Carbofuran use and abuse in Kenya: Residues in soils, plants, water courses and the African white-backed vultures (*Gyps africanus*) found dead

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ABSTRACT

The increasing number of incidences of alleged wildlife poisoning with Furadan in Kenya have sparked off a strong lobby fronted by wildlife conservationists against Furadan use in the country and prompted this study. The worst case scenario was in 2004 in Athi River where a massive number of 187 African white-backed vultures (*Gyps africanus*) and hyenas were found dead at a spot where poisoning was suspected to have occurred through a Furadan-laced camel carcass bait. The study was initiated by the Peregrine Fund ─ Africa Project and the objective was to provide evidence for Furadan exposure, its misuse and involvement in vulture poisoning and potential impact on areas near two wildlife conservancies in two most affected districts. The study found evidence for ready availability of Furadan 5G in local veterinary retail shops and its illegal misuse by pastoralists and farmers against wildlife to protect their animals and crops. Analysis of soil, water and plants taken from the farms and water sources by High Perfomance Liquid Chromatography (HPLC) and Gas Liquid Chromatography-Mass Spectrometry (GC-MS) found residues of carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran indicating that Furadan was used extensively in farming causing residual environmental distribution and contamination and posing risks to small birds and mammals. Forensic analysis of residues in beaks, feet and crop content of the dead vultures as well as in a laced camel carcass bait and soil samples from one site of poisoning also showed carbofuran and its two metabolites supporting allegations of Furadan involvement in wildlife poisoning and high mortality cases of African white-backed vultures (*Gyps africanus*) in Kenya.

Keywords: Furadan; poisoning; wildlife; environmental contamination; Kenya.

1. Introduction

Carbofuran (2, 3-dihydro -2, 2-dimethyl-7-benzofuranyl-N-methylcarbamate) is the active compound in Furadan. Furadan, is a widely used systemic and contact insecticide, acaricide and nematicide which has broad spectrum of activity against many agricultural pests sold in various formulations with varying concentrations of carbofuran. Formulations of carbofuran include silica-based granules of Furadan 3G, 5G, 10G, or 15G, the number referring to the percentage of active ingredient (a.i.) by weight in the formulation. Another granular formulation Furadan CR-10 consists of dried granulated corn cob. Liquid formulations such as 350ST are also sold. Granular insecticides were designed for convenience and safety of the person applying the product and to provide timed (controlled) release of the chemical. Carbofuran has a relatively moderate mammalian toxicity (oral LD_{50} 8 - 11 mg/kg in rats) but is very toxic to invertebrates and birds with quite low avian Furadan LD_{50} values ranging from 0.238 mg/kg, in whistling ducks, to 5.62 mk/kg, in European starling, *Stumus vulgaris* (Hodgson et al. 1991; Hopkins and Scholz 2006; Mineau 1993; Mineau et al. 1999; Mineau 2001). Field studies with silica-based carbofuran granules of Furadan 3G, 5G, 10G and 15G as well as CR-10 have shown high mortalities in birds (Mineau and Collins 1988; Mineau et al 1999; Mineau 2003). The planting machinery has been found to leave a substantial number of granules exposed on the surface of the soil after application. This has been found mainly in row crops such as corn and field crops such as oilseeds (Mineau 2003). Several cases of poisoning through food-chain transfer, secondary exposure and illegal poisoning with laced baits have been reported (Allen et al. 1996; Crocker 2005; Elliott et al. 1996; Mineau 2003; Vyas et al. 2003; Vyas et al. 2005). Acute uptake of carbofuran, just like other carbamates, through accidental exposure can result in acute toxicities and fatalities even in humans (Hayes 1982; Ecobichon 1993). Therefore, despite its efficacy as an agricultural pesticide, Furadan usage has been restricted or banned in many countries including the USA and Canada.

In Kenya, Furadan is still being imported mainly for seed mixing to control soil dwelling and foliar feeding insects, for treatment of barley seeds (3G and 350ST), for seed dressing in rice, bananas, beans, vegetables and coffee (5G) and for application at the rate of 0.5-4 kg a.i /Ha to control soil insects and nematodes and early foliar feeding insects in coffee, bananas, pineapples, pyrethrum and maize (10G) (PCPB 1992). Approximately more than 23 tonnes of granular Furadan and more than 15,000 litres of Furadan concentrate are imported annually into the country (PCPB 1992). According to the national pesticide control regulations, up to 10% a.i. Furadan formulations are allowed into the country for restricted use by informed users only (Otieno 2009). However, a recent survey showed that Furadan is sold freely over the counter in many veterinary retail shops without any form of restrictions (Otieno 2009). Furadan usage is also expanding to other very vital crops such as in maize and wheat farming. Due to its ready availability and low price, Furadan is also being misused as an acaricide for tick control and for control of small mammals such as moles (Ogada and Keesing 2009; Otieno 2009). Its marketing and application in Kenya is currently presenting a number of problems in areas where its usage is prevalent.

Furadan poisoining in Kenya has affected birds, hyenas, camels, lions and hippos since 2003, supposedly involving indirect or direct poisoning (KWS 2009). In Athi River, 187 African white-backed vultures (*Gyps*

africanus) and hyenas were found dead near a laced camel carcass in 2004 where Furadan was suspected to have been used (Otieno 2009). The increasing number of reported cases of Furadan poisoning of wildlife have sparked off a strong lobby against its importation and use in the country, fronted by wildlife conservationists and the National Museum of Kenya. The vulnerability of vultures to Furadan poisoning is at a high level and the real risk of extirpation of vultures and other less common bird species in Kenya is eminent. Recent studies have shown the rapid decline of vultures over a three-year period in Laikipia district in Central Kenya, where raptors were observed to have declined by more 40% over the period from 2001-2003 and vultures and Bateleurs *Terathopius ecaudatus*, accounted for most of the decline (Ogada and Keesing 2009). Vulture sightings declined by 77% during the same period. The rapid decline was attributed to consumption of Furadan-laced baits which the pastoralists are using to kill large predators that attack their livestock (Ogada and Keesing 2009). Some of the reported cases of wildlife poisoning by Furadan in Kenya can be seen in figure 1 (Fig. 1). Although other toxic pesticides such as dicofol (Vyas 1999), fensulfothion and diazinon (Elliott et al. 1996; Frank et al. 1991) and non-steroidal anti-inflammatory drugs such as diclofenac (Prakash 2004) have been involved in bird poisoning cases in other countries such as India, the Kenyan cases have mainly been linked with Furadan misuse. Furadan threat to wildlife, especially birds, has also been reported in South Africa and Uganda, with many cases involving indirect poisoning of different vulture species (Otieno 2009; VEU 2005).

Carbofuran degradation and metabolism is rapid and occurs within the organisms and in plants, soil and water through the Phase I 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 (Ecobishon 1993; Hodgson et al. 1991). Carbofuran degradation also occurs in various matrices through different routes involving chemical reactions such as hydrolysis, oxidation and reduction as well as through microbial activity (Ecobishon 1993; Hodgson et al. 1991; Lalah et al. 2001). Its two main metabolites, 3-hydroxycarbofuran and 3-ketocarbofuran, known to be equally toxic, have been detected in various environmental matrices (Ecobishon 1993; Hassall 1990; Hayes 2001; Lalah et al. 2001). In flooded soils, most carbofuran residues remain in the top 10 cm layer and in the surface water (Lalah and Wandiga 1996). The granules can be carried away from the site of application and be distributed into the aquatic environment, providing a potential route of exposure to fish, mammals and birds. Cases of duck poisoning in paddy rice irrigation schemes have been reported in Kenya since 1990's (Lalah and Wandiga 1996; Otieno 2009). Treated seeds left in the field are another source of exposure to birds that feed on such grains and to scavengers such as vultures through indirect exposure and food chain transfer (Bishop et al. 2000a; Bishop et al. 2000b; Mineau et al. 2005; Richards et al. 2005). Birds that sift waterlogged sediment in search of food are also exposed to the granules in the fields (Mineau 1993; Mineau et al. 1999; Mineau et al. 2005).

To provide evidence for Furadan exposure and its involvement in poisoning of the African white-backed vultures (*Gyps africanus*) in the most affected areas in Kenya, a study was initiated in 2007 in Isiolo and Laikipia districts where Furadan is widely used to control agricultural pests in maize, potatoes, beans and vegetables. The main aim of the study was to provide forensic evidence for Furadan involvement in the massive deaths of vultures by monitoring carbofuran usage, its residue concentrations and their environmental distribution and contamination

of water points, soils and plants in selected sites in the two districts and in tissue samples of dead birds, to establish potential routes of exposure.

Fig. 1

2. Materials and methods

The study involved a survey conducted to gather information on Furadan usage and to provide evidence of Furadan misuse in the two areas and carbofuran residue analysis in samples of soil, plants and water taken from selected farms and water sources in the two affected areas as well as collected dead vulture tissue samples to provide evidence for Furadan usage and its involvement in vulture poisoning.

2.1 Description of the study area and sampling sites

The study was done in Laikipia and Isiolo districts. The sampling sites were located within longitudes 37°30′E and 40°E and latitudes 0°6′N and 0°20′N. Lewa Wildlife Conservancy (LWC) in Isiolo district and Gallman Memorial Wildlife Conservancy (GMWC) in Laikipia district were the two wildlife conservancies targeted in the study. LWC is home to a number of different species of wildlife under protection. In this conservancy there are continuous threats from illegal wildlife poisoning by pastoralists and numerous cases of bird and lion mortalities have been reported. LWC is also surrounded by small scale farmers most of whom are found in Manyangalo and Ngare Ndare forest and are also suspected in Furadan poisoning of stray animals to protect their crops. Adjacent to the conservancy flow two rivers, namely River Ngare-Ndare and Ngare Sirgoi and there are a few ponds (the water points for the animals) located within the protected area. Small scale irrigation farming takes place along the river banks. For soil and maize plant samples, there were six sampling sites randomly located in the farms around the conservancy (Table 1). For water samples, two major ponds within the conservancy and the two rivers were used (Table 1). GMWC, also known as Ol Ari Nyiro, is surrounded by pastoralists and crop farmers. The area where GMWC is located is relatively more fertile and farmers have comparatively larger farms (>3.5 acres) and clearly, pesticides are used in the farms for better harvests. GMWC has a number of ponds and dams located within and without it. Soil and maize plant samples were taken from the farms and the water samples were taken from dams and ponds as given in Table 1. The catchment characteristics and agricultural activities of the two districts are summarized in Table 2. The terrain of the two areas is such that when it rains the runoff flows into the ponds making them potentially highly polluted with pesticide residues. The samples were taken in one rainy season (October 2007) and one dry season (June 2008). Samples of vulture feet, beaks, blood and crop content were obtained from various areas including Tsavo, Athi River, Kilimanjaro and Masalani in other districts (not described here) as explained in the following section 2.3.

Table 1

Table 2

2.2 Survey on carbofuran usage in Isiolo and Laikpia districts

A survey was conducted by administering 85 questionnaires to the farmers, pastoralists and the conservationists in the two districts. This included 48 and 37 respondents in Isiolo and Laikpia districts, respectively. The survey method adopted was the use of semi-structured questionnaires with open and close-ended questions combined with face-to-face interviews to gather additional information. Field visits were conducted in the affected areas and oral interviews with stakeholders including companies selling Furadan to farmers, wildlife conservationists (including those from the National Museum of Kenya (NMK) and the two major wildlife conservancies (Lewa and Gallman Memorial)), the Pest Control Products Board of Kenya (the pesticide regulating authority in Kenya) and the National Environmental Management Authority (NEMA) were conducted. The questionnaire included information on the amount of Furadan used, the frequency and times of application in the year and methods of disposal of the un-used chemicals and used containers. Questionnaires also contained information on the amounts of Furadan supplied and purchased in a year and which specific regions were supplied with the pesticide as well as information on labelling, legal issues, illegal use for poisoning wildlife and reasons for poisoning. Other aspects that were included in the survey covered quality control checks i.e. if there were any other carbamates used in the two areas, any other pesticides (either organochlorine or organophosphates) that were prevalently used in the two areas as well as any possible pesticide combinations which may have included carbofuran in their formulations. ProSurvey Means package was used for data analysis.

2.3 Sampling in Isiolo, Laikipia, Kilimanjaro, Tsavo, Naivasha, Masalani and Athi River

Weathered vulture feet of dead birds (16 samples from each site) weighing approximately 25 grams each were collected from Lewa conservancy (Isiolo), Mbirikani (Laikipia) and Kilimanjaro ranches, respectively, for analysis. The samples were placed in a cool icebox and transported to Maseno University Chemistry laboratory for analysis. Samples of muscle of suspected Furadan-laced carcasses (2 samples from Tsavo and Athi River, respectively), beaks (48 samples from Lewa, Mbirikani and Kilimanjaro), blood and crop content (4 samples each from Simon Thomsett of Athi River conservancy) of dead vultures were also provided for analysis (Table 3). Feet (4 samples) of dead open-billed Stork birds from Masalani, Tana River District, were obtained from Lewa Conservancy (Table 3). We wish to clarify here that no living birds were used in this study and all samples taken for analysis were of birds found already dead.

Stratified random sampling was used to collect soil, water and plant samples. About 100 grams of soil samples were collected from 30 different randomly picked spots within the farms near LWC and GMWC, respectively. Triplicate soil samples were also taken from a site where the suspected Furadan-laced camel meat

poisoning occurred for forensic investigation. In soil sampling, an auger was used to get a scoop of approximately 100 grams of topsoil up to a depth of 2 cm. Samples were wrapped in an aluminium foil and transported in an icebox to the laboratory. Two litres each of water samples were collected randomly, upstream and downstream of rivers Ngare-Ndare and Ngare-Sirgoi, respectively. The water samples were taken by dipping a brown 2.5-litre-Winchester bottle into the water and then filling it up with surface water. In Laikipia district, 2-litre water samples were collected from the four dams and the two ponds, respectively (Table 1). The water samples were then kept in brown bottles and transported in an icebox to the laboratory for analysis. Five hundred (500) grams of plant samples were collected randomly from 12 different spots within the agricultural farms (Table 1). The samples were kept in an icebox for transportation to the laboratory.

2.4 Pesticide residue analysis

Pure analytical pesticide standard mixture containing carbofuran and its two metabolites 3-hydroxycarbofuran and 3-ketocarbofuran (10 mg/L in acetonitrile, purity > 99.9%) was obtained from the Institute of Ecological Chemistry, Helmholtz Zentrum, Munich, Germany. Pesticide residue analysis-grade solvents including dichloromethane, acetone, methanol and HPLC water were obtained from Kobian (K) Ltd, Nairobi. Anhydrous sodium sulphate (for drying samples), florisil (for column clean-up) and activated charcoal were also obtained from Kobian (K) Ltd, Nairobi. Thimbles and filter papers used during extraction were pre-extracted first using 250 mL dichloromethane for 8 hours in a Soxhlet apparatus.

The analytical procedure involved solvent extraction of homogenized samples, clean-up on a solid phase extraction column and analysis using reverse-phase HPLC with UV detection based on other methods reported earlier (Argauer et al. 1995; Kawamoto and Makthata 2003; Lalah and Wandiga 1996; Takino et al. 2004; Vyas et al. 2005; Yang et al. 1996). Accurately weighed 25 grams of weathered feet and beaks of the dead birds were cut using a pair of scissors. Each foot (below the distal end of the tarsometatarsus) and beak was cut further into approximately 0.6 cm pieces using scissors before homogenization in a pestle and mortar before solvent extraction. Each sample was extracted 3 times in a glass conical flask with 50 mL of acetone: dichloromethane (1:1, volume) followed by filtration using Whatman filter paper No 1 (Vyas et al. 2005). The extracts were combined and reduced to 2 mL in a rotary evaporator at about 20° C (Lalah and Wandiga 1996) before clean-up. Clean up was done in a glass column with a teflon stopcock and glass wool plug at the bottom, 4 g of florisil, and 2 g of anhydrous sodium sulphate at the top. Then 10 mL of dichloromethane was added to condition the prepared column. The sample extract (2 mL) was added to the top and eluted with 10 mL dichloromethane, then with 10 mL dichloromethane:acetone (95:5,volume) and then finally with 10 mL acetone/dichloromethane (10:90 volume) (Vyas et al. 2005). For plants and carcass sample extracts, 2g activated charcoal was added at the top of the column for decolorizing the plant pigments and removal of carcass lipids. The eluates were pooled, reduced to dryness in a rotary evaporator and then re-dissolved in 2 mL methanol for HPLC analysis. The same procedure was followed for crop and carcass tissue samples with appropriate modification.

The soil samples were air-dried in the laboratory at room temperature in darkness then 25 g weighed for analysis. To achieve satisfactory recovery, samples were further dried by mixing, separately, with 20 g of anhydrous sodium sulphate before homogenization in a mortar with pestle followed by sieving through a 2-mm mesh. The homogenized soil sample was placed in pre-cleaned thimbles, extracted in a Soxhlet for 4 hours with 130 mL mixture of dichloromethane and acetone (10:3 volume) and then the dichloromethane extract concentrated in a rotary evaporator to about 2 mL at 20ºC before clean-up as described above.

Five hundred (500) grams of air-dried plant samples were macerated, homogenized with 2 g Na_2SO_4, and extracted in a Soxhlet apparatus for 4 hours with 150 mL solvent mixture of dichloromethane and acetone in the ratio of 10:5 (volume). The extract was concentrated in a rotary evaporator to about 2 mL at 20ºC before clean-up as described above. The water samples (500 mL each) were, separately, partitioned with dichloromethane in a 1-litre glass separatory funnel, shaking with 100 mL dichloromethane for 15 min, and then repeating with 50 mL and 60 mL dichloromethane, respectively. The organic extracts were pooled and concentrated in a rotary evaporator to 2 mL at 20°C. Two grams of sodium sulphate was added to dehydrate the extracts before filtration. Clean-up was done as described above.

2.5 HPLC and GC-MS Analysis

Carbofuran residues were analyzed both qualitatively and quantitatively by an Agilent 1100 series HPLC model equipped with a UV/VIS detector at λ max = 254 nm and fitted with a Supelco C_{18} cartridge reverse phase column (250 × 4.6 mm ODS 5 µm). HPLC-grade solvent: acetonitrile/ water (4:1, volume) was used as the mobile phase at a flow rate of 1 mL/min. For recovery efficiency, 0.5 µg of carbofuran standard mixture was added to 500 mL of water, 25 g of soil, 500 g of plants and 25 g of the animal tissue of control samples, respectively, for analysis through the same procedure. The % recoveries were: carbofuran- 85±7.10 (water), 78±3.22 (plants), 90±6.72 (soil), 84±3.44 (animal tissue); 3-hydroxycarbofuran- 80±6.45 (water), 75±2.11 (plants), 88±4.20 (soil), 85±4.40 (animal tissue); 3-ketocarbofuran- 90±3.44 (water), 89±4.60 (plants), 86±2.33 (soil) and 92±3.42 (animal tissue). Solvent background residue concentration levels and carbofuran standard residue detection limits were determined. Carbofuran residues were identified by comparing the retention times with those of the standards and quantified by extrapolation of corresponding sample peak areas with those from standard calibration curves prepared using carbofuran standard solutions. For calibration curves, standard solutions of concentrations ranging from 0.01 to 2 mg/L and injection of 1 µL into the HPLC were used and peak areas of standard solution were plotted against corresponding concentrations. The limit of detection was taken as 3 times the detector noise level. For quality control, the precision of the methods used in this study was established by HPLC injection of the same sample in triplicate. The accuracy of the method was also ensured by running blank solvents and standards (every six injections) between the injections. Control samples for water, soil and plants from adjacent fields where no pesticides were applied were run but in all the cases there were no detectable levels of carbofuran, 3-ketocarbofuran and 3-hydroxycarbofuran. Detection limits were 0.001µg/g and 0.001µg/g for carbofuran in water and soil samples, respectively. Selected 20 samples of extracts including beaks (4 samples), feet (4 samples), carcass (2 samples),

water (2 samples), soil (2 samples), plants (2 samples), blood (2 samples) and crop content (2 samples) were taken to KEPHIS laboratory, Nairobi, for GC-MS analysis to confirm presence of carbofuran and its metabolites in the matrices by mass-spectrometry.

3. Results

3.1 Results of survey from the farmers and pastoralists

From the survey study, 65% of the 48 respondents in Isiolo reported using Furadan for agricultural purposes and 4% indicated that they had used it to poison the animals which had strayed out of the conservancy. In Laikipia, 54% of the 37 respondents reported using Furadan for agricultural purposes and 20% indicated that they had used it as a poison to kill wildlife such as stray dogs, hyenas, baboons and jackals which kill their livestock and destroy crops. A large proportion (70%) of farmers in Laikipia responded that it was not easy to quantify the deaths of wildlife caused by Furadan but some reported that destruction of crops and cattle by stray wildlife happens every year. In both districts, in oral interviews, the respondents reported that they preferred to use Furadan to poison wildlife because of its efficacy and fast action against targeted predators that came out of the confines of the conservancies. The wildlife conservationists believed that all cases of wildlife poisoning by farmers and pastoralists in the two districts were done as a way of avenging and deterring the killings of livestock and destruction of crops and the survey did establish this. Although in questionnaire the respondents indicated that they had used Furadan to poison the animals, none of the farmers was willing to accept openly the use of Furadan in wildlife poisoning during oral interviews, supposedly due to fear of being found breaking the laws. No respondent reported any knowledge of Furadan use to kill vultures but some admitted that the vultures could get exposed through the Furadan-laced carcass baits. It was established that Furadan was mainly used in the two districts as a pesticide to control soil dwelling and foliar feeding pests in maize and horticultural farms.

The survey established that there were other alternative pesticides in the market such as methomyl (90% w/w), ethotrop in form of Mocap GR10 (supplied by Bayer Crop Science), fenamiphos in form of Nemacur 400EC and bio-pesticides like azadirachtin in form of Nimbecidine and *Paecilomyces lilacinus* (Bio-nematon), but all farmers still preferred use of carbofuran in controlling pests. The survey also found that the Kenya Wildlife Service (KWS) and the private conservancies were involved in activities aimed at preventing wildlife poisoning through monitoring, awareness creation within the communities and initiating community-based development projects. On pesticide regulation, the survey found that the agrochemical stockists visited in both districts stocked and sold Furadan in varied sizes ranging from 100g to 200g plastic cans, with labels in English and Kiswahili, especially during the planting seasons. The stockists visited did not admit any knowledge of purchase of Furadan with the intention to poison wildlife. Referring to the PCPB regulations (http://www.pcpb.org.ke), we concluded that the national regulations on Furadan use were not adhered to as it was being sold, without restriction, to uninformed users and was being used without engagement of licensed pest control operators. Other details of the survey results have been published in a thesis (Otieno 2009).

3.2 Results of residue analysis.

The concentration levels of carbofuran and its two major metabolites 3-hydroxycarbofuran and 3-ketocarbofuran in the vulture tissue samples and in soil, water and plant samples taken from the two areas are summarized in Tables 3-6. The data obtained provided empirical evidence of carbofuran exposure in *Gyps africanu*s vultures in Kenya. The data also confirmed Furadan usage and environmental contamination of soil and water sources in the two districts. The concentrations of the metabolites, 3-ketocarbofuran and 3-hydroxycarbofuran were detectable even in vulture crop samples which had been preserved in formaldehyde since October 2005. Analysis by GC-MS for some of the samples confirmed carbofuran and its two metabolites in the matrices as analysed by HPLC. The concentrations in environmental matrices such as water were also of concern, and provided enough evidence to warrant further investigation and action in the two districts to support the current campaign and awareness against Furadan usage in Kenya. Although the levels in water, soil and plants showed environmental risk, exposure, and potential harm to a range of wildlife, they are probably not a major risk to vultures that are usually known to be killed mainly by laced carrion. However, vultures also drink water and bathe in it and therefore risk of exposure through such circumstances cannot be ruled out completely.

Table 3

Table 4

Table 5

Table 6

Table 7

4. Discussion

4.1 Vulture tissues

The results of the concentrations of carbofuran and its two metabolites in bird tissue samples are given in Table 3. The vulture tissue analysis in this study for forensic investigation is supported by similar forensic analyses which have been reported various insecticide residues in bird poisoning cases (Frank et al. 1991; Stroud and Adrian 1996). Forensic analysis of weathered feet of brown-headed cowbirds (*Molothrus attar*) and Eastern screech owls (*Otus asio*) by GC-MS have been reported (Vyas et al. 2003; Vyas et al. 2005). Analysis of other matrices including crop, stomach and gizzard content as well as liver and blood by HPLC and by acetyl-cholinesterase inhibition assay have

also been reported in other countries (Henderson et al. 1994; Slotta-Bachmayr et al. 2004; Vyas 1999). In general, the presence of residues on the feet and beaks do not imply lethality but serve as evidence for exposure before death (Stroud and Adrian 1996). In addition, other factors such as pesticide toxicity, environmental half-life and history of the field situation are considered (Allen et al. 1996; Henderson et al. 1994; Vyas et al. 1999; Vyas et al. 2005). Detailed accounts and limitations in sampling and analysis of bird tissue for forensic investigations are well discussed in literature (Henderson et al. 1994; Martin and Forsyth 1996; Mineau and Collins 1988).

In this study, it was not easy to get fresh birds' tissues for analysis, and therefore the feet and beaks were collected for analysis without being absolutely certain how long the samples had stayed in the field after death. The feeding nature of vultures and other scavenging birds is such that they step on the prey as they eat. This action might take several minutes during which the pesticide can be absorbed through the feet. This explains the presence of carbofuran and its metabolites on the birds' feet. Muscle samples from the laced camel carcass accidently found by conservationists during their routine monitoring in Athi – River showed the presence of the two metabolites of carbofuran indicating Furadan lacing on the bait. The concentrations obtained were low, in the parts per billion levels, which indicated that the laced camel carcass could have been retrieved from the field several days after the baiting when the residue concentrations had decreased due to dissipation and degradation. The possible mechanism of spread of contamination on laced carcass is explained elsewhere (Otieno 2009). For secondary exposure 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 present on the carcass or in the dead animal (Brown 1997). However, it was not possible to make a definite conclusion on cause-effect toxicity because we did not know for how long samples had weathered in the field before analysis, nor did we know the rate of degradation of carbofuran on carcass. However, the fact that carbofuran is very highly toxic to birds (Mineau and Tucker 2002a; Mineau and Tucker 2002b; Vyas et al. 2005) and that it is the chemical of choice for illegal poisonings resulting in several avian mortalities in many countries supports possibility of Furadan use and cause of death of the vultures analysed. The ranges of concentrations of residual carbofuran and its two metabolites in the beak and in the crop samples indicated that Furadan consumption could have been sufficient to cause adverse effects because granular Furadan formulation contains only traces in the range of 5-10% a.i. of carbofuran. Only one granule of Furadan 3% a.i. is enough to kill a mallard duck (Mineau and Tucker 2002a; Mineau and Tucker 2002b). We therefore estimated that if the acute oral toxicity (LD_{50}) for *Gyps africanus*, whose natural total body weight lies in the range from 4.2 ─ 7.2 kg, were 5.62 mk/kg of Furadan (as reported for the European sterling, *Stumus vulgaris*), then its acute oral toxicity (LD_{50}) for the active compound carbofuran, would be in the range of 0.29 ─ 0.59 and so the concentrations reported for the two metabolites, 3-hydroxycarbofuran (0.081─ 0.093 mg/kg of tissue) and 3-ketocarbofuran (0.179 ─ 0.219 mg/kg of tissue), in the crop content were quite high and could point to Furadan exposure and probable cause of death of the vultures.

Carbofuran is susceptible to hydrolysis and oxidation and therefore the elevated temperatures and high moisture conditions experienced in Kenyan forests coupled with changes in pH could facilitate its rapid breakdown into toxic 3-hydroxycarbofuran and 3-ketocarbofuran and this could account for the presence of these metabolites even *in vivo* (Ecobishon 1993; Raminderjit et al. 2000). The way the dead birds were found, lying dead, near the

laced camel carcass bait also indicated possible carbamate poisoning from the laced carcass meat (Vyas 1999). Vultures are not usually the target species but their feeding habits, ability to spot carcass miles away and travel far distances looking for carcasses make them quite vulnerable. The threat to *Gyps africanus* vulture species is further exacerbated by the fact that they are long-lived raptors with low reproductive rate, laying one egg at a time, making it sensitive to decrease in adult number (Slotta-Bachmayr et al. 2004). Under natural circumstances, they have high adult survival which somehow compensates for low annual offspring production. However, the death of over twenty vultures in one poisoning incidence can have a significant negative impact on the demographic viability of the local population. The undercover nature of the practice of poisoning in Kenya makes it often very difficult to document poisoning cases affecting them and therefore the number of deaths being reported by the conservationists could just be underestimates of the real scenario (Hendersen et al. 1994; Vyas 1999). Provision of ante-dotes such as atropine sulphate (often administered against carbamate poisoning) and rehabilitation have not been practised in Kenya.

4.2 Water, soil and plant samples

The concentrations of carbofuran and its two metabolites detected in water, soil and plant samples are shown in Tables 4, 5 and 6, respectively. Apart from being adjacent to the conservancies the sampling sites for water were located within the catchments with good agricultural activities likely to contribute to residue contamination to wildlife and humans. Agrochemical application in farming in the upper parts of the two rivers in Isiolo district which rely heavily on irrigation using river water could contribute to run-off of pesticide residues. More residues were detected during the rainy season in the water samples (Table 4). The concentration of carbofuran and its two metabolites were higher in Laikipia water samples than Isiolo. Over 70% of large-scale farmers (data not shown here) who use Furadan are in Laikipia district. There was significant difference ($p<0.05$) in mean concentrations of carbofuran and its metabolites in the two sampling seasons in both regions under study. The mean concentration levels of carbofuran in both Isiolo and Laikipia were above the US allowable freshwater contaminant level of 40μg/L and the European Union drinking water limit of 1μg/L and this indicated high risk for drinking purposes depending on the residue spatial distribution (Otieno 2009).

For the soil samples taken from the site where two lions were poisoned and over twenty vultures were found dead, the results showed presence of carbofuran metabolites in low concentrations. This result was consistent because some of the respondents in the survey had reported that there could have been spraying of Furadan on the camel carcass with an aim of killing the predators at the site. Pesticide residues can reach the soil surface from contact with laced carcass or during lacing through spreading or spraying. In the other soil sampling sites, randomly selected within the farms, the concentrations of carbofuran and its metabolites were detectable and indicated usage of Furadan in the two districts (Table 5). Although the levels of residues found in soil in the two regions in this study were low, contamination of water through run-off and secondary transfer through soil organisms to other smaller birds are still possible. The concentration of carbofuran was found to be higher in soil during the wet season than during dry season which is consistent with the fact that carbofuran dissolves easily and can be found in the soil matrix within a short time after application. Furadan 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 exposure and poisoning, however, this has also repeatedly given rise to extensive bird mortality in organisms that sift through soil (Mineau 1993). Field monitoring immediately after pesticide application, especially following rainfall, has revealed many cases of avian and small mammalian mortality in large farms in other countries following pesticide application including mortality cases of ducklings sifting through contaminated puddles for food and other small bird species feeding on contaminated locusts (Mineau 1993; Vyas et al. 2003).

The plant samples showed presence of carbofuran and its two metabolites in both Isiolo and Laikipia (Table 6). Carbofuran, like other carbamates, is a systemic pesticide which means that the plant absorbs it through the roots, and from there distributes it throughout its various organs mainly the vessels, stems and leaves where insecticidal concentration are attained. Carbofuran can get absorbed by the plant roots so that within 7 - 10 days after application it is found in the plant leaves (Crocker 2005) although the residue levels often decline due to breakdown. The breakdown in plant tissue is rapid initially but slows down so that many residues ultimately persist for longer than predicted by first order kinetics (Crocker 2005). It is therefore expected that it could find its way into the vulture food chain when they eat herbivores though at low concentrations. It is feasible that such exposure route through food chain transfer would present some acute toxicity problems. A well documented case of hawks eating insects feeding on plants when seeds had been treated with insecticides such as disulfoton and a similar one where bird kills occurred after eating earthworms present in contaminated soils have been reported (Mineau et al. 1999). There was significant difference ($P< 0.05$) in mean concentrations of carbofuran and its metabolites in plants in the dry and wet seasons in both regions. Previous studies by Raminderjit et al. (2000) reported that 3-hydroxycarbofuran in sugarcane plant remained higher and persisted longer than that of the parent compound. The concentration levels of carbofuran and its metabolites in this study compared well with their concentrations in *Zea mays* after 117 days from the time of application as given in the Handbook for Chemical Risk Assessment (Table 7) (Eisler 2000).

5. Conclusions

The detectable levels of carbofuran and its metabolites, 3-ketocarbofuran and 3-hydroxycarbofuran in water, soil and plant samples indicated that Furadan was used extensively in areas near the wildlife conservancies in both Laikipia and Isiolo and that there was environmental distribution and exposure of residues in water which posed risks when used for domestic purposes or as drinking water for the animals. 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 and terrestrial mammals.

Continued availability and usage of Furadan in Laikipia and Isiolo districts poses risks to the prevalent African white-backed vulture (*Gyps africanus*) principally through illegal poisoning. Furadan exposure in vulture was indicated by the presence of carbofuran and its two metabolites in bird tissue samples and in laced camel meat carcass bait. Residue analysis of bird tissue samples including feet, beak, crop for forensic investigations on Furadan poisoning should include the two carbofuran metabolites as they appear to persist long and sometimes occur in

higher concentrations than the parent compound. This is significant in tropical conditions where carbofuran degradation at the site of application can be quite rapid.

The underlying reason behind wildlife poisoning is the ever increasing and unresolved human-wildlife conflict in the affected areas. Wildlife poisoning has been further exacerbated by continuous environmental degradation, climate change and increase in human population which have put a lot of pressure on available grazing land thus pushing the pastoralists to move closer to and even illegally encroach into the wildlife conservancies in search of pasture and water. However, the survey established that there have been very positive mitigation actions through provision of education, awareness creation and compensation to the affected local community by the Kenya Wildlife Service and the private wildlife conservancies and these efforts are very commendable and should be encouraged. The survey study found that it is becoming too difficult to control misuse of Furadan to kill wildlife because its use in agriculture in the two areas has become increasingly popular and therefore it would be safer to remove Furadan from the market or ban it altogether nationally.

The preliminary results from this study contributed to wildlife conservation efforts which have culminated into mounting pressure on responsible national authorities to ban Furadan. Due to these efforts, Juanco Kenya Ltd, the sole distributor of Furadan in Kenya and FMC Corporation of USA have resolved to temporarily withdraw Furadan from the Kenyan market pending further scientific evidence of its threat to wildlife. The matter is also now being discussed at higher levels in government and in parliament.

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Table 1
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 dam

LWC: Lewa conservancy, GMWC: Gallman Memorial wildlife conservancy.

Table 2
Characteristics and agricultural activities of the two catchments.

	Isiolo	Laikipia
Climatic conditions	Rainfall is spread into the two seasons (aver 580.2 mm annually) Classified as arid and semi-arid area.	Cool temperate climate with both dry and rainy season (600mm -1200mm per year). Classified as semi arid to high potential area.
Location	Longitudes: 36° 50'E and 40° E and latitudes: 0° 05' S and 2° N.	LongitudeS: 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, where samples were taken). 120, 500 Km^2
Economic activities	Mainly pastoralism, tourism and arable farming.	Agriculture, pastoralism, ranching
Type of farming	Small scale mixed farming	Small scale mixed farming and large scale commercial farms
Size of farms	Average of 0.5 acre per individual;	Average of 3.5 - 16 acre
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 Furadan per 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	Maize stalk borer, maize aphid, leaf miner, root nematodes, cutworms
Non-target organisms	Predator wildlife e.g. lions, elephants, jackals and scavenging birds e.g. vultures	Predator wildlife e.g. lions, elephants, jackal and scavenging birds e.g. vultures
Application rate	0.5-4 Kg a.i /Ha	0.5-4 Kg a.i /Ha

Table 3
Mean (±s.d.) concentrations (mg/kg dry weight) of detected residues in bird tissue samples

Site	carbofuran	3-ketocarbofuran	3-hydroxycarbofuran
Beak samples			
Isiolo	0.060±0.010	0.067±0.002	0.146±0.001
Laikipia	bdl	bdl	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
Feet samples			
Isiolo	0.0500±010	0.180±0.010	0.018±0.001
Laikipia	bdl	0.030±0.006	0.040±0.010
Kilimanjaro	bdl	0.090±0.016	0.046±0.001
*__Feet__	bdl	0.116±0.022	0.084±0.014
Mean conc	0.025	0.240	0.073
Crop samples			
Naivasha	bdl	0.199±0.020	0.087±0.006
Mean conc	bdl	0.199	0.087
Muscle samples			
Tsavo	bdl	bdl	bdl
Athi River	bdl	0.080±0.002	0.096±0.005
Mean conc	bdl	0.040	0.048
**__Soil__	0.01±0.004	0.800±0.21	0.115±0.020

n = 48 beak and feet samples, respectively; n= 2 for Furadan-laced carcass muscle samples; n=4 for crop samples; bdl: below detection limit,
*__Feet__ samples of dead open-billed Stork birds from Masalani, Tana River district, **__soil__ samples from site of poisoning with laced camel carcass bait.
No residues were found in blood (data not shown here).

Table 4
Mean (±s.d.) concentrations (mg/L) of carbofuran and its metabolites in water samples from Isiolo and Laikipia districts

Regions	Season I	Season II	Regional mean
Carbofuran			
Isiolo	0.016±0.002	0.060±0.011	0.011
Laikipia	1.062±0.420	0.121±0.017	0.592
3-ketocarbofuran			
Isiolo	0.032±0.018	0.105±0.039	0.068
Laikipia	0.323±0.18	0.727±0.10	0.525
3-hydroxycarbofuran			
Isiolo	0.114±0.010	0.122±0.024	0.118
Laikipia	0.437±0.18	0.856±0.090	0.646

Table 5
Mean (±s.d.) concentrations (mg/kg dry weight) of carbofuran and its metabolites in farm soil samples

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
3-ketocarbofuran			
Isiolo	0.239±0.120	0.729±0.280	0.484
Laikipia	0.158±0.062	0.467±0.177	0.313
3-hydroxycarbofuran			
Isiolo	0.191±0.073	0.676±0.057	0.433
Laikipia	0.207±0.074	1.181±0.190	0.694

Table 6
Mean (±s.d.) concentrations (mg/kg dry weight) of carbofuran and its metabolites in plant samples

Regions	Season I	Season II	Regional mean
Carbofuran			
Isiolo	0.197±0.010	0.001±0.005	0.099
Laikipia	0.417±0.121	0.122±0.017	0.269
3-ketocarbofuran			
Isiolo	0.112±0.012	0.177±0.110	0.145
Laikipia	0.185±0.040	1.098±0.230	0.641
3-hydroxycarbofuran			
Isiolo	0.136±0.100	0.257±0.130	0.196
Laikipia	0.237±0.151	0.761±0.169	0.499

Table 7
Comparing concentrations (mg/kg dry weight) of carbofuran and metabolites found in this study (whole plant samples) with values reported for various parts of *Zea mays* plant 117 days after application.

	carbofuran	3-ketocarbofuran	3-hydroxycarbofuran
[*]*Zea mays*			
Leaves	0.43	0.40	4.57
Stalks	0.24	0.00	0.04
Cobs	0.04	<0.02	<0.02
Kernels	0.00	<0.01	0.02
Plants this study			
Whole plant samples (range)	0.12 - 0.54	0.11 - 1.33	0.04 - 0.93

[*]*Source: Eisler 2000.*

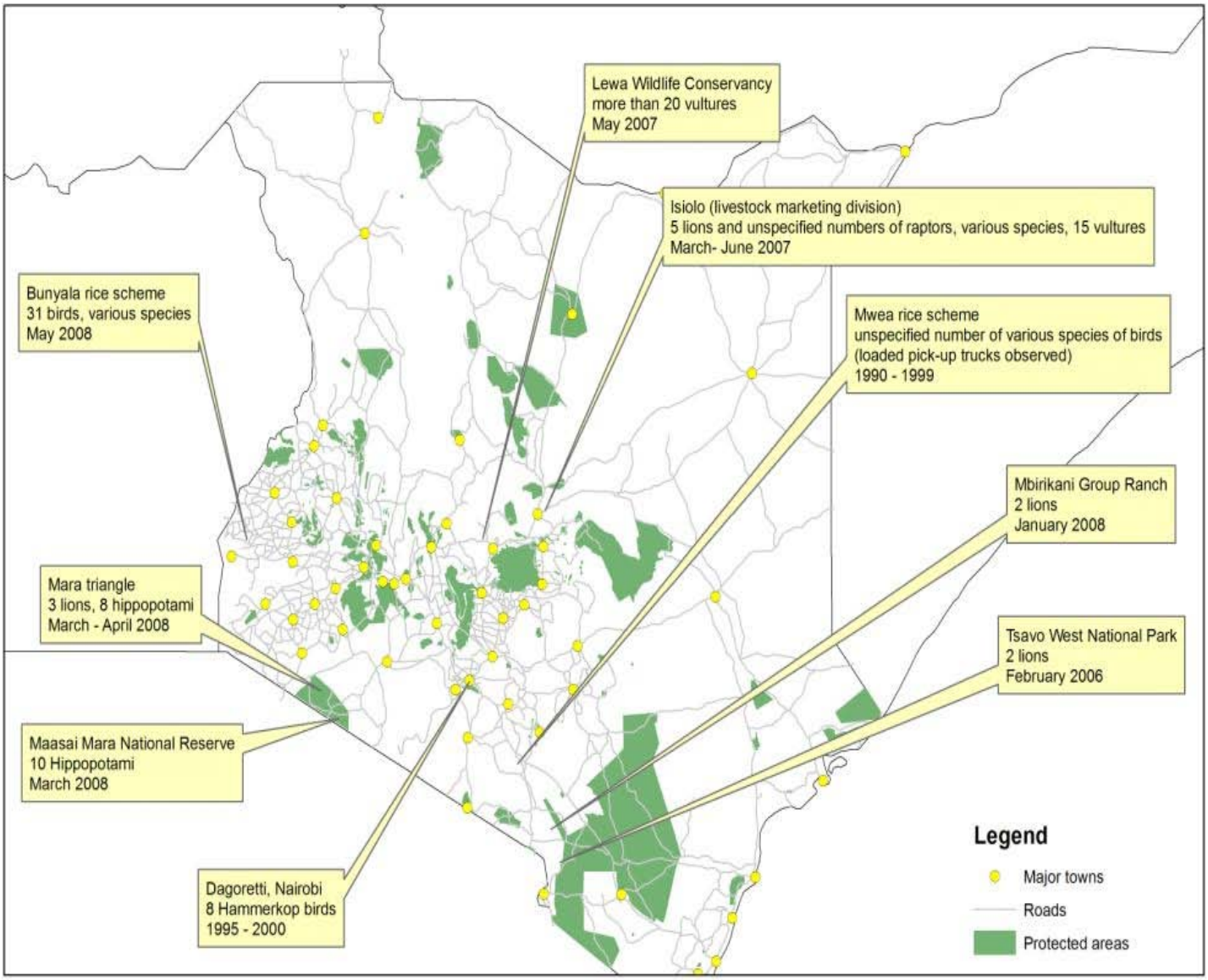

Fig. 1. Reported incidences of Furadan poisoning of wildlife in Kenya (*Adapted from Seamus report to FMC Corporation, Furadan Taskforce meeting July 2009, Wildlife Direct, Nairobi).*