductive rate laying one egg at a time. They have high adult survival which somehow compensates for low annual offspring production. These characteristics make this species highly sensitive to a decrease in adult survival (Slotta-Bachmayr *et al.*, 2004). For this reason a mass death vultures in one go present significant negative impact on the demographic viability of this species. The under cover nature of the practise makes it often very difficult to document poisoning cases affecting wildlife and specifically the *gyps* species. About 70% of the respondents say that it has not been easy to quantify the deaths of livestock and destruction of crops because most cases go unreported as seen in Table 4. In Table 5, out of all conservationists respondents interviewed 55.6% reported that cases of poisoning are independent of the seasons and therefore occur anytime there was destruction or kill by the predator wildlife

Although the private wildlife conservancies have tried to plough back a bit of their revenues by putting up a number of projects like health centres in the communities to help reduce the negative attitude of the communities towards wildlife, poisoning has still continued. According to the results in Table 5, the conservationists also agree with the farmers that indeed the human-wildlife conflict has been there for along time and they have made efforts to reduce it by educating the farmers on appropriate action to take when a problem occurs. Many projects including education projects, health centres, establishment of business enterprises, involving the elders in the management of conservancies, employing the youths from the communities around the conservancies have been put up to reduce the negative attitude of the farmers towards wildlife and for them to realize the need to conserve them. The ground informers reported that indeed the projects have reduced the negative attitude towards wildlife conservation but they still hold their livestock so dear to them that nothing would stop them from avenging their killing.

Table 5: Tabulated information gathered from the conservationists in Isiolo and Laikipia

	Isiolo	*Laikipia*	*Total percentage*
Number of conservationists	**5**	**4**	9
Percentage respondents	55.6	44.4	100
Cases of wildlife deaths			
Many cases	55.6	44.4	100
Few cases	0	0	
None	0	0	
Possible causes of deaths			
Natural	22.2	22.2	44.4
Suspected poisoning	33.4	22.2	55.6
Season where there are many deaths			
Rainy	11.1	11.1	22.2
Dry	11.1	11.1	22.2
Any time	33.4	22.2	55.6
Wildlife that die commonly			
Predators	11.1	11.1	22.2
Scavenging birds	11.1	11.1	22.2
Predators and scavenging birds	33.4	22.2	55.6
All the species	0	0	
Reported cases of crop destruction/livestock kills			
Yes	55.6	44.4	100
No	0	0	0
How reported cases are handled			
Compensation	0	0	0
Not compensated	44.5	33.30	77.8
Round up destructive wildlife	11.1	11.1	22.2
Reported cases of carbofuran poisoning			
Yes	55.6	44.4	100
No	0	0	0
Action taken against those found			
Arrest and prosecute	55.6	44.4	100
Do nothing	0	0	0
Have the cases been reported to PCPB			
Yes	55.6	44.4	100
No	0	0	0
PCPB action			
Educate the farmers/pastoralists	22.2	11.1	33.3
Investigate	11.1	22.2	33.3
Do nothing	22.3	11.1	33.4

The results of the information gathered from the conservationists indicate that cases of misuse of carbofuran as a poison had been reported to the Pests Control products board. The PCPB has over the years educated the farmers on the need to use the pesticide for the purpose it is intended.

Table 6: Tabulated responses from the stockists in Laikipia and Isiolo Districts.

	Isiolo	*Laikipia*	*Total percentage*
Number of respondents	5	7	12
Percentage respondents	41.7	58.3	100
Products stocked			
Seeds	8.4	0	8.4
Pesticides	0	0	
Other agrochemicals	0	0	
All the above	33.3	58.3	91.6
Pesticides stocked	0	0	
Organophosphates	0	0	
Carbamates	0	0	
Pyrethroids	0	0	
All the above	41.7	58.3	100
One that sells faster.			
Organophosphates	16.7	16.7	33.4
Carbamates	25.0	25.0	50
Pyrethroids	8.3	8.3	16.6
Most effective pesticides			
Organophosphates	8.3	16.7	25
Carbamates	8.4	8.3	16.7
Pyrethroids	25.0	33.3	58.3
Are you aware of any restricted pesticide			
Yes			
No	41.7	58.3	100
Estimated tin of 200 g sold in a day.			
0-5	8.3	16.7	25
6-10	23.0	33.3	56.3
Above 10	8.4	8.3	16.7
Are you aware of any other use of carbofuran			
Yes			
No	41.7	58.3	100
Are you aware of wildlife poisoning using carbofuran?			
Yes	8.3	0	8.3
No	33.4	58.3	91.7
Alternatives of carbofuran			
Yes	41.7	58.3	100
No			
How effective are the alternatives?.			
Very effective	33.3	41.7	75
Moderately effective	8.3	16.7	25
Less effective	0	0	

According to stockists respondents in Isiolo carbofuran was the preferred choice of pesticide amongst the farmers and the same report was received in Nanyuki, Nyahururu and Kinamba in Laikipia District. Although there are other alternatives in the market like methomyl 90% w/w, mocap GR10 supplied by Bayer crop science, Nemur 400EG and other bio-pesticides like nimbecidine and bio-nematon (*Paecilomyces lilacinus* 1.15% WP), farmers still prefer use of carbofuran to control pests. In this study, the survey report showed that stockists in both districts stock carbofuran in varied sizes ranging from 100 g pack to 200 g pack as captured in Plate 8 in Appendix II. According to the stockists this pesticide sells fairly fast during planting seasons and it is basically used as per the instruction on the labels which is written in both English and Kiswahili. Stockists seemed not aware of its use as a poison although some admitted that it can work as a poison given that it can kill living organism. None wanted to confirm that it can be used as a poison for the stray wildlife. This was perhaps because no one wanted to be associated with poisoning of wildlife. According information posted on the website of the Pest Control Product Board (PCPB) (PCPB, 2009) which is a statutory organization of the Kenya Government established with a broad mandate of regulating the trade and use of pesticide, carbofuran combination below 10% is restricted. This means that restricted pesticides are extremely toxic and should only be handled by trained, experienced and well equipped Pest Control Operators who are licensed by the board to undertake this function. The PCPB had strict advise to the general public in the advertisement in the Daily Nation newspaper dated March 12th, 2008 that any Grain stockist, transporters and farmers who wants to use the restricted pesticide must engage the services of the licensed pest control operators. Unfortunately this was not the case in the field and anybody has an access to the pesticide freely and over the counter without any prescription and direction. Tables 4, 5 and 6 give a summary of the responses to pertinent questions that were addressed in the questionnaire in Appendix III which sought to know the level sells and distribution of carbofuran by the stockists.

4.2 Results and discussion from the analytical work

4.2.1 Levels of carbofuran and its degradable products in the vultures' tissues and Lion carcass samples

Results from the experimental work recorded in Table 7 shows the presence of carbofuran residues of significantly low mean concentration (0.025 ppm) at $p < 0.05$ in Mbirikani group Ranch compared with Isiolo and Laikipia. Beak samples from Isiolo recorded high mean concentration of carbofuran (0.060 ppm) that showed significant difference ($p < 0.05$) with the mean concentration of the beak samples (0.04 pm). Beak samples from Laikipia had non detectable levels of carbofuran residues. This could mean that there were no carbofuran residues retained in the beak during the feeding process. The results also indicated the presence of 3-ketocarbofuran (0.067 ppm) and 3-hydroxycarbofuran (0.146 ppm) in the beak samples from Isiolo. Beak samples from Mbirikani group ranch had significantly high mean concentration ($p < 0.05$) of 3-Ketocarbofuran (0.487 ppm) compared to the beak samples from Isiolo. The levels of carbofuran detected in beaks samples were low as recorded in Table 3 but this simply indicate that the vultures were exposed to carbofuran pesticide just before they died. The presence of carbofuran residues in the beak could be due vultures' using their beaks repeatedly to break and tear the flesh of a poisoned carcass. During this time, small amount of carbofuran residues are retained on it, a fact that was confirmed by the presence of low concentration of carbofuran and its two metabolites in the beaks. According to Table 6, mean concentration of 0.025 ppm of carbofuran, 0.240 ppm of 3-ketocarbofuran and 0.073 ppm of 3-hydroxycarbofuran were found on the feet samples in Isiolo, Mbirikani and Laikipia sites. Feet samples from Isiolo had significantly high mean concentration of carbofuran (0.05 ppm) at $p < 0.05$ with no detectable levels in feet samples from both Laikipia and Mbirikani.

Table 7: Mean concentration (ppm) of carbofuran residues in the vultures' beaks, feet, crop content and Lion carcass muscles from different sites (means ± s.d)

Site	**carbofuran**	**3-ketocarbofuran**	**3-hydroxycarbofuran**
Beak samples			
Isiolo	0.060 ± 0.010	0.067 ± 0.002	0.146 ± 0.001
Laikipia	nd	nd	0.014 ± 0.001
Kilimanjaro	0.020 ± 0.005	0.487 ± 0.012	0.016 ± 0.003
Mean conc	0.04	0.185	0.059
CV%	19.24	1.04	2.62
LSD (P<0.05)	0.001	0.002	0.002
Feet samples			
Isiolo	0.050 ± 010	0.180 ± 0.010	0.018 ± 0.001
Laikipia	nd	0.030 ± 0.006	0.040 ± 0.010
Kilimanjaro	nd	0.090 ± 0.016	0.046 ± 0.001
Mean conc	0.025	0.240	0.073
CV%	19.24	1.04	2.62
LSD (P<0.05)	0.001	0.002	0.002
Crop content samples			
Naivasha	nd	0.199 ± 0.020	0.087 ± 0.006
Mean conc	nd	0.199	0.087
CV%	19.24	1.04	2.62
LSD (P<0.05)	0.001	0.002	0.002
Muscle samples			
Tsavo	nd	nd	nd
Athi River	nd	0.080 ± 0.002	0.096 ± 0.005
Mean conc	nd	0.040	0.048
CV%	19.24	1.04	2.62
LSD (P<0.05)	0.001	0.002	0.002
Blood samples	nd	nd	nd
Mean conc.	nc	nc	nc
Gut samples			
Athi River	0.221 ± 0.11	0.423 ± 0.173	0.574 ± 0.231
Mean Conc.	0.221	0.423	0.574

Note: The beaks and feet samples of vultures (*Gyps africanus*) were collected in October 2007 from Isiolo and Laikipia Districts. In November 2007 blood ,birds' crop and gut samples from vulture carcass were collected from Simon Thomsett a conservationist based in Athi River (Naivasha October 2005 poisoning). In August 2008 more feet and beak samples were collected from Seamus based in Mbirikani Ranch for analysis. The s.d denotes standard deviation n = 4, CV denotes coefficient of variation and LSD denotes Least significant difference, ND denotes not detected and NC denotes not calculated. Detection limit = 0.01ppm.

It is important to note that the residue levels from the feet and beaks do not necessarily imply a lethal dermal or oral exposure but are evidence that point to the insecticide to which the bird was exposed and report the minimum insecticide concentration that was initially on the foot (Stroud and Adrian, 1996). The presence of the pesticide residues on the feet can further be supported by the fact that the reduced epidermal layers in the stratum corneum of the skin and lack of an efficient barrier layer composed of hyalinized keratin appear to be the ways in which avian skin contributes to increased sensitivity of birds to dermal exposure to insecticides (Stettenheim, 1972). According to Martin and Forsyth (1998), despite the tougher, scalier skin of bird feet relative to that of most of the feathered parts of their bodies, feet are no means impermeable to the uptake of insecticides. Feeding nature of vultures and any other scavenging birds is such that they step on the prey as long as they eat it. This might take several minutes during which the pesticide is absorbed through the skin. This explains the presence of carbofuran and its two metabolites on the birds' feet though in low concentrations.

Muscle samples from carcasses collected from Athi–River and Tsavo National park were also analysed because several vultures had been found lying next to carcass and results reported no presence of carbofuran but 3-ketocarbofuran and 3-hydroxycarbofuran were detected at an average concentration of 0.040 ppm and 0.048 ppm as observed in Table 7 based on dry weight respectively. There were no detectable levels of carbofuran residues in the muscle samples from Tsavo National park and Athi-River ranch. The presence of metabolites could be attributed to presence of carbofuran on the dead carcasses as poison bait to kill other scavenging animals. Although samples from outside the study area were analysed, the results were meant to provide evidence of the on-going poisoning of wildlife by use of carbofuran pesticide. Other cases of wildlife poisoning where use of carbofuran was merely suspected are captured in the Appendix I.

The blood samples poisoned vultures had no detectable levels of carbofuran and its two metabolites although there was significantly high mean concentration of carbofuran residues (0.221 ppm), 3-ketocarbofuran (0.423 ppm) and 3-hydroxycarbofuran (0.574 ppm) at $p < 0.05$ in as seen Table 7 the gut tissues compared to other tissue samples. This could be due to the fact that poisons concentrate and enter into the system through the upper part of the gastrointestinal tract. Analysis of this part of the vulture could explain why there was a high mean concentration of the carbofuran residues and its two metabolites here.

Vultures crop content collected from dead African white-backed vultures (*Gyps africanus*) in Naivasha on October, 28th 2005 which had been kept by a renowned conservationists were also analysed. The results showed no level of carbofuran but high concentration of 3-ketocarbofuran (0.119 ppm) and of 3-hydroxycarbofuran (0.087 ppm). The presence of the metabolites in the crop content demonstrates that the bird was exposed to this pesticide just before death. Given its history of toxicity to birds and confession from some farmers about its use as a poison, it is possible that it could have been responsible for the death of these vultures. It is important to note that the degree of toxicity will depend on the amount and the type of tissue ingested by the vultures. The lethal dose for a vulture would generally be much lower than the amount ingested by the dead animal (Brown, 1997). As result vultures would easily die after feeding on carcasses with even small amount of carbofuran. The ability of vultures to move quickly immediately after the death of an animal (before degradation takes place) coupled with their preference for viscera makes them susceptible to secondary poisoning. Vultures that ingest poisoned tissues are acutely intoxicated, become comatose and are generally discovered lying dead beside the poisoned carcass (Brown, 1997). Those that can fly do not go far away but are found staggering around the field adjacent to the carcass. This is quite typical of carbofuran poisoning since it is acutely toxic (Brown, 1997). However, carbofuran poisoning is completely reversible by use of atropine sulphate but this should be done soon after exposure in order to save the lives of the affected animals (Amdur *et al*, 1991).

It was not easy to track the carcasses of all the birds and wildlife that had just died from poisoning due to challenges that included expanse land around the conservancies, the hostility and suspicion of the pastoral community and fear of being attacked by the bandits and cattle rustlers. However, the conservationists played a big role in helping to gather some of the samples for the study even though most of the samples had undergone decomposition. This challenge was also reported by Mineau and Collins (1988) in a related study where they regretted the difficulty in arriving at a scientifically defensible estimate of the actual number of poisoned birds because of the high rate of scavenging.

Due to spontaneity of the deaths and inability to accurately keep track of the poisoning times, some samples were collected from outside the study area. This move also helped in understanding how widespread the issue of wildlife poisoning is in the country. Despite the challenges in the detection of the degradation products in the vultures and animal tissue this study provides remarkable evidence to the misuse of carbofuran as a poison. The presence of metabolites is due to the degradation of carbofuran which is a neutral ester of carbamic acids as observed in the degradation pathway in Figure 2. It is susceptible to hydrolysis and can also undergo oxidation to give degradation products as explained in section 2.4. Increase in temperature in the tropical region coupled with increase in pH facilitates their breakdown into non-toxic 3-hydrocarbofuran and 3-ketocarbofuran (Amdur *et al.,* 1991; Murkehejee *et al.*, 2006). This explains why concentrations of the metabolites in the animal tissue collected and all other environmental sample extract investigated here are higher than the parent compound (Carbofuran).

From the survey results recorded in Table 4, 55.6% of the respondents said that the deaths of wildlife are independent of the seasons and therefore it is not easy to relate the application of carbofuran in the farms to the deaths. This could confirm poisoning through laced carcass as the possible cause of the deaths.

Vultures are threatened world over. Related studies in Mediterranean countries have reported that the use of poisoned baits to control predators is a frequent practise that affects several other raptor species such as Beaded vultures (*Gypaesus barbarus*) in France, Spanish Imperial Eagle (*Aquia adalberts*) and cinereous vultures (*Aegypius Monochus*) in Spain (Slotta-Bachmayr, 2004). In another study in India, cases of poisoning by use of diclofenac were reported to have caused rapid decrease in their population (Prakash, 2004). In UK, WIIS (2003) reported that insecticides are commonly detected in raptors with carbofuran, mevinphos and aldicarb as the most common compounds. But all these cases are currently under surveillance with mitigation measures put in place except in Kenya. The data gathered here will therefore form a strong background for the ecological risk assessment.

4.2.2 Water samples

Water samples were analysed to find the possible routes of exposure of the pesticide to vultures given that they are found at the helm of the food chain. Water samples from the the Upper-Ngare Sirgoi in Isiolo recorded significantly high ($p < 0.05$) mean level of carbofuran residues (0.038 ppm) than any other site in this region during the rainy season as shown in Table 8. This could be attributed to the application of carbofuran pesticide in the adjacent irrigated farms. The mean concentration reduced considerably down stream to 0.016 ppm during the rainy season. This could be attributed to ability of carbofuran to hydrolyse in water to form 3-hydoxycarbofuran and 3-ketocarbofuran and also its high solubility in water (700 mg/L at 20°C) (EXTOXNET, 2001a). There was also a general decrease in the concentration of carbofuran residues from the rainy season to the dry season. Similar concentration variation was observed in River Ngare-Ndare both upstream and down stream. The ponds A and B both within the conservancy recorded non-detectable levels of carbofuran perhaps because there was no direct drainage into the ponds from the farms. There was significant difference ($p < 0.05$) between the mean concentration of carbofuran in the two sampling season with the rainy season recording higher values. This could be attributed to sampling which was done a few weeks after application of the pesticide.

The sites in Laikipia recorded significantly higher ($p < 0.05$) mean levels of carbofuran compared to the sites in Isiolo. Ol Moran ponds recorded higher mean levels of carbofuran (1.823 ppm) in the rainy season than any other site. This could have been as a result of more agricultural activities around the pond than the other sites. The levels showed significant difference ($p < 0.05$) between the seasons with the rainy season recording in higher values. No carbofuran was detected in both upper and lower sections of the river. Perhaps all the carbofuran residues may have been hydrolysed and washed away. It is important to note that sampling sites were adjacent to the conservancies and were located within the catchments with intense agricultural activities likely to influence residue contamination in the aquatic environment. Some of the main agricultural activities around the area of study include using the river water to irrigate maize and horticultural farms in the upper parts of the two rivers in Isiolo District which also rely heavily on agrochemical application.

Table 8: Seasonal mean concentration of carbofuran residues (ppm) in water samples in different sites in the area under study (mean ± s.d)

	CARBOFURAN			
	Site	Season	Isiolo	Laikipia
	1	1	0.038 ± 0.005	0.992 ± 0.199
		2	0.016 ± 0.002	0.018 ± 0.03
	2	1	0.016 ± 0.003	1.034 ± 0.314
		2	0.010 ± 0.005	0.070 ± 0.010
	3	1	0.026 ± 0.010	0.747 ± 0.178
		2	0.050 ± 0.010	0.023 ± 0.010
	4	1	0.015 ± 0.002	0.546 ± 0.038
		2	0.060 ± 0.02	0.012 ± 0.001
	5	1	nd	1.232 ± 0.300
		2	nd	0.430 ±0.012
	6	1	nd	1.823 ± 0.478
		2	nd	0.237 ± 0.111
	7	1	nd	nd
		2	nd	nd
	8	1	nd	nd
		2	nd	nd
Mean conc.			**0.011**	**0.592**
CV %				**4.93**
LSD ($p < 0.05$)		**0.045**		**0.045**
Interaction ABC			**0.031**	

Nd–Non detectable, nc–not calculated CV–coefficient of variation Detection limit = 0.01ppm
n=3
Sites in Isiolo: 1-Upper-Sirgoi, 2-Lower Sirgoi, 3-Upper Ngare-Ndare, 4-Lower Ngare Ndare, 5-Pond A within the conservancy, 6- Pond B within the conservancy.
Sites in Laikipia: 1-Kinamba ponds, 2-Pond B within the conservancy 3-Pond A within the conservancy, 4-Donyo ponds 5-Mutarakwa, 6-Ol- Moran ponds. 7-Upstream of Makutan River, 8-Downstream part of Makutan River

From the survey, carbofuran is used mostly in both districts to control pests and viruses which include maize streak virus, stem borers, aphids, leaf worms, cabbage aphids, leaf hoppers and through run-off the pesticide residues are probably washed back to the river. For this reason it would easily be dissolved into water system as opposed to dry season when there is little surface water run-off. There was significant interaction at $p < 0.05$ between the sites seasons and regions.

Table 9: Seasonal variation in mean concentration of 3-ketocarbofuran residues (ppm) in water samples in different sites in the area under study (mean ± s.d)

3-HYDROXYCARBOFURAN				
	Site	Season	Isiolo	Laikipia
	1	1	0.183 ± 0.031	0.752 ± 0.113
		2	0.194 ± 0.024	1.022 ± 0.192
	2	1	0.114 ± 0.031	0.403 ± 0.101
		2	0.186 ± 0.014	0.487 ± 0.067
	3	1	0.172 ± 0.033	0.576 ± 0.042
		2	0.282 ± 0.046	0.377 ± 0.180
	4	1	0.218 ± 0.026	0.334± 0.047
		2	0.068 ± 0.022	0.220 ± 0.070
	5	1	nd	nd
		2	nd	1.482 ± 0.58
	6	1	nd	0.355 ± 0.101
		2	nd	1.546 ± 0.421
	7	1	nd	nd
		2	nd	nd
	8	1	nd	nd
		2	nd	nd
Mean conc.			0.118	0.646
CV %			2.14	
LSD ($p < 0.05$)		**0.025**		**0.025**
Interaction ABC			**0.017**	

Nd- not detectable, ns- not sampled, CV–coefficient of variation Detection limit = 0.01 ppm n=3

Sites in Isiolo: 1-Upper-sirgoi, 2- Lower Sirgoi, 3-Upper Ngare-Ndare, 4-Lower Ngare Ndare, 5-Pond A within the conservancy, 6-Pond B within the conservancy

Sites in Laikipia: 1-Kinamba ponds, 2-Pond B within the conservancy 3-Pond A within the conservancy, 4-Donyo ponds 5-Mutarakwa, 6-Ol-Moran ponds. 7 Upstream of Makutan River, 8-Downstream part of Makutan River

Carbofuran is hydrolysed in water to form 3-hydroxycabofuran and 3–ketocarbofuran as the major metabolites. Tables 9 and 10 gives the levels of both degradation products found in water samples in different sites in both regions of study. There was higher mean concentration of 3-ketocarbofuran and 3-hydroxycarbofuran in all the sites compared to the carbofuran levels. Pond A and Pond B in Lewa wildlife conservancy had no detectable levels of the metabolites. This could be due to low usage of carbofuran in this region and no direct drainage to the ponds. Generally the sites in Laikipia recorded significantly higher ($p < 0.05$) levels of the metabolites compared to Isiolo. This could be due the higher concentration of carbofuran registered earlier which probably broke down these compounds.

Table 10: Seasonal variation in mean concentration of 3-ketocarbofuran residues (ppm) in water samples in different sites in the area under study (mean ± s.d)

	Site	Season	Isiolo	Laikipia
	3-KETOCARBOFURAN			
	Site	Season	Isiolo	Laikipia
	1	1	0.086 ± 0.011	0.274 ± 0.039
		2	0.423 ± 0.072	0.764 ± 0.171
	2	1	0.013 ± 0.001	0.359 ± 0.049
		2	0.088 ± 0.021	0.742 ± 0.028
	3	1	0.052 ± 0.014	0.236 ± 0.018
		2	0.077 ± 0.020	0.734 ± 0.023
	4	1	0.025 ± 0.003	0.148 ± 0.022
		2	0.040 ± 0.010	0.427 ± 0.173
	5	1	nd	0.406 ± 0.117
		2	nd	0.800 ± 0.256
	6	1	0.013 ± 0.001	0.516 ± 0.111
		2	nd	0.894 ± 0.321
	7	1	nd	nd
		2	nd	nd
	8	1	ns	nd
		2	ns	nd
Mean conc.			0.068	0.525
CV %				**2.01**
LSD (p < 0.05)		**0.018**		**0.018**
Interaction ABC			**0.012**	

Nd- not detectable, ns- not sampled - CV–coefficient of variation detection limit = 0.01 ppm n = 3

Sites in Isiolo: 1-Upper-sirgoi, 2-Lower Sirgoi, 3-Upper Ngare-Ndare, 4-Lower Ngare Ndare, 5-Pond A within the conservancy, 6-Pond B within the conservancy

Sites in Laikipia: 1-Kinamba ponds, 2-Pond B within the conservancy 3-Pond A within the conservancy, 4-Donyo ponds 5-Mutarakwa, 6- Ol-Moran ponds. 7-Upstream of Makutan River, 8-Downstream part of Makutan River

Furthermore there was significant difference in mean region concentration of carbofuran and its detected metabolites at $p < 0.05$ between Laikipia and Isiolo water samples.

Laikipia water samples recorded significantly high mean region carbofuran concentration (0.592 ppm) at $p < 0.05$ compared to Isiolo water samples (0.011 ppm) as indicated in Table 11 This could be explained from survey data obtained from the field which indicated that over 70% of large-scale farmers who use carbofuran are in Laikipia districts.

Table 11: Mean concentration (ppm) of carbofuran residues in water samples from the Isiolo and Laikipia in both wet and dry seasons (means ± s.d)

Regions	Season I	Season II	Regional mean Conc
Carbofuran			
Isiolo	0.016 ± 0.002	0.060 ± 0.011	0.011
Laikipia	1.062 ± 0.420	0.121 ± 0.017	0.592
Means seasons	0.539	0.064	
CV%	4.93		
LSD ($p < 0.05$)	0.045		
Interactions	0.063		
3-ketocarbofuran			
Isiolo	0.032 ± 0.018	0.105 ± 0.039	0.068
Laikipia	0.323 ± 0.18	0.727 ± 0.10	0.525
Means seasons	0.177	0.416	
CV%	2.01		
LSD ($p < 0.05$)	0.018		
Interactions	0.025		
3-hydroxycarbofuran			
Isiolo	0.114 ± 0.010	0.122 ± 0.024	0.118
Laikipia	0.437 ± 0.18	0.856 ± 0.090	0.646
Means seasons	0.276	0.489	
CV%	2.14		
LSD ($p < 0.05$)	0.025		
Interactions	0.034		

Note that the first sampling (season I) was done during the rainy season of (October, 15^{th} -26^{th} 2007) and the second sampling (season II) was done during the dry season (June, 9^{th}-20^{th} 2008). S.d denotes standard deviation ($n = 24$),

This higher concentration of carbofuran and its metabolites in water samples from Laikipia could therefore be as a result of its higher rate of application by farmers during the planting season. On this basis there was a significant interaction at $p < 0.05$ between the season and the mean region concentrations.

The result is consistent with the findings of a study which reported that carbofuran is highly soluble and hydrolyse easily in water (Lalah *et al.*, 2001).There was also significant mean seasonal variation at $p < 0.05$ in carbofuran concentration where the wet season registered higher mean concentration than the dry season.

Average concentrations of 0.032 ppm and 0.114 ppm for 3-ketocarbofuran and 3-hydroxycarbofuran respectively were found in the wet season for Isiolo region. These mean concentrations increased significantly at $p < 0.05$ to 0.105 ppm and 0.122 ppm for 3-ketocarbofuran and 3-hydroxycarbofuran respectively, in the dry season. In Laikipia the average concentration of 3-ketocarbofuran and 3-hydroxycarbofuran in water samples collected from the ponds were found to be 0.323 ppm and 0.437 ppm respectively, in the wet season with an increase in the dry season. Generally the results showed significant variation at $p < 0.05$ in the concentration of metabolites from the wet to dry season. This higher concentration of metabolites supports the study carried outLalah and Wandiga's (1996a) which reported that carbofuran is highly soluble in water, non-persistent and degrades rapidly under flooded conditions. The metabolites once formed tend to persist in the environment longer than the parent compound. This explains why there was higher mean concentration of metabolites in dry season than wet season. The results show that there was significant difference ($p < 0.05$) in mean concentration of carbofuran and it's metabolites in water samples in the two sampling seasons in both regions under study.

In water most of the residues are found in the top 10 cm layer presenting great danger to fish and other water organisms (Lalah and Wandiga, 1996a). The results concurs with the study results by El-Kabana *et al.* (2000) which reported that degradation of carbofuran occurs fairly fast because of its high solubility which can account for the low concentrations detected in high intensity agricultural areas where concentration levels of the residue would be expected to be high. The mean concentration of carbofuran in water samples (0.011 ppm) in Isiolo fell below the US allowable contaminant level which is 0.04 ppm an indication that it can not present any serious risk to the `humans and wildlife in this matrix. This could therefore not be a potential route of exposure to the vultures and other wildlife species. However, the average levels of carbofuran in water samples in Laikipia was 0.592 ppm far above US allowable contaminant level (0.04 ppm) (EPA, 2009). This could possibly present a lot of risk to humans and wildlife exposed to this water though this is dependent on the spatial distribution

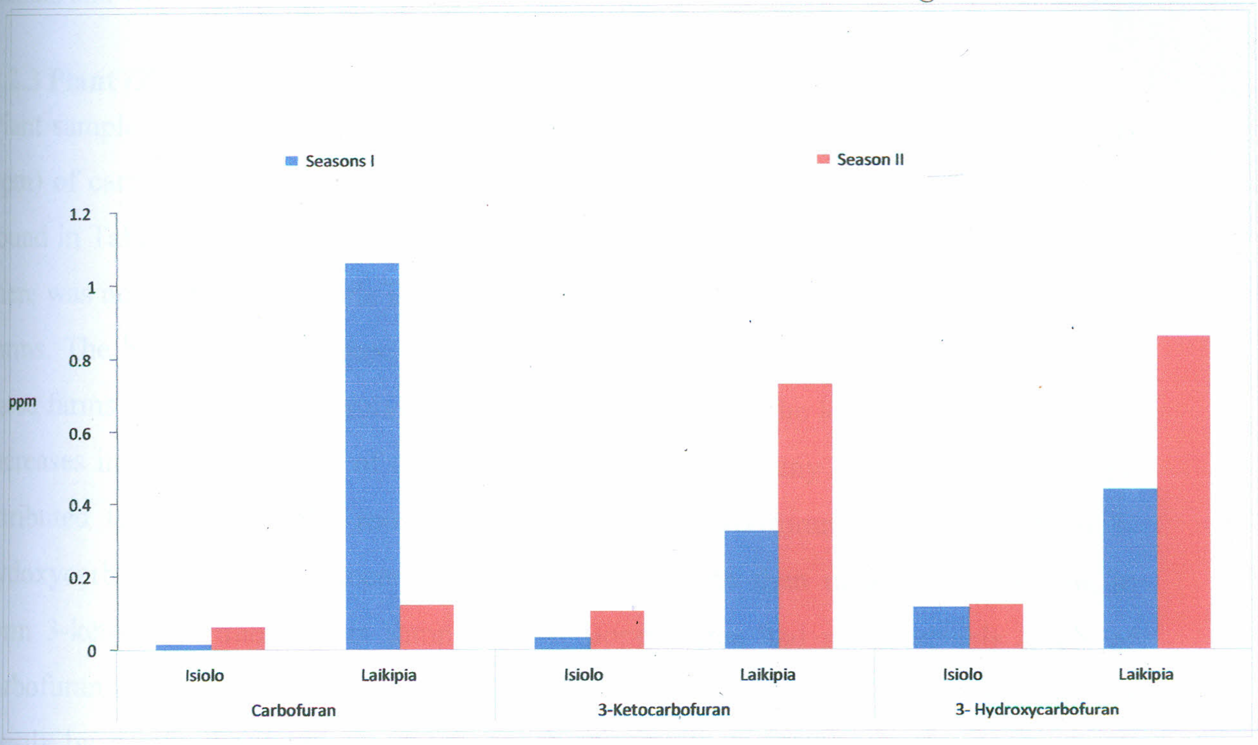

Figure 7. Regional and seasonal variations in concentration levels of carbofuran and its metabolites in water samples

Figure 7 clearly gives the seasonal variation in concentration levels of carbofuran and its metabolites in the area of study. As may be observed the mean carbofuran concentration is higher in Laikipia (1.062 ppm) in the wet season compared to Isiolo (0.016 ppm). This could be attributed to the higher usage of carbofuran in Laikipia than it is in Isiolo which mostly experiences short rains and is also less fertile. High solubility of carbofuran in water especially when it rains after application further supports these results. In the dry season the level of carbofuran went down in both regions as the mean concentration of 3-ketocarbofuran and 3-hydrocarbofuran went up from between 0.177-0.276 ppm to between 0.416 ppm-0.489 ppm, respectively.

The results of this study is supported by the report of study done by Nicosia *et al*. (1991) who also detected low concentration of carbofuran in the Sacramento River. The levels were reported to be below Maximum contaminant levels. The results of this study were further supported by the results reported by Kawamoto and Makihata (2003) who detected fairly low values (0.002 ppm-0.250 ppm) from River Hyogo Prefecture water which was adjacent to a paddy field. The levels of carbofuran in Laikipia was significantly higher than the recommended levels by USEPA and may present risks to human and wildlife

4.2.3 Plant *(Zea mays)* samples.

Plant samples from Ngare Ndare farms showed significantly higher mean levels (1.031 ppm) of carbofuran at $p < 0.05$ than any other site in Isiolo during the rainy season as found in Table 12. However, the levels decreased during the dry season to an extent that there was non-detectable levels in some sites like Manyangalo Loperua and Ngare-Ndare farms. The high levels in Ngare Ndare could be due to intense agricultural activities in these farms as supported by survey data. These decreases in the levels of carbofuran and increases in the mean levels of degradation products from rainy to dry season could be attributed to uptake by the roots and hydrolysis of carbofuran in plants to form 3-hydoxycarbofuran and 3-ketocarbofuran. This metabolite then undergoes oxidation to form 3-ketocarbofuran. Plants samples from Borana area had no detectable levels of carbofuran and its two metabolites (see Tables 12 and 13) because it is an area inhabited mostly by pastoralist. Little agricultural activities that require application of pesticides take place here.

Plants samples from Ol Moran farms had significantly high mean levels ($p < 0.05$) of carbofuran (1.0112 ppm) from the leaves in the rainy season. Karia-ni farms had low levels of carbofuran (0.022 ppm) in the rainy season. There was a general decrease in the mean levels of carbofuran in all the sampled sites in the two areas of study.

Table 12: Seasonal variation in mean concentration of carbofuran residues (ppm dry weight) in plant samples in different sites in the area under study (*Zea mays* leaves)

	CARBOFURAN			
	Site	Season	Isiolo	Laikipia
	1	1	0.050 ± 0.001	0.451 ± 0.091
		2	nd	0.060 ± 0.010
	2	1	0.015 ± 0.002	0.032 ± 0.011
		2	0.070 ± 0.040	0.021 ± 0.001
	3	1	0.091 ± 0.032	0.022 ± 0.002
		2	nd	0.070 ± 0.02
	4	1	0.035 ± 0.011	0.028 ± 0.005
		2	nd	0.012 ± 0.002
	5	1	1.031 ± 0.117	0.857 ± 0.369
		2	nd	0.446 ± 0.141
	6	1	nd	1.112 ± 0.327
		2	nd	0.237 ± 0.101
Mean conc.			**0.099**	**0.269**
CV %			**13.44**	
LSD ($p < 0.05$)		**0.074**	**0.074**	
Interaction ABC			**0.052**	

Nd- not detectable CV–coefficient of variation, LSD-Least significant difference Detection Limit = 0.01 ppm

Sites in Isiolo: 1-Manyangalo farms, 2-Ngare Ndare forest, 3-Loperua farms, 4-Meru central farms, 5-Ngare Ndare farms, 6- Borana area

Sites in Laikipia: 1-Kinamba farms, 2-Makutano farms, 3- Karia-ini farms, 4-Donyo farms 5. Mutarakwa farms 6.-Ol Moran farms.

3-hydroxycarbofuran was detected in all the sties in Isiolo and Laikipia. In the wet season, Ngare Ndare farms registered higher concentration values (0.508) than all the other sites. The high values could have been due to high concentration of carbofuran in the wet season, an observation that would be attributed to hydrolysis and oxidation of the parent compound. The decrease in mean concentration could be due to further oxidation of 3-hydroxycarbofuran to 3-ketocarbofuran.

Table 13: Seasonal variation in mean concentration of carbofuran residues (ppm dry weight) in plant samples in different sites in the area under study (*Zea mays* leaves)

	3-HYDROXYCARBOFURAN			
	Site	Season	Isiolo	Laikipia
	1	1	0.016 ± 0.124	0.066 ± 0.014
		2	0.980 ± 0.04	0.274 ± 0.123
	2	1	0.045 ± 0.032	0.134 ± 0.081
		2	0.146 ± 0.117	0.712 ± 0.312
	3	1	0.150 ± 0.011	0.112 ± 0.014
		2	0.028 ± 0.010	0.674 ± 0.243
	4	1	0.095 ± 0.002	0.441 ± 0.220
		2	0.385 ± 0.191	0.662 ± 0.291
	5	1	0.508 ± 0.231	0.257 ± 0.116
		2	nd	0.700 ± 0271
	6	1	nd	0.416 ± 0211
		2	nd	1.546 ± 0379
Mean conc.			**0.196**	**0.499**
CV %				**9.8**
LSD (p < 0.05)		**0.04**		**0.011**
Interaction ABC			**0.07**	

Nd- not detectable CV –coefficient of variation, LSD- Least significant difference, detection limit = 0.01 ppm

Sites in Isiolo: 1-Manyangalo farms, 2-Ngarendare forest, 3-Loperua farms, 4-Meru Central farms, 5-Ngare Ndare farms, 6-Borana area

Sites in Laikipia: 1-Kinamba farms, 2-Makutano farms, 3-Karia-ini farms, 4-Donyo farms 5.-Mutarakwa farms 6.Ol Moran farms.

3-Ketocabofuran was similarly detected in the plant samples from all the sites in Isiolo and Laikipia except in Borana area as shown in Table 14. There was a general decrease in the concentration from rainy season to dry season especially in Laikipia District. This could have been due to the hydrolysis of 3-ketocarbofuran to much less toxic 3-ketocarbofuran-7-phenol, a terminal residue which is not likely to be detected in plants above trace levels (Eisler, 1985).

Table 14: Seasonal variation in mean concentration of 3-ketocarbofuran (ppm dry weight) in plant samples in different sites in the area under study (*Zea mays* leaves)

	3-KETOCARBOFURAN			
	Site	Season	Isiolo	Laikipia
	1	1	0.018 ± 0.006	0.025 ± 0.006
		2	0.363 ± 0.102	0.521 ± 0.125
	2	1	0.126 ± 0.014	0.051 ± 0.011
		2	0.303 ± 0.045	0.615 ±0.180
	3	1	0.092 ± 0.011	0.074 ± 0.016
		2	0.132 ± 0.017	1.367 ± 0.224
	4	1	0.077 ± 0.020	0.033 ± 0.012
		2	0.262 ± 0.019	0.826 ± 0.231
	5	1	0.362 ± 0.038	0.648 ± 0.126
		2	nd	2.364 ± 0.435
	6	1	nd	0.280 ± 0.039
		2	nd	0.894 ± 0.168
Mean conc.			**0.145**	**0.641**
CV %				**10.7**
LSD ($p < 0.05$)		**0.04**		**0.013**
Interaction ABC			**0.09**	

Nd-not detectable CV–coefficient of variation, LSD-Least significant difference detection limit =0.01 ppm
Sites in Isiolo: 1-Manyangalo farms, 2-Ngarendare forest, 3-Loperua farms, 4-Meru Central farms, Ngare Ndare farms, 6-Borana area
Sites in Laikipia: 1-Kinamba farms, 2-Makutano farms, 3-Karia-ini farms, 4-Donyo farms 5-Mutarakwa farms 6-Ol Moran farms.

Generally, the presence of carbofuran in the plants could be attributed to carbofuran's systemic. This means that the plant absorbs it through the roots, and from here the plant distributes it throughout its organs mainly the vessels, stems and leaves where insecticidal concentration are attained (Eisler, 1985; Lalah *et al*., 2003; Lea and Mladen, 1999). In this regard it's expected that it could find its way into the vultures' food chain through scavenging of herbivores a fact that could be ruled out from the low mean concentration of carbofuran (0.099–0.269 ppm) and its metabolites in the plant samples from Isiolo and Laikipia as observed from Table 15.

According results presented in Table 15, plant samples showed significant seasonal variation in concentration of carbofuran at $p < 0.05$ in the wet season (0.539 ppm) and the dry season (0.064 ppm) in both Isiolo and Laikipia. This variation could be attributed to intense use of the pesticide during planting stages. Laikipia (0.592 ppm) registered significantly high regional mean carbofuran concentration at $p < 0.05$ compared to Isiolo (0.011 ppm). From the survey results, Laikipia region has a higher acreage under farming compared to Isiolo and this could explain this difference in concentration. There was higher concentration of carbofuran in plants in the first sampling (October) than it was in June because most crops were still green in October as opposed to June. This could be attributed to systemic characteristic carbofuran which means that after root uptake the pesticide moves to the leaves where it has high insecticidal concentration (Caro *et al.*, 1976). It is important to note that carbofuran could be diluted and metabolised by the plants in the long term by plants growth and this explains the significantly low mean concentration at $p < 0.05$ of carbofuran in the plant leaves. From the variation in the concentration of carbofuran and its two degradation products it is clear that there was significant interaction at $p < 0.05$ between the sites, seasons and regions with the concentrations varying within the sites and season in different regions.

Table 15: Mean concentration (ppm dry weight) of carbofuran residues in plant samples in Isiolo and Laikipia during both wet and dry seasons (*zea mays* leaves) (mean ± s.d)

Regions	Season I	Season II	Regional mean conc.
Carbofuran			
Isiolo	0.197 ± 0.010	0.010 ± 0.005	0.099
Laikipia	0.417 ± 0.121	0.122 ± 0.017	0.269
Means seasons	0.307	0.061	
CV%	13.444		
LSD (p<0.05)	0.074		
Interactions	0.104		
3-ketocarbofuran			
Isiolo	0.112 ± 0.012	0.177 ± 0.110	0.145
Laikipia	0.185 ± 0.040	1.098 ± 0.230	0.641
Means seasons	0.149	0.637	
CV%	1.07		
LSD (p<0.05)	0.013		
Interactions	0.018		
3-hydroxycarbofuran			
Isiolo	0.136 ± 0.100	0.257 ± 0.130	0.196
Laikipia	0.237 ± 0.151	0.761 ± 0.169	0.499
Means seasons	0.186	0.509	
CV%	0.98		
LSD (p<0.05)	0.011		
Interactions	0.014		

Note that the first sampling (season I) was done during the wet season of (October, 15th - 26th 2007) and the second sampling (season II) was done during the dry season (June 9th -20th, 2008). (n=18),

The significant mean seasonal variation ($p < 0.05$) in concentration of carbofuran and the degradation products in plants has been supported in previous study by Raminderjit *et al.* (2000). In this study 3-hydroxycarbofuran in sugarcane plant remained higher and persisted longer than that of the parent compound a result that is also reported in this study.

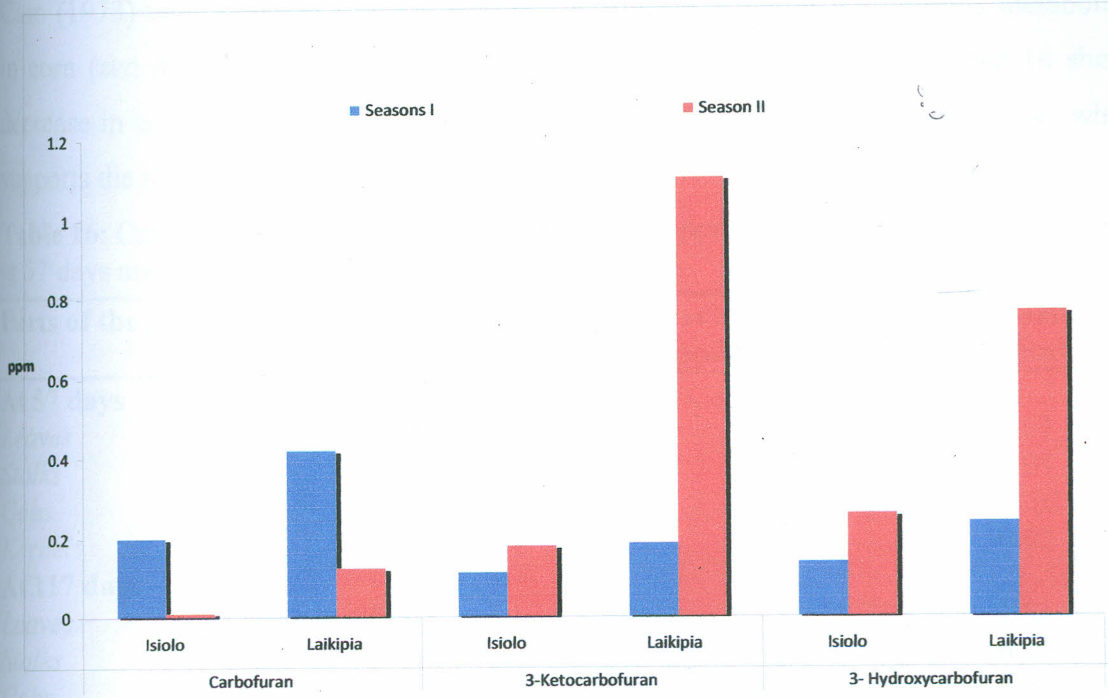

Figure 8: Seasonal variation in concentration levels of carbofuran residues in plants (*Zea mays* leaves)

It is clear from the chart (Figure 8) that the mean concentration of carbofuran is higher in the wet season compared to the dry season. This could be attributed to its ability to be absorbed by the plant roots so that within 7-10 days after application the pesticide is found in the plant leaves. This is a characteristic of all systemic pesticide, carbofuran being one of them.

Infact in the dry, season the concentration of carbofuran in Isiolo is far below the average region concentration compared to the concentration of the same in Laikipia. This may have been as a result of higher level of usage of the pesticide in Laikipia than it is in Isiolo. There was significant interaction at $p < 0.05$ between the seasons and the regions.

Since carbofuran hydrolyses easily in tropical environment the concentration of metabolites seemed to have increased in the dry seasons compared to the wet season. Previous studies by Crocker (2005) reports that the residues often decline due to breakdown, however, this happens rapidly to begin with but the rate of loss slows downs so that many residues ultimately persist for long. This study supports the results presented in this study where the concentration of the metabolites was found to be higher than the parent compound. The results compare well with the results of the study by Turner and Caro (1973) which sought to establish the distribution of carbofuran and its metabolites in corn (*zea mays*) in different times after application. The results in Table 16 shows decrease in concentration of carbofuran and its metabolites over a period of time, which supports the results of this study.

Table 16: Carbofuran and its degradation products in ppm dry weight in corn (*Zea mays*) at 57 days and at 117 days

Parts of the plants	Carbofuran (ppm)	3-ketocarbofuran (ppm)	3-hydroxycarbofuran (ppm)
At 57 days			
Leaves	0.43	0.40	4.67
Stalks	0.24	0.00	0.04
Cobs	0.04	<0.02	<0.02
Kernels	0.00	<0.01	0.02
At 117 days			
Leaves	0.21	0.34	1.51
Stalks	0.03	0.00	0.05
Cobs	0.06	0.00	0.00
Kernels	<0.01	<0.01	0.00
Plants in this study			
Maize leaves 2009	0.099-0.269	0.145-0.61	0.196-0.499

Source: Turner and Caro, 1973

The concentration of carbofuran and its metabolites seem to be more concentrated in the leaves than other parts of the plants as was also observed in the current study. This data is further supported by reports from related studies by Zan and Chantara (2007) and Terrakun and Reungsang (2005). The studies reported that fruits, vegetables and leaves from the fields where carbofuran pesticide was applied had higher concentration of carbofuran residues compared to their roots. This could be due to systemic action of carbofuran. Caro *et al.* (1976) also found out that carbofuran was readily absorbed by roots and transported via plant fluids to the areas of greatest transpiration like the leaves. These studies explain how use of carbofuran in the farms to control pesticide could present a potential risk to the non-target organism.

4.2.4 Soil samples

The results presented in Table 17 showed significant mean seasonal variation at $p < 0.05$ between wet (0.310 ppm) and dry season (0.014 ppm) with the wet season registering higher values for carbofuran concentration. Laikipia on the other hand recorded significantly high mean regional concentration for carbofuran (0.176 ppm) at $p < 0.05$ compared to Isiolo (0.146 ppm). This could be attributed to Laikipia being agriculturally productive and therefore uses high percentage of this pesticide in the farms. The higher concentration of carbofuran in wet season could also be attributed to the sampling that was done soon after application and possibly due to its ability to dissolves easily in water. It is possibly because of this that carbofuran residues are found in the soil matrix within the shortest time after application. The level of carbofuran reduced in the dry season and this was attributed to the action of microbial degraders and metabolism of the pesticide in the soil matrix. This could be due to the possible oxidation of 3-hydroxycarbofuran to 3-ketocarbofuran (Hassall, 1990).

The result of this study supports previous studies done on soils where the disappearance of pesticide from soil under the field conditions was found to show a typical pattern with an initial disappearance experienced immediately after application, followed by a second phase when the dissipation rate was much slower or almost constant and the pesticide residue tended to bind to the soil matrix (Lalah *et al*, 2001; McGarvey, 1993). Statistically there was significant interaction at $p < 0.05$ between the seasons and the regions.

The concentration of carbofuran is significantly lower ($p < 0.05$) than that of its metabolites since it's attacked by micro organisms at different sites. Chemical transformation processes of carbofuran in soil are influenced by soil characteristics such as pH, temperature, clay content, organic matter, moisture content, presence of micro-organism and the type of functional groups attached to the pesticide molecule (Lalah *et al.*, 2001). This could explain the significantly high concentration of metabolites than the parent compound in the soil samples in the dry season. In addition carbofuran degrades fairly fast in tropical soils (Lalah *et al.*, 2001) and that is possibly why there was higher concentration of metabolites in the range of 0.199 ppm–0.598 ppm than the parent compound which ranged between 0.014 ppm–0.310 ppm. The levels of carbofuran range

0.146 ppm–0.176 ppm found in soil in this study in the two regions are too low to present any risk to both human and wildlife and therefore can not be considered as the potential route of exposure to birds. However, one cannot rule out long term potential impact on soil and especially water ecosystems which do not have outlet drainage channels.

Table 17: Means concentration (ppm dry weight) of carbofuran residues in soil samples in Isiolo and Laikipia in dry and rainy season (mean ± s.d)

Compound	Season 1	Season II	Regional mean
Carbofuran			
Isiolo	0.276 ± 0.045	0.015 ± 0.005	0.146
Laikipia	0.344 ± 0.030	0.013 ± 0.010	0.176
Means seasons	0.310	0.014	
CV%		7.46	
LSD ($p < 0.05$)		0.036	
Interactions		0.052	
3-ketocarbofuran			
Isiolo	0.239 ± 0.120	0.729 ± 0.280	0.484
Laikipia	0.158 ± 0.062	0.467 ± 0.177	0.313
Means seasons	0.199	0.598	
CV%		12.13	
LSD ($p < 0.05$)		0.146	
Interactions		0.205	
3-hydroxycarbofuran	0.191 ± 0.073	0.676 ± 0.057	0.433
Isiolo	0.207 ± 0.074	1.181 ± 0.190	0.694
Laikipia	0.199	0.928	
Means seasons			
CV%		0.61	
LSD ($p < 0.05$)		0.011	
Means seasons		0.014	

Note that the first sampling (season I) was done during the rainy season of (October, 15th - 26th 2007) and the second sampling (season II) was done during the dry season (June, 9th -20th 2008). S.d denotes standard deviation (n = 18)

The first rapid phase of disappearance is greatly influenced by the rainfall especially if it comes immediately after application and this point further justifies the decrease in mean carbofuran concentration from 0.310 ppm in wet season to 0.014 ppm in soil sampled in dry season. Figure 9 represents a clear regional and seasonal variation in concentration levels for carbofuran for easy understanding. Previous studies done by Lalah *et. al.*

(2001) reports that carbofuran is adsorbed and rapidly metabolised in soil giving a large number of metabolites. This is enhanced through water which provides a reaction medium and is mostly common during rainy season. Results from the soil sample extracts showed that they contained carbofuran residues which steadily declined an observation that is supported by Raminderjit *et al.* (2000) in his study. Fairly persistence characteristic of carbofuran in soil is also influenced by the soil organic matter and soil type. The clay and black cotton type of soil in Isiolo and Laikipia could have influenced the adsorption hence the persistence of carbofuran in the soil from rainy to dry season.. In a related study by Greenhalgh and Belanger (1981), carbofuran residues have been found to accumulate in clay soil to an extent that 3.8 ppm residues were detected after two successive annual treatments. About 8.7 ppm was also detected in clay-organic soil sampled late in the crop season (Greenhalgh and Belanger, 1981)

Figure 9 gives a clear picture of the seasonal and regional variations. Generally the concentration of carbofuran seems to be higher in both Isiolo and Laikipia in the wet season compared to the dry season.

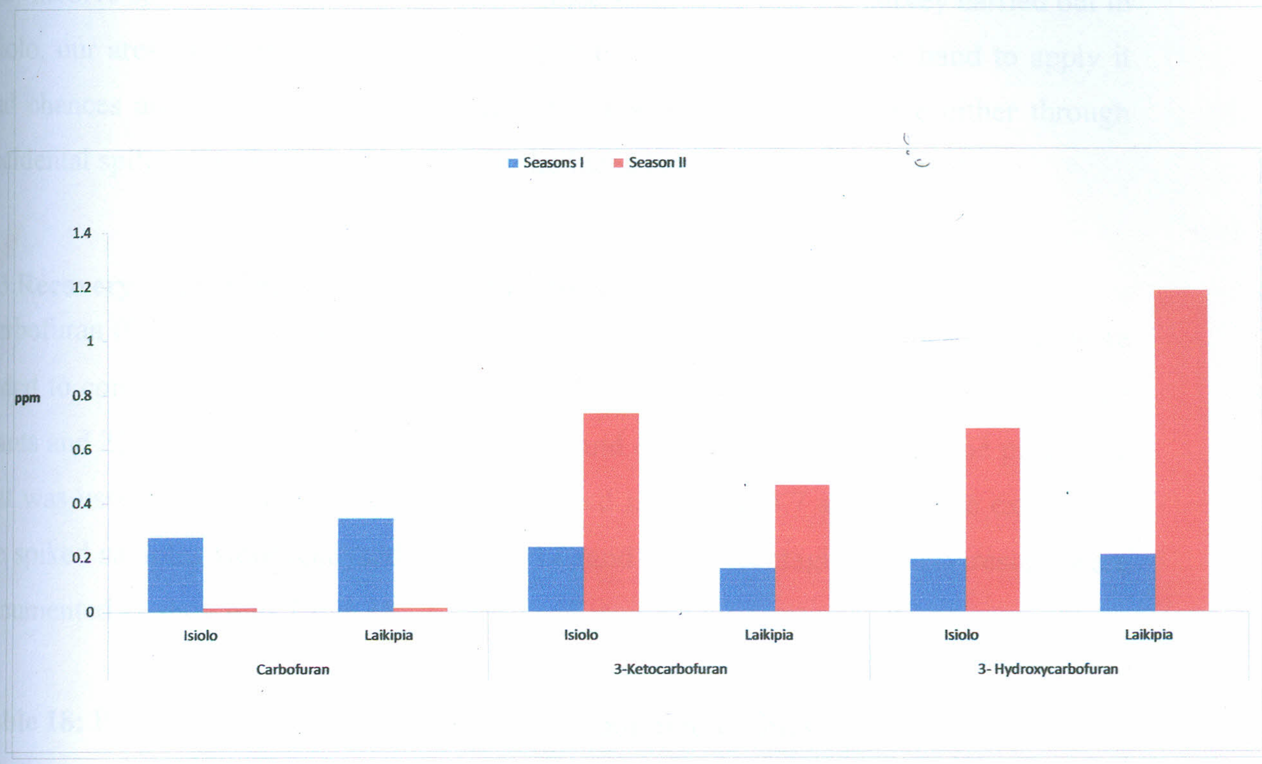

Figure 9: Seasonal and regional variation in concentration of carbofuran and its metabolites.

However, in the dry season the concentration of the metabolites seem to have increased as can be seen clearly from the chart perhaps because of their ability to persist slightly longer than the parent compound. Carbofuran is generally applied over the seed furrow before planting, during planting or even after planting and in all the cases the granules must be incorporated in the soil about 3 cm to 5 cm in the bands around the plants or in the soil. In-furrow application is meant to reduce cases of poisoning, however, this has also repeatedly given rise to extensive bird mortality in other controls (Mineau *et al.*, 1993). Despite these earlier findings in Canada, the current survey and study did not come across cases of field-application poisoning. On this basis intentional poisoning seem to be the most likely route of exposure in the area under study. Significant part of this pesticide remains adsorbed in form of bound residues in the soil where it is not easy for vultures to get exposed to it unless the granules adhere to the earthworms through which scavenger can get exposed.

According to Vyas *et al.* (2003), carbofuran may be topically applied on large baits and may involve spreading it over the carcass surface something that the survey carried out in Isiolo, our area of study, revealed. The perpetrators use whatever is at hand to apply it and chances are that this pesticide could have reached the soil surface either through accidental spillage or through contact with the laced baits or poisoned animals.

4.3 Recovery of carbofuran and its metabolites

Carbofuran (0.5 μg), 3-ketocarbofuran (0.5 mg) and 3-hydroxycarbofuran (0.5 mg) were added to control samples which included, 500 ml of water, 25 g of soil, 500 g of maize plants and 25 g of each animal tissue analysed and extracted through the same procedure that was used to extract the samples as described in section 3.7 and 3.8 The extracts of the spiked samples were analysed using HPLC and the percentage recovery results were documented as shown in Table 18.

Table 18: Percentage analytical recoveries of carbofuran residues

Compound	River water	Plants (*Zea mays* leaves)	Soil	Animal tissue
Carbofuran	85 ± 7.10	78 ± 3.22	90 ± 6.72	84 ± 3.40
3-hydroxycarbofuran	80 ± 6.45	75 ± 2.11	88 ± 4.20	85 ± 4.40
3-ketocarbofuran	90 ± 3.44	89 ± 4.60	86 ± 2.33	92 ± 3.42

n=3

River water for control analysis was obtained from which is a tributary of Ewaso Ngiro River which transverse a short distance into Isiolo. Plants and soil samples were obtained from the arid parts of Isiolo (Mirti Division) where no farming takes place. Muscles from the slaughtered goat carcass were used though it was not possible to get control samples for vultures' beaks, blood, feet, crop and the gut. The control samples were collected from the area with no cases of pesticide use.

CHAPTER FIVE

5.0 CONCLUSION

Among the pesticides used by farmers in the two regions under study, carbofuran pesticide is the most preferred choice. It is used by both small scale farmers and large farmers to control pests in their farms. Misuse of carbofuran as a poison to kill predator animals and consequently its presence in the food chain could be the main cause of the death of vultures reported in the area under the study. Confessions by some farmers that carbofuran is used as laced on carcasses or crops to avenge the killing of their livestock and crop destruction is an evidence of misuse. The main reason behind the poisoning is the ever increasing and unresolved human-wildlife conflict in the affected areas. The poisoning has further been worsened by the continuous environmental degradation and upsurge in population which has put a lot of pressure on the grazing land. Other effects include expansion of settlement, increase in land under crop production and increasing heads of livestock which have displaced wildlife and contributed immensely to the on going conflict.

The concentration of carbofuran was found to be higher during the rainy season a time that it was expected to be applied in the farms. High solubility of carbofuran in water explains its high concentration and its immediate presence in environmental matrices in the rainy season. However, the concentration reduced in the dry season as most of it was expected to have been taken up by plants and others broken down into degradation products.

Carbofuran, 3-ketocabofuran and 3-hydroxycarbofuran were detected in the *zea mays* leaves samples analysed from both areas under study. Their presence in the environment demonstrates that carbofuran was used extensively in the area under study. The reported concentration in plants and soil samples were too low to be considered as potential routes of exposure to the vultures though they presented high risk to the wildlife and humans in this region. The low levels could have been due to possible degradation of the pesticide which accounted for low mean concentration especially in high intensity agricultural area like Laikipia where high levels were expected. The presence of carbofuran residues in plants and more specifically the possible presence in edible parts like roots and leaves could present risks to human.

Surface soil contamination was also high and posed risks through run-off into the dams and rivers as well as through secondary poisoning of small birds. There was possibility of carbofuran granules applied in soil to adhere to earthworms through which the scavenging birds could easily get exposed to it and die.

In addition the average level of carbofuran in water in Laikipia was found to be above the US Maximum Allowable Limit of 0.04 ppm and this may pose serious risks to the users which include humans and wildlife.

Vultures' tissue and part of poisoned carcass analysed recorded presence of carbofuran and its two metabolites. The presence of the residues demonstrates that the wildlife was exposed to this pesticide before their deaths and could have possibly be the cause of their deaths. The data gathered in this study can therefore form enough background for ecological risk assessment and may be used for lobbying and advocacy for enhanced application of policies and good practices of use of carbofuran in Kenya.

Possible exposure from environmental matrices as a cause of death is ruled out because of the spontaneous deaths reported and low mean concentration of carbofuran and its degradation products in the environmental matrices, However, carbofuran is highly toxic to birds even at low concentration of 0.3 ppm and therefore the environmental distribution and exposure of residues in water posed a great risk to human and wildlife continuous monitoring is necessary. Use and misuse of carbofuran in the area of study is responsible for the death of vultures through secondary poisoning.

Soil, *zea mays* plant leaves and water are contaminated with carbofuran and its residues however the average concentration levels in water samples from Laikipia are significantly high and above the US Maximum Allowable Levels of 0.04 ppm.

The presence of metabolites in the samples detected by HPLC and confirmed by GC-MS can now be considered significant step in forensic investigation since metabolites appear to persist in even higher concentrations than the parent compound

From the preliminary report of this study and concerns from other conservationists, Juanco Kenya Ltd the sole distributor of carbofuran in Kenya and FMC Corporation of USA, resolved to withdraw carbofuran from the market pending further scientific evidence that it is being misused.

5.1 RECOMMENDATION

In view of these residue results and the survey conducted, the following recommendations can be made. According to the Pesticide Control Products Board, carbofuran 5% is listed as one of the restricted pesticides. Under the pesticide control Act cap 346 Laws of Kenya, restricted pesticides are extremely toxic and should be handled by trained, experienced and well-equipped operators who are licensed by the board. This is not the case in the field as the pesticide is purchased freely over the counter in complete disregard to this regulation. There is need to educate farmers on the need to use the pesticide for the purpose it is intended for. Most of the pesticides used in the tropics originate from the developed countries and under the current legislation, the export may be done even if the pesticide registration is prohibited or severely restricted in the exporting country parathion and carbofuran. This matter of concern that needs to be urgently addressed as it could be the reason behind introduction of toxic pesticide in our Kenyan market. There is need for harmonized policy and legal framework for management of the farmers, wildlife and use of pesticides.

Care must be taken when using water during the rainy especially in Laikipia where the level of carbofuran was found to be higher. This concern applies to water from the stagnant water points like the ponds with direct drainage and no outlet.

Apart from water samples in Laikipia, soil and plants samples recorded low levels of carbofuran which might not pose any risk to wildlife and even human. However, there are other less toxic pesticides in the market that can also be used like.

The presence of carbofuran residues in animal tissues demonstrate that carbofuran was used to poison the animals and of course the vultures through secondary poisoning. In this respect it is recommended that legal policies be put in place to minimize human-wildlife conflict. There is need to employ integrated or holistic approach to address such conflict situations as this is the source of the poisoning problem. For this awareness creation among farmers and pastoralists in the districts is absolutely necessary

The perpetrators of poisoning activities should be educated about the importance of wildlife to the socio-economic status of the community and the country at large and be advised on the ways of living with wildlife. Integration of conservation and economic development based on sustainable exploitation of wildlife resources needs to be put in place. Few conservancies have started development projects within the communities to make them understand and appreciate wildlife. These efforts should be supported because they will reduce the hostility of the communities around the conservancy towards wildlife.

The pesticide industry has recently introduced into the market the organic pesticides which are currently gaining farmers, conservationists and environmentalists confidence because of their low toxicity and environmental friendliness. Significantly reducing the use of carbofuran could avoid serious problems and at the same time help encourage farmers to use less hazardous pesticide. If pesticide like carbofuran continues to be available then there will be no incentive to use less hazardous means of pest management. Continuous monitoring the impact of this pesticide on wildlife is necessary and incidents of poisoning that are caused by misuse should be investigated with an aim of prosecuting the offenders.

5.2 Recommendation for further studies.

There is little information regarding the toxicity of the degradation products of carbofuran. It is therefore recommended that toxicities studies be done for the main metabolites like 3-ketocarbofuran and 3-hydroxycarbofuran.

High carbofuran residues have been detected in the maize plant leaves. It is therefore important that the levels of carbofuran be determined in the edible parts of plants like leaves for vegetables and even fruits to establish if the levels are within the maximum allowable limits because they present a potential risk to the consumers.

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