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Carbofuran and its Toxic Metabolites Provide Forensic Evidence for Furadan Exposure in Vultures (*Gyps africanus*) in Kenya

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Abstract Forensic analysis of carbofuran residues in weathered tissue samples for evidence of Furadan exposure in vultures (*Gps africanus*) by HPLC gave concentration (mg/Kg dry tissue weight) ranges of bdl – 0.07 (carbofuran), bdl – 0.499 (3-ketocarbofuran) and 0.013–0.147 (3-hydroxycarbofuran) in beaks, bdl–0.65 (carbofuran), 0.024–0.190 (3-ketocarbofuran) and 0.017–0.098 (3-hydroxycarbofuran) in feet, 0.179–0.219 (3-ketocarbofuran) and 0.081–0.093 (3-hydroxycarbofuran) in crop content, 0.078–0.082 (3-ketocarbofuran) and 0.091–0.101 (3-hydroxycarbofuran) in muscle of a laced carcass and 0.006–0.014 (carbofuran), 0.590–1.010 (3-ketocarbofuran) and 0.095–0.135 (3-hydroxycarbofuran) in soil sampled from a poisoning site. These compounds were confirmed by GC-MS. The results showed that HPLC combined with GC-MS is suitable for forensic analysis of carbofuran residues in bird tissue samples and that forensic investigation should include its two toxic metabolites, 3-hydroxycarbofuran and 3-ketocarbofuran.

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Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl-*N*-methylcarbamate) is a widely used systemic and contact insecticide, acaricide and nematicide with a broad spectrum of activity against many agricultural pests. It has been reported to have relatively high mammalian toxicity (oral LD_{50} 8–11 mg/kg in rats) and to be very toxic to invertebrates, fish and birds and should therefore be handled with a lot of care to avoid environmental contamination and incidental exposure (Eisler 1985; Hodgson et al. 1991; Trotter et al. 1991; Mineau 1993, 2001). Acute uptake of carbofuran through accidental exposure can result in acute toxicities and fatalities even in human (Hayes 1982; Ecobischon 1993). It has been used worldwide for control of pests in sugarcane, sugarbeet, maize, rice and coffee and is very effective in controlling rice pests such as green leafhoppers, brown planthoppers, stemborers and whorl maggots. Other pests which are resistant to organophosphorous insecticides (OP's) e.g. white flies, leafminers, ants, mealy bugs, scale insects, cockroaches, wasps and aphids are also effectively controlled by carbofuran. Carbofuran has rapid action against both nymphs and adults killing them within 20 min (Suett 1986). The mode of action of carbofuran in target and non-target organisms is through inhibition of the acetylcholine esterase (AChE) resulting in accumulation of acetylcholine at the junction of the nerve cell and the receptor sites which finally causes the nerve to fire continuously leading to tremors, convulsions and death (Ecobischon 1993; Hayes 2001). This process involves binding of the phenyl ring moiety of the pesticide molecule to AChE and carbamylation to give an unstable covalently bound enzyme-pesticide (inhibitor) complex in a reversible

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reaction step, eventually followed by decarbamylation through hydrolysis to release the inhibited actetylcholine-esterase enzyme and a hydroxylated pesticide molecule (Hodgson et al. 1991; Ecobischon 1993). It is the significantly fast rate of decarbamylation of the inhibited enzyme that is responsible for the slight reversibility of the inhibition of the AChE in carbamates. In contrast, the decarbamylation process is so much slower with OP's, which also act by inhibiting the AChE, making the inhibition mechanism be referred to as irreversible (Hill 1989; Ecobischon 1993; Hill and Fleming 1982; Brown 1997; Mineau 2003). The slight reversibility of inhibition by carbamates often presents problems and uncertainty during their AChE-inhibition assays (Mineau and Tucker 2002a, b; Hopkins and Scholz 2006). The metabolism of carbofuran is rapid and occurs within the organisms involving Phase I and II P_{450} systems and by conjugation with substrates including glutathione, glucuronic acid, glutamic acid and glycine, leading to more polar metabolites that are excreted (McRae 1989; Hodgson et al. 1991). Carbofuran metabolism also occurs in the environment and in various matrices through different routes involving chemical reactions including hydrolysis, oxidation and reduction as well as microbial activity (McRae 1989; Hassall 1990; Lalah et al. 2001). The main metabolites of carbofuran that have been found in various matrices are 3-hydroxycarbofuran and 3-ketocarbofuran which are more polar but equally toxic to target and non-target organisms (Eisler 1985; Hassall 1990; Ecobischon 1993; Hayes 2001; Lalah et al. 2001). Other known metabolites of carbofuran include carbofuran phenol, 3-hydroxy-7-carbofuran phenol and 3-keto-7-carbofuran phenol (through phenyl-ring oxidation/reduction and hydroxylation reactions) and *N*-hydroxymethylcarbofuran and 3-hydroxy-*N*-hydroxymethylcarbofuran (through methyl hydroxylation reactions), respectively (Hassall 1990).

Despite its efficacy as an agricultural pesticide, carbofuran and its common technical formulation Furadan is known to be very toxic to non-target species especially birds with some of the reported cases of Furadan oral LD_{50}'s being 0.238 mg/kg (whistling ducks), 0.51 mg/kg (mallard), 1.3 mg/kg (house sparrow) (Mineau 1993, 2001; Mineau et al. 1999; Hopkins and Scholz 2006). A number of bird poisoning and high mortality cases involving Furadan have been reported previously in various countries including USA and Canada (Allen et al. 1996; Elliott et al. 1996; Mineau 2003; Crocker 2005). These cases include poisoning through food-chain, through secondary exposure as well as direct poisoning using laced baits (Vyas et al. 2003, 2005). Based on its high acute toxicity and threat to birds, its use has been restricted or banned in these countries (USEPA 2002). However, Furadan is still imported into Kenya particularly for seed dressing at the rate of 0.5–4 kg a.i /Ha to control soil-dwelling and foliar-feeding insects. It is marketed as Furadan 3G granules (3% a.i. for treatment of wheat and barley seeds using seed treatment equipment; restricted use), Furadan 5G (5% a.i. for seed dressing in rice, banana, beans, vegetables, coffee; applied manually) and Furadan 10G (10% a.i. applied by granular applicators to control soil insects and nematodes and early foliar-feeding insects in coffee, bananas, pineapples, pyrethrum nurseries and maize) (PCPB 1992). Furadan 350ST liquid is also marketed in Kenya for dressing barley seeds. Approximately more than 23 tonnes of granules and more than 15,000 l of liquid concentrate are imported annually (PCPB 1992). According to the Pest Control Products Board of Kenya (PCPB), the national pesticide regulating authority in Kenya, Furadan, with up to 10% a.i., is allowed into the country for restrictive use, only by informed users (Otieno 2009). However, a recent survey showed that Furadan is sold freely over the counter in many veterinary shops without any proper monitoring or adherence to the stipulated restrictions on its sale and application and therefore its presence in the Kenyan market presents great risks to both the environment and its users (Otieno 2009). Carbofuran has high water solubility and can leach easily from points of application to contaminate surface and groundwater (Lalah and Wandiga 1996; Lalah et al. 2001). Furadan granules can easily be exposed to small birds, mammals and invertebrates in agricultural fields as well as large predators and scavengers through food chain transfer (Eisler 1985; Lalah and Wandiga 1996; Bishop et al. 2000a, b; Mineau et al. 2005; Richards et al. 2005).

Furadan threat to wildlife, notably birds, has been reported in South Africa and Uganda with many cases involving both direct and indirect poisoning of different vulture species (VUE 2005; Otieno 2009). In Kenya, Furadan poisoning has affected birds, hyenas, camels, lions and hippos, through indirect and direct poisoning since 2003, with one particular case in 2004 where 187 African white-backed vultures (*Gyps africanus*) and hynenas were killed near Athi River (KWS 2009; Otieno 2009). The large number of reported cases of recent Furadan poisoning through misuse by farmers and pastoralists in Kenya have sparked off a strong lobby fronted by wildlife conservationists for Furadan ban in the country (Ogada and Keesing 2009; Otieno 2009). Vultures, because of their scavenging habits and ability to sight Furadan-laced carcass baits from a long distance, are at the greatest risk of extirpation. Recent research studies have shown a rapid decline of vulture numbers over a 3-year period in Laikipia district, Central Kenya, where raptors were found to have declined by more 40% over the period from 2001 to 2003 and

vultures including Bateleurs (*Terathopius ecaudatus*) accounted for most of the decline (Ogada and Keesing 2009). Vulture sightings declined by 77% during the same period (Ogada and Keesing 2009). Some of the reported cases of wildlife poisoning by Furadan in Kenya can be seen in Fig. 1. Although other toxic pesticides such as dicofol, fensulfothion and diazinon (Frank et al. 1991; Elliott et al. 1996) and antibiotics such as diclofenac (Prakash 2004) have been involved in bird poisoning cases in other countries like India, the Kenyan cases have been found to be mainly due to Furadan misuse, especially in agricultural areas where Furadan is used extensively.

To provide evidence for carbofuran exposure and involvement of Furadan in poisoning of the African white-backed vultures (*Gyps africanus*) in the most affected areas in Kenya where Furadan is used in farming to control agricultural pests in maize, potatoes, beans and vegetables, a study was initiated in 2007 in Isiolo and Laikipia districts. The main aim of the study was to provide strong documentary evidence for Furadan involvement in the massive deaths of vultures in the two districts and to establish potential routes of exposure to vultures. The study involved determination of concentration levels of carbofuran and its metabolites in tissue samples of poisoned birds and soil samples taken from a site where a carcass with bait poison had been, at one time placed, resulting in massive death of vultures. The results of the analysis are reported in this publication.

Materials and Methods

Lewa Wildlife Conservancy (LWC) in Isiolo district and Gallman Memorial Wildlife Conservancy (GMWC) in Laikipia district were selected as sites for the study. LWC is home to a number of different species of wildlife under protection. LWC is also surrounded by small scale farmers most of whom are found in Manyangalo and Ngare-Ndare forests. Small scale irrigation farming takes place along the banks of rivers Ngare-Ndare and Ngare-Sirgoi which flow adjacent to the conservancy. There are persistent threats of wildlife poisoning by pastoralists and crop farmers living around the conservancy to avenge and deter the killings of their livestock and destruction of their crops by wildlife resulting in numerous cases of wildlife mortalities. These cases of poisoning are believed to be due to direct use of Furadan-laced baits to poison predator animals such as lions, hyenas and hippos. As a consequence, scavenger birds notably vultures are directly or indirectly, through food chain transfer, exposed, resulting in high mortalities. Although this illegal activity has been reported to government authorities, there has been doubt about the exact cause of the deaths due to lack of scientific evidence for

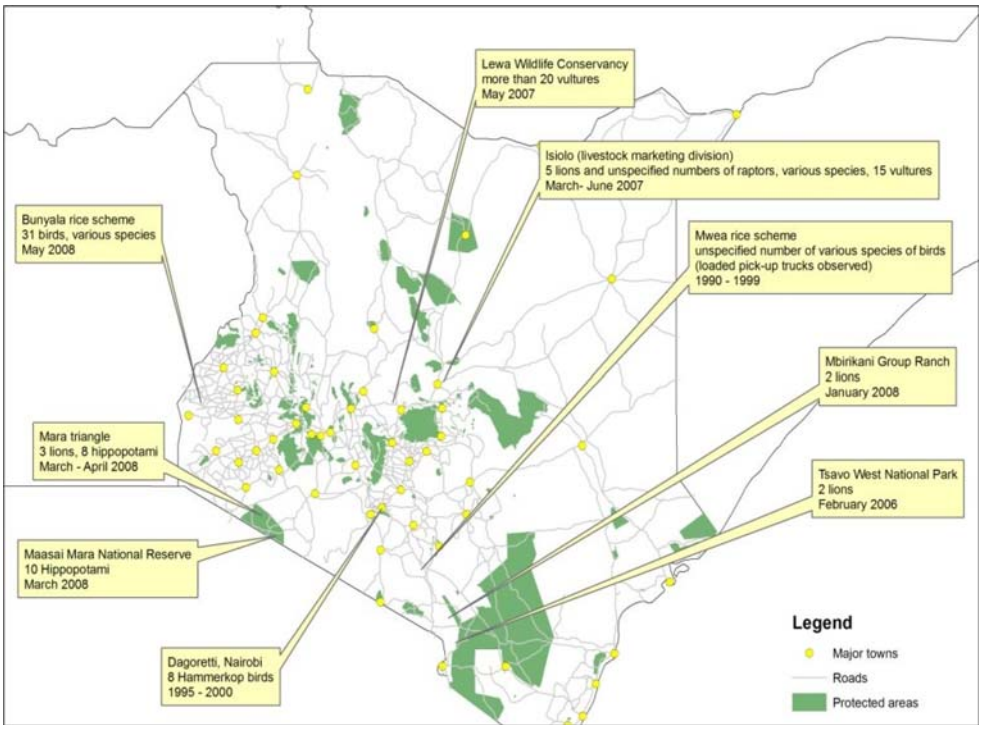

Fig. 1 Reported incidences of Furadan poisoning of wildlife in Kenya (Adapted from Seamus report to FMC Corporation, Furadan Taskforce meeting July 2009, Wildlife Direct, Nairobi)

Furadan involvement. GMWC, formerly Ol Ari Nyiro, in Laikipia district is also surrounded by pastoralists and crop farmers. The area is relatively very fertile and farmers have comparatively larger farms (>2.5 Ha) and pesticides are used in the farms for better harvests. Although nobody accepted direct use of Furadan to poison wildlife because of the fear of the legal consequences, it was established in a recent survey that Furadan is available in the area and is widely used to control agricultural pests. The two study sites lie within longitudes 37°30′E and 40°E and latitudes 0°15′N and 0°20′N. Sampling of vulture body parts for analysis was done in the rainy season (October 2007) when planting was starting and therefore use of Furadan was at its highest peak and in the dry season (June 2008).

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, acetonitrile and HPLC water were obtained from Kobian (K) Ltd. Anhydrous sodium sulphate (for drying samples), Florisil (for column clean-up) and activated charcoal which was used for removal of lipids and colour from animal tissues were also obtained from Kobian (K) Ltd, Nairobi, and were pre-extracted with *n*-hexane in a Soxhlet apparatus for 8 h before use. Thimbles and filter papers used during extraction were pre-extracted first using 250 mL dichloromethane for 8 h in a Soxhlet apparatus.

It was not easy to find fresh samples of dead or dying birds for analysis because of the expansiveness of the land and the secrecy of illegal baiting against wildlife. Therefore, weathered vulture feet from dead poisoned birds, weighing approximately 25 g each, beaks and Furadan-laced carcass samples were obtained from Lewa wildlife conservancy, Mbirikani and Kilimanjaro ranches, respectively. The samples were wrapped in aluminium foil, placed in a cool icebox and transported to Maseno University laboratory for analysis. Samples of blood and crop content of dead vultures, preserved in formalin since October 2005, were provided by Simon Thomsett (Athi River conservancy). Feet of dead open-billed Stork birds from Masalani, Tana River district, were obtained from Lewa conservancy. About 100 g of soil samples (in triplicate) from a site where two lions and twenty vultures had been found dead and Furadan poisoning was highly suspected, were collected and wrapped in aluminium foil. An auger was used to scoop approximately 100 g of topsoil up to a depth of 2 cm.

In the laboratory, the analytical procedure used involved solvent extraction of homogenized samples, clean-up on a solid phase extraction column and analysis using reverse-phase high performance liquid chromatography (HPLC) with an ultra violet (UV) detector. This procedure was chosen after a review of previous methods available in literature (Argauer et al. 1995; Lalah and Wandiga 1996; Yang et al. 1996; Pogacnik and Franko 1999; Kawamoto and Makthata 2003; Takino et al. 2004; Vyas et al. 2005). Accurately weighed 25 g, separately, of weathered feet and beaks of the dead birds were cut into small pieces using a pair of scissors. Each foot (below the distal end of the *Tarsome tatarsus*) and beak was cut further into approximately 0.6 cm pieces using scissors before homogenization in a pestle and mortar to facilitate chemical extraction. Each sample was extracted 3 times in a glass separatory flask with 50 ml of acetone:dichloromethane (1:1, volume) followed by filtration using Whatman filter paper No. 1 (Vyas et al. 2005). The crop and blood (preserved separately in 20 mL formalin since 2004) and mashed carcass samples were also extracted in the same way. The extracts were combined and reduced to 5 ml in a rotary evaporator at about 20°C before clean-up (Lalah and Wandiga 1996). Clean up was done in a glass column containing 4 g of Florisil and 2 g of anhydrous sodium sulphate at the top with Teflon stopcocks and glass wool plug at the bottom. For crop, blood and carcass tissue sample extracts, 2 g activated charcoal, for removal of lipids, was added at the top of the column. Before clean-up, the prepared column was first conditioned by adding 10 mL of dichloromethane. The sample extract (5 mL) was then added to the top of the column and eluted with 10 ml dichloromethane. This was followed by 10 mL dichloromethane:acetone (95:5, volume) and then finally by 10 mL acetone/dichloromethane (10:90, volume) (Vyas et al. 2005). The eluates were pooled, reduced to dryness in a rotary evaporator at low temperature and then re-dissolved in 5 mL methanol for HPLC analysis. The soil samples were air-dried in the laboratory at room temperature in darkness then 25 g samples taken for analysis. 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. Each homogenized soil sample was placed in a pre-cleaned thimble and extracted in a Soxhlet for 4 h with 130 ml mixture of dichloromethane and acetone (10:3, volume). The extract was concentrated in a rotary evaporator at 20°C to about 5 mL before clean-up as described above.

Carbofuran residues were analyzed both qualitatively and quantitatively by HPLC using an Agilent 1100 series model made in Japan equipped with an UV 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) as the mobile phase at a flow rate of 1 ml/min. For recovery efficiency, 0.5 µg of carbofuran standard mixture was spiked to 25 g of control samples, respectively, for analysis

through the same procedure. The extracts were cleaned- up in the glass column, analysed using HPLC and the % recoveries calculated. Solvent background residue concentration levels and carbofuran standard residue detection limits were determined. Carbofuran residues in the sample extracts were identified by comparing the retention times with those of the pure standards and quantified by extrapolation of corresponding sample peak areas with those from standard calibration curves prepared using pure carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran standard solutions, respectively. 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. Peak areas of standard solutions were plotted against corresponding concentrations. The limit of detection was taken at 3 times the detector noise level. For quality control, the precision of the methods used in this study was established by HPLC injections of the samples in triplicate. The accuracy of the method was also ensured by running blank solvents and standards (every six injections) between the sample injections. The detection limit was 0.001 µg/g. The percentage recoveries of the pesticide residues are given in Table 1. Selected samples of extracts were taken to Kenya Plant Health Inspectorate (KEPHIS) laboratory in Nairobi, for gas chromatography-mass spectrometry (GC-MS) analysis to confirm the identity of carbofuran and its metabolites.

Results and Discussion

The analytical results provided data on concentration levels of carbofuran and its two major metabolites 3-hydroxycarbofuran and 3-ketocarbofuran in the vulture tissue samples analysed with recoveries above 75% (Tables 1, 2) and provided empirical evidence of Furadan exposure and poisoning of *Gyps africanu*s vultures in Kenya. The concentrations of the metabolites, 3-ketocarbofuran and 3-hydroxycarbofuran were relatively high even in vulture crop samples which had been preserved in formaldehyde since October 2005. These results indicate that HPLC analysis of carbofuran and its metabolites in tissue and environmental sample extracts, after careful solvent extraction of the residues from the matrices, provides a reliable method for forensic investigation of exposure and poisoning in birds. Analysis by GC-MS for a few selected samples confirmed the presence of carbofuran and its metabolites in the matrices. The concentrations of carbofuran in the soil samples were below detection limits but the two metabolites 3-ketocarbofuran and 3-hydroxycarbofuran were detected at low concentrations and provided enough evidence for Furadan exposure at the suspected poisoning site. Earlier, in a survey conducted in the area where the poisoning site was located, some respondents had reported that Furadan had been sprayed on camel meat carcass bait with an aim of killing the predators and this was confirmed by our results. Although we could not confirm the existence of the granules, we still concluded that the poisoning with laced baits was done with granular Furadan since granular Furadan products were sold in the veterinary stores in the two districts but there were no liquid concentrate products found in the stores. Granules of Furadan are solid, the size of dietary grits taken by small birds to help in digestion in the crop (Mineau 1993). Technical carbofuran (>95% pure) is coated onto the solid granules which releases it to the environment or target. Therefore, if the granules impinge on fresh meat which is still wet, the active ingredient (technical carbofuran) which has a high water solubility of 700 mg/L would then diffuse from the granules, get dissolved and then spread all over on the carcass meat which would then

Table 1 Analytical (%) recoveries of residues

Compound	Soil	Animal tissue
Carbofuran	90 ± 6.72	84 ± 3.40
3-Hydroxycarbofuran	88 ± 4.20	85 ± 4.40
3-Ketocarbofuran	86 ± 2.33	92 ± 3.42

n = 3

Table 2 Mean (± SD) concentrations (mg kg^{-1} dry weight) of detected residues in bird tissue samples and their statistical analysis

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
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[a]	bdl	0.116 ± 0.022	0.084 ± 0.014
Crop samples			
Naivasha	bdl	0.199 ± 0.020	0.087 ± 0.006
Muscle samples			
Tsavo	bdl	bdl	bdl
Athi River	bdl	0.080 ± 0.002	0.096 ± 0.005
Soil[b]	0.01 ± 0.004	0.800 ± 0.21	0.115 ± 0.020

bdl below detection limit

[a] Feet samples of dead open-billed Stork birds from Masalani, Tana River district

[b] Soil samples from site of poisoning with laced camel carcass

become highly contaminated and can kill a number of predators, depending on the size of the carcass meat. Ingestion of the granules can also occur, causing instant deaths. Therefore, during the feeding on the carcass, it is possible for the birds and other predators to get in contact with residues of carbofuran and its metabolites and the most likely parts of their body are the feet and beaks which can provide evidence in special cases where fresh tissue samples such as brain, blood, crop, gizzard, gut and intestines (the gastrointestinal tract), which are often used in forensic analysis, are not available (Mineau 1993; Vyas et al. 2005). The results also showed that for forensic investigations on carbofuran poisoning especially using weathered samples, analysis of its metabolites is very critical, as they appear to persist longer and even occur in higher concentrations than the parent compound. This is significant in tropical conditions where carbofuran degradation and dissipation from the site of application can be quite rapid. The degradation, metabolism and dissipation of carbofuran can occur in the soil or in the tissue matrices at rates controlled by environmental conditions, notably pH, moisture and temperature (Mineau 1993; Lalah et al. 2001). Soil contamination from laced carcass was also experienced by Vyas et al. (2003). Previous studies done by Lalah et al. (2001) reported that carbofuran is rapidly adsorbed and metabolised in soil giving a large number of metabolites. This is enhanced through water which provides a reaction medium and is mostly common during the rainy season. Numerous cases of poisoning of waterfowl species and other predator and scavenger birds in agricultural fields, especially following rainfall and formation of puddles and floods, through various food chain transfer mechanisms involving earthworms, grass hoppers, crickets, frogs, mice and voles have been reported in USA and Canada (Mineau 1993; Mineau and Tucker 2002a, b). Our study indicated the presence of residues in the soil and if there is localized concentration it is possible to present risk at the site of application. These results indicate the importance of analysing soil samples and other environmental matrices from poisoning sites for forensic evidence of Furadan exposure. Mineau (1993), Stroud and Adrian (1996), Brown (1997), Trudeau and Cartier (2000), Mineau (2002, 2003) and Mineau and Tucker (2002a, b) describe in detail the methods and procedures to follow when looking for forensic evidence for Furadan poisoning. Although the metabolism of carbofuran is known to be rapid giving various metabolites, the detection of metabolites such as 3-ketocarbofuran and 3-hydroxycarbofuran, reported in this study, has not been reported before in forensic investigations (Mineau 1993).

Wildlife forensic laboratories have previously analyzed insecticide residues on feet if they are available (Frank et al. 1991; Stroud and Adrian 1996; Vyas et al. 2003, 2005). According to Vyas et al. (2003), diazinon and chlorpyrifos applied on lawns were found on the feet of brown-headed cowbirds (*molothrus attar*) that were weathered for 1 month. In another report, Vyas et al. (2005) demonstrated the use of GC-MS for analysis of carbofuran in weathered feet of Eastern screech owls (*Otus asio*), 28 days following treatment, after solvent extraction with dichloromethane/acetone and filtration. In a related study done in Croatia, analysis of 15 Griffon vultures found carbofuran 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). A forensic investigation done by Vyas et al. (2005) found carbofuran residues on the owl's feet after 28 days following exposure showing that in absence of conventionally analyzable matrices due to decomposition the feet or claws can be used since they are retained intact even after decomposition of the birds' carcasses. In general, the presence of carbofuran residues on the feet do not necessarily imply a lethal dermal exposure but serves as an evidence of the insecticide to which the bird was exposed and indicates the minimal insecticide concentration that was initially 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 residues on the feet can provide evidence of the cause of death (Vyas et al. 2005). A lot of care must then be taken to secure the feet within the shortest time possible after the death (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. In Kenya the environmental conditions are adverse and therefore a lot of degradation is expected especially in the conservancies which are forested areas and may be wet from dew and moisture especially in the morning. In a study by Raminderjit et al. (2000) the concentration of 3-hydroxycarbofuran was found to be higher and persisted longer than that of the parent compound in plant tissue samples.

In this study, it was not easy to track carcass and get fresh birds' tissues for analysis due to usual challenges which include expansiveness of the land around the conservancies, the hostility and suspicion of the pastoral community and the fear of being attacked by bandits and cattle rustlers in the areas. Similar challenges and sampling difficulties during forensic investigations have been reported in other countries (Mineau and Collins 1988; Crocker 2005). The feet and beaks were therefore collected for analysis although it was not absolutely certain how long

the samples had stayed in the field after exposure. The results of the residues detected in the beaks and feet here indicate that the vultures were exposed to this pesticide before they died. The residue levels from the feet and the beaks do not necessarily imply a lethal dermal or oral dosage but are evidence of carbofuran exposure to the birds (Stroud and Adrian 1996). According to Martin and Forsyth (1996), despite the tougher, scalier skin of bird feet relative to that of most of the feathered parts of their bodies, feet are by no means impermeable to uptake of insecticides. The feeding nature of vultures and any other scavenging birds is such that they step on the prey while they are eating. This might take several minutes during which the pesticide can be absorbed through the skin. This explains the presence of carbofuran and its metabolites on the birds' feet.

In 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). In absence of carbofuran toxicity data for vulture species, it was not possible to predict cause–effect from our results. In this investigation, the amount of residues of the metabolites detected in the laced-carcass was lower than the Furadan LD_{50} value of 1.9 mg/kg (Eastern Screech-Owl) (Mineau 1993) but it was difficult to extrapolate and make conclusions on cause–effect toxicity because we did not know for how long samples had weathered in the field before analysis and the rate of degradation of carbofuran on carcass. Vultures would easily die after feeding on carcasses with even small amounts of carbofuran, depending on statistical and spatial distribution of the residues on the carcass tissue. In cases of Furadan poisoning, vultures that ingest poisoned tissues are acutely intoxicated, become comatose and are generally discovered lying dead beside the poisoned carcass due to its high neurotoxicity (Brown 1997) and the massive deaths of vultures reported indicated carbamate poisoning.

Crop content collected from the dead African white-backed vulture (*Gyps africanus*) in Naivasha on 28th October 2005 showed no level of carbofuran but significantly high concentrations of 3-ketocarbofuran and 3-hydroxycarbofuran as shown in Table 2. The ranges of concentrations of carbofuran and its two metabolites in the beak and in the crop samples indicated that Furadan consumption could have been high because Furadan formulation contains only traces in the range of 5%–10% a.i. of carbofuran. The acute oral toxicity value for carbofuran in vultures was not available but Mineau (1993) gave a Furadan LD_{50} of 1.9 mg/kg for Eastern Screech-Owl which we used to estimate exposure level. The acute oral toxicity value of 1.9 mg/kg would translate into a value in the range of 0.1–0.2 mg/kg of carbofuran (a.i.) for the bird and therefore this indicates that the amounts of 3-hydroxycarbofuran (in the range 0.081–0.093 mg/kg of tissue) and 3-ketocarbofuran (0.179–0.219 mg/kg tissue), detected in the crop, were significantly high and more definitively pointed to Furadan exposure. Poisoning by the laced bait was therefore highly likely responsible for the deaths as was further confirmed by a few revelations during a survey. The presence of a Furadan-laced carcass bait in the field forms a death chamber for the vultures and other scavenging birds that eventually find their way there. Vultures are not the target species but their feeding habits, ability to spot carcass miles away and to 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 presents a significant negative impact on the demographic viability of this species. The undercover nature of using pesticide-laced baits makes it often very difficult to document poisoning cases directed towards them and it is most likely that cases which have been reported by the conservancies are only just a tip of the iceberg. Provision of ante-dotes such as atropine sulphate (often administered against carbamate poisoning) and rehabilitation to reverse poisoning effects for conservation (Sanchez-Fortun and Barahona 2001) have not been well practised in Kenya. The only case where administration of atropine in a confirmed case of Furadan poisoning through blood sample analysis led to recovery and release of poisoned adult Ruppells, lappet-faced and white backed vultures in April 2004 was reported by Simon Thomsett of Athi-River. A suitable and rapid forensic analytical procedure such as reported in this paper can improve monitoring and rehabilitation of affected wildlife. According to literature, vultures are threatened world over (Allen et al. 1996; Brown 1997; Mineau et al. 1999, 2005). In Mediterranean countries, the use of poisoned baits to control predators is a frequent practice that affects several species including the beaded vultures (*Gypaesus barbarus*) in France, the Spanish Imperial Eagle (*Aquia adalberts*) and cinereous vultures (*Aegypius Monochus*) in Spain (Slotta-Bachmayr et al. 2004). In India, cases of vulture poisonings by use of diclofenac have been reported to have caused a rapid decrease in their population (Prakash 2004). The Asian vulture crisis is a clear demonstration of the far-flung impact of wildlife poisoning if the problem is not addressed in good time (Vyas et al. 2003).

The significantly high concentration levels of carbofuran and its metabolites, 3-ketocarbofuran and 3-hydroxy-

carbofuran, found in various matrices demonstrate that Furadan was involved in poisoning of vultures. Continuous availability and usage of Furadan in Laikipia and Isiolo districts poses risks to the threatened African white-backed vulture (*Gyps africanus*). Furadan exposure and its involvement in the death of over twenty vultures were indicated by the presence of carbofuran metabolites in the suspected Furadan-laced camel meat carcass and in soil samples taken from the site of the poisoning episode. The results of this study showed that HPLC combined with GC-MS are useful for forensic analysis of carbofuran residues in birds and the investigations should include its two toxic metabolites, 3-hydroxycarbofuran and 3-ketocarbofuran.

The preliminary results from this study contributed to wildlife conservation efforts which have culminated into mounting pressure on responsible authorities to ban Furadan in Kenya. 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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