TECHNICAL UNIVERSITY OF KENYA

International Workshop on Poison Investigation and Vulture Conservation in Africa and Andalucia

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Participant:

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Profile

Academic and Research Interests:

- Lecture topics: analytical/environmental/inorganic chemistry (electronic structure and bonding, transition metals and coordination chemistry).

Research areas:

- Trace organic contaminants such as PAH's, PCBs, dioxins, pesticide residues and p-nonylphenols: sources, environmental distribution, fate in aquatic media and in aquatic food chain; exposure to aquatic organisms and human.
- Pesticides: - application and effectiveness; behavior and fate in soil and water environments; residual lifetimes, effects on catchments and non-target organisms; impact on wildlife and birds; application and efficacy in malaria vector control.
- Heavy metals and other inorganic contaminants: sources, environmental distribution and speciation; coordination chemistry: role of dissolved organic matter in transport and bioavailability of contaminants in freshwaters; water quality; validation of methods of analysis and speciation studies of Hg, As and Sn.

INTEGRATED ENVIRONMENTAL MONITORING EXPERIENCE TO ASSESS VULTURE POISONING

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Background Information

- Kenya's population of approx 45 million people with a projected fast population growth rate of 2.56% in 2014; 18 million income < 1.25 US$ per day.
- The main activities are: Agriculture (contributes 27% GDP compared with 1% for UK and USA) and therefore impact of agrochemicals more significant; Tourism (a major foreign exchange earner).
- Endowed with abundant rich flora and fauna, lakes, rivers etc.
- Wildlife diversity and population very unique and very critical in its economic development.
- The country is lucky to have an increasing number of generally educated people with one of the best school enrolments in Africa.

Background Information contd.

- Adequate infrastructure in form of institutions and laws
- But; poor implementation of policies partly due inadequate capacity and specialized trained manpower, especially in the areas of science and technology.
- e.g. education – existing vocational training facilities, middle level colleges and universities (but lack of proper implementation hampers utilization of these facilities)
- For the country to achieve sustainable socio-economic development, there is need for environmental monitoring and research.
- This would need development of infrastructural capacity, development in scientific skills and necessary tools used for environmental research and monitoring.

Problems with Agrochemicals

- With expansion in agriculture and agro-based activities, increase in application of agrochemicals in form pesticides and chemical fertilizers is expected.
- Leave toxic residues that negatively affect the environment, the people, wildlife and other species.
- Due to rapid increase in population and need for agricultural expansion, human-wildlife conflicts have recently arisen.
- This has led to illegal misuse of pesticides on wildlife by pastoralists and farmers.
- See Table 1.
- All these issues have severe impacts on important species and can lead to their high mortalities and extinction.

Importation of pesticides in Kenya (PCPB)

Insecticides: for agriculture/food crops, veterinary and public health vector control

fungicides: mainly for large scale farming such as potatoes and coffee farming.

Herbicides: large scale farming such as tea and sugarcane farming.

Trend: unpredictable; depends on foreign exchange & status of economy/ agriculture.

Table 1

Category	2006/7		2007/8		2008/9		2009/10	
	Quantity in tones	Value '000 000 Kshs	Quantity in tones	Value '000 000 Kshs	Quantity in tones	Value '000 000 Kshs	Quantity in tones	Value '000 000 Kshs
Insecticide	2475	1181	2887	3909	2995	2079	3181	2493
Fungicide	3190	1251	2651	602	2340	3153	2415	3874
Herbicide	1859	324	2289	206	2933	944	1840	939
Others	1225	362	1330	191	1413	1167	1396	918
Total	**8749**	**3443**	**9157**	**4908**	**9681**	**7343**	**8832**	**8224**

Monitoring the threat of Pesticides to Raptors (African Gyps Vulture species) in Kenya: case studies

INTRODUCTION

- Raptors including vultures are meat eaters with a hooked beak for cutting and tearing meat.
- Many raptors will either catch live prey while others prefer scavenging for carrion.
- Raptors are valuable indicators of the health of the environment.
- Thriving populations generally means that there is plenty of food available & the rest of the food chain is healthy.
- Two case studies of environmental monitoring were done in 2008 and 2012, respectively, for forensic proof of illegal poisoning of Vultures in Isiolo and Laikipia districts in Kenya and demonstration of pesticide use and impact on aquatic food chain in an intensively cultivated horticultural farming near Lake Naivasha.

Introduction contd

- Raptors bring economic benefits to the country through ecotourism hence significantly contribute to the country's GDP.
- One raptor species, the Vulture, is also important in cleaning environment.
- Despite this, raptors are on a rapid decline and if not checked a few species will face extinction.

Reasons for decline

- Habitat loss, Electrocution
- Accidents of windmills, prosecution
- Poisoning: *pastoralists and crop farmers.*
- Chronic poisoning and changes in growth and development

However, poisoning by chemicals (pesticides) remains widespread and disastrous near wildlife conservancies. In 2004, 187 species (vultures (white-backed *Gyps Africanus*) and hyenas, reported killed by poisoning in Athi River (carbofuran?)

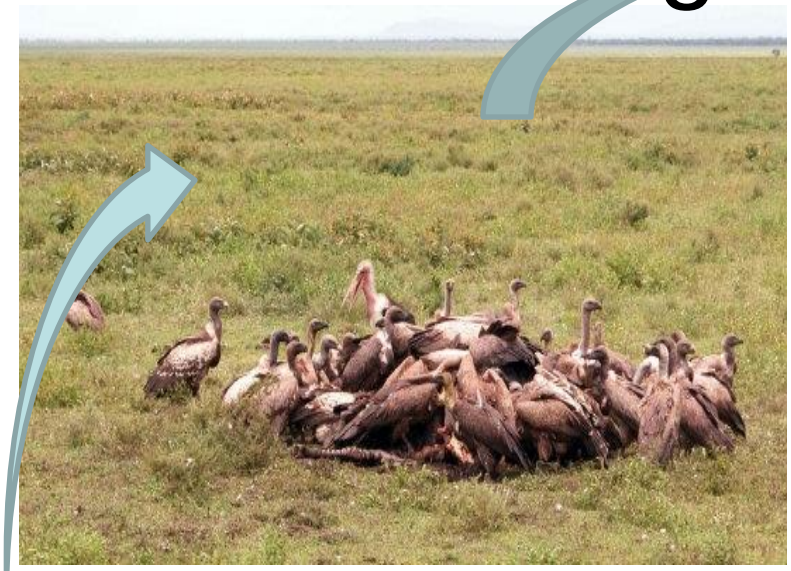
Decline in raptors in agricultural areas (Laikipia): 40% decline from 2001 to 2003; mostly vultures and bataleurs are affected (Ogada *et al*. 2009).

- Presence of pesticides & other Endocrine Disruptors in the food chain contribute to rapid decline. Residues in food chain in lakes in Kenya.

In the first case study: Though pesticides were widely implicated, there was no documented forensic evidence to show which pesticides were responsible (Furadan was suspected). Wildlife conservationists in Kenya initiated a study (funding from Peregrine Fund, USA etc.)

In the second case study: Intensive application of pesticides (chlorpyrifos, carbofuran etc) is practised and there was need to develop rapid analytical techniques for determination of residues in fish, water and sediment.

Poisoning incidents in Kenya



Vultures are exposed to poisoned lions/contaminated carcass

- **Lions/predator animals are targeted for poisoning as a revenge/baits laced with acutely toxic pesticide – carbofuran (granules of Furadan (5%a.i.)).**
- **Vultures then scavenge on poisoned carcass & because of their feeding habits they get poisoned thro secondary exposure resulting into decline in population.**
- **Although human survey implicated Furadan, there was no scientific evidence to show that they were exposed to it before death.**

Photo of dead vultures in Isiolo (*Courtesy of Lewa wildlife conservancy)* Acute toxicity of carbofuran; fulvous whistling ducks LD_{50} 0.238 mg/kg, Mallard 0.51 mg/kg and House sparrow LD_{50} 1.3 mg/kg (Anonymous 2007a).

Additional poisoning Scenario

African fish-eagle preying on fish

Contaminated tilapia fish

Flower farms use enormous amount of pesticides

- **Pesticides runoff & direct discharge of wastewater results into;**
- **Contamination of fish thro' bioaccumulation of lipophilic pesticides residues.**

Objectives of the Studies

1. Determine the possible toxic pesticides responsible for mortality of vultures *Gyps Africanus* through forensic analysis of carcass and other tissues (Target: carbofuran and its residues).

2. Determine through laboratory analysis the presence and levels of likely toxic pesticides in fish, which are commonly preyed on by the African-fish Eagle (target: areas of intensive use of chlorpyrifos).

Justification of the Studies:

1. In Kenya, alleged cases of misuse of Furadan as a poison to solve the human-wildlife conflict had been on the increase.

2. Data generated from the intended forensic investigation will form a strong scientific evidence to call for a ban of this pesticide in order to save and promote wildlife conservation

3. Cases of reported concerns impact of agrochemicals including pesticides applied in farms located close to the lake, involving both occupational exposure accidents and discharge into the lake had been raised.

FIRST CASE STUDY SITE

- Monitoring Laikipia and Isiolo Districts in Central Kenya.
- Furadan 5g was supected; found by survey and in vet stores. (Active ingredient: Carbofuradan).
- Carbofuran (AChE inhibitor) is an effective systemic and contact pesticide used both as insecticide and nematicide, cheap widely used in Kenya previously.
- Used to control pests in rice, maize, cabbages, coffee and other crops.
- Doesn't biaccumulate, non-persistent and works within 20 minutes after application, very toxic to invertebrates, birds and can also be acutely toxic to human.
- Its impact and many studies had been done in US, UK and Canada, involving both normal use and misuse.
- USEPA (1991) report says that carbofuran has been responsible for more birds mortalities than any other pesticide (Anonymous, 2007a).
- Due to this USEPA cancelled the registration of carbofuran in 2006.
- Focus on production and use shifted to India and African

THE SECOND CASE STUDY SITE

- Lake Naivasha is located on the floor of the Eastern Rift Valley and is ~160 km^2 in area.
- Due to climate-related factors and economics reasons, the nature of agriculture in the lake basin has changed from livestock rearing, ranching and sisal-cultivation to irrigated farming, mainly of horticultural crops
- The observed change in land use pattern has seen increase in pest population, distribution and resistance and intensive application of pesticides [2].
- Agrochemical usage in floriculture and vegetables has increased in farming mainly by irrigation of water diverted from the lake.
- L. Naivasha catchment, a big agro-industry: Kenyan flower export accounts for ~50% of all the flowers used in UK.
- However, discharge of waste water from greenhouses and agricultural runoff contributes to pollution of the lake.
- Studies show that application of pesticides, has led to detection of their residues in ground water, natural surface (lake) water, sediment as well as fresh water organisms [5].
- Due to the magnitude of the application of these pesticides in the catchment of L. Naivasha a potential problem exists for a considerable quantity of their residues to find their way into lake thus the need for continuous environmental monitoring..

The Second Case Study site contd.

Chlorpyrifos-ethyl and diazinon used intensively and known to be potential endocrine disruptor & easily bio-concentrate in different non-target fish and other aquatic organisms [6].

Use of similar agrochemicals esp. herbicides and insecticides in other large-scale agro establishments in Kenya.

Literature Review

- American Birds Conservancy, Defender of Wildlife and Bird Conservation Alliance, carbofuran has been the greatest threat. (Anonymous, 2007a).
- The death of vultures could be as a result of direct ingestion, inhalation or feeding on baits that are laced with highly toxic insecticides (Nimish et al., 2005).
- To establish the cause-effect relationship - the dead birds must be recovered.
- If poisoning is suspected then biochemical and chemical analysis are conducted (Nimish et al., 2005).
- For rapid screening, brain cholinesterase activity is measured for both OPs and carbamates poisoning.
- Chemical residue analysis of the GI tract, crop and gizzard. (Hill and Fleming, 1982).

Literature review contd

- Scavenging, decomposition and pesticide degradation may render these conventional matrices unsuitable for analysis (Vyas, 1999; Vyas et al., 2003).
- Feet or claws and beaks can used even after decomposition of the birds' carcass (Nimish et al., 2005).
- Carbofuran metabolises by hydrolysis, oxidation and conjugation to form other metabolites (Lalah and Wandiga, 1996). Major metabolites: 3-hydroxy carbofuran, 3-ketocarbofuran, carbofuran phenol
- Carbofuran persists moderately in the soil with half-life of a few weeks in tropical but this can be up to 378 days in temperate soil conditions (Lalah et al 2001).

METHODOLOGY

- Field visits and consultation with stakeholders farmers, wildlife conservationists, PCPB and KWS.
- Literature survey and questionnaires.
- Documentation of all data from the visits.
- Chemical analysis of residues in samples by HPLC, GC-MS.
- Statistical data analysis.

Analytical Methodology

- For carbofuran: collection soil, water, carcas and bird tissue samples from the poisoning sites in farms, water near conservancies

Fish ***Niloticus leucosticus,*** *sediment and water* samples from Lake Naivasha were obtained and levels of bioconcentrated pesticides residues determined.

Focus was on Chlorpyrifos in muscle and gills; levels can be more in organs

- Analysis of carbofuran and its most common metabolites in soil was done using Lalah *et al*. 1996 method, in Maseno University.

Fish samples were extracted and analyzed in Helmholtz Zentrum Muenchen (Wan *et al*., 2001; Kristenson *et al*., 2006)

Materials and Methods

Carcass of Vultures found

Beaks and claws cut off from poisone Vultures

Crashed and ground according to method used by Vyas et al. 2005

Carbofuran-HPLC-UV analysis at 254 nm- GC-MS at Kephis.

Sample Extraction

- 25 grams weathered feet, GI tract and crop cut to approx 0.6 cm pieces then crashed.
- Each Sample extracted 3 times with 1:1 acetone: dichloromethane (Vyas et al., 2005).
- Reduced to 5 ml in a rotary evaporator at about 20° C
- 25 grams of soil weighed, mixed with 20g anhydrous sodium sulphate and homogenized in a mortar with pestle then sieved for extraction in a Soxhlet extractor for 4 hours with 130 ml mixture of DCM and acetone; (10:3 volume) (Lalah and Wandiga).
- 500 g of plants samples macerated, homogenized, and extracted using soxhlet apparatus. 150 ml solvent mixture of DCM and acetone (10:5) will be used.
- 500 ml of water partitioned with dichloromethane in a 1-litre separatory funnel, gentle shaking with 100 ml DCM for 15 min, and then repeating with 50 ml and 60 ml DCM respectively.
- Extract pooled and concentrated in a rotary evaporator. 2 g of sodium sulphate will be added and then filtered.

Clean-up and instrument analysis

- Clean-up performed using 4g florisil in small glass columns.
- Eluted with 10 ml DCM, then 10 ml DCM /acetone (95:5,volume) and 10 ml acetone/DCM (10:90 volume).
- Pooled and reduced to dryness in a stream of nitrogen then dissolved in 5 ml methanol.
- Determined Qualitatively and quantitatively by HPLC-UV reverse phase ODS column.
- Mobile phase acetonitrile; water 4:1v/v, HPLC grade at a flow rate of 1 ml min-1.
- Confirmation of residues by GC-MS.
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- **For Naivasha study:** fish, water and sediment sample extraction and clean-up using SPE and MSPD and analysis by micro-HPLC DAD.

Results for tissue and soils analysis

Site	Carbofuran	3-Ketocarbofuran	3-Hydrocarbofuran
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	0.050 ± 0.010	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
Crop samples			
Naivasha	bdl	0.199 ± 0.020	0.087 ± 0.006
Athi River	bdl	0.080 ± 0.002	0.096 ± 0.005
Soil[b] n = 14	0.01 ± 0.004	0.800 ± 0.021	0.115 ± 0.020

Results in mg/kg dry wt; LOD= 0.005 for carbofuran, 0.008 for 3-Ketocarbofuran & 0.006 for 3-Hydroxycarbofuran (ppm)

[b] *Soil samples from site of poisoning with laced camel carcass n= 32*

Table 2 Levels of chlorpyrifos residues in fish

Season	Mean	Kasarani	Oserian	Sher Karuturi	Overall Seasonal Mean
Wet	Mean Conc.	0.0028 **bdl-0.0089**	0.0032 **bdl-0.0087**	3.0 **bdl-0.0087**	**0.0030^{A}**
	Mean Lipid	2.6 **bdl-9.3**	3.2 **bdl-11.7**	3.8 **bdl-8.9**	3.2^{A}
	Mean Size	206.4 **121-250**	158.4 108-250	186.1 100-250	191.2^{A}
Dry	**Mean Conc.**	0.0012 **bdl-0058**	0.0012 **bdl-0.0048**	0.0009 **bdl-0.008.7**	**0.0011^{B}**
	Mean Lipid	1.4 **0-4.7**	2.3 **bdl-9.3**	2.2 0-11.2	2.0^{B}
	Mean Size	148.8 **100-232**	178.5 **105-234**	180.3 **100-212**	147.9^{B}
Mean Site Conc.		**0.0020^{A}**	**0.0022^{A}**	**0.002^{A}**	**LSD; p < 0.05 1.4**

Season F = 30.15 p < 0.0001; LOD = 0.001mg/Kg wet weight; Lipid is %, conc in mg/kg wet wt

Summary of results

- The use of Furadan was confirmed through a survey conducted during the study
- Soil, plants and water samples from the farms, two rivers flowing through the farms, ponds and dams near wildlife conservancies showed contamination with various concentrations of carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran, by HPLC and confirmation with GC-MS (Otieno et al. 2010a).
- Varying concentrations of carbofuran, 3-ketocarbofuran and 3-hydroxycarbofuran residues detected by HPLC in beaks, feet of vultures and soil sample from poisoning site.
- Varying concentrations of 3-ketocarbofuran and 3-hydroxycarbofuran detected in crop content and laced muscle carcass from site of poisoning.
- Residues of chlorpyrifos and diazinon found in water, sediment and muscle and gills of fish samples from various parts of Lake Naivasha.
- Only chlorpyrifos detected in fish muscle and gills in samples from various parts of the lake.
- Use of ELISA kit for chlorpyrifos was compared and tested for water and sediment for rapid screening.

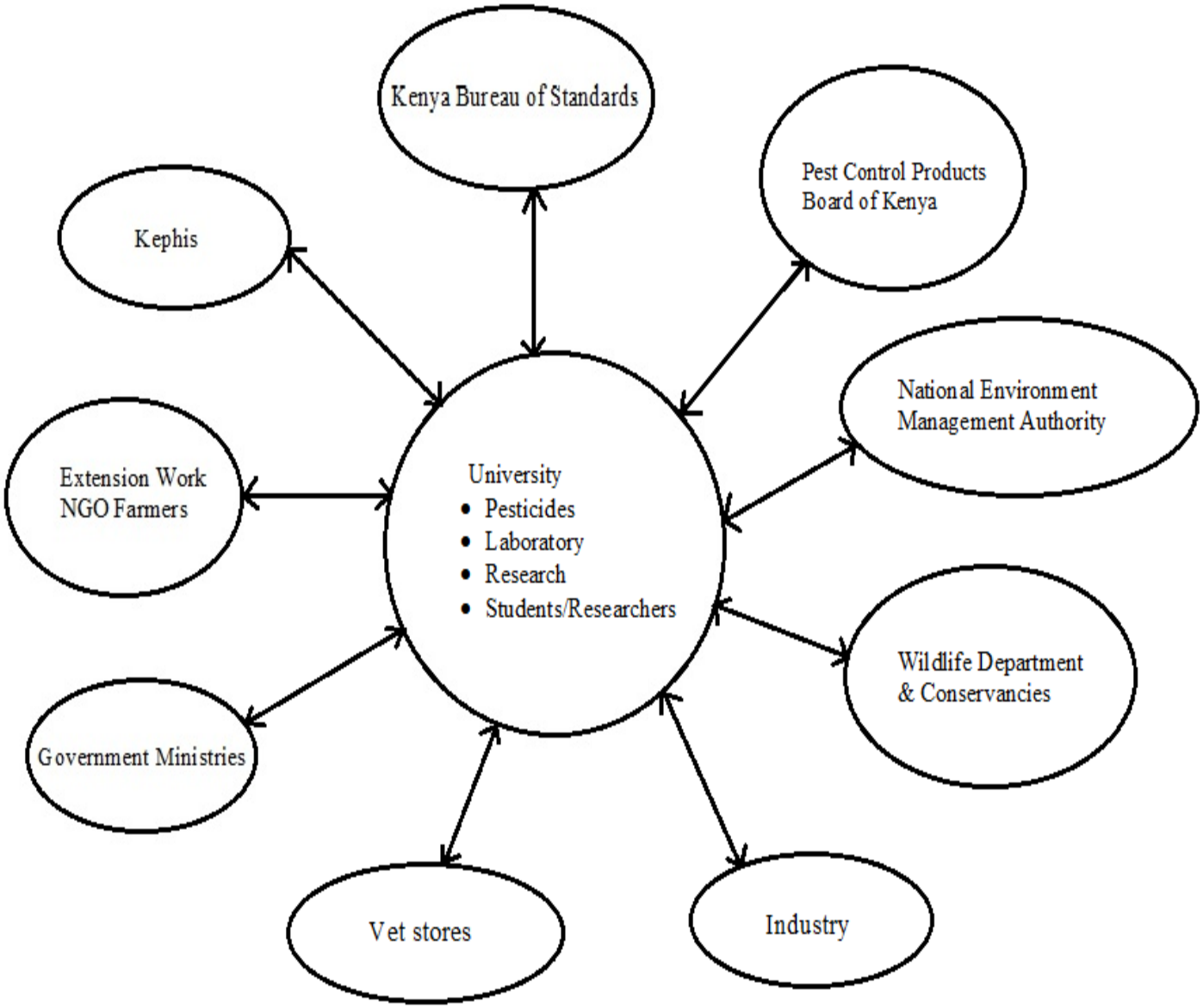
Conclusion and recommendation

- Regular monitoring of pesticide use and impact is required for protection of Wildlife and vulnerable species such as the *Gyps Africanus* species, aimed at detection of misuse and chronic exposure.

- Strengthening of institutions and capacity building in all forms scientists, scientific instruments and tools and knowledge for environmental monitoring.

- Strengthening collaboration networks locally and internationally.

- We have identified a chart we can use for effective collaborations and that makes it easy to identify various needs


Kenya Bureau of Standards
Pest Control Products Board of Kenya
Kephis
National Environment Management Authority
Extension Work NGO Farmers
University
Pesticides
Laboratory
Research
Students/Researchers
Wildlife Department & Conservancies
Government Ministries
Vet stores
Industry

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THANK YOU