Journal of Environmental Science and Health Part B (2010) **45**, 137–144
Copyright © Taylor & Francis Group, LLC
ISSN: 0360-1234 (Print); 1532-4109 (Online)
DOI: 10.1080/03601230903472058

Soil and water contamination with carbofuran residues in agricultural farmlands in Kenya following the application of the technical formulation Furadan

PETER O. OTIENO[1], JOSEPH O. LALAH[1], MUNIR VIRANI[2], ISAAC O. JONDIKO[1]
and KARL-WERNER SCHRAMM[3]

[1]*Department of Chemistry, Maseno University, Maseno, Kenya*
[2]*Ornithology Section, Department of Zoology, National Museum of Kenya, Nairobi, Kenya*
The Peregrine Fund, Boise, Idaho, USA
[3]*Helmholtz Zentrum, German National Research Centre for Environmental Health, Munich, Germany*

This study was undertaken to determine the concentrations of carbofuran residues in water, soil and plant samples from selected sites in the farmlands in Kenya and to demonstrate the impact of Furadan use on the local environment. Soil, water and plant samples obtained from agricultural farmlands where the technical formulation Furadan has been used extensively showed high environmental contamination with concentrations of carbofuran and its two toxic metabolites 3-hydroxycarbofuran and 3-ketocarbofuran, separately, ranging from 0.010–1.009 mg/kg of dry surface soil, 0.005–0.495 mg/L in water samples from two rivers flowing through the farms and bdl-2.301 mg/L in water samples from ponds and dams located close to the farms. Maize plant samples contained these residues in concentrations ranging from 0.04–1.328 mg/kg of dry plant tissue. The significantly high concentration levels of carbofuran and its metabolites, 3-ketocarbofuran and 3-hydroxycarbofuran, found in various matrices demonstrate that Furadan was used extensively in the two areas 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 animals in two wildlife conservancies where the dams and ponds are located. Surface soil contamination was also high and posed risks through run-off into the dams and rivers as well as through secondary exposure to small birds and mammals.

Keywords: Furadan; agricultural soil; water; dams; carbofuran residues; environmental contamination.

Introduction

Carbofuran (2, 3-dihydro -2, 2-dimethyl-7-benzofuranyl-N-methylcarbamate) is a widely used systemic and contact insecticide, acaricide and nematicide which has 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 very toxic to invertebrates and birds and should therefore be handled with a lot of care.[1] Acute uptake of carbofuran, just like other carbamates, through accidental exposure can result in acute toxicities and fatalities even in human.[2,3] 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, stem-borers 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 minutes.[4] The metabolism of carbofuran is rapid and occurs within the organisms and in plants, soil and water. In organisms, *in vivo* metabolism of carbofuran occurs 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.[1−3] Carbofuran metabolism also occurs in various environmental matrices through different routes involving chemical reactions such as hydrolysis, oxidation and reduction as well as through microbial activity.[5] Different types of enzymes, bacteria and fungi in soil and in aquatic environments are responsible for both incomplete and complete degradation of carbofuran and several microorganisms including *Pseudomonas, flavobacterium*, *Achromobacter*, *Arthrobacter*,

---

Address correspondence to Joseph O. Lalah, Department of Chemistry, Maseno University, Maseno, Kenya; E-mail: josephlalah57@yahoo.com
Received October 9, 2009.

Sporocytophaga and *Corynebacterium* have been identified as discussed in other reports.[5,6] The main metabolites of carbofuran which have been found in various matrices are 3-hydroxycarbofuran and 3-ketocarbofuran.[3,5,6] They have been found to be more polar but equally toxic to target and non-target organisms.[7,8] 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.[7] Despite its efficacy as an agricultural pesticide, carbofuran in form of its 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).[9–12] A number of bird poisoning and high mortality cases involving Furadan have been reported previously in various countries including USA and Canada.[13–16] These cases include poisoning through food-chain, through secondary exposure and direct poisoning using laced baits.[17,18] Based on its high acute toxicity and threat to birds and other wildlife, its use has been restricted or banned in these countries.

In Kenya, Furadan is still imported mainly for use in seed dressing at the rate of 0.5–4 kg a.i/Ha for control of 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 in control of soil insects and nematodes and early foliar feeding insects in coffee, bananas, pineapples, pyrethrum nurseries and maize).[19] Furadan 350ST liquid is also marketed and used in dressing barley seeds. Approximately more than 23 tonnes of granules and more than 15,000 litres of concentrate are imported annually.[19] According to the Pest Control and Products Board of Kenya (PCPB), the national pesticide regulating authority in Kenya, Furadan, up to 10% a.i. is allowed into the country for restrictive use by informed users only.[20] However, a recent survey showed that Furadan is sold freely over the counter in many veterinary shops and that there is no proper monitoring or restrictions on sale and application.[20] Although Furadan was previously (between 1960's–1990's) mainly used in rice farming its application in other agricultural areas such as in maize and wheat farming has recently increased in Kenya.[20] Its marketing and usage in the country is currently presenting a number of potential non-target toxicity problems in areas where its usage is prevalent. Previous studies at Ahero paddy rice fields in Kenya indicated that in flooded soils most carbofuran residues tend to stay in the top 10 cm layer of the soil and surface water in the first three weeks after application due to its moderately high water solubility[21] and that it can leach easily. The granules can also be carried away from the surface of the soil where they are applied and be distributed into the aquatic environment. Therefore, its presence in the rice irrigation farms and in canals provides a route of exposure to fish, mammals and birds and problems of duck poisoning cases in rice irrigation schemes have been reported in Kenya since 1990's.[20,21] 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.[22–25] Application of Furadan in flooded soil has presented great risk to birds that sift waterlogged sediment in search of food. Poisoning of waterfowl from carbofuran granules exposure in flooded or partly flooded fields has been reported in other countries.[9] More problems have arisen primarily in acidic soils, presumably on account of longer half-life of carbofuran and its granules under those conditions.[9,21] In Kenya, Furadan poisoining events affecting birds, hyenas, camels, lions and hippos, supposedly involving indirect or direct poisoning by Furadan, have been reported since 2003. In particular, 187 African white-backed vultures (*Gyps africanus*) and hyenas were found dead near Athi River in 2004 where Furadan was supposedly used with laced meat.[20] Recent research studies showed the rapid decline of vultures over a three-year period in Laikipia district, Central Kenya, where raptors were observed to have declined by more 40% over the period from 2001–2003 and vultures including Bateleurs (*Terathopius ecaudatus*) accounted for most of the decline.[26] Vulture sightings declined by 77% during the same period. The rapid decline was attributed to consumption of poisoned baits with Furadan which the pastoralists are using increasingly to kill large predators that attack their livestock.[26]

To investigate the environmental distribution of carbofuran residues in agricultural farmlands, a study was initiated in 2007 in Isiolo and Laikipia districts where Furadan is used to control agricultural pests in maize, potatoes, beans and vegetables. The main aim of the study was to determine the concentrations of carbofuran residues in water, soil and plant samples from selected sites in the farmlands in the two districts and to show the impact of Furadan use on the local environment.

Materials and methods

Description of the study area and sampling sites

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. At this conservancy there is a continuous threat from wildlife poisoning by pastoralists and farmers living around the conservancies. LWC is also surrounded by small-scale farmers most of whom are found in Manyangalo and Ngare–Ndare forests. Small-scale

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	Donyo dam
3 Loperua farm	Upper Ngare Ndare	Mutarakwa farm	Pond A within GMWC
4 Meru central farm	Lower Ngare Ndare	Ol Moran farm	Pond B within GMWC
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.

irrigation farming takes place along the river banks. Adjacent to the conservancy flow two rivers namely River Ngare-Ndare and Ngare Sirgoi and a few ponds (the water points for the animals) are located within the protected area. For soil and plant samples, there were six sampling sites in farms around the conservancy (Table 1). For water, two major ponds within the conservancy and the two rivers were sampled (Table 1). GMWC, formerly Ol Ari Nyiro, in Laikipia is surrounded by pastoralists and crop farmers. The area is relatively very fertile and some farmers have comparatively larger farms (>2.5 Ha) and pesticides are used in the farms for better harvests. GMWC has a number of ponds located within and without it. The terrain is such that when it rains the runoff flows into the ponds making them potentially highly polluted with pesticide residues. Soil and plant samples were taken from various farms both within and outside the GMWC conservancy and the water samples were taken from four dams and two ponds (Table 1). The sampling sites lie within longitudes 37°30′E and 40°E and latitudes 0°15′N and 0°20′N. Environmental sampling was done in one rainy season (Season 1, October 2007) and one dry season (Season 2, June 2008).

Chemicals and reagents

Pure analytical pesticide standard mixture containing carbofuran and its two metabolites 3-hydroxycarbofuran and 3-ketocarbofuran (10 $\mathrm{mgL^{-1}}$ 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 high performance liquid chromatography (HPLC) water were obtained from Kobian Kenya 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 plants and animal tissues were also obtained from Kobian (K) Ltd, Nairobi, and were pre-extracted with n-hexane in a Soxhlet apparatus for 8 hours before use. Thimbles and filter papers used during extraction were pre-extracted first using 250 mL dichloromethane for 8 hours in a Soxhlet apparatus.

Sampling and laboratory residue analysis

About 100 grams of soil samples were collected from 30 different randomly picked spots within the farms at LWC and GMWC, respectively. 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 aluminium foil and transported in the icebox to the laboratory for analysis. Two litres each of water samples were collected randomly upstream and downstream from rivers Ngare-Ndare and Ngare-Sirgoi, respectively. In Laikipia district, 2-litre water samples were collected from the four dams and the two ponds, respectively. The water samples were then kept in brown bottles and transported in the icebox to the laboratory for analysis. Five hundred (500) grams of maize plant samples were collected randomly from 10 different spots within the agricultural farms (Table 1). The samples were kept in the icebox and transported to the laboratory for analysis.

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 ultraviolet light (UV) detection. This procedure was chosen after review of previous methods available in literature.[18,21,27,28] The soil samples were air-dried overnight in the laboratory at room temperature in darkness then 25 grams weighed. 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 samples were 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 5 mL at 20°C before clean-up. Clean up was done in glass columns with teflon stopcocks and glass wool plug at the bottom, 4 g of florisil and 2 g of anhydrous sodium sulphate at the top. For plant sample extracts, 2 g activated charcoal was added at the top of the column for decolorizing the plant pigments. Ten 10 mL of dichloromethane was added to condition the prepared column. The sample extract (2 mL) was added to the top and was 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).[18] The eluates were pooled, reduced to dryness in a rotary evaporator and then re-dissolved in 5 mL methanol for HPLC analysis. Five hundred (500) grams of air-dried plant samples were macerated, homogenized with 2 g Na_2SO_4, and extracted in a Soxhlet apparatus 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 5 mL at 20°C 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 gently with 100 mL dichloromethane for 15 min, and then repeating with 60 mL and 50 mL dichloromethane, respectively. The organic extracts were pooled and concentrated in a rotary evaporator to 5 mL at 20°C. Two grams of sodium sulphate was added to dehydrate the extracts before filtration. Clean-up was done as described above.

HPLC and GC-MS analysis

Carbofuran residues were analyzed both qualitatively and quantitatively by HPLC using an Agilent 1100 series model made in Japan equipped with an Ultra-Violet/Visible light (UV/VIS) detector at λ max = 254 nm and fitted with a Supelco C_{18} cartridge reverse phase column (250 × 4.6 mm ODS 5μ m), HPLC grade solvent: acetonitrile/water (4:1, volume) as the mobile phase at a flow rate of 1 mL min^{-1}. For recovery efficiency, 0.5 μg of carbofuran standard mixture was added to control samples of 500 mL of water, 25 g of soil and 500 g of plants, respectively, for analysis following the same procedures described above. The extracts were analysed using HPLC and the % recoveries were calculated. 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 mgL^{-1} and injection of 1μL 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 injections. Control samples for water, soil and plants from adjacent farms without chemicals were run but in all the cases there were no detectable levels of carbofuran, 3-ketocarbofuran and 3-hydroxycarbofuran. Detection limits were 0.001 μgL^{-1}, 0.001 μgg^{-1}, respectively. The percentage recoveries of the pesticide residues are given in Table 2. A few selected samples were taken to KEPHIS laboratory, Nairobi, for gas chromatography-mass spectrometry (GC-MS) analysis to confirm presence of carbofuran and its metabolites in the matrices by mass-spectrometry.

Table 2. Analytical (%) recoveries of residues.

compound	*River water*	*plants*	*soil*
Carbofuran	85 ± 7.10	78 ± 3.22	90 ± 6.72
3-hydroxycarbofuran	80 ± 6.45	75 ± 2.11	88 ± 4.20
3-ketocarbofuran	90 ± 3.44	89 ± 4.60	86 ± 2.33

n = 3.

Statistical analysis

Summary statistics (minimum, maximum, arithmetic means, and standard deviations) were derived. Analysis of variance ($P \leq 0.05$) and coefficient of variation were generated to determine the seasonal and site variations in mean residue concentration levels and to determine the relationship between the mean concentration levels of carbofuran (and its metabolites) in different sites and seasons.

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 soil, water and plant samples taken from the two areas, with recoveries above 75% (Tables 3–5). The data 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 high in soil, water and plants samples showing the metabolism of carbofuran in the matrices. These results indicate that HPLC analysis of carbofuran and its metabolites in environmental sample extracts after careful extraction of the residues from the matrix provides a reliable method for analysis of environmental exposure. Analysis of a few of the samples by GC-MS was done at the Kenya Plant Health Inspectorate (KEPHIS) laboratory in Nairobi and confirmed the identity of carbofuran and its two metabolites in the matrices. The concentrations in water were high and provided enough evidence to show the carbofuran menace to animals using the ponds and the two rivers. The results showed that for determination of environmental contamination from Furadan use, analysis of the two carbofuran 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 dissipation from the site of application can be quite rapid.

Table 3. Seasonal, regional and site concentrations (mean ± sd) of carbofuran and its metabolites (in mgL^{-1}) in water samples from Isiolo and Laikipia districts in Kenya.

site	*season*	*carbofuran*	*3-ketocarbofuran*	*3-hydroxycarbofuran*
Isiolo				
1	1	0.038 ± 0.005	0.086 ± 0.011	0.183 ± 0.031
1	2	0.016 ± 0.002	0.423 ± 0.072	0.194 ± 0.024
2	1	0.016 ± 0.003	0.013 ± 0.001	0.114 ± 0.031
2	2	0.010 ± 0.005	0.088 ± 0.021	0.186 ± 0.014
3	1	0.026 ± 0.010	0.052 ± 0.014	0.172 ± 0.033
3	2	0.050 ± 0.010	0.077 ± 0.020	0.282 ± 0.046
4	1	0.015 ± 0.002	0.025 ± 0.003	0.218 ± 0.026
4	2	0.060 ± 0.020	0.040 ± 0.010	0.068 ± 0.022
5	1	bdl	bdl	bdl
5	2	bdl	bdl	bdl
6	1	bdl	0.013 ± 0.001	bdl
6	2	bdl	bdl	bdl
Laikipia				
1	1	0.992 ± 0.199	0.274 ± 0.039	0.252 ± 0.113
1	2	0.018 ± 0.030	0.764 ± 0.171	1.022 ± 0.192
2	1	1.034 ± 0.0314	0.359 ± 0.049	0.403 ± 0.101
2	2	0.070 ± 0.010	0.742 ± 0.028	0.487 ± 0.067
3	1	0.747 ± 0.178	0.236 ± 0.018	0.576 ± 0.042
3	2	0.023 ± 0.010	0.734 ± 0.023	0.377 ± 0.180
4	1	0.546 ± 0.038	0.148 ± 0.022	0.334 ± 0.047
4	2	0.012 ± 0.001	0.427 ± 0.173	0.220 ± 0.070
5	1	1.232 ± 0.300	0.406 ± 0.117	bdl
5	2	0.430 ± 0.012	0.800 ± 0.256	1.482 ± 0.580
6	1	1.823 ± 0.478	0.516 ± 0.111	0.355 ± 0.101
6	2	0.237 ± 0.111	0.894 ± 0.321	1.546 ± 0.421
	Mean (Isiolo)	0.011	0.068	0.118
	Mean (Laikipia)	0.592	0.525	0.646
	CV%	4.93	2.01	2.14

Note: for the names of the sites, refer to Table 1.
bdl: below detection limit, n = 3.

Pesticide residues in water samples

Rivers Ngare-Ndare and Ngare-Sirgoi in Isiolo and Kinamba dam and a dam inside the Gallman Memorial W C, chosen for this study, are adjacent to the conservancies located within the catchments where good agricultural activities are likely to contribute to residue contamination. Some of the main agricultural activities around the area of study include maize, beans, potatoes and vegetable farming. Some agricultural activities in the upper parts of the two rivers in Isiolo district which rely heavily on agrochemical application involved irrigation using river water and these could contribute to run-off of pesticide residues. More residues were detected during the rainy season in the water samples (Table 3). The concentration of carbofuran and its two metabolites were higher in Laikipia water samples than Isiolo. Its water solubility and rapid degradability accounts for the decrease in concentration of carbofuran downstream. The metabolism of carbofuran in the environment accounts for the two metabolites detected in the water. Generally the results showed a marked increase in the concentration of metabolites from the wet to dry season. The metabolites once formed tend to persist in the environment longer than the parent compound and 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 contaminant level (40 μgL^{-1}) and the European Union drinking water limit (1 μgL^{-1}) and this indicated high risk for drinking purposes depending on spatial contamination.[29] Carbofuran has been reported to be persistent in surface water and can contaminate canals, streams and lakes.[21] Carbofuran has also been reported to have very high ubiquity score in ground water in other countries and has been detected in ground water at a comparable mean concentration of 0.0231 mg L^{-1} in 43.2% of samples in Punjab in India.[30] It has been detected in streams at about 0.001 mg L^{-1} and in ground water in the range 0.001–0.030 mg L^{-1} in USA.[31,32] Due to its high water solubility, carbofuran runoff from fields where it had been applied caused localised fish kills in other areas.[33] Concentrations

Table 4. Mean concentrations (mgkg^{-1} dry weight) of carbofuran and its metabolites in plant samples and their statistical analysis.

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
Means seasons	0.307	0.061	
CV%		13.444	
LSD (<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 (<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 (<0.05)		0.011	
Interactions		0.014	

Table 5. Mean concentrations (mgkg^{-1} dry weight) of carbofuran and its metabolites in soil samples and their statistical analysis.

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 (<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 (<0.05)		0.146	
Interactions		0.205	
3-hydroxycarbofuran			
Isiolo	0.191 ± 0.073	0.676 ± 0.057	0.433
Laikipia	0.207 ± 0.074	1.181 ± 0.190	0.694
Means seasons	0.199	0.928	
C%		0.61	
LSD (<0.05)		0.011	
Means seasons		0.014	

in flooded soils in British Columbia where it has been applied has also shown that puddle water and soil can contain high concentrations of 1.7 mg L^{-1} and 1.96 mg L^{-1}, respectively, with massive deaths of water birds such as ducks and mallards.[9] This indicates that immediately following rainfall, extensive runoff to nearby water sources can cause very high risks to any aquatic organisms, given its low LC$_{50}$ values for various fish in the range of 0.13–1.42 mg/L, based on 72–96 hr tests.[34] Our results therefore show that the water sources, including rivers Ngare Ndare and Ngare Sirgoi, in Isiolo and Laikipia where Furadan is being used intensively are quite polluted and their ecology is threatened in comparison with those reported cases in other countries. There is also high potential toxicity threat to animals that drink from the dams and ponds within the conservancies in Laikipia where more Furadan is used in farming.

Pesticide residues in plant samples

Plant samples showed presence of carbofuran and its two metabolites in both Isiolo and Laikipia (Table 4). Carbofuran 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, of stems and leaves where insecticidal concentration are attained. It is therefore expected that it could find its way into other organisms through the food chain though at low concentrations. There was higher concentration of carbofuran in plants in the first sampling (October) than in June because most crops were still green in October as opposed to June and therefore high insecticidal concentration was expected in the leaves. 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.[35] reported that 3-hydroxycarbofuran in sugarcane plant remained higher and persisted longer than that of the parent compound. Carbofuran like other systemic pesticides can get absorbed by the plant roots so that within 7–10 days after application it is found in the plant leaves.[16] Studies by Crocker[16] reported that the residues often decline due to breakdown but this happens rapidly at the beginning and then slows down so that many residues ultimately persist for longer than predicted by first-order kinetics of dissipation. Caro et al·[36] found that the highest concentration of carbofuran was in the leaves with very little in the ears of corn and estimated that 0.14% of applied carbofuran was taken up by the plant. Eisler[37] reported that the principal metabolite is 3-hydroxycarbofuran which is hydrolysed to 3-ketocarbofuran and this is further rapidly hydrolysed to 3-ketocarbofuran-7-phenol, a much less toxic metabolite unlikely to be detected above trace levels in plants. The concentration levels of carbofuran and its metabolites in this study compared well with their concentrations in *Zea mays* after 117 days from time of application as given in the Handbook for Chemical Risk Assessment (Table 6).[37] The concentrations found in the plants taken from the farms

Table 6. Comparing concentrations (mgkg^{-1} 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.[37]

would therefore be potentially toxic to cattle depending on their ADI but fortunately the farmers would never allow cattle or any other herbivores in their farms.

Pesticide residues in soil samples

The concentrations of carbofuran and its two metabolites were significantly high in the surface farm soil and indicated usage of Furadan in the two districts (Table 5). Since the levels of residues found in soil in the two regions are significant, contamination of water through run-off and secondary transfer through soil organisms to other smaller species such as birds is possible. The concentration of carbofuran was found to be higher in surface soil during the wet season than during the dry season since this pesticide dissolves easily and can be found bound in the soil matrix within the shortest time after application. Previous studies done by Lalah et al.[5] 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. 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.[15] 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 in other countries.[9] Our study indicated presence of residues in the soil and, if there is localized concentration, it is possible to present risk at the sites of application in the farms. Both regional and seasonal variation ($P < 0.05$) in mean concentration levels of carbofuran, 3-ketocarbofuran and 3-hydroxycarbofuran, respectively, were found for soil samples from the various sites in the two regions. Furadan granules left on soil surface have been found to cause high mortality in organisms that sift for food from the soils such as small birds through direct exposure by consumption of the granules (as in song birds) or indirect exposure by consumption of contaminated invertebrates, notably earthworms.[9] Although no granules were found in the soil during sampling, the concentrations of carbofuran and its metabolites in the soil in the farms indicate significant threats through leaching and runoff to water sources when it rains or through contamination of the soil organisms.

Conclusion

The significantly high concentration levels of carbofuran and its metabolites, 3-ketocarbofuran and 3-hydroxycarbofuran, found in various matrices, demonstrate that Furadan was used extensively in the two areas especially in Laikipia 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 mammals. The results of this study showed that HPLC combined with GC-MS is useful for analysis of carbofuran residues in samples obtained from areas where Furadan has been used and the investigations should include its two toxic metabolites, 3-hydroxycarbofuran and 3-ketocarbofuran.

Acknowledgments

This study was kindly funded by The Pelegrine Fund, through the Africa Project. The authors also wish to thank Simon Thomsett of Athi River conservancy, Ian Craig, Chege, Joanne, Richard and all staff of Lewa Conservancy, Kuki Gallman and Philip Ochieng of Gallman Memorial Wildlife Conservancy, Mbirikani and Kilimanjaro ranches, Darcy Ogada and all staff of the National Museum of Kenya, technical staff of Chemistry Department, Maseno University. We also thank the German Academic Exchange Service (DAAD) for a visiting fellowship to J.O. Lalah for research at Bayreuth University, Germany, which enabled the preparation of the manuscript.

References

[1] Hodgson, E.; Roe, R.M.; Motoyama, N. Pesticides and the Future: Toxicological studies of risks and benefits. Rev. Pestic. Toxicol. **1991**, *1*, 3–12.

[2] Hayes, W.J. Jr. Metabolism of pesticides. In *Pesticides Studied in Man*; Williams, I.H. and Wilkins, J.P.G. Eds.; McMillan: Baltimore, 1982.

[3] Ecobischon, D.J. Toxic Effects of pesticides. In *Casarett and Doull's Toxicology: The Basic Sciences of Poisons*, 4th Ed; Amdur, M.O., Doull, J., Klaasen, C.D., Eds.; Macmillan Press: New York, 1993; pp 565–589.

[4] Suett, D.L. Effects of formation of dimethylamine and diethylamine in soil treated with pesticides. Crop Protect.**1986**, *5*, 16–167.

[5] Lalah, J.O.; Kaigwara, P.N.; Getenga, Z.; Mghenyi, J.M.; Wandiga, S.O. The major environmental factors that influence rapid disappearance of pesticides from tropical soils in Kenya. Toxicol. Environ. Chem. **2001**, *81*, 161–197.

[6] McRae, I.C. Microbial metabolism of pesticides and structurally related compounds. Rev. Contam. Toxicol. **1989**, *109*, 1–34.

[7] Hassall, K. *The Biochemistry and uses of pesticides*, 2nd Edition; Macmillan Press Ltd: Hong Kong, 1990; pp 140–143.

[8] Hayes, W. *Principles and methods of toxicology*, 4th Ed.; Taylor and Francis: London, 2001; pp 587–591.

[9] Mineau, P. The hazard of carbofuran to birds and other vertebrate wildlife. Canadian Wildlife Service Technical Report No. 177. Headquarters, Canadian Wildlife Service Environment Canada: Ottawa, Canada, 1993; 96 pp.

[10] Mineau, P.; Fletcher, M.R.; Glaser, L.C.; Thomas, N.J.; Brassard, C.; Wilson, L.K.; Elliot, J.E.; Lyon, L.; Henny, C.J.; Bollinger, K.; Porter, S.L. Poisoning of raptors with organophosphorous and carbamate pesticides with emphasis on Canada, US and U.K. J Raptor Research **1999**, *33*(1), 1–37.

[11] Mineau, P. Pesticide Acute Toxicity: Reference Values for Birds, Rev. Environ. Contam. Toxicol. **2001**, *13*, 71–80.

[12] Hopkins, A.W.; Scholz, L.N. Ecotoxicolgy of anticholinesterase pesticides: data gaps and research challenges. Environ. Toxicol. Chem. **2006**, *25*(5), 1185–1186.

[13] Allen, G.T.; Veach, J.K.; Stroud, R.K.; Vendel, C.G. Winter poisoning of coyotces raptors with Furadan-laced carcass baits. J. Wildlife Diseases **1996**, *32*, 385–389.

[14] Elliot, J.E.; Langlier, K.M.; Mineau, P.; Wilson, L.K. Poisoning of bald eagles and red-tailed hawks by carbofuran and fensulfothion in the Fraser Delta of British Columbia. Canada. J. Wildlife Diseases **1996**, *32*, 486–491.

[15] Mineau, P. 2003. Avian Species. In *Encyclopedia of Agrochemicals*; Plimmer, J.R., Gammon, D.W., Ragsdale, N.N., Eds.; John Wiley & Sons Inc.: New York, 2003; pp 1–27.

[16] Crocker, D.R. Estimating the exposure of birds and mammals to pesticides in longer-term risk assessments. Ecotoxicol. **2005**, *14*, 833–851.

[17] Vyas, N.B.; Spann, J.W.; Albers, E.; Patterson, D. Pesticide-laced predator baits: considerations for prosecution and sentencing. Environ. Lawyer **2003**, *9*, 589–608.

[18] Vyas, N.B.; Spann, J.W.; Hulse, C.S.; Bauer, W.; Olson, S. From the Field: Carbofuran detected on weathered raptor carcass feet. Wildlife Society Bull. **2005**, *33*, 1178–1182.

[19] PCPB. Pest Control Products Board (PCPB) of Kenya annual report 1992. Ministry of Agriculture: Nairobi, Kenya, 1992.

[20] Otieno, P.O. Monitoring carbofuran residues in Laikipia and Isiolo districts in Kenya for ecological risk assessment. MSc thesis, Department of Chemistry, Maseno University, Maseno, Kenya, 2009.

[21] Lalah, J.O.; Wandiga, S.O. Distribution and dissipation of carbofuran in a paddy field in Kano plains of Kenya. Bull. Environ. Contam. Toxicol. **1996**, *56*, 584–593.

[22] Bishop, C.A.; Ng, P.; Mineau, P.; Quinn, J.S.; Strugger, J. Effects of pesticide spraying on chick growth, behaviour, and parental care in tree swallows (Tachycineta bicolour) nesting in an apple orchard in Ontario, Canada. Environ. Toxicol. Chem. **2000**, *19*, 2286–2297.

[23] Bishop, C.A.; Collins, B.; Mineau, P.; Burgess, N.M.; Read, W.F.; Risley, C. Reproduction of carvity-nesting birds in pesticide-sprayed apple orchards in southern Ontario, Canada, 1988–1994. Environ. Toxicol. Chem. **2000**, *19*, 588–599.

[24] Richards, N.L.; Mineau, P.; Bird, D.M. A risk assessment approach to DDE exposure based on the case of the Eastern Screech-owl (*Megascops asio*) in apple orchards of southern Quebec, Canada. Arch. Environ. Contam. Toxicol. **2005**, *49*, 403–409.

[25] Mineau, P.; Downes, C.M.; Kirk, D.A.; Byane, E.; Csizy, M. Patterns of bird species abundance in relation to granular insecticide use in the Canadian prairies. Ecoscience **2005**, *12*, 267–278.

[26] Ogada, D.L.; Keesing, F. Rapid decline of vultures over a three-year period in Laikipia, Central Kenya. J. Raptor Res. (submitted) **2009**.

[27] Yang, S.S.; Goldsmith, A.I.; Smetena, I. Recent advances in the residue analysis of N-methylcarbamate pesticides. J. Chromatog. A **1996**, *754*, 3–16.

[28] Takino, M.; Yamaguchi, K.; Nakahara, T. Determination of carbamate pesticide residues in vegetables and fruits by liquid chromatography-atmospheric pressure photoionization-mass spectrometry and atmospheric pressure chemical ionization-mass spectrometry. J Agric. Food Chem. **2004**, *52*, 727–735.

[29] Pogacnik, L.; Franko, M. Determination of Organophosphate and carbamate pesticides in spiked samples of tap water and fruit juices by a senor with photothermal detection. Biosensors and Bioelectronics **1999**, *14*, 569–578.

[30] Tariq, M.H.; Afzal, S.; Hussain, I. Pesticides in shallow ground water of Bahawalnagar, Muzafargarh, D.G. Khan and Rajan Pur districts of Punjab, Pakistan. Environ Int. **2004**, *30*, 471–479.

[31] Cohen, S.Z. Pesticides in ground water in the United States: Monitoring, modelling and srisks, the US perspective. Environ. Sci. Health, Part B **1986**, *31*(3), 345–352.

[32] Kimbrough, R.A.; Litke, D.W. Pesticides in streams draining agricultural and urban areas in Colorado. Environ. Sci. Technol. **1996**, *30*, 908–916.

[33] Trotter, D.M.; Kent, R.A.; Wong, M.P. Aquatic fate and effect of carbofuran. Crit Rev. Environ. Contam. **1991**, *21*(2), 137–176.

[34] Eisler, R. Carbofuran hazards to fish, wildlife and invertebrates: A synoptic review. US Fish Wildlife Service, Biology and Reproduction. **1985**, *85*(1.3), 36pp.

[35] Raminderjit, S.B.; Ranjinder, L.K.; Ranjit, S.D. Estimating of Residues of carbofuran and its metabolites in sugarcane and soil by derivation with 1- Flouro-2, 4-Dinitribenzene and GC with NPD. J. AOAC Int. **2000**, *83*, 569–573

[36] Caro, J.H.; Taylor, A.W.; Freeman, H.P. Comparative behaviour of dieldrin and carbofuran in the field. Archives Environ Contam. Toxicol. **1976**, *3*, 437–447.

[37] Eisler, R. *Handbook of chemical risk assessment*; Volume 2; Lewis Publishers: Washington DC, 2000.