Missouri
Department of
Natural Resources

UCFWO SOP: Removal of Liver Tissue from Passerines for Analysis of Metal Residues

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I. General

This standard operating procedure (SOP) outlines methods to collect liver tissue from small birds (e.g., passerines) for analysis of metal residues. The tissue preparation and metal residue analysis of liver tissue is outlined by the analytical facilities responsible for the analysis. This SOP is in addition to established procedures for bird collection and initial measurements on those birds. The SOP for bird collection is identified in the sampling and analysis plan or protocol that identified this procedure.

This SOP requires that birds be euthanized. Permissible euthanasia procedures include applying gentle pressure on the sternum of the bird until movement stops.

II. Equipment

Copies of approved State and Federal permits for bird collection
Powder free gloves
Cryogenic tubes
Work table and chairs
Ethanol
Alconox
Reagent-grade nitric acid
Squirt bottles
Deionized water
Dissecting tools (scalpel, dissecting forceps (stainless), dissecting scissors (stainless))
Dissecting tray
Work lights (head or table mounted)
Liquid Nitrogen and dewer with sample holders or dry ice and cooler
Permanent black ink pins
Data forms
Paper towels
Kimwipes

III Dissection and tissue collection procedure

Wear powder-free gloves for labeling and handling of vials, and for the dissection procedure. Label and preweigh a cryogenic vial to the nearest 0.001 g. Ensure balance is level with a stable zero (no sample or vial). All handling of vials must be with gloved hands. Place the dead bird on its back on the dissecting tray. Gently blow on the breast of the bird to expose the unfeathered area along the sternum. Spray ethanol on this area to dampen the feathers and sterilize the area. Cut the skin from the posterior region of the sternum to the abdomen with a scalpel. Cut the musculature with dissecting scissors at the edge of the breast muscles to expose the peritoneal cavity. Gently lift the breast from the cardiac region and cut the connective tissue with the scalpel or scissors. Collect blood from the heart if required by other protocols or standard procedures. Remove the majority of the breast with dissection scissors. The bird's left liver lobe can be removed rapidly by cutting the vasculature and connective tissue with clean (decontaminated) dissection scissors. The bird's right lobe contains the gall bladder and must be removed carefully to avoid contaminating the liver with bile. The right lobe with gall bladder must be removed together by cutting the vasculature and connective tissue. Place the right lobe (with gall bladder) on a clean Kimwipe, clamp the proximal end of the gall bladder with forceps, and remove the gall bladder with dissection scissors. Note on data sheets if any visible bile from the gall bladder contacts the liver. Place liver pieces into the labeled, and preweighed cryogenic vial, and freeze immediately on liquid nitrogen or dry ice. Before freezing, verify the sample ID on the vial and data sheet match. Remove other tissues as indicated in the sampling and analysis plan or study protocol.

Dissecting tools will be dedicated to specific procedures. Dissecting tools used to expose the internal organs will not be used to remove tissues. Dissection tools will be decontaminated prior to each use (see below).

Deceased birds will be disposed of properly as indicated on state and federal bird collection permits.

III. Dissection tool cleaning procedure

All dissecting tools and trays contacting bird tissue must be cleaned prior to use with another bird. The solutions used for cleaning include a weak alkanox soap solution, 5% nitric acid solution, and deionized water, all in squirt bottles. The weak alkanox soap solution is sprayed on the tool and the edges of the tool are gently scrubbed with a kimwipe. The tool is sprayed with deionized water to wash soap off. The tool is rinsed with nitric acid solution, then rinsed with deionized water and placed back onto a clean kimwipe at the dissecting area.

The alkanox solution is made in one liter increments by placing approximately one gram of alkanox in the prelabeled bottle, and diluting with tap water. The nitric acid solution is also made in one liter increments by filling the bottle 50% full with deionized water, adding 50 ml of reagent-grade concentrated nitric acid, then filling the bottle to the neck with deionized water. DO NOT add acid first, a violent reaction will result when the deionized water is added.

VIII. Sample handling and documentation

Liver tissue samples will be placed into cryogenic tubes, then the sample tube will be placed into liquid nitrogen or on dry ice. The sample ID on the cryogenic tube and data sheet will be verified for accuracy prior to placing into liquid nitrogen. The sample ID will be verified against the data sheet prior to starting procedures on the next bird. The liver samples will be stored in the office freezer upon return to the office. Samples will be shipped on dry ice to the analytical laboratory.

Data sheets will be placed into a dedicated three-ringed binder immediately after being completed. Data sheets will be photocopied weekly and copies placed in an archive three-ringed binder that is kept at the UCFWO

IX. Safety

Powder-free gloves will be worn during the procedure to avoid contaminating samples and as a safety procedure for field technicians.

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