# Proceedings of the Wildlife Toxicology Workshop

3-7 October 2022
Naivasha, Nairobi and Juja, Kenya


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Photos

DAY 1


Dr Dominik Fischer, Zoo Wuppertal teaching Avian Emergency Response Medicine, WRTI, Naivasha, Kenya.

Raptor rehabilitation case study, hands-on emergency medicine practice, sample collection and necropsy training at the Kenya Bird of Prey Trust's rehabilitation facility, Naivasha, Kenya.

DAY 2


Opening introductions (Martin Odino, The Peregrine Fund), stakeholder meeting, and research discussions on Kenyan veterinary toxicology needs (Dr. Rafael Mateo, IREC), KWS headquarters, Nairobi.

DAY 3

Introductions, toxicology and chemistry lectures, introduction to an analytical lab, JKUAT, Juja (Drs Elijah Ngumba and Anthony Gachanja teaching about mass spectrometry).

DAY 4

Collecting, processing, and testing avian brain samples for evidence of poison exposure, JKUAT, Juja.

DAY 5


Analyzing results from brain tissues for evidence of poison using multiple methods and closing remarks where attendees talked about what they gained from the workshop and hopeful next steps, JKUAT, Juja.

## Summary

A 5-day workshop addressed important aspects in the fight against illicit wildlife poisoning. The aims of the workshop were to 1) improve analytically sound detection in wildlife toxicology, 2) expand scientific capacity of wildlife authorities to better address national law enforcement, and 3) review veterinary interventions to save poisoned wildlife and experimental plans for laboratories that test suspected poisoned samples, or that have the mandate to do so. This workshop brought together the major stakeholders, namely wildlife veterinarians from Kenya Wildlife Service (KWS) and private KWS-affiliated veterinarians, the Wildlife Research and Training Institute (WRTI), vulture conservation NGOs, and six Kenyan laboratories.

The workshop was held in three locations in Kenya: Naivasha, Nairobi and Juja, and included 51 professionals. On day one, wildlife veterinarians gathered at the WRTI headquarters at Naivasha and then visited Kenya Bird of Prey Trust's raptor rehabilitation facility for a hands-on training about clinical procedures in avian medicine for saving poisoned birds.

Next, a day-long meeting brought together key stakeholders from the government such as KWS, WRTI, the Government Chemist and Directorate of Veterinary Services, public universities such as Jomo Kenyatta University of Agriculture and Technology (JKUAT) and University of Nairobi (UoN), and conservation organisations such as The Peregrine Fund, Smithsonian Institution, Kenya Bird of Prey Trust, San Diego Zoo Wildlife Alliance, and BirdLife International. Objectives were to address the main challenges regarding laboratory testing of poisoned wildlife samples. Three major challenges were identified, 1) cost of testing samples at the Government Chemist, 2) length of time required to test samples at the Government Chemist, and 3) acknowledgement of a range of Kenyan laboratories that have the equipment and capacity to test samples, but not the legal mandate. Discussions involving Senior Managers of government institutions focused on forming a Technical Working Group, led by KWS, to find solutions for solving these challenges. A concurrent session reviewed best practice methods for collecting, storing and transporting suspected poisoned wildlife samples from the field to the lab.

The final three days focused on training 15 laboratory technicians from the Government Chemist, UoN, JKUAT, WRTI forensics and genetics lab, KWS veterinary diagnostic laboratory and Central Veterinary Laboratories. Lab professionals learned new methods for testing a range of chemicals used to poison wildlife. Another important goal was achieved, which was to enhance communication and networking between laboratories and veterinarians, including improving knowledge about the different capacities and equipment available in each laboratory.

Key takeaways:

1) Interest by wildlife veterinarians in developing more skills in avian medicine and having bird-focused veterinarians
2) The need for a Technical Working Group led by KWS to address key policy issues and mandates
3) Stakeholder mapping identified roles and responsibilities of different organizations in the investigation and prosecution of wildlife poisoning events, and potential areas for collaboration and strengthening of ties between these organizations
4) Laboratory personnel and veterinarians are now better connected and there's an increased understanding of the challenges faced by each

5) Laboratory technicians learned new and simple techniques to detect carbamate poisoning, which is the most common class of chemicals used to poison wildlife in Africa

## Introduction

Wildlife poisoning is rife in Africa and poses a serious threat to iconic African species such as lions, elephants, rhinos, hyenas and vultures. In East Africa highly toxic pesticides are frequently used to poison wildlife and the motivations for poisoning wildlife include conflict with predators that kill livestock, to eliminate crop damaging animals, and to harvest animals for food, particularly birds and fish. Because poisons kill indiscriminately they devastate entire scavenger communities as well as the intended target species, while also posing a serious environmental hazard. Poisoning of water sources (e.g. lakes, waterholes, rivers) to kill damage-causing animals such as elephants or to harvest fish is one of the most environmentally destructive practices, endangering not only wildlife, but also humans and their livestock (Ogada 2014).

Tackling wildlife poisoning requires a multi-pronged approach that addresses the socio-economic drivers of poisoning, the lack of awareness about the dangers of poison use, and also the criminal element whereby perpetrators are infrequently apprehended and even less frequently prosecuted or convicted. To date there have been very few (<10) convictions of perpetrators of wildlife poisoning in Kenya despite the fact that over the past 10 years there have been 6067 recorded mortalities (African Wildlife Poisoning Database 2022), a number which represents the tip of the iceberg because most wildlife poisoning incidents are never reported. In two regions of Kenya 121 lions are known to have been poisoned during 2001-2011, yet laboratory testing of these samples was rare (Frank et al. 2011).

Steps are being taken to address the socio-economic drivers leading to wildlife poisoning, including initiatives to build predator-proof bomas (corrals) and erecting electric fences to deter crop-raiding elephants. Local NGOs are engaged at the grass-roots level to create awareness about the dangers of using poisons to kill wildlife. The largest remaining gap is to improve analytically sound detection and capacity in wildlife toxicology to address the lack of arrests and convictions of perpetrators to serve as a deterrent to wildlife poisoning (Lalah et al. 2011).

Over the last decade samples from suspected poisoned wildlife have reached the few equipped laboratories in Nairobi with limited results. Major impediments to obtaining positive results include lack of proper training in sample collection, storage and transport, and laboratory techniques from how best to screen samples for a range of toxins, how to conduct more focused testing to identify culprit poisons, and a basic knowledge of toxicology in order to interpret test results. There are also limitations in terms of equipment and supplies for individual field personnel (veterinarians) and laboratories, which requires a plan for how and where certain tests will be conducted (Lalah et al. 2011).

We conducted a 5-day training and intellectual exchange program in Kenya that brought together international experts in veterinary medicine, government policy, wildlife toxicology and their field and laboratory-based counterparts in Kenya in order to improve local capacity to detect pesticides and other toxins that are used illicitly to poison wildlife.

### Objectives

1) To increase the laboratory capacity of six Kenyan labs on issues of wildlife toxicology. Each of the labs has a different level of available equipment and trained personnel. We also know that wildlife poisoning is a wide-scale problem and by including a range of analytical laboratories and their specific expertise/government mandate we will stimulate discussions and information sharing about this issue across a wide scope of government-mandated institutions in Kenya.
2) To conduct an interactive training with field-based personnel (Kenya Wildlife Service and affiliated veterinarians and vet technicians) together with laboratory staff in best practice methods for collecting, storing and transporting suspected poisoned wildlife samples from the field to the lab. Only authorized personnel are allowed to collect wildlife samples and they have limited access to even minimal field equipment.
3) To improve knowledge among KWS vets/vet techs about treatment, handling and longer-term care of raptors that have been poisoned, but survive.
4) To enhance communication among Kenyan labs to facilitate sample testing when equipment, supplies and capacity are limiting factors. Laboratories face similar challenges in terms of capacity and support, and fostering a communications network between them will allow them to support and rely on each other in the face of these challenges.

## Workshop Proceedings

### Day one: Veterinary training, 3 October, KWS Training Institute and Kenya Bird of Prey Trust, Naivasha

**Attendance:** 9 wildlife veterinarians/vet techs, 8 NGO staff, 4 trainers, 1 Kenya Vet Board representative, Total 22 people

**Official Opening remarks:** Dr Francis Gakuya, WRTI

**Official Opening remarks:** Dr Edward Kariuki, Kenya Wildlife Service

**Official Opening remarks:** Dr Darcy Ogada, The Peregrine Fund

### Morning session

Led by Dr Dominik Fischer, Zoo Wuppertal at the teaching facility at WRTI, this presentation focused on avian medicine and clinical treatment for poisoned birds. The theoretical session focused on:

- Quick examination of an avian patient: normal clinical values and abnormal clinical presentation
- Patient stabilization: First aid, shock treatment, and rehydration
- Further examination, sample collection, and diagnostic test selection
- Common problems, differentials (poisons and others), and specific critical care treatment
- Safe restraint, transport, husbandry, feeding and care of raptors
- Additional threats to raptors, particularly electrocution

## Quick examination of an avian patient

Raptors hide the signs of illness for as long as possible. Observe first from distance and check:

- Observe the bird’s body posture (legs, wings, head and neck), and motion
- Eye opening (wide open or slit-shaped (lemon) eye?)
- How it responds to you and the environment (consciousness)
- Is its head drooping? (signs of weakness and depression, commonly in poisonings and severe shock)
- Are the wings symmetrical and held close to the body?
- Is it holding its head level and still or is there an uncontrolled tremor?
- Is it placing its full weight on both legs or just one?
- Are there obvious distortions/mal-alignments in any limbs?
- Is there any blood or abnormal discharges?
- Are flies present?
- How is the breathing?

**Vital parameters** in medium-sized to large raptors are:

- Body Temperature: 39.5 – 40.5 °C
- Breathing rate: 35-200/min (in small birds higher as in larger birds)
- Heart rate: 60-200/min (in small birds higher as in larger birds)
- Mucous membranes: pink to grayish (species specific differences)
- Reflexes (lid reflex, corneal reflex

## Competent and safe restraint

Restrain the body and always the head (especially in vultures) and the feet (less important in vultures, but important in eagles and hawks). You may use leather gloves to facilitate a safe handling.

Apply only minimal pressure on the sternum and the neck, to not disturb respiration. Staff should be aware and recognize possible “signs to euthanize ” such as seizure, rigidity, tremor, head arching back, gasping for breath, and stopped breathing.

Don’t be too busy talking to notice what is happening to your patient.

Safe handling techniques for large vultures. In this case a Rüppell's Vulture at the Kenya Bird of Prey Trust's rehabilitation facility. Note that the wings are held between the right arm and the body of the fixator, but there is no pressure to the sternum in order to not jeopardize breathing (photo: Dr. Dominik Fischer).

## First Aid / Treatment

- Birds get sicker and die quicker than mammals and smaller birds die quicker than larger ones
- Stress is a certain risk factor for diseases and it might be associated with travel (heat, poor ventilation, handling by humans, proximity to other species). Try to keep stress as low as possible
- First and most important aim is to treat shock, which can be expected in all sick and trauma patients

  → But do not do too much too soon
  → Priority is First Aid (A, B, C) including rehydration of the raptor

**A** = Airway management (respiration: place the bird's body in ventral position, beak deeper than body level, ensure that mouth & throat are empty)
**B** = Blood supply (circulation: try to rehydrate the bird as soon as possible)
**C** = Central nervous system (keep the bird alert)

## Cardio pulmonary reanimation (CPR) in birds

- Place the bird in ventral recumbency with beak below body level
- Cardiac massage (frequency 80-100/min) with index finger at the thoracic aperture, and parallel pressure on the sternum and/or synchronous movement of the wings
- CPR: breathing : massage = 30:2

## Assisted breathing

1. Place the bird in ventral recumbency with beak below body level
2. Clear airways and endotracheally intubate ** and fix the tube using tape/bandage
3. Breath every 2 seconds, using the valve-tubus and the ventilator bags

* Sealing of the glottis using bandage material on top of the endotracheal tubes is better suited than using the "cuff" (air ballon), as the cuff may cause pressure necrosis at the trachea.

The figure left shows the oral cavity of a Rüppell's Vulture. Note the epiglottis (tracheal opening) in the center directly behind the tongue). The esophagus is behind the epiglottis on the right side of the neck (photo: Dr. Dominik Fischer).

### Give a brief cursory examination for critical conditions and treat accordingly

- Critical hemorrhage (i.e. ongoing or significant blood loss)
- Respiratory distress (a bird which is struggling to breath or has obviously noisy breathing)
- Circulatory collapse (pale membranes in the mouth, poor capillary refill i.e. >5 seconds)
- Other critical injuries
  → Unless the patient needs immediate euthanasia, the best option is treat for shock, repeat in 2 hours and give a full clinical examination at 2-4 hrs

### What is "shock"

In traumatized or ill birds blood vessels may begin to leak and fluid is lost from the circulation. Thus, circulation is less efficient and vital organs (e.g. brain, heart and kidneys) no longer have a sufficient/good blood supply. Birds become dehydrated and may show a high heart rate (tachycardia), pale mucus membranes and a flat pulse. Moreover, a sticky / wrinkled skin, enophthalmos, cold legs, and shivering may be present.

### Treatment of shock

1. Fluid therapy
2. Warmth (but not over heating)
3. Accommodation (quiet & dark) and rest for 2 hours

→ Never feed a bird until it is rehydrated (and shock is effectively treated)
(wait ~24hrs to feed a large vulture and 8-12 hrs in a bird with less than 1kg body weight)

## Fluid therapy - ways to rehydrate a bird

1. **Oral rehydration (PO)**
   → invasive procedure: knowledge and experience required
   to avoid putting it down the trachea, as this will result in death
   → Never introduce fluids unless you are certain the tube is in
   the esophagus
   → 1-2% of body weight in ml repeated after 2 hours
   → application via tubing or gavage

The figure shows the oral rehydration via stomach tube in a Griffon Vulture. It must be ensured that the tube is placed in the esophagus on the right side of the trachea prior to fluid application (photo: Dr. Neil Forbes).

2. **Subcutaneous fluid therapy (SC)**
   → safest and minimal stressful method
   → Fluid must be sterile, administered in an aseptic manner (part the feathers and
   surgically
   → prepare the skin with spirit or disinfectant
   → application between the shoulder blades or at the femoral web between leg
   and chest; max. 40-70 ml at one site
   → Small volumes can be easily be given with syringe and needle, larger volumes
   are better given with a butterfly catheter attached to a syringe
   → When administering, especially in smaller birds, risk of the bird wriggling and
   the tip of needle penetrating the air sac and thus drowning the bird is possible

The figures show a butterfly catheter attached to a syringe (left) and the subcutaneous fluid application in a Griffon Vulture (middle & right) (photos: Dr.Neil Forbes).

3. **Intravenous fluid therapy (IV)**
   → Much more effective than PO or SC, especially in severely dehydrated patients
   → Jugular veins, metatarsal veins and ulnar veins are routinely accessed
   → Fluids can be given by bolus (e.g. 20ml/kg on admission) repeat after 2 hours,
   then 10ml/kg/hr, or as dictated by daily fluid need

→ Collapsed or shocked vultures tolerate drips well. So long as they will tolerate it, best given by drip/a ‘giving set’.

→ For a 10 kg bird, using a standard (20 mL) giving set and assuming 10% dehydrated, the drip rate should be one drop every 3.5 seconds for the first four hours, reducing to one drip every 5 seconds thereafter, until the end of day three after admission.

The figures show the jugular vein in a Griffon Vulture (left: photo Dr.Dominik Fischer) and the intravenous application, the previous disinfection and the placement and fixation of an intravenous catheter in the metatarsal vein of a vulture (middle and two pictures on the left, photos: Dr.Neil Forbes).

4. **Intraosseous fluid therapy (IO)**
   → potential alternative if IV is impossible, e.g. in severely dehydrated birds
   → Sites: proximal tibiotarsus or distal ulna
   → potentially more painful and a certain risk of (serious) infections

Intraosseous placement of a cannula/syringe needle in the left tibio-tarsus of a dead red-tailed hawk and a bone preparation of a red-tailed hawk (photo: Dr. Dominik Fischer).

## Fluid demand / amount

**a) Mild (~ 10%) dehydration:**
→ Give 100ml/kg in the first 24 hours
→ Then 75ml/kg in the second 24 hours
→ Repeated 75ml/kg on the third day

→ So for a 10kg Cape or Lappet faced vulture this would be:
1000ml on day one, 750ml on day two, and 750ml on day three

**b) Medium (~ 15%) dehydration:**

→ Give 125ml/kg in the first 24 hours

→ Then 87.5ml/kg in the second 24 hours
→ repeated 87.5ml/kg on the third day
→ So for a 10kg Cape or Lappet faced vulture this would be:
1250ml on day one, 875ml on day two, and 875ml on day three

**c) Severe/significant (~ 20%) dehydration:**

→ Give 150ml/kg in the first 24 hours

→ Then 125ml/kg in the second 24 hours
→ Repeated 125ml/kg on the third day
→ So for a 10kg Cape or Lappet faced vulture this would be:
1500ml on day one, 250ml on day two, and 1250ml on day three

## Further examination & taking samples:

- Do not give an injured bird a full clinical examination (physical exam) until you have treated the shock for at least 2 hours
- Duration of handling and restraint, must be absolutely minimized
- Have everything readily prepared!
- Examine the bird according to priority:
  - Head (Beak, eyes, ears, nose and sinuses, mouth, tongue and throat, skull)
  - Neck (crop and trachea)
  - Body (abdomen, sternum / pectoral muscle, skeleton)
  - Cloaca
  - Extremities (wings & legs)
  - Skin & feathers

**Sternum:**
Check the sternum for Body Condition Score, assessing the prominence of the keel bone (carina) and the muscles around the more prominent it is, the less muscle is present, i.e. the thinner the bird.

**Wings:**

- Check the propatagium (the soft aerofoil which runs between shoulder and wrist on each wing)
- Check if here is similar tension in both wings
- Check each joint (flex and extend- shoulder, elbow, carpus/metacarpae - joint by joint)
- Check the bones (run your fingers down either side of humerus, ulna/radius, metacarpus)
- Check the wing plumage (e.g. for fret bars and ectoparasites)

**Back and legs:**

- Palpate down the back and check the tail to ensure the spine is straight
- Locate each hip – upon the pelvis, flex and extend
- Check each joint (flex and extend - hip joint, stifle joint, intertarsal joints, digits - joint by joint)
- Check the bones (run your fingers down either side of femur, tibio-tarsus, tarso-metatarsus, digits)
- Check underneath each foot (e.g. for bumblefoot)
- Check and locate the preen gland just in front of the insertion point of the central deck feathers.
- The tuft of feathers on top should have oil in it, if not give a firm squeeze at the base.
- Check the cloaca - typically there will be a mute (fecal) sample in the transport box, collect some of this and do a fecal parasite check.

**Further diagnostics - which are mostly not applicable in the field - include:**

- Hematology
- Blood chemistry
- Plasma/serum protein electrophoresis
- Endoscopy
- Radiography (X-ray)
- Ultrasonography (Ultrasound)
- Computed tomography (CT)
- Magnetic resonance imaging (MRI)

**Record keeping:**

- Each patient should be identified
- Source, when, who, history
- Weight, condition
- Clinical examination outcome
- Tests, treatments
- Where housed, with whom
- Progress
- Outcome

## Poisoned birds - General rules

1. If one or more sick, collapsed, dead, uncoordinated birds is found
   → Immediate action is required
2. If you attend the site - preserve it! (forensic)
3. Beware of human toxicity effect (wear personal protection clothing, gloves, mask)
4. Prevent any carnivores from eating the carcass(es)
5. Contact law enforcement
6. Any live birds treat as a priority

**In general, clinical signs of poisoning in raptors may include:**

- Sudden death
- Stupor
- Severe depression
- Loss of responsiveness
- Sudden onset of blindness
- Collapse
- Prostration
- Hypersalivation
- Constricted pupils (miosis)
- Diarrhea
- Vomition
- Respiratory distress
- Weakness
- Muscle twitching
- Inco-ordination, Ataxia
- Seizures
- Tremors

**NSAID toxicity (Non-steroidal anti-inflammatory drugs)**

- Apart from Metacam – all NSAIDs can be toxic: especially Diclofenac Sodium (Diclofenac, Voltaren), but also ketoprofen (Ketofen), phenylbutazone (Tomanol, Phenylarthite, Equipa-lazone, and Fenylbutazone), flunixin (Finadyne, Cronyxin, Pyroflam, Hexasol), vedaprofen (Qaudrisol), and carprofen (Rimdayl Aquous)
- Main clinical sign is dehydration (from slight to severe) with weakness, drooping head, depressed appearance (spaced out), and wings held slightly out from the body
- Therapy:
    1.) Fluid therapy via a drip at twice maintenance rates is critical to reduce the severe dehydration.
    2.) Allopurinol at 30 mg/kg BID PO, to reduce blood uric levels, but nothing reverses renal damage

**Heavy metal toxicity (lead intoxication)**

- Sources are ingested lead particles (e.g. from lead shot, rounds, ammunition, fishing lead)
- Clinical signs include green feces, weakness of legs and drooping wings, tremors, incoordination, fits and coma.
- Birds show weak or paralyzed legs with toes knuckled over and various other central nervous signs. Characteristically the bird is seen sitting on its hocks grasping one foot in the other.
- Vultures with lead poisoning are often thin / emaciated, very weak and unable to feed for themselves.
- Diagnosis is mainly based on the presence of lead particles in the gastrointestinal tract on x-ray, or an elevated blood lead level on testing (normal: <10 ug/dl (= <0.48 umol/L); exposure: 10-20 ug/dl (0.48 – 0.96umol/L) (no clinical significance), positive and need of

therapy: > 20 ug/dl (= 0,96 umol/L). Blood lead levels are often elevated in raptors in the absence of particulate lead in the gut, which is consequent to the consumption of ballistic fragments (i.e. fragments of hunter's bullets). There is good evidence that many vulture trauma cases have elevated lead levels. Especially power line injuries and other illnesses, seem to be associated with long-term low levels of lead toxicosis.

- Treatment:

  1) Try to remove lead from the gastro-intestinal tract (surgically if needed)

  2) Chelation therapy
     - EDTA – 35 mg/kg twice daily by IM or IV for 5 days (Alternative DTPA)
     - Intensive fluid therapy

  3) Chelation therapy, is not continued beyond five days after the particles are removed, as it may cause renal damage. If nervous signs persist, blood lead level test should be repeated 7 days after cessation of EDTA and chelation therapy should be continued if levels remain elevated. In all lead poisoning cases, fluid therapy (preferably via IV drip) is important to reduce the likelihood of kidney failure and concurrently treat any seizure activity.

## Agro-chemical toxicities

- Organophosphates (e.g. Fenthion, Methamidophos, Diazinon, Chlorfenvinphos, Fenamiphos, Cadusafos, …)
- Carbamates (e.g. Aldicarb, Carbofuran (granular and liquid), Methomyl, E605, …)
- Clinical signs and prognosis include pupil constriction (miosis), vomiting, tremors, paralysis, hypothermia and hypersalivation

| **Organophosphates** | **Carbamates** |
|---|---|
| **Fenthion** (onset approx. 30min): Birds may fly for half an hour before toxic effects ground them; recovery can take as long as eight months, but prognosis is very poor | **Aldicarb** (immediate onset of signs): Birds are usually found within a few metres of source. The prognosis is extremely poor |
| **Methamidophos** (rapid onset of signs in 5 min): Birds are typically found within 50m of source; prognosis is very poor | **Carbofuran** (granular) (onset of signs 5-30 min): Usually sub-lethal concentrations are ingested so prognosis is less guarded; birds may reach nests where they & the chicks die |

| | |
|---|---|
| **Diazinon** (very rapid onset of signs in 3-5 min): Birds will be found within 100m of the source; prognosis is fairly good with no long term effects | **Carbofuran** (liquid) (immediate onset of signs): Birds are usually found at the source; prognosis is poor. |
| **Chlorfenvinphos** (rapid onset of signs in 5 min): No long term effects, prognosis is poor | **Methomyl** (immediate onset of signs):Birds are usually found at the source, the prognosis is extremely poor with no survival |
| **Fenamiphos** (rapid onset of signs): There are virtually no survivors; birds are found within 100m of the source; prognosis is very poor | |
| **Cadusafos** (slow onset of signs, up to 30 min): Birds can move very far from the source; cases can recover but signs reoccur, in particular when birds lose weight; prognosis very poor & survival unlikely | |
| **Emergency therapy*** Rapid availability and administration of the correct antidote is imperative. If not treated correctly within 24-48 hours, therapy is often ineffective (>24 hours, therapy may make clinical signs even worse - In this event treatment should be stopped): | |
| **Organophosphates** | **Carbamates** |
| 1.) If the patient is strong enough to cope with **oral fluids by gavage tube**, give this mix orally (PO):<br>20ml/kg of saline, electrolyte (or **water**)<br>+ 10ml/kg **liquid paraffin** (mineral oil)<br>+ 1-2g/kg of **activated charcoal** | 1.) If the patient is strong enough to cope with **oral fluids by gavage tube**, give this mix orally (PO):<br>20ml/kg of saline, electrolyte (or **water**)<br>+ 10ml/kg **liquid paraffin** (mineral oil)<br>+ 1-2g/kg of **activated charcoal** |

| | |
|---|---|
| 2.) Administer **Pralidoxime Chloride (= 2 PAM)**<br>→ 50 mg/kg once intravenously (IV) or intramuscularly (IM)<br>→ repeated after 6 hrs if necessary<br>→ then repetition every 24 hrs as needed<br>(This antidote is difficult to get, if treatment has not started within 24 hours, therapy may make clinical signs worse. In this event PAM treatment should be stopped, but Atropine maintained.) | |
| 3.) Administer **Atropine** IV or IO<br>→ start with 1mg/kg<br>→ double the dose (2, 4, 8, 16 mg/kg) and repeat every 5 minutes, until either the hypersalivation ceases or the pupils dilate<br>→ then place 20-30% of the last dose in a drip and give over 8 hours.<br><br>Failing this administer Atropine at 25mg/kg IM<br><br>If the bird improves after Atropine, but later deteriorates, repeat the Atropine, keep repeating until deterioration doesn't occur anymore. | 2.) Administer **Atropine** IV or IO<br>→ start with 1mg/kg<br>→ double the dose (2, 4, 8, 16 mg/kg) and repeat every 5 minutes, until either the hypersalivation ceases or the pupils dilate<br>→ then place 20-30% of the last dose in a drip and give over 8 hours.<br><br>Failing this administer Atropine at 25mg/kg IM<br><br>If the bird improves after Atropine, but later deteriorates, repeat the Atropine, keep repeating until deterioration doesn't occur anymore. |
| 4.) Supportive care: sling as necessary, control seizures, fluid therapy IV (if not possible IO or SC -see above) and later nutritional support | 3.) Supportive care: sling as necessary, control seizures, fluid therapy IV (if not possible IO or SC -see above) and later nutritional support |

* If the poison is unidentified (organophosphate and carbamate unclear) use Atropine at 25 mg/kg plus 2 PAM at 50 mg/kg and repeated as necessary (i.e. if clinical signs improve then deteriorate again). Typically Atropine treatment will be required every 2-4 hours, whilst if PAM is also used this can be repeated every 8 hours.

**Electrocution**

- >34% of vulture mortality in South Africa is as a result of power line collisions or electrocution
- Electrocution: External wounds may seem slight, but extensive charring of soft tissues may occur internally

- Therapy: Fluid therapy, pain relief, wounds are difficult to manage with a significant charring – inside out. Keep wounds moist and apply analgesia and antibiosis and wait

**Bleeding (haemorrhage)**

- Maximum loss 1% of its body weight. Any patient lost >1% requires IV fluids and in severe cases a blood transfusion may be required.
- Apply firm digital pressure to site of bleeding and keep finger in place, for 2x as long as necessary
- Use haemostatic swabs, powders (e.g. glucose or talcum powder) or even cobwebs and as last resort - tourniquets.

**Zoonosis**

Mind the potentially zoonotic pathogens from birds such as avian influenza, West Nile virus (WNV), Newcastle Disease (ND), Q fever, *Cryptococcus, Chlamydia, Salmonella, Campylobacter, Mycobacterium avium, E. coli oder Yersinia.*

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## Afternoon session

Led by Dr Dominik Fischer, Simon Thomsett, and Shiv Kapila and undertaken at the Kenya Bird of Prey Trust's rehabilitation facility. This session included an overview of the rehab facility, including a tour of the facilities aviaries, summary of operations, and then a visit inside the vulture aviary to get the wildlife veterinarians up close with the resident vultures.

For the practical session each vet worked on a fresh domestic duck carcass to gain or reinforce skills in:

- Airway management: tips and tricks using supplies available including modified endotracheal tubes
- Drug and fluid administration using several different techniques to address confounding factors such as an animal not able to stand up or have accessible veins.
- Wound management and broken wing stabilization
- Necropsy and gross anatomy review with sample collection
- Heads were saved for further diagnostic work-up training

The workshop was accredited by the Kenya Veterinary Board and participants (vets) were awarded 32 CPD points. Fifteen practical kits containing equipment and supplies required to triage and treat poisoned raptors were also prepared by the workshop organizers and distributed to KWS (and affiliated) veterinarians after the workshop.

## Day two: Stakeholders meeting, 4 October, KWS Headquarters, Nairobi

**Attendance:** 12 wildlife veterinarians/vet techs, 16 laboratory professionals, 8 NGO staff, 3 trainers, 2 KWS Security Department, 1 Kenya Vet Board representative

**Official Opening remarks:** Dr Francis Gakuya, WRTI

**Official Opening remarks:** Dr Darcy Ogada, The Peregrine Fund

**Morning session: stakeholder presentations**

*Wildlife poisoning in Kenya*
Martin Odino, The Peregrine Fund
Key reasons for poisoning in Kenya include to acquire food and in retaliation against problem animals. Poisoning hotspots (10s to 1000s+ dead animals) are rice schemes and wetlands, and areas surrounding national parks/reserves and conservancies. Poisoning is the biggest threat to African vultures, many species of which are critically endangered or endangered. Mitigation efforts currently underway by local conservation NGOs are aimed at reducing carnivore conflict through training communities to build predator-proof bomas, training rangers to identify and respond to poisoning incidents, GPS tracking of vultures to identify poisoning sites, rescue & rehabilitation of poisoned wildlife, and increasing grassroots networking to better respond to poisonings.

*Intentional and accidental poisoning of wild and domestic animals in Spain*
Rafael Mateo, Institute for Game and Wildlife Research
Spain, which is similar in size to Kenya, has a lot of cases of wildlife poisoning and as a result has developed considerable expertise in investigating scenes, testing samples, and developing successful court cases. This presentation provided an overview of the steps involved in building testing capacity, investigating cases, and the regional jurisdiction in Spain. A map showed the network of toxicology laboratories and reported number of cases and their regional variation. An overview of which species and chemicals were most involved was provided. Analytical screening methods, available equipment, and the outcome of legal actions were discussed. This presentation showed the level of management and agency coordination required if countries such as Kenya are to increase prosecutions of poisoners.

*Government Chemist's Department role in the provision of analytical and forensic services*
Dr Samuel Gachuhi, Government Chemist Department
GCD provide analytical and forensic laboratory services in administration of justice, public health and environmental services. GCD has three regional branches and two main sections. Forensics includes: Forensic Toxicology, Criminalistics and Illicit Drugs, and Forensic Biology (Serology & DNA). Analytical section includes: Food and Drugs, Water and Environmental, and Clinical Toxicology. The majority of their forensic toxicology cases are human-related. Samples required and considerations about sample collection were discussed. From 2019 to Sep 2022 GCD handled 68 veterinary cases where a wide range of poisons were detected including carbamates, organophosphates, heavy metals, aflatoxins and oils. External challenges are: inappropriate/poor sample collection, packaging and transportation, poor coordination between agencies, and limited background information to assess how to prioritize sample testing. Internal challenges are acquisition of quality standards, reagents, limited analytical equipment and rapid maintenance and repairs of equipment.

*Department of PHPT in Public Health*

Lucy Waweru, University of Nairobi

The Department of Public Health, Pharmacology and Toxicology is one of the departments in the Faculty of Veterinary Medicine of the University of Nairobi. The department is involved in teaching, research, consultancy, and diagnostic services. Testing methods include ELISA, microscopy, culture, serology, PCR, TLC, HPLC, and gas chromatography. They also undertake toxicological studies for PCPBs (in vivo).

*Introduction Wildlife Research and Training Institute*

Tevin Onyango and Antoinette Miyunga, WRTI

WRTI began in July 2020 with a mandate to undertake and coordinate wildlife research and training in Kenya. WRTI's Veterinary Science and Laboratories Department coordinates and undertakes veterinary research and disease surveillance and is home to the Wildlife Forensics and Genetics Laboratory, which investigates illegal trade in wildlife and wildlife products. In future they plan to build capacity in morphological analysis, toxicology, pathology, pharmacology and entomology.

*Central Veterinary Laboratories*

Dr. Timothy Lesuuda, Central Veterinary Laboratories

National Veterinary Laboratory provides laboratory diagnostic services to safeguard animal and human health, improve animal welfare, increase livestock productivity, and ensure safe, high-quality livestock and their products for food security, and domestic and international trade. CVL has seven main departments: Transfer & Receiving Facility, Acarology, Bacteriology, Chemistry, Pathology, Molecular and Virology. They conduct residue testing in animal foods, quality control of veterinary drugs, analysis of tissue samples for poisoning, and residue analysis. Challenges are a lack of consumables, mostly standards, and the calibration and maintenance of their GC-MS/MS machine.

*Laboratory Equipment Status*

Prof Anthony Gachanja, Jomo Kenyatta University of Agriculture and Technology

There must be investment in equipment to undertake scientific analysis in order to ensure reliability of results. Do we have the equipment and instruments required to confidently tackle the questions asked in suspected wildlife poisoning incidents? Instruments are very expensive, initial investment for a mass spectrometry (MS) machine can be high, up to 1 million USD. Specialist training is required and maintenance, servicing and upkeep are challenging. Need to address, at management level, the philosophy of having instruments if investments are not sufficient to keep them in operation. Also, need to address the serious challenge of having specialized personnel to run the instruments and maintain them.

*Collaborations and Conservation in Kenya*

Dr Caroline Moore, San Diego Zoo Wildlife Alliance

SDZWA has supported conservation work in Kenya for over 17 years, and has focused on increasing collaborations with KWS, community conservancies, and NGOs in the last six years. In northern Kenya SDZWA supports work on hirola, leopard, giraffe, black rhino, elephant and vultures. Objectives are specific for each project, but broadly focus on disease investigation, population assessment, human-wildlife conflict, habitat requirements, illegal trade, translocation, community support, capacity development, population monitoring and toxicology. SDZWA initiatives in Kenya are based upon a network of close partnerships and collaboration.

Challenges include animal and environmental sample collection and testing for disease monitoring during population health programs with partners, linking wildlife research with policy support, and wildlife toxicology interpretation given the lack of reference data available for most wildlife species.

## Afternoon parallel sessions

**Session 1** Stakeholder mapping involving Senior Management

*Kenya Wildlife Service, WRTI, CVL, Government Chemist*
Dr James Hassell, Smithsonian's Global Health Program & Prof Anthony Gachanja, Jomo Kenyatta University of Agriculture and Technology
Stakeholder mapping was conducted to map the primary actors, processes and governance by which wildlife poisoning events are investigated and brought to prosecution in Kenya and identify challenges facing this process from the perspective of sample collection, storage, transport and analysis. Results of the participatory mapping activities are presented in **Figure 1** and **Table 1.**

Three key challenges were identified; *i)* a lack of uniform protocols for the collection of data, and collection, packaging and submission of samples collected by veterinarians at suspected poisoning events; *ii)* accessible sources of funding for wildlife forensic toxicology, and a lack of clarity as to the criteria for that funding being used; *iii)* access to expertise and resources in laboratories that lie outside the chain of custody for forensic cases. From discussions that took place around these challenges emerged the recommendation for a technical working group on wildlife poisoning to be formed. The group suggested that KWS would be best placed to coordinate this working group, which would be mandated to comprehensively review and make recommendations to strengthen the existing protocols and legal framework within which wildlife poisoning events are investigated. Initial efforts would need to focus on developing terms of reference and a governance structure, identifying funding support, and engaging representatives from relevant stakeholders who would form the membership of the working group.

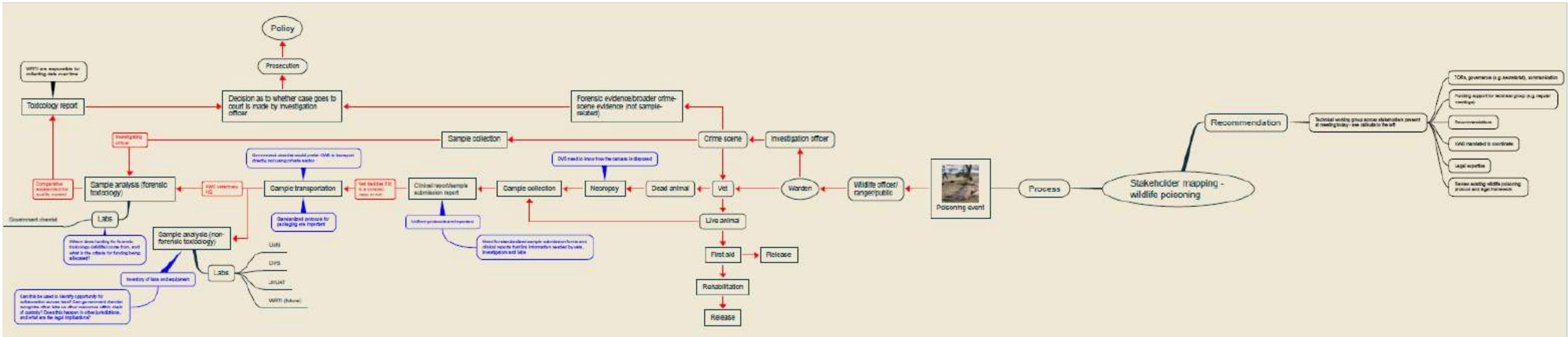


**Figure 1: Stakeholder mapping for response to wildlife poisoning events.** Red arrows represent flow of information between stages of the process. Blue boxes represent potential barriers to determining the cause of poisoning that were identified by the group.

**Table 1: Stakeholders involved in response to wildlife poisoning events.**

| Organization | Role |
|---|---|
| **KWS** | Rangers are often first responders to the scene<br>Vets are notified by the warden, and responsible for collecting clinical samples, conducting necropsies and providing a clinical report<br>Designated crime scene officers are responsible for collecting forensic evidence for prosecution of wildlife crimes |
| **WRTI** | Scientists are responsible for collecting data with which to monitor the impacts of poisoning on conservation efforts |
| **NEMA** | KWS officers will normally engage during an investigation - should be fully involved in the investigative process.<br>Should also be included for the perspective of contamination of the environment (e.g. water bodies), and ensuring appropriate disposal of toxic waste materials |
| **Local administration (Office of the President)** | Assistant County Commissioner provides locally-relevant intelligence to KWS vets and officers |
| **Government Chemist** | Is mandated to conduct and report on forensic toxicology |
| **University laboratories** | Have capacity to conduct forensic toxicology, but are not mandated for these purposes |
| **DVS** | Poisoned carcasses can be disposed of at DVS where there is an incinerator. Have capacity to conduct forensic toxicology, but are not mandated for these purposes. |
| **KVB & VMD** | Should be involved in investigation process if there is evidence of the negligent use of a veterinary drug |
| **PPB** | Should be involved in investigation process if there is evidence of the negligent use of a human drug |
| **PCPB** | Should be involved in investigation process if there is evidence of the negligent use of an agricultural product |
| **Ministry of Health** | Will be alerted by KWS if there is the potential for secondary poisoning of humans |

**Session 2** Chain of Custody, Field Investigation and Report Writing involving veterinarians, laboratory technicians and NGO staff

*Kenya Wildlife Service Chain of Custody*
Stephen Ondieki, Security Department, KWS
Chain of custody is a careful documentation of evidence to an alleged crime that establishes who had contact with the evidence, data and time evidence was handled, circumstances for evidence being handled, what, if any, changes were made to the evidence, and the condition of the exhibit. There is a procedure to establish a chain of custody that involves collecting and examining evidence using legal techniques and reporting information obtained. Chain of custody must be sufficient to show that the object in a courtroom is the same one that was involved in the events on trial. KWS has a chain of custody form to maintain an ongoing record of custody for each object collected from a scene. The importance of proper handling and management of court exhibits cannot be over emphasized. Without proper and convincing evidence the prosecution will not be able to provide proof beyond a reasonable doubt.

*Field Investigation of Wildlife Poisonings and Content of the Toxicological Reports*
Rafael Mateo, Institute for Game and Wildlife Research
Good field investigation is critical to the success of poisoning cases. The first things to consider are contextual information – are there any reasons for poisoning of wildlife in this case, types of poisoning - intentional vs. accidental, and most criminal cases involve conflicts between humans and wildlife. Investigation involves: detection of dead animals (fieldwork), identification of the cause of death (necropsy and toxicological analysis), interpretation of the results (lethal dose), was poisoning intentional/illegal or accidental/legal, and identification of the person(s) who poisoned (legal actions, preventative measures). The report of the crime scene should include all necessary information about the case including: location (GPS coordinates), date and hour, full description of the scene (photos), habitat and information about historic conflicts/use of poisons/availability of chemicals. Deciding which samples to collect is based on evidence at the scene and necropsy findings, e.g. how fast did the animal die? There needs to be an understanding of the mode of action of different types of chemicals. For most poisons collecting the gastric content and the liver are priorities for testing, though other tissues such as blood and brain can be helpful. Samples can be collected using glass or plastic, and in tubes/vials or Ziploc bags. Ensure labeling is with permanent marker. Samples should be frozen, but without a freezer acetone can be used to store samples at room temperature. Interpretation of results, i.e. did this amount of chemical kill this animal, is dependent upon knowing the full volume of stomach contents and other descriptive data about the animal, the reference values for the species including lethal dose (LD), and calculating the suspected amount of poison the animal consumed. The final step is writing a toxicological report that describes the testing methods, results, and an interpretation of the results including references.

Kenya-specific wildlife poisoning concerns were discussed including what human-wildlife conflicts lead to poisoning events, what are the targeted and non-targeted species of concern, and what possible solutions may help reduce wildlife poisoning events.

## Day three: Overview of laboratory methods, 5 October, JKUAT, Juja

**Attendance:** 6 wildlife veterinarians, 18 laboratory professionals, 4 NGO staff, 2 trainers, 2 KWS Security Department, 1 Kenya Vet Board representative

**Official Opening remarks:**

The workshop was officially opened by Prof Waititu, the Dean of the faculty of Physical Sciences at the conference hall at SAJORC center. He thanked the organizers of the workshop for recognising the facilities available at JKUAT, taking note that collaboration is the way forward and more so in this important area of toxicology and wildlife poisoning for Kenya and Africa.

**Morning session**
*Laboratory Investigation of Wildlife Poisoning*
Rafael Mateo
Different classes of poisons mostly require different laboratory tests. Initial steps for all poisons are: visual inspection, homogenization, extraction, filtration, evaporation and then 'clean up' or actual testing for specific poisons. Use of Quechers allows the preparation of a large number of samples in less time and it is useful for a wide range of poisons. It is important to track trends over time in poisons used, regional variation, species poisoned, and whether poisoning was accidental, intentional, or could not be detected. Very important to understand all the different classes of poisons used to kill wildlife, their modes of action, and the testing procedures for each.

**Afternoon session**
Overview of laboratory equipment and basic procedures for the benefit of wildlife veterinarians and NGO staff. Non-laboratory staff were shown gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) machines used for testing poisoning samples. We discussed the pros and cons of using each machine and which type of samples were better suited to each machine.

## Day four: Laboratory techniques, 6 October, JKUAT, Juja

**Attendance:** 3 wildlife veterinarians, 13 laboratory professionals, 2 NGO staff, and 2 trainers

The morning was spent in the lab conducting sample collection and preparation using brain samples from domestic ducks and a case of known exposure. This was in preparation for a two-part analysis, with one test looking for the actual chemical of interest in the brain tissue using GC-MS (quantitative using a reference library) and LC-MC (qualitative due to the lack of standards) and the second part looking at the toxicity impact of the chemical of interest in the brain. This was a case study with known positive cases, therefore the analysis focused on two chemicals of interest. It was discussed at length that if this was a real case much more exploratory analysis would be needed but the principles of testing remain the same. Participants were split into two groups and asked to make reference standard curve solutions. The MS tests were prepped and run over night.

In the afternoon two often-used pesticide groups, namely organophosphates and carbamates, which both kill quickly by inhibiting acetylcholinesterase enzymatic (AchE) activity, were discussed. How this mechanism of action (cholinesterase inhibition) causes well recognized clinical signs such as paralysis diarrhea, urinary incontinence, tremors, confusion, ataxia (clumsy involuntary movements) and seizures was reviewed. To test if a sample of brain tissue has inhibited AChE activity, brain tissue with the enzyme, a substrate (a chemical the enzyme cuts up), and a buffer were added together. If the AChE is working the mixture turns yellow; if the AChE is inhibited by a poison, the sample stays opaque. The amount of yellow is measured by a spectrophotometric reader, and correlates to the amount of AChE activity. References of what ‘normal’ AChE activity is in the brain (and blood) per species is needed for ideal interpretation. For example, birds often have 20x more activity naturally than mammals. These tools are available in most labs and easy to run.

Methods to differentiate poisons were tested. It is well established that carbamates do not create a chemical bond with AChE and therefore if you add more buffer to the brain/substrate mixture, you can effectively wash out the inhibitory poison and the sample will turn yellow over time. The second group of poisons, organophosphates, bind strongly to AChE but if you add a drug called 2-PAM, the 2-PAM will unbind the poison and re-activate the AChE, and the sample will turn yellow over time. Formulas and data analysis were reviewed to calculate AChE inhibition and recovery.

## Day five: Laboratory techniques and wrap-up, 7 October, JKUAT, Juja

**Attendance:** 2 wildlife veterinarians, 15 laboratory professionals, 2 NGO staff, and 2 trainers

**Morning session**

Final results of anticholinesterase testing were presented to the group after which there was a presentation about methodology using different lab equipment.

*Method development GS-MS and LC-MS*

Dr Elijah Ngumba

This presentation discussed the following topics.

- How do I get my sample into the LC?
- How do I transport my sample through the LC?
- How do I transfer my sample onto the column?
- How do I choose my column?
- How do I separate my analytes?
- How do I choose my mobile phase?
- How do I detect my analytes?
    - If, MS, which mass analyser is best?
    - How to ionise analytes?

Also discussed was what to consider when developing methods for GC-MS. Steps for method validation and when this should be done, and method calibration using external vs internal methods.

**Afternoon session**

Wrap-up session was led by Dr Eunice Nyawade, representing the chairman, Chemistry Department of JKUAT. Participants provided positive feedback on the laboratory training and thanked the workshop organizers and supporters for the training. Certificates were given to laboratory professionals who attended all three days of the training.

## The way forward

Based upon feedback provided during and after the workshop, and through a course evaluation, the following suggestions were raised.

### Kenya Wildlife Toxicology Capacity Building

Kenya Wildlife Toxicology Technical Working Group (TWG): This TWG will meet and ensure gaps identified during the stakeholders meeting are recognized, addressed, and funding sources found as needed. NGOs and research teams will work with the TWG to ensure wildlife toxicology capacity building happens in a sustainable and coordinated effort so pressing issues can be addressed. Examples could include sourcing quality standards, new technique training (e.g. cyanotoxin testing) can be perused via network connections with laboratories actively doing the technique of interest, and incorporating vulture poisoning prevention support into on-gong community events.

During workshop presentations and discussions there were a number of challenges raised as follows:

- Lack of veterinary capacity in many areas of the country
- Lack of veterinary capacity related to avian medicine
- Who pays for samples to be tested and is this standardized throughout KWS?
- Laboratories may lack the standards, reagents and proper equipment necessary for testing
- There can be a long turnaround time for samples to be tested at Government Chemist
- It is not clear where to take samples for snakebite and cyanobacteria
- For Chain of Custody there can be different people involved (vets, Security Dept, police) who have different levels of knowledge and there can be a lack of clear leadership
- It is not always clear who is responsible for taking samples to the lab
- Lack of cold storage in the field and during transport to labs
- Who transports samples and pays for this?
- There's a number of people/organisations that still require training

Many of these aspects have been included in the TWG mapping exercise and plan (see above section on day two)

### Veterinary aspects

Workshops: For future workshops participants requested more training about avian physiology, and raptor medicine, surgery and anesthesia. It was suggested that it would be ideal if Simon Thomsett gives a lecture on the Kenyan perspective (working with KWS, field techniques etc.). It was highlighted that some of the techniques are not practical in a field situation. There should be a dedicated afternoon for poisoning response training. Sample collection training with field teams most likely to see a crime scene first (rapid responders, rangers, KWS veterinarians).

Equipment and Supplies: Toolkits for rapid sample collection, preservation, and storage techniques were requested. Set up sustainable pipelines for veterinary medical supplies for poison response and recovery as needed.

Research: Wildlife population level research to identify current state of wildlife toxicology cases and threats. Increased support for wildlife monitoring of raptors to identify if poisoning events are preventing population stability and/or the possibility of species recovery plans. Support discussions with teams doing vulture research across Africa, especially for migratory birds.

Rehabilitation and Release: Establish collaborations with zoos and Kenya rehabilitation centers to support raptor health before, during and after poisoning events.

### Laboratory aspects

Kenya Wildlife Toxicology Workshop: There was an interest to continue such workshops to keep the momentum going and broaden the overlap between Kenya's extensive chemistry expertise and applying it to toxicology.

Advance Wildlife Toxicology Workshop: Participants are looking for a workshop to spend more time on the equipment alongside experts, particularly GC-MS and LC-MS, for training to cover testing the same sample using different methods, including TLC, and for data analysis and interpretation of results. Future trainings should also include quantification methods for pesticide and drug samples. In terms of sample preparation, there should be training on how to extract carbamates for GC-MS and there should be a range of polar and non-polar molecules to be analysed to overcome the challenges of missing the polar compounds during extraction.

Wildlife Toxicology Exchange Program: Participants requested help with facilitating them to visit advanced toxicology laboratories for continued education, e.g in Spain. This idea could be expanded to to include veterinary aspects, including Smithsonian's National Zoo and San Diego Zoo Wildlife Alliance, additional forensic laboratories including National Fish and Wildlife Forensics Laboratory and Patuxent Wildlife Research Center, and diagnostic labs such as California Animal Health & Food Safety Lab System and MSU Veterinary Diagnostic Laboratory.

Laboratory Collaboration Support: Support communication across laboratories in and outside of Kenya where expertise in new laboratory analysis, maintenance tips and tricks, and consumables can be shared as needed to ensure toxicology testing is an available tool to fight against wildlife poisoning events.

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