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Waste and Environmental Services

Standard Operating Procedure

for **PROCESSING BIOTA SAMPLES FOR ANALYSIS**

APPROVAL SIGNATURES:

Subject Matter Expert:	**Organization**	**Signature**	**Date**
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1.0 PURPOSE AND SCOPE

This procedure describes the methods of processing (i.e., preparing samples for analysis) foodstuff (produce, eggs, honey, fish, and game animals) and non-foodstuff (vegetation, mice, bees, birds) biota samples for the analysis of radionuclides, target analyte list (TAL) elements, and PCBs. Detection levels for radionuclide and TAL elements and method specs are given in Attachment 1.

2.0 BACKGROUND AND PRECAUTIONS

2.1 Background

This document establishes the basic requirements for processing biota samples for analysis and applies to all personnel performing laboratory procedures described in this document. Work performed under this procedure by LANL personnel will occur only after required training to applicable documents has been completed and documented.

2.2 Precautions

See Hazard Review (Attachment 2) for any precautions during processing of samples.

3.0 EQUIPMENT AND TOOLS

• Cutting boards and knives; • Balance; • Glass beakers (50-mL, 100-mL, 1-L, and 2-L volumes); • Aluminum foil; • Hot-mitts/pot holders; • Hot plate; • Watch glass; • Plastic wrap (*e.g.*, Saran wrap™); • Ice cubes; • Small paper bags;	• Wiley mill with a 40-mm screen; • Drying and ashing ovens; • Polyethylene bottles (20-mL and 500-mL volumes; one for each sample); • Ziplock™ bags (gallon size) and labeling pens; • Chain-of-custody tape; • Laboratory notebook; • Personal Protective Equipment (e.g., safety glasses, safety shoes, lab coat, rubber gloves, cut-resistant [Kevlar] gloves when using knife, and face shield when cutting up game samples).

4.0 STEP-BY-STEP PROCESS DESCRIPTION

4.1 Processing Biota Samples for Tritium Analysis

Analyst	1.	Don safety glasses, lab coat, and heavy rubber gloves.
	2.	Water is distilled from a sample and collected. There are various distillation vessels to achieve this, but a simple setup is as follows. Place a 100-mL beaker upside-down in the center of a 1L sample beaker, with a 50-mL beaker right-side-up on top of it. Then place samples in the 1L beaker. Refer to Schematic of Distillation Setup (Attachment 3).
	3.	Cover the top of the large 1L beaker with a watch glass and seal with plastic wrap.

	4.	To aid in condensation of the water sample, fill a beaker with ice and place it on top of the watch glass.
	5.	Place the sample on a hot plate, warming at a low temperature until water begins to condense on the watch glass. Be certain that the condensation drips into the 50-mL sampling beaker. **CAUTION!! Hot plate and glassware will become hot! Use care when handling these items.**
	6.	Collect about 15mL of distillate from each sample, and carefully place sample into labeled 20-mL polyethylene bottles.
	7.	Seal each bottle with chain-of-custody tape, and record each sample on the appropriate chain-of-custody form.
	8.	Place all tritium samples and the chain-of-custody form into a labeled Ziplock™ bag and refrigerate. Maintain chain-of-custody on the samples (see chapter *Chain-of-custody for samples*).

4.2 Processing Biota Samples for TAL Analysis

Analyst	**Produce and Vegetation**	
	1.	<u>Produce</u> Remove approximately 100 g (fresh weight) of produce from each composite sample and rinse as though being washed for human consumption. Pat the produce dry with paper towels, and cut it into pieces to facilitate oven drying. Place samples into labeled paper bags. <u>Native Vegetation</u> Remove approximately 100 g (fresh weight) of vegetation from each composite sample and cut or break vegetation into smaller pieces to facilitate oven drying. (Note: native vegetation is not washed.) Place individual samples into labeled paper bags.
	2.	Place paper bags into drying oven and dry the samples in the beakers at 75°C for a minimum of 48 hours, or up to 5 days for certain sample types.
	3.	After initial drying, weigh the samples to the nearest 0.01 g. Continue drying and weighing the beakers each day until sample weights are constant (+10%) in two successive weighings—samples are dry.
	4.	Remove the samples from the oven, and grind each through a 40mm screen using a Wiley mill. Wear safety glasses, lab coat, and heavy rubber gloves when using mill. Clean after use with a small vacuum cleaner. **CAUTION**: Do not operate mill unless you have received personal instruction from a previously trained group member.
	5.	Place ground samples into labeled 20-mL polyethylene bottles, and then seal the bottles with chain-of-custody tape.
	6.	Place the sealed bottles into a labeled Ziplock™ bag
	7.	Record all samples on a chain-of-custody form, and maintain proper chain-of-custody

		on the samples until submitted to the analytical laboratory. See chapter *Chain-of-custody for samples.*
		Fish, Game Animals, Mice and Birds
	1.	Fish Clean and rinse fish with water. Game Animals Thoroughly wash muscle, bone, and organs to remove excess blood and/or debris. (Wear face mask when cutting up game samples.) Use paper towels to pat-dry.
	2.	Fish Remove a 10-g (fresh weight) sample of meat (fillet) from fish. Game Animals Remove a 10-g (fresh weight) sample of muscle, bone, or organ. Mice Mice are submitted whole. Birds Birds are submitted whole
	3.	Put samples into individually labeled Ziplock™ bags and place in freezer. Keep samples frozen until analysis.
	4.	Record all samples on a chain-of-custody form, and maintain proper chain-of-custody on the samples. See chapter *Chain-of-custody for samples.*
4.3	**Processing Biota Samples for Radiochemical Analysis**	
Analyst	1.	Prepare the sample beakers by labeling (with pencil) and weighing the 2-L beaker to determine the tare weight and record this value in the laboratory notebook.
	2.	Place ~ 500 to 2,000 g of produce (sliced), fish (head and viscera removed), game animal (muscle, bone, or organ meat), vegetation, honey, mice (whole body), and birds (whole body) into labeled 2-L tared beakers and weigh to the nearest 0.01 g to determine gross weight.
	3.	Record the fresh weight of the samples (subtract the tare weight from the gross weight) in the laboratory notebook.
	4.	Cover each beaker with vented aluminum foil. Poke holes into the aluminum foil to aid the venting processes and place in the drying oven. Carefully note or map the placement-order of the beakers in the lab notebook (this is important because during the ashing process the labeling may burn off.)
	5.	Dry the samples in the beakers at about 75°C a minimum of 48 hours, or up to 5 days for certain sample types.
	6.	After initial drying, weigh the samples to the nearest 0.01 g. Continue drying and weighing the beakers each day until sample weights are constant (+10%) in two successive weighings—samples are dry.

	7.	Remove dry samples from the oven and weigh them to the nearest 0.01 g. Subtract the original tare weight from this gross weight to calculate the dry weight of each produce sample. Enter this data in the laboratory notebook.
	8.	Place samples in the ashing oven, carefully note placement of beakers, and burn the samples for about 5 days to an ash. During ashing, raise the temperature slowly and step-wise from 75°C to 500°C to avoid explosive combustion of the organic materials in the early stages of the process.
	9.	After ashing is complete (sample should look white) and samples have cooled, reweigh the samples to the nearest 0.01 g. Calculate ash weights by subtracting tare weights from gross ash-weights. Record all data and calculations in the laboratory notebook.
	10.	Transfer each ash sample to a 500-mL polyethylene bottle and label the bottle.
	11.	Seal the bottles with chain-of-custody tape and record all samples on a chain-of-custody form. See chapter *Chain-of-custody for samples.*
4.4	**Processing Fish Samples for Organic (PCB) Analysis**	
Sampler and Analyst	1.	Record fish weight and length.
	2.	Gut and separate viscera (organs, fatty deposit).
	3.	Cut a fillet sample containing both meat and skin.
	4.	Put fillet sample in pre-labeled 500mL amber screw-top jars and place in refrigerator or freezer until analysis.
	5.	Record all samples on a chain-of-custody form, and maintain proper chain-of-custody on the samples. See chapter *Chain-of-custody for samples.*
4.5	**Processing Mice and Bird Samples for Organic (PCB) Analysis**	
Sampler and Analyst	1.	Place whole body mice and bird samples into pre-labeled 500mL amber screw-top jars and place in refrigerator or freezer until analysis.
	2.	Record all samples on a chain-of-custody form, and maintain proper chain-of-custody on the samples. See chapter *Chain-of-custody for samples.*
4.6	**Maintaining Custody of Samples**	
Sampler and Analyst	1.	Document chain-of-custody for all samples used to demonstrate compliance.
	2.	Verify the possession and handling of samples is traceable at all times. [NOTE: A sample is considered in custody if it is one of the following: • In one's physical possession; • In one's view after being in one's physical possession; • In one's physical possession and then locked up so that no one can tamper with it; or • Kept in a secure area where access is restricted to authorized and accountable personnel only. A secured area is an area that is locked (e.g., a room, cooler, vehicle, or refrigerator).]

	3.	If the area cannot be secured, use a custody seal to secure the area or the sample container.
4.7	**Transferring Custody of Samples**	
Sampler and Analyst	1.	Whenever samples are transferred into the custody of another person or organization, complete the "relinquished by/received by" and "date" sections of the form. [NOTE: These sections of the form must provide a complete history of custody of the samples from collection to transfer to the analytical laboratory.]
4.8	**Broken Chain-of-Custody**	
Sampler and Analyst	1.	Whenever there is a break in the chain-of-custody of a sample, document the failure by initiating a deficiency report.
	2.	Document the occurrence, evaluate the potential impact (if any) on the samples, and propose a fix to prevent recurrence.
4.9	**Emergency Actions to Take in the Event of Control Failure**	
Sampler and Analyst	1.	Perform First Aid for cuts, as appropriate.
	2.	For all injuries, provide first aid and see that the injured person is taken to Occupational medicine (only if immediate medical attention is not required) or to the nearest hospital.
	3.	Notify the individual's supervisor and group office as soon as possible.
4.10	**Records**	
Sampler and Analyst	1.	Submit the following records generated by this procedure to the Records Processing Facility: • Completed Chain of Custody form. • Copy of Shipping Manifest.

5.0 PROCESS FLOW CHART

Flow chart is to be included at a later date.

6.0 ATTACHMENTS

Attachment 1 Reporting limits for radionuclides and TAL elements required for analysis.

Attachment 2 Hazard Review for Processing Samples for the Foodstuffs and Nonfoodstuffs Monitoring Program.

Attachment 3 Schematic of Distillation Setup.

7.0 REVISION HISTORY

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Revision No. *[Enter current revision number, beginning with Rev.0]*	Effective Date *[DCC inserts effective date for revision]*	Description of Changes *[List specific changes made since the previous revision]*	Type of Change *[Technical (T) or Editorial (E)]*
0	10/4/96	New Document	T
1	3/99	Reformatted in accordance with LIR300-00-01, Safe Work Practices.	E
2	4/01	Added new Section 9.0, Training.	T
3	4/02	Change in directorate.	E
4	4/03	Team name change to Environmental Surveillance.	E
5	7/13/04	Updated and reformatted document to conform with MAQ procedures.	E
6	11/20/04	Add instructions for oven temperature setting and ventilation, added HR to replace HCP.	T
0	1/30/08	Changed title, renumbered, and reformatted to WES Division.	E

Using a CRYPTOCard, click here to record "self-study" training to this procedure.

If you do not possess a CRYPTOCard or encounter problems, contact the ERSS training specialist.

Appendix 1.

MDC for Radionuclides and Reporting limits for metals and method specs for BIOTA (tissue)

Element	**MDC or Reporting limits**	**Media**	**Method**	**MDC or RL**	**Special considerations**
H-3	0.50 pCi/mL	water	906.0 LSC	0.50 pCi/mL	5ml min volume with long count. 10ml volume uses normal count.
Am-241	0.01 pCi/g	ash	ASTM-D3972-mod Alpha spec	0.01 pCi/g	7 gram minimum, with 1000 min. count. (note 3g gives 0.03 MDC)
Cs-137	1 pCi/g	ash	901.1 mod Gamma	1 pCi/g	8 gram minimum, with 1000 min count
Sr-90	0.05 pCi/g	ash	ASTM-D5811-mod GFP	0.05 pCi/g	5 gram minimum with 1000 min count
Pu isotopes	0.01 pCi/g	ash	ASTM-D3972-mod Alpha spec	0.01 pCi/g	7 gram minimum, with 1000 min. count. (note 3g gives 0.03 MDC)
U isotopes	0.01 pCi/g	ash	ASTM-D3972-mod Alpha spec	0.01 pCi/g	7 gram minimum, with 1000 min. count. (note 3g gives 0.03 MDC)
			SW 6010	RL (ppm) 1 gram aliquot	RL (ppm) 5 gram aliquot (if feasible *)
Al	2 ppm	tissue	ICP	10	2
Ba	0.05 ppm	tissue	ICP	0.2	0.04
Be	0.02 ppm	tissue	ICP	0.1	0.02
Ca	10 ppm	tissue	ICP	50	10
Cr	0.1 ppm	tissue	ICP	0.5	0.1
Co	0.1 ppm	tissue	ICP	0.2	0.04
Cu	0.05 ppm	tissue	ICP	0.2	0.04
Fe	1 ppm	tissue	ICP	5	1
Mg	10 ppm	tissue	ICP	50	10
Mn	0.1 ppm	tissue	ICP	0.2	0.04
Ni	0.1 ppm	tissue	ICP	0.5	0.1
K	500 ppm	tissue	ICP	50	10
Na	5 ppm	tissue	ICP	50	10

V	0.1 ppm	tissue	ICP	0.5	0.1
Zn	0.1 ppm	tissue	ICP	0.5	0.1
Hg	0.01 ppm	tissue	Cold vapor AA	0.01	NA
			SW 6020	RL (ppb) 1 gram aliquot	RL (ppb) 5 gram aliquot (if feasible *)
Sb	5 ppb	tissue	ICPMS	30	6
As	30 ppb	tissue	ICPMS	200	40
Cd	5 ppb	tissue	ICPMS	30	6
Pb	8 ppb	tissue	ICPMS	50	10
Se	20 ppb	tissue	ICPMS	100	20
Ag	2 ppb	tissue	ICPMS	10	2
Tl	3 ppb	tissue	ICPMS	20	4

ATTACHMENT 2: HAZARD REVIEW FOR PROCESSING AND SUBMITTING SAMPLES FOR THE FOODSTUFFS MONITORING PROGRAM

Hazard Review for Processing and Submitting Samples for the Foodstuffs and Nonfoodstuffs Monitoring Program	Records Use only 

Work Tasks/Steps	**Hazards, Concerns, and Potential Accidents; Likelihood/Severity**	**Controls, Preventive Measures** (e.g., safety equipment, administrative controls, etc.)	**Hazard Level** (from IMP 300-00-00, Hazard Grading Matrix)
Steps to process samples in chapter *Processing samples for organics analysis*: use electrical appliances such as hot plates	Burns from hot surfaces (occasional/moderate = low)	Wear safety glasses, lab coat, and rubber gloves. Be familiar with the operator's manuals for each piece of equipment.	Low
Handle hot glassware when removing from ovens or hot plates	Hot glass (occasional/moderate = low)	Use hot mits when handling hot equipment or parts.	Low
Handle hot glassware—breakage is possible	Broken glass edges can cause cuts (occasional/moderate = low)	Wear safety glasses, lab coat, and cut-resistant (Kevlar) gloves	Low
Process samples in steps in chapter *Processing samples for tritium analysis*	Splattering of hot water (occasional/moderate = low)	Wear safety glasses, lab coat, and heavy rubber gloves.	Low
Turn on oven and raise temperature (steps in chapter *Operating the drying/ashing oven*	Smoke and smell can cause irritation to other building residents (occasional/moderate = low)	Ensure hood fan is turned on to create negative pressure in lab room—any time oven is to be used.	Low

Work Tasks/Steps	**Hazards, Concerns, and Potential Accidents; Likelihood/Severity**	**Controls, Preventive Measures** (e.g., safety equipment, administrative controls, etc.)	**Hazard Level** (from IMP 300-00-00, Hazard Grading Matrix)
Steps to dry and ash samples in chapter *Operating the drying/ashing oven*	Burns from hot surfaces of drying and ashing ovens (occasional/ moderate = low)	Use hot-mitts or pot holders when working with the ovens, hot plates, or hot beakers.	Low
Grind samples using the Wiley Mill in chapter *Processing samples for heavy metal analysis*	The Wiley Mill (remote/ negligible = minimal)	Receive instruction on use of mill. Wear safety glasses, lab coat, and heavy rubber gloves. Be familiar with the operator's manual.	Low
Cut and process samples in steps in chapters *Processing samples for tritium analysis* and *Processing samples for heavy metal analysis*	Use of knives (improbable/moderate = minimal)	When knives are being used, wear cut-resistant (Kevlar) gloves to prevent injuries	Low
Cut and process samples in steps in chapter *Processing samples for tritium analysis*	Ergonomic injuries (repetitive motion) (remote/negligible = low)	Take a short break every hour.	Low
Cut and process samples in steps in chapter *Processing samples for tritium analysis*	Exposure to potential blood-borne pathogens (improbable/critical = low)	Wear a face shield.	Low

Wastes or Residual Materials

Take all waste foodstuffs and animal parts directly to the County Landfill. Do not dispose in dumpsters at TA-21.

Emergency Actions to Take in Event of Control Failure

For cuts and burns, perform First Aid as appropriate. Go to hospital for serious injuries. Go to HSR-2 for evaluation. Notify supervisor ASAP.

Appendix 3.

Schematic of Distillation Setup

For processing samples for tritium analysis