PROTOCOL FOR TAKING SAMPLES IN THE FIELD FROM DEAD ANIMALS (PRESUMABLY POISONED) FOR TOXICOLOGICAL ANALYSIS

Project: Bright Future for Black Vulture in Bulgaria

LIFE14 NAT/BG/000649

Action: A5

INTRODUCTION

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1.1 COLLECTION OF SAMPLES

The products used for poison baits are often highly toxic insecticides. In general, the finding of any artificial food piece in the environment should lead to suspicion of it being poisoned bait.

The discovery of dead animals does not always mean that the cause of death was poisoning, however, the cadaver should be collected, whether it is classified as species of interest, common species or domestic animal (**all species, or parts of them are relevant for detection of a poisoning case**).

First we must remember that the products used are highly toxic so care should be taken in their collection. It is very important to collect all baits and dead animals found since it can be important evidence for the investigation process of the presumably poisoning incident.

It is strongly recommended sending the whole body to the lab because that is where the best samples in the search for a possible poison are selected. If not possible, then a veterinarian with experience in wildlife necropsy should perform the necropsy following the instructions of the corresponding section.

COLLECTION OF POISON BAITS, CARCASSES, AND OTHER SAMPLES FROM THE NATURAL ENVIRONMENT

Handling the toxic baits or poisoned dead animals can be dangerous, and besides, if samples are incorrectly taken one may invalidate any administrative or judicial action later one. In order to avoid that the following instructions must always be respected:

- **For collection of samples always wear nitrile gloves and mask FP3**. The products used have a high toxicity and may act through the skin or be inhaled (Image 1).
- All baits and cadavers that are found in the inspection area must be collected. All can be evidence of a crime and very important for any eventual judicial case. Removing of the poison baits or cadavers from the field is also important to avoid new cases of poisoning.
    - **Bait**: wrap each piece in aluminium foil (do not use plastic, especially when poison is observed at the exterior of the bait, as it can interfere with the toxicological analysis) (Image 2), and insert it in a plastic container or plastic bag (one for each bait) (Image 3). Label/number each container, put it in a bag (see section 1.3) and seal. If several samples/baits are collected they can be placed into the same bag for transport to the laboratory (Image 4).
    - **Toxic substances**: Often poison bags or bottles can be found during the inspections, mainly in indoor inspections. These compounds/substances should be collected and a portion should be sent to the laboratory for analysis. Use a closed container (Image 3). Label and number it, put it in a bag (see section 1.3) and seal.
    - **Corpse:** regardless whether it is a fresh or skeletonized (Image 5 and 6) corpse, packaging must be double (see section 1.3): the corpse should be placed in a bag (Image 4) (one per corpse) and this bag into another to prevent the accidental release of fluids from the corpses autolysis. Then labelled/numbered and sealed.
        - If some residues are observed: baits or vomiting in the mouth or beak (Image 13), it is recommended to collect in aluminium foil and include it then in a closed container (procedure like for baits) (Image 7). It is also possible to cover the head with aluminium foil; in this way it is avoided to handle the remains found in the mouth.
    - **Soil sample**: if suspicious substances (poison alike) are observed on the ground (e.g. vomiting or toxic residues) (Image 16), collect and insert the material in plastic container (Image 7), number (see section 1.3) and seal.

It is also advisable to collect soil samples under the corpse, 5 cm deep. (Image 8).

- **Entomofauna**: insects consume the corpse, so they are very important for the toxicological analysis, as they can contain the poison ingested by the animal inside. Collect insects (Image 9) in a closed container (Image 7). Number, pack (see section 1.3) and seal.
- **Vomit**: same as bait, put it in a container or plastic bag. (Image 16). Number each container, put it in a bag (see section 1.3) and seal.

1.2 SAMPLES FROM NECROPSY

It is strongly recommended to submit the whole body (corpse) to the lab, as this is where the best samples for the detection of poisons are selected accordingly. There are special cases or circumstances (very large animals) in which it is not possible to send the whole body to the lab. Only in these cases the necropsy should be performed by a veterinarian with experience with wildlife and forwarded the following samples to the laboratory (securely packaged in tightly closed cans (Image 7), labelled and sealed (see section 1.3)):

- **Stomach contents** (complete): the most important part for the detection of poison sample. Place it in a tightly sealed container. (Image 10).
- **Liver and kidney** (complete): place them separately in tightly closed containers (Image 11 and 12).
- **Oral content**, **oesophagus** (generally different samples of stomach digestive system):
  - If remains of baits or vomit in the mouth are present, collect in foil and include in a container then close.
  - Scrape the entire digestive tract, from the mouth into the oesophagus and put it in a container.
  - For birds: collect the contents of the ventricle-proventriculus (Image 14), and place in a closed container. If content cannot be observed scratch as in the previous case.
- **Claws**: The claws may contain traces of possible bait, so collection is recommended. Cut full claws (**don't try to open them**) wrap in foil and then in a container (Image 15).

1.3 LABELING AND PACKAGING OF SAMPLES AND THEIR SUBMISSION BY THE FOR TRANSPORT OF HAZARDOUS TOXIC AND BIOLOGICAL MATERIAL ACCORDING TO THE NATIONAL LAW

All samples should be frozen as soon as possible - prior to transport (to the lab) to avoid possible degradation of the poison, thus facilitating its detection, identification and quantification in the laboratory.

As indicated in each section, all samples must be labelled. These numbers or codes will be the same that appears in the records (field forms) and in the chain of custody.

To meet the requirements for transport of material with toxic / infectious risk category B, according to the P650 of existing European legislation these steps for the packaging of the samples have to be respected:

- All samples must be sent in **double green bag tightly closed and all these have to go into the sealed container (big one)** (Image 18).
- On the bottom of the container, there must be an **absorbent material** (if there is a spill). Paper towels can be used for example.
- The container on the outside must have white “diamond” shaped sticker saying: "**Exempt Animal Specimen**".

The green bags and the container should be properly sealed (Image 4 and 18). These seals must also be recorded in the records (forms) and chain of custody that accompany the samples.

DETAILED INFORMATION ON PACKAGING, LABELLING AND DOCUMENTS FOR SUBSTANCIES “CATEGORY B (infectious)”

The packaging must meet the requirements of P650:

a) Packaging / containers must be of good quality, strong enough to withstand shocks and loadings during the transport, including transhipment between cargo units and between transport units for manual or mechanical handling. Packaging / containers have to be made and closed in way not be deform under normal conditions of transport (pressure, vibrations or change in temperature of humidity).

   Packaging / container must consist of three elements: 1) a primary container, 2) a secondary packaging; and 3) external packaging of which either the secondary packaging / well packaging / external packaging has to be rigid. At least one

surface of the packaging / external packaging must have a minimum size of 100mm x 100mm.

b) The primary containers are placed in a secondary package. Under normal conditions of transport the primary container shouldn't break or leak contents into the secondary packaging. The secondary packaging shall be secured in the external packaging with suitable softening material.

c) For liquid substances:

Primary containers and secondary packaging must be water-resistant. The primary receptacle or the secondary packaging must withstand without leakage, an internal pressure of 95 kPa (0.95 bar).

If several fragile primary receptacles are placed in a single secondary packaging, the primary container shall be individually wrapped or separated to prevent contact between them. Absorbent material between the primary containers and secondary packaging is also needed. The absorbent material shall be in sufficient quantity so that it can absorb the entire contents of the primary containers so that the release of the liquid substance will not compromise the integrity of the softening material of the external package.

d) For solid substances:
Primary containers and secondary packaging must be leak-proof.
If several fragile primary receptacles are placed in a single secondary packaging, the primary receptacles shall be individually wrapped or separated to prevent contact between them.
When in doubt about the presence of residual fluid in the primary receptacle during transport, packaging suitable for liquids, including absorbent materials should be also used.

e) Labelling:
The name, address and phone number of the recipient
Requirements for storage temperature (optional).
The proper shipping name ("BIOLOGICAL SUBSTANCE, CATEGORY B") next to the following rhomboid mark:

Annex: figures/images

Image 1. Basic personal protective equipment (nitrile gloves, masks FP3)

Image 2. Bait in aluminium foil.

Image 3. Bait in aluminium foil and sealed plastic bag and container

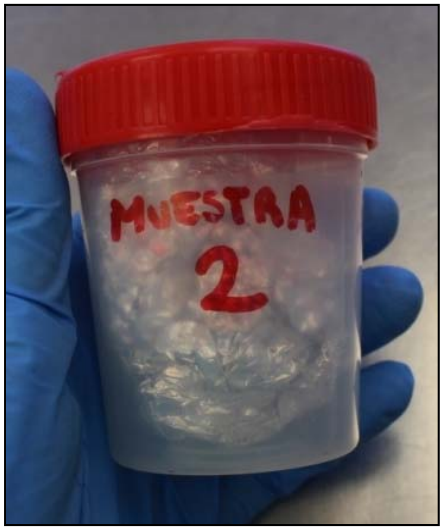


Image 4. Sealed bag for transfer of corpses and containers with samples

Image 5. Fresh corpse

Image 6. Skeletonized corpse

Image 7. Containers for submission of samples

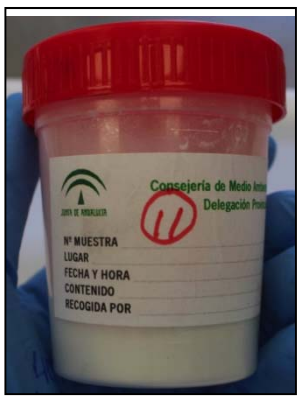
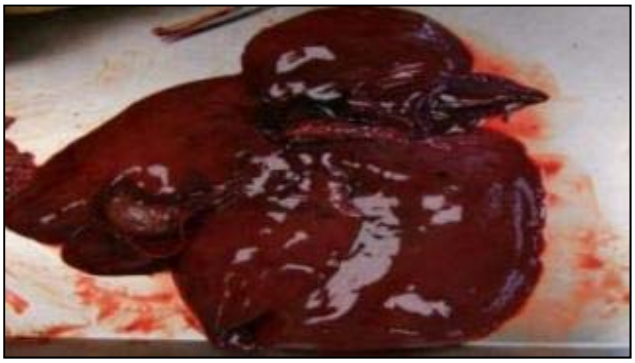


Image 8. Entomofauna and soil below the cadaver

Image 9. Entomofauna

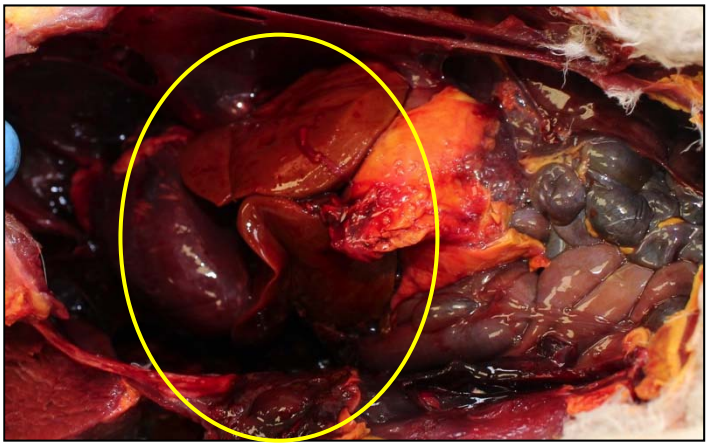
Image 10. Stomach contents

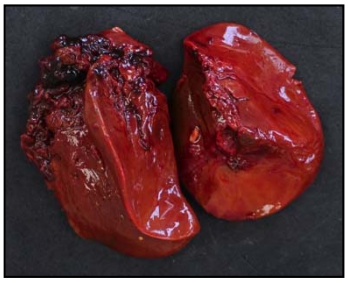
Image 11. Liver	Image 12. Kidneys



e.g. Egyptian Vulture necropsy

Liver





Kidneys

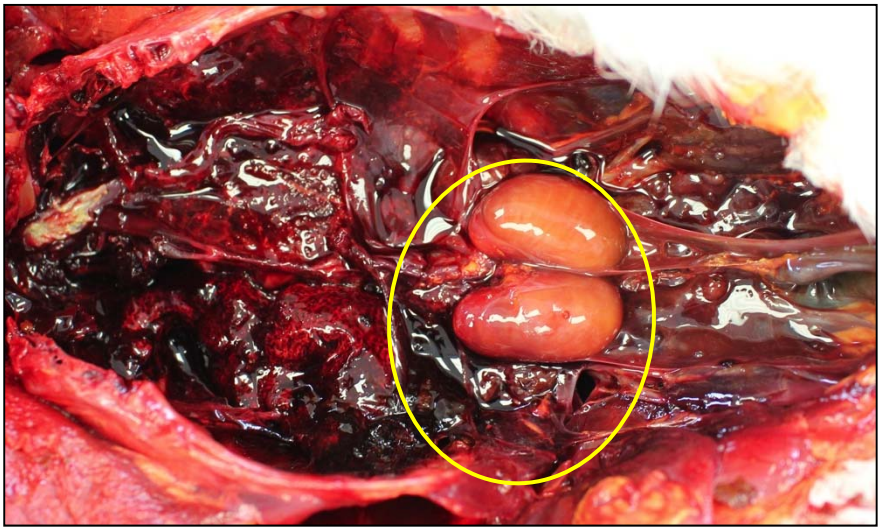


Image 13. Remains of bait in the peak

Image 14. Ventricle

Image 15. Claws

Image 16. Vomit

Image 18. Sealed container (the big one).