STANDARD OPERATING PROCEDURE
For
Processing Fish & Other Biota Tissue Samples Intended for Contaminant Analysis

SOP #: Fish Process **REVISION #: 1.0** **DATE: August 2002**

MassDEP

Massachusetts Department of Environmental Protection
Division of Environmental Analysis
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TABLE OF CONTENTS

LIST OF REVISIONS

Rev. #	Date	Description of Revision	Page #
0	May 2002	None	-
1.0	August 2002	Assorted revisions/updates	Throughout document

LIST OF TABLES

Page

Not Applicable

1.0 SCOPE AND APPLICATION

This standard operating procedure (SOP) is based on the U.S. Environmental Protection Agency guidance document for fish sampling and analysis (USEPA, 1993) and covers the preparation of whole finfish (fish), shellfish, and lobster and other crustacean specimens after receipt and login at the MA DEP Senator William X. Wall Experiment Station (WES) through preliminary homogenization prior to contaminant analysis (i.e., for trace metals, organochlorine pesticides, and PCB Aroclors and congeners). Fish preparation SOPs for special analyses (i.e., VOCs, and SVOCs) are located on the WES w:\dea-qap shared drive. Fish samples received from the MA DEP Division of Watershed Management (DWM) are usually received at WES as filleted and/or prepared samples and are not covered by this SOP (see separate SOP – MA DEP, 2001).

2.0 SUMMARY OF METHOD

Prior to digestion or extraction for contaminant analysis, whole fish, shellfish and lobster and other crustacean specimens (i.e., received preferably on ice and not frozen) are processed as follows: 1) various physiological parameters are determined and recorded; 2) whole fish are filleted, shellfish are shucked, and lobsters and other crustaceans are picked; 3) the edible tissues are homogenized, packaged, and labeled; and 4) the prepared tissue samples are finally stored at -10 to -20°C.

3.0 DEFINITIONS

Not Applicable

4.0 INTERFERENCES

Not Applicable

5.0 SAFETY

5.1 Standard laboratory personal protective equipment (i.e., laboratory coat, eye protection, and gloves) must be worn at all times. The Material Safety Data Sheet (MSDS) for isopropanol is readily available to all laboratory staff.

5.2 All cutting, shucking, and homogenizing equipment must be used according to accepted safe practices and the manufacturer's instructions.

6.0 APPARATUS AND EQUIPMENT

6.1 Cutting board composed of glass or PTFE. Alternatively, the cutting board can be made of other materials (e.g., wood) if it is covered with heavy-duty aluminum foil that is changed after filleting, shucking, or picking each specimen.

6.2 Storage containers of molded high-density polyethylene (HDPE), such as VWR Scientific 44333 series containers. These containers are lot tested by storing blank tissues in them and have been found not to contaminate samples for the current suite of analytes (i.e., for mercury, arsenic, selenium, cadmium, lead, copper, chromium, organochlorine pesticides, and PCB Aroclors and congeners) and by the methods currently employed. For organic contaminants, the containers are also lot tested by adding 10 mL of hexane to a container, leaving the hexane in the container

for 2 hours, and then analyzing the hexane for organochlorine pesticides, and PCB Aroclors and congeners by GC/ECD (i.e., Modified AOAC Method 983.21).

6.3 Filleting knives of high quality, corrosion resistant stainless steel. These have been found not to contaminate samples for the current suite of analytes and by the methods currently employed.

6.4 Shucking knives of high quality, corrosion resistant stainless steel. These have been found not to contaminate samples for the current suite of analytes and by the methods currently employed.

6.5 Small surgical scissors of high quality, corrosion resistant stainless steel. These have been found not to contaminate samples for the current suite of analytes and by the methods currently employed.

6.6 Forceps of high quality, corrosion resistant stainless steel. These have been found not to contaminate samples for the current suite of analytes and by the methods currently employed.

6.7 Scalpels, with replaceable blades, of high quality, corrosion resistant stainless steel. These have been found not to contaminate samples for the current suite of analytes and by the methods currently employed.

6.8 Spatulas of high quality, corrosion resistant stainless steel. These have been found not to contaminate samples for the current suite of analytes and by the methods currently employed.

6.9 Knife sharpening equipment.

6.10 Permanent markers suitable for writing on the HDPE storage containers with ink resistant to moisture, heat, and cold.

6.11 Blender: Waring 1 Liter and 4 Liter, or Black and Decker Handy Chopper Plus, or equivalent base unit; 500-mL stainless steel or glass, 1-Liter stainless steel or glass, or 4-Liter stainless steel cups; or Black and Decker Handy Chopper blending cups and assemblies, or equivalent, as appropriate for the size of the sample.

6.12 Fish measuring board: A board-mounted measuring rule upon which a fish can be placed ventrally. This rule must be capable of measuring to the nearest 1mm.

6.13 Vernier calipers capable of measuring to the nearest 0.05 mm.

6.14 Small envelopes (e.g., coin envelopes) used to store scales or otoliths, optional.

6.15 A balance (Mettler Toledo Model BD6000) capable of weighing to the nearest gram for larger tissue samples and another balance (Mettler Toledo Model BD1201) capable of weighing to the nearest 0.1 gram for smaller samples (e.g., lobster tomalley).

7.0 REAGENTS AND CONSUMABLE MATERIALS

7.1 ASTM Type I reagent water

7.2 Detergent solution (Micro™ 2% solution in water or equivalent)

7.3 Pesticide-grade isopropanol

7.4 Heavy-duty aluminum foil

7.5 Nitrile gloves

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Fish, shellfish, and lobster and other crustaceans are collected by the MADEP Division of Watershed Management (DWM) and MA Division of Marine Fisheries (DMF), and submitted to WES for analysis. Sample collection and management procedures employed by these two groups are described elsewhere (MA DEP, 2001; MA DMF, 2002).

9.0 QUALITY CONTROL

Not Applicable

10.0 CALIBRATION AND STANDARDIZATION

10.1 Calibration of the balances in Section 6.15 is performed according to the procedures described in the WES Laboratory Quality Assurance Plan.

11.0 PROCEDURE

11.1 All fish processing procedures described below are performed in the WES Sample Preparation Clean Laboratory. Analysts must wash hands with soap and water, and put on nitrile gloves prior to commencing any fish processing procedure.

11.2 Finfish

11.2.1 Prior to the filleting process and between each specimen or sample composite, the cutting board, knives, and any other equipment to be used must be washed with the detergent solution, rinsed thoroughly with tap water, rinsed with pesticide-grade isopropanol, and then rinsed with ASTM Type I reagent water. Rinsing boards between specimens or composites may be omitted if the board is covered with heavy-duty aluminum foil, which is replaced between each specimen/composite (preferred method).

11.2.2. Fresh whole fish specimens submitted to WES must be iced immediately. Fish must remain on ice or refrigerated until processed. Upon arrival or shortly thereafter, all specimens must be inspected carefully to ensure that they have not been compromised in any way. Any specimen deemed unsuitable for analysis must be identified and immediately reported to the collector. The following physiological parameters must then be measured and recorded immediately for each fish – weight (see Section 11.2.4), length (see Section 11.2.5), and any morphological abnormalities – unless previously recorded in the field and submitted with the specimens.

11.2.3 Frozen whole fish specimens submitted to WES must be placed immediately in a suitable freezer at -10 to -20°C until processed. When ready to prepare a frozen specimen, remove it from the freezer and allow it to thaw only enough to be worked (approximately 2 hours). Alternatively, place frozen specimen in a refrigerator and allow it to thaw overnight. The fillets should still be stiff and ice crystals should still be present in the tissues. Inspect the specimens carefully to ensure that they have not been compromised in any way. Any specimen deemed unsuitable for analysis must be identified and immediately reported to the collector. Physiological parameters must then be measured and recorded as described above in Section 11.2.2.

11.2.4 Sample weighing: The wet weight must be determined for each whole fish specimen. Specimens must be weighed on a balance that has been properly calibrated and that provides adequate accuracy and precision to meet the data quality objectives of this program. Calibration must be checked before, at the end of, and after every 20 specimens during a weighing session. Weights must be recorded in a laboratory notebook or fish processing record to the nearest one gram, unless otherwise specified.

Specimens must be weighed on a foil-lined balance tray. Care must be taken with frozen specimens to be sure fluids are not lost. A clean, tared, non-contaminating container may be used for this purpose.

11.2.5 Specimen lengths are obtained by placing the fish onto a fish measuring board, head butting up to the stop. Take care to ensure that the specimen is flat on the board and that the measurement is taken at the correct tail fin location for the species being measured. Lengths should be recorded in a laboratory notebook or fish processing record to the nearest 1mm, unless otherwise specified.

11.2.6 Physiological abnormalities visible to the naked eye, such as tumors, fin rot, abnormal coloration, mouth deformities, parasites, etc., are also recorded in a laboratory notebook or fish processing record at this time.

11.2.7 At this point, each fish specimen is placed on the cutting board and cleansed of all mucous (slime), dirt or other foreign matter before proceeding. Pass the blade of a knife gently over the fish from head to tail, cleaning the knife as needed, until the entire side of the fish is cleaned. If necessary, clean the cutting board before doing the other side of the fish. Repeat process for other side. Clean the cutting board before proceeding, if necessary.

11.2.8 Age determination by scale or otolith (optional):

11.2.8.1 Scales, typically five to ten, are removed from the appropriate area of the fish specimen. A fisheries biologist familiar with that species should be consulted since the scale collection site may be species specific. Remove the scales by laying the fish flat on the cutting board and scraping the correct area from the tail end towards the head with the cutting edge of the cleaned knife blade. Slide the knife blade under the loosened scales to pick them up and transfer scales to a small, properly labeled, envelope and then seal the envelope. Minimally, the envelope must be labeled with the identifying sample field number.

11.2.8.2 Otoliths are removed by laying the fish flat on the cutting board and cutting diagonally tail-ward from the top of the eye to the top of the head into the inner ear, making sure not to damage the otolith. Widen the opening sufficient to insert a pair of forceps by bending the head and body apart. Some experience is necessary and this procedure may require the supervision of an experienced fisheries biologist familiar with the technique and species. Grasp the otolith with the forceps, remove, and place in a small, properly labeled, envelope and seal the envelope. Minimally, the envelope must be labeled with the identifying sample field number.

11.2.9 Sex determination (optional): When the person performing this procedure is not the one doing the filleting, the sex determination should occur prior to filleting; otherwise, the sex determination can be made at the time of filleting. To observe the gonads,

make an incision on the ventral surface of the body from a point immediately anterior to the anus to a point immediately posterior to the pelvic fins. In some specimens, a second incision may be necessary from the first incision toward the dorsal fin. Fold back the resulting flap and observe the gonads. Ovaries generally appear whitish to greenish to golden brown with a granular texture. Testes generally appear creamy white with a smooth texture. Spawning stage may also be noted at this time. Some experience is required, especially with immature specimens and the spawning stage; this procedure may require the supervision of a fisheries biologist familiar with this technique and the species.

11.2.10 Scaling (optional): If the fish specimen has scales and the fillet is to be tested with skin on, scaling is required; otherwise, this step is generally bypassed. To control cross-contamination, a separate set of utensils and cutting boards must be used for scaling and filleting. Alternatively, the cutting board may be covered with heavy-duty aluminum foil prior to scaling with the foil and scales removed after scaling and prior to filleting. If the same knife is used for scaling and filleting, the knife must be thoroughly cleaned as described in Section 11.2.1 before proceeding to the filleting step.

Scale the fish by laying it flat on the cutting board and scraping from tail to head with the cutting edge of the knife, taking care to not penetrate the skin. Turn the fish specimen over and repeat process for other side. Check to insure that all of the scales have been removed. Rinsing of the fish specimen with ASTM Type I reagent water may be necessary to remove all the scales.

11.2.11 Filleting:

11.2.11.1 Lay the fish specimen on the cutting board and with the knife, make a shallow cut through the skin from the top of the head to the base of the tail.

11.2.11.2 Make a cut behind the entire length of the gill cover. Cut through both the skin and flesh to the bone.

11.2.11.3 If not already present, make a shallow cut along the belly from the base of the gill cover to the tail. Do not cut into the gut cavity as this may contaminate the fillet.

11.2.11.4 Hold onto the skin at the top of the fish with your non-cutting hand. Carefully slide tip of knife between the tissue and the bone structure, lifting fillet as you cut. For flat fish, such as flounder, once you have cut to the middle of the fish, turn the fish 180 degrees and repeat the process from the ventral side of the fish until the entire fillet is separated from the bone structure.

11.2.11.5 Make a cut vertically through the skin and tissue to the bone structure at the base of the tail to detach the fillet.

11.2.11.6 Turn fish over and repeat steps 11.2.11.1 through 11.2.11.5. Visually inspect the internal organs, especially the gastrointestinal tract, for possible rupture and contamination of the fillets. Record any visible organ rupture.

11.2.11.7 To remove the skin from the fillet, place the fillet skin side down onto the cutting surface. Be sure to have the tail portion of the fillet towards you. Holding the skin at the tail end, carefully slide filleting knife between the skin and the muscle to separate. When there is sufficient skin separated, grasp the skin as you are sliding the knife along the skin muscle interface. Take

care to not cut through the skin. Repeat for the other fillet. When compositing samples, make sure that all equipment is clean before processing the next specimen for the composite. If necessary, rinse equipment with ASTM Type I reagent water before proceeding to the next fish specimen.

11.2.12 Homogenizing fillets:

11.2.12.1 Place both right and left fillets into a labeled tared blender cup previously cleaned using the procedure in Section 11.2.1 and appropriate for the fillet size. Large fillets may need to be cut into smaller pieces before placing into the blender cup. Composite samples are prepared by combining equal weights (± 10%) of tissue from each of the specimens to be combined into one sample; except as otherwise requested. Weigh blender cup and subtract tare weight to determine the wet weight of the fillet sample; record the fillet sample wet weight.

11.2.12.2 Firmly place lid on blender cup and place cup on correct blender base. Turn on blender starting at low speed and increasing the speed as necessary. Do not blend for longer than 1 minute at a time. The blender assembly may overheat and seize and/or the fish tissue temperature may increase above acceptable levels if blended continuously for longer periods. When you no longer hear the tissue blending, stop blender and scrape tissue from the sides of the blender cup to the bottom of the cup with a stainless steel spatula previously cleaned using the procedure in Section 11.2.1. Repeat blending and scraping until tissue is homogenous. The tissue should be homogenized to a paste-like consistency. Large quantities of tissue may strain the blender; to prevent overheating of the tissue, the blender cup (with tissue) should be set aside or refrigerated for a couple of minutes during blending.

11.2.12.3 Place homogenized tissue into a labeled HDPE storage container and analyze immediately or store in a freezer at -10 to -20°C until analyzed. After analysis is completed, the homogenized tissue sample is held in the freezer at -10 to -20°C (archived) as long as required by the program that submitted the sample for analysis.

11.3 Shellfish

11.3.1 Prior to the shucking process and between each specimen or sample composite, the cutting board, knives, and any other equipment to be used must be washed with the detergent solution, rinsed thoroughly with tap water, rinsed with pesticide-grade isopropanol, and then rinsed with ASTM Type I reagent water. Rinsing boards between specimens or composites may be omitted if the board is covered with heavy-duty aluminum foil, which is replaced between each specimen/composite (preferred method).

11.3.2 Fresh shellfish specimens submitted to WES must be iced immediately. Shellfish must remain on ice or refrigerated until processed. Upon arrival or shortly thereafter, all specimens must be inspected carefully to ensure that they have not been compromised in any way. Any specimen deemed unsuitable for analysis (e.g., dead or crushed shellfish) must be identified and immediately reported to the collector. The following physiological parameters must then be measured and recorded immediately for each

shellfish – length (see Section 11.3.5) and any morphological abnormalities – unless previously recorded in the field and submitted with the specimens.

11.3.3 Frozen shellfish specimens submitted to WES must be placed immediately in a suitable freezer at -10 to -20°C until processed. When ready to prepare a frozen specimen, remove it from the freezer and allow it to thaw only enough to be worked; the body tissues and body fluids should still be stiff and ice crystals should still be present in the tissues. Inspect the specimens carefully to ensure that they have not been compromised in any way. Any specimen deemed unsuitable for analysis (e.g., crushed shell) must be identified and immediately reported to the collector. Physiological parameters must then be measured and recorded as described above in Section 11.3.2.

11.3.4 Rinse all shellfish with ASTM Type I reagent water to remove mud and other debris from the outside of the shell.

11.3.5 Measure the shell length of each shellfish using a metric caliper.

11.3.6 Shuck each shellfish specimen of the sample into a labeled tared blender cup previously cleaned using the procedure in Section 11.3.1. Take the shucking knife and insert it between the shell halves, sever the adductor muscles and pry open the shell halves. Scrape body tissues and fluids with shucking knife into the blender cup. Note that the byssal threads from mussels should be removed with a knife or scissors before shucking and should not be included in the composite sample. Rinse shucking knife with ASTM Type I reagent water between shellfish specimens in a composite. Repeat until all shellfish specimens have been shucked for the sample; record the number of shellfish specimens that were included in the composite sample. Weigh blender cup and subtract tare weight to determine the wet weight of the composite sample; record the composite sample wet weight.

11.3.7 Homogenizing tissue: Firmly place lid on blender cup and place cup on the correct blender base. Turn on blender starting at low speed and increasing the speed as necessary. Do not blend for longer than 1 minute at a time. The blender assembly may overheat and seize and/or the blended tissue temperature may increase above acceptable levels if blended continuously for longer periods. When you no longer hear the tissue blending, stop blender and scrape tissue from the sides of the blender cup to the bottom of the cup with a stainless steel spatula previously cleaned using the procedure in Section 11.3.1. Repeat blending and scraping until tissue is homogenous. The shellfish composite should be homogenized to a paste- to soup-like consistency. Large quantities of tissue may strain the blender; to prevent overheating of the tissue, the blender cup (with tissue) should be set aside or refrigerated for a couple of minutes during blending.

11.3.8 Place homogenized tissue into a labeled HDPE storage container and analyze immediately or store in a freezer at -10 to -20°C until analyzed. After analysis is completed, the homogenized tissue sample is held in the freezer at -10 to -20°C (archived) as long as required by the program that submitted the sample for analysis.

11.4 Crustaceans

11.4.1 Prior to the picking process and between each specimen or sample composite, the cutting board, knives, and any other equipment to be used must be washed with the detergent solution, rinsed thoroughly with tap water, rinsed with pesticide-grade isopropanol, and then rinsed with ASTM Type I reagent water. Rinsing boards

between specimens or composites may be omitted if the board is covered with heavy-duty aluminum foil, which is replaced between each specimen/composite (preferred method).

11.4.2 Fresh crustacean specimens submitted to WES must be iced immediately. Crustacean specimens must remain on ice or refrigerated until processed. Upon arrival or shortly thereafter, all specimens must be inspected carefully to ensure that they have not been compromised in any way. Any specimen deemed unsuitable for analysis must be identified and immediately reported to the collector. The following physiological parameters must then be measured and recorded immediately for each crustacean – body length and any morphological abnormalities – unless previously recorded in the field and submitted with the specimens. For lobsters, it must be noted if the specimen is cull or double cull (i.e., number of front claws absent) and if the shell is paper, thin, soft, or hard.

11.4.3 Frozen crustacean specimens submitted to WES must be placed immediately in a suitable freezer at -10 to -20°C until processed. When ready to prepare a frozen specimen, remove it from the freezer and allow it to thaw only enough to be worked (approximately 2 hours). Alternatively, place frozen specimen in a refrigerator and allow it to thaw overnight. The body tissues and body fluids should still be stiff and ice crystals should still be present in the tissues. Inspect the specimens carefully to ensure that they have not been compromised in any way. Any specimen deemed unsuitable for analysis (e.g., crushed shell) must be identified and immediately reported to the collector. Physiological parameters must then be measured and recorded as described above in Section 11.4.2.

11.4.4 Picking crustaceans:

11.4.4.1 Tissue from the edible portions of the crustacean specimen are picked and homogenized. For example, edible tissues for lobster typically include the tail and claw meat, and the tomalley (i.e., hepatopancreas). Gonads (i.e., testes and ovaries) must also be removed and analyzed if consumed by the local population. The tomalley and gonads may be combined with the meat tissue (record that it has been added to the homogenate) or they can be analyzed separately – the latter is the preferred approach unless otherwise specified by the agency submitting the samples to WES for analysis (see Section 11.4.7).

11.4.4.2 Separate specimen into the following sections: claw and knuckles, body (cephalothorax), and tail.

11.4.4.3 Remove the meat from the claw and knuckles by first separating the claw from the knuckles. Separate the smaller section of the claw from the larger one, remove any tissue from the cartilage, and place into a labeled tared blender cup previously cleaned using the procedure in Section 11.4.1. Pour any liquid held in the claw sections into the blender cup. Scrape tissue from the larger claw section and from inside the smaller claw section with a cleaned (see procedure in Section 11.4.1) stainless steel spatula, and add to the blender cup. Note that these sections may need to be cut open with cleaned surgical scissors in order to access the claw meat tissue.

11.4.4.4 Remove knuckle meat by separating the knuckles and scraping out the tissue with a spatula, and add to blender cup. Note that knuckles may need to be cut open with the scissors in order to access the meat tissue.

11.4.4.5 Remove the tomalley, and if applicable, the gonads by holding the ventral and dorsal halves in separate hands and pulling the halves apart. The tomalley and gonads are located near the head above the gill chamber; generally they will be in the ventral portion but if the sample has been frozen they may be attached to the dorsal section. Scrape tissue (separate the tomalley, and if applicable, the gonads) into separate labeled tared sample storage containers (see Section 6.2), making sure not to include any other tissue, and then blend using a cleaned stainless steel spatula; or if requested by the data end user, add the tomalley and gonad tissue to the meat sample from Section 11.4.4.4.

11.4.4.6 Remove telsons and then extract meat from the tail section. Using the surgical scissors, cut along the mid ventral line from the posterior to the anterior. Push the meat out the anterior end with the spatula. Add to the blender cup from Section 11.4.4.4.

11.4.4.7 Repeat Steps 11.4.4.2 through 11.4.4.6 for other lobster specimens making up the composite samples; record the number of lobster specimens that were included in the composite samples. Weigh blender cups and subtract tare weight to determine the wet weight of the composite samples – i.e., tail and claw meat, tomalley, and if applicable, the gonads.

11.4.5 Homogenizing tissue: Firmly place lid on blender cup and place cup on correct blender base. Turn on blender starting at low speed and increasing the speed as necessary. Do not blend for longer than 1minute at a time. The blender assembly may overheat and seize and/or the blended tissue temperature may increase above acceptable levels if blended continuously for longer periods. When you no longer hear the tissue blending, stop blender and scrape tissue from the sides of the blender cup to the bottom of the cup with a stainless steel spatula previously cleaned using the procedure in Section 11.4.1. Repeat blending and scraping until tissue is homogenous. The tissue should be homogenized to a paste- to soup-like consistency. Large quantities of tissue may strain the blender; to prevent overheating of the tissue, the blender cup (with tissue) should be set aside or refrigerated for a couple of minutes during blending.

11.4.6 Place homogenized tissues (i.e., tail and claw meat, tomalley, and if applicable, the gonads) into separate labeled HDPE storage containers; analyze immediately or store in a freezer at -10 to -20°C until analyzed. After analysis is completed, the homogenized tissue samples are held in the freezer at -10 to -20°C (archived) as long as required by the program that submitted the sample for analysis.

11.4.7 If a total edible portion composite is requested to be analyzed, mix equal percent of the homogenized tail and claw meat, tomalley, and if applicable, gonad weights (e.g., 25% each of the tail-claw meat, tomalley, and gonad homogenate weights) in a labeled blender cup previously cleaned using the procedure in Section 11.4.1. Homogenize thoroughly and store as described in Sections 11.4.5 and 11.4.6, respectively. In addition to the analysis of the total edible portion composite, analyze separately the tail and claw meat, tomalley, and gonad composites.

12.0 DATA ANALYSIS AND CALCULATIONS

Not Applicable

13.0 METHOD PERFORMANCE

Not Applicable

14.0 POLLUTION PREVENTION

Refer to the WES Environmental Management System (EMS) policy and SOPs regarding pollution prevention.

15.0 WASTE MANAGEMENT

14.1 WES laboratories fully comply with all applicable federal, state, and local environmental regulations. WES is also committed to protecting the air, water, and land by minimizing and controlling all chemical releases from fume hoods, biological safety cabinets, and bench operations. Refer to the WES Environmental Management System (EMS) policy and SOPs regarding waste management.

14.2 Isopropanol is used in this method to clean cutting boards and utensils. Waste isopropanol is collected in sealed waste containers. Once the waste containers reach capacity, they are transferred to the WES hazardous waste storage room where they are emptied into a waste solvent drum. Within 180-days of waste accumulation, the waste solvent drum is transported off the premises by a licensed hazardous waste management contractor. Under the WES EMS, a chemical inventory database has been developed to track purchases and use of solvents and other hazardous materials, and the waste generated by the use of these chemicals.

14.3 Waste finfish, shellfish, and crustacean specimens and tissues are placed in plastic bags and held frozen until the day that the WES dumpster is picked up by our solid waste contractor. On the morning of the dumpster pick-up, all available waste specimen/tissue bags are placed in the dumpster.

16.0 REFERENCES

U.S. Environmental Protection Agency (USEPA). 1993. *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Vol.1 – Fish Sampling and Analysis.* EPA 823-R-93-002. USEPA, Office of Science and Technology, Office of Water, Washington, DC

Massachusetts Department of Environmental Protection (MA DEP). 2001. *Standard Operating Procedure for Fish Monitoring – Fish Collection and Preparation (CN 040.1).* MA DEP, Division of Watershed Management, Worcester, MA

Massachusetts Division of Marine Fisheries (MA DMF). 2002. *Seafood Monitoring and Field Sampling Work Plan – New Bedford Harbor Superfund Site.* MA DMF, Gloucester, MA

17.0 TABLES AND VALIDATION DATA

Not Applicable