SPECIMEN HANDLING AND QUALITY ASSURANCE FOR THE RUTGERS SAMPLE PREPARATION PHASE OF THE AMCHITKA SCIENCE PLAN

J. Burger Original Draft 8-5-04 Revised 9-30-04

1. Program Description

1.1. The overall objective of the Rutgers Sample Preparation Phase is to receive, prepare, and send specimens to the analytical laboratories, and maintain appropriate Chain of Custody and tracking records.

There are two main analytic streams:

Analysis at INEEL will be as follows:

Soft tissue (fish,bird, invertebrates):
1) I-129
2) gamma suite (Am-241, Cs-137, Co-60, U-238, Eu-152)

Bone (fish,bird)
1) gamma suite (Am-241, Cs-137, Co-60, U-238, Eu-152)
2) Sr-90
3) Tc-99
4) U-234, 235, 236, 238,,
5) Pu-238, 239, 240

Kelp
1) I-129
2) gamma suite (Am-241, Cs-137, Co-60, U-238, Eu-152)
3) Sr-90
4) Tc-99
5) U-234, 235, 236, 238
6) Pu-238, 239, 240

A subset of these will also be analyzed at Vanderbilt for the same analytes for interlaboratory comparison. The separate Vanderbilt stream will focus on Cs-137 and other relevant gamma emitters (about 300 additional samples including quality control samples, and interlaboratory comparison).

For the INEEL stream there is a screening phase which will be followed by a re-assessment.

The Rutgers Laboratory Preparation phase has several components:

1.2. Receive specimens (in designated coolers).

1.3. Sort specimens appropriately and place them in freezers.

1.4. Track physical specimens while at Rutgers, and maintain records of shipment to analytical laboratories.

1.5. Select specimens for compositing.

1.6. Composite, homogenize, and prepare specimens for shipment.

1.7 Recode samples.

1.8 Pack and ship samples.

2. Purpose and Scope

2.1. The purpose of this document is to document policy, and methods for sample receipt, handling, preparation and shipment, and to describe quality assurance/quality control procedures for the Rutgers laboratory preparation phase.

3. Responsibilities

3.1. Program Director (J. Burger)

a. Establish policies and procedures

b. Monitor and conduct training sessions

c. Monitor specimen tracking, data collection, sample preparation, and sample management.

d. Monitor daily/weekly/monthly sample flow

e. Assign laboratory and other personnel

f. Work with V.Vyas and D. Kosson to track and monitor specimen flow to analytical laboratories.

g. Work with INEEL to ensure specimen flow follows agreed-upon procedures.

h. Oversee distribution of samples to analytical laboratory, and emaili notification of laboratories of sample shipment.

i. Provide V. Vyas with a list of all newly created archive specimens (derived from the compositing process of g and r samples).

j. Inform PI (C. Powers) and Analytical Coordinator (D. Kosson) and V. Vyas, about any significant changes in protocols or procedures.

k. Review data and update plans.

l. Maintain an inventory of all Controlled Copies of this laboratory manual.

m. Provide V. Vyas and D. Kosson with a table of all sample description, and re-coded sample identifiers for laboratory analysis.

3.2. Quality Assurance/quality control Director (J. Burger)

a. Oversee and monitor QA/QC procedures.

b. Develop use of Chain of Custody forms, modify as necessary. In this case, implement use of INEEL Chain of Custody forms.

c. Conduct QA/QC training of all personnel.

d. Interface with other project personnel and labs to ensure QA/QC between and among laboratories (INEEL, Vanderbilt).

e. For the laboratory sample preparation phase, QC is meant to include: calibration and documentation of balances at the beginning of each day, implementation and documentation of cross-contamination protocol (re: Volz), and accurate logging of samples to be shipped in each batch to analytical laboratories. QA is the totality of steps and procedures that document that the quality control program, Chain of Custody, data logging, and data transfer within and among laboratories is performed. Burger will regularly meet with laboratory staff to ascertain that procedures are in place and followed, identify any potential changes, and periodically review laboratory notebooks with technicians to assure that procedures outlined in this document are followed.

3.3, Specimen Tracker (A. Chaluseriu/S. Burke)

a. Become familiar with all protocols and procedures.

b. Work with Program Director (J. Burger) and CRESP data manager (V. Vyas) to develop specimen tracking procedures.

c. Track physical specimens in freezers and laboratory

d. Maintain original records of composites, and transmit duplicates to CRESP data management (V. Vyas) and Vanderbilt (D. Kosson).

e. Retrieve specimens as needed to form composites.

f. Primary responsibility for packing and shipping.

3.4. Laboratory Personnel (S. Burke, R. Shubert Chakavarty)

a. Become familiar with all protocols and procedures.

b. Maintain equipment and order supplies as needed.

c. Conduct appropriate laboratory procedures for sample preparation and adherence to QA/QC

d. Maintain appropriate laboratory notebooks, following established procedures.

e. Provide other personnel with timely information about any potential laboratory problems or work improvements.

4. PROCEDURES

4.1. Safety Precautions

a. Health and safety considerations come first. Training

and work will adhere to guidelines and standards set forth by Rutgers Environmental Health Services.

b. Appropriate gloves and protective eyewear will be used

c. Laboratory personnel will be informed of procedures for safe handling of chemicals and potential chemical and radiologic hazards. All chemicals will be treated with due care.

d. All specimens involved in this study have been screened on the ship with hand-held monitors, and do not pose a radiation hazard to laboratory personnel. Radioactivity of spiked specimens provided by INEEL-RESL will be below a level of health concern. In case of any spill or leakage of samples from INEEL-RESL, Rutgers REHS will be notified immediately, and all work will cease until REHS has cleared the laboratory for further work.

e. Periodic monitoring of laboratory samples, surfaces, supplies and equipment will be conducted.

-This will involve initial ambient sampling of the laboratory and its environment (to include 10 randomly selected sites in and around the Rutgers laboratories).

-Laboratory surfaces will be monitored with wipe samples at the start and the end of each day. These samples will be placed in vials and sealed for later counting (per D. Volz).

f. Any accidents or health problems will be reported immediately to the Project Director and QA director.

4.2. Training

a. Training with laboratory procedures is the responsibility of J. Burger, with QA/QC training conducted by M. Gochfeld.

b. All laboratory personnel will be trained prior to initiation of work. Only trained personnel will be involved in laboratory procedures.

4.3 Receipt of Specimens

a. Purpose: CRESP biological specimens are stored in coolers (ice-chests), located in a freezer in a Newark Storage facility. Groups of coolers will be delivered to Rutgers in stages, and specimens will be transferred to freezers at Rutgers for preparation, prior to shipment to analytical laboratories.

b. Coolers will be requested, according to needs for laboratory preparation (An Initial list of Coolers shipped from the Second Ocean Explorer Expedition can be found in Appendix A).

c. Shipment of coolers from Newark facility to Rutgers for the initial inventory will be handled by Henry Mayer.

d. Opening of coolers and inventory of specimens will be overseen by J. Burger and V. Vyas. Laboratory personnel will help

with all phases. After completion of the inventory, coolers will be requested as needed and samples will be deposited in the freezers at Rutgers, and followed by the Rutgers specimen tracker.

e. An initial step in the CRESP project is to inventory all coolers shipped from the Ocean Explorer and the NOAA trawler. The inventory of the coolers from the second expedition will be completed by mid-September. The coolers that contained largely unprocessed specimens were inventoried in early August and the remaining coolers from the second expedition will be inventoried by mid-September. All coolers will be brought to Rutgers and assorted according to type: birds, fish, invertebrates, kelp. In the second stage, all fish samples (except for archive samples) will remain at Rutgers because they are the first to be prepared for analysis, and others will be returned to Newark until needed. V. Vyas will maintain records of the specimens remaining in the coolers, and which ones have remained at Rutgers (their physical locations will be tracked by the specimen tracker).

Many of the coolers contain G samples, defined as those that contain muscle, liver and bone from one individual in one specimen bag prior to compositing.

f. Archive Samples: Coolers containing archived specimens will be opened in subsequently for the purpose of completing the CRESP inventory, and will be returned to storage (except where needed for inter-laboratory comparisons). This will allow us to keep track of specific archive specimens should they be needed for compositing and for larger samples needed for interlaboratory comparisons.

g. NOAA coolers contain fish collected on the NOAA trawler. When needed for sample preparation, they will be brought to Rutgers.

4.4. Sample tracking

a. There are two types of tracking: physical specimen tracking within the laboratory (which freezer have which specimens and retrieval of specimens as needed for compositing) and data tracking from the Rutgers laboratory to analytical laboratories and back.

b. Specimen tracking within the laboratory will be assigned to the Specimen Tracker overseen by J. Burger. This will require tracking specimens as to location and type so that a given specimen can be found at any time for processing to assure a smooth flow of samples.

c. A data tracking system for composites to be shipped to the analytical laboratories (INEEL, Vanderbilt) has been set up by V. Vyas, who will manage the electronic data base during and following analysis. There will also be laboratory notebook documentation of

sample distribution. Data tracking communicated to the analytical laboratories will include coded sample identification, wet weight of initial sample, and any quantity of distilled water added for processing or making samples up to designated volume.

d. All specimens shipped from Rutgers to both analytical laboratories will use the INEEL Chain of Custody to ensure consistency among laboratories and to fulfill the INEEL requirements.

e. An inventory of samples shipped will be electronically transmitted to the receiving laboratory, to D. Kosson, M. Stabin, M. Gochfeld, and C. Powers, and will be maintained in the CRESP data management system (V. Vyas). Shipment schedules will be coordinated with the appropriate laboratory manager at INEEL and Vanderbilt to ensure that samples are expected. Each shipment going to INEEL will also be accompanied by their Radioanalytical services Analysis Request Form.

4.5. Specimen selection

4.5.1. There are four levels of specimen selection.

a. Selection of species and locations for analysis

b. Selection of the order of specimens to be prepared in the laboratory.

c. Selection of composites for preparation

d. Selection of individuals (g samples containing liver, muscle and bone) for composites (some of which were made on the ship.

4.5.2. Selection of species for chemical analysis was completed in a 2-day meeting (2-3 August) with the PI, J. Burger, D. Kosson, M. Gochfeld, M. Stabin, D. Volz, and others based on an initial list provided by J. Burger (see Sample Selection Justification document, Appendix B).

4.5.3. Species selection for Screening Analysis was based on biological importance (for Aleuts, commercial fisheries, marine ecosystem assessment), distribution among sites (Milrow, Long Shot, Cannikin, Kiska), and sufficient abundance for compositing. The screening phase will cover the trophic levels: kelp, invertebrates, fish, birds. Species selected for Radiological Screening Analysis at INEEL are:

KELP

Alaria fistulosus
Alaria nana
Fucus

INVERTEBRATES

Green Urchin
Rock Jingle

FISH
Rock Greenling
Pacific Cod
Sculpin (Yellow Irish Lord)
Black Rockfish
Atka Mackerel
Walleye Pollock
Ocean Perch

BIRDS
Eider eggs (Common Eider)
Adult Gull (Glaucous-winged Gull)
Young Gull (Glaucous-winged Gull)
Tufted Puffin
Pigeon Guillemot

Additional samples will be analyzed for various gamma emitters at Vanderbilt. Soft tissue will be analyzed for all the above, and bone for fewer selected species.

4.5.3.1. Key species to analyze after we are sure of methods and have reviewed screening results include Eagle, Octopus, Brown Crab, Halibut, and Sea Lion. Based on a re-assessment after the screening phase, additional samples of certain species will be analyzed at INEEL to enhance power for statistical analysis.

4.5.3.2. An initial specimen flow chart for the INEEL samples (developed by D. Kosson/M. Stabin, reviewed by Burger/Gochfeld/Powers) will be used to design the flow of laboratory work. (Kosson draft dated 8-22-04).

4.6. Composite selection for the full radionuclide analysis at INEEL. Terminology: each of the four sites (Long Shot, Milrow, Cannikin, Kiska) had 4 to 6 transects, and each transect had 2 to 4 depth stations). Each site had an intertidal station. Selection of composites for the initial screening (1 from each of Milrow, Long Shot, Cannikin, Kiska, where appropriate) will be conducted by V. Vyas/J. Burger using a random-number, non- replacement process for those species from the diving transects (Alaria, Green Urchin, Rock Greenling), to ensure that not all are from the same station (transect, depth).

-For Puffins and Guillemots and mobile fish, the initial selection will combine Long Shot and Cannikin, and selection will

be random.

- For species with g samples, where physical compositing has not been completed, specimens will be identified for compositing, and composite numbers will be assigned in advance, to allow random selection. J. Burger/M. Gochfeld will select the individuals for these future composites, and V. Vyas/J.Burger will select the composite for full screening analysis using the random method outlined above.

4.7. An initial chart of samples to be selected for Cesium and other gamma analysis at Vanderbilt will be developed by J. Burger/M.Gochfeld in conjunction with D. Kosson and V. Vyas.

4.8. Specimens to be prepared each day will be selected by Burger/Gochfeld depending on the needs of the analytical laboratory and constraints of the preparation laboratory.

a. All steps will be recorded in a laboratory notebook (Burger team), and specimen tracking of composites (and archived specimens) will be entered into the CRESP computer data base (V. Vyas).

b. Laboratory notebooks will be initialed daily by personnel involved in each phase of sample preparation. Dr. Burger will review the laboratory notebooks periodically.

4.9. Soft Tissue and handling

a. Apparatus:

-appropriate safety equipment (gloves, safety glasses).
-dissection tools,
-appropriate balances and calibration weights
-several homogenizers with different blades
-aluminum foil
-laboratory containers (125 ml plastic bottles with screw tops)
-shipping containers
-acetone, nitric acid, deionized water, cleaning solution
-Chain of Custody forms
-filter paper for wipe samples

b. Preparation of final composites: Specimens to be composited have been or will be selected based on species, location, size and age.

c. Specimens will be removed from freezers for defrosting

d. Composites will be assembled mainly from ‘g’ specimens (with r samples added where needed). Field composites already assembled (‘C samples) will follow the same procedure (beginning in step e). The ‘g’ specimens will be dissected with acid-washed

instruments, with component parts placed on aluminum foil.

e. Components will be weighed and trimmed to 25 $\pm$ 1.0 gram (where possible depending upon available material). Depending upon the preferred final ash weight, composite samples are expected to be about 100 g (although less may be required for some species and matrices) in one nalgene jar for I-129, gamma counting and Sr-90, and 15 g in another container for Tc-99.

f. Homogenization. Each sample will be homogenized until a uniform blend is achieved. Based on preliminary methods development, this may require the addition of up to 25 ml DI water to allow for sufficient homogenization (volume added will be recorded in the notebook). Any addition of DI water will be documented to nearest 0.1 ml. For most specimen types this requires homogenizing individual samples, and then blending.

g. The five homogenates will be combined and then blended in a homogenizer for 10 min or until a uniform blend is achieved. When fewer than 5 individuals are used, an equivalent amount of tissue will be composited to produce the about 100 g (or less, depending upon availability of sample), and the 15 g sample. Both the weight of the initial homogenated sample, and the amount of water will be tracked. Where possible, no DI water will be added.

h. The main Homogenate (about 100 g or less) will be placed in DI rinsed and tared 125 ml bottles, labelled with the new code number, and taped according to INEEL protocol. The 15 g sample will be placed in bottles provided by INEEL (or to their specifications), using the procedure just described. Original and new code number will be recorded in the notebook.

i. The final weight of the residues will be recorded, and the samples will be placed in the agreed-upon jars. Both tare and final weight will be recorded. Samples will be frozen for later shipment. Any excess composited material will be saved as Composite r samples.

j. Excess sample, as was the procedure on the boat, will be placed in a separate bag with a Chain of Custody, given a code, and archived. Although for large specimens (i.e. Cod, some Halibut) there will be archival material (packed individually), it is expected that for small organisms there will be no excess material. Thus it is critical to save samples in the analytical stream that have not been destroyed during analysis.

k. Chain of Custody

1.-Original Chain of Custody forms will be retained in the laboratory, along with a copy of the new Chain of Custody that will go forward with the specimen (the INEEL Chain of Custody will be used for all samples sent to INEEL and Vanderbilt).

2-Copies of the Chain of Custody will be maintained

in the CRESP data base by V. Vyas.

3-New Chain of Custody forms will reflect the needs of the originating laboratory and analytical laboratory, and will be the INEEL form. Where possible, these will be maintained electronically.

4.10. Bone and Kelp Tissue Specimen handling

a. Apparatus includes a sonicator, microwave oven, the heavy duty soft tissue homogenizers, and special bone homogenizers. Hearing protection is required when using the sonicator.

b. Preparation of final composites: specimens to be composited will be selected based on species, location, size and age.

c. Specimens will be defrosted.

d. Individual bones will be cleaned with a combination of sonication and hand-picking following microwaving for 1-3 minutes.

e. Bones will then be crushed, weighed, and composited (normally 3-5 individuals), to make composites.

f. Optimally, 2 samples of bone will be prepared: a 100 g sample (homogenized to small pellets or powdered form) and a 2 g sample, each in separate nalgene jars. Where less is available, quantities would be reduced accordingly.

g. Optimally, 3 samples of kelp will be prepared: a 100 g sample, and two 10 g samples. Final quantities depend upon sample availability and upon drying/ashing weight methods development.

g. Crushed bone or kelp will be placed in DI rinsed and tared 125 ml bottles (and in smaller bottles provided or specified by INEEL for the 10 g samples), and procedures will follow those for soft tissue described above.

4.11. Procedures prior to (and after) any compositing.

a. Premise: It is essential to prevent cross contamination of all samples, at all stages.

b. A hand-held counter will be used between all composites to screen laboratory working surfaces. These screens will be recorded according to the Protocol developed by D. Voltz in collaboration with J. Burger/M. Gochfeld.

c. During all dissections and composites, all tissues will be placed on fresh aluminum foil to maintain integrity.

d. Between every composite preparation, all laboratory equipment and laboratory surfaces will be cleaned.

1.In the dissection and homogenization steps all glassware, utensils and counters will be acid washed (HNO_3) and rinsed with deionized water between each procedure.

e. At the end of the day, wipes of the laboratory surface

will be made, and these wipes placed in separate bottles, and labelled with the surface and date.

4.12. Inclusion of Spiked Samples

a. It is Standard Operating procedure (SOP) for spiked samples and blanks to be included in material sent to analytical laboratories.

b. Spiking of samples will be done by the INEEL-RESL laboratory and sent to Rutgers along with a paired blank, for re-coding and inclusion in samples subsequently sent to INEEL and Vanderbilt. These samples will be blended to the same consistency as other samples by RESL.

c. Pending methods development, bone and kelp samples will be sent to the INEEL laboratory for preparation of paired blanks and spikes (to achieve consistency), and returned to Rutgers for inclusion in samples subsequently sent to INEEL and Vanderbilt.

d. The RSEL laboratory will prepare the soft tissue (fish) samples for paired spikes and blanks using fish they purchase in commercial markets.

4.13. New Coded Sample Numbers

a. All composite specimens leaving the Rutgers laboratory will have a new number that does not identify location or species but does identify specimen type.

b. The new code will be assigned by J. Burger, M. Gochfeld and V. Vyas, in collaboration. An example of the coding system follows:

Tissue type-species type-number

B-A-1 where B = bone, A = species A (e.g. Halibut), 1 = first sample. All numbers will be sequential (i.e. no duplicate ID numbers)

c. Information necessary for analytic laboratories includes: type (bone, soft tissue, kelp), and species type (all fish and birds will be assigned a letter code so that type of species can receive special attention if required.

d. For tracking purposes, V. Vyas will generate a new data file which does not include the original sample identifier. A file correlating the coded sample numbers AND the original sample identifiers information will be maintained by V.Vyas, and coded information will be available only to J. Burger, M. Gochfeld, D. Kosson, V. Vyas and C. Powers.

4.14. Shipment

a. Shipment of all biological specimens (including spikes) for analysis will originate from Rutgers in insulated packages. Specimens will be shipped in 125 ml nalgene wide mouthed jars, enclosed in a container which contains a list of the sample numbers, and is sealed with a chain of custody form. The shipping boxes will contain frozen specimens, with blue ice (not dry ice). This will be placed in an outer shipping container and a packing list describing the contents as "Biological Samples for Laboratory Analysis" with a list of sample numbers) will be taped to the exterior.

b. Records of all shipments will be routinely emailed to D. Kosson, C. Powers, and V. Vyas, as well as the appropriate laboratory (Elias, Stabin). The receiving laboratories will email confirmation of receipt to Vyas, Kosson, Stabin, Gochfeld, Powers, and Burger.

5. Inventory Control

5.1. An ultimate goal is to have an inventory that includes the processed specimens (currently in the log books and in an electronic data base maintained by V. Vyas), as well as the unprocessed specimens and archived specimens.

5.2. The inventory process should be completed by late-September, and V. Vyas will produce an inventory list from specimens in all coolers.

5.3. When coolers were opened, specimens were recorded in a notebook for transfer into an electronic version. V. Vyas will track composites and g specimens (and a samples located in archive coolers).

APPENDIX A. INITIAL COOLERS STORED IN NEWARK (J Burger 8-4-04)

CODES: c = composites
g = muscle, bone, liver from one individuals for compositing
a = archive (usually fish muscle or kelp).
r = replicates (will be added to composites where additional mass is needed)

PRINCIPLES: To minimize the possibility of losing significant samples, we did not pack all the same species from the same location in one cooler. This list does NOT include coolers shipped by Jim Weston (NOAA trawler: Coolers 50-58).

LIST OF COOLERS (with main contents)

1. g for fish/birds
2. g for fish/birds
3. r for kelp
4. bird eggs/g for fish
5. g for fish/birds
6. g for fish/birds
7. r for kelp
8. r for kelp/g for fish
9. g for fish/birds/Aleut foods
10. r for kelp
11. g/r for birds/fish
12. g for birds/fish
13. r for algae/urchins
14. r for kelp
15. c for fish/algae
16. g for fish/birds/kelp
17. UNUSED NUMBER
18. c/g for fish/kelp
19. c for kelp
20. c for kelp
21. c/g for fish/urchins/limpets/mussels
22. eagle to be dissected

23-30: Archive coolers (not distinguished during packing)

40-48: Mainly unprocessed dive specimens, certain Aleut foods, and voucher specimens

40: urchins/jingles
41. chitons and kelp

42. jingles/kelp
43. jingles/kelp
44. fish/eider eggs
45. jingles/
46. chitons
47. jingles/chitons
48. r urchins

APPENDIX B: SELECTION OF SAMPLES FOR SCREENING

The overall objective of the Screening Analysis is to provide a broad base of data on a wide range of radionuclides to serve as a base for understanding the occurrence (and an indication of origin) of radionuclides in a wide range of organisms at different trophic levels in the marine ecosystem, for foods that are consumed by the Aleuts, and for organisms that are harvested commercially. This initial screen will serve as a basis for a additional analyses, depending upon the screening results, the relative importance of the organisms to as Aleut and commercial foods and to the marine ecosystem, and the availability and distribution of specimens across sampling areas.

The rationale for our choice of organisms for screening was based on our initial three-pronged approach (Aleut foods, commercial fisheries, marine food web) and the availability of organisms within the marine ecosystem around Amchitka and the Kiska reference site. Within these constraints, organisms were selected based on their mobility (Table 1) and life history traits (Table 2).

Our rationale for the number of organisms to be screened was a function of mobility (Table 3). When organisms were sedentary, we chose to screen 1 composite each from the three Amchitka test shots and 1 composite from Kiska. When organisms were mobile, we chose to screen 1 composite from the Bering side and 1 from the Pacific side of Amchitak (in the region of the test shots). When organisms were highly mobile we chose to screen 1 composite from Amchitka and 1 from Kiska (Table 2).

Organisms that are very important to the human food chain (i.e. Halibut, King Crab, Octopus) but were collected in much smaller numbers will be examined during the next phase of radionuclide analysis, as will organism that are key to marine food web (i.e. Eagle, other kelp or invertebrates).

TABLE 1. MOBILITY TRAITS INFLUENCING SELECTION OF SCREENING SPECIES

MOBILITY	IMPORTANCE	SPECIES
Sedentary	Provides an indication of point exposure	Fucus Alaria nana Alaria fistuloa
Locally mobile	Integrates exposure over a small area	Sea Urchin Rock Jingle Black Rockfish Rock Greenling Glaucous-winged Gull
Mobile	Provides an indication local movement within a few km of designated site	Yellow-Irish Lord Ocean Perch Walleye Pollock Tufted Puffin Pigeon Guillemot Common Eider Brown King Crab
Migratory	Provides an indication of regional exposure	Atka Mackerel Pacific Cod

TABLE 2: RATIONALE FOR SPECIES SELECTION FOR SCREENING ANALYSIS

PRIMARY PRODUCERS: The following species are all primary producers in the marine ecosystem, are sedentary (and thus represent local exposure), and are the base of food chains. There is good representation of the sedentary species from the four study sites (Milrow, Long Shot, Cannikin, Kiska), and for the mobile species from Amchitka and Kiska.

Alaria fistulosus - This kelp occurs at several depths, representing the subtidal environment.

Alaria nana - This kelp occurs mainly in the intertidal.

Fucus - This brown algae occurs in the intertidal, and there is reference data from other places.

INVERTEBRATES: Invertebrates are often the primary consumers in marine ecosystems, are eaten by organisms higher on the food chain, and are fairly sedentary representing local exposure. They are also eaten by the Aleut people.

Green Urchin - Urchins were abundant in most of the diving transects at 15, 30 and 60 feet and thus represent good coverage of the marine floor environment. They are a primary food of Sea Otters, a species of concern. They are also eaten by Eiders and Gulls (based on the literature and on stomach contents we examined). And they are considered a delicacy by Aleuts.

Rock Jingle - They are less abundant, but are sedentary.

VERTEBRATES: Vertebrates are often secondary or tertiary consumers, and have different degrees of mobility. The species selected, at some stage in their life cycle, are all eaten by Aleuts and some are part of commercial fisheries.

FISH:

Rock Greenling - This is a sedentary species, each male maintaining a small territory, hence representing local exposure, that lives in the kelp zone. It is eaten by Aleuts (as are its eggs), and is eaten by fish higher on the trophic chain, such as Cod and Gulls.

Black Rockfish - This is a relatively sedentary species (representing local exposure) that lives in the kelp zone and just

outside the kelp zone. It is eaten by Aleuts and is a little higher on the food chain than the Rock Greenling.

Sculpin (Yellow Irish Lord) - This is a less sedentary (but not migratory) species that is larger than Black Rockfish, eats invertebrates, and is an Aleut food.

Atka Mackerel - This is a deep water, bottom fish that is relatively low on the food chain, but is of commercial value and is migratory.

Pacific Cod - This fish can reach 50-60 pounds, and eats smaller fish, such as Rock Greenling and Atka Mackerel, as well as Octopus, squid, fish eggs, and crabs (all found in our specimen's stomachs). It is both a preferred fish for the Aleut people and a major commercial species. It is mobile to migratory.

Ocean Perch - Top level predator of commercial interest that is mobile.

Walleye Pollock - This predatory fish is a major commercial species that is mobile.

Halibut - This fish is a top-level predator, can reach large sizes (up to 500 pounds) and advanced ages, and is highly prized both by Aleuts and commercial fisheries, and is migratory.

BIRDS (all are year round residents on or near Amchitka):

Eiders - Common Eiders are hunted extensively by Aleuts and their eggs are also eaten. It represents a low trophic level for birds, eating mussels, snails, and urchins.

Gulls - Glaucous-winged Gull eggs are considered a delicacy by Aleuts, and gulls represent an omnivorous species. We found urchins, starfish, and fish (including Dolly Varden and Greenlings) in their stomachs. Since there are nesting colonies at each of the test sites, and they normally feed within 5 miles of their colony, they represent local exposure. They do not migrate and so represent longer term exposure in the vicinity of Amchitka. They also can live to be 30 + years old.

Young Gull - There were nesting colonies adjacent to each of the 3 test shot areas, and on Kiska. Since parents feed their young entirely from local foods (usually within 5 miles of nesting colonies), they represent local exposure.

Tufted Puffin - They eat entirely fish of small to intermediate sizes. They are less localized to test shots, and represent local exposure within a local area. Birds were moving back and forth from the Long Shot to the Cannikin shoreline.

Pigeon Guillemot - They eat mainly small fish and invertebrates, and are localized to the sides of islands during the breeding season. Birds were moving back and forth from the Long Shot to the Cannikin shoreline.

TABLE 3. RATIONALE FOR SELECTION OF SCREENING NUMBERS.

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1. Where possible, one sample from each of the four study sites (Milrow, Long Shot, Cannikin, Kiska) for species that are sedentary or are locally mobile will be selected for screening (N = 4 for screening purposes).

Fucus	Sea Urchin
Alaria nana	Rock Jingle
Alaria fistulosa	Black Rockfish
Glaucous-winged Gull	Rock Greenling
(adults, chicks)	Yellow-irish Lord

2. Species that are mobile within a few km of a designated site will be examined from both sides of Amchitka (Bering Sea/Pacific Ocean), and from Kiska (N = 3).

Common Eider (eggs)
Pigeon Guillemot
Tufted Puffin

3. Species that are highly mobile or migratory will be examined from Amchitka and from Kiska (N = 2)

Ocean Perch
Atka Mackerel
Walleye Pollock

4. Where specimens were available for both the inshore sampling and the NOAA trawl, species will be screened from both sampling methods. This was true only for Pacific Cod, and will include both sides of Amchitka as well as Kiska from the nearshore sampling, and from Amchitka and Kiska for the NOAA trawl (N = 5).

TABLE 4: SPECIMENS FOR THE INEEL SCREEN OF SOFT TISSUE **.

Kelp: TOTAL = 12
1. Alaria fistulosa - 4
2. Alaria nana - 4
3. Fucus - 4

Fish: TOTAL = 23
1. Black Rockfish - 4
2. Ocean Perch - 2
3. Atka Mackerel - 2
4. Rock Greenling - 4
5. Walleye Pollock - 2
6. Pacific Cod - 5
7. Sculpin - 4

Invertebrates: TOTAL = 8
1. Sea Urchin - 4
2. Rock Jingle - 4

Birds TOTAL = 17
1. Common Eider eggs - 3
2. Tufted Puffin - 3
3. Glaucous Gull
   adult - 4
   young - 4
4. Pigeon Guillemot - 3

** When the sample size is 4, one composite will be selected from near Milrow, Long Shot, Cannikin, and Kiska. When the sample size is 3, one composite will be selected from the Bering side of Amchitka, the Pacific side of Amchitka (near the test shot region), and from Kiska. When the sample size is 2, one composite each will be selected from around Amchitka and from Kiska.